

The Hydroperoxide Lyase Branch of the Oxylipin Pathway and Green Leaf Volatiles in Plant/Insect Interaction

Marco Taurino, Stefania De Domenico, Stefania Bonsegna and Angelo Santino*

Institute of Sciences of Food Production C.N.R. Unit of Lecce, via Monteroni, 73100, Lecce, Italy

Abstract

The lipoxygenase (LOX) catalysed peroxidation of polyunsaturated fatty acids (PUFA) is a key starting point in defence mechanisms common to plant, animal and at least some microorganisms. In human, the peroxidation of arachidonic acid finally leads to the biosynthesis of important defence effectors such as leukotrienes and lipoxins. In plant, the most common substrates of lipoxygenases are represented by linoleic (C18:2) and linolenic (C18:3) acids. Differently from auto-oxidation reactions which produce a huge number of hydroperoxide derivatives, the oxidation reaction catalysed by LOXs is positional and stereo-specific. Indeed, only 9- or 13-hydroperoxides (with a prevalence of the S stereoisomers) are produced from these substrates. PUFA hydroperoxides are *per se* signal molecules or can be used as substrates for a number of enzymatic reactions carried out by other enzymes of the LOX pathway. At the end, an array of volatile and non volatile compounds, collectively known as phyto-oxylipins are produced upon specific stress signals, by the contribution of a multitude of enzymes localised in different subcellular compartments. Thanks to the excellent work of several groups of scientists around the world, our knowledge on the contribution of the oxylipin pathway on plant defence mechanisms dramatically increased in recent years.

In the present contribution, we'll focus our attention on the hydroperoxide lyase branch of the plant oxylipins pathway, responsible for the synthesis of volatile aldehydes, alcohols and other related compounds which are important constituents of fruit aromas and the green leaf volatiles.

Keywords: Green leaf volatiles; Hydroperoxide lyase; Oxylipins; Volatile aldehydes

The CYP74 Cytochrome P450 Family

CYP74 enzymes differ from other P450 enzymes, in that they have an atypical reaction mechanism that requires neither oxygen nor an NADPH-reductase, and as a consequence have extraordinarily high turnover numbers. Hydroperoxide lyase (HPL) is a member of this CYP74 sub-family and has an important role either in oxylipin metabolism and plant defence. The enzyme cleaves hydroperoxides, formed from the oxygenation of polyunsaturated fatty acids by the action of lipoxygenase (LOX), into an array of volatile and non-volatile products that have direct and indirect defence properties. HPL has similar substrate specificity to other classes of CYP74 enzymes, allene oxide synthase (AOS) and divinyl ether synthase (DES). Unlike HPL, which cleaves hydroperoxides, AOS transforms them into allene oxides, which are finally converted into jasmonic acid; whereas DES converts them into divinyl ethers, showing antifungal properties (Figure 1).

Plant HPLs are Involved in Stress Induced Biosynthesis of Volatile Aldehydes

Hydroperoxide lyases (HPL) catalyse the cleavage of hydroperoxides into short chain aldehydes and α -oxo fatty acids (Figure 1). Some HPLs show a strict specificity for 13-hydroperoxides with consequent production of (Z)-3-hexenal and 12-oxo-(Z)-9-dodecenoic acid starting from 13-hydroperoxy-derivatives of linolenic acids [1,2].

Aldehydes produced by HPL can be further converted to the corresponding alcohols by alcohol dehydrogenase, acetylated by acetyltransferase or isomerised with the consequent production of other volatile compounds i.e. (Z)-3-hexenol, (E)-2-hexenal and (Z)-3-hexenyl acetate. All these volatile compounds are important constituents of the green leaf volatiles (GLV) and are rapidly released by plant in response to mechanical damage or herbivores attack [1,2].

Together with 13-HPL, 9/13-HPLs have been reported. They were initially thought to be restricted to the *Cucurbitaceae* family, even though their occurrence in other plant species such as *Medicago* spp., rice, almond and grape have been later reported [3-7]. 9/13-HPL is able to use both 9- and 13-hydroperoxides. Starting from 9-hydroperoxy-derivatives of linolenic acids, (E,Z)-2,6-nonadienal and C9-oxo-acids are synthesised. Differently from 13-HPL, whose expression is restricted to green tissues, 9/13 are also expressed in underground tissues as in the case of rice HPL (OsHPL1) and the *Medicago truncatula* 9/13-HPL (*MtHPLF*) [4,6].

