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Mitigation of cucumber autotoxicity in hydroponic culture using microbial strain

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Abstract

Effects of a microbial suspension (bacterial strain) on the autotoxicity of cucumber plants grown by hydroponic culture with or without addition of 2,4-dichlorobenzoic acid (DCBA) to the nutrient solution were investigated. The growth and fruit yield of cucumber plants significantly decreased on the addition of DCBA (10 μmol /liter) to the nutrient solution. The growth, however, recovered upon addition of the microbial strain. The yield reduction of cucumber plants for non-renewal of the nutrient solution was also checked when the strain was added to the nutrient

solution at two weeks after the initial harvest. This result suggested that microorganisms, if added to the nutrient solution at the reproductive growth stage of cucumber, can catabolize autotoxic substances from root exudates into nontoxic substances and this results in an increase in fruit yield of cucumber plants.

Key Words: Autotoxicity; Cucumber (*Cucumis sativus* L.); Hydroponics; Microorganism; Root exudates; 2,4-Dichlorobenzoic acid (DCBA)

1. Introduction

Closed hydroponics is a system used for plant cultivation in areas with environmental hazards (Van Os, 1995) in which the nutrient solution is not released into the surrounding environment but recycled (Ruijs, 1994). However, in this system plants may suffer from autotoxicity due to an accumulation of toxic exudates in the nutrient solution (Yu et al.1993). It is not easy to remove the growth inhibitors without renewing the nutrient solution, which is expensive and not practical.

In closed hydroponic culture without renewal of the nutrient solution, we found that the fruit yield of cucumber plants decreased significantly in the late reproductive stage

(two weeks ahead of final harvest) and the growth was recovered by the biweekly renewal of nutrients or supplementation of activated charcoal to the nutrient solution (Asao et al., 1998a). This inhibition has been attributed to the autotoxicity from root exudates (Yu et al., 1993). The autotoxicity of cucumber also differs among cultivars (Asao et al., 1998b). Fruit harvesting of a susceptible cucumber cultivar grown in a closed nutrient flow system was prolonged by grafting onto a non-autotoxic cultivar (Asao et al., 1999a). Thus, cucumber root exudates from a closed hydroponic system were analyzed and among a number of growth inhibitors detected (Asao et al., 1999b; Pramanik et al., 2001), 2,4-Dichlorobenzoic acid (DCBA) was the strongest inhibitor.

Microorganisms can degrade chemical substances in soil and water (Markus et al., 1984; Nanbu, K., 1990; Sundin et al., 1994). Van den Tweel et al. (1987) reported that 2,4-dichlorobenzoate was degraded through reductive dechlorination by microorganisms. Recently, we found that the inhibitory effect of 2,4-Dichlorobenzoic acid on cucumber seedlings could be reversed using strains of microorganisms (Asao et al., 2001). However, the effects of such strains on cucumber reproductive growth in the presence or absence of DCBA have yet to be elucidated.

Therefore, in this study we investigated the effects of microbial strains on the autotoxicity of cucumber plants grown with or without DCBA in the nutrient solution.

2. Materials and Methods

Cultivation of cucumber plants with or without microbial strain in presence of DCBA

The DCBA-degrading microorganism (microbial strain) was isolated and screened from soil in Aichi prefecture (Asao et al., 2001). Nutrient solutions with DCBA (10 mg/liter) and sucrose (1 g/liter) were prepared and sterilized by autoclave. A 200 ml volume of sterile nutrient solution was inoculated with the DCBA-degrading microorganism and shaken continuously by machine at 25 ℃ for nine days to have stock microbial suspension.

Cucumber (*Cucumis sativus* L. var. Shougoin-aonaga-fushinari) plants were grown in a green house by hydroponics at different concentrations of DCBA with or without addition of DCBA-degrading microorganisms to the nutrient solution. On 23 August 2000, one week-old cucumber seedlings raised in vermiculite were transplanted into plastic tanks (34 × 54 × 20cm) containing 50 liters of continuously aerated (3.8 liter/min.) 75 % Enshi nutrient solution having an electrical conductivity (EC) of 2.0 dS/m.

Eighteen seedlings were planted with urethane foam as support in each container. On 5 September, three seedlings with four leaves were transplanted to each container containing 50 liters of the same nutrient solution. The solutions were prepared at concentrations of 0(control), 2 or 10 μmol/liter of DCBA with or without bacterial suspension in the nutrient solution. The solutions were renewed biweekly. Three plants were planted in each container with three repetitions.

At the 15-leaf stage, the apical buds of cucumber plants were plucked to maintain 15 leaves on the main stem. The terminal buds of all the developing primary, and secondary branches were removed keeping only one node in each branch. The mean air and water temperature during the experiment ranged from 23.0 to 25.5℃and from 23.3 to 34.4℃, respectively. At the end of the experiment, data were recorded on plant growth, dates of anthesis in male and female flowers, number of healthy female flowers, and harvested fruit number.

