The glossyhead1 Allele of ACC1 Reveals a Principal Role for Multidomain Acetyl-Coenzyme A Carboxylase in the Biosynthesis of Cuticular Waxes by Arabidopsis

Shiyou Lü, Huayan Zhao, Eugene P. Parsons, Changcheng Xu, Dylan K. Kosma, Xiaojing Xu, Daiyin Chao, Gregory Lohrey, Dhinoth K. Bangarusamy, Guangchao Wang, Ray A. Bressan, and Matthew A. Jenks*

Center for Plant Stress Genomics and Technology, King Abdullah University of Science and Technology, Thuwal 23955–6900, Kingdom of Saudi Arabia (S.L., H.Z., D.K.B., G.W., R.A.B.); Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, Indiana 47907–2054 (E.P.P., X.X., D.C., G.L., R.A.B.); Biology Department, Brookhaven National Laboratory, Upton, New York 11973–5000 (C.X.); Department of Plant Biology, Michigan State University, East Lansing, Michigan 48824 (D.K.K.); and United States Arid Land Agricultural Research Center, United States Department of Agriculture–Agricultural Research Service, Maricopa, Arizona 85138 (M.A.J.)

A novel mutant of Arabidopsis (Arabidopsis thaliana), having highly glossy inflorescence stems, postgenital fusion in floral organs, and reduced fertility, was isolated from an ethyl methanesulfonate-mutagenized population and designated glossyhead1 (gsd1). The gsd1 locus was mapped to chromosome 1, and the causal gene was identified as a new allele of Acetyl-Coenzyme A Carboxylase1 (ACC1), a gene encoding the main enzyme in cytosolic malonyl-coenzyme A synthesis. This, to our knowledge, is the first mutant allele of ACC1 that does not cause lethality at the seed or early germination stage, allowing for the first time a detailed analysis of ACC1 function in mature tissues. Broad lipid profiling of mature gsd1 organs revealed a primary role for ACC1 in the biosynthesis of the very-long-chain fatty acids (C20:0 or longer) associated with cuticular waxes and triacylglycerols. Unexpectedly, transcriptome analysis revealed that gsd1 has limited impact on any lipid metabolic networks but instead has a large effect on environmental stress-responsive pathways, especially senescence and ethylene synthesis determinants, indicating a possible role for the cytosolic malonyl-coenzyme A-derived lipids in stress response signaling.

Cuticular waxes are lipidic compounds that coat essentially all aerial plant organs and play an important role in protecting plants from many forms of environmental stress (Kosma et al., 2009). In addition, plant waxes also define a promising source of high-energy compounds for conversion to biofuels (Jetter and Kunst, 2008). Wax synthesis is thought to involve multisubunit cytosolic elongase complexes that repeatedly condense malonyl-CoA with a growing fatty acyl-CoA chain to produce very-long-chain fatty acids (VLCFAs; C20:0 or longer), which themselves are further modified to alkanes, primary alcohols, aldehydes, esters, or similarly derived species-specific aliphatics (Kunst and Samuels, 2009). The malonyl-CoA substrates for these VLCFA-generating reactions are synthesized by a cytosolic multidomain protein called Acetyl-Coenzyme A Carboxylase1 (ACC1). The ACC1 protein is derived from a single polypeptide, unlike the prokaryote- and plastid-specific acetyl-CoA carboxylase that exists as a multisubunit complex from the products of multiple genes. The ACC1-catalyzed reaction is thought to serve as the committed and rate-limiting step in the synthesis of VLCFAs utilized in the synthesis of not only cuticular waxes but also seed storage triacylglycerides, suberin, and sphingolipids and in the synthesis of malonated derivatives of 1-aminocephalopane-1-carboxylic acid, α-amino acids, and flavonoid glycosides (Liu et al., 1983, 1984; Nikolau et al., 2003; Sasaki and Nagano, 2004; Taguchi et al., 2010).

Although plant cuticular waxes are, arguably, the major plant lipids derived from VLCFAs, direct ACC1 involvement in wax synthesis has not been demonstrated. One factor limiting such work is that all previously reported acc1 mutant alleles cause embryo abortion, early germination, or seedling lethality and thereby restrict analyses of ACC1 function in mature tissues. Certain weak ACC1 mutant alleles reported by
Torres-Ruiz et al. (1996) can, in fact, produce flowering plants, but only under strict in vitro conditions where normal cuticle synthesis is not necessary, and these plants are still highly disfigured. Nonetheless, previous reports support the hypothesis that ACC1 is involved in wax synthesis. For example, certain mutations in the ACC1 gene (e.g. gurke [sk], pasticcino3 [pas3], and embryo lethal22 [emb22]) cause postgenital fusions in embryo tissues (Torres-Ruiz et al., 1996; Faure et al., 1998; Baud et al., 2004; Kajiwara et al., 2004), a trait exhibited by mature organs of many cuticle mutants (Chen et al., 2005; Zheng et al., 2005). This postgenital fusion phenotype can be mimicked in wild-type plants sprayed with quizalofop, a specific inhibitor of multidomain ACC1 (Dehaye et al., 1994). Other ACC1 mutant alleles (acc1-1 and acc1-2) suppress the synthesis of VLCFAs specifically in the seed triacylglycerol (TAG) pool just before embryo abortion (Baud et al., 2003). Since these mutants are embryo lethal, it could not be determined whether ACC1 might also influence the synthesis of VLCFAs required for wax production by mature organs. However, RNA interference suppression of ATP-citrate lyase (ACL), the enzyme solely responsible for generating cytosolic acetyl-CoA, did in fact cause a reduction in cuticular waxes on mature Arabidopsis organs (Fatland et al., 2005), indicating that deficiencies in the substrate for ACC1 could reduce waxes. Altogether, these results support the postulate that ACC1 plays a role in wax synthesis, yet direct evidence is still lacking.

We report here the visual screening of an ethyl methanesulfonate (EMS)-mutagenized population to uncover a novel mutant having very glossy inflorescence stems as well as postgenital fusion in floral organs and reduced fertility. The mutation was mapped using PCR-based markers, and the candidate gene was identified as a new allele of ACC1. This, to our knowledge, is the first discovered mutant allele of ACC1 that does not cause lethality at the seed or early germination stage of development. We designated this unique mutant glossyhead1 (gsd1) because it exhibits a rounded- or fiddlehead-like floral structure similar to the fiddlehead mutants (Lolle et al., 1998), and we provide direct evidence that ACC1 has a primary function in the biosynthesis of plant cuticular waxes.

