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Reduced Growth in *Eustoma* under High Nutrient and Phytotoxic Organic Acid Concentrations and Recovery through Hot Water Conditioning of Continuously Cropped Soil

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Abstract. **Stunted vegetative growth and delayed or absent flowering are commonly observed in eustoma (*Eustoma grandiflorum*) when cultivated continuously in the same greenhouse soil. These effects are likely caused by the excessive accumulation of soluble salts and/or phytotoxic organic acids in the soil. This study aimed to clarify the mechanism of continuous cropping obstacles and formulate prevention measures of eustoma. Seedlings of eustoma 'Croma III White' were grown hydroponically with 0%, 25%, 50%, 75%, 100% (full), 125%, 150%, 175%, or 200% strength of Johnson's solution. Plant height, leaf area, and shoot dry weight increased steadily as solution strength increased from 25% to 125% [solution electrical conductivity (EC) of 2.4 dS·m^{-1}] and then gradually decreased as solution strength further increased from 125% to 200% (solution EC of 3.8 dS·m^{-1}). When grown hydroponically in 200% strength Johnson's solution, plant height, leaf area, and root length increased with increasing equimolar mixtures of organic acids, including maleic acid, benzoic acid, malic acid, and hydroxybenzoic acid, up to 1.2 to 1.6 mM and decreased thereafter. Node number and the percentage of flower bud visibility declined beyond 1.6 mM organic acid mixtures. Plants with 2.0 and 2.4 mM organic acid mixtures had the lowest net photosynthetic rate, stomatal conductance, transpiration, and intercellular carbon dioxide concentration. Plants had normal growth and produced flower buds when the continuously cropped soil was preconditioned with 100 °C reverse-osmosis water before planting.**

Eustoma (*Eustoma grandiflorum*) is an important and popular cut flower crop with a stable market demand. Nevertheless, the growth performance of eustoma was poor when grown successively for multiple years on the same land (Asao et al. 2007). The reasons for eustoma continuous cropping obstacles remain unclear and could be associated with improper soil nutrient status and/or autotoxicity.

Eustoma plants require high nutrient levels (Frett et al. 1988; Marchese et al. 2005) and continue to accumulate dry matter and minerals after the flower bud has become visible (de Camargo et al. 2004; Ushio and Fukuta 2010). The nutrient level is usually kept relatively high during vegetative growth (Alvarado-Camarillo et al. 2018; Hernández-Pérez et al. 2016; Mendoza-Villarreal et al. 2015), and salt accumulation in soil could be a problem without proper leaching (Mendoza-Villarreal et al. 2015).

Autotoxicity can occur after plants have been cultivated continuously for years on the same land or grown hydroponically without renewing the nutrient solution (Asao et al. 2001). Plant exudates causing growth inhibition in the successive monoculture have been documented for many horticultural crops (Asao and Asaduzzaman 2012; Weir et al. 2004). Growth inhibitors such as maleic acid, benzoic acid, malic acid, and hydroxybenzoic acid were detected in root exudates of eustoma grown in a closed hydroponic system (Asao et al. 2007).

Mitigation strategies for autotoxicity in eustoma have been previously reported. Asao et al. (2007) demonstrated that the reduced growth of eustoma after prolonged cultivation in a field might be avoided by amending the soil with activated charcoal at a rate of 600 kg·ha^{-1}. Foliar spray of histidine can promote growth and early flowering during autotoxicity in a closed hydroponic system, although the timing and doses of amino acid application remain unclear (Mondal et al. 2015). Eustoma growers are eager to find more easily obtained, economical, and practical alternatives. Soil conditioning by oven heating, steam heating, or hot water treatment has been reported to alleviate soil sickness caused by abiotic or biotic factors. Nie et al. (2007) heated the soil used to grow aerobic rice for 10 consecutive seasons at 60, 90, 120, or 150 °C for 12 h, and increased growth was observed for the 90 °C treatment. A similar treatment of the ground soil might overcome the stunted growth of eustoma after continuous cropping.

The objectives of this study were to evaluate the effects of salinity by supplying nutrient solution from low to high strengths, organic acids with potential autotoxicity under high salinity, and hot water and volume for conditioning the field soil on eustoma growth and flowering.

