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2,4-D and NAA supplementation mitigates autotoxicity of strawberry in hydroponics

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Abstract

In order to mitigate the autotoxicity in growing plants in closed hydroponic systems, the effects of foliar applications of 2,4-dichlorophenoxyacetic acid (2,4-D) and 1-naphthaleneacetic acid (NAA) on the growth of strawberry were investigated. Although the growth of strawberry plantlets was not affected by the auxin treatments in the fresh nutrient solution, the auxin treatments recovered the growth in the used nutrient solution. Benzoic acid, a compound reportedly accumulating in the reused nutrient solution of strawberry hydroponics, resulted in a significant decrease in the growth of strawberry plantlets at 50 μM concentration, compared to the growth in the nutrient solution without benzoic acid. Mitigation of the growth inhibition caused by the previously used nutrient solution or addition of the high concentration of benzoic acid in the fresh solution was demonstrated by immersing strawberry leaves in the auxin solutions (0.45 and 4.5 μM 2,4-D or 5.4 and 54.0 μM NAA) for two seconds before transplanting. The number of flowers and harvested fruits, and the fruit yield of strawberry plants grown in the greenhouse for about 33 weeks were reduced by the non-renewing the nutrient solutions. These values recovered in the 5.4 μM NAA treatment and were not significantly different from the control (renewal of the nutrient solution). These results suggested that reductions in the number of flowers and the yield of strawberry in closed hydroponic systems appear to be related to the allelochemicals exuded by the plant itself. The auxin such as NAA would avoid the growth reduction of strawberry caused by autotoxicity. The 5.4 μM NAA treatment may be the most effective for alleviating autotoxicity of strawberry and increasing the yield.

Key words: Autotoxicity, 2,4-dichlorophenoxyacetic acid (2,4-D), hydroponics, 1-naphthaleneacetic acid (NAA), strawberry (*Fragaria* × *ananassa* Duch.).

Introduction

Closed hydroponics is a system used for plant cultivation in environmentally sensitive areas (Van Os, 1995) where the nutrient solution is not released into the surrounding environment, but recycled (Ruijs, 1994). For such a reason, recently, closed hydroponics has been considered for strawberry cultivation (Takeuchi, 2000; Oka, 2002; Koshikawa and Yasuda, 2003). However, in a closed hydroponic system, plants could suffer autotoxicity, due to the accumulation of toxic exudates from the roots themselves in the nutrient solution (Yu *et al.*, 1993).

We suggested that the autotoxicity of strawberry in a closed hydroponic culture was caused by root exudates such as benzoic acid (Kitazawa *et al.*, 2005). The autotoxicity of strawberry reduced the shoot and root growth, number of flowers and harvested fruit per plant, and most notably, the fruit enlargement was inhibited. The vegetative and reproductive growth inhibition caused by root exudates was mitigated by the addition of activated charcoal into the nutrient solution (Yu and Matsui, 1993; Asao *et al.*, 1998; Sato, 2004). However, the activated charcoal adsorbed Fe-EDTA in the nutrient solution and subsequently caused Fe deficiency in plants (Yu *et al.*, 1993).

Phenolic compounds disrupt the balance of endogenous hormones in plants (Rice, 1984; Asao *et al.*, 2001). Some of substituted benzoic acids or the compounds having a structure similar to benzoic acid have been considered as anti-auxin (Van *et al.*, 1951; Keitt and Baker, 1966; Karabaghli-Degron *et al.*, 1998). It is known that auxin controls several fundamental functions including hormonal regulations for the plant development (Lomax *et al.*, 1995; Hobbie, 1998; Berleth and Sachs, 2001). Callis (2005) reported that the fruit expansion and maturation of strawberry depends on auxin. Thus, benzoic acid exuded from the strawberry roots may reduce the auxin activity and inhibit the fruit enlargement. Auxin treatments could support the recovery from inhibited fruit enlargement of strawberry, which was caused by autotoxicity in a closed hydroponic culture. In this study, we investigated the effects of the 2,4-dichlorophenoxyacetic acid (2,4-D) and 1-naphthaleneacetic acid (NAA) treatments on the growth inhibition of strawberry caused by autotoxicity.

