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Caffeic acid affects early growth, and morphogenetic response of hypocotyl cuttings of mung bean (*Phaseolus aureus*)

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Summary

Caffeic acid (CA) is one of the most common cinnamic acids ubiquitously present in plants and implicated in a variety of interactions including allelopathy among plants and microbes. This study investigated the possible interference of CA with root growth and the process of rhizogenesis in hypocotyl cuttings of mung bean (*Phaseolus aureus* = *Vigna radiata*). Results indicated that CA (0–1000 μM) significantly suppressed root growth of mung bean, and impaired adventitious root formation and root length in the mung bean hypocotyl cuttings. Further investigations into the role of CA in hampering root formation indicated its interference with the biochemical processes involved in rooting process at the three stages – root initiation (third day; RI), root expression (fifth day; RE), and post-expression (seventh day; PE) – of rhizogenesis. CA caused significant changes in the activities of proteases, peroxidases (PODs), and polyphenol oxidases (PPOs) during root development and decreased the content of total endogenous phenolics (TP) in the hypocotyl cuttings. The enhanced activity of PODs and PPOs, though, relates to lignification and/or phenolic metabolism during rhizogenesis; yet their protective role to CA-induced stress, especially during the PE phase, is not ruled out. At 1000 μM CA, where rooting was significantly affected, TP content was very high during the RI phase, thus indicating its non-utilization. The study concludes that CA interferes

Abbreviations: CA, caffeic acid; PE, post-expression stage (seventh day); PODs, peroxidases; PPO, polyphenol oxidases; RE, root expression stage (fifth day); RI, root initiation stage (third day); RL, root length; SL, seedling length; SW, seedling dry weight; TP: total phenolics

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with the rooting potential of mung bean hypocotyl cuttings by altering the activities of PODs and PPOs and the endogenous TP content that play a key role in rhizogenesis.
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Introduction

Phenolic acids are secondary metabolites that form a diverse group of ubiquitously distributed hydroxybenzoic and hydroxycinnamic acids in plants. They have been widely implicated in negative plant–plant interactions including allelopathy (Rice, 1984). They constitute an important class of allelochemicals that release into the soil from plants through various mechanisms such as root exudation, leachation, and residue decomposition (Rice, 1995). Upon release, they play a multitude of ecological and physiological roles. For example, they inhibit plant growth (Rice, 1984), alter mineral uptake (Lyu and Blum, 1990), disrupt membrane permeability (Baziramakenga et al., 1995), cause stomatal closure and induce water stress (Barkosky and Einhellig, 1993), affect photosynthesis and protein synthesis (Baziramakenga et al., 1997), and alter enzyme activities (Rohn et al., 2002; Doblinski et al., 2003).

Caffeic acid (3,4-dihydroxycinnamic acid; $C_9H_8O_4$; hereafter CA), a type of carboxylic acid, is one of the most common cinnamic acids isolated from a variety of crops, weed residues, as well as other plants (Rice, 1995). It inhibits the growth of fungi (Harrison et al., 2003), bacteria (Bowles and Miller, 1994), and plants (Rice, 1984). It is a potent root growth inhibitor (Vaughan and Ord, 1990; Baleroni et al., 2000; Barkosky et al., 2000) and disrupts plant–water relationships and photosynthesis in leafy spurge, *Euphorbia esula* (Barkosky et al., 2000). It alters root activity either by interfering with absorption of K (Glass, 1974) and P_i (Glass, 1975) or by depolarizing root membrane (Glass and Dunlop, 1974). Although studies are available regarding the growth inhibitory and physiological effects of CA, yet little or no information is available about its effect on the process of rhizogenesis and activities of associated enzymes. Keeping this in mind, a study was undertaken to determine the effect of CA on early growth of mung bean (*Phaseolus aureus* = *Vigna radiata*), morphogenetic response in hypocotyl cuttings of mung bean, and changes in the activities of some associated enzymes – proteases, polyphenol oxidases (PPOs), and peroxidases (PODs) – and endogenous total phenolics (TP) at root initiation (third day; 48 h; appearance of root primordia; RI), root expression (fifth day; 120 h; appearance of roots; RE), and post-expression (seventh day; 168 h; PE) stages of rhizogenesis.

Materials and methods

Materials

CA (MW = 180.16) of technical grade (>98% purity) was purchased from Hi-Media Laboratories Ltd., Mumbai, India. For dose–response bioassay and morphogenetic studies, certified seeds of mung bean (*P. aureus* Roxb. = *V. radiata* (L.) Wilczek var. SML-32) were used. A stock solution of 1000 μM CA was prepared in distilled water after dissolving the requisite amount in ethanol and making the final volume with water. The concentration of ethanol in the final solution was ~0.15%. The same volume of ethanol was added to distilled water to serve as control. The stock solution of CA was diluted to obtain concentrations of 100, 10, and 1 μM. All CA solutions were buffered with phosphate buffer and adjusted to pH 6.0 to eliminate the effect due to very low pH. The used concentrations of CA are ecologically relevant since phenolic acid concentrations in the soil generally vary between 0.01 and 0.1 mM (Rice, 1984; Kuiters, 1990; Baleroni et al., 2000).

CA bioassay

The effect of CA (1, 10, 100, and 1000 μM) on early growth of mung bean was studied under laboratory conditions in a Petri dish bioassay (five Petri dishes per treatment and 25 seeds in each). Mung bean seeds imbibed in the respective solution of CA or distilled water (as control) for 8 h were placed equidistantly on Whatman no. 1 filter paper moistened with 7 mL of respective treatment solution in a 15 cm diameter Petri dish. These were maintained in a completely randomized manner in an environmentally controlled growth chamber maintained at 25 ± 2 °C temperature, ~75% relative humidity, and a 16 h/8 h light/dark photoperiod of 180 μmol m^{-2} s^{-1}. After 7 d, root length (RL) and seedling length (SL) (from root tip to shoot tip) and seedling weight (SW) were determined. The experiment was repeated and the data presented as means of both.

Rooting of mung bean hypocotyl cuttings

Mung bean seeds imbibed in distilled water for 8 h were allowed to grow for 7 d in 30 cm × 20 cm enamel

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