Materials and Methods

All of the experiments were conducted in a greenhouse situated at the Tainan District Agricultural Research and Extension Station in Taiwan (23°03′35.1″N, 120°20′27.5″E).

Expt. 1: Effects of various nutrient concentrations. Eustoma 'Croma III White' seedling plugs, each with three to four pairs of leaves, were thoroughly rinsed with deionized water to remove the root medium. Plants were then placed in nutrient solutions in 4-L plastic containers fitted with Styrofoam lids with holes to support the plants, with five plants per container. Each container was fitted with an air pump (Pro6000; Shiruba, Taichung, Taiwan) to aerate the nutrient solution. Plants were supplied with Johnson's solution (Johnson et al. 1957) at 0% (reverse osmosis water), 25%, 50%, 75%, 100% (full strength), 125%, 150%, 175% or 200% (double strength) strength. The nutrient solution pH was adjusted to 6.2 ± 0.2 following the methods described by Kirkby and Mengel (1967) and measured with a pH meter (ST3100; OHAUS, Parsippany, NJ, USA). The corresponding electrical conductivity (EC) values of each solution measured with a conductivity meter (inoLab® Cond7310; WTW, Wilheim, Germany) were 0, 0.6, 1.0, 1.6, 2.1, 2.4, 2.9, 3.4, and 3.8 dS·m^{-1}, respectively. The nutrient solutions were replaced weekly.

Expt. 2: Effects of various concentrations of organic acids with high-strength nutrient solution. Eustoma 'Croma III White' seedling plugs, each with three to four pairs of leaves, were treated with 200% strength Johnson's solution containing 0 (as the control), 0.4, 0.8, 1.2, 1.6, 2.0, or 2.4 mM (combined) of an organic acid mixture. This organic acid mixture consisted of maleic acid, benzoic acid, malic acid, and hydroxybenzoic acid (Sigma, St. Louis, MO, USA), with equal molarity. These acids have been identified from root exudates of eustoma (Asao et al.

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2007). Nutrient solutions were replaced weekly. The youngest fully expanded leaf pair was used to measure the photosynthetic parameters. A portable photosynthesis system (LI-6800; LI-COR, Lincoln, NE, USA) was used to measure net photosynthesis (*Pn*), stomatal conductance (*gs*), transpiration (E), and intercellular carbon dioxide (CO_2) concentration (Ci) under 700 μmol·m^{-2}·s^{-1} photosynthetic photon flux (*PPF*). Air flow was set at 600 μmol·s^{-1}. Water vapor and CO_2 entering the system were absorbed by desiccant (WA Hammond Drierite Co., Xenia, OH, USA) and soda lime (LI-COR). CO_2 entering the leaf chamber was provided by a CO_2 cartridge (LI-COR), and the concentration was set at 400 μmol·mol^{-1}. Leaf temperature was 32.5 to 36.2 °C. Relative humidity within the leaf chamber was 64.3% to 84.2%, and vapor pressure deficit ranged from 1.0 to 2.2 kPa.

The average noon *PPF* was 831 μmol·m^{-2}·s^{-1}, and the day and night temperatures during the experiments were 25 to 34 °C and 18 to 23 °C, respectively. Plant growth was measured 42 d after treatments, and the time (in days) to the appearance of visible flower buds was recorded. Plant height was measured from the base to the top of plant. Number of nodes formed from the first true leaf pair to the topmost visible leaf pair was recorded. Leaf area was determined by an area meter (LI-COR 3100; LI-COR). The length of the longest root was measured. Shoots and roots were oven-dried at 70 °C for 72 h to determine for dry weights.

The two experiments described were arranged in a completely randomized design, with three replications per treatment and five plants per replication. Linear and quadratic regression analyses were performed and presented using Sigma Plot 12.0 programming (SPSS, Chicago, IL, USA).

Expt. 3: Effects of water temperature and volume. Soil samples were collected from a greenhouse of a local grower who had continuously grown eustoma on the same land for 8 years. The soil was composed of 58% sand, 22% loam, and 20% clay (sandy loam). A 1 soil: 5 water test showed that the EC was 1.5 dS·m^{-1}, whereas a 1 soil: 1 water test showed that the pH was 7.9. The Mehlich-3 extraction method (Mehlich 1984) revealed that the soil contained 640, 982, 5632, and 1034 mg·kg^{-1} of P, K, Ca, and Mg, respectively.

