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 (Colletet 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 (Sulmone 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-dichlorophenoxyacetic acid and 4.0 mg dm⁻³ of N-(2-furanyl methyl)-1H-purine-6-amine (Oliveira et al., 1995), maintained at 32°C during a 16h photoperiod and 15 μmol m⁻²s⁻¹ irradiance (provided by fluorescent tubes 20 W). Cali 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 cm³ portions of cold isopropanol and this was followed by re-extraction with two portions of 20 cm³ of chloroform-methanol solution (2:1), both fraction were pooled and 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 cm³) were heated under reflux for 30 min. After, the methanol was evaporated up to approximately 10 cm³, which was completed with addition of 50 cm³ 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⁻¹) up to pH 2 and fatty acids were extracted with ethyl ether, after the water was removed with anhydrous sodium sulfate and ethyl ether distilled yielding the free fatty acids. The samples, 2 drops of concentrated HCl and 5 cm³ of anhydrous methanol were heated under reflux for 5 min, after 10 cm³ 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 *Cyperus peruvianus*

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Plant shoots (%)</th>
<th>Callus tissue (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>2.36</td>
<td>2.10</td>
</tr>
<tr>
<td>C18:1</td>
<td>2.36</td>
<td>nd</td>
</tr>
<tr>
<td>C18:2</td>
<td>3.90</td>
<td>32.20</td>
</tr>
<tr>
<td>C18:3</td>
<td>2.12</td>
<td>1.12</td>
</tr>
<tr>
<td>C20:1</td>
<td>nd</td>
<td>2.24</td>
</tr>
<tr>
<td>C20:3</td>
<td>35.10</td>
<td>nd</td>
</tr>
<tr>
<td>C20:4</td>
<td>nd</td>
<td>1.96</td>
</tr>
<tr>
<td>C22:0</td>
<td>nd</td>
<td>13.00</td>
</tr>
<tr>
<td>C22:3</td>
<td>7.88</td>
<td>4.20</td>
</tr>
<tr>
<td>C22:1</td>
<td>tr</td>
<td>nd</td>
</tr>
<tr>
<td>C22:2</td>
<td>nd</td>
<td>37.80</td>
</tr>
<tr>
<td>C20:1</td>
<td>2.36</td>
<td>0.42</td>
</tr>
<tr>
<td>C20:3</td>
<td>3.15</td>
<td>0.56</td>
</tr>
<tr>
<td>Total of SFA</td>
<td>56.87</td>
<td>53.60</td>
</tr>
<tr>
<td>Total of PUFA</td>
<td>2.36</td>
<td>42.00</td>
</tr>
</tbody>
</table>

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 min⁻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 (C18:2) was the major component or unsaturated fatty acids in the callus culture while in the plants palmitic acid (C16:0) was the main constituent (Table 1). In the plant shoots, stearic acid (C18:0) was detected in higher proportion but oleic acid (C18:1) was present in traces amount; oleic acid was absent in callus tissues.

Myristic acid (C14:0) was detected in higher amount in callus tissues than in shoot of the plants and short chain fatty acid such as decanoic acid (C10:0) was present in more or less equal proportion in both tissues. Unusual fatty acids such as pentadecanoic acid (C15:0), palmitoleic acid (C16:1) and heptadecanoic acid (C17:0) were detected in small amounts only in callus tissues. Long chain fatty acid (C20:0) and (C22:0) 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; Chiu 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. peruvianus* 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; Chiu et al., 2001; Su et al., 2004).

The linoleic acid produced by callus culture of *C. peruvianus* is an economically important factor since linoleic 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 C₁₈:3 (18.6%), C₁₈:1 (11.3%), C₁₆:0 (13.6%), C₁₆:1 (11%), C₂₀:0 (7.1%) and C₂₁:4 (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-term callus culture of *C. peruvianus* is a good model for the biochemistry studies of C₁₈:2 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 health and environmental.

References


