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Specific inhibition of plant very-long-chain fatty acid elongases by herbicides

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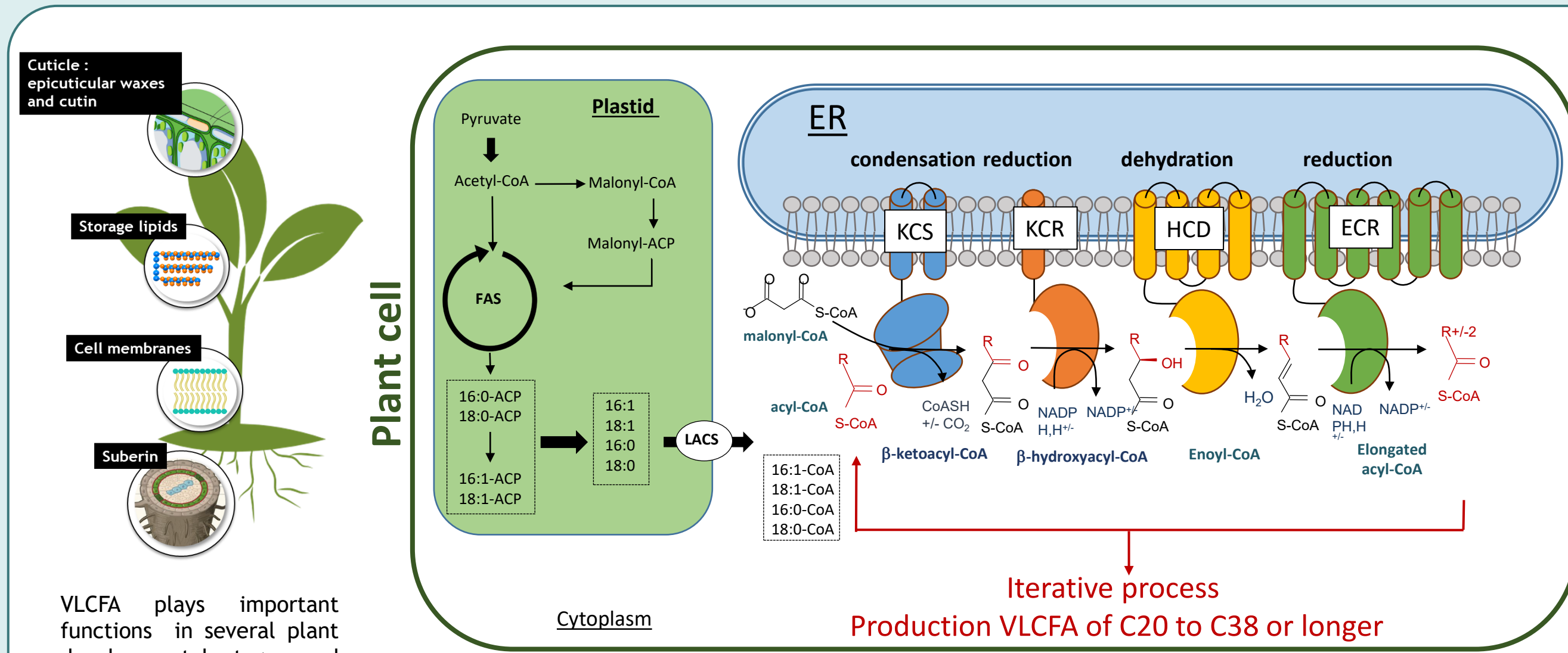
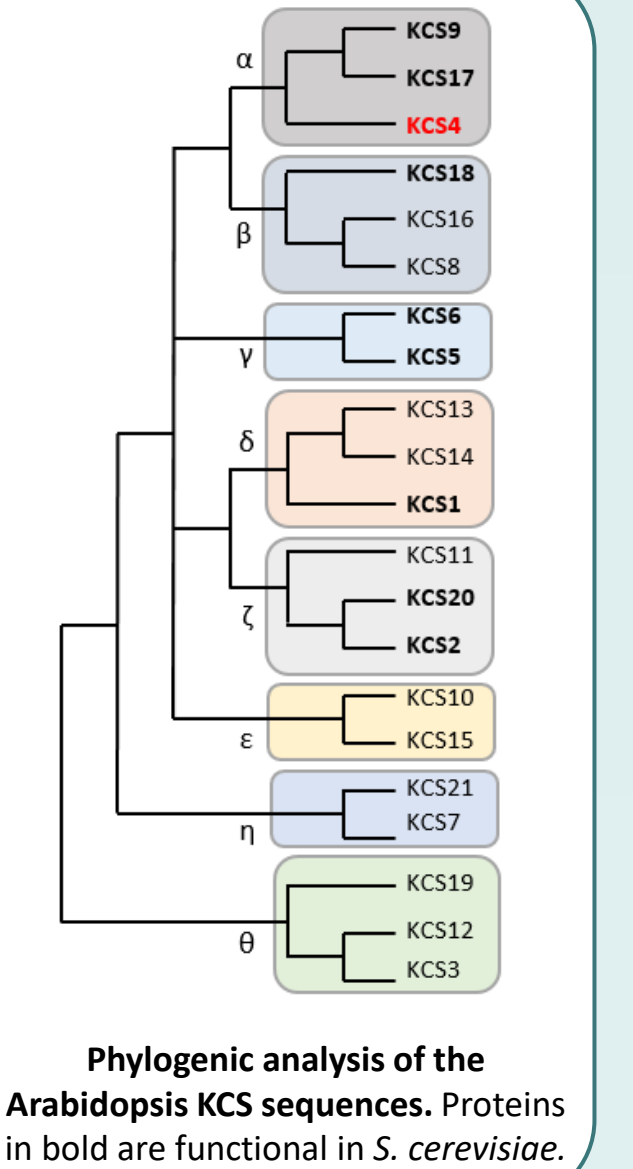


