ABSTRACT

Acyl-CoA:lysophospholipid acyltransferases (LPLAT) are considered to be the key enzymes involved in the remodeling process. They are responsible for editing of acyl groups in phospholipids and cytoplasmic acyl-CoAs pool. LPLAT enzymes are able to conduct two distinct reactions. In the *forward* reaction these enzymes catalyze the addition of an acyl groups to the lysophospholipids resulting in production of a specific phospholipid. In the *backward* reaction, they are responsible for a reverse process, which may have impact on the supplementation of acyl-CoAs pool with newly synthesized or modified fatty acids. As a consequence, this pool may be a source of substrates for other acyltransferases reactions, for instance synthesis of storage lipids or editing of the membrane lipids. Nevertheless, despite potentially, essential role of LPLAT enzymes in the remodeling process, research on their participation in this mechanism is scarce.

Due to deficit in knowledge in this field, the major aim of my doctoral thesis was to describe the remodeling process occurring in the tissues of oil plant – *Camelina sativa*. The prime objective was to determine the effectiveness of this process in seeds and leaves considering factors such as: seed maturity, plant cultivation conditions and external temperature shocks/fluctuations. Currently, *C. sativa* is becoming the model plant for studying lipid metabolism, yet it has not been fully characterized. Consequently, the first part of my experiments focused on the characterization of biochemical parameters of LPLAT enzymes present in different tissues of this plant. Moreover, the obtained results allowed for a deeper characterization of the LPEAT enzymes. LPEAT encoding genes were cloned, analyzed and inserted into yeast system, in which their preferences and activity were tested.

Primarily, research focused on *C. sativa* seeds. The obtained results showed that LPLAT enzymes are described by different biochemical parameters, but each of them exhibits the highest preference toward acyl-CoAs with 18-carbon unsaturated fatty acids. Analysis of contribution of different classes of LPLAT enzymes in the remodeling process revealed that LPCAT enzymes are able to transfer almost all polyunsaturated fatty acids synthesized in phosphatidylcholine pool to the cytoplasmic acyl-CoA pool. Further experiments provided answers to questions about the involvement of other LPLATs in the acyl editing process in seeds: LPAAT enzymes can transfer fatty acids from phosphatidic acid (PA) to acyl-CoA pool extremely quickly, while remodeling of the phosphatidylethanolamine (PE) pool *via* LPEAT enzymes takes much more time. The efficiency of fatty acids transfer from PA and PE to

cytoplasmic acyl-CoA pool during the whole seed development reached 5% and 2%, respectively, of all the fatty acids present in the lipids of mature seeds.

Conducted research also proved that the biochemical properties and efficiency of the remodeling process depend on the plant cultivation conditions. This part of the research clearly showed that the results from the experiments focusing on the lipid biochemistry conducted on plant derived from *in vitro* condition do not reflect the effects observed for plant cultivated *in vivo.* Therefore, such results cannot be directly applicable in real growth environment.

Last part of the study focused on the impact of different temperatures on the LPLAT enzymes. It led to an extremely significant discovery about physiological role of the LPEAT. These enzymes turned out to act as sensors which respond to temperature changes. The results of these experiments indicate that LPEAT enzymes can modify their substrate preferences and regulate the composition of PE pool, depending on the temperature conditions. Additionally, to confirm the above discovery, genes encoding individual LPEAT isoenzymes have been cloned and their biochemical characteristic and substrate preference in yeast system have been verified. During these experiments it has been also noticed that *Cs*LPEAT1 prefers the utilization of 18:1-LPE, while the *Cs*LPEAT2 prefers 18:2-LPE as acyl acceptor. Moreover, only the tested isoenzymes of *Cs*LPEAT2 were able to utilize very long chain acyl donors. The cloned sequences were also used for comparative and phylogenetic analysis. Phylogenetic analyses revealed that *CsLPEAT1c* and *CsLPEAT2c* originated from *Camelina hispida*, whereas other variants of *CsLPEAT* originated from *Camelina neglecta*. The expression analysis showed that genes encoding isoenzymes of *Cs*LPEAT1 are mainly expressed in seeds, while encoding CsLPEAT2b are predominantly expresses in leaves, what judging by the substrate specificity of these isoenzymes, may correlate with the different functions of these organs.