RESULTS

Identification of the gsd1 Mutant

Visual screens of an Arabidopsis EMS-mutagenized population uncovered a mutant exhibiting a glossy stem (Fig. 1, A and B) and flower buds that were smaller and fused together to produce a fiddlehead shape (Fig. 1, D, F, and G), phenotypes not present in wild-type plants (Fig. 1, C and E). The leaf size, number, and shape as well as flowering time were similar between the wild type and the gsd1 mutant (Fig. 1, C and D). This mutant was designated gsd1 because of its unique glossy stem and flower bud clusters that fused to form a head-like (fiddlehead) morphology. The gsd1 mutant expressed reduced fertility that could not be restored with high humidity. Rather than suppressing pollen hydration during stigma interactions, as occurs in other cuticle mutants (Chen et al., 2003; Lü et al., 2009), the gsd1 mutant’s reduced fertility is likely due to its highly bent (crooked) stamen filaments (data not shown) and its highly fused sepal, which appear to entrap and inhibit anthers from reaching the stigma (Fig. 1H). Nonetheless, gsd1 will sometimes produce a few seed-bearing siliques.

The gsd1 Mutation Causes Dramatic Alterations of the Cuticle

The total amount of all wax chemical constituents on gsd1 inflorescence stems was decreased to 39.8% of wild-type levels (Table I). The alkane, secondary alcohol, and ketone wax classes on gsd1 stems were the most reduced wax classes relative to the wild type (Table I). And of these classes, this reduction could be attributed primarily to 83%, 76%, and 69% reductions of C29 alkane, C29 secondary alcohol, and C29 ketone, respectively (Fig. 2A). Stem aldehydes were also changed in gsd1, with the C14 and C26 aldehydes being increased to 218% and 201% of the wild-type amounts, respectively, whereas the C30 aldehydes decreased to 33% of the wild-type amounts. The amounts of stem C22, C24, and C26 primary alcohols increased to 201%,
255%, and 137% of the wild type, respectively, whereas the C_{22} and C_{30} primary alcohols decreased to 72% and 36% of wild-type levels, respectively. Although low in abundance on wild-type plants, the amounts of stem C_{22} and C_{24} fatty acids increased to 374% and 137%, respectively, of wild-type levels, whereas the C_{28} and C_{30} fatty acids decreased by 53% and 80%, respectively, of wild-type levels, whereas the C_{28} and C_{30} fatty acids decreased by 53% and 80%, respectively, of wild-type levels, whereas the C_{28} and C_{30} fatty acids decreased by 53% and 80%, respectively, (Fig. 2A; Table I). A significant increase was observed for all five detected stem ester constituents, resulting in gsd1 mutants having over 119% more total esters than the wild type (Fig. 2A; Table I). Leaf wax composition of gsd1 showed similar proportional changes to those in the stem, although the changes in total amounts were much less. Total leaf waxes were reduced to less than the wild type (Fig. 2A; Table I). A significant increase was observed for all five detected stem ester constituents, resulting in gsd1 mutants having over 119% more total esters than the wild type (Fig. 2A; Table I). Leaf wax composition of gsd1 showed similar proportional changes to those in the stem, although the changes in total amounts were much less. Total leaf waxes were reduced to 33.3% of wild-type levels, with the major alkanes reduced most quantitatively (Fig. 2B; Table I).

The other major lipid constituent of Arabidopsis stem cuticles is cutin. As a polymer, cutin does not dissolve in normal solvents used in tissue preparation for transmission electron microscopy (TEM). As such, TEM can be used to visualize the basic cuticle membrane ultrastructure. The gsd1 cuticle membrane was slightly reduced in thickness, more osmiophilic, and the cuticle membrane was slightly reduced in thickness, more osmiophilic, and more osmiophilic, and more osmiophilic, respectively. The other major lipid constituent of Arabidopsis stem cuticles is cutin. As a polymer, cutin does not dissolve in normal solvents used in tissue preparation for transmission electron microscopy (TEM). As such, TEM can be used to visualize the basic cuticle membrane ultrastructure. The gsd1 cuticle membrane was slightly reduced in thickness, more osmiophilic, and more osmiophilic, respectively. The major cutin monomer, the C_{18:2} dioic acids, were increased to 120% of wild-type levels, whereas the C_{18:3} dioic acids and 18-OH C_{18:0} were decreased by 28% and 20%, respectively (Fig. 4A). The C_{16:0} dioic acids were increased by approximately 100% in gsd1 stems, while 16-OH C_{16:0} and 10,16-OH C_{16:0} were not changed significantly (Fig. 4A). There was no significant difference observed in 18-OH C_{18:1} and C_{18:1} dioic acids between gsd1 and the wild type. The C_{16:0} and C_{18:2} dioic acids were most elevated on stems, explaining in large part why the gsd1 mutants had over 20% more total stem cutin monomers than the wild type (Fig. 4A). In gsd1 leaves, the most abundant monomer C_{18:2} dioic acids were not changed significantly; however, C_{16:0} dioic acids were increased by approximately 23% (Fig. 4B). The monomers on gsd1 leaves that were significantly reduced relative to monomers on wild-type leaves were the 10,16-OH C_{16:0}, 18-OH C_{18:0}, and C_{18:0} dioic acids, being reduced by 55%, 45%, and 43%, respectively, (Fig. 4B). The total amount of cutin monomers per unit leaf area was unchanged (Fig. 4B).

gsd1 Has Reduced Amounts of VLCFAs in Seed Lipids

Compared with the wild type, the amount of C_{20:1} fatty acid derived from TAGs in mature gsd1 seeds was decreased to 60% of wild-type levels (with the other VLCFAs trending lower), whereas the C_{18:1} was increased in gsd1 by 40% (Fig. 5A). Other seed acids were not changed significantly in gsd1 (Fig. 5A). These reductions in seed VLCFAs of the gsd1 mutant had no obvious effects on embryo development, germination, or seedling development. By comparison, the total fatty acid profiles for rosette leaves (containing no VLCFAs) exhibited very little change from wild-type profiles (Fig. 5B).

The gsd1 Mutant Exhibits Increased Suberin Monomers in Roots

Even though the gsd1 mutant allele was first isolated as causing a cuticle mutation of aerial organs, the ACC1 pro:GUS reporter and quantitative reverse transcription-PCR revealed that ACC1 was also expressed in roots (Supplemental Fig. S1, B, C, and I), leading us to speculate that the gsd1 mutation could have effects on the synthesis of the VLCFA of the root suberin polyester. The aliphatic suberin monomers in roots of gsd1 and wild-type plants were examined to show significant increases in the total amount of VLCFA-derived C_{34:0} acid suberin monomer of 46% (Fig. 6). In contrast, the other VLCFAs, C_{22:0} and C_{24:0} ω-HC acid, decreased by 35.7% and 18.3%, respectively. The gsd1 ω-HC acids C_{16:0} and C_{18:1} increased by 167.5% and 24.4%, respectively, the dioic acids C_{16:0} and C_{18:1} increased by 175.1% and 26.7%, respectively, and the C_{18:0} primary alcohol increased by 22.9%. Overall, there was an increase in the total amount of aliphatic suberin monomers of 30.9% in gsd1 over wild-type levels (Fig. 6). Other aliphatic constituents on gsd1 were unchanged relative to the wild type (Fig. 6).