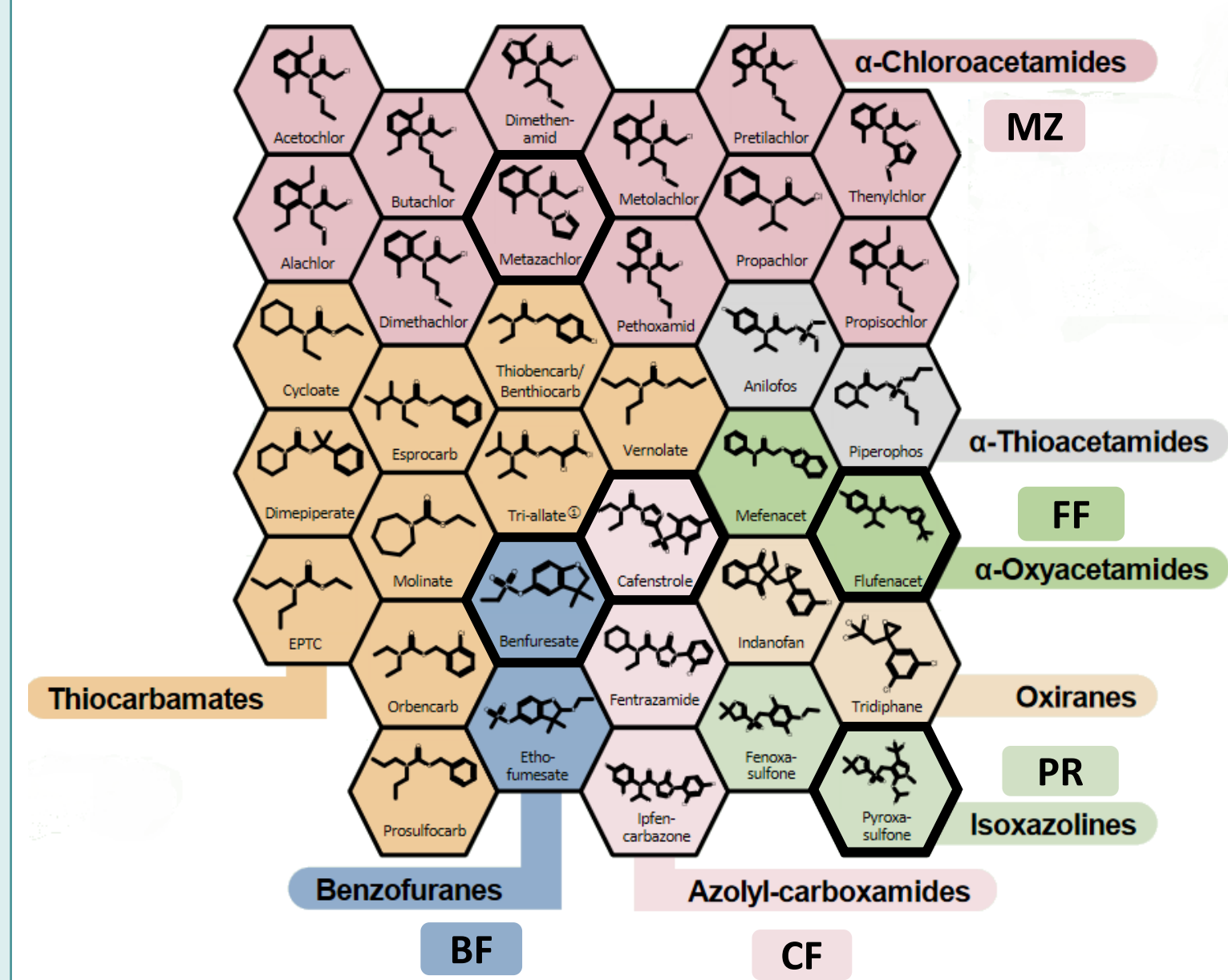
Figure adapted from Batsale et al., 2021

