Lipases of germinating seeds of oilseed plants, in particular seeds of jojoba – the only plant accumulating wax esters

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The presented study was carried out on three species of oilseed plants: *Simmondsia chinensis* (jojoba), *Crabe abyssinica* (crambe) and *Camelina sativa* (false flax). Jojoba is the only plant known to store wax esters instead of triacylglycerols in its seeds. Crambe and false flax are cultivated oilseeds plants which, due to the relatively small use of their oils in the food industry, were selected in the" Industrial Crops producing added value Oils for Novel chemicals" (ICON) - an international research project partially founded by the European Union - for the potential production of wax esters (after appropriate genetic modifications). The study contributed to the ICON project research.

Jojoba seed wax esters are composed of very long-chain monounsaturated fatty acids and fatty alcohols and account for up to 60% of its seed weight. During the germination of jojoba seeds, the first stage of wax esters mobilisation is catalysed by lipases. Lipases also catalyse the first step in the mobilisation of triacylglycerols during the germination of seeds of other oilseeds plants. To date, none of the genes encoding jojoba lipase(s) have been cloned, and the lipases of germinating jojoba seeds, as well as lipases of germinating seeds of other oilseeds plants, have been relatively poorly characterised. The main aim of this work was to characterise the lipases of germinating seeds of the three oilseeds plants listed above; focusing especially on their specificity towards wax esters.

Microsomal fractions from germinating seeds of the above-mentioned plant species were used in the study. In the first stage of the research, it was shown that the activity of lipases present in microsomal fractions obtained from germinating seeds at various stages of their germination was to some extent correlated with the intensity of mobilisation of storage lipids of these seeds. In addition, studies conducted on germinated jojoba seeds have shown that as germination progresses, the content of oleosins, proteins characteristic of oil bodies, increases in the tested microsomal fractions. Therefore, we have concluded that the lipases of the studied microsomal fractions originate from membrane remnants of degraded oil bodies. The study on the substrate specificity of lipases of germinating seeds of all three species *(S. chinensis, C. abyssinica and C. sativa*) showed that there are no major differences in the ability of these lipases to hydrolyse wax esters. Moreover, lipases of all the tested species display a higher affinity to triacylglycerols than to wax esters (including those from germinating jojoba seeds). Lipases of all three tested species were also able to synthesise wax esters in vitro. In terms of biochemical properties, the lipases of the germinating seeds of the tested oilseeds plants showed both similarities and differences. All of them were most active in the reaction medium with a pH of 7-8. However, differences could be found in their response to a lower or a higher pH. All of them were also activated by Ca2+ ions; however, Mg2+ ions stimulated only the lipase activity of jojoba seeds. Lipases of germinating jojoba and *C. abyssinica* seeds showed the highest activity at a temperature of about 60 °C, while lipases of *C. sativa* seeds reached their maximum activity already at a temperature of 30 °C. Additional differences were noted in their responses to sub- and above-optimal temperatures. During seeds germination of transgenic *C. sativa* plants storing wax esters, it was shown that these wax esters were mobilised, but their mobilisation was slightly delayed compared to the mobilisation of triacylglycerols of those seeds that were mobilised faster and in earlier stages of germination.