The phylogenetic analysis carried out on a number of plant HPLs (Figure 2) clearly confirms the presence of these two distinct groups inside plant HPLs.

Endocellular Localisation of HPLs

Since CYP74 enzymes compete for the same substrates, their endocellular localisation is an important control point in the biosynthesis of oxylipins.

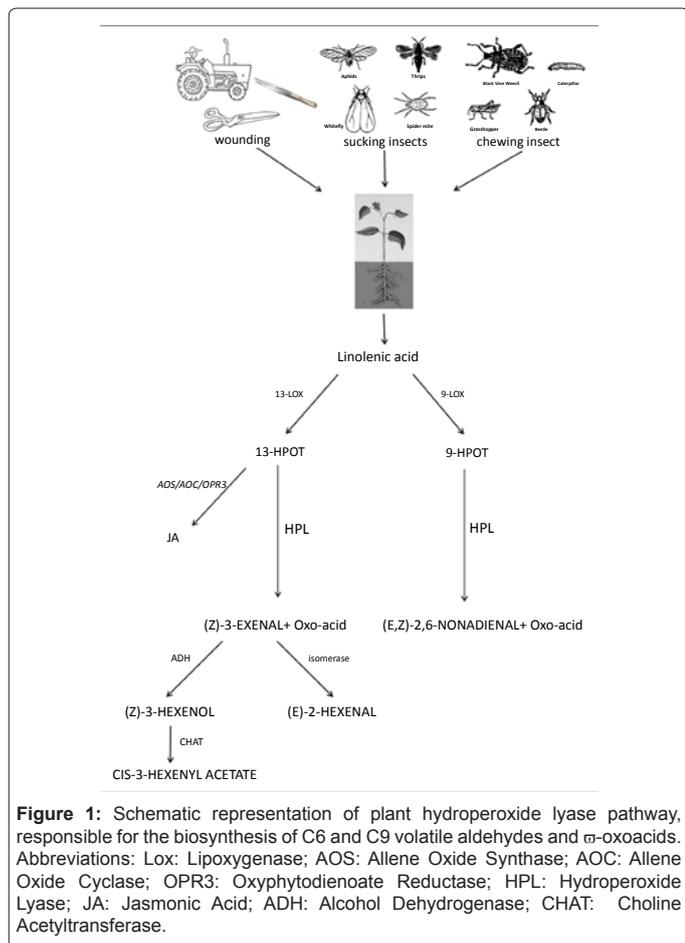
A plastidial localisation was clearly demonstrated for HPLs and

*Corresponding author: Angelo Santino, Institute of Sciences of Food Production, C.N.R. Section of Lecce, via Monteroni, 73100, Italy, Tel: +390832422606; Fax: +390832422620; E-mail: angelo.santino@ispa.cnr.it