Materials and methods

Effect of auxin treatments on the growth of strawberry plantlets in the nutrient solution used for strawberry culture: Strawberry (*Fragaria* × *ananassa* Duch. cv. 'Toyonoka') plantlets, each with 4 leaves, were used for the experiment. 10 strawberry plantlets were transplanted into a formed urethane block inside a plastic container (17 × 29 × 9.5 cm) containing 3 L of the nutrient solution freshly prepared or previously used for a strawberry hydroponic culture (continuously used for 8 months). The major ion concentrations (NO_3^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+} and Fe^{3+}) in the

used nutrient solution were adjusted to be as close as possible to a half strength of 'Enshi' nutrient solution (Hori, 1966). The full-strength nutrient solution contains the following amounts of salts per 1000 L of tap water: 950 g of $Ca(NO_3)_2 . 4H_2O$; 810 g of KNO_3; 500 g of $MgSO_4 . 7H_2O$; 155 g of $NH_4H_2PO_4$; 3 g of H_3BO_3; 2 g of $ZnSO_4 . 7H_2O$; 0.05 g of $CuSO_4 . 5H_2O$; 0.02 g of $NaMoO_4 . 2H_2O$. We used a reflectometer (RQflex2; Merck, Darmstadt, Germany) for NO_3^-, the molybdenum blue molecular absorption spectrometric method (Murphy and Riley, 1962) for PO_4^{3-} and an atomic absorption spectrometer (Z-5010; Hitachi, Tokyo, Japan) for K^+, Ca^{2+}, Mg^{2+} and Fe^{3+} analyses. The solutions were not renewed during the two week experiment.

The leaves of strawberry plantlets were immersed in aqueous solutions of 2,4-D at 0 (control), 0.45 and 4.5 μM, and NAA at 0 (control), 5.4 and 54 μM with 0.4% ethanol for 2 seconds before transplanting. The containers were placed in a growth chamber at 25° with a light intensity of 74-81 μmol s^{-1} m^{-2} and a 16 h photoperiod provided by the white fluorescent tubes. The plantlets were grown for 2 weeks, and then the number of leaves per plant, the dry weights (DW) of shoots and roots, and maximum root length per plant were measured.

Effects of auxin treatments on the growth of strawberry plantlets in nutrient solution with benzoic acid: Strawberry plantlets, each with 4 leaves, were used for this experiment. A half strength 'Enshi' nutrient solution containing benzoic acid at 0 (control), 5 or 50 μM was prepared (EC=1.36 dS m^{-1}). Only the leaves of strawberry were immersed in aqueous solutions of 2,4-D at 0 (control), 0.45 and 4.5 μM, and NAA at 0 (control), 5.4 and 54 μM with 0.4% ethanol for 2 seconds before transplanting. The growth conditions were same as the previous experiment. To minimize the effect of microbial degradation of the organic acids (Sundin and Waechter-Kristensen, 1994), the solutions in the containers were renewed every 3 or 4 days. The plantlets were grown for 2 weeks, and then the number of leaves per plant, the DW of shoots and roots, and maximum root length were measured.

Effects of non-renewal of nutrient solution and auxin treatments on strawberry growth and yield in greenhouse: Strawberry plantlets, each with 5 leaves, grown in foamed urethane blocks as a support, were transplanted on 21 October, 2003, into plastic containers (54 × 34 × 20 cm) filled with 30 L of continuously aerated (3.8 L min^{-1}) half strength 'Enshi' nutrient solution in the Shimane University greenhouse. 6 ml of the aqueous solutions of 2,4-D at 0, 0.45 and 4.5 μM, and NAA at 0, 5.4 and 54 μM with 4% ethanol were sprayed on leaves every 2 weeks. These nutrient solutions were not renewed throughout the experiment period. A control treatment that the nutrient solutions were renewed at 2-week intervals was prepared. 3 plantlets were planted in each container, and 4 containers were used for each treatment as replicates. Nutrient concentrations (NO_3^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+} and Fe^{3+}) in the solution were adjusted at 2-week intervals as close as possible to be the initial concentration on the basis of chemical analyses by the same methods described previously. In all treatments, the EC and pH in the nutrient solutions ranged from 1.28 to 1.38 dS m^{-1} and 6.67 to 7.57, respectively. Pollination was aided by vibrating the plants with a soft brush at 2-day intervals. The fruits were collected when ripe. The mean air and water temperature during the experiment ranged from 7.1 to 31.2°, and from 7.4 to 28.3°, respectively. At the end of the experiment (10 June, 2004), the number of leaves per plant, the fresh weight (FW) and DW of the shoots, the DW of roots, and maximum root length per plant were measured. During cultivation, the number of flowers, flower clusters per plant, and beginning dates of harvest, mean fruit weight per plant, the number of harvested fruits, and yield per plant were recorded.

