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rapeseed genome. Taking advantage of the presence of a HindIII restriction site only present in the CE8 coding region, an RT-PCR assay coupled with a digestion step was used to measure the relative expression of the CE7 and CE8 mRNAs. In both cultivars, CE7 mRNA was the predominant isoform expressed throughout the developmental period of the seed studied.

Immunodetection of CE during seed development

Western blots were performed using anti-CE antibodies prepared against a CE recombinant protein [11]. In both cultivars, the immunoblots revealed two proteins of 60 and 67 kDa from the earliest stages of seed development. These proteins may correspond to ubiquitous condensing enzyme proteins involved in fatty acid production [11]. However, an additional 57-kDa protein was detected only in the 5–8 WAP HEAR samples.

In conclusion, our results suggest that: (i) the CE gene codes for a native 57-kDa protein; (ii) this protein occurs only in the Gaspard cultivar; (iii) this protein is probably the active CE enzyme and (iv) the mutations leading to the LEAR phenotype may induce a disregulation of the expression of the CE gene at the translational or post-translational levels.

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Biochemical and molecular characterization of corn (Zea mays L.) root elongases
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Abstract

Root surfaces are protected against the soil environment by the deposition of lignin and suberin. In order to obtain more insight into the regulation of root suberin biosynthesis, elongases from primary roots of corn (Zea mays L.) seedlings were characterized. Elongase activities (acyl-CoA and ATP-dependent) were located in the microsomal fraction of the root cells. C,, C,, and C,, fatty acids were detected as primary products of elongases. Preferred substrates of the acyl-CoA elongases were C,, a-, CoA and C,, a-, CoA. Applying a molecular approach, using PCR and degenerate primers derived from the sequences of known leaf and seed 3-ketoacyl-CoA synthases (KCSs), catalysing the first step of very-long-chain fatty acid synthesis, the cDNA of a putative root KCS was obtained showing high homology to known leaf and seed KCSs at the DNA and amino acid levels. Thus, our approach provides the first direct evidence for the presence and the activity of root elongases in Z. mays. Ongoing research is focusing on the molecular analysis and the regulation of KCS expression in roots in reaction to different environmental stimuli.

Key words: ketoacyl-CoA synthase, suberin, very-long-chain fatty acid.
Abbreviation used: VLCFA, very-long-chain fatty acid.
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**Introduction**

Suberized and lignified hypodermal and endodermal cell walls form the apoplastic interface between roots and the soil environment [1]. Passive uptake of water and dissolved ions is controlled by these cell-wall structures [2]. It has been shown recently that the lipophilic biopolymer suberin is involved in regulating transport properties of the root apoplast [3]. Suberin is a lipophilic biopolymer composed of linear, long-chain, aliphatic compounds belonging to different substance classes (primary fatty acids, primary alcohols, ω-hydroxy fatty acids, diacids and 2-hydroxy fatty acids) with chain lengths varying between \( C_{16} \) and \( C_{20} \) [4]. Suberin biosynthesis requires two different biochemical pathways [4]. The first involves the elongation of fatty acids giving rise to very-long-chain fatty acids (VLCFAs) [5]. The second involves the functionalization of VLCFAs, probably by hydroxylases [6], giving rise to the characteristic suberin monomers. Elongase activities resulting in VLCFAs have been analysed in detail in developing leaves where wax synthesis is occurring [7] and in developing seeds where VLCFAs are stored chiefly as triacylglycerols [8]. In order to obtain more insight into root suberin biosynthesis we characterized root elongases on the biochemical and molecular levels.

**Biochemical characterization of root elongases**

Primary roots (2–4 cm long) of corn (Zea mays L.) seedlings germinated for 3 days on moist filter paper were harvested. The preparation of the microsomal fraction of roots and the biochemical assay, measuring elongase activities in roots, were basically carried out as described in detail in [9]. Roots were ground in Hepes buffer (0.08 M, pH 7.2, 0.32 M sucrose and 10 μM β-mercaptoethanol) and subjected to different centrifugation steps (1. 10,000 g; 2. 100,000 g). In standard experiments, 30 μg of microsomal proteins were incubated at 30 °C for 1 h in the presence of the cofactors NADPH (500 μM), NADH (500 μM), MgCl₂ (1 mM) and dithiothreitol (2 mM). \(^{14}C\)-Labelled malonyl-CoA (19 μM, 53.1 Ci·mol⁻¹) and stearoyl-CoA (10 μM) were used as substrates. Labelled fatty acids were extracted after saponification by 2 ml of chloroform and separated according to their chain length by reversed-phase TLC. Amounts of radioactivity used for the calculation of enzymatic activities were determined by liquid scintillation counting.

Preliminary experiments measuring elongase activities in different cell fractions (total cell extract, cell walls, cytosol and microsomes) showed that elongase activity leading to VLCFAs was basically located in the microsomal fraction. Elongase activities in standard assays using 30 μg of microsomal protein were between 0.67 and 1.33 nM·mg⁻¹·h⁻¹, which was about 10 times lower than elongase activities reported for etiolated corn coleoptiles [9]. Primary elongated products were \( C_{20} \), \( C_{22} \) and \( C_{24} \) fatty acids. Optimal concentrations were 100 μM for malonyl-CoA and 10 μM for stearoyl-CoA. Stearoyl-CoA concentrations higher than 100 μM strongly inhibited elongase activities. The most important cofactor for the elongase was NADPH, since enzymatic activities decreased by 50% when NADPH was missing. Preferred substrates of corn root elongase are

![Figure 1](https://example.com/figure1.png)

**Figure 1**

Substrate specificity of corn (Z. mays L.) root elongases

30 μg of microsomal protein were used in the elongase assay. Results represent three replicates with S.D.
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Figure 2

Dendrogram of the ketoacyl-CoA synthase gene family based on the amino acid sequences
Alignments were created with Clustal X and resulting trees were generated using TreeView (R.D.M. Page, 2000). GenBank accession numbers: AtKCS1 (AAC99312); SbKCS (AAD25560); FIDDLEHEAD (AAC14526); ATCUT1 (AAD37122); BnFAE1 (AA872178); BjFAE1 (CAA71989); AtFEA1 (AAA70154). Species identification: At, Arabidopsis thaliana; Zm, Z. mays; Sb, Sorghum bicolor; FIDDLEHEAD, A. thaliana; Bn, Brassica napus; Bj, Brassica juncea.

Figures were C₁₈₋₉-CoA and Cₑ₀₋₉-CoA. Unsaturated acyl-CoAs such as C₁₈₋₁₀-CoA and C₁₈₋₁₁-CoA and acyl-CoAs having shorter (C₁₂₋₉₋C₁₆₋₀) or longer chain lengths (C₂₂₋₉₋C₂₄₋₀) were poorly elongated (Figure 1).

Molecular characterization of root elongases

Using degenerate primers designed from known ketoacyl-CoA synthases [7,8] and reverse-transcriptase PCR, a putative ketoacyl-CoA synthase primarily expressed in corn roots was obtained. Homology on the amino acid level to ketoacyl-CoA synthases cloned from leaves [7] and seeds [8] from different plant species was between 63 and 72% (Figure 2), strongly supporting our conclusion that the obtained PCR product is a ketoacyl-CoA synthase expressed in corn roots.

Concluding remarks

We demonstrated the existence of elongase activities in corn roots. Enzymatic parameters of corn root elongases were determined and a putative ketoacyl-CoA synthase was cloned. Future work will concentrate on the analysis of expression patterns and biochemical activities of root elongases in response to different environmental stress factors such as drought, toxic compounds (e.g. heavy metals) and pathogen attack.

References


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