Very-long-chain fatty acids (fatty acids with more than 18 carbons) have crucial physiological and structural roles in plants. VLCFA are produced by the fatty acid elongase (FAE complex) which is an integral ER membrane enzymatic complex with multiple components. If the biochemical mechanism of elongation is now well characterized, little is known about the specific proteins involved in this process. Especially biochemical and genetic studies in *Arabidopsis* led to the idea that multiple elongase complexes with distinct chain-length specificities perform sequential and parallel reactions to produce the wide range of VLCFA found in plants. Using CRISPR-CAS9 technology, we established an expression platform to reconstitute the different *Arabidopsis* FAE complexes in yeast and characterize the substrate specificity of the 21 *Arabidopsis* KCS candidates.

Herbicides inhibiting the synthesis of VLCFA (HRAC group K3), such as flufenacet, pyroxasulfone or metazachlor, play an important role in weed management strategies, especially in cereals. The mode of action of these molecules was first identified based on the typical phenotype observed in treated plants suggesting an inhibition of VLCFA synthesis (Trenkamp et al., 2014). To better understand the mechanism responsible for the selective action of K3 herbicides within the plant kingdom, in particular between dicotyledonous and monocotyledonous plants, we expressed and characterized KCS from *A. thaliana*, *Zea mays*, *Setaria viridis*, *Brassica Napus* in *Saccharomyces cerevisiae* and examined the VLCFAs profiles of plant elongase expressing strains in the presence of various herbicides.