Results

Bioassays in the fresh and used nutrient solutions: In the fresh nutrient solution, the number of leaves and the DW of shoots were not significantly different among all five treatments (Table 1). In the used nutrient solutions, the number of leaves and the DW of shoots decreased to 39 and 32% of control values, respectively, and these recovered by the auxin treatments. The DW of roots was not significantly different among all treatments. The maximum root length was not significantly different among all treatments in the fresh nutrient solution. In the used nutrient solution, however, the maximum root length decreased to 45% of control value, and was recovered to the level not significantly different from that in the control, by the 5.4 μM NAA treatment.

Table 1. Effects of used nutrient solution and auxin treatment on the growth of strawberry plantlets

Nutrient solution[z]	Auxin treatment (μM)		Number of leaves plant^{-1}	DW of shoots plant^{-1} (g)	DW of roots plant^{-1} (g)	Maximum root length (cm)
Fresh	— (Control)		5.1a[y]	0.078a	0.047a	13.1a
Fresh	2,4-D	0.45	5.8a	0.067a	0.043a	12.0ab
Fresh	2,4-D	4.5	6.0a	0.070a	0.045a	10.7abcd
Fresh	NAA	5.4	5.8a	0.079a	0.049a	11.5abc
Fresh	NAA	54.0	5.8a	0.073a	0.050a	13.1a
Used	— (Control)		2.0b	0.025b	0.039a	5.9e
Used	2,4-D	0.45	5.2a	0.058a	0.042a	8.8cde
Used	2,4-D	4.5	5.2a	0.056ab	0.047a	9.5bcd
Used	NAA	5.4	6.2a	0.070a	0.048a	10.8abcd
Used	NAA	54.0	4.8a	0.048ab	0.049a	8.2de

[z]Fresh: the fresh nutrient solution. Used: nutrient solution used for strawberry culture but nutrient concentration adjusted to Fresh.
[y]Values in a column followed by a different letter differ significantly by Tukey's test (P=0.05).

Table 2. Effects of added benzoic acid in nutrient solution and auxin treatment on the growth of strawberry plantlets

Benzoic acid (μM)	Auxin treatment (μM)		Number of leaves plant^{-1}	Dry wight of shoots plant^{-1} (g)	Dry wight of roots plant^{-1} (g)	Maximum root length (cm)
0[z]	(Control)		5.5a[y]	0.098a	0.060a	11.4a
0	2,4-D	0.45	4.6a	0.112a	0.068a	12.7a
0	2,4-D	4.5	6.1a	0.080a	0.059a	10.9a
0	NAA	5.4	4.7a	0.111a	0.071a	12.7a
0	NAA	54.0	5.6a	0.118a	0.069a	12.8a
0	(Control)		5.5a	0.098a	0.060a	11.4a
5	-		5.6a	0.107a	0.056a	11.3a
5	2,4-D	0.45	6.0a	0.073b	0.054a	10.6a
5	2,4-D	4.5	7.1a	0.100a	0.060a	12.4a
5	NAA	5.4	5.3a	0.110a	0.064a	12.5a
5	NAA	54.0	5.6a	0.086a	0.054a	10.8a
0	(Control)		5.5a	0.098a	0.060ab	11.4ab
50	-		5.0a	0.075b	0.055b	10.6b
50	2,4-D	0.45	5.4a	0.090ab	0.056b	10.1b
50	2,4-D	4.5	6.0a	0.107a	0.068a	10.1b
50	NAA	5.4	5.4a	0.102a	0.059b	11.3ab
50	NAA	54.0	6.0a	0.095a	0.051b	12.6ab

[z]Benzoic acid 0 μM with non-auxin treatment was control at each concentration.
[y]Values in a column followed by a different letter differ significantly by Tukey's test (P=0.05).