Cucumber cultivation with microbial suspension in absence of DCBA

The same cultivation procedure was followed without addition of DCBA to the nutrient solution in another set of

experiments. On 26 June, three cucumber seedlings with four leaves were transplanted to each container containing the nutrient solution without DCBA.

During culture, the water level of containers was kept constant by regularly adding tap water. Nutrient contents (NO_3^-, $H_2PO_4^{2-}$, K^+, Ca^{2+}, Mg^{2+} and Fe^{3+}) of the solutions were adjusted to the initial concentrations at two-week intervals by adding required amount of the nutrients on the basis of chemical analyses using an atomic absorption spectrometer (Shimadzu AA-630) and ion meter (HORIBA D-23). In all the treatments, the EC and pH in the nutrient solution ranged from 1.4 to 2.8 dS/m and 6.4 to 7.9, respectively. The microbial suspension was supplied to the nutrient solution added a) at planting, at the plucking of apical buds and at two weeks after initial harvest, b) at the plucking of apical buds and at two weeks after initial harvest, and c) at two weeks after initial harvest. No DCBA was added. An additional cultivation with biweekly renewal of the nutrient solution in the absence of the microbial suspension or DCBA was set up to serve as a control. Three plants were planted in each container and three containers were used for each treatment.

At the 15-leaf stage, the apical buds of cucumber plants were removed to maintain 15 leaves on the main stem.

The terminal buds of the branches were removed keeping only one node on each branch. The mean air and water temperature during the experiment ranged from 24.9 to 31.3℃ and 25.8 to 32.1℃, respectively. Data were recorded as mentioned for the preceding experiment.

3. Results

Effects of DCBA with microbial suspension on the growth and yield of cucumber.

Cucumber plants were grown in hydroponics using different concentrations of DCBA with or without the addition of microorganisms to the nutrient solution. Results reveal that the length of the main stem and primary branches decreased with the increase in DCBA concentration (Table 2). The dry weight of stem, leaf, root and primary branches of plants grown with DCBA (10μmol/liter) was also decreased by about 60%, 30%, 26% and 32% of that without DCBA, respectively. This growth inhibition was significantly recovered by the addition of soil microorganisms to the nutrient solution. This indicates that the microbes efficiently degraded the added DCBA in the nutrient solution and thus, restored the inhibitory effect of DCBA on the plants.

The date of male flower anthesis was unaffected by the

addition of DCBA and the microbial suspension. However, the presence of DCBA at a concentration of 10 μ mol/liter shifted the date of female flower anthesis and harvesting time by about 5 and 16 days, respectively. Addition of the microbial suspension to the nutrient solution enhanced early flowering and fruit setting in the cucumber plants treated with DCBA. The number of healthy female flowers and the harvested fruit number per plant also decreased as the DCBA concentration increased, and this decrease was significantly compensated by the microbial suspension.

Effects of microbial suspension on cucumber growth in the absence of DCBA.

The suspension of DCBA-degrading microorganisms was added once at two weeks after the initial harvest, twice upon plucking the apical buds and at two weeks after the initial harvest, and three times at the beginning of the culture, on plucking the apical buds and at two weeks after the initial harvest. There was no significant difference in the growth of cucumber except in the dry weight of roots and fruit number (Table 3). Root dry weight increased by about 43 % with the addition of the suspension of DCBA-degrading microorganisms once at two weeks after the initial harvest. The treatments did not affect the dates of antheses in male

and female flower, the beginning of harvest, or the number of flowering female flowers per plant. The harvested fruit number per plant was the lowest (14.2/plant) for non-renewal of the nutrient solution. The number of fruits recovered from 14.2 to 17.4 on addition of the suspension to the nutrient solution once at two weeks after the initial harvest.

4. Discussion

2,4-Dichlorobenzoic acid is one of the growth inhibitors found in cucumber root exudates (Pramanik et al., 2000) and we found that DCBA is a most effective inhibitor of the growth of cucumber plants (Asao et al., 1999b). In these experiments we also found that DCBA strongly retarded the growth of cucumber plants (Table 2). However, this inhibition was significantly recovered by the addition of DCBA-degrading microbes (Asao et al., 2001) into the nutrient solution. This result reveals that the microbial strain appreciably deactivated the inhibitory action of DCBA including the other inhibitors in cucumber root exudates in the nutrient solution and thus, the cucumber plant growth was enhanced. The recovery of growth, especially the dry weight of roots and branches, in the plants grown with the microbial suspension, was about 3 times higher than that of cucumber grown with DCBA alone at a concentration of 10 μ

mol/liter. Consequently, dates of male and female flower anthesis, and initial harvest were several days earlier. Numbers of healthy female flowers as well as fruits also significantly increased on the addition of the microbial suspension.

