BIOLOGIA PLANTARUM 43 (2): 313-316, 2000

BRIEF COMMUNICATION

Lipid accumulation during canola seed germination in response to cinnamic acid derivatives

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Abstract

The objective of this research was to investigate how ferulic and *p*-coumaric acids affect lipid and fatty acid composition during canola (*Brassica napus* L.) seed germination. Data showed that both compounds increased total lipid and fatty acid contents in the cotyledons during germination. The largest accumulation in lipids occurred at 1.0 mM *p*-coumaric acid with an increase in all unsaturated fatty acids. The results suggest that allelochemicals interfere in canola seed germination by reducing lipid mobilization.

Additional key words: allelopathy, *Brassica napus* L., ferulic acid, lipid mobilization, *p*-coumaric acid.

Higher plants regularly release organic compounds by volatilization from their surfaces and through leaf leachates and root exudates. Decomposition products are often added to the soil matrix and some of these have been reported as agents of plant-plant interactions, a phenomenon termed allelopathy (Rice 1984). The magnitude of the impact of allelochemicals on plant depends on the type of compound, concentration and stability of that compound in the soil, and plant resistance and sensitivity to that compound (Einhellig 1995). Cinnamic acid derivatives are commonly found in soils, at concentrations between 0.01 and 0.1 mM, and affect germination and seedling growth of various species at concentrations up to 10.0 mM (Whitehead 1964, Whitehead *et al.* 1982, Macias 1995). Deleterious or toxic effects of cinnamic acids on plant growth are well documented (Lyu and Blum 1990, Vaughan and Ord 1990, Bergmark *et al.* 1992, Booker *et al.* 1992, Baziramakenga *et al.* 1994) but the physiological or biochemical roles of many of them are mostly unknown.

Canola (*Brassica napus* L.) is cultivated as an important oil crop in many parts of the world. The development of cultivars with low erucic acid content in the oil and low glucosinolate content in the meal has made canola a valuable source of good-quality oil for people and nutritional protein for animals (Chen and Heneen 1992). Recently, canola has been cultivated after the soybean but crop yields have not been satisfactory. There are suspicions that root residues, aerial parts, and seeds of soybean which contain cinnamic acid derivatives such as ferulic acid (FA) and *p*-coumaric acid (*p*-CA) (Ramakrishna *et al.* 1989, Sathiyamoorthy 1990) may influence the germination and development of canola. As reported above, the effects of phenolic acids on seedling growth in several species are known but information of these effects on canola seed has not been reported to date. Therefore, the present investigation was conducted to evaluate how cinnamic acid derivatives affect lipid and fatty acid composition of cotyledons during canola seed germination.

Canola seeds (*Brassica napus* L. cv. Hyola 401, obtained from *Zeneca Co.*, Alberta, Canada) were surface sterilized in a solution of 2 % (v/v) sodium hypochlorite for 5 min and washed thoroughly with deionized water.

Received 14 September 1999, *accepted* 20 December 1999.
Abbreviations: FA - ferulic acid, *p*-CA - *p*-coumaric acid, FAME - fatty acid methyl esters.
Acknowledgments: C.R.S. Baleroni is grateful to Capes, Brazil for providing a scholarship.
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For each treatment a batch of 50 seeds was spread in 11 × 11 cm plastic boxes containing two layers of *Whatman No. 1* filter paper moistened with 8 cm^3 of a full-strength nutrient solution (Hoagland and Arnon 1950) with phenolic acid at different concentrations (0.01 to 1.0 mM). The controls were wetted with nutrient solution. The initial pH of all solutions was adjusted to 6.0 with 17 mM potassium phosphate buffer. The boxes were incubated in a germination chamber (*Tecnal TE 400*, São Paulo, Brazil), in darkness (temperature of 25 ± 0.2 °C and relative humidity of 70 - 80 %). The moment when the solution was added to the dry seeds was taken as the zero time of germination. At different times, cotyledons were detached from the roots and oven dried at 75 °C. After 24 h, the cotyledons were stored in a desiccator and the dry matter determined immediately. Experiments were made in aseptic conditions and during germination, fungal infection was absent from all samples tested.

