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Autotoxicity of root exudates from taro

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Abstract

Effects of chemicals exuded from taro roots in hydroponic culture on the growth and yield of taro were investigated. Taro plants grown in the nutrient solution without activated charcoal (AC) had significantly lower leaf numbers (90%) and shoot dry weights (67%) than those grown with AC. The corm yield per plant also decreased by 34% in the nutrient solution without AC. The allelochemicals adsorbed by the AC were extracted and analyzed by GC-MS. The identified compounds included lactic acid, benzoic acid, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, vanillic acid, succinic acid, and adipic acid. The allelopathic potentials

of these compounds were evaluated with taro plantlets as a test material. Results indicated that almost all the compounds were inhibitory to the growth of taro plantlets. But benzoic acid was the strongest inhibitor. All of these suggest that root exudates from the taro plant itself is one of the causes of problems in taro culture.

Key Words: Autotoxicity; Allelochemicals; Hydroponics; Root exudates; Taro

1. Introduction

Taro plants (*Colocasia esculenta* Schott) do not grow well if cultivated consecutively for years on the same land (Takahashi, 1984). Rotation with other crops for at least three years (Miyoshi et al., 1971a), in combination with organic matter and soil disinfectants (Murota et al. 1984), has been suggested to improve the yield of taro. However, even in a fixed crop rotation system, there was a great difference in the growth and yields of taro plants. This depends upon the kinds of crops in rotation, and the order in which they were rotated. In combination with burdock, the yield of taro was equal to or more than that of taro in the first-year planting, and the extent of corm injury was slight (Murota et al. 1984).

Harmful microbes (Atumi, 1956, 1957; Atumi and Nakamura, 1959; Nagae et al., 1971) and nematodes (Miyoshi et al., 1971b; Oashi, 1973; Matsumoto et al., 1973, 1974) in the soil are the main causes of damage in the successive culture of taro. However, Takahashi (1984) suggested that unknown factors were also involved. Also, Miyaji et al. (1979) found that taro residues in soils after harvest were inhibitory to its growth.

Tsuzuki et al. (1995) evaluated the physicochemical properties of soils cultivated with taro either alone or in combination with other crops for years, and found little difference in carbon and nitrogen contents, available phosphoric acid, or even nematode contents. Methanol extracts of taro residues alone or of soils with taro residues were found to strongly inhibit the elongation of hypocotyls and radicle growth of turnip. The foregoing results reveal that growth inhibitors from taro were connected with replanting problems.

Our laboratory has established used hydroponic culture system to assess autotoxicity in crop plants (Asao et al., 1999; Pramanik et al., 2000). Thus, an attempt was made to identify the chemicals exuded by taro roots and to evaluate the allelopathic effects of these exudates on the growth and yield of taro through this established hydroponic culture

system.

2. Materials and Methods

Plant cultivation

Taro cv. Aichi-Wase was used for this experiment. Corms were planted in a plastic tray (32×47×7cm) containing vermiculite on 15 March 1999 in Shimane University green house. On 28 May, at the third leaf stage, taro plantlets were transplanted into larger plastic containers (34 × 54 × 20cm) containing vermiculite (Fig.1). Below each planted container, another container filled with 30 liters of continuously aerated (3.8 liter/min.) 75% Enshi nutrient solution with electrical conductivity (EC) of 2.0 dS/m was placed (Hori, 1966). Full-strength nutrient solution contains the following amounts of salts per 1000 liters of tap water: 950 g of $Ca(NO_3)_2 \cdot 4H_2O$; 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 $ZnSO_4 \cdot 7 H_2O$; 0.05 g of $CuSO_4 \cdot 5 H_2O$; and 0.02 g of $NaMoO_4$. Two small air filters each packed with 100 g of activated charcoal (AC), (Takeda Chemical Industry Co., Type GH2C, 4-8 mesh), were attached to an air pump of solution container. The same aeration system was maintained for the nutrient solution without AC. The AC