Plastic pots (9-cm diameter) were filled with 600 mL (620–630 g) dry soil and irrigated with 100, 200, or 300 mL reverse-osmosis water, each at 25, 50, and 100 °C. Data loggers (HOBO Temperature Data Logger-U23-003; Onset Computer Co., Bourne, MA, USA) were used to record the changes of soil temperature of pots given 100 °C water at the three volumes. Sensors were placed at the bottom of the pot below the soil, with one per pot. After irrigation treatments, seedling plugs of eustoma 'Croma III White', each with three to four pairs of leaves, were then planted in these pots containing the conditioned soil. Plants were grown in a glasshouse with air temperatures of 19 to 36 °C and average noon *PPF* of 748 μmol·m^{-2}·s^{-1}.

Additional tests were conducted twice on 11 Mar 2019 (18–35 °C, 602 μmol·m^{-2}·s^{-1} noon *PPF*) and 28 Feb 2020 (18–36 °C, 560 μmol·m^{-2}·s^{-1} noon *PPF*) using the same 9-cm pots, and the continuously cropped soil was conditioned with 300 mL reverse-osmosis water at 25, 50, or 100 °C. Seedling plugs of eustoma were subsequently transplanted into these pots.

All plants were grown for 56 d in a glasshouse and fertilized with 5 g of 14N–5.2P–10.8K slow-release fertilizer (Hi-Control 14–12–13; JCAM-AGRI Co., Tokyo, Japan). Growth data similar to those in Expts. 1 and 2 were collected. There were three replications per treatment and five plants per replication. The differences between water temperature and irrigation volume were analyzed by the least significant difference at $P < 0.05$ using CoStat 6.4 (CoHort Software; Monterey, CA, USA).

Results and Discussion

Expt. 1: Effects of various nutrient concentrations. The results showed that plant height, leaf area, shoot dry weight, and shoot-to-root ratio of eustoma 'Croma III White' increased as Johnson's solution strength increased up to 100% to 125% (2.1–2.4 dS·m^{-1}) and exhibited a slight decrease with further increasing nutritional strength (Fig. 1A, C, D, and G). The node number was lowest in plants with reverse-osmosis water only and did not differ significantly between 25% (0.6 dS·m^{-1}) and 200% (3.8 dS·m^{-1}) strengths (Fig. 1B). Both the root length (Fig. 1E) and root dry weight (Fig. 1F) decreased as the nutrient solution strength increased from 0% to 200%. Plants with reverse-osmosis water only did not produce any visible flower buds after treatments for 42 d, whereas other treatments resulted in similar days to flower bud visibility (Fig. 1H).

Nutrient-deficient eustoma exhibited stunted shoot growth and did not produce visible flower buds, but more root growth and a lower shoot-to-root ratio occurred (Fig. 1). Depending on cultivars (Yashiro 1994) and environments (Chen et al. 2022), eustoma requires a certain number of leaves or nodes before flower bud formation. Nutrient deficiency resulted in fewer nodes and may delay or prevent

