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Accumulation of phytotoxic organic acids in reused nutrient solution during hydroponic cultivation of lettuce (*Lactuca sativa* L.)

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Abstract

Proper nutrient solution management in the root zone is the first consideration for the adoption of a closed hydroponic system. Plant roots often exude numerous organic acids, which are known to inhibit growth. To investigate the accumulation of these phytotoxic organic acids as root exudates, lettuce (*Lactuca sativa* L.) was hydroponically grown in reused nutrient solution. Organic acids were extracted with diethyl ether from the reused nutrient solutions (RNS), root residues, and activated charcoal (AC) then quantified by GC/MS. Five individual organic acids were identified from the root residues and seven from the reused nutrient solutions. After 90 days of lettuce cultivation, in the treated AC in 3RNS, benzoic, phenylacetic, cinnamic, *p*-hydroxybenzoic, lauric, phthalic, vanillic, palmitic, and stearic acids were identified. In contrast, little or no organic acids were detected in the 3RNS treated with AC (3RNS/AC). Artificially applied pure organic acids ranging from 25 to 200 μM inhibited lettuce growth in a concentration-dependent manner. Lettuce growth was also greatly reduced in the nutrient solutions containing a externally applied, simulated mixture of the organic acids as in the 3RNS. Lettuce growth was not inhibited following the addition of AC (2.5 g/L) to the nutrient solution containing the mixture of organic acids. Our results demonstrated that organic acids were accumulated in reused nutrient solutions and were phytotoxic to lettuce growth. Also, this study showed that the addition of AC reduces the phytotoxic effects by eliminating the organic acids.

Keywords: *Lactuca sativa* L.; Growth inhibition; Reused nutrient solution; Activated charcoal; GC/MS; Organic acid; Root exudate

1. Introduction

The hydroponic nutrient solution requires frequent adjustments based on chemical analysis of inorganic elements or immediate measurement of each element concentration with multi-ion sensors. Disease and pest build-up in recycled nutrient solutions are another serious problem, and thus disinfection and filtration of the reused solutions are necessary. There are many recycling schemes, such as continuous maintenance of solution composition, efficient filtration and disinfection methods, which have been systemized in many ways (Adams, 1992; Menzies and Bélanger, 1996). Replant failure in hydroponics due to the accumulation of phytotoxic organic acids in the nutrient solution is another problem, and should not be overlooked. Plant roots exude many kinds of organic acids into the external rhizosphere under various conditions, and these organic compounds are known to inhibit growth (Rice, 1984).

According to Yu and Matsui (1993, 1994), the growth of tomato and cucumber were reduced by successive cultivation with recycled nutrient solutions due to the presence of several organic acids in reused nutrient solution. To eliminate these phytotoxic organic acids from the nutrient solution and to mitigate autotoxicity in hydroponic culture, several methods using activated charcoal (AC) or microorganisms have been tried (Asoa et al., 2004b). It has been shown that the use of AC in reused nutrient solutions effectively eliminated several phytotoxic substances (Asao et al., 1998, 1999a,b; Yu and Matsui, 1994; Yu et al., 1993).

Numerous phytotoxic organic acids have been identified from plant roots or the root zone (Al Saadawi et al., 1983; Guenzi and McCalla, 1966; Perez and Ormeno-Nunez, 1991; Rice, 1984; Tang and Young, 1982; Whitehead, 1964; Yamane et al., 1992). The organic acids present in soil or substrate are

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most commonly studied in relation to allelopathy. Phytotoxic phenolic compounds such as ferulic, *p*-hydroxybenzoic, *p*-coumaric, protocatechuic, salicylic, syringic, and vanillic acids have been shown to accumulate through the prolonged cultivation of cucumber in a peat-bark substrate (Politycka et al., 1984). The water soluble monomeric phenolic substances and polyphenols were also identified in soils under several coniferous and deciduous tree species. Ferulic, *p*-coumaric, vanillic, protocatechuic, syringic, and benzoic acid were found to be major compounds among them (Kuiters and Denneman, 1987). Similarly, the extract with diethyl ether from residual nutrient solution contained benzoic, phthalic, sinapic, and palmitic acids as phytotoxic substances, and the extract from residual AC contained additional organic acids such as 4-hydroxybenzoic, phenylacetic, vanillic, ferulic, caffeic, and 2-hydroxy-3-phenyl-propanoic acids in hydroponic cultivation of tomato (Yu and Matsui, 1993). A total of 12 phytotoxic compounds have been identified from nutrient culture systems. Among them, 3-hydroxyhydrocinnamic, benzoic, phenylacetic, and hydrocinnamic acids were the major rhizospheric compounds having growth inhibitory activities (Tang and Young, 1982).

