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IDENTIFICATION OF A LIPASE INVOLVED IN JASMONIC ACID BIOSYNTHESIS IN *NICOTIANA ATTENUATA*

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Jasmonic acid (JA) is a multifunctional growth regulator widely distributed in the plant kingdom that modulates anther dehiscence, fruit ripening, root growth, tendril coiling, and plant resistance to insects and pathogens. The first step of JA biosynthesis is catalyzed by a lipase that hydrolyzes plastidial membrane lipids releasing free fatty acids. The identification of two Arabidopsis plastidial phospholipases, DAD1 and DGL provided genetic evidence that this is a critical step in the activation of JA biosynthesis.

We identified three putative functional homologues of DAD1 and DGL in *N. attenuata*: PLA1, PLA2 and PLA3 that were functionally characterized by Virus Induced Gene Silencing (VIGS) to investigate their involvement in JA biosynthesis.

After elicitation by wound and fatty acids coniugates (FACs), leaves of PLA2 and PLA3 VIGS-silenced plants accumulated similar amounts of JA and JA-Isoleucine (JA-Ile) compared to control plants. In contrast, PLA1 silenced plants showed significant reductions of >80% in JA and JA- levels.

The full-length *PLA1* cDNA was obtained by 3' and 5' rapid amplification of cDNA ends (RACE). The sequence analysis revealed the presence of conserved lipase-3 domain with a catalytic triad consisting of glutamate-histidine-serine and of a putative signal peptide for plastid targeting.

To assess whether PLA1 encodes for an active glycerolipid acyl-hydrolase, we expressed PLA1 as a recombinant protein in bacteria and analyzed its activity towards different glycerolipid substrates. The enzyme hydrolyzed acyl groups of phospholipid (PC), galactolipid (MGDG) and triacylglycerol (triolein) with similar rates that ranged from ~1,5 to ~2 μg h-1 μg protein-1.

The subcellular localization of PLA1 was assessed by fusing the gene to EGFP by its C-terminus and cloned under regulation of the CaMV35S promoter. As controls, EGFP and a fusion EGFP protein carrying the first 273 amino acids containing the predicted plastid transit peptide of Lipoxygenase3 (LOX3) were used. *N. attenuata* leaves were infiltrated with Agrobacteria carrying the corresponding constructs and EGFP expression in mesophyll cells was analyzed by laser confocal microscopy. Protoplasts transformed with PLA1-EGFP and with LOX3-EGFP showed co-localization of a GFP green fluorescence and chlorophyll red autofluorescence, consistent with the predicted plastidial localization of PLA1, in contrast with protoplasts transformed with EGFP that showed a diffused green fluorescence characteristic of cytosolic localization that did not overlap with chlorophyll red autofluorescence.

These results indicate that the PLA1 protein is a plastidial lipase that catalyzes the release of fatty acids from chloroplast membranes and supplies linolenic acid (18:3) for *de novo* JA production in *N. attenuata* leaves.