Experiments to clarify the influence of root exudates and microbes on cucumber plant growth were conducted with or without biweekly renewal of the nutrient solutions (Table 3). Results revealed that the root growth and fruit number of the cucumber plants grown with biweekly renewal of nutrient solution were significantly increased than those grown without renewal of nutrient solution. The addition of the microbes to the nutrient solutions also increased the growth of cucumber plants compared to the non-renewal of nutrient solution. However, the microbial suspension added to the nutrient solutions in vegetative stage (at the start of the culture or the plucking of apical buds) did not make significant yield difference from non-renewed solution culture. Addition of DCBA-degrading microbial suspension applied once at two weeks after the initial harvest was effective enough to recover yield reduction of cucumber from autotoxicity. DCBA causing autotoxicity in the cucumber was detected in their root exudates only in the reproductive stage (Pramanik et al., 2000). Apparently it indicates that

the growth inhibitors including DCBA would have sufficiently accumulated in the nutrient solution through cucumber root exudation at the reproductive stage. The degrader (applied two or three times) probably became a source of nutrients for other microorganisms. In this case, the degrader did not dominate more than other microorganisms. However, when supplied once at two weeks after the start of harvest, the degrader did not become a nutrient source for other microorganisms and degraded the DCBA exuded from cucumber. This was why the DCBA exuded from cucumber sustained the microbial activity.

In conclusion, DCBA-degrading microorganisms, if added to the nutrient solution, may degrade DCBA including other growth inhibitors exuded from cucumber roots and avoid autotoxicity in cucumber resulting increase the fruit yield. Addition of the microbial suspension in the reproductive stage of cucumber plants appears to degrade the growth inhibitors efficiently. However, the timing of degrader addition to the nutrient solution for efficient mitigation of cucumber autotoxicity needs further study.

References

Asao, T., Umeyama, M., Ohta, K., Hosoki, T., Ito, N. and Ueda, H., 1998a. Decrease of yield of cucumber by non-

renewal of the nutrient hydroponic solution and its reversal by supplementation of activated charcoal (in Japanese, with English summary). J. Jpn. Soc. Hort. Sci. 67, 99-105.

Asao, T., Ohtani, N., Shimizu, N., Umeyama, M., Ohta, K. and Hosoki, T., 1998b. Possible selection of cucumber cultivars suitable for a closed hydroponics system by the bioassay with cucumber seedlings(in Japanese, with English summary. Soc. High Tech. Agr. 10,92-95.

Asao, T., Shimezu, N., Ohta, K. and Hosoki, T., 1999a. Effect of rootstocks on the extension of harvest period of cucumber (*Cucumis sativus* L.) grown in non-renewal hydroponics(in Japanese, with English summary. J. Jpn. Soc. Hort. Sci. 68, 598-602.

Asao, T., Pramanik, M.H.R., Tomita, K., Ohba, Y., Ohta, K., Hosoki, T., Matsui, Y., 1999b. Influences of phenolics isolated from the nutrient solution nourishing growing cucumber (*Cucumis sativus* L.) plants on fruit yield(in Japanese, with English summary). J. Jpn. Soc. Hort. Sci. 68, 847-853.

Asao, T., Taniguchi, H., Suyama, K., Yamamoto, H., Itoh, K., Tomita, K., Taniguchi, K. and Hosoki, T., 2001. Reversibility of the action of 2,4-Dichlorobenzoic acid on cucumber seedlings

by strains of soil microorganisms (in Japanese, with English summary). J. Jpn. Soc. Hort. Sci. 70, 393-395.

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

Markus, A., Klages, U., Krauss, S. and Lingens, F., 1984. Oxidation and dehalogenation of 4-chlorophenylacetate by a two-component enzyme system from *pseudomonas* sp. Strain CBS3. J. Bacteriol. 160, 618-621.

Nanbu, K., 1990. Fate of pesticides in soil (in Japanese). Plant Protection 11, 477-482.

Pramanik, M. H. R., Nagai, M., Asao, T. and Matsui, Y., 2000. Effects of temperature and photoperiod on the phytotoxic root exudates of cucumber (Cucumis sativus) in hydroponic culture. J. Chemical Ecology 28,1953-1967.

Ruijs, M. N. A., 1994. Economic evaluation of closed production systems in glasshouse horticulture. Acta Hort 340, 87-94.

Sundin, P. and Waechter-Kristensen, B., 1994. Degradation of phenolic acids by bacteria from liquid hydroponic culture of tomato. pp. 473-475, *in* P. C. Struik et al. (eds.). Plant Production on the Threshold

of a New Century. Kluwer Academic, Dordrecht.

Van den Tweel, W. J. J., Kok, J. B. and de Bont, J. A. M., 1987. Reductive dechlorination of 2,4-dichlorobenzoate to 4-chlorobenzoate and hydrolytic dehalogenation of 4-chloro, 4-bromo-, and 4-iodobenzoate by Alcaligenes denitrificans NTB-1. Appl. Environ. Microbiol. 53,810-815.

Van Os, E. A., 1995. Engineering and environmental aspects of soilless growing systems. Acta Hort. 396, 25-32.

Yu, J. Q., Lee, K. S. and Matsui, Y., 1993. Effects of the addition of activated charcoal to the nutrient solution on the growth of tomato grown in the hydroponic culture. Soil. Sci. Plant Nutri. 39, 13-22.