Insertional mutant analysis reveals that long-chain acyl-CoA synthetase 1 (LACS1), but not LACS8, functionally overlaps with LACS9 in Arabidopsis seed oil biosynthesis

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SUMMARY

Triacylglycerols (TAGs) are major storage materials that accumulate in developing seeds and serve as carbon and energy reserves for germination and growth of the seedling. One of the critical reactions in TAG biosynthesis is activation of fatty acyl chains to fatty acyl CoAs, catalyzed by long-chain acyl CoA synthetases (LACSs). Of the nine LACSs identified in Arabidopsis, only LACS9 is known to reside in the plastid, the site of de novo fatty acid synthesis, and is considered the major LACS isoform involved in plastidial fatty acid export for TAG formation. Because the lacs9 null mutant did not show any detectable phenotype, it was hypothesized that at least one additional LACS enzyme must be active in the plastid. Expression analyses to identify potential plastid-localized LACSs involved in TAG biosynthesis revealed that, in addition to LACS9, isoforms LACS1, LACS2, LACS4 and LACS8 are transcribed in the seed. LACS8 showed the highest expression level in the embryo and a high sequence similarity with LACS9, and was therefore characterized further and shown to be associated with the ER, not the plastid. Furthermore, disruption of LACS8 in the lacs8 mutant and lacs8 lacs9 double mutant, and over-expression of LACS8, did not affect the seed fatty acid content. In contrast, 11 and 12% decreases in fatty acid content were detected in lacs1 lacs9 and lacs1 lacs8 lacs9 seeds, respectively, indicating that LACS1 and LACS9 have overlapping functions in TAG biosynthesis. This result is surprising because, unlike LACS9, LACS1 is localized in the ER and has been shown to be involved in cuticular lipid synthesis.