Lipids of cotyledons were extracted in a mortar with chloroform:methanol (2:1, v/v), filtered in *Whatman* paper, and total lipid concentration from each sample was assayed using the sulfo-phospho-vanillin method (Knight *et al.* 1972). Fatty acid methyl esters (FAME) were prepared by transmethylation according to the procedure of the ISO (1978), using 2 M KOH in methanol and *n*-heptane. FAME was analyzed using a *Shimadzu 14A* (Tokyo, Japan) chromatograph equipped with a flame ionization detector and fused silica capillary column (50 m × 0.25 mm × 0.20 µm of 20 M *Carbowax*). The column was operated isothermally at 190 °C, the injector at 220 °C and the detector at 230 °C. Flows of nitrogen, hydrogen and of synthetic air were 30, 30, and 300 cm^3 min^{-1}, respectively. Identification of fatty acids was made by comparing the relative retention times of FAME peaks from samples with standards from *Sigma Chemical Co.* (St. Louis, USA). Peak areas were determined by the CG-300 computing integrator (*CG Instruments*, São Paulo, Brazil).

The experimental design had four replications for the evaluation of lipid and fatty acids. Each plot was represented by two plastic boxes with fifty seeds each one. Data was analyzed using the *InStat*® package (Version 1.12a, *GraphPAD Software*, San Diego, USA). Statistical significance of the difference between parameters was evaluated by means of Student's *t*-test. The phenolic compounds and FAME used in this investigation were purchased from *Sigma Chemical Co.* All other reagents were of analytical grades.

The lipid content gradually decreased in seeds germinated without phenolic acids, showing mobilization of these reserves and the use of their products in root and seedling growth (Fig. 1*A*). After 60 h and 120 h germination, concentrations of total lipid decreased by about 42 and 67 %, respectively. Lipid concentrations decreased also significantly ($P < 0.05$) under the action of phenolic acids; after 60 h the corresponding values were 43 % (with FA) and 52 % (with *p*-CA) higher than that of control. Similarly, after 120 h lipid contents in the cotyledons were 46 % (with FA) and 85 % (with *p*-CA) higher than that in control. The effects of both compounds were significantly different from the control as checked by Student's *t*-test ($P < 0.001$). Concentrations below 1.0 mM also modified cotyledon lipid content (Fig. 1*B*). At 60 h germination, FA and *p*-CA (0.1 and 0.5 mM, but not 0.01 mM) significantly ($P < 0.05$) increased lipid content in the cotyledon. After 120 h, 1.0 mM *p*-CA had more significant effect on lipid content than FA.

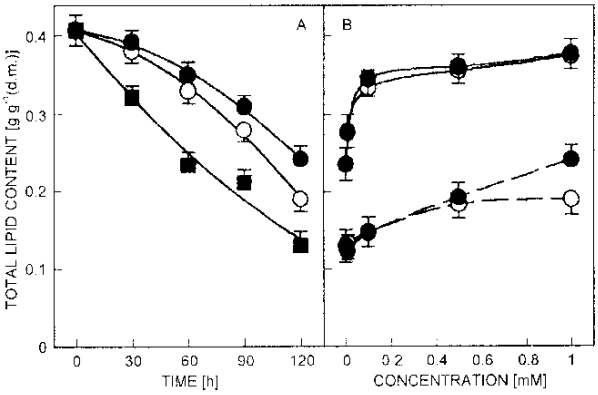

Fig. 1. Effect of phenolic acids (FA and *p*-CA) on cotyledons lipid content during canola seed germination. *A*: - canola seeds were germinated at 25 °C, in darkness, without (*squares*) and with 1.0 mM FA (*open circles*) or 1.0 mM *p*-CA (*closed circles*). *B*: - seeds were germinated at different concentrations (0.01 to 1.0 mM). *Solid lines* - 60 h germination and *broken lines* - 120 h germination. Mean values ± SE (n = 4).