### Table 1. Cuticular wax composition of inflorescence stems of wild-type Col-0, gsd1, Col-0/ler-0, gsd1/acc1-3, and gsd1/gk-SC and of leaves of Col-0 and gsd1

| Organ                  | Total Load | Fatty Acids | Aldehydes | 1-Alcohols | Alkanes | 2-Alcohols | Ketones | Esters |
|------------------------|------------|-------------|-----------|------------|---------|------------|---------|--------|
| Inflorescence stems    |            |             |           |            |         |            |         |        |
| Col-0                  | 1,899.6 ± 67.6 | 54.1 ± 9.1 | 73.6 ± 6.1 | 219.8 ± 3.0 | 941.7 ± 41.7 | 83.5 ± 8.7 | 491.9 ± 20.1 | 35.2 ± 6.0 |
| gsd1                   | 756.0 ± 74.6 | 39.0 ± 5.5  | 60.0 ± 4.6 | 193.0 ± 18.3 | 204.4 ± 23.1 | 29.9 ± 4.1 | 152.6 ± 14.8 | 77.1 ± 9.8  |
| Col-0/ler-0            | 2,000.4 ± 121.3 | 43.0 ± 5.5  | 163.9 ± 12.1 | 241.6 ± 17.4 | 971.6 ± 42.3 | 70.6 ± 6.5 | 505.3 ± 23.3 | 75.4 ± 10.7 |
| gsd1/acc1-3            | 1,575.1 ± 81.5 | 32.0 ± 11.9 | 123.4 ± 6.0 | 235.1 ± 24.1 | 626.7 ± 33.9 | 89.1 ± 8.6 | 374.5 ± 11.6 | 94.2 ± 7.2  |
| gsd1/gk-SC             | 553.5 ± 21.5 | 18.2 ± 1.5  | 63.8 ± 0.8  | 140.7 ± 11.9 | 121.9 ± 4.8  | 15.3 ± 2.0  | 98.8 ± 5.4  | 94.8 ± 18.1 |
| Rosette leaves         |            |             |           |            |         |            |         |        |
| Col-0                  | 147.5 ± 7.8  | 8.6 ± 1.3   | 2.5 ± 0.5  | 27.5 ± 0.6  | 106.5 ± 5.0   | –         | 1.9 ± 0.3   | –         |
| gsd1                   | 98.3 ± 2.3   | 8.5 ± 1.3   | 2.9 ± 0.3  | 25.1 ± 1.0  | 60.8 ± 2.4   | –         | 1.0 ± 0.1   | –         |

Values shown are means (µg dm⁻²) ± se total wax amounts and coverage of individual compound classes (n = 3–4).
The gsd1 Mutation Increases Cuticle Permeability

Whole rosettes from the wild type and the gsd1 mutant were submerged in 0.05% toluidine blue for 2 min. Results showed clearly that both adaxial and abaxial surfaces were much more permeable (i.e. absorbed blue stain more rapidly through their cuticles) than the wild type (Fig. 7, A and B). Since viewing with the light microscope did not reveal the stomata-specific entry of this stain (data not shown), the increased transport of blue stain likely occurs via the cuticular pathways of gsd1 that are more diffusive than the wild type. A wilting assay in the dark (stomata assumed closed) revealed that aerial tissues of gsd1 reached the wilting point much faster than the wild type after excision, apparently due to more rapid water loss through the epidermis with closed stomata (Fig. 7, C and D). Water loss rates of excised rosettes in the dark (stomata assumed closed) were measured to show that the gsd1 mutant rosettes exhibited a higher water loss rate than wild-type rosettes (Fig. 7E), an indication that gsd1 had increased cuticular permeability to water. A chlorophyll-leaching experiment was also conducted in which rosette leaves from both gsd1 and wild-type plants were submerged in 80% ethanol and the change over time in chlorophyll concentration in the solution was determined. The results showed that chlorophyll was extracted at a much faster rate through the leaf epidermis of gsd1 than the wild type (Fig. 7F).

Mapping of the Causal DNA Polymorphism in gsd1

The gsd1 mutant was backcrossed with wild-type ecotype Columbia (Col-0) twice, and the segregation ratio of the wild type to the glossy mutant was approximately 3:1 (265:90 = 2.9444:1, \( \chi^2 \) test on 355 plants; \( P < 0.05 \)), indicating recessive inheritance. To map the causal gene, gsd1 was crossed with Landsberg
erecta (Ler-0) to create an F2 mapping population. At first, we used a small population containing 76 F2s for rough mapping and localized the gsd1 allele on chromosome 1 between two simple sequence length polymorphism (SSLP) markers, F5.5M and F18.6M. An enlarged population containing 1,000 F2s was then employed for fine mapping. In the rough mapping interval, we developed seven PCR-based markers, including SSLP, cleaved-amplified polymorphic sequence (CAPS), and derived (d)CAPS, and narrowed down the causal gene to a 260-kb genomic region between markers F13.46MB and F13.72MB (Fig. 8A). We were unable to develop more polymorphic markers in this region after many tries, potentially due to the candidate gene’s location so close to the centromere. According to The Arabidopsis Information Resource 9 annotation, the identified region contained 22 transposons and 19 predicted functional genes (Supplemental Table S2). However, only one gene encoding ACC1 was highly expressed in the epidermis (Suh et al., 2005). The entire open reading frame of ACC1 was then sequenced to reveal a G-to-A (G256A; in the first exon) transition in the gsd1 mutant (Fig. 8B). Although this point mutation in gsd1 had no effect on ACC1 expression at the transcript level (data not shown), it is predicted to cause a single amino acid substitution from Glu to Lys (E to K), which is within a highly conserved region of the eukaryotic ACC1 protein’s biotin carboxylase domain (Fig. 8, C and D).

Complementation Tests of gsd1 with Other ACC1 Alleles

To confirm that the polymorphism of ACC1 in the gsd1 mutant confers the observed gsd1 phenotype, allelism tests were performed between gsd1 and three other mutant ACC1 alleles, gk-SC, emb22, and acc1-3. Because homozygous gk-SC, emb22, and acc1-3 mutants express embryo lethality under normal growth conditions, heterozygous F1 plants of gk-SC, emb22, and acc1-3 were hybridized with the nonlethal homozygous gsd1. The heterozygous plants carrying two unique mutant alleles at the ACC1 locus, gsd1/gk-SC, gsd1/emb22, and gsd1/acc1-3, were confirmed by molecular genotyping using allele-specific primers and sequencing (Supplemental Table S1; data not shown). When immersed in toluidine blue stain, both the 10-d-old gsd1 seedlings from agar plates and the leaves of 6-week-old greenhouse-grown gsd1 plants absorbed more stain than the wild-type control (Fig. 9, A, B, G, and H). Similar results were observed in the seedlings

**Figure 3.** TEM scans of the cuticle layer of inflorescence stems of wild-type Col-0 (A) and the gsd1 mutant, having a more irregular, osmiophilic cuticle (B). Bars = 0.2 μm.