Bioassay in the presence of benzoic acid: The number of leaves was not significantly affected by the foliar auxin treatments, regardless of the benzonic acid concentration (Table 2). The DW of shoots and roots, and maximum root length were significantly not affected by the auxin treatment in the nutrient solution containing 0 and 5 μM benzoic acid, except for the shoot DW in the 0.45 μM 2,4-D treatment combined with 5 μM benzoic acid in the nutrient solution. With 50 μM benzoic acid without auxin treatments, the DW of shoots decreased to 77% of the control value. All auxin treatments increased the DW of shoots to the level similar to the control. The values of DW of roots in the 4.5 μM 2,4-D treatment was greater than the control value and those in the other auxin treatments. Maximum root length was not significantly affected by the auxin treatments in the nutrient solution containing 50μM benzoic acid.

Effects of non-renewal of the nutrient solution on the growth of strawberry plants: In non-renewed nutrient solution treatment, the number of leaves, FW and DW of shoots decreased significantly to 35, 33 and 37% of control values, respectively (Table 3). These values recovered by the auxin treatments. Regardless of the auxin treatment, the DW of roots was not significantly different among the non-renewed nutrient solution treatment although the DW of roots tended to increase by the auxin treatment. The maximum root length was not significantly different among all treatments.

The number of flowers decreased significantly to 75% of the control value in the non-renewed nutrient solution treatment without auxin treatment (Table 4). It recovered by the 4.5 μM 2,4-D, 5.4 μM NAA, and 54 μM NAA treatments to 90, 99 and 91% of the control values, respectively. The number of flower clusters, the beginning dates of harvested fruit, and the mean fruit weight per plant were not significantly different among all treatments. In the non-renewed nutrient solution without auxin treatment, the number of harvested fruit and the yield decreased significantly to 56 and 58% of the control values, respectively. However, a significant recovery of fruit number and yield was found in the auxin treatments. The 5.4 μM NAA treatment had both fruit number and yield similar to those of the control.

Table 3. Effects of non-renewal of nutrient solution and auxin treatment on the growth of strawberry plants

Nutrient solution	Auxin treatment (μM)		Number of leaves plant^{-1}	Fresh weight of shoots plant^{-1}(g)	Dry weight of shoots plant^{-1}(g)	Dry weight of roots plant^{-1}(g)	Maximum root length(cm)
Renewed[z]	(Control)		31.0a[y]	108.9a	31.0a	7.8b	48.5a
Non-renewed	-		11.0b	35.6b	11.5b	8.5ab	31.5a
Non-renewed	2,4-D	0.45	38.0a	97.7a	28.9a	11.2a	51.0a
Non-renewed	2,4-D	4.50	26.5a	97.4a	27.9a	10.7a	40.0a
Non-renewed	NAA	5.40	25.5ab	121.1a	33.5a	10.3ab	46.5a
Non-renewed	NAA	54.0	32.0a	83.3ab	25.3a	10.7a	46.0a

[z]Complete renewal of the nutrient solution every other week (control).
[y]Values in a column followed by a different letter differ significantly by Tukey's test (P=0.05).

Table 4. Effects of non-renewal of nutrient solution and auxin treatment on number of flowers and flower clusters, fruit weight, harvested fruits and yield on strawberry plants

Nutrient solution	Auxin treatment (μM)		Number of flowers plant^{-1}	Number of flower clusters plant^{-1}	Beginning dates of harvested fruit (month day^{-1})	Mean fruit weight per plant (g)	Number of harvested fruits plant^{-1}	Yield per plant (g)
Renewed[z]	— (Control)		30.5a[y]	6.0a	2/24a	11.9a	49.5a	588.6a
Non-renewed	—		22.8c	4.0a	2/24a	12.5a	27.5c	340.8c
Non-renewed	2,4-D	0.45	23.5c	5.5a	2/24a	12.2a	40.0b	477.0b
Non-renewed	2,4-D	4.50	27.3b	5.5a	2/24a	12.9a	39.0b	500.3ab
Non-renewed	NAA	5.40	30.3a	5.0a	2/24a	13.0a	43.0ab	549.6ab
Non-renewed	NAA	54.00	27.8ab	5.5a	2/24a	12.9a	38.3b	492.7ab

[z]Complete renewal of the nutrient solution every other week.
[y]Values in a column followed by a different letter significantly by Tukey's test (P=0.05).

