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Autotoxicity in beans and their allelochemicals

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ABSTRACT

The autotoxicity of *Pisum sativum*, *Phaseolus vulgaris*, and *Vicia faba* were investigated in hydroponics either with or without activated charcoal (AC) addition. Growth and yield of the three beans were significantly reduced when grown in the culture solution without AC addition. In *P. sativum* plants grown in non-renewed culture solution without AC, the number of pods, pod fresh mass, number of seeds, and seed fresh mass were reduced by about half compared with those with AC. The number of pods plant^{-1} and fresh mass of pods^{-1} plant in *P. vulgaris*, as well as pod number in *V. faba*, were decreased significantly to 49–67% without AC addition. The identified allelochemicals were benzoic, salicylic, and malonic acids in the root exudates of *P. vulgaris* and lactic, benzoic, *p*-hydroxybenzoic, vanillic, adipic, succinic, malic, glycolic, and *p*-hydroxyphenylacetic acids in *V. faba*. Bioassay of the identified allelochemicals revealed that benzoic, salicylic, and malonic acids significantly reduced the growth of *P. vulgaris* even at low concentrations. In *V. faba*, benzoic acid at 50 μM significantly reduced root length, and shoots fresh and dry mass by over 81% of those of the control, whereas adipic and *p*-hydroxyphenylacetic acids decreased root length to 87 and 88% of that of the control, respectively.

1. Introduction

Beans are grain legumes that belong to the family Leguminosae, which includes food and forage legumes. Bean plants are cultivated primarily for their seeds, which are harvested at maturity and are rich in protein and energy. They are used either for animal feed or for human consumption. The major grain legumes are *Pisum sativum*, *Vicia faba*, *Lens culinaris*, *Glycine max*, *Phaseolus vulgaris*, *Lupinus* spp., and *Cicer arietinum*. These grain legumes are generally intercropped with cereals to enhance crop yield, increase nitrogen use efficiency, and reduce weed infestation and the occurrence of plant disease (Willey, 1979; Jensen, 1996; Hauggaard-Nielsen et al., 2001, 2008). Among the grain legumes, some edible beans are used as vegetables and intensively cultivated in the same farmland year after year. The production of these common bean plants and other perennial legumes declines in replanting conditions owing to autotoxicity, a form of intraspecific allelopathy that occurs when a plant species releases chemical substances that inhibit or delay germination and growth of the same plant species (Putnam, 1985; Miller, 1996; Singh et al., 1999). Allelopathy has been investigated in some beans such as in *P. sativum* (Kato-Noguchi, 2003), *Mucun pruriens* (Fujii et al., 1991), *Glycine max* (Huber and Abney, 1986; Xiao et al., 2006; Yan and Yang, 2008), and *Cicer arietinum* (Yasmin et al., 1999). L-DOPA and cynamidine has been found to be potential allelochemicals identified in *Mucuna pruriens* and *Vicia villosa*, respectively (Fujii, 2003). It has been found that, in addition to common beans, several other species within the Leguminosae family contain secondary plant products that have allelopathic potential (Rice, 1984). In field experiments, it has been reported that residues and extracts of pea plants suppressed the growth and population size of several plant species (Purvis, 1990; Schenk and Werner, 1991; Tsuchiya and Ohno, 1992; Akemo et al., 2000). Phytotoxic substances in *P. sativum* root exudates have been reported by several researchers (Hatsuda et al., 1963; Yu and Matsui, 1999) and, recently, pisatin has been identified as an inhibitory chemical from its shoots (Kato-Noguchi, 2003). Aqueous leachates of dry shoot of *P. vulgaris* that contain phenolics showed allelopathic effects on several crop species (Nava-Rodríguez et al., 2005). Autotoxicity due to root exudates found to be involved in growth reduction in *Glycine max* monocropping, which decreased plant biomass and root triphenyl tetrazolium chloride-reducing activity as well as seedlings after exposure to root exudates, exhibited higher activities of superoxide dismutase and guaiacol peroxidase (Xiao et al., 2006).