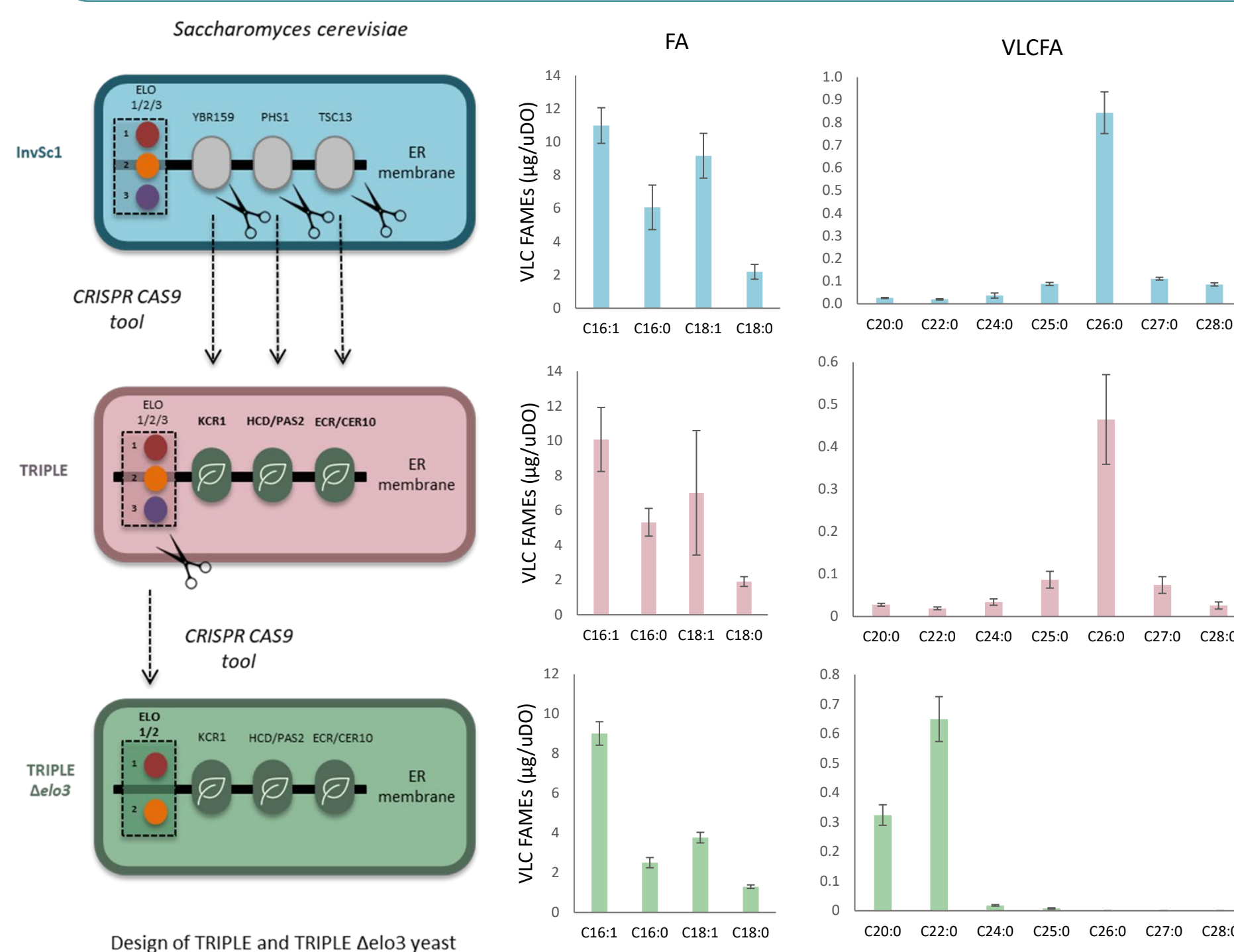


Inhibition of Very Long-Chain Fatty Acid Synthesis by herbicides



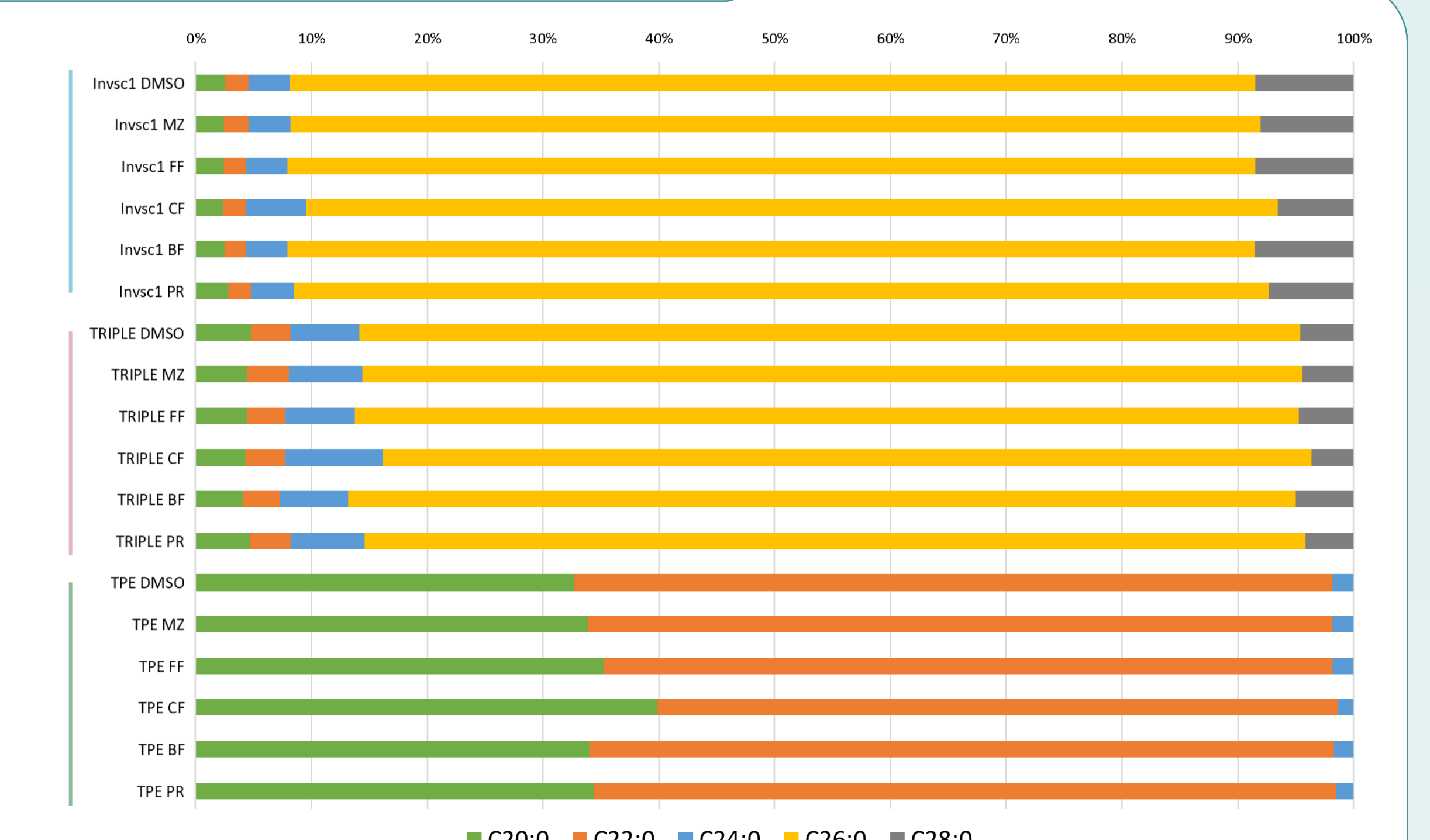
Structures of K3 herbicides (HRAC Mode of Action Classification 2022). Herbicides circled in bold have been tested as potential VLCFAE inhibitors in plant elongase-expressing yeast strains.

