A mechanism that plants have adopted to survive in a terrestrial, aerial environment is the production of waxes deposited into and on the cuticular layer. The cuticle acts as the first protective barrier against UV radiation and bacterial and fungal attacks, and it regulates nonstomatal water loss. Cuticular waxes are a mixture of several compounds, such as very-long-chain fatty acids (VLCFA; >18C), fatty aldehydes, primary and secondary alcohols, ketones, and esters. The proportion of these compounds differs among plant species and even among the different tissues of an individual plant. VLCFA, the precursor of most wax components, is produced by elongation of fatty acids (C16 or C18) through the addition of two-carbon moieties from the donor malonyl-CoA at each step in a series of four reactions (condensation, reduction, dehydration, and second reduction) catalyzed by the fatty acid elongation (FAE) system. Other components of the wax are produced by modifications of elongated fatty acids, either through decarbonylation, which produces aldehydes, secondary alcohols, alkanes, and ketones, or through acyl reduction, which forms primary alcohol, which in turn combines with free fatty acids to give wax esters (reviewed in Post-Beittenmiller, 1996).

In addition to epidermal cells, on which a cuticle is generally present, specialized cells of reproductive organs also produce fatty acids. For example, cuticle is present on the cell walls of differentiated epidermal cells, such as the stigmatic papillae (Lolle and Pruitt, 1999). The outer wall of the pollen, or the exine, is composed of sporoollenin, a very stable polymer containing long-chain fatty acids and phenylpropanoids (Guilford et al., 1988), which are probably synthesized by the activity of a FAE complex. Exine deposition must be under the control of a highly regulated mechanism that determines a specific pattern characteristic for each plant species. Although both tapetum and microspores contribute to exine formation, genes encoding lipid biosynthetic enzymes were found mainly in the tapetum (Piffanelli et al., 1997).

\[ ms2 \] mutants of Arabidopsis form aborted pollen without an exine. The MS2 gene is expressed in the tapetum and encodes a putative fatty acid reductase (Aarts et al., 1997). In some plants, a lipidic pollen coat or trypHEME is laid onto the exine wall. This is made up of substances secreted or released in the localule when tapetal cells disintegrate, and it fills the cavities between the baculae of the exine. The pollen coat has been well characterized in Brassicaceae. It includes proteins and is highly enriched in neutral esters containing mostly medium- and long-chain saturated fatty acids, free fatty acids, waxes, and other hydrocarbons (Piffanelli et al., 1997). In Arabidopsis, lipid analysis of the trypHEME has also shown the presence of very-long-chain wax esters that are essential for pollen hydration (Preuss et al., 1993). The pollen coat, therefore, is considered essential for hydration but not for adhesion, because pollen mutants deprived of a trypHEME adhere by means of an exine to the papillary cells (Zinkl et al., 1999).

### MUTANTS AND GENES FOR WAX BIOSYNTHESIS

Defects in the wax layer are easily detectable because mutant plants have a glossy appearance. This characteristic has become an accurate genetic marker, and several mutants affected in wax biosynthesis have been isolated and studied in some plant species. In Arabidopsis, 21 independent cerf (cer) loci seem to be involved in regulating wax production, and for 20 cer mutants, relative changes in the amount of the wax compounds were compared with levels in the wild type (Koornneef et al., 1989; Hannoufa et al., 1993). Interestingly, the cer1, cer3, cer6, cer8, and cer10 mutants also exhibited reduced fertility if they were kept under conditions of low humidity. In the past few years, some CER genes have been cloned, and sequence analysis of these clones has further elucidated their function in wax biosynthesis.

Novel Arabidopsis cer1 mutant alleles were generated using the I/dSpm transposon tagging system (cer1-m; Aarts et al., 1995) or by ethyl methanesulfonate treatment (cer1-147; Huelskamp et al., 1995). Both mutants showed the typical glossy phenotype characteristic of the cer mutants as well as conditional male sterility. The latter phenotype has been thoroughly described by Preuss et al. (1993) for another cer mutant, cer6-2 (or pop-1), which is allelic to the first cer6-1 mutant identified by Koornneef et al. (1989). cer1-m, cer1-147, cer6-1, and cer6-2 are all male sterile if grown under relatively dry conditions, but fertility is restored if plants are grown under high humidity.