**Figure 4.** Cutin monomer composition of inflorescence stems and rosette leaves of wild-type Col-0 and the gsd1 mutant. The C16 and C18 labels on the x axis represent the 16- and 18-carbon acid chains, respectively, whereas the number preceding the “OH” indicates chain insertion point(s). Dioic represents dioic acid. The number of double bonds is indicated after the colon. Monomer amounts are expressed as μg dm⁻² stem (A) and leaf (B). Mean ± SD; n = 4. * P ≤ 0.05, ** P ≤ 0.01.
of gsd1/emb22 and gsd1/gk-SC heterozygotes relative to their respective wild-type controls, Col-0 and Col-0/Ler-0 F1 (Fig. 9, C and E). However, leaves of the 6-week-old plants of gsd1/emb22 and gsd1/gk-SC growing in the greenhouse exhibited much higher rates of toluidine blue stain absorption than the homozygous gsd1 (Fig. 9, G and H). This observation indicated that emb22 and gk-SC do not complement the phenotype of gsd1 and thus confirmed that the gsd1 allele at the ACC1 locus is responsible for the observed phenotypes of the gsd1 mutant. In contrast, however, seedlings and 6-week-old leaves of gsd1/acc1-3 absorbed toluidine blue at a rate more similar to the corresponding wild-type control (Col-0/Ler-0 F1), indicating an elevated level of functional complementation (Fig. 9, D, F, G, and H).

Most wax mutants exhibit visibly glossy or semiglossy stems (rather than fully glaucous stems, as does the wild type) and have altered wax crystallization patterns on their inflorescence stem surfaces. Scanning electron microscopy (SEM) was used to demonstrate that gsd1 stems had a much lower density of wax crystals than stems of the wild type and a slight reduction in the proportion of the most dendritic crystals (Fig. 10, A and B). In whole flowering plants, gsd1/gk-SC and gsd1/emb22 mutants had more glossy stems and fewer microscopic wax crystals than even gsd1, whereas gsd1/acc1-3 exhibited a semiglaucoous stem wax coating and slightly higher wax crystal density than all mutant variants, with that density being slightly less than in the wild type (Fig. 10). These wax crystalline phenotypes were consistent with the wax composition, with the mutant variants showing similar reductions in total wax amount to the observed reductions in wax crystals (Fig. 11; Table I; data not shown for gsd1/emb22).

Organ- and Tissue-Specific Expression of ACC1

We constructed an ACC1 pro::GUS construct for the transformation of wild-type Arabidopsis plants to monitor expression patterns of ACC1 at different developmental stages. We observed that ACC1 is constitutively expressed throughout development (Supplemental Fig. S1), being especially highly expressed in the cotyledon and root radicles early in development (Supplemental Fig. S1, A and B), with strong signals also detected in whole seedlings, roots, cotyledons, and true leaves (Supplemental Fig. S1C). In mature plants, ACC1 was strongly expressed in inflorescences, siliques, and cauline leaves (Supplemental Fig. S1, E and I). In stems, the expression levels of ACC1 varied along the whole stem length, with the highest expression at the top of the stem and the weakest expression at the base of the stem (Supplemental Fig. S1, E, F, and I). ACC1 is also highly but not specifically expressed in the epidermal layer (Supplemental Fig. S1G). These results are consistent with those of Suh et al. (2005), who showed based on microarray analysis that the expression of ACC1 is most highly expressed in epidermis, with epidermis-to-stem expression ratios of 4.2-fold in the younger stem (top) portion and 2.3-fold in the older stem (base) portion.

Microarray Analysis of the gsd1 Transcriptome

All genes showing at least 2-fold or more change in transcript abundance in gsd1 compared with the Col-0 wild-type parent are shown in Supplemental Table S3 (up-regulated in gsd1) and Supplemental Table S4 (down-regulated in gsd1). In gsd1, 290 genes were up-regulated by 2-fold or more (Fig. 12A), whereas 115 genes were...
genes were down-regulated by 2-fold or more relative to the wild-type control (Fig. 12B). Even though ACC1 is assumed to function in creating substrates for numerous lipid pathways, the number of lipid pathway-associated genes whose expression was altered by the gsd1 mutation was small. Only three genes associated with lipid pathways in gsd1 were up-regulated 2-fold or more (1-aminocyclopropane-1-carboxylate oxidase [AT1G12010], GDSL-motif lipase/hydrolase [AT2G19050], and glycerophosphoryl diester phosphodiesterase [AT5G41080]), and only eight lipid genes were down-regulated, these being genes involved in lipid transfer or the metabolism of phospholipids or jasmonates (Fig. 12; Supplemental Tables S3 and S4). The major class of genes impacted by the gsd1 mutation was associated with stress (abiotic and biotic) tolerance and response, with gsd1 exhibiting 58 up-regulated stress-associated genes and 15 down-regulated stress-associated genes (Fig. 12B). As for the transcription of other gene classes in gsd1, many hormone response-associated genes showed altered regulation, with 17 genes showing up-regulation and eight showing down-regulation (Fig. 12). Most other genes whose expression was modified 2-fold or more

Figure 7. Whole plant rosette permeability assays of wild-type Col-0 and the gsd1 mutant. A and B, Toluidine blue staining of 3-week-old plantlets as viewed from the adaxial surface (A) and the abaxial surface (B) reveals less blue coloration in the wild type (left) than in gsd1 (right). C and D, Three-week-old wild type (left) and gsd1 (right) rosettes were excised from roots (C) and then subjected to 8 h of dehydration (D), revealing the rapid wilting phenotype of gsd1. E and F, The rate of water loss (E) and chlorophyll leaching (F) of 3-week-old plants. Similar permeability characteristics were observed in older leaves and inflorescence tissues. Mean ± so; n = 10, P ≤ 0.05.