Yeast engineering - herbicides specifically inhibited the *Arabidopsis* KCS enzymes



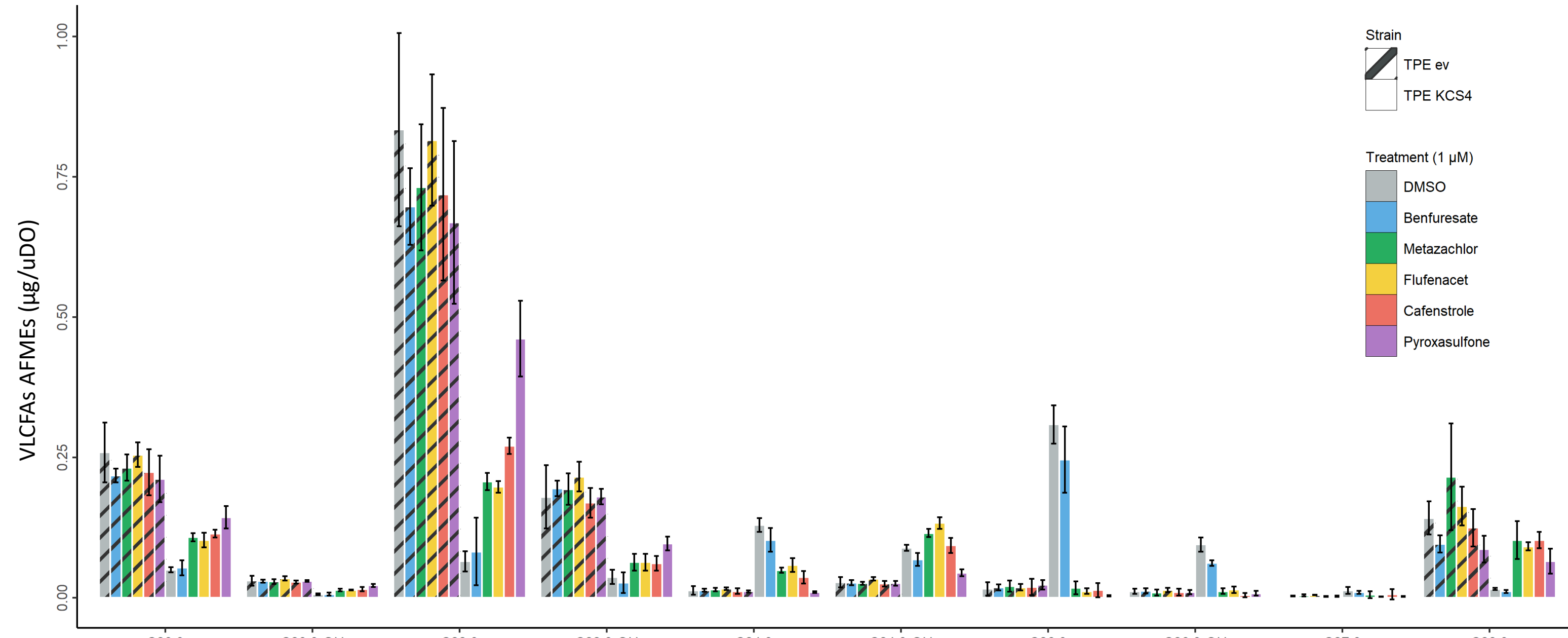
Design of TRIPLE and TRIPLE Δ elo3 yeast strains from wild-type Invsc1 yeast strain

CRISPR Cas9 technology was used to replace the yeast last three enzymes involved in fatty acid elongation (YBR159, PH51 and TCS13) by their *Arabidopsis* orthologues (KCR, HCD/PAS2 and ECR/CER10) and to delete the gene of the elongase ELO3 which elongates C22:0 to C24:0 and C26:0.



