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Fatty Acids Production from Plants and Callus Cultures of *Cereus peruvianus* Mill. (Cactaceae)

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Abstract: Callus culture of *Cereus peruvianus* provides an alternative method to unsaturated and unusual fatty acids production. Analysis of fatty acids composition of the lipids extracted from hypocotyls-induced calli showed that linoleic acid ($C_{18:2}$) was the major component of unsaturated fatty acids in the callus culture. Unusual fatty acids such as pentadecenoic acid ($C_{15:1}$), palmitolenic acid ($C_{16:1}$) and heptadecanoic acid ($C_{17:0}$) were also detected in callus tissues. The long-time callus culture of *C. peruvianus* is a good model for the biochemistry studies of $C_{18:2}$ rich tissues, for unusual fatty acid production and for metabolic engineering of plants.

Key words: Cactus, tissue culture, unsaturated fatty acids, unusual fatty acids

Introduction

Plants, unlike animal, are rich in unsaturated fatty acids and there is evidence that some of these have potential applications in the food, pharmaceutical, paint, detergent and plastic industries (Harborne, 1998; Somerville *et al*., 2000). The polyunsaturated fatty acids, in particular, are clinically useful in human disorders (Harbige, 2003; Clore *et al*., 2004; Astorg, 2004) and are recognized as essential nutrients in the human diet (Collett *et al*., 2001; Kelly *et al.,* 2003). Polyunsaturated fatty acids have been recommended as a therapeutic measure in preventive medicine (Wang *et al*., 2004; Diniz *et al*., 2004).

Many of the unusual fatty acids found in nature have also important industrial uses (Somerville *et al*., 2000). However, the plants producing them are often poorly suitable for high-production agriculture. Because the plants are plastic enough to adapt to a range of environmental regimes (Evans *et al*., 1988), contrasting biochemical and physiological characteristics can be found in tissues of the same specie of plant that are cultured to different habitats. The differential biochemical and physiological characteristics can produce qualitative and quantitative changes of compounds and in this case a same extraction protocol can not be used to the same specie cultured in different regions of the continents.

This problem can be bypass by *in vitro* tissue culture. *In vitro* tissue culture is a beneficial strategic because the compounds can be obtained under control environmental, regardless of climatic changes and soil conditions (Collin, 2001). Living plants generally present various concentrations of the larger compounds, which may depend on the specific time of the plant harvest (Salmore and Hunter, 2001; Puricelli *et al*., 2002; Ralphs and Gardner, 2001).

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Unsaturated fatty acids, as well as longer chain than 18 hydrocarbons, have been obtained from *Cereus peruvianus* plant species (Dembitsky and Rezanka, 1996; Rezanka and Dembitsky, 1998). Previous studies have shown that *C. peruvianus* callus tissue have been explored for *in vitro* production of alkaloids (Oliveira and Machado, 2003) and polysaccharides (Machado *et al*., 2004). This callus culture may also be a potential unsaturated fatty acids source. Then, in the current study callus culture of *C. peruvianus* was used as an alternative method to unsaturated fatty acids production.

Material and Methods

Biological Material

The shoots of adult plants and the long-term callus culture of *C. peruvianus* were used for lipid extraction and characterization. The shoots were collected from plants maintained for 15 years on the campus of the State University of Maringá (Maringá, PR., Brazil). The long-term culture callus tissues were obtained from hypocotyls in MS medium containing B5 vitamins, 0.8% agar (Select Agar-Invitrogen Life Technologies), 3% sucrose, 4.0 mg dm^{-3} of 2.4-dichlorophenoxiacetic acid and 4.0 mg dm^{-3} of *N*-(2-furanylmethyl)-1H-purine-6 amine (Oliveira *et al*., 1995), maintained at 32°C during a 16h photoperiod and 15 μmol $m^{-2}{\cdot}s^{-1}$ irradiance (provided by fluorescent tubes 20 W). Calli were generated from pieces of hypocotyls stalks of six seedlings that grew from seeds collected from a single plant (Oliveira *et al*., 1995). The calli (14 years old) have been subcultured in fresh medium at 30-60 days interval.