Figure 8. Map-based cloning of the gsd1 allele. A, The mutation was mapped between SSLP makers F5.5MB and F18.6MB using 76 F2 plants. Fine mapping narrowed gsd1 down to a 260-kb region between markers F13.46MB and F13.72MB using 1,000 F2 plants. Numbers above the horizontal line are the number of recombinants between the indicated marker and gsd1. B, The location of the gsd1 mutation in the ACC1 (At1g36160) open reading frame. The structure of the ACC1 gene and the positions of the point mutations identified in gsd1, gk-SC, emb22, and acc1-3 are shown. Black boxes represent exons, whereas black lines stand for introns and untranslated regions. Nucleotide positions are relative to the translational start site (+1). C, The gray, dark gray, and black boxes show the biotin carboxylase (BC) domain, biotin-carboxyl carrier (BCC) domain, and carboxyl transferase (CT) domain, respectively. D, Amino acid sequence alignments of a conserved motif of all ACCases. Letters shaded by black or dark gray (red in the online version) indicate positions where amino acids are highly conserved. The asterisk indicates the conserved amino acid residue mutated in gsd1. AtACC1, Arabidopsis ACCase 1 (At1g36160); GgACC1, Gallus gallus ACCase (J03541); HsACC1, Homo sapiens ACCase 1 (U19822); ScACC1, Saccharomyces cerevisiae ACC1 (CAA96294); TaACC1, Triticum aestivum ACCase (U10187); ZmACC1, Zea mays ACCase (U19183). [See online article for color version of this figure.]
resembled fundamental metabolic (housekeeping) genes (Fig. 12).

The most highly induced gene in gsd1 was **SENESCENCE-ASSOCIATED GENE1** (**SEN1**), showing a 30.8-fold induction (Supplemental Table S3). A UDP-glucuronosyl/UDP-glucosyltransferase family protein (AT3G11340)-encoding gene was also highly induced in gsd1, with 24.5-fold induction (Supplemental Table S3). The gene encoding 1-aminocyclopropane-1-carboxylate oxidase (the main rate-limiting enzyme in ethylene synthesis) increased 14.3-fold in gsd1 over Col-0 (Supplemental Table S3). Transcripts for a Gly-rich protein, a nitrate transmembrane transporter (**NRT2.6**), and an unknown protein were the next most highly induced after ACC1, these being induced 13.8-, 12.7-, and 12.6-fold, respectively (Supplemental Table S3). The other genes showing up-regulation were two glutathione S-transferases, being induced 11.2- and 8.9-fold, respectively (Supplemental Table S3). The genes showing the greatest down-regulation were annotated as encoding an invertase/pectin methylesterase inhibitor family protein and β-1,3-GLUCANASE, showing 11.6- and 11.5-fold reduction, respectively (Supplemental Table S4). An unknown protein was the next most down-regulated transcript, being reduced 9.0-fold (Supplemental Table S4).

**DISCUSSION**

The Arabidopsis **gsd1** mutation represents a unique weak allele of **ACC1** that does not cause seed, embryo, or early seedling lethality, as reported for other **ACC1** mutant alleles (Baud et al., 2003, 2004; Kajiwara et al., 2004), a condition that allows the impact of **ACC1** on mature organs to be assessed here, to our knowledge, for the first time. We show that **gsd1** causes reduced glaucousness (less visible waxiness) of the inflorescence stem, postgenital fusion, and reduced fertility, similar to some other reported wax mutants (Chen et al., 2003; Zheng et al., 2005; Kurdyukov et al., 2006; Panikashvili et al., 2009). In addition, **gsd1** exhibits a large reduction in the total wax amount, expressed mainly as a reduction in very-long-chain alkanes, secondary alcohols, and ketones. Wax synthesis involves a series of condensation reactions that add 2-carbons from malonyl-CoA to a growing acyl-CoA chain to create the VLCFA (C20:0 or longer) precursors of most waxes. Whereas previous studies in yeast and

![Figure 9](image1)

**Figure 9.** Permeability of cuticles of gsd1 and gsd1-derived heterozygotes to toluidine blue stain. A to F, Permeability of seedling leaf cuticles of wild-type Col-0 (A), gsd1 (B), heterozygous gsd1/emb22 (C), Col-0/Ler-0 (D), heterozygous gsd1/gk-SC (E), and heterozygous gsd1/acc1-3 (F) to toluidine blue. The term “heterozygous” as used here indicates two distinct mutant alleles at the **ACC1** locus. G and H, Permeability of leaf adaxial (G) and abaxial (H) surfaces of wild-type Col-0, gsd1, gsd1/emb22, Col-0/Ler-0, gsd1/acc1-3, and gsd1/gk-SC to toluidine blue stain.

![Figure 10](image2)

**Figure 10.** SEM results showing stem epicuticular wax crystals of wild-type Col-0 (A), gsd1 (B), gsd1/emb22 (C), gsd1/gk-SC (D), Col-0/Ler-0 (E), and gsd1/acc1-3 (F). Bars = 5 \( \mu \text{m} \).
other systems showed that the cytosolic ACC1 protein generates the malonyl-CoA needed for VLCFA synthesis (Baud et al., 2003; Sasaki and Nagano, 2004; Tehlivets et al., 2007), the results here reveal, to our knowledge for the first time, ACC1’s critical function in generating malonyl-CoA precursors for plant wax synthesis (Fig. 13).

Whereas seed TAGs are dominated by C₁₆ and C₁₈ acids, these seed oils also possess VLCFAs having 20 and 22 carbons, thought to be synthesized by a seed-specific acyl-CoA-condensing enzyme, FAE1 (Kunst et al., 1992). Previous studies suggest that seed FAE1 utilizes malonyl-CoA derived from ACC1, since the ACC1 mutant alleles pas3, gk-SC, acc1-1, and acc1-2 exhibit lower amounts of seed C₂₀:₀, C₂₀:₁, C₂₀:₂, and C₂₂:₁ acids (Baud et al., 2003, 2004). The gsd1 mutation causes similar reductions in seed VLCFAs as well as similar increases in the C₁₈:₁ acids, just like these other ACC1 mutant alleles. These results indicate that ACC1 provides VLCFA substrates for both wax and seed oil synthesis.

Cutin and suberin are the plant polyesters whose major monomers are derived from plastidic C₁₆ and C₁₈ acids (Fig. 13). Cutin does not contain significant amounts of VLCFA, although suberin has both C₂₀ and C₂₂ monomers. However, the total amount of these VLCFA-derived suberin monomers is not affected in the gsd1 mutant relative to the wild type, indicating that the weak gsd1 allele either is not severe enough to limit the overall production of these VLCFA suberin monomers or that these suberin monomers derive from a separate elongation pathway, such as that defined by the ELO-like family of condensing enzymes (Dunn et al., 2004; Paul et al., 2006). Malonyl-CoA can also be produced in roots by malonyl-CoA synthetase (Chen et al., 2011), but whether this occurs at high enough levels to solely supply the VLCFA monomer pathways of suberin is unknown. It is interesting that ACC1 transcript abundance is very low in wild-type roots (Supplemental Fig. S1I; Baud et al., 2003), supporting the proposition that ACC1 likely plays a minor role, if any, in the synthesis of VLCFAs destined for suberin. Nevertheless, changes in the proportional distributions of VLCFA monomers observed in gsd1 suberin, as well as the overall increase in the total amount of long-chain C₁₆ and C₁₈ monomers in gsd1 suberin, indicate that ACC1 activity does, in fact, have some direct or indirect effect on suberin synthesis. The elucidation of basic suberin synthetic pathways, and the possible involvement of ACC1 in suberin synthesis, require further study.

