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STUDY OF THE FUNCTIONAL REDUNDANCY OF ARABIDOPSIS KCS FROM THE CLADE α

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Plant very-long-chain fatty acids (VLCFA, $\geq 20C$) are important molecules with crucial physiological and structural roles. VLCFA are precursors for various lipids such as membrane phospholipids and sphingolipids, epidermal cuticular waxes, root suberin monomers or seed triacylglycerols. Plant VLCFA are therefore involved in different mechanisms, such as membrane trafficking, cell division and differentiation, non-stomatal water loss prevention and energy storage. VLCFA are produced by the fatty acid elongase (FAE), an integral endoplasmic reticulum membrane multi-enzymatic complex consisting of four core enzymes. The 3-ketoacyl-CoA synthase (KCS) catalyzes the first reaction of the elongation mechanism and determines the substrate specificity of each elongation cycle, whereas the other three enzymes have broad substrate specificities and are shared by all FAE complexes. Consistent with the co-existence of multiple FAE complexes, performing sequential and/or parallel reactions to produce the broad chain-length-range of VLCFA found in plants, twenty-one KCS genes have been identified in the genome of *Arabidopsis thaliana*. We previously showed that Arabidopsis KCS from the clade α (KCS4, KCS9 and KCS17) mainly produce VLCFA with 24 and 26 carbons in both yeast and tobacco systems (Batsale et al., 2022). The role of KCS9 and KCS4 has already been investigated in single Arabidopsis mutants, which lipids pools were only slightly affected in their VLCFA content (Kim et al., 2013; Kim et al., 2021). These results suggest that the synthesis of the various lipids containing C24 and C26 VLCFA relies on the functional redundancy of several AtKCS genes coexpressed in different plant tissues. In order to overcome such redundancies and dissect further the role of each KCS of the Arabidopsis KCS family cluster α in different lipid pathways and tissues, double and triple T-DNA mutants for *KCS4*, *KCS9* and *KCS17* genes have been generated. Lipidomic analyses were performed in tissues in which *KCS4*, *KCS9* and *KCS17* are simultaneously or differentially expressed: roots, vegetative and reproductive tissues, seed coat. These analyses emphasized exacerbated lipid phenotypes in different tissues and different lipid pools compared to the changes observed in single mutants. Our results suggest that the synthesis of the various lipids containing C24 and C26 VLCFA relies on the functional redundancy of AtKCS from cluster α and their specific spatiotemporal expression in different tissues. This work sheds light on the genetic and biochemical redundancy of fatty acid elongation in plants.

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