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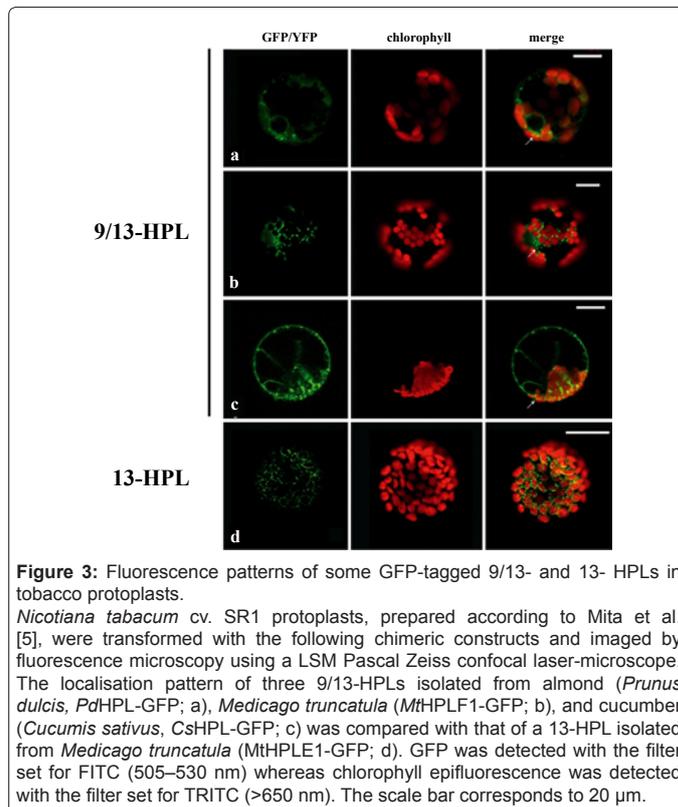
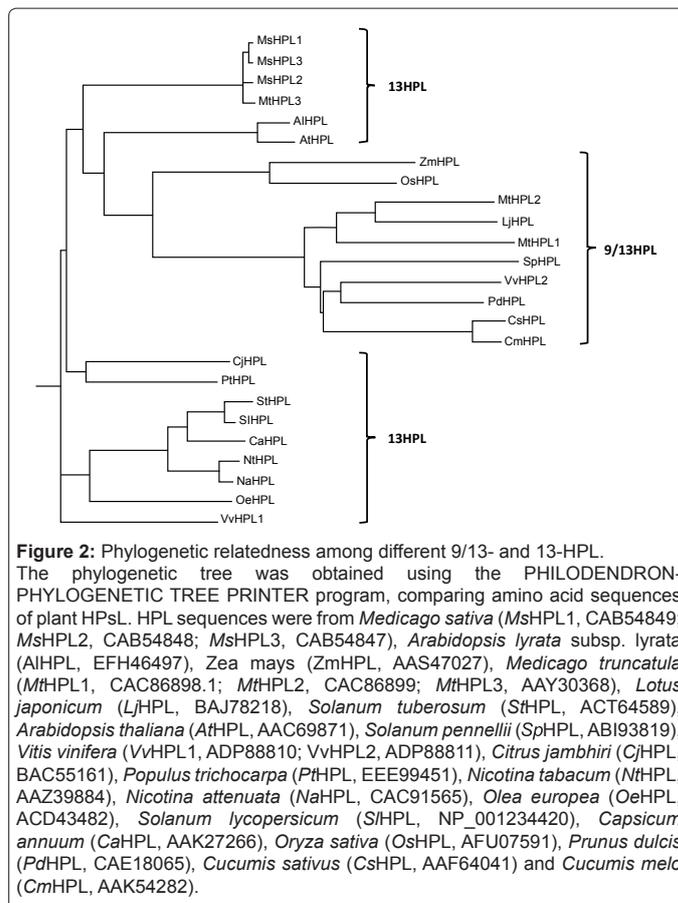
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AOSs showing a strict specificity towards 13-hydroperoxides. As far as the members of CYP74C and D subfamilies, which do not show strict substrate specificity, their localisation is not currently completely elucidated. Some 9/13 HPLs, tagged with the fluorescent marker GFP, were visualised by confocal analysis (Figure 3), after a transient expression in tobacco protoplasts. When compared with that of *M. truncatula* 13-HPL which shows a clear chloroplast localisation, a different localisation for 9/13-HPLs appears evident. Indeed, 9/13-HPLs showed a prevalent microsomal or cytosolic distribution [5,6]. Notably, these enzymes appeared also to be specifically associated with punctuate spots (indicated by arrows, in figure 3) corresponding to lipid droplets. Biochemical analyses reported in previous works [5,6] confirmed confocal microscopy analyses. We hypothesised that such association with lipid droplets could result in the protein conformational changes required for full activation of these enzymes [1,6].

Physiological Roles of Oxylipins Produced via the HPL Branch

The products of HPL catalysis are important constituents of green leaf volatiles (GLV). Their levels are very low in intact plants but increase rapidly after wounding or insect feeding. GLV function as stress signal in the same and in neighbouring plants [8-12], triggering the expression of defence genes [13-15]. Recently, Zhang et al. showed that exposing *A. thaliana* plants to Lima bean volatiles induced considerable transcriptomic changes [12]. Results indicated that either the ethylene or jasmonate signaling pathways were up-regulated (the latter with a weaker response) whereas salicylic acid pathway did not.