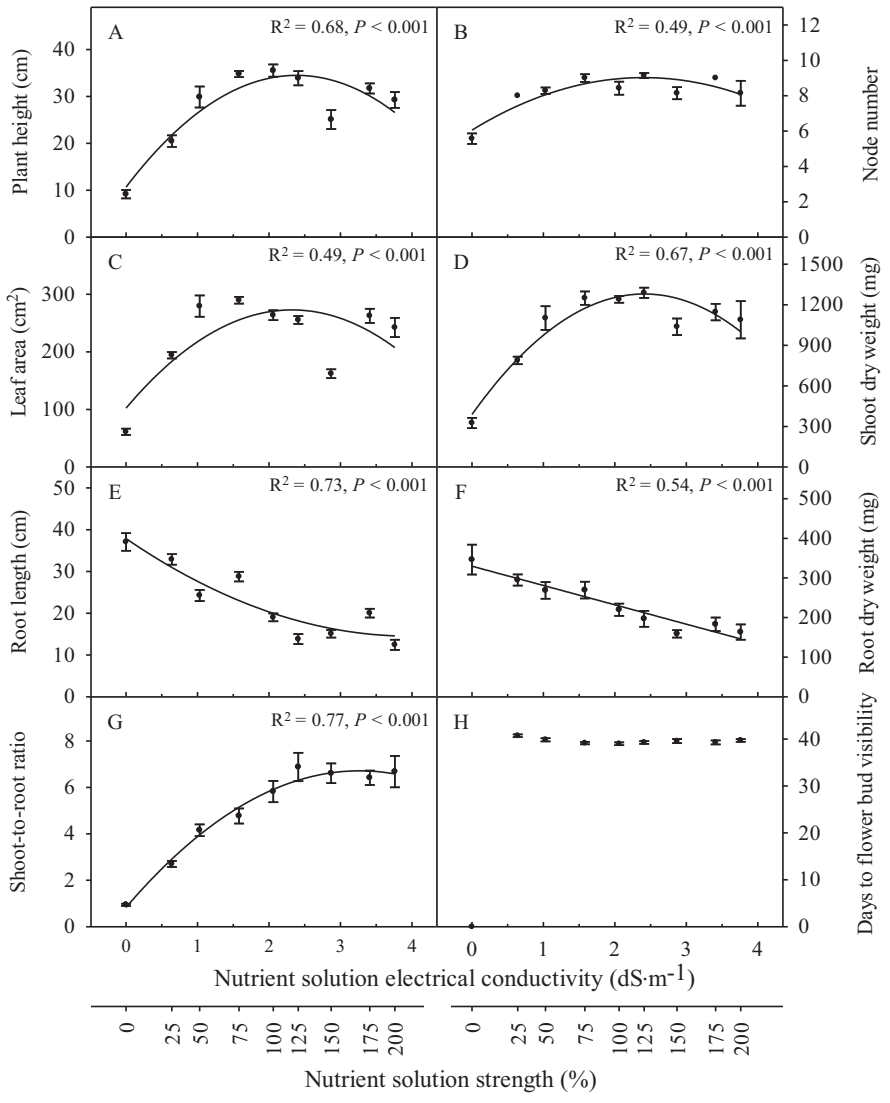

Fig. 1. Effect of the nutrient solution electrical conductivity (EC) on plant growth and days to visible flower bud of eustoma 'Croma III White'. Bars indicate the *SEM*. An additional x-axis is given to indicate Johnson's solution strength.

flowering. Preferential partitioning of photosynthetic carbon to the roots, which serves to decrease the shoot-to-root ratio, has been well-documented for plants under nitrogen or nutrient deficiency (Chen et al. 2018; Hermans et al. 2006; Yeh et al. 2000).

Peak shoot growths were obtained when supplied with 75% to 125% Johnson's solution with a solution EC ranging from 1.6 to 2.4 dS·m^{-1}. Plants given high nutrition levels (125%–200% strength of Johnson's solution, 2.4–3.8 dS·m^{-1}) exhibited only slightly reduced growth (Fig. 1), suggesting high salt-tolerance. Our results were consistent with previous reports indicating that eustoma, depending on salinity sensitivity, could be grown profitably when irrigated with saline water with EC of 4.1 to 7.4 dS·m^{-1} (Ashrafi and Rezaei Nejad 2018). In our study, plants with a high nutrient level up to 200% strength of Johnson's solution (3.8 dS·m^{-1}) still produced visible flower buds (Fig. 1) and did not express continuous cropping disorder, nutrient deficiency, or toxicity symptoms.

Expt. 2: Effects of various concentrations of organic acids with high-strength nutrient solution. Plant growth responses were determined with 200% strength of Johnson's solution containing several organic acids. Plant height, leaf area, and root length increased with increasing concentrations of the organic acid mixture up to 1.2 to 1.6 mM and then decreased thereafter (Fig. 2A, C, and E).

All plants had similar node numbers and produced visible flower buds at organic acid mixture concentrations of 1.2 mM or lower, whereas node numbers and the percentage of visible flower buds declined at higher organic acid mixture concentrations (Fig. 2B and H). No visible flower bud was observed with the 2.4 mM organic acid mixture concentration (Fig. 2H).