Several organic acids have also been identified from root exudates of cucumber: benzoic, *p*-hydroxybenzoic, 2,5-dihydroxybenzoic, 3-phenylpropionic, cinnamic, *p*-hydroxycinnamic, myristic, palmitic, and stearic acids, as well as *p*-thiocyanatophenol and 2-hydroxybenzothiazole, all of which, except 2-hydroxybenzothiazole, were toxic to the growth of lettuce (Yu and Matsui, 1994). Asao et al. (1999a) also recently identified residual nutrient solution in four different organic acids: benzoic, *p*-hydroxybenzoic, 2,4-dichlorobenzoic, and phthalic. Among them, 2,4-dichlorobenzoic acid exhibited the strongest inhibitory activity. Several organic acids adsorbed in AC were identified during hydroponic cultivation of cucumber (Pramanik et al., 2000). Those were benzoic acid and its derivatives, cinnamic acid derivatives, and fatty acids presumably from plants as root exudates (Siqueira et al., 1991; Vaughan and Ord, 1991) or by decomposition of plant residues (Kuiters and Sarink, 1986; Patrick, 1971). Asao et al. (2001, 2004a) also elucidated species differences in the susceptibility to autoxicity and the identification of lactic, benzoic, *m*-hydroxybenzoic, *p*-hydroxybenzoic, vanillic, adipic, succinic acids in eight leafy vegetables.

Although lettuce is widely cultivated in greenhouses using the nutrient film technique (NFT) for hydroponic systems in Korea, nutrient solutions have not been properly changed, adjusted or analyzed during cultivation. In many situations, nutrient solutions are renewed after a single use because of a new start of cultivation, and the used nutrient solution is drained out. However, if the phytotoxic organic acids that accumulate in the nutrient solutions are effectively eliminated, nutrient solution reuse could be more generally adopted in hydroponic cultivation.

Lettuce is known to be sensitive to phytotoxic substances, which could be present in the raw water or released by roots and microorganisms (Ortega et al., 1996), and some reports have been published regarding lettuce growth in relation to the autotoxic potentials and the identification of phytotoxic root exudates. However, few reports are released on the simultaneous monitoring of root exudate concentration during subsequent reuse of nutrient solution in hydroponic cultivation of lettuce. Therefore, the objectives of this study were to identify and monitor the putative phytotoxic organic acids in reused nutrient solution, root residues, and AC during successive hydroponic cultivation of lettuce. Furthermore, amelioration of the growth reduction was attempted through AC treatment of the reused nutrient solutions.

2. Materials and methods

2.1. Plant material

Lettuce (*Lactuca sativa* L. cv. Chungchima) seeds were sown in respective 288 cells of a polyurethane sponge. Emerged seedlings with fully developed cotyledons were transferred to pre-planting trays for about a week before transplanting. The plants at 3–4 leaf stage were then transplanted for hydroponic cultivation.

2.2. Hydroponic system

Three cultivation beds coupled with 200 L nutrient solution tanks were used for preparation of the reused nutrient solution. Each system consisted of a 300 cm × 61 cm × 6 cm styrofoam bed and 72 transplanted lettuce seedlings on transplanting panels. The lettuce plants were cultivated for 25–30 days. The pH and electrical conductivity (EC) of the used nutrient solutions were adjusted to the initial level for the next cultivation. In this way, the once reused nutrient solution (1RNS), the twice reused nutrient solution (2RNS), and the thrice reused nutrient solution (3RNS) were simultaneously obtained.

For the AC treatment in the respective reused nutrient solution (RNS), 44 cm × 34 cm × 19 cm plastic trays containing 20 L nutrient solution were used. Nine lettuce seedlings were transplanted to the transplanting panel and the tray was shielded from light with insulating aluminum film. To bioassay lettuce seedling growth as affected by applying pure organic acid standards into fresh nutrient solution, smaller trays were used. Twelve lettuce seedlings at the cotyledon stage were transplanted to a 3 mm thick styrofoam panel. The panels were floated in 21 cm × 15 cm × 10 cm plastic trays filled with 2 L of the prepared nutrient solution. The nutrient solutions were aerated with a 5 W air pump for 20 min/h. Powder type AC (Sigma Co.) was suspended directly in the nutrient solution at 2.5 g/L into 3RNS or organic acid-treated nutrient solutions to evaluate the elimination effect.

2.3. Nutrient solution

Yamazaki's lettuce solution (Yamazaki, 1982) was used for all experiments. The Yamazaki's solution was composed of 404 KNO_3, 236 $Ca(NO_3)_2{\cdot}4H_2O$, 57.5 $NH_4H_2PO_4$, and 123 mg/L $MgSO_4{\cdot}7H_2O$ as macroelements. Microelement