In comparison to pathogens, insects are highly complex organisms showing complex and different behaviours. Plants have evolved specific mechanisms to sense insect pressure, walking and eating. A number of reports showed that mechanostimulation of plant tissues trigger the expression of defence genes of the jasmonate pathway. A second layer of perception occurs when insects disrupt plant tissues integrity. Wounding is *per se* another important signal able to elicit defence responses, leading to the so called wound-induced resistance. Finally, plants are also able to detect herbivores more specifically by the recognition of specific compounds released during feeding. Indeed, a number of studies have shown that insect oral secretions are able to potentiate wound induced response [16,17]. Therefore, the emerging scenario indicates that wounding, direct feeding or volatiles released by injured plants elicit different stress signals. A recent report showed that either generalist or specialist caterpillars were able to greatly reduce the levels of two main HPL products, i.e. (Z)-3-hexenol and (Z)-3-hexenyl acetate, but increased the levels of jasmonate in *A. thaliana* plants [18]. By converse, either mechanical wounding or aphids feeding elicited the same compounds. Notably, the expression levels of jasmonate biosynthesis genes were up-regulated by insect feeding which resulted in a burst of jasmonate [18]. The authors hypothesised that these changes in signaling dynamics might be due to insects induced transcriptional modifications of critical genes in the HPL branch.

Together with GLV, which can be considered a blend of different volatile compounds, single volatile compounds, i.e. (Z)-3-hexenyl acetate or nonanal, can also elicit defence responses in neighbouring plants [19-21].

GLV primed plants are able to respond more rapidly or more effectively to a subsequent attack [15,22,23]. With this strategy, plants avoid extensive reprogramming and biochemical investments, which can affect both plant physiology and agronomic performances. A recent report showed that the white-backed planthopper (*Sogatella furcifera*) but not the brown planthopper (*Nilaparvata lugens*) was able to induce resistance to bacterial blight in rice [24]. In this case, resistance was mediated by a specific HPL isoform encoded by *OsHPL2*. Over-expression of *OsHPL2* in rice plants resulted in higher resistance to bacterial blight. Furthermore, rice plant exposed to (E)-2-hexenal were more resistant to the same pathogen [24]. A direct antifungal and insecticide effects of volatile aldehydes was also reported in previous works [5,25].

Furthermore, signalling mediated by GLV is faster than vascular system and reach other parts of the plant lacking vascular connections with the damaged tissue or located close to it but on different branches [22,23].

Finally, GLV have been shown to have an important role in tritrophic interactions. HPL over-expressing *Arabidopsis* plants, showing increased levels of GLV, were more attractive to the parasitic wasp *Cotesia glomerata*, leading to higher mortality of herbivores, as in the case of larvae of the cabbage white butterfly, *Pieris rapae*. The same plants showed a higher resistance to the gray mold *Bortrytis cinerea*. On the contrary, antisense suppression of HPL, resulted in lower levels of GLV, lower attraction of parasitoid and higher susceptibility to pathogen [26]. Wei et al. demonstrated that plants use (Z)-3-hexenol, a universally induced GLV, to attract *Opius dissitus* wasps. This compound helps wasps to locate their preys, as the pea leafminer *Liriomyza huidobrensis* [27].

Concluding Remarks

Plants utilise hormones and hormone-like compounds as signals to communicate their stress status from local damaged tissues to distal organs within the same plant and to other neighbouring plants. Some of these signalling molecules, as jasmonates, can be systemically translocated, others, as GLV or methyl jasmonate function as airborne signals. The jasmonate signaling cascade is considered a key regulator of plant defence response to arthropods, herbivores and pathogens. However, it's becoming evident the existence of a complex tuning among different signaling cascades interacting each others on the basis of specific stress signals released by plant and host. The integration of signals from different signaling pathway has the important role to integrate the initial perception (insect walking/feeding) with later events (release of specific insect elicitors) and translate them into an appropriate defence response.

The use of different, tightly controlled signals and different diffusion systems permit the rapid transmission of the stress signal to all the tissues and the reduction of inappropriate expression of costly systemic defences. Recent reports showed the ecological significance of GLV emitted by injured plants on intact neighbouring plants and the impact of primed defence on their fitness and agronomic performances.

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