Asao et al. (2001) identified benzoic acid from root exudate in potted marigold (*Calendula officinalis*) and found that 100 μM benzoic acid increased, but that 400 μM resulted in reduced shoot fresh weight and root dry weight. Root exudates such as 50 to 400 μM maleic acid, benzoic acid, and *m*-hydroxybenzoic acid reduced eustoma growth (Asao et al. 2007). Our results showed reduced growth of eustoma under high-nutrient solution EC with high concentrations of maleic acid, benzoic acid, malic acid, and *m*-hydroxybenzoic acid but stimulated growth under low concentrations (Fig. 2). Roots and basal shoots are often left intact in the greenhouse field after harvesting eustoma cut flowers. This indicates that allelopathic chemicals from plant exudates combined with the accumulated salts would have accumulative adverse effects on eustoma growth, such as that resulting from the extension of continuous cropping.

The general trends of changes in photosynthetic parameters and growth in response to organic acid mixture concentrations were similar. The peak *Pn*, *g*s, E, and Ci were observed in plants grown with an organic acid mixture concentration of 1.6 mM (Table 1). Plants with organic acid mixture concentrations of 2.0 or 2.4 mM had the lowest *Pn*, *g*s, E, and Ci, indicating a stomatal limitation. Similar studies have reported that benzoic acid can reduce *Pn*, *g*s, E, and Ci in *Cucumis sativus* (Yu et al. 2003), and that hydroxybenzoic acid caused stomatal closure in *Glycine max* (Barkosky and Einhellig 2003). Decreased photosynthesis and growth have also been reported for peach seedlings when drenched with benzoic acid solution, but it was a dose-dependent reduction (Zhu et al. 2017).

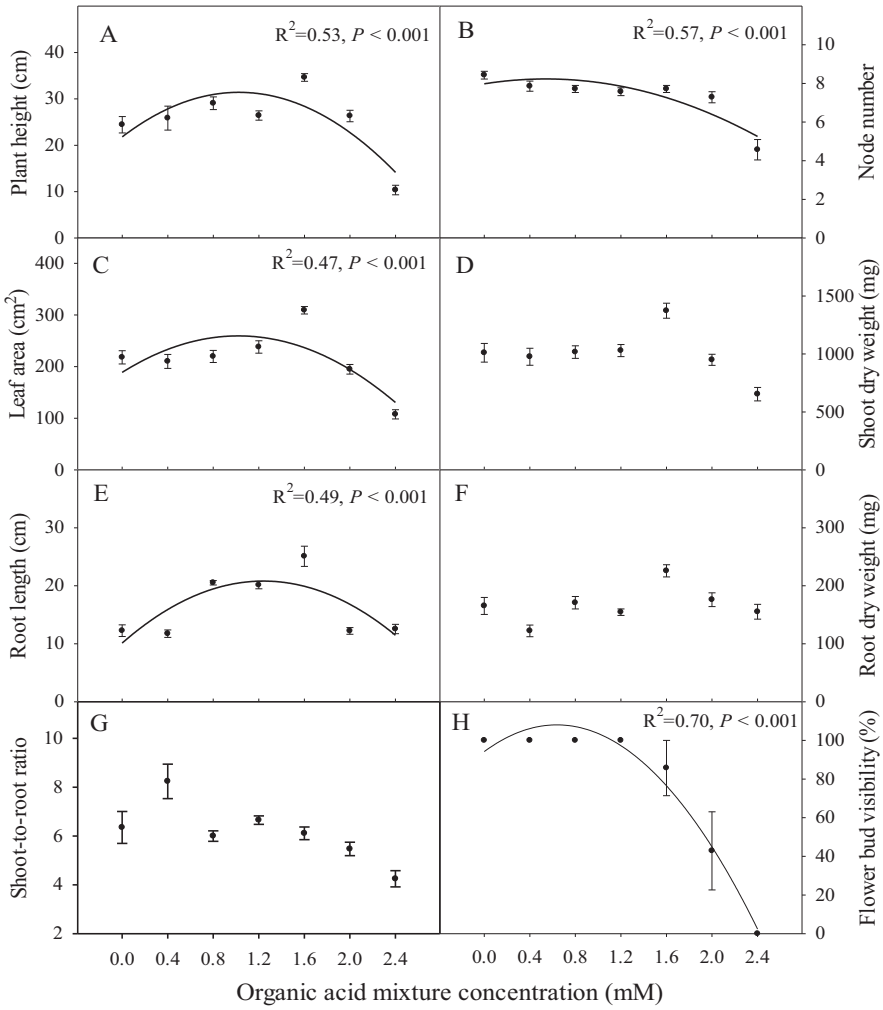

Fig. 2. Effect of organic acid mixture concentration on plant growth and percentage of flower bud visibility of eustoma 'Croma III White' with 200% strength of Johnson's solution. Bars indicate the *SEM*.