Lipids Extraction

The lyophilized callus and plant tissue (1 g) were extracted by maceration with two 20 cm^3 portions of cold isopropanol and this was followed by re-extraction with two portions of 20 cm^3 of chloroform-methanol solution (2:1), both fraction were pooled an solvents were eliminated in rotative evaporator.

The lipids extracts were separated in neutral and polar lipids by column chromatography on silicagel in ethereal solution, the neutral lipids passed through, leaving the phospho and glycolipids adsorbed. The neutral lipids ethereal fraction was evaporated yield 7.7 mg (0.77%) in the callus neutral lipids (CNL) and 3.9 mg (0.39%) in the plant neutral lipids (PNL).

Saponification of Neutral Lipids

The samples of CNL (3.88 mg) and PNL (1.8 mg), KOH (0.5 g) and methanol (50 cm^3) were heated under reflux for 30 min. After, the methanol was evaporated up to approximately 10 cm^3, which was completed with addition of 50 cm^3 of water. The unsaponificated matter was extracted from alkaline mixture, with ethyl ether.

Methylation of Fatty Acids

The alkaline solutions were acidificated with diluted HCl (10%, cm^3 m^{-3}) up to pH 2 and fatty acids were extracted with ethyl ether, after the water was removed with anhydrous sodium sulfate and ethyl ether distillated yielding the free fatty acids. The samples, 2 drops of concentrated HCl and 5 cm^3 of anhydrous methanol were heated under reflux for 5 min, after 10 cm^3 of water was added to reaction and the methyl fatty acids were extracted with hexane. The hexane fractions were dried with anhydrous sodium sulfate and evaporated.

Gas Chromatography – Mass Spectrometry Analysis

The methyl esters were analyzed after preparation using a Shimadzu GC/MS model QP 2000, EI 70 eV. The analytical column (SE-30) was a 50 m × 0.25 mm fused-silic capillary coated

Table 1: Fatty acids proportion extracted from the shoot and callus tissue culture of *Cereus peruvianus*

Fatty acids	Plant shoots (%)	Callus tissue (%)
$C_{10:0}$	2.36	2.10
$C_{10:1}$	2.36	nd
$C_{14:0}$	3.90	32.20
$C_{15:0}$	2.12	1.12
$C_{15:1}$	nd	2.24
$C_{16:0}$	35.10	nd
$C_{16:1}$	nd	1.96
$C_{17:0}$	nd	13.00
$C_{18:0}$	7.88	4.20
$C_{18:1}$	tr	nd
$C_{18:2}$	nd	37.80
$C_{20:0}$	2.36	0.42
$C_{22:0}$	3.15	0.56
Total of SFA	56.87	53.60
Total of PUFA	2.36	42.00

Nd, not determined; tr, traces < 0.1%
SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid

with 0.25 μm film thickness of and was temperature-programmed for samples from 50 to 260 °C at 5°C m^{-1}, then held at 220°C for 5 min. A 1:50 split injection ratio was used with He as the carrier gas.

Results and Discussion

A comparative study of fatty acids composition of the lipids extracted from the shoot and callus tissues derived from hypocotyls plant parts of *C. peruvianus* showed that linoleic acid ($C_{18:2}$) was the major component or unsaturated fatty acids in the callus culture while in the plants palmitic acid ($C_{16:0}$) was the main constituent (Table 1). In the plant shoots, stearic acid ($C_{18:0}$) was detected in higher proportion but oleic acid ($C_{18:1}$) was present in traces amount; oleic acid was absent in callus tissues.