Successive culture of the same crop on the same land for years cause soil sickness or replanting injuries (Hirano, 1940; Bonner and

Abbreviations: AC, activated charcoal; EC, electrical conductivity; h, hour; GC–MS, gas chromatography–mass spectrometry; M, molar; DE, diethyl ether; EA, ethyl acetate; EI, electron impact; FM, fresh mass; DM, dry mass; HPLC, high performance liquid chromatography; rpm, revolutions per minute; μM, micro molar.

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Galson, 1944; Tsuchiya, 1990) resulting reduction in both crop yield and quality. This phenomenon is evidenced in agricultural cropping system especially in the production of horticultural crops (Young, 1984; Grodzinsky, 1992). It leads to the resurgence of disease pest, exhaustion of soil fertility, and developing chemical interference in the rhizosphere referring to allelopathy (Takahashi, 1984; Young, 1984; Hegde and Miller, 1990). Similar to successive culture, in closed hydroponics phytotoxic chemicals accumulated in the culture solution leading to the occurrence of autotoxicity and it was investigated in *Cucumis sativus* (Yu and Matsui, 1994, 1997), *Citrullus lanatus* (Kushima et al., 1998; Hao et al., 2007), *Colocasia esculenta* (Asao et al., 2003), *Fragaria ananassa* (Kitazawa et al., 2005), *Solanum lycopersicum* (Yu and Matsui, 1993), and *Lactuca sativa* (Lee et al., 2006). In this phenomenon, root exudates hamper the plant growth mainly by hampering water and mineral uptake. Previous studies have shown that allelochemicals released from plant roots play an important role in replant injuries of crops. Autotoxicity of root exudates is an important feature for understanding replanting problems in agroecosystem as it represents one of the largest direct inputs of allelochemicals into the rhizosphere environment with potent biological activity and great variation in chemical components (Inderjit and Weston, 2003). The synthesis and exudation of allelochemicals, along with increased overall production of root exudates, is typically enhanced by stress conditions that the plant encounters such as extreme temperature, drought and UV exposure (Pramanik et al., 2000; Inderjit and Weston, 2003). The removal of the inhibitory chemicals from soils or culture solution can permit continued crop cultivation in the same land for years. Hydroponic culture technique has the facility of trapping and isolating the chemicals released through plant roots. Elimination of these growth inhibitors from recycling culture solution is desirable from the viewpoint of conservation-oriented agriculture. Therefore, many researchers suggested addition of AC to the culture solution to improve growth and yield significantly by adsorbing organic compounds (mainly phenolics), for example in *Fragaria ananassa* (Kitazawa et al., 2005), *Colocasia esculenta* (Asao et al., 2003), *Cucumis sativus* (Yu and Matsui, 1994; Asao et al., 1998a, 1999, 2000), several leafy vegetables (Asao et al., 2004), and some ornamentals (Asao et al., 2007a).

The study of autotoxicity in commonly grown beans would provide useful knowledge of sustainable crop production. Thus, identification of the allelochemicals from bean root exudates, evaluation of their phytotoxicity, and their removal would facilitate the maintenance of profitable crop production. Previously, we found evidence of autotoxicity in *Lathyrus ordoratus*, a leguminous crop (Asao et al., 2007b); in this study, we investigated autotoxicity in three beans, namely, *P. sativum*, *P. vulgaris*, and *V. faba* as well as their allelochemicals, using hydroponic culture. The phytotoxicity of the identified allelochemicals was evaluated using seedling growth bioassay of the test plants.

2. Materials and methods

2.1. Plant materials

Bean plants viz. *P. sativum* cv. Kurume-yutaka, *P. vulgaris* cv. Taibyou-morokko, and *V. faba* cv. Nintoku-1-sun were used in this experiment.

