Interactive effects of lettuce (*Lactuca sativa* L.), irradiance, and ferulic acid in axenic, hydroponic culture

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**Abstract**

Ferulic acid (FA) is released by living roots and by decaying plant material and is involved in chemical interactions between plants. Effects of FA on plant growth and root development of lettuce (*Lactuca sativa* L. cv. Grand Rapids) cultivated in axenic nutrient solution were studied in two factorial experiments. Root and shoot growth was impeded when 200 μM *trans*-FA was added to the nutrient solution and the light intensity was in the range of 250–380 μmol m⁻² s⁻¹. Root growth showed a stronger response to FA than did shoot growth. At 200 μM, FA strongly inhibited root hair formation and reduced mean lengths of primary, secondary and tertiary roots, but stimulated primary and secondary root branching. Both isomerization to the *cis* isomer and the presence of the plant reduced the concentration of *trans*-FA in the nutrient solution during the two weeks exposure period. A third experiment was conducted to assess the influence of irradiance on the phytotoxicity of FA. At a light intensity of 489 μmol m⁻² s⁻¹, or in the presence of microorganisms, the concentration of FA in the nutrient solution was lowered and the phytotoxic effects were reduced.

**Introduction**

Closed, hydroponic systems, where the nutrient solution is recirculated to reduce the release of excess water and nutrients to the environment, are often utilized for lettuce production in greenhouses in Scandinavia. Lettuce, however, is sensitive to phytotoxic agents (Ortega et al., 1996) which could be present in the raw water or released by plant roots, by microorganisms, or from organic substrates. Yu and Matsui (1993) found that plant growth was reduced by phenolic compounds which had been collected from the nutrient solution when tomato was grown in closed, hydroponic culture. One of the phenolic acids identified was ferulic acid (FA), which had been previously identified by Politycka et al. (1984) in re-used solid substrates found to be phytotoxic to cucumber plants.

In plants, FA is important as a precursor for lignification (Higuchi, 1981), and it is also bound to the cell walls of several monocots and of certain dicots (Fry, 1986; Hartley, 1987). FA and other phenolic acids are released from plants as root exudates (Siqueira et al., 1991; Vaughan and Ord, 1991; Schulz et al., 1994) or by decomposition of plant residues (Patrick, 1971; Kuiters and Sarink, 1986).

Phenolic acids have been suggested to be involved in allelopathic interactions, and addition of phenolic acids to the root medium has been shown to reduce plant growth and nutrient uptake (Siqueira et al., 1991;
Einhellig, 1995). Membrane perturbations have been suggested as their initial mode of action, triggering subsequent changes in plant-water relationships, mineral uptake, chlorophyll content, photosynthesis, carbon flow, and phytohormone activity (Einhellig, 1995). Threshold concentrations for phytotoxic effects of phenolic acids present in the root environment are commonly found in the range of 100–1000 μM (Siqueira et al., 1991). However, with the exception of Harms et al. (1969a,b) and the work of Vaughan and co-workers (e.g. Vaughan and Ord, 1990; 1991), most studies on the effects of phenolic acids on plant growth and nutrient uptake have been conducted under non-sterile conditions. Many microorganisms are able to metabolize phenolic acids (Siqueira et al., 1991; Rosazza et al., 1995; Sundin et al., 1995), and hence may suppress their phytotoxic effects (Vaughan et al., 1983; 1993). Threshold concentrations for toxic effects of phenolic acids also depend on plant species (Einhellig and Eckrich, 1984; Schulz et al., 1994) and developmental stage (Waters and Blum, 1987), and on environmental factors like temperature (Einhellig and Eckrich, 1984), nutrition (Vaughan and Ord, 1990; Vaughan et al., 1993) and, in particular, pH (Blum et al., 1985). Little is known about the effects of light intensity on threshold concentrations of phenolic acids. In greenhouses, light levels are highly dependent on weather conditions. In Sweden, the amount of supplemental light recommended when lettuce is cultivated during the dark period of the year is 70 Wm⁻² using high-pressure sodium lamps (Mats Johansson Kron, personal communication), which is approximately equivalent to 350 μmol m⁻² s⁻¹. Harms et al. (1969a) reported a higher plant uptake of phenolic acids at higher light levels. Since light also stimulates the activities of enzymes involved in phenolic metabolism (McClure, 1979), effects on threshold concentrations are likely.

In the present work, we aimed to investigate under axenic conditions 1) the threshold concentrations for effects of FA on growth and root development of lettuce, 2) the influence of the plant on the content of FA in the nutrient solution, and, finally, 3) the influence of light intensity on the inhibitory effects of FA.

Materials and methods

Experimental design

Experiment I was a factorial experiment with 0, 100 and 1000 μM FA in the nutrient solution and two harvest times. In Experiment II, the effects of five FA concentrations (0, 25, 50, 100 and 200 μM) were tested. Experiments I and II were conducted twice. Experiment III was a factorial experiment where culture flasks with or without plants were exposed to three light levels and two FA treatments (0, 200 μM).

Plant cultivation

Seeds of lettuce (Lactuca sativa L. cv. Grand Rapids) were immersed in 10% H₂O₂ for 30 min followed by rinsing in sterile, ultra-pure water (Elgastat maxima, Elga Ltd.). The surface-sterilized seeds were placed on 10% Tryptic Soy Agar (TSA). Six days after sowing, sterile seedlings were transferred singly to the autoclaved (121 °C, 20 min) cultivation systems consisting of a 500 ml conical culture flask closed with a cotton plug (Caspersen, 1997). Each flask contained 200 ml of a sterile filtered (0.2 μm Cellulose acetate filter, Sartorius) nutrient solution containing 10 mM KNO₃, 4.5 mM Ca(NO₃)₂, 1.0 mM MgSO₄, 1.0 mM KH₂PO₄, 1.0 mM Na₂HPO₄, 1.25 mM NH₄Cl, 40 μM Fe-EDTA, 5 μM MnSO₄, 4 μM ZnSO₄, 30 μM H₃BO₃, 0.75 μM CuCl₂ and 0.5 μM Na₂MoO₄ (adapted from Sonneveld and Straver, 1989). Nutrients and FA (4-hydroxy-3-methoxycinnamic acid, SIGMA) were dissolved in ultra-pure water and pH was adjusted to 6.0 by H₂SO₄. At the start of the experiments, only trans-FA was detected in the nutrient solution.

The culture flasks were distributed randomly on trolleys in a climate chamber with a photoperiod of 18 h provided by fluorescent tubes (VHA Sylvania Cool White 215 W). Twenty-four flasks, evenly distributed on the trolleys were selected for weekly measurements of light intensity with a quantum sensor (Li190SA, Lambda Instr. Corp., Lincoln, Neb., USA). The mean irradiance±SD at the top of the flasks was 304±45 μmol m⁻² s⁻¹ (Experiment I) and 377±50 μmol m⁻² s⁻¹ (Experiment II) PAR (400–700 nm). In Experiment III, irradiances were 246±30, 365±27 and 489±46 μmol m⁻² s⁻¹ at the low, medium and high light intensity, respectively. Day temperature was 21.7±0.5 °C and night temperature was 17.8±0.5 °C (Experiments I and II), and 21.0±0.2 °C and 18.6±0.2 °C (Experiments III). The atmosphere in each flask...