Myristic acid ($C_{14:0}$) was detected in higher amount in callus tissues than in shoot of the plants and short chain fatty acid such as decanoic acid ($C_{10:0}$) was present in more or less equal proportion in both tissues. Unusual fatty acids such as pentadecanoic acid ($C_{15:1}$), palmitolenic acid ($C_{16:1}$) and heptadecanoic acid ($C_{17:0}$) were detected in small amounts only in callus tissues. Long chain fatty acid ($C_{20:0}$) and ($C_{22:0}$) were detected in higher proportion in plant shoots. The callus culture had a higher proportion of unsaturated fatty acids than saturated fatty acids while usual unsaturated fatty acids was not detected in shoot of the *C. peruvianus* plants (Table 1).

The differential fatty acids proportion for the lipids from *C. peruvianus* shoots and callus culture may be explained by differential metabolic flux in callus tissues growing in culture medium. Influence of the culture medium in pattern of fatty acids production in callus culture is known in others plant species (Halder and Gadgil, 1984; Lopez *et al*., 1999; Chiou *et al*., 2001). This is a positive aspect of the callus culture since unsaturated fatty acid production could be increased after medium optimization by addition of supplement on callus medium and with the establishment of suspension callus cultures. A relationship between culture medium containing tyrosine and total alkaloid production was reported in *C. peruvinaus* callus culture (Oliveira and Machado, 2003); callus increased the capacity for alkaloid synthesis by 11%. Optimization for production of fatty acids *in vitro* has been obtained by manipulation of nutrient sources of culture medium and by different *in vitro* maintenance conditions of the callus tissues (Hirano *et al*., 1997; Chiou *et al*., 2001; Su *et al*., 2004).

The linoleic acid produced by callus culture of *C. peruvianus* is an economically important factor since linolenic acid have been used to synthesis of conjugated linoleic acid, which present pharmaceutical industrial applications (Guo and Sun, 2004; Yang and Liu, 2004). Anti proliferation

effect and anti tumor ability of conjugated linoleic fatty acid have been reported by Agatha *et al*. (2004) and Wang *et al*. (2004) studies.

In *C. peruvianus* we found that hypocotyls-induced calli are capable to producing significant amounts of unsaturated and unusual fatty acids, which are found in lower proportions or absent in shoot of adult plants. Furthermore, a differential fatty acid proportion and composition was obtained from *C. peruvianus* shoots maintained on the campus of the State University of Maringa when compared to fatty acid composition detected in *C. peruvianus* plants maintained in a different region of the continent under different environmental conditions (Dembitsky and Rezanka, 1996). Six major fatty acids have been identified including $C_{16:0}$ (18.6%), $C_{16:1}$ (11.3%), $C_{18:0}$ (13.6%), $C_{18:1}$ (11%), $C_{24:0}$ (7.1%) and $C_{24:1}$ (5.7%) by Dembitsky and Rezanka (1996) study.

The plants of the different regions had different fatty acid composition but the long-term callus culture from hypocotyls could have similar fatty acid pattern when isolated and grown under identical *in vitro* conditions. A stable and repeated growth of the callus under standard conditions (Oliveira *et al*., 1995) has been observed after more a hundred subculture cycles (14 years with periodic subculture at 30-60 days-interval). There are, therefore, indications that callus tissue culture of *C. peruvianus* form a stabilized cell line, which, according to Bougard *et al*. (2001), could be regarded as such when growth parameters could be repeated during three consecutive subculture cycles in stable culture conditions. Genetic stability of the *C. peruvianus* callus culture has been observed since unchanged esterase isozyme pattern in hundreds callus have been detected in long-term callus culture maintained in their original culture conditions (unpublished results).

Thus, the long-time callus culture of *C. peruvianus* is a good model for the biochemistry studies of $C_{18:2}$ rich tissues and for metabolic engineering of plants. Metabolic engineering of plants is now routinely possible to introduce gene required for synthesis and/or modification of different types of fatty acids (Suh *et al*., 2002; Kinney *et al*., 2002; Thelen and Ohlrogge, 2002) since gene for many of the important enzymes involved in processes have been cloned (Budziszewski *et al*., 1996; Murphy, 1999a, b). A further perspective to callus tissues of *C. peruvianus* is the introduction of gene involved in the expression of fatty acid modification. Transformed *C. peruvianus* cell line may represent an attractive biological system for producing unsaturated and unusual fatty acids since they offer economic and qualitative benefits. *In vitro* culture of transformed cell line will take advantage of the existing infrastructure for transgenic plants cultivation, processing and storage and overcome the potential risks for human heath and environmental.