was used to trap the chemicals exuded from taro plants and was replaced by fresh AC at two-week intervals until harvesting for efficient adsorption of the chemicals. The used AC was either immediately extracted with alkaline methanol or stored at 4 °C for later extraction. $FeSO_4 \cdot 7H_2O$ (0.75 g) was added to each solution container at two-day intervals since the AC absorbed Fe-EDTA, and Fe^{2+} was rapidly oxidized to Fe^{3+} and less available for plants. During cultivation, the water level of the solution containers was kept constant by regularly adding tap water. Nutrient concentrations (NO_3^- PO_4^{2-}, K^+, Ca^{2+}, Mg^{2+} and Fe^{3+}) of the solution were adjusted as close as possible to the initial concentration at two-week intervals on the basis of chemical analyses with an atomic absorption spectrometer (Shimadzu AA-630) and ion meter (Horiba D-23). Five plantlets were planted in each container and three containers were used for each treatment (plants with or without AC). At the end of this experiment, measurements were made of the longest leaf stalk, maximum leaf length and width, leaf number per plant, shoot dry weight and corm yield.

GC-MS analysis of root exudates in AC.

The ACs used to trap the exuded organics from taro roots were desorbed three times using a mixture of methanol

(100 ml) and 0.4 M aqueous NaOH (100ml). Thus, each batch of AC (200 g) was gently shaken with the mixture for 12 hr at room temperature with an electric shaker. The extracts were combined and filtered. The filtrates were neutralized and concentrated to 25 ml by a rotary vacuum evaporator at 40 °C. Organic compounds in the concentrate were extracted according to Yu and Matsui (1993). The concentrated solution was adjusted to pH 2.0 with 4 M HCl, extracted three times with 35 ml of refined diethyl ether (DE), and another three times with 35 ml of ethyl acetate (EA). DE2 and EA2 are the ether and ethyl acetate extracted fractions at pH 2.0, respectively. The DE2 and EA2 fractions were dried over anhydrous $CaSO_4$ and concentrated to 5 ml each in a rotary evaporator at 40 °C.

All the fractions (DE2 and EA2) extracted from AC were analyzed with a gas chromatograph coupled to a mass spectrometer (GC-MS, Hitachi M-80B) before or after methylation. Fraction DE2 gave a number of peaks in the gas chromatogram, whereas the EA2 fraction gave only a few detectable peaks. An aliquot of the concentrated DE2 fraction (1 or 2 ml) was diluted in a 50 ml ether solution, treated with diazomethane, and concentrated in a rotary evaporator before being bubbled with a N_2 stream in a water bath at 35 °C. One microliter of the concentrated sample

was injected into a GC-MS unit coupled with a capillary column (GL Science, TC-5, 60m). Helium was used as the carrier gas at a pressure of 0.8 kg/cm^2. The initial column temperature was held at 100 °C for 2 min and then raised at 5 °C/min to a final temperature of 260 °C with isotherm for 10 min. The injector temperature was held at 270 °C. The ionization voltage and temperature in the electron impact (EI) mode were 70 eV and 250 °C, respectively.

Bioassay with identified chemicals.

Aqueous solutions of the identified acids at concentrations of 0 (control) or 400 μmol/L were prepared with a 75 % Enshi nutrient solution (EC 2.0 dS/m). The test solutions were added to flasks (capacity about 420ml) wrapped in black polyethylene to avoid light to the roots. One taro plantlet cv. Aichi-Wase at the second leaf stage was transplanted to each flask with urethane foam as support. The planted flasks were placed in a growth chamber at 25℃with a light intensity of 74～81 μmol/s/m^2 and 16 hr photoperiod. To minimize the effect of aeration and the microbial degradation of organic acids (Sundin and Waechter-Kristensen, 1994) on the bioassay, we renewed the test solutions in the planted flask every three or four days. The taro plantlets were grown for 26 days and then

the fresh and dry weights of shoots, number of leaves, longest root length and root dry weight were measured. Each treatment was replicated 15 times. We carried out further bioassays following the same procedure with benzoic and adipic acids at concentrations of 0 (control), 25, 50, 100 200 and 400 μmol/L. In this case, the taro plantlets were grown for 20 days.

3. Results

Effects of non-renewal of the nutrient solution on the growth and yield of taro.