After 60 h, FA and *p*-CA (0.1 mM or 1.0 mM) altered unsaturated (but not saturated) fatty acids content (Fig. 2). Although polyunsaturated (linoleic and linolenic) acid contents were affected by FA and *p*-CA, the most significant increases occurred in oleic acid content. The corresponding values were 55 and 60 % with 0.1 mM FA or *p*-CA respectively, and, even, 83 and 97 % with 1.0 mM FA or *p*-CA in comparison to the control ($P < 0.001$). No significant difference ($P > 0.05$) occurred among the allelochemicals for any fatty acid. Effects of phenolic acids on fatty acid content were different over time. At 0.1 mM FA or *p*-CA (Fig. 2*C*, 120 h), fatty acid contents were not modified significantly. However, unsaturated fatty acid contents (Fig. 2*A*, 60 h) were significantly ($P < 0.001$) affected by the compounds. 1.0 mM FA increased oleic, linoleic and linolenic acid contents less than *p*-CA (Fig. 2*B*, *D*).

Results of the present investigation suggest that lipid accumulation induced by FA and *p*-CA may be due to the reduction in the mobilization of these reserves. Although the mechanisms involved are still unknown, these modifications may be important in explaining allelopathic

effects caused by phenolic acids. Among countless other actions, allelochemicals suppress seed germination, cause injury to root growth, or inhibit seedling growth (Einhellig 1995). At cellular level, these compounds alter membrane properties into which they can be incorporated (Shann and Blum 1987), modify their fluidity (Liu and Lovett 1993), affect certain enzymes (Politycka 1996, Sert *et al.* 1997/98) and, consequently, the energy metabolism (Baziramakenga *et al.* 1995, Maffei *et al.* 1999). Preliminary results (unpublished data) showed that both allelochemicals reduced the canola germination percentage. They inhibited primary root elongation, the seedlings had fewer lateral roots and tended to grow horizontally. After 120 h germination, the cotyledon dry matter increased suggesting reduction in reserve mobilization. Effects on specific enzymes that act on lipid mobilization remain to be explored and, in this context, further studies regarding the actions of phenolic acids on lipases and isocitrate lyase (Baleroni *et al.* 1997) would yield useful information.

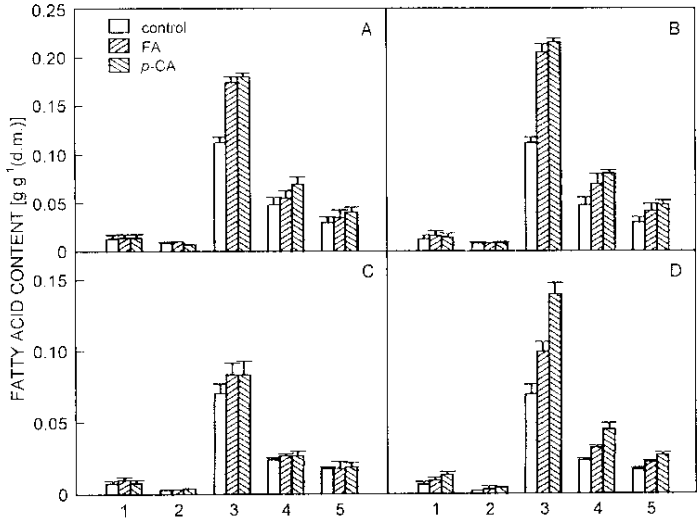

Fig. 2. Effect of phenolic acids on fatty acid content in the cotyledons after 60 h (*A*, *B*) and 120 h (*C*, *D*) germination. Canola seeds were germinated at 25 °C, in darkness, with 0.1 mM (*A*, *C*) and 1.0 mM (*B*, *D*) FA or *p*-CA. *1* - palmitic, *2* - stearic, *3* - oleic, *4* - linoleic, and *5* - linolenic acid. Mean values ± SE (n = 4).

References

Baleroni, C.R.S., Ferrarese, M.L.L., Costa, S.C., Souza, N.E., Ferrarese-Filho, O.: Isocitrate lyase activity and mobilization of lipids and carbohydrates in cotyledons of canola. - Rev. Bras. Fisiol. Veg. **9**: 189-192, 1997.

Baziramakenga, R., Sunard, R.R., Leroux, G.D.: Effects of benzoic and cinnamic acids on growth, mineral composition, and chlorophyll content of soybean. - J. chem. Ecol. **20**: 2821-2833, 1994.

Baziramakenga, R., Leroux, G.D., Simard, R.R.: Effects of benzoic and cinnamic acids on membrane permeability of soybean roots. - J. chem. Ecol. **21**: 1271-1285, 1995.