The gsd1 mutation inhibits the synthesis of VLCFA precursors for waxes and TAGs but enhances the overall synthesis of cutin, suberin, and seed fatty acid monomers that arise from the long-chain acids (i.e. C₁₆ and C₁₈ acids). This appears to be the result of gsd1’s partial blockage of the endoplasmic reticulum-based elongation of long to VLCFAs through the

Figure 11. Cuticular wax composition on stems of Col-0/Ler-0, gsd1/acc1-3, and gsd1/gk-SC. Wax coverage is expressed as μg dm⁻² surface area. The genotype of each sample is shown at the top left corner of each panel. Each wax constituent is designated by carbon chain length and is labeled by chemical class along the x axis. Mean ± sd; n = 4.
reduced production of malonyl-CoA. This blockage apparently leads to the accumulation of excessive C\textsubscript{16} and C\textsubscript{18} acyl chains, which are shunted into cutin, suberin, and TAG biosynthetic pathways. Since malonyl-CoA is membrane impermeable (Liedvogel, 1986; Fatland et al., 2002; Schwender and Ohlrogge, 2002), it is unlikely that plastid-derived malonyl-CoA itself could be shunted into cytosolic pathways to account for increased amounts of C\textsubscript{16} and C\textsubscript{18} constituents of gsd1 lipids. Further studies are needed to elucidate how changes in ACC1 activity affect the distribution of the long-chain acids throughout these lipid metabolic networks as well as the role of ACC1 in the synthesis of other VLCFA-derived lipids in mature plant tissues, such as sphingolipids and malonated derivatives of 1-aminocyclopropane-1-carboxylic acid, D-amino acids, and flavonoid glycosides.

The gsd1/emb22 and gsd1/gk-SC heterozygotes possessed inflorescence stems that were visibly glossier and cuticles that were more permeable than even the homozygous gsd1, indicating a clear dosage effect of the alleles. Whereas the emb22 and gk-SC mutants produced heavily truncated nonfunctional transcripts, we show that gsd1 produces a full-size transcript. As such, the emb22 and gk-SC mutants likely do not express any ACC1 protein, whereas gsd1 most likely produces an ACC1 protein having reduced activity relative to the wild type. In contrast, the gsd1/acc1-3 heterozygote showed almost complete complementation. Kajiwara et al. (2004) reported that acc1-3 carries a mutation (G929A, in the third exon) within the biotin carboxylase domain that causes a single amino acid substitution from Gly to Asp. The gsd1 mutation (G256A, in the first exon), which caused a single amino acid substitution from Glu to Lys (E to K), occurs in this same biotin carboxylase domain (Fig. 8, C and D). It is known that as a part of its normal function, ACC1 forms homodimers in the cytoplasm (Tong, 2005). One possible explanation for the near complementation in gsd1/acc1-3 is that the gsd1 and acc1-3 alleles each produce protein products that combine to form a functional ACC1 homodimer complex, wherein monomer defects are compensated by the other. Alternatively, the multiple promoters on ACC1 create the potential for alternative splicing at the 5’ end in a way that affects protein regulation (Mao et al., 2003; Tong, 2005; Podkowski et al., 2003). Whether the gsd1 or acc1-3 mutation is far enough upstream to impact transcription start sites or 5’ splicing is also unknown. A more complete explanation for how gsd1/ acc1-3 complementation occurs, whether by protein rearrangement, effects on substrate binding or carboxylation, alternative splicing, or some other mechanism, requires further study.

Multiple transcription networks are impacted when ACC1 activity is changed by the gsd1 mutation. A total of 290 genes representing diverse cellular processes showed higher transcript abundance than in the wild type, and 114 genes were down-regulated. Antisense down-regulation of ACL that generates acetyl-CoA showed very similar effects on global gene expression to gsd1 (Fatland et al., 2005). In both gsd1 and the antisense ACLA lines, fewer lipid-associated genes than expected are altered. The gsd1 mutant showed only three lipid genes up-regulated and eight lipid genes down-regulated, whereas antisense ACLA showed only 11 lipid genes up-regulated and no lipid genes down-regulated. None of these findings, however, are predicted to have a direct impact on VLCFA-derived lipid pathways. Since acetyl-CoA and malonyl-CoA are key substrates to many cytosolic lipid pathways, it is unclear why the transcriptome of downstream lipid pathways is not more affected. Apparently, ACC1-associated lipid metabolism is regulated mainly at the posttranscriptional level, not at the level of gene transcription. Further studies are needed.
The gene expression network most impacted by the gsd1 mutation was that associated with environmental stress responses. The number of stress-associated genes responding in gsd1 was 58 up-regulated and 15 down-regulated, a response similar to that of antisense ACLA, which exhibited 62 up-regulated and three down-regulated stress-associated genes (Fatland et al., 2005). Why the suppression of acetyl-CoA and malonyl-CoA precursors shows effects on so many stress-associated pathways is unclear. Possibly, the change in malonyl-CoA precursors creates a so-called “metabolic stress,” a condition wherein the deficiency of these precursor pools causes an imbalance in many fundamental stress-linked metabolic networks. As the ACC1 promoter is known to contain many environmental stress response motifs (Figueroa-Balderas et al., 2006), a direct linkage to environmental or metabolic stress responses seems likely. Malonyl-CoA (or acetyl-CoA) itself could serve as a signal in stress responses or else could serve as a substrate for the synthesis of lipid-based signals in stress responses such as jasmonic acid (Mang et al., 2009). Although gsd1 plants possess a more permeable cuticle that could cause tissue dehydration in water-limiting environments, all of the plants examined here were well watered and otherwise did not experience stress. That gsd1 experienced an environmental stress not felt by the wild type is highly unlikely. Furthermore, 17 genes encoding hormone response determinants were up-regulated, while eight hormone response-associated genes were down-regulated in gsd1. Since the stress response is tightly linked to hormonal regulation, these results are consistent with the proposition that multiple stress signaling networks are activated by the lipidic changes occurring in the gsd1 mutant.