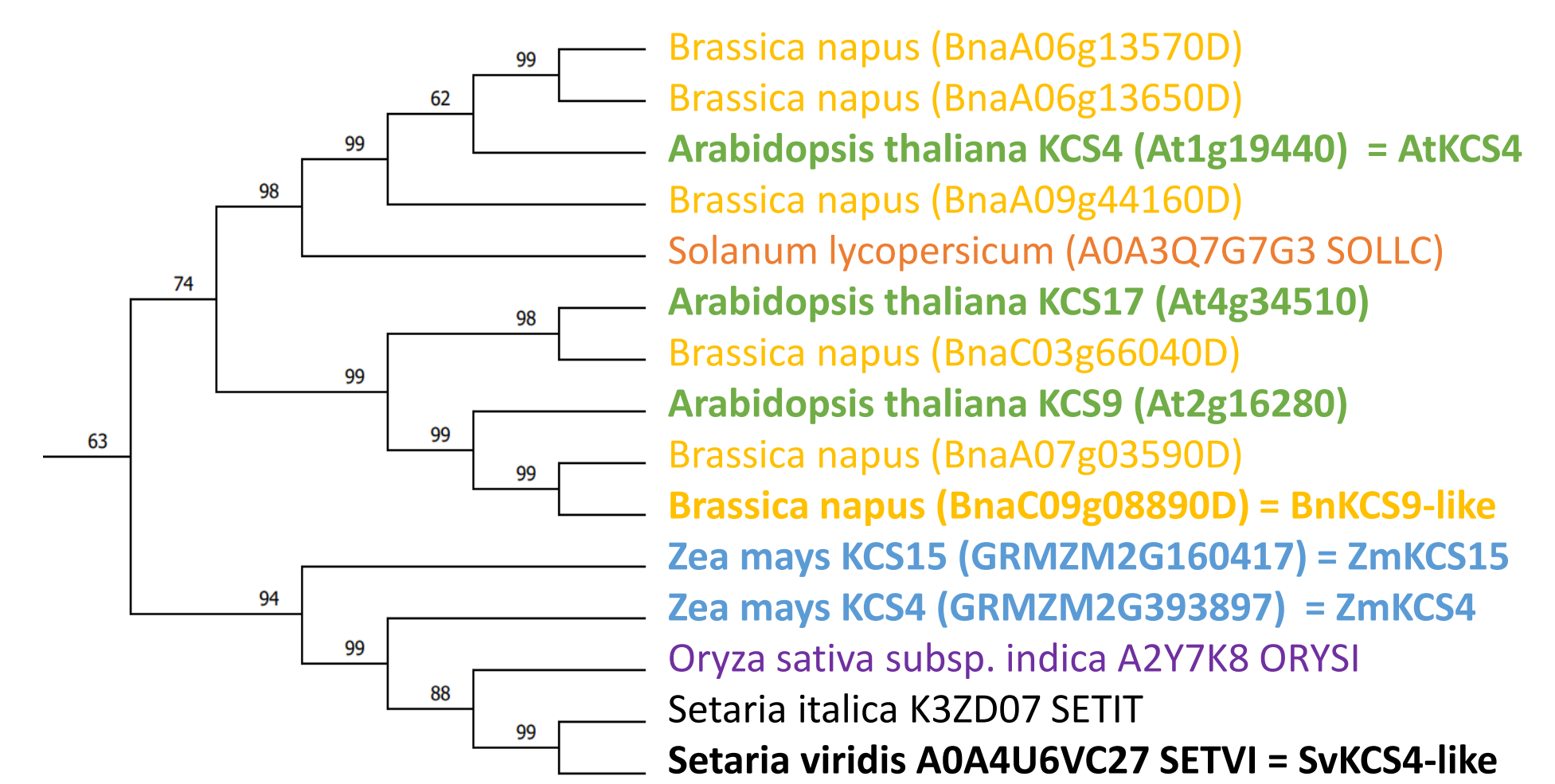
FAMES profiles of Invsc1, TRIPLE and TRIPLE Δ elo3 (TPE) collections treated with different herbicides (10 μ M). Values are the average of five replicates. "DMSO" corresponds to the negative control treatment. Herbicides do not modify the FAMES profiles on Invsc1, TRIPLE and TRIPLE Δ elo3 (TPE) collections. Herbicides do not inhibit the activity of *Arabidopsis* KCR, HCD and ECR enzymes.