References

Agatha, G., A. Voigt, E. Kauf and F. Zintl, 2004. Conjugated linoleic acid modulation of cell membrane in leukemia cells. Cancer Lett., 209: 87-103.

Astorg, P., 2004. Dietary n-6 and n-3 Polyunsaturated fatty acids and prostate cancer risk: A review of epidemiological and experimental evidence. Cancer Causes Control, 15: 367-386.

Bougard, F., A. Gravot, S. Milesi and E. Gontier, 2001. Production of plant secondary metabolites: A historical perspective. Plant Sci., 161: 839-851.

Budziszewski, G.J., K.P. Croft and D.F. Hildebrand, 1996. Uses of biotechnology in modifying plant lipids. Lipids, 31: 557-569.

Chiou, S-Y., W-W. Su and Y-C. Su, 2001. Optimization production of polyunsaturated fatty acids in *Marchantia polymorpha* cell suspension culture. J. Biotechnol., 85: 247-257.

Clore, J.N., J.S. Stillman, J. Li, S.J. O'Keefe and J.R. Levy, 2004. Differential effect of saturated and polyunsaturated fatty acids on hepatic glucose metabolism in humans. Am. J. Physiol. Endocrinol. Metab., 287: 358-365.

Collett, E.D., L.A. Davidson, Y.Y. Fan, J.R. Lupton and R.S. Chapkin, 2001. n-6 and n-3 polyunsaturated fatty acids differentially modulate oncogenic *Ras* activation in colonocytes. Am. J. Physiol. Cell Physiol., 280: 1066-1075.

Collin, H.A., 2001. Secondary product formation in plant tissue cultures. Plant Growth Regulation; 34: 119-134.

Dembitsky, V.M. and T. Rezanka, 1996. Molecular species of wax esters in *Cereus peruvianus*. Phytochemistry, 42: 1075-1080.

Diniz, Y.S., A.C. Cicogna, C.R. Padovani, L.S. Santana, L.A. Faine and E.L. Novelli, 2004. Diets rich in saturated and polyunsaturated fatty acids: Metabolic shifting and cardiac health. Nutrition, 20: 230-234.

Evans, J.R., S. von Caemmerrer and W.W. Adams, 1988. Photosynthesis: carbon reactions, In: Ecology of Photosynthesis in Sun and Shade, pp: 195-249. CSIRO, Mlbourne.

Guo, Z. and Y. Sun, 2004. Sovent-free enzymatic synthesis of 1,3-diconjugated linoleoyl glycerol optimized by response surface methodology. Biotechnol. Prog., 20: 619-622.

Halder, T. and V.N. Gadgh, 1984. Comparison of fatty acid patterns in plant parts and respective callus cultures of *Cucumis melo*. Phytochemistry; 23: 1790-1791.

Harbige, L.S., 2003. Fatty acids, the immune response and autoimmunity: a question of n-6 essentially and the balance between n-6 and n-3. Lipids, 38: 323-341.

Harborne, J.B., 1998. Organic Acids, Lipids and Related Compounds. In: Phytochemical Methods – A Guide to Modern Techniques of Plant Analysis, pp: 151-186. Chapman & Hall, London.

Hirano, M., N. Sudah, K. Abe and R. Teruo, 1997. Polyunsaturated fatty acid production by Ribes rubrum calli. J. Ferment. Bioeng., 83: 608-611.

Kelly, O., S. Cusack, C. Jewell and K.D. Cashman, 2003. The effect of polyunsaturated fatty acids, including conjugated linoleic acid, on calcium absortion and bone metabolism and composition in young growing rats. Br. J. Nutr., 90: 743-750.