The strongest up-regulated gene in gsd1 is SEN1, a gene whose expression is often used as a marker of senescence (Xiao et al., 2010). The gene encoding 1-aminocyclopropane-1-carboxylic acid oxidase (ACO), a rate-limiting enzyme in ethylene synthesis, is also highly induced. Ethylene is a critical plant growth regulator, serving as a signal of many stress responses and inducing senescence (van Loon et al., 2006; Lin et al., 2009). Previous studies report that VLCFAs of C24:0 can activate ethylene biosynthesis by inducing the accumulation of ACO transcripts (Qin et al., 2007). Even though the longer C26, C28, and C30 acids are lower in gsd1, the C22 and C24 acids on the gsd1 inflorescence stem (although among the low-abundance constituents in Arabidopsis) are actually increased by 374% and 137%, respectively. Whether the higher C22 and C24 fatty acids on gsd1 were responsible for inducing the transcription of ACO, SEN1, or related genes is unknown. Interestingly, the gsd1/gk-SC and gsd1/emb22 heterozygotes with the most severe nonlethal ACC1 deficiency phenotypes exhibited clear visible signs of early senescence in flower buds and leaves (data not shown), an indication

---

**Figure 13.** A model pathway describing flux through primary aliphatic lipid metabolic pathways in plants. The red X indicates the location of normal ACC1 activity and the site of the gsd1 metabolic lesion. ACP, Acyl carrier protein; FAS, fatty acid synthase; KAS, 3-ketoacyl-acyl carrier protein synthase; MCAT, malonyl-CoA:ACP acyltransferase, SAD, stearoyl-ACP desaturase.
that ACCO induction in ACC1 mutants likely increased ethylene synthesis.

In summary, the gsd1 mutant reveals new insight into the role of ACC1 in lipid synthesis, linking ACC1 to a primary function as a provider of malonyl-CoA precursors for cuticular wax synthesis in plants. Other lipids that do not require VLCFAs were comparably unaffected, except for their synthesis being enhanced due perhaps to the shunting of lipid precursors. Studies of the transcriptome revealed a unique connection between ACC1 and abiotic and biotic stress response networks, raising the likelihood that ACC1-derived lipids may serve as important stress-responsive signaling compounds.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The gsd1 mutant was obtained by visual screens of an Arabidopsis (Arabidopsis thaliana) EMS-mutagenized population. Seeds of gk-SC (CS18148) were obtained from the Arabidopsis Biological Resource Center (www. arabidopsis.org). Seeds of emh22 and acc1-3 were kindly provided by Dr. Masao Tasaka (Kajiwara et al., 2004). The gsd1 mutant was backcrossed to the wild-type Ler-0 and advanced to the F2 generation for genetic analysis. Arabidopsis wild-type and mutant seeds were stratified for 3 to 4 d at 4 °C, followed by germination in complete darkness. Water loss rates were recorded over 180 min and measured as a percentage of the initial weight of fully hydrated rosettes.

Assessment of Cuticle Permeability

The toluidine blue staining protocol was adapted from a previously described method (Tanaka et al., 2004). Three-week-old plants and 7-week-old leaves were immersed for 5 min in 0.05% toluidine blue and rinsed with water. For water loss measurements, plants were transferred from a growth room to a dark room for at least 4 h. The whole shoots of 3-week-old plants with root system detached were weighed every 20 min using a microbalance with a 10-rpm rotation speed in a hybridization oven (Agilent). After the 17-h incubation, the arrays were washed using low-stringency wash buffer 1 (Agilent) at room temperature for 1 min followed by a high-stringency wash using wash buffer 2 (Agilent) at 37°C. The arrays were air dried and scanned using the high-resolution array scanner (Agilent) with the appropriate settings for one-color gene expression arrays. The signal intensities were extracted from the scanned images with the aid of Feature extraction software 10.7.1.1 (Agilent) and subjected to background subtraction and spatial detrending. The outliers and the abnormal features were flagged, and the data were normalized using intraarray percentile shift normalization.
(threshold of 75 and above) and median-based interarray normalization. GeneSpring GX (Agilent) was used to calculate the intensity ratios and fold changes. All genes with \( P < 0.05 \) and change above 2-fold were chosen for the Gene Ontology enrichment analysis.

**Supplemental Data**

The following materials are available in the online version of this article.

**Supplemental Figure S1.** The expression pattern of ACC1 in various Arabidopsis tissues.

**Supplemental Table S1.** Primers used in this study.

**Supplemental Table S2.** Genes located in the mapping region (260 kb), including transposons and functional genes.

**Supplemental Table S3.** Genes whose transcript abundance is up-regulated at least 2-fold in gsd1 flowers.

**Supplemental Table S4.** Genes whose transcript abundance is down-regulated at least 2-fold in gsd1 flowers.

**ACKNOWLEDGMENTS**

We are grateful to Dr. Masao Tsaka (Nara Institute of Science and Technology) for providing emb22 and aci1-3 seeds. We also thank Debra Sherman and Chia-Ping Huang of the Purdue University Electron Microscopy Center for support.

Received August 8, 2011; accepted September 21, 2011; published September 23, 2011.

**LITERATURE CITED**

Aharoni A, Dijkstra S, Jetter R, Thones E, van Arkel G, Pereira E (2004) The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in Arabidopsis. Plant Cell 16: 2463–2480

Baud S, Bellec Y, Miquel M, Bellini C, Caboche M, Lepiniec L, Faure JD, Rochat C (2004) gurk and pasticcino mutants affected in embryo development are impaired in acetyl-CoA carboxylase. EMBO Rep 5: 515–520

Baud S, Guyon V, Kronenberger J, Wuilleme S, Miquel M, Caboche M, Lepiniec L, Rochat C (2003) Multifunctional acetyl-CoA carboxylase 1 is essential for very long chain fatty acid elongation and embryo development in Arabidopsis. Plant J 33: 75–86

Bonaventure G, Beisson F, Ohlrogge J, Pollard M (2004) Analysis of the substrate specificity of the enzymes and sensitivity towards aryloxyphe-nolic herbicides. Eur J Biochem 225: 425–443

Jetter R, Kunst L (2008) The epidermis-specific extracellular BODYGUARD controls cuticle development and morphogenesis in Arabidopsis. Plant Cell 19: 321–339

Kajiwara T, Furutani M, Hiba K, Tazaka M (2004) The GURKE gene encoding an acetyl-CoA carboxylase is required for partitioning the embryo apex into three subregions in Arabidopsis. Plant Cell Physiol 45: 1122–1128

Kosma DK, Bourdoux B, Bernard A, Parsons EP, Liu S, Joubès J, Jenks MA (2009) The impact of water deficiency on leaf cuticle lipids of Arabidopsis. Plant Physiol 151: 1918–1929

Kunst L, Samuels L (2009) Plant cuticles shine: advances in wax biosynthesis and export. Curr Opin Plant Biol 12: 721–727

Kunst L, Taylor DC, Underhill EW (1992) Fatty acid elongation in developing seeds of Arabidopsis thaliana. Plant Physiol Biochem 30: 425–434

Kurdyukov S, Faust A, Nawrath C, Bär G, Voss H, Efremova N, Franke R, Schreiber L, Saedler H, Mérats JP, et al. (2006) The epidermis-specific extracellular BODYGUARD controls cuticle development and morphogenesis in Arabidopsis. Plant Physiol 142: 909–918

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-DDCT) method. Methods 25: 402–408

Lolle SJ, Hsu W, Pruitt RE (1998) Genetic analysis of organ fusion in Arabidopsis thaliana. Genetics 149: 607–619

