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# Profiling candidate genes involved in wax biosynthesis in *Arabidopsis thaliana* by microarray analysis

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## Abstract

Plant epidermal wax forms a hydrophobic layer covering aerial plant organs which constitutes a barrier against uncontrolled water loss and biotic stresses. Wax biosynthesis requires the coordinated activity of a large number of enzymes for the formation of saturated very-long-chain fatty acids and their further transformation in several aliphatic compounds. We found in the available database 282 candidate genes that may play a role in wax synthesis, regulation and transport. To identify the most interesting candidates, we measured the level of expression of 204 genes in the aerial parts of 15-day-old *Arabidopsis* seedlings by performing microarray experiments. We showed that only 25% of the putative candidates were expressed to significant levels in our samples, thus significantly reducing the number of genes which will be worth studying using reverse genetics to demonstrate their involvement in wax accumulation. We identified a beta-keto acyl-CoA synthase gene, At5g43760, which is co-regulated with the wax gene CER6 in a number of conditions and organs. By contrast, we showed that neither the fatty acyl-CoA reductase genes nor the wax synthase genes were expressed in 15-day-old leaves and stems, raising questions about the identity of the enzymes involved in the acyl-reduction pathway that accounts for 20% of the total wax amount.

*Keywords:* Microarray; Wax synthesis; *Arabidopsis thaliana*

## 1. Introduction

Water is a crucial prerequisite for plant life. During the evolution process, physiological, anatomical and morphological adaptation has taken place in plants in order to maintain a water status suitable for survival even under adverse environmental conditions. Indeed, specialized structures like well-developed cuticles and stomata are present in fossil specimens of the very earliest terrestrial plants known [1,2]. Both structures are required to optimize photosynthetic gas exchange under the constraint of loss of water to a dry atmosphere [3,4].

Plants living in an aerial environment should develop a barrier against uncontrolled water loss. Such a barrier should be efficient, translucent for photosynthetically active radiation, flexible and self-healing. The plant cuticle, which controls the movement of water between the outer cell wall of the epidermis and the atmosphere adjacent to the plant, combines all these properties: it is a rather thin (0.02 – 10 μm thick) membrane consisting of a polymer matrix (cutin), polysaccharides and associated solvent-soluble lipids (cutic-

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ular waxes) [5]. Cutin is a three-dimensional polymer of mostly C16 and C18 hydroxy fatty acids cross-linked by ester bonds [6–10].

Cuticular wax is a general term for complex mixtures of very long chain aliphatics lipids, but it also includes triterpenoids and minor secondary metabolites, such as sterols and flavonoids. The physical and chemical properties of cuticular wax determine vital functions for plant. Indeed, besides playing a major role in protecting the aerial parts of the plants from uncontrolled water loss [3,4], it protects plants against ultraviolet radiation and helps minimize deposits of dust, pollen and air pollutants [11]. In addition, surface wax is believed to play important roles in plant defence against bacterial and fungal pathogens [12] and has been shown to participate in a variety of plant–insect interactions.

A large number of enzymes is required to carry out the formation of saturated very-long-chain fatty acids (VLCFAs) with predominant chain lengths from 20 to 32 carbons [13], and their further transformation in several aliphatic compounds that constitute the wax layer (Fig. 1). Two principal wax biosynthetic pathways coexist in the epidermal cells of plants: an acyl reduction pathway, which produces primary alcohols and wax esters, and a decarbonylation pathway, leading to the formation of aldehydes, alkanes, secondary alcohols and ketones [14].

Several wax-deficient mutants have been isolated in different plant species [11,14–16]. The mutant loci in *Arabidopsis thaliana* are termed *eceriferum* (*cer*), and 22 independent *cer* loci have been identified in this plant model. The corresponding mutants display an abnormal wax composition and/or a global decrease in wax constituents [17–20].

In the past few years, five *CER* genes have been cloned (*CER1*, *2*, *3*, *5* and *6*), but the biological function of the corresponding protein is only known with certainty for *CER5* and *CER6*, which encode, respectively, an ABC transporter and a condensing enzyme of the elongase complex [11,21].

Further to the strategy of using *Arabidopsis cer* mutants, some other genes encoding proteins involved in wax biosynthesis have been cloned and characterized. A mutation of the *GLOSSY 8* (*GL8*) locus in maize results in decreased levels of wax components longer than C24. It has been later demonstrated that the corresponding gene encodes a reductase involved in VLCFAs synthesis [22,23]. The wax synthase (fatty acyl-coenzyme A: fatty alcohol acyltransferase), which catalyzes the final step in the synthesis of linear esters, has been characterized and partially purified from developing jojoba embryo. This led to the cloning of the corresponding gene [24]. Very recently, it has been demonstrated that WIN1/SHINE1, an *Arabidopsis thaliana* ethylene response factor-type transcription factor, can up-regulate wax production in leaves and stems when overexpressed [25,26].

Our current knowledge is thus confined to a very limited number of genes from a series of different plants, and several strategies have been developed to discover other

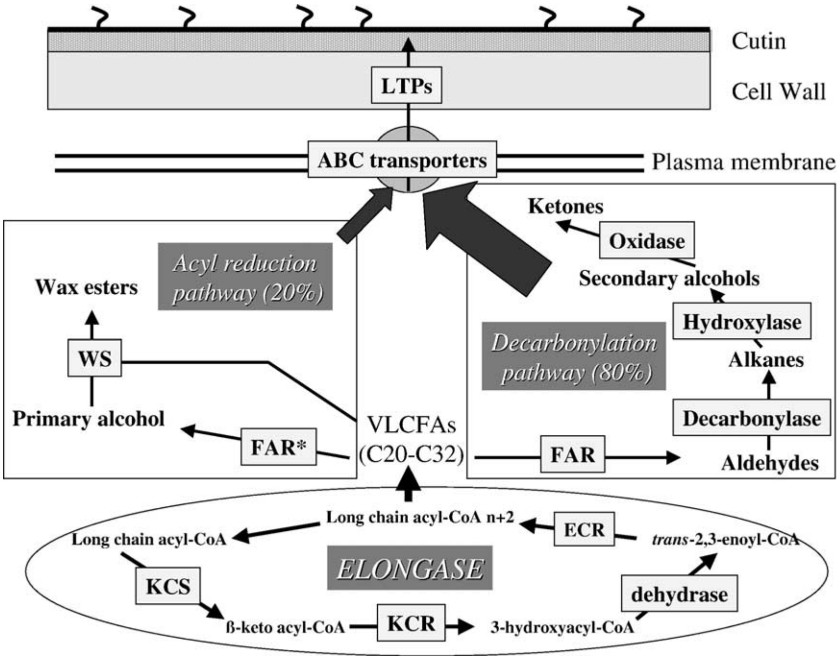

Fig. 1. Schematic representation of the wax biosynthetic pathways in *Arabidopsis*. Possible metabolic pathway for wax biosynthesis and transport in *Arabidopsis* stems and leaves. The VLCFAs are synthesized by Elongases, which are multi sub-units complex. Then, they are converted to different components through the acyl reduction pathway (20% of the metabolic flux) and the decarbonylation pathway (80% of the flux). The different classes of molecules cross the plasma membrane, most probably through ABC transporters, and then travel up to the plant surface. It has been proposed that this transport involves LTPs to some degree. In the case of the fatty acyl-CoA reductase (FAR*) of the acyl reduction pathway, the aldehyde is not released from the enzyme before its further transformation in primary alcohol. This FAR* is therefore distinct from the one involved in the decarbonylation pathway.