AtKCS4 is specifically inhibited by a set of herbicides



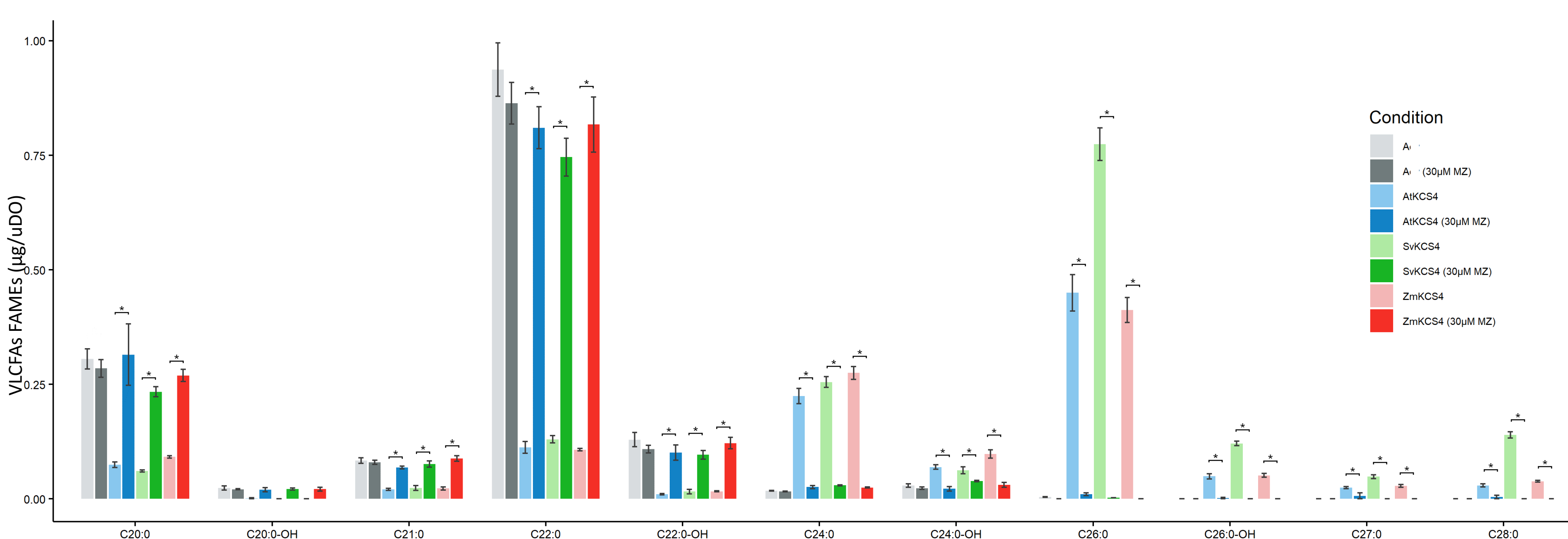
FAMES profiles of the AtKCS4 in TRIPLE Δ elo3 strain treated with different herbicides. Relative quantification based on the internal standard C17:0 (20 μ g/mL). Values are the average of five replicates. "EV" corresponds to the negative control strain containing the empty vector and "DMSO" corresponds to the negative control treatment.

