The effect of temperature on hairy root cultures of *Catharanthus roseus*: Growth, indole alkaloid accumulation and membrane lipid composition

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**Abstract.** Cultivation of *Catharanthus roseus* hairy root cultures at different temperatures was found to have an effect on growth rate and indole alkaloid content as well as lipid composition. When lowering the temperature, the roots responded by increasing the degree of unsaturation of cellular lipids, which was mainly due to an increased proportion of linolenic acid in the main lipid classes. The modifications in lipid composition were obviously necessary for the roots to retain the proper cell membrane fluidity at each temperature. Despite of changes in membrane lipids, no effect on the distribution of indole alkaloids between the roots and the medium could be detected. Instead, the level of alkaloid accumulation showed a clear increase with lowering temperature.

**Abbreviations.** PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PG, phosphatidylglycerol; CL, cardiolipin; DGD, digalactosyldiglyceride; PI, phosphatidylserine; PG, phosphatidylglycerol; NL, neutral lipids; DU, degree of fatty acid unsaturation.

**Introduction**

Temperature-induced changes in membrane lipid composition of plants and their role in temperature adaptation have been studied extensively in a wide variety of biological systems (Levitt 1980; Neidleman 1987). Often, plant tissues have been observed to contain higher amounts of unsaturated acids at reduced temperatures. Furthermore, changes in fatty acid chain length or an increase in phospholipid content have been reported in plants when exposed to low temperature. The actual role of cell membrane modifications in temperature adaption is however still obscure, and many contradictory results on the subject have been published (Willemeit 1979; Raison 1980). Plant cell cultures have also been used with varying results to study the effect of temperature on membrane lipids (Gawer et al. 1983; Radwan et al. 1978; Rebeille et al. 1980). In our laboratory, temperature responses of *Catharanthus roseus* cell suspension cultures were studied recently, and the cultivation temperature was found to affect both the degree of fatty acid unsaturation and the mean fatty acid chain length of membrane lipids (Toivonen et al. 1992). Hairy root cultures, obtained through genetic transformation of dicotyledonous plants by *Agrobacterium rhizogenes*, have proved to be genetically more stable than suspension cultures. As they grow well without growth hormones and have a metabolism resembling that of intact plant roots (Hamill 1987), they may offer a suitable *in vitro* system for studying plant lipid metabolism. In the present work, our aim was to find out whether the temperature-induced changes observed in cell cultures appear also in hairy root cultures of *C. roseus*. Furthermore, knowing that culture temperature affects suspension cultures (Courtin and Guern, 1980; Morris 1986), we were interested in the effects of cultivation temperature on growth and indole alkaloid content of hairy root cultures of *C. roseus*, as this has not been studied previously. The uptake of alkaloids across the tonoplast into the vacuoles for storage has been suggested to be an active, energy-requiring mechanism (Deus-Neumann and Zenk 1984). Therefore, particularly the distribution of alkaloids between the tissue and the medium was examined at different temperatures, in order to find out, if a correlation exists between temperature-induced changes in membrane lipids and alkaloid release.

**Materials and Methods**

**Hairy root culture.** Hairy root cultures of *Catharanthus roseus* (L.) G.Don cv. Little Delicata were established and maintained as described by Toivonen et al. (1989, 1991). Clone No 8 was used in the present studies.

**Culture experiments at different temperatures.** To study the effect of growth temperature on *C. roseus* hairy roots cultures, ca.0.7 g of root tips were inoculated into 40 ml of liquid 1/4B50 medium (one-fourth dilution of the standard B5 (Gamborg et al. 1968), bat containing optimized phosphate and nitrate concentrations (Toivonen et al. 1991), 40 g sucrose/l, 45 mg (NH₄)₂SO₄/l and 0.5g/l carbenicillin (Sigma). The cultures were incubated at 19.5°C, 24°C or 32°C on a gyratory shaker (60 rpm) in the dark. During the cultivation, samples (two parallel flasks) were withdrawn for the analysis of growth and lipid composition until the stationary phase of growth was reached.

**Analysis of growth.** Growth was measured as increase of both fresh and dry weight. The roots were harvested by filtration, washed twice with distilled water, frozen and lyophilized.

**Fatty acid analysis.** To determine the fatty acid composition of the samples, ca. 20 mg of lyophilized and powdered roots were weighed, suspended in an excess of saponification reagent and analysed as described by Toivonen et al. (1992). All the fatty acid analyses were performed in duplicate the calculated values thus being mean values of four separate analyses (two parallel samples both analysed in duplicates).
The standard deviation (n=4) was always <0.7% for the relative amounts and <0.08% of root dw for the total absolute amounts of fatty acids. The degree of unsaturation in the lipids was calculated as $\Delta\text{mole} = (1.0(%\text{monoenoic acid}) + 2.0(%\text{dienoic acid}) + 3.0(%\text{trienoic acid})) / 100$ and the mean fatty acid chain length was calculated as the the ratio of C16:C18.

**Lipid extraction and analysis.** The lipids were extracted from the lyophilized and powdered roots as described by Toivonen et al. (1992)

**Indole alkaloid analysis.** Indole alkaloids were extracted and analysed as described previously by Toivonen et al (1991). Ibogain (C.Roth GmBdH) was used as an internal standard.

**Results and Discussion**

**Growth and fatty acid content of hairy root cultures at different temperatures**

The growth of *C. roseus* hairy root cultures was affected by the cultivation temperature, the doubling times being 3.2d, 4.4d and 8.7d at 32°C, 24°C and 19.5°C, respectively (Fig.1a). At 32°C the growth was thus fastest, but the roots were brownish and formed callus. However, the final biomass yield was about the same (11-12 g/l) at all the temperatures studied.

The total fatty acid content of the hairy roots during the cultivation at different temperatures is illustrated in Fig.1b. In every case, the fatty acid content of the roots was highest in the beginning of the period of active growth and decreased towards the end of the growth cycle. This was obviously due to a decline in the proportion of meristematic, actively dividing root cells rich in endoplasmic reticulum. Even at the end of growth the fatty acid content of the hairy root tissue was somewhat higher than that of intact plant roots (0.94% of root dw), which could also be explained by a difference in the content of meristematic regions.

Irrespective of the temperature used the fatty acid pattern of the hairy root cultures was found to be similar to that of intact plant roots: Palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and linolenic acids (C18:3) accounted for more than 95% of the total amount of fatty acids, and stearic acid (C18:0) could always be detected in small quantities. Laurie (C12:0), myristic (C14:0), arachidic (C20:0), behenic (C22:0) and C16:0 dicarboxylic acid also occurred occasionally as traces. As seen

![Fig.1](a) The growth of hairy root cultures of *C. roseus* and b) the total fatty acid content of the roots (% of root dry weight) at 19.5°C, 24°C and 32°C. The standard deviation for total fatty acid analysis was <0.08% of root dry weight at each point.

![Fig.2](a) The relative fatty acid composition (% of the total fatty acid content) of *C. roseus* hairy root cultures during the growth cycle at a) 19.5°C, b) 24°C and c) 32°C. □ = C16:0, ○ = C18:0, ▲ = C18:1, ● = C18:2 and △ = C18:3. The standard deviation was <0.7% at each point.