In wild-type Arabidopsis plants and other members of Brassicaceae, a conversion of the trypHEME forms a foot of lipids and proteins between the pollen grain and the papilla; this occurs when the pollen lands on the stigma. Through this foot, hydraulic contact is established, and pollen hydrates and consequently germinates a pollen tube. Pollen from cer1 and cer6 mutant lines did not germinate on wild-type stigmas, but wild-type pollen germinated perfectly on the stigmas of mutant plants.
Furthermore, when grown in vitro, mutared pollen germinated a tube that grew similar to that of the wild type. The inability of cer6-2 to germinate on the stigma was interpreted as a failure in hydration caused by the lack of tryphine in this mutant, particularly by the lack of C29 and C30 VLCFAs. When wild-type mentor pollen was used in a mixed pollination with mutant pollen, the tryphine carried by the former could rescue the functionality of the latter (Preuss et al., 1993). Interestingly, cer1-m, cer6-1, and another cer6 mutant, cer6-2654 (Huelskamp et al., 1995), retain the tryphine, although this has smaller lipid droplets than wild-type pollen, whereas cer1-147 has a tryphine without lipids. This mutant and cer6-2654 can also be rescued in a mixed pollination using pollen from wild-type Brassica rapa, a species very closely related to Arabidopsis (Huelskamp et al., 1995).

The cer1-m mutant tagged with IdDspm was used to clone the genomic flanking DNA corresponding to the CER1 gene. Sequence analysis revealed homology with fatty acid desaturases and an al-kane hydroxylase and the presence of an iron binding motif. This, together with the fact that cer1 mutants are blocked in the conversion of C30 aldehydes to C29 alkanes, strongly supports the idea that the CER1 protein acts as a catalytic iron-containing component of a fatty aldehyde dehydrogenase enzyme.

The analysis of waxes of several cer mutants (Hannoufa et al., 1993) established that in cer2 Arabidopsis plants, the total amount of wax was ~40% of that found in wild-type plants, with a particular reduction in aldehydes, alkanes, secondary alcohols, and ketones that was two or four carbons shorter than that in wild-type plants. This finding is in agreement with a block in elongation from C28 to C30. These characteristics are also found in cer6 mutants. In addition, Preuss et al. (1993) showed that cer2, like cer6-1 and cer6-2, is a conditional male-sterile mutant affected in pollen hydration. Unlike cer6-2 but like cer6-1, cer2 has a tryphine with reduced lipid droplets.

The CER2 gene has been cloned by chromosomal walking (Xia et al., 1996) and by T-DNA tagging (Negruk et al., 1996). The deduced CER2 protein shares sequence similarity with GL2, the product of the Glossy2 gene of maize, which is also involved in cuticular wax accumulation (Tacke et al., 1995). On the basis of the wax composition in cer2 mutants, it was suggested that CER2 encodes an elongase activity. Unfortunately, the sequence analysis in this case does not help to elucidate the function of this gene, because no similarities were found with enzymes required for fatty acid biosynthesis.

Among all of the cer mutants, cer3 and cer6 show the most extreme defects in leaf wax accumulation, and a novel cer3 mutant, cer3-2186 (Huelskamp et al., 1995), was shown to be defective in hydration, like cer1 and cer6. This phenotype was also investigated by cross-pollinating cer1, cer3, and cer6 mutant plants with one another. If the CER1, CER3, and CER6 genes produce different end products required for pollen hydration, hydration should be rescued in some combinations. This was not the case, demonstrating that all three genes are required for one class of products. Moreover, the cer3-2186 mutant exhibited a tryphine indistinguishable from that of the wild type, which indicates that conditional male sterility is not related to the structure of the tryphine. The Arabidopsis CER3 gene has been cloned by T-DNA tagging (Hannoufa et al., 1996), and its deduced protein contains a nuclear localization signal, indicating that CER3 is a regulatory protein.

THE CER6 GENE

The cer6 mutant was the first to be characterized as being defective in pollen hydration, and it was originally named pop1 (for pollen—pistil interaction1; Preuss et al., 1993). To date, it is also the only mutant that has been characterized with respect to the lipid composition of the pollen tryphine. Some of the characteristics of this mutant have been discussed above in connection with cer1. On pages 2001–2008 of this issue of THE PLANT CELL, Fiebig et al. describe the cloning by positional mapping of the CER6 gene from Arabidopsis. In this article, the authors unequivocally demonstrate that the CER6 gene is located on chromosome 1 at 107 centimorgans (cM), and they show by sequence comparison that it is identical to a previously cloned gene, CUT1, that was mapped erroneously to 35.98 cM on chromosome 1 (Millar et al., 1999). CUT1 was identified among a group of expressed sequence tags corresponding to a family of FAE1-related genes, and when silenced in transformed Arabidopsis plants by cosuppression, it produced a phenotype identical to that of the cer1, cer2, and cer6 mutants. Despite the wrong map position, the characterization of this gene strongly supported the idea that CUT1 encodes a condensing enzyme.