2.2. Plant cultivation either with or without AC

Seeds of the beans under study were germinated on vermiculite on a plastic tray with tap water. The seedlings were transplanted to plastic containers (50 cm × 60 cm × 21 cm) in the greenhouse of Shimane University (Fig. 1). Twelve plants were planted in each container and three containers were used for each treatment (plants either with or without AC) following a randomized block design. The container was filled with 50 l of 75% Enshi nutrient solution with electrical conductivity (EC) of 2.0 dS m^{-1} (Hori, 1966). Full-strength nutrient solution contains the following amounts of salts 1000 l^{-1} 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 $ZnSO_4{\cdot}7H_2O$; 0.05 g of $CuSO_4{\cdot}5H_2O$; 0.02 g of $NaMoO_4$; and 25 g of NaFe-EDTA. The nutrient solution in the containers were continuously aerated (3.8 l min^{-1}) using air pumps with two small air filters each packed with 100 g of AC (Type Y-4P, 4-8 mesh, Ajinomoto Fine Techno Co., Kawasaki, Japan). The same aeration system was maintained for the nutrient solution without AC. The AC was used to trap the chemicals exuded from the plants and was replaced by fresh AC at 2-week intervals until the end of the experiment 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 2-day intervals since the AC that absorbed Fe-EDTA and Fe^{2+} was rapidly oxidized to Fe^{3+} and less available for the 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+}) in the solution were adjusted as close as possible to the initial concentration at 2-week intervals on the basis of chemical analyses with an atomic absorption spectrometer (AA-630, Shimadzu Co., Kyoto, Japan), a spectrophotometer (UVmini-1240, Shimadzu Co., Kyoto, Japan), and an ion meter (D-23, Horiba, Kyoto, Japan). The pH of the nutrient solutions ranged from 5.7 to 7.1 irrespective of either with or without AC addition. At the end of the experiment, plant length, fresh and dry mass of shoots, dry mass of roots, root length, numbers of pods and seeds, and fresh mass of pods and seeds were recorded.

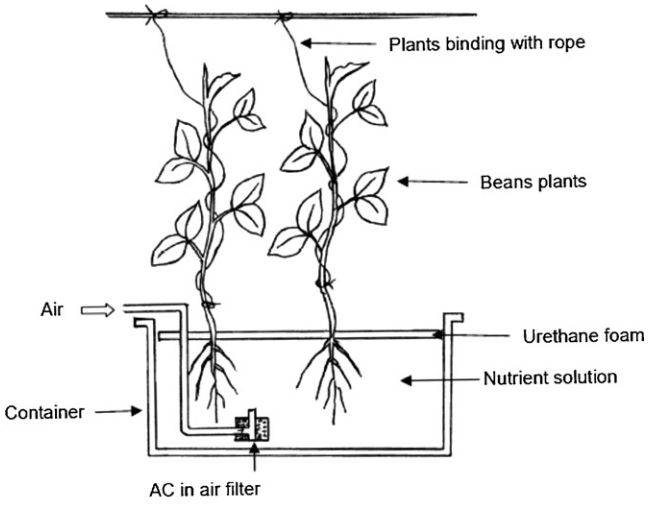

Fig. 1. Hydroponic system used for bean plant cultivation.

2.3. GC–MS analysis of root exudates adsorbed in AC

The AC used to trap the exudates (organics) were desorbed three-times using 200 ml 1:1 (v/v) methanol (100 ml):0.4 M aqueous NaOH (100 ml) (Pramanik et al., 2001). Each batch of AC (200 g) was gently shaken with the mixture for 12 h at room temperature (25 °C) with an electric shaker (20 rpm). The three extracts (600 ml) were combined and filtered through Whatman (No. 6) filter paper. The filtrates were neutralized with 6 M HCl and concentrated to 25 ml in a rotary vacuum evaporator at 40 °C. Organic compounds in the concentrate were then extracted according to Yu and Matsui