Expt. 3: Effects of water volume and temperature. Regardless of water temperature, leaching through pots was observed when irrigated with 300 mL water only, with ∼50 mL of leachate. Soil temperature at 10 cm below the soil surface (bottom of the soil column in pot) increased to a maximum of 40, 43, and 45 °C at 30 min after irrigating with 100, 200, or 300 mL 100 °C water, respectively (Fig. 3), and a maximum of 38 °C at 40 min after irrigating with 300 mL 50 °C water (data not shown).

Both water volume and water temperature significantly affected shoot and root dry weights (Table 2). Irrigation with 100 or 200 mL water did not result in leaching through the pot and did not significantly improve

Table 1. Effect of the organic acid mixture concentration on the net photosynthetic rate (*Pn*), stomatal conductance (*g*s), transpiration rate (E), and intercellular carbon dioxide concentration (Ci) of the fully expanded leaves of eustoma 'Croma III White' grown with 200% Johnson's solution at day 42 after treatment.

Organic acid concn (mM)	*Pn* (μmol CO_2·m^{-2}·s^{-1})	*g*s (mol H_2O·m^{-2}·s^{-1})	E (mmol H_2O·m^{-2}·s^{-1})	Ci (μmol·mol^{-1})
0.0	15.1 ± 2.5 ab[1]	0.22 ± 0.08 ab	4.0 ± 1.2 abc	250.1 ± 24.1 ab
0.8	15.0 ± 1.0 ab	0.37 ± 0.29 ab	5.3 ± 3.0 a	278.9 ± 45.1 ab
1.2	16.2 ± 1.7 ab	0.23 ± 0.08 ab	3.3 ± 1.1 abc	244.4 ± 36.3 ab
1.6	17.9 ± 0.2 a	0.43 ± 0.12 a	4.7 ± 1.5 ab	296.2 ± 16.5 a
2.0	14.4 ± 1.3 b	0.15 ± 0.04 b	1.6 ± 0.5 c	211.5 ± 32.9 bc
2.4	13.8 ± 0.9 b	0.14 ± 0.03 b	1.7 ± 1.3 c	210.5 ± 26.7 bc

[1] Data are means ± *SD*. Mean separation with columns according to the least significant difference at $P < 0.05$.

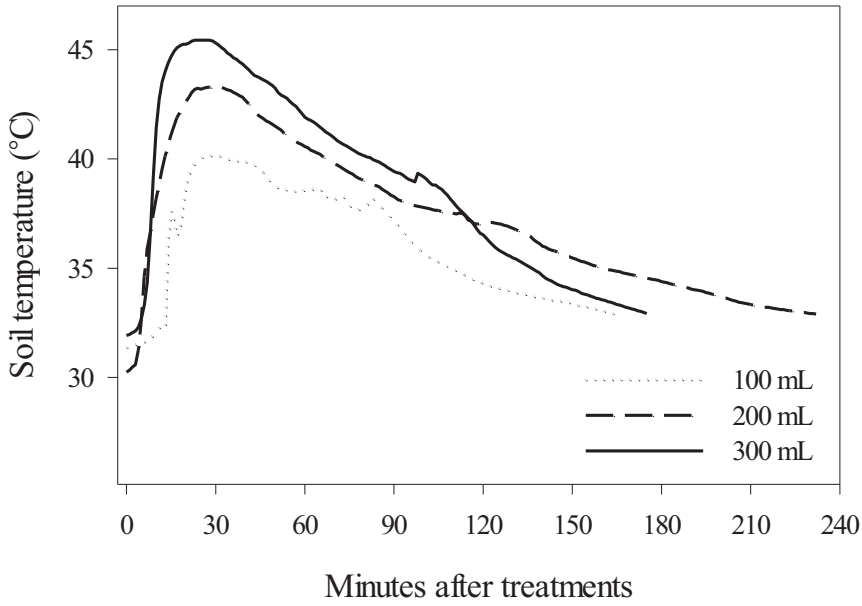

Fig. 3. Changes of soil temperature (10 cm below soil surface) after irrigating with different volumes of 100 °C water.