Fiebig et al. also show here that on chromosome 1 at 38 cM, there is instead a novel gene that shares sequence homology with exons 1 and 2 of CER6. Sequence analysis of the cer6-1 and cer6-2 mutant alleles also highlighted an interesting point. cer6-1 is produced by the deletion of a codon, whereas cer6-2 is produced by an A-to-C transversion that changes a His residue to a Pro in a peptide region that is not conserved among condensing enzymes. Moreover, the phenotype of the cer6-2 mutant plants was stronger than that of other cer6 mutants, suggesting the cer6-2 may be a null allele (Preuss et al., 1993).

The most surprising result described in this article derives from the complementation analysis. The authors observed that the glossy phenotype and male sterility were not rescued equally after the introduction of the CER6 gene in cer6-2 mutants. Some of the trans-
formants with restored fertility still had defective stem wax. This is the first report that the two phenotypic effects of the cer mutations can be separated. Even more interesting is the finding of a dominant intragenic suppressor mutation that converts the Pro residue in the cer6-2 mutated allele to a Ser residue. Again, this mutation is in a nonconserved region among condensing enzymes, thus diminishing the possibility that the active site of the enzyme is affected. It would be interesting to visualize a folding model for these three peptides. Pollen of suppressor plants regains an abundant trypine with lipid droplets that contain the C29 and C30 VLCFAs, although not at the wild-type level.

This brings us back to the question of whether (and how much) trypine and lipids are necessary to recover pollen fertility, because some alleles of four independent CER loci have trypine and some lipids, yet are male sterile.

A NEW ROLE FOR EXTRACELLULAR LIPIDS

The Fiddlehead gene, which is implicated in lipid biosynthesis but is not related to the CER genes, was recently cloned and shown to share similarities with FAE1 and CHS (Pruitt et al., 2000). fdh mutants are disturbed in epidermal fusions and interactions, exhibiting fused organs, pollen germination, and tube growth on epidermal cells. Epidermal cell walls and cuticles in these mutants are more permeable and show an altered composition of lipids in crude cell wall fractions (Lolle et al., 1997). Based on these observations, the authors formulated a model in which localized changes in the permeability of the cell wall and the cuticle modify the interaction between epidermal cells. This model is supported by a recent report (Sieber et al., 2000) that describes alterations in the cuticle, organ fusions, and pollen tube growth on leaves of transformed Arabidopsis plants expressing a fungal cutinase. Interestingly, though, the chemical wax composition of transgenic plants was identical to that of wild-type plants.

Pruitt et al. (2000) suggested that the properties of the cuticle are modified to accommodate cell-cell interactions by altering its lipid composition. The long-chain lipids of the trypine may indirectly serve a similar function in pollen-pistil interactions. For example, they may act as a solvent to release particular compounds of the trypine or the papillar cells. We have demonstrated that a class of triacylglycerols (which are absent in the trypine of Brassica spp and Arabidopsis) are sufficient to establish pollen interaction with pistils and leaves (Wolters-Arts et al., 1998). Nevertheless, in our experiments, we always used untreated wild-type pollen, which carries its own components for the interaction. One of these components could be a protein such as oleosin, which has been shown to be necessary for the hydration of Arabidopsis pollen (Mayfield and Preuss, 2000). We discussed the possibility that triacylglycerols (and perhaps other lipids) act as a matrix in which water is distributed or that they modify the membrane permeability of the pollen (at the site of tube formation) and/or the stigma. The latter hypothesis (modification of permeability) is closer to the model of Pruitt et al. (2000) and does not attribute a specific action to the lipids; rather, it creates a suitable physicochemical environment for the interaction to occur.

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REFERENCES


IN THIS ISSUE

CUT1, an Arabidopsis gene required for cuticular wax biosynthesis and pollen fertility, encodes a very-long-chain fatty acid condensing enzyme. Plant Cell 11, 825–838.

Molecular cloning and characterization of the CER2 gene of Arabidopsis thaliana. Plant J. 9, 137–145.


**Complex Waxes**
M. Mariani and M. Wolters-Arts
*Plant Cell* 2000;12;1795-1798
DOI 10.1105/tpc.12.10.1795

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