Bergmark, C., Jackson, W.A., Volk, R.J., Blum, U.: Differential inhibition by ferulic acid of nitrate ammonium uptake in *Zea mays* L. - Plant Physiol. **98**: 639-645, 1992.

Booker, F.L., Blum, U., Fiscus, E.L.: Short-term effects of ferulic acid on ion uptake and water relations in cucumber seedlings. - J. exp. Bot. **43**: 649-655, 1992.

Chen, B.Y., Heneen, W.K.: Inheritance of seed colour in *Brassica campestris* L. and breeding for yellow-seeded *Brassica napus* L. - Euphytica **59**: 157-163, 1992.

Einhellig, F.A.: Allelopathy. Current status and future goals. - In: Inderjit, Dakshini, K.M.M., Einhellig, F.A. (ed.): Allelopathy. Organisms, Processes and Applications. Pp. 1-24. American Chemical Society, New York 1995.

Hoagland, D.R., Arnon, D.I.: The water-culture method of growing plants without soil. - California agr. exp. Sta. Circ. **347**, Berkeley 1950.

ISO - International Organization for Standardization - Animal and vegetables oils. Preparation of methyls esters of fatty acids. Methods ISO 5509, 1978.

Knight, J.A, Anderson, S., Rawle, J.M.: Chemical basis of the sulfo-phospho-vanillin reaction for estimating total serum lipids. - Clin. Chem. **18**: 199-202, 1972.

Liu, D.L., Lovett, J.V.: Biologically active secondary metabolites of barley. II. Phytotoxicity of barley allelochemicals. - J. chem. Ecol. **19**: 2231-2244, 1993.

Lyu, S.W., Blum, U.: Effects of ferulic acid, an allelopathic compound, on net P, K, and water uptake by cucumber seedlings in a split-root system. - J. chem. Ecol. **16**: 2429-2439. 1990.

Macias, F.: Allelopathy in the search for natural herbicides models. - In: Inderjit, Dakshini, K.M.M., Einhellig, F.A. (ed.): Allelopathy. Organisms, Processes and Applications. Pp. 311-329, American Chemical Society, New York 1995.

Maffei, M., Bertea, C.M., Garneri, F., Scannerini, S.: Effect of benzoic acid hydroxy and methoxy ring substituents during cucumber (*Cucumis sativus* L.) germination. I. Isocitrate lyase and catalase activity. - Plant Sci. **141**: 139-147, 1999.

Politycka, B.: Peroxidase activity and lipid peroxidation in rests of cucumber seedlings influenced by derivatives of cinnamic and benzoic acids. - Acta Physiol. Plant. **18**: 365-370, 1996.

Ramakrishna, M.B.V., Mital, B.K., Gupta, K.C., Sand, A.N.: Determination of phenolic acids in different soybean varieties by reversed phase high performance liquid chromatography. - J. Food Sci. Technol. **26**: 154-155, 1989.

Rice, E.L.: Allelopathy. - Academic Press. New York 1984.

Sathiyamoorthy, P.: Identification of vanillic and *p*-coumaric acid as endogenous inhibitors of soybean seeds and their inhibitory effect on germination. - J. Plant Physiol. **136**: 120-121, 1990.

Sert, M.A., Ferrarese, M.L.L., Bernadelli, Y.R., Kelmer-Bracht, A.M., Bracht, A., Ishii-Iwamoto, E.L.: Effects of ferulic acid on L-malate oxidation in isolated soybean mitochondria. - Biol. Plant. **40**: 345-350, 1997/98.

Shann, J.R., Blum, U.: The utilization of exogenously supplied ferulic acid in lignin biosynthesis. - Phytochemistry **26**: 2977-2982, 1987.

Vaughan, D., Ord, B.: Influence of phenolic acids on morphological changes in roots of *Pisum sativum*. - J. Sci. Food Agr. **52**: 289-299, 1990.

Whitehead, D.C.: Identification of *p*-hydroxybenzoic, vanillic, *p*-coumaric, and ferulic acids in soils. - Nature **202**: 417-418, 1964.

Whitehead, D.C., Dibb, H., Hartley, R.D.: Phenolic compounds in soil as influenced by the growth of different plant species. - J. appl. Ecol. **19**: 579-588, 1982.