Results revealed that plants grown without AC had experienced significant shoot growth retardation compared to those grown with AC. The leaf numbers and shoot dry weights of the plants grown without AC decreased to about 90% and 67% of those grown with AC, respectively (Table 1). Addition of AC to the nutrient solution also improved yield significantly. The total yield per plant without AC decreased to about 34% compared to that on the addition of AC (Table 2). Larger corms were harvested from the nutrient solution with AC.

Phytotoxins in root exudates of taro.

Analysis of the extracted taro root exudates with GC-MS gave more than thirty peaks (Fig. 2). Based on the comparison of retention times and mass spectra with those of authentic samples, seven peaks were assigned as methyl esters of lactic acid, benzoic acid, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, vanillic acid, succinic acid, and adipic acid.

Bioassay.

The allelopathic potential of the identified compounds, was evaluated using taro plantlets as the test material. Benzoic acid at 400 µmol/L induced severe growth inhibition of shoots and roots, while adipic acid at the same concentration reduced only dry weight of roots (Table 3). Thus, we further evaluated growth inhibition potential of benzoic acid and adipic acid at concentrations ranged from 0 to 400 µmol/L using taro plantlets. Both acids significantly inhibited the growth of plantlets (Table 4). Benzoic acid induced growth retardation even at 50 µmol/L and growth decreased with increasing concentration of the acid. Benzoic acid at the highest concentration of 400

μmol/L reduced fresh weight, shoot dry weight, root length and root dry weight in taro plants to 54 %, 53 %, 54 %, and 75 % of control values, respectively. Adipic acid only at 400 μmol/L reduced fresh weight of shoot and root length. Lower concentrations of this acid did not affect shoot or root growth.

4. Discussion

As the nutrient concentrations and growth environment in the hydroponic cultures of taro plants were apparently identical, the significant growth differences between the plants grown with and without AC could be attributed to the variation in the chemical composition of the nutrient solution. These chemicals would have exuded from taro roots. Tsuchiya and Ohno (1992) indicated that water extracts from soils used consecutively for taro cultivation over a period of years inhibited the growth of lettuce. Since the same phenomenon was observed even when the extracts were autoclaved, it was considered that the inhibition was caused by allelochemicals rather than by harmful soil microorganisms. There have been many reports that taro residues exhibited an allelopathic effect on plant growth (Miyaji et al., 1979; Tsuzuki et al., 1995 ; Pardales

and Dingal, 1988). It was made clear here that the vegetative growth and corm yield of taro plants were decreased in the non-renewed culture solution, and the loss was recovered by adding AC to the nutrient solution. This result suggests that the chemicals exuded from taro roots had induced the inhibition of growth and reduced yield. This inhibition was prevented by the adsorption of the exuded allelochemicals in AC.

The substances adsorbed on the AC were extracted, analyzed and some of them identified as phenolic and aliphatic acids although many compounds in the root exudates are yet to be detected. The allelopathic potential of each identified compound was evaluated and found that almost all the compounds inhibited the growth of taro plantlets (Table 3 & 4). Benzoic induced significant growth inhibition in taro plantlets even at concentration of 50 µmol/L. Inhibitory effects of phenolic acids (Pramanik et al., 2001) and aliphatic acids (Yu and Matsui, 1997) to plant growth have been well recognized. In a bioassay, Blum (1996) found that 30 % reduction of absolute leaf expansion brought about at 0.23 µmol of fenolic acid per gram soil, while it required only 0.05 µmol in the presence of 0.06, 0.17, and 0.04 µmol of *p*-coumaric, *p*-hydroxybenzoic, and vannilic acids per gram of soil,

respectively. Thus, mixture of allelochemicals can below their inhibitory levels. This indicates that taro plants exude a number of compounds (Fig.2) into its surroundings and those inhibit the growth taro plants by synergistic or additive actions.

In conclusion, taro roots exude a number of allelochemicals including aromatic acids such as benzoic acid and aliphatic acids such as adipic acid which inhibit the growth of taro plants by additive or synergistic actions. Benzoic acid induced strongest inhibition. Thus, the decline in yield on the successive culture of taro would appear to be related to the allelochemicals exuded from the taro plant itself.

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