

Biosynthesis of Very Long Chain Polyunsaturated Fatty Acids in Chicory

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Introduction:

Very long chain polyunsaturated fatty acids (VLCPUFAs) are declining in reserves, since they are produced by oily fish. VLCPUFAs are important in human nutrition because they are responsible for the production of inflammatory mediators such as prostaglandins, the regulation of cholesterol synthesis and its transport, and the maintenance of cellular membranes. This research aims to produce VLCPUFAs in edible plants.

Methods:

1. Cotyledons from 14d-old seedlings of five cvs. of *Cichorium intybus* were transformed by *Agrobacterium tumefaciens* carrying genes for Δ^9 elongase, Δ^8 desaturase, Δ^5 desaturase and Δ^9 elongase + Δ^8 desaturase.
2. PCR and RT-PCR were performed on DNA and RNA respectively, extracted from leaves of putatively transformed plants.
3. GC was performed on methylated extracts of freeze dried leaves.
4. Cell suspensions were initiated from Δ^9 + Δ^8 transgenic plants showing high gene expression. Suspensions in either shoot regeneration medium (SM= MS-based + 1 mg/l BAP and 0.1 mg/l IAA) or root formation medium (RM= MS-based + 0.1 mg/l IBA) were treated with different concentrations of acetyl salicylic, ascorbic, caffeic, citric, jasmonic, nicotinic and salicylic acids to determine the effect of these elicitors on growth and VLCPUFAs biosynthesis.

Results:

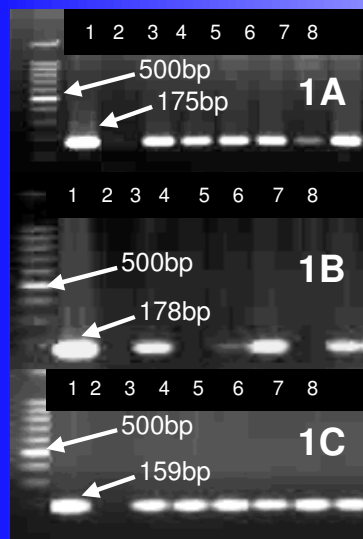


Figure 1: Amplification of A, the Δ^9 elongase, B, the Δ^8 desaturase and C, the Δ^5 desaturase genes in leaf DNA extracts.

Lane 1: positive control, lane 2: negative control. Lanes 3-8 (Figure 1A and C) and lanes 3, 6 and 8 (Figure 1B) are representative examples of transgenic plants.

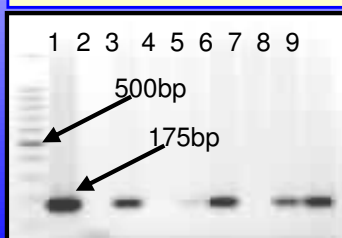


Figure 2: RT-PCR for expression of the Δ^9 elongase gene in leaves.

Lane 1: positive control, lane 2: negative control. Lanes 3, 5, 6, 8 and 9 are representative examples of transgenic plants expressing the Δ^9 elongase gene. Lanes 4 and 7 did not show any expression

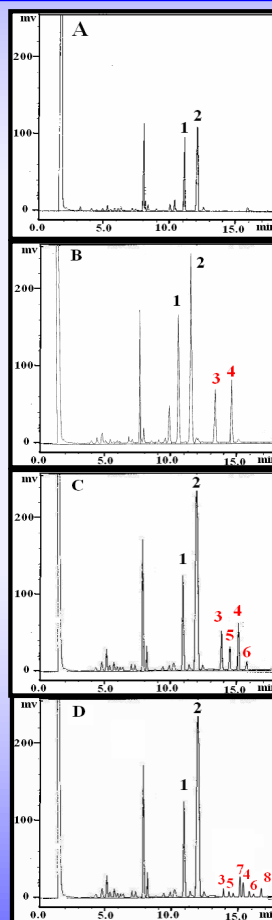


Figure 3: GC profiles of fatty acid methyl esters in chicory cv. Pan di Zucchero. Fatty acids were extracted from a non-transformed plant (A), and transgenic plants expressing Δ^9 elongase activity (B), both Δ^9 elongase and Δ^8 desaturase activities (C) and Δ^9 elongase, Δ^8 and Δ^5 desaturase activities (D).

- 1: Linoleic acid.
- 2: α -Linolenic acid.
- 3: Eicosadienoic acid.
- 4: Eicosatrienoic acid.
- 5: Dihomo- γ -linolenic acid.
- 6: Eicosatetraenoic acid.
- 7: Arachidonic acid.
- 8: Eicosapentaenoic acid.

Conclusions:

- Transgenic plants of chicory were morphologically similar to non-transformed plants.
- $\omega 3$ and $\omega 6$ fatty acids were produced in transgenic chicory plants.
- Different elicitors affect the growth rate differently in cell suspensions of transgenic plants.

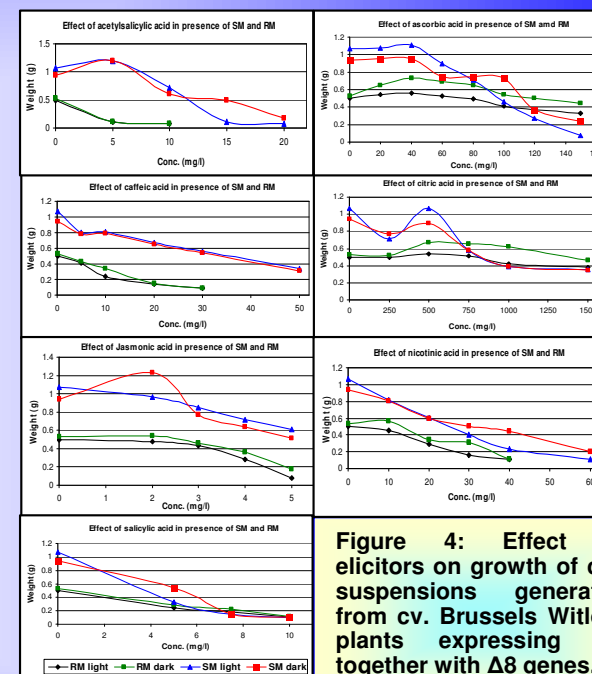


Figure 4: Effect of elicitors on growth of cell suspensions generated from cv. Brussels Witloof plants expressing Δ^9 together with Δ^8 genes.

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