Table 2. Effects of water temperature and volume on shoot and root dry weights and percentage of visible flower bud of eustoma 'Croma III White' grown in continuously cropped soil.

Treatment		Dry wt (mg)		Flower bud
Water temp. (°C)	Volume (mL)	Shoot	Root	visibility (%)
25	100	666.4 ± 126.8 d[i]	207.5 ± 11.3 e	0
	200	760.9 ± 130.9 d	225.5 ± 71.3 e	0
	300	828.6 ± 075.6 cd	259.5 ± 30.9 de	0
50	100	724.1 ± 072.6 d	234.5 ± 47.1 e	0
	200	806.2 ± 095.9 d	238.3 ± 46.0 e	0
	300	1033.8 ± 133.8 c	328.3 ± 28.9 cd	100
100	100	1390.3 ± 174.8 b	396.6 ± 76.5 c	0
	200	1673.6 ± 227.1 a	484.9 ± 97.9 b	0
	300	1775.5 ± 203.9 a	575.6 ± 77.9 a	100

[i] Data are means ± *SD*. Mean separation with columns according to the least significant difference at $P < 0.05$.

shoot and root dry weights when compared with the 300 mL water treatments. Plants had increased growth and produced flower buds when leaching the continuously cropped soil with 300 mL of 50 or 100 °C reverse-osmosis water. However, the shoot dry weight was lower in plants that received 50 °C reverse-osmosis water compared with 100 °C reverse-osmosis water. Plants had maximum dry weight with visible flower buds after being irrigated with 300 mL of 100 °C reverse-osmosis water (Table 3), indicating the importance of soil heating. Generally, soil organic acids do not dissolve completely in water, whereas low-molecular-weight (46 to a few 100 Dalton) organic acid, such as malic and maleic acids, are more soluble compared with the high-molecular-weight (a few hundred to million Dalton) soil organic acids (Adeleke et al. 2017). Maleic acid exhibits high solubility in water of 78.8 g per 100 mL of water at 25 °C and 392.6 g per 100 mL of water at 97.5 °C (Zeitsch 2000). Our results indicated that leaching with 300 mL of 100 °C water could have a synergistic effect to mitigate continuous cropping obstacles through reducing excessive salts and soil organic acids (Table 3). Nie et al. (2007) also reported plant height, leaf area, and total biomass of rice increased after the continuous cropped soil was treated with heat at 90 °C for 12 h and 120 °C for 3 h.

Table 3. Effect of leaching water temperature on shoot and root dry weight of eustoma 'Croma III White' grown in continuously cropped soil.

	Dry wt (mg)		
Water temp. (°C)	Shoot	Root	Flower bud visibility (%)
	Expt. duration 11 Mar 2019–6 May 2019		
25	461.6 ± 50.8 c[i]	100.5 ± 5.5 b	0
50	540.2 ± 38.2 b	131.5 ± 11.5 a	0
100	607.8 ± 39.6 a	132.0 ± 29.1 a	100
	Expt. duration 28 Feb 2020–24 Apr 2020		
25	559.9 ± 85.3 b	120.0 ± 13.6 b	0
50	671.9 ± 225.5 b	150.4 ± 39.8 ab	0
100	1160.8 ± 356.8 a	183.5 ± 56.3 a	100

[i] Data are means ± *SD*. Mean separation with columns and experimental duration according to the least significant difference at $P < 0.05$.

In conclusion, stunted growth of eustoma may be caused by high soil or hydroponic solution EC and its root exudates under successive monocropping. The root zone EC value for hydroponic solution should be kept below 2.4 $dS \cdot m^{-1}$. Under salinity stress, organic acids at low concentrations could be beneficial to plant growth; however, accumulation of allelopathic chemicals and their synergistic effects could severely reduce eustoma growth and flowering. Plant residues should be removed after harvesting eustoma cut flowers because the soil organic acids usually originate from root and plant litter decomposition (Adeleke et al. 2017). The rhizosphere with complex interaction between root exudates, chemicals, and microbes can alter successive plant growth. Excessive accumulations of salts and phytotoxic organic acids are likely responsible for continuous cropping obstacles in eustoma. Hot water leaching treatment can be a readily accessible and practical solution to improve eustoma growth in continuously cropped soil.

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