Kinney, A.J., E.B. Cahoon and W.D. Hitz, 2002. Manipulating desaturase activities in transgenic crop plants. Biochem. Soc. Trans., 30: 1099-1103.

Lopez, M.C., I.R. Sánchez-Mendoza and N. Ochoa-Alejo, 1999. Comparative study of volatile components and fatty acids of plants *in vitro* cultures of parsley (*Petroselinum crispum* Mill. Nym ex Hill). J. Agric. Food Chem., 47: 3292-3296.

Machado, F.A.P.S.A., A.J.B. Oliveira, C.A. Mangolin, L. Gobbi-Filho and M.F.P.S. Machado, 2004. Polysaccharide production from callus cultures of *Cereus peruvianus* Mill. (Cactaceae). Crop Breed. Applied Biotechnol., 4: 313-316.

Murphy, D.J., 1999a. Manipulation of plant oil composition for the production of valuable chemicals. Progress, problems and prospects. Adv. Exp. Med. Biol., 464: 21-35.

Murphy, D.J., 1999b. Production of novel oils in plants. Curr. Opin. Biotechnol., 10: 175-180.

Oliveira, A.J.B. and M.F.P.S. Machado, 2003. Alkaloid production by callous tissue cultures of *C. peruvianus* (Cactaceae). Applied Biochem. Biotechnol., 104: 149-155.

Oliveira, S. A., M.F.P.S. Machado, A.J. Prioli and C. A. Mangolin, 1995. *In vitro* propagation of *Cereus peruvianus* Mill. (Cactaceae). *In Vitro* Cell. Dev. Biol. Plant, 31: 47-50.

Puricelli, L., G. Innocenti, G. Delle Monache, R. Caniato, R. Filippini and E.M. Cappelletti, 2002. *In vivo* and *in vitro* production of alkaloids by *Haplophyllum patavinum*. Nat. Prod. Lett., 16: 95-100.

Ralphs, M.H. and D.R. Gardner, 2001. Alkaloid levels in duncecap (*Delphinium occidentale*) and tall larkspur (*D. barbeyi*) grown in reciprocal gardens: Separating genetic from environmental influence. Biochem. Syst. Ecol., 29: 117-124.

Rezanka, T. and V.M. Dembitsky, 1998. Very-long-chain alkyl esters in *Cereus peruvianus* wax. Phytochemistry, 42: 1145-1148.

Salmore, A.K. and M.D. Hunter, 2001. Elevational trends in defense chemistry, vegetation and reproduction in *Sanguinaria canadensis*. J. Chem. Ecol., 27: 1713-1727.

Somerville, C., J. Browse, J.G. Jaworski and J.B. Ohlrogge, 2000. Lipids. In: Biochemistry . Molecular Biology of Plants. Buchanan, B.B., W. Gruissem and R.L. Jones (Eds.). American Society of Plant Physiologists, Rockville, Maryland, pp: 456-527.

Su, X.Q., K.N. Antonas and D. Li, 2004. Comparison of n-3 polyunsaturated fatty acid contents of wild and cultured Australian abalone. Intl. J. Food Sci. Nutr., 55: 149-154.

Suh, M.C., D.J. Schultz and J.B. Ohlrogge, 2002. What limits production of unusual monoenoic fatty acid in transgenic plants? Planta, 215: 584-595.

Thelen, J.J. and J.B. Ohlrogge, 2002. Metabolic engineering of fatty acid biosynthesis in plants. Metab. Eng., 4: 12-21.

Wang, X.L., B.Q. Chen, Y.M. Yang, Y.M. Zheng and J.R. Liu, 2004. The effect of conjugated linoleic acid on the ability of murine macrophage in killing tumor cells. Zhonghua Yu Fang Yi Xue Za Zhi, 38: 107-110.

Yang, T.S. and T.T. Liu, 2004. Optimization of production of conjugated linolei acid from soybean oil. J. Agric. Food Chem., 52: 5079-5084.