Liu Y, Hoffman NE, Yang SF (1983) Relationship between the malonation of 1-aminocyclopropane-1-carboxylic acid and D-amino acids in mung bean hypocotyls. Plant Physiol 83: 437–441

Liu Y, Su LY, Yang SF (1984) Stereoselectivity of 1-aminocyclopropane-1-carboxylate malonyltransferase toward stereoisomers of 1-amino-2-ethyl-cyclopropane-1-carboxylic acid. Arch Biochem Biophys 235: 319–325

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-DDCT) method. Methods 25: 402–408

Mao J, Chirala SS, Wakil SJ (2003) Expression of cytosolic and plastid acetyl-coenzyme A carboxylase genes in young wheat plants. Plant Physiol 131: 763–772

Mang HG, Laluk KA, Parsons EP, Kosma DK, Cooper BR, Park HC, AbuQamar S, Boccongelli C, Miyazaki S, Consiglio F, et al. (2009) The Arabidopsis RESURRECTION1 gene regulates a novel antagonistic interaction in plant defense to biotrophs and necrotrophs. Plant Physiol 151: 290–305

Mao J, Chirala SS, Waiyi SJ (2003) Human acetyl-CoA carboxylase 1 gene: presence of three promoters and heterogeneity at the 5′-untranslated mRNA region. Proc Natl Acad Sci USA 100: 7515–7520

Nikolau BJ, Hohlgren JB, Wurtele ES (2003) Plant biotin-containing carboxylases. Arch Biochem Biophys 414: 211–222

Panikashvili D, Shi JX, Schreiber L, Aharoni A (2009) The Arabidopsis DCR encoding a soluble BAHD acyltransferase is required for cutin polyester formation and seed hydration properties. Plant Physiol 151: 1773–1789

Paul S, Gable K, Beaudoin F, Cao E, Jaworski J, Napier JA, Dunn TM (2006) Members of the Arabidopsis FAE1-like 3-ketoacyl-CoA synthase gene family substitute for the Elop proteins of Saccharomyces cerevisiae. J Biol Chem 281: 9018–9029

Podkowski J, Jelenska J, Sirikachomkit A, Zuther E, Hasselkor R, Gorlicki P (2003) Expression of cytosolic and plastid acetyl-coenzyme A carboxylase genes in young wheat plants. Plant Physiol 131: 763–772

Qin YM, Hu CY, Pang Y, Kastaniotis AJ, Hiltunen JK, Zhu YX (2007) Saturated very-long-chain fatty acids promote cotton fiber and Arabidopsis cell elongation by activating ethylene biosynthesis. Plant Cell 19: 3692–3704

GSD1/ACC1 Function in Wax Biosynthesis by Arabidopsis thaliana. Plant Cell Physiol 45: 1122–1128

Lin Z, Zhang S, Grierson D (2009) Recent advances in ethylene research. J Exp Bot 60: 3311–3336

Mao J, Chirala SS, Wakil SJ (2003) Expression of cytosolic and plastid acetyl-coenzyme A carboxylases. Arch Biochem Biophys 281: 9018–9029

Panikashvili D, Shi JX, Schreiber L, Aharoni A (2009) The Arabidopsis DCR encoding a soluble BAHD acyltransferase is required for cutin polyester formation and seed hydration properties. Plant Physiol 151: 1773–1789

Paul S, Gable K, Beaudoin F, Cao E, Jaworski J, Napier JA, Dunn TM (2006) Members of the Arabidopsis FAE1-like 3-ketoacyl-CoA synthase gene family substitute for the Elop proteins of Saccharomyces cerevisiae. J Biol Chem 281: 9018–9029

Podkowski J, Jelenska J, Sirikachomkit A, Zuther E, Hasselkor R, Gorlicki P (2003) Expression of cytosolic and plastid acetyl-coenzyme A carboxylase genes in young wheat plants. Plant Physiol 131: 763–772

Qin YM, Hu CY, Pang Y, Kastaniotis AJ, Hiltunen JK, Zhu YX (2007) Saturated very-long-chain fatty acids promote cotton fiber and Arabidopsis cell elongation by activating ethylene biosynthesis. Plant Cell 19: 3692–3704

Plant Physiol. Vol. 157, 2011 1091
Sasaki Y, Nagano Y (2004) Plant acetyl-CoA carboxylase: structure, biosynthesis, regulation, and gene manipulation for plant breeding. Biosci Biotechnol Biochem 68: 1175–1184

Schwender J, Ohlrogge JB (2002) Probing in vivo metabolism by stable isotope labeling of storage lipids and proteins in developing Brassica napus embryos. Plant Physiol 130: 347–361

Suh MC, Samuels AL, Jetter R, Kunst L, Pullard M, Ohlrogge J, Beisson F (2005) Cuticular lipid composition, surface structure, and gene expression in Arabidopsis stem epidermis. Plant Physiol 139: 1649–1665

Taguchi G, Ubukata T, Nozue H, Kobayashi Y, Takah M, Yamamoto H, Hayashida N (2010) Malonylation is a key reaction in the metabolism of xenobiotic phenolic glucosides in Arabidopsis and tobacco. Plant J 63: 1031–1041

Tanaka T, Tanaka H, Machida C, Watanabe M, Machida Y (2004) A new method for rapid visualization of defects in leaf cuticle reveals five intrinsic patterns of surface defects in Arabidopsis. Plant J 37: 139–146

Tehlivets O, Scheuringer K, Kohlwein SD (2007) Fatty acid synthesis and elongation in yeast. Biochim Biophys Acta 1771: 255–270

Tong L (2005) Acetyl-coenzyme A carboxylase: crucial metabolic enzyme and attractive target for drug discovery. Cell Mol Life Sci 62: 1784–1803

Torres-Ruiz RA, Lohner A, Jürgens G (1996) The GURKE gene is required for normal organization of the apical region in the Arabidopsis embryo. Plant J 10: 1005–1016

van Loon LC, Geraats BP, Linthorst HJ (2006) Ethylene as a modulator of disease resistance in plants. Trends Plant Sci 11: 184–191

Xu C, Moellering ER, Pan J, Benning C (2008) Mutation of a mitochondrial outer membrane protein affects chloroplast lipid biosynthesis. Plant J 54: 163–175

Xiao S, Gao W, Chen QE, Chan SW, Zheng SX, Ma J, Wang M, Welti R, Chye ML (2010) Overexpression of Arabidopsis acyl-CoA binding protein ACBP3 promotes starvation-induced and age-dependent leaf senescence. Plant Cell 22: 1463–1482

Zheng H, Rowlad O, Kunst L (2005) Disruptions of the Arabidopsis enoyl-CoA reductase gene reveal an essential role for very-long-chain fatty acid synthesis in cell expansion during plant morphogenesis. Plant Cell 17: 1467–1481