Discussion

The inhibition of vegetative growth in strawberry was reported as a result of autotoxic root exudate due to non-renewal of the nutrient solution resulted from the autotoxic root exudates (Kitazawa *et al.*, 2005). There were some reports that phytotoxic substances accumulated in the nutrient solution that was used for hydroponic culture (Yu and Matsui, 1993; Asao *et al.*, 1999). In our study, the growth of strawberry plantlets was inhibited in the nutrient solutions that were previously used for the strawberry culture (Table 1). This nutrient solution may contain potential substances inhibiting strawberry growth. Although the growth of strawberry plantlets was not affected by the auxin treatments in the fresh nutrient solution, the auxin treatments recovered the growth of strawberry plantlets in the used nutrient solution. It has been known that auxin controls several fundamental functions of the plant development such as cell expansion and division, lateral root formation, vascular differentiation, and shoot elongation (Lomax *et al.*, 1995; Hobbie, 1998; Berleth and Sachs, 2001). Thus, our results suggested that the exudates from strawberry roots inhibited vegetative growth of strawberry plantlets, which could be alleviated by the auxin treatments. The 5.4 μM NAA treatment appeared to be the most effective in ameliorating the inhibited vegetative growth of strawberry plantlet grown in the non-renewed nutrient solution.

Kitazawa *et al.* (2005) reported that benzoic acid was the strongest inhibitor of vegetative and reproductive growth in strawberry. Shann and Blum (1987) have reported that cucumber plants absorbed phenolic acid. It was suggested that phenolic compounds disrupt the balance of endogenous hormones in plants (Rice, 1984). Thus, benzoic acid exuded from the roots may be absorbed and disrupt the balance of endogenous auxin in strawberry. In our study, the effects of auxin on growth inhibition caused by benzoic acid added into the nutrient solution were investigated. The 50 μM benzoic acid resulted in a significant decrease in the growth of strawberry plantlets, compared to the strawberry growth in the nutrient solution without benzoic acid (Table 2). The inhibition of the DW of shoots caused by addition of benzoic acid could be avoided by the auxin treatments. Some of substituted benzoic acids or the compounds having a benzoic acid like structure such as *trans*-cinnamic acid (Van *et al.*, 1951), chlorobenzoic acids (Keitt and Baker, 1966), and 2,3,5-triiodobenzoic acid (Karabaghli-Degron *et al.*, 1998), are considered anti-auxin. Thus, our results suggest that benzoic acid as anti-auxin caused the reduced growth of strawberry, and the addition of auxin could ameliorate the growth inhibition.

The strawberry plantlet grown in the non-renewed nutrient solution resulted in a significant decrease as compared to the renewed nutrient solution (control) (Table 3). Vegetative growth of strawberry was probably inhibited by root exudates in the non-renewed nutrient solution. The growth inhibition could be avoided by the auxin treatments, especially in the 5.4 μM NAA treatment.

Asao *et al.* (2001) reported that the number of flowers in cucumber was unaffected and the number of harvested fruit decreased by autotoxicity. In our study, there were no significant differences in the number of flower clusters, the beginning dates of harvested fruit, and mean fruit weight in strawberry between the renewed and non-renewed nutrient solution (Table 4). The number of flowers and harvested fruit, and fruit yield were reduced by the non-renewing the nutrient solutions. These growth parameters recovered in the 5.4 μM NAA treatment and were not statistically different from the control (renewal of the nutrient solution). Callis (2005) reported that fruit expansion and maturation of strawberry depended on auxin. It was suggested that auxin affected the reproductive growth such as expression (Galum *et al.*, 1965) and formation (Kondo *et al.*, 1999; Reinhardt *et al.*, 2000) of flower. Similar to these findings, our study suggests that the number of flowers and harvested fruit of strawberry could be reduced by autotoxicity, and an auxin may have some effects to ameliorate autotoxic growth inhibition.

In conclusion, reductions in the number of flowers and the yield of strawberry in a closed hydroponic system appears to be related to the allelochemicals exuded by the strawberry plant itself. The auxin such as NAA would avoid the growth reduction of strawberry caused by autotoxicity. The 5.4 μM NAA treatment may be the most effective for alleviating autotoxicity of strawberry and increasing the yield.

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