AtKCS4 orthologs expressed in *S. cerevisiae*

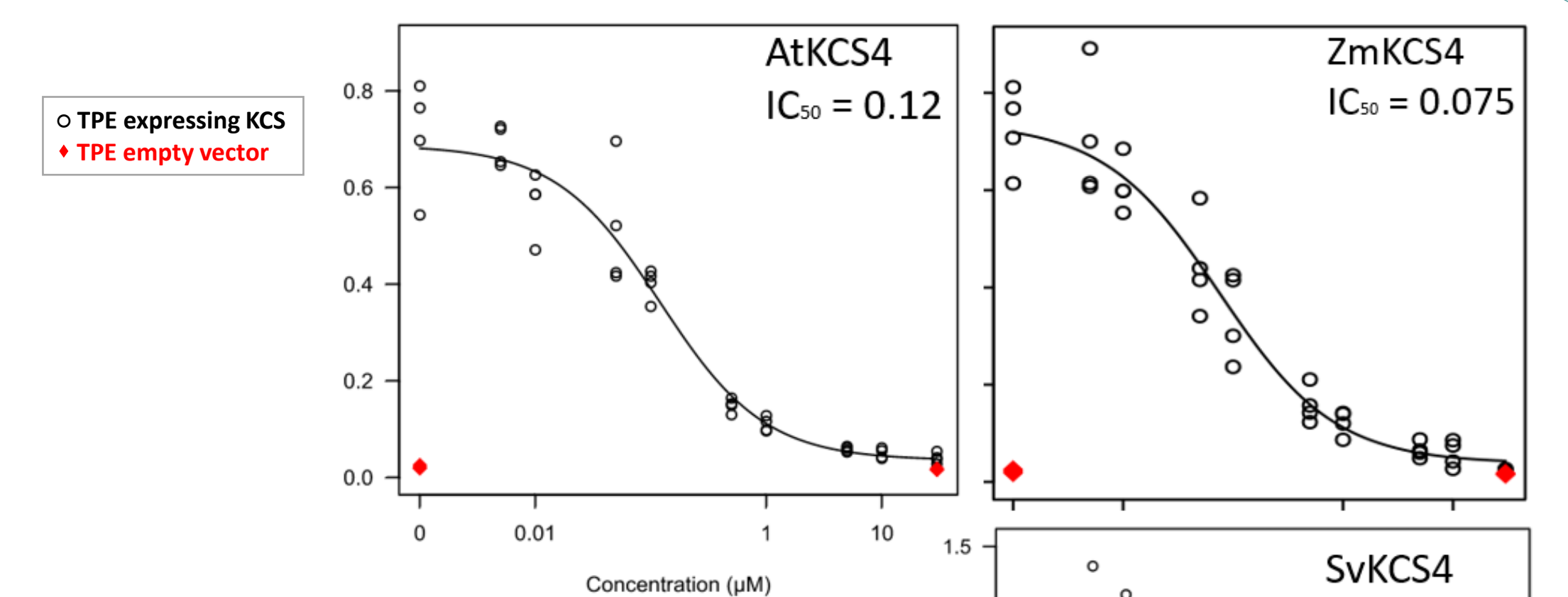


Phylogenetic analysis of the KCS sequences. The protein sequences were compared using the multiple alignment program MUSCLE. The MEGA package was used for the construction of the phylogenetic tree and comparison of parsimony and distance (Neighbor Joining algorithm) methods. The robustness of the tree was assessed by the bootstrap method on 1,000 replicates. Genes in bold are functional in *S. cerevisiae*.

Inhibition by metazachlor of plant KCS4-like expressed in *S. cerevisiae*



FAMES profiles of the AtKCS4 in TRIPLE Δ elo3 strain treated with metazachlor (30 μ M). Relative quantification based on the internal standard C17:0 (20 μ g/mL). Values are the average of five replicates. "EV" corresponds to the negative control strain containing the empty vector.



Dose-response curve generated from the sum of C24:0, C26:0 and C28:0 quantity (μ g/ μ DO) for AtKCS4, ZmKCS4 and SvKCS4-like expressed in TRIPLE Δ elo3 with metazachlor treatment and IC50 calculation. The curve is based on the average of five replicates, the IC50 corresponds to the concentration at which the inhibition is equal to 50%.

IC50 leads to measure the effectiveness of a given compound to inhibit a biological function. No difference in sensitivity between the monocotyledonous and dicotyledonous KCS tested here.

Conclusion

	KCS1	KCS2	KCS4	KCS5	KCS6	KCS9	KCS17	KCS18	KC20
Genetic background	TRIPLE Δ elo3	TRIPLE Δ elo3	TRIPLE Δ elo3	TRIPLE Δ elo3	TRIPLE Δ elo3	TRIPLE Δ elo3	TRIPLE Δ elo3	TRIPLE Δ elo3	TRIPLE Δ elo3
Benfuresate (1 μ M)	+?	-	-	-	-	-	-	-	-
Metazachlor (1 μ M)	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Flufenacet (1 μ M)	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Cafenstrole (1 μ M)	-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Pyroxasulfone (1 μ M)	-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-

	SvKCS4-like	ZmKCS15	ZmKCS4	BnKCS9-like	BnKCS20-like
Genetic background	TRIPLE Δ elo3	TRIPLE Δ elo3	TRIPLE Δ elo3	TRIPLE Δ elo3	TRIPLE Δ elo3
Pyroxasulfone (10 μ M)	+	+	+	+	+
Metazachlor (10 μ M)	+	+	+	+	+

Inhibition pattern of different elongases expressed in *S. cerevisiae* by group K3 herbicides. Results of five replicates. +/- corresponds to partial inhibition, + corresponds to total inhibition, - corresponds to no inhibition

Using two heterologous systems, we were able to determine that the nine active AtKCS enzymes were inhibited by metazachlor, flufenacet, cafenstrole and pyroxasulfone herbicides. Activity of AtKCS was not inhibited by benfuresate. This study also shows that pyroxasulfone and metazachlor specifically inhibited several active KCS enzymes from different plants species (*Zea mays*, *Setaria viridis* and *Brassica Napus*). However the IC50 calculation allowed us to determine that there is no difference in specificity between monocotyledonous and dicotyledonous KCS.



Batsale, M.; Bahammou, D.; Fouillen, L.; Mongrand, S.; Joubes, J.; Domergue, F. Biosynthesis and Functions of Very-Long-Chain Fatty Acids in the Responses of Plants to Abiotic and Biotic Stresses. *Cells* 2021, 10, 1284. <https://doi.org/10.3390/cells10061284>
Trenkamp S, Martin W, Tietjen K. Specific and differential inhibition of very-long-chain fatty acid elongases from *Arabidopsis thaliana* by different herbicides. *Proc Natl Acad Sci U S A*. 2004;101(32):11903-11908. doi:10.1073/pnas.0404600101