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CHARACTERISATION OF AN ALMOND 9-HYDROPEROXIDE LYASE TARGETED TO LIPID BODIES

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Oxylipin metabolism represents one of many defence mechanisms employed by plants. The products of the pathway are biologically active both in defence signalling and as direct anti-microbial agents. Some of them are also significant in determining the organoleptic characteristics of food, through their flavour and aroma properties.

Most of the phyto-oxylipins so far identified are synthesised via the lipoxygenase (LOX) pathway. LOXs catalyse the hydroperoxidation of polyunsaturated fatty acids such as linoleic (C18:2) or linolenic acid (C18:3) and produce 9- or 13- hydroperoxides. 9- and 13-hydroperoxides are very reactive compounds and are further metabolised to an array of different oxylipins by the action of the other enzymes downstream located in the pathway, including hydroperoxide lyase (HPL).

HPL converts fatty acid hydroperoxides into aldehydes and oxoacids; in the case of 13-hydroperoxides and 13-HPL the products are 6-carbon aldehydes that are believed to have a signalling function and also play a direct role in plant defence. At present it is unknown if the products of 9-HPL can function as signal molecules and induce the expression of other genes.

Although the subcellular location of the enzymes of the early part of the pathway is well established, there is relatively little information on the subcellular distribution of HPL. In order to investigate on the subcellular localization of HPL, we used an almond HPL cDNA, previously isolated and identified as a 9-HPL, and prepared a set of green fluorescent protein (GFP)-tagged HPL fusions used to transform tobacco protoplasts. Confocal laser scanning microscopy analysis revealed that the almond HPL is targeted to the endomembrane system and to spherical bodies that were selectively stained with Nile Blue A thus indicating that they are strictly related to oil bodies.

This localization has been compared with a GFP based marker which localizes in oil bodies (oleosin-GFP) and with other markers targeted to other compartments, directly associated to endoplasmic reticulum, but independent from oil bodies such as neutral vacuoles (GFP-Chi).