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Chapter- 11

Green Leaf Volatiles: A Crucial Mediator of Plant

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Abstract:

Plants have evolved different defense strategies to counter biotic and abiotic threats coming from their surroundings. The role of different emitted volatile organic compounds (VOCs) in plant defense has been the field of active research in the last decades. Green leaf volatiles (GLVs), emitted from the vegetative parts of the plant body have appeared as the utmost crucial mediator in defense and plant-to-plant communications. GLVs are formed through the oxidation of polyunsaturated fatty acids (PUFAs) by the action of lipoxygenase (LOX) enzyme. The plasma membrane has been the source of all PUFAs in GLV biosynthesis. The enzyme hydroperoxide lyase (HPL), performs a crucial role in GLV formations by producing different volatile aldehydes. Upon herbivory, plants are found to release more amount of GLVs which can able to elicit the expression of different defense-related genes, and thus indirect defense against the herbivory can be achieved. Emitted GLVs can induce the defense mechanism in neighbouring plants by priming method. GLVs also showed antagonistic effects on invading phytopathogens, especially against the invading fungi. Despite its tremendous potential as a defense mediator the molecular mechanisms of GLV uptake and perception in plants have not been well understood.

Introduction:

Plants are known to emit a myriad of volatile compounds in their surroundings from both above and below-ground parts of their plant body. Among these volatile compounds, floral volatiles are the most common and well-studied due to fragrance and flavour. Chemical nature of floral volatiles, their emission pattern, and the regulation of emission have been investigated thoroughly in several plant species in the last decade. Recently, emitted volatiles from other vegetative organs including below-ground root systems have attracted considerable attention of researchers around the world for their tremendous effect on plant life. Green leaf volatiles (GLVs), derived by the oxidation of polyunsaturated fatty acids (PUFAs) from the vegetative parts of the plant body, perform a crucial role in plant communication, protection, and stress management. GLVs are made up of C6 and C9 aldehydes, alcohols, and their esters (Baldwin et al., 2006; Dudareva et al., 2006) and are found to be released from almost all plant species. Plants emit GLVs due to mechanical damage, pathogen attack, and in abiotic stress such as heat, high light, and heavy metal stress (Ameye et al., 2018).

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Polyunsaturated fatty acids, the precursors of GLVs are generally released from the plasma membrane by the action of specific lipases whose identities have not been established till now. Released polyunsaturated fatty acids are oxygenated by the enzyme lipoxygenase (LOX) to form fatty acid hydroperoxides, acting as substrates for the enzyme hydroperoxide lyase (HPL). Natural flavouring agents and odorants are in use worldwide on a massive scale in different products due to the consumer's demand, especially for their health issues. GLVs are performing appropriately as valuable natural flavouring agents for different products throughout the world. GLVs are produced on a large scale for commercial purposes through biotechnological intervention by isolating different genes, crucial for GLVs biosynthesis from different plant species (ul Hassan et al., 2015).

Biosynthesis:

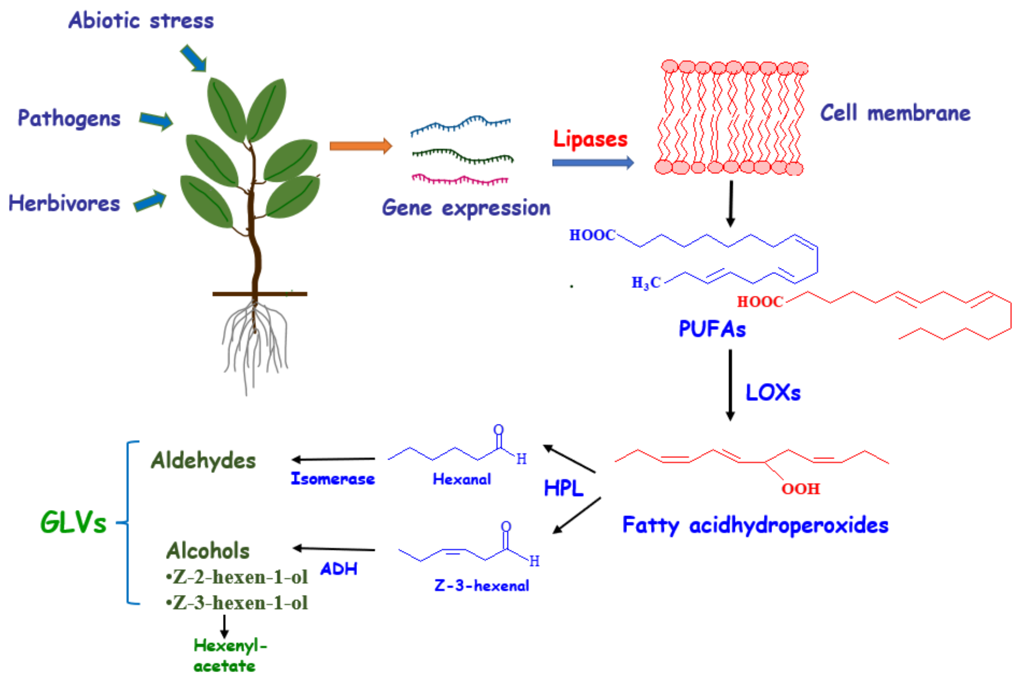

Figure 1. Biosynthesis of green leaf volatiles (GLVs) in plants (ul Hassan et al., 2015). PUFAs- polyunsaturated fatty acids, LOXs- lipoxygenases, HPL- hydroperoxide lyase, ADH- alcohol dehydrogenase.

Biosynthesis of GLVs is started by the formation of PUFAs, such as linoleic acids (LA) and α-linolenic acid (ALA), which have been produced from plasma membrane by specific lipases. The identities of specific lipases for GLVs formation are still not well established. So far, two phospholipase D (*OsPLDα4* and *OsPLDα5*) from rice have been found, related to GLVs formation. Antisense expressions of *OsPLDα4* and *OsPLDα5* in rice have significantly reduced the emission of GLVs along with reduced formation LA and Jasmonic acid (Qi et al., 2011). Released PUFAs are subjected to an enzymatic reaction, catalyzed by a monomeric, nonheme iron-containing enzyme family known as lipoxygenases (LOXs). Members of this enzyme family catalyze the addition of molecular oxygen into PUFAs to form fatty acid hydroperoxides. Several LOX genes have been reported from different plant species (Ameye et al., 2018) and are mostly found to be localized in chloroplast (Bannenberg et al., 2009; Porta et al., 2008). Plant LOXs can be distributed into two broad groups: Non-plastidic 9-LOX, categorized as type-1; Chloroplast localized 13-LOX, designated as type-2. Formed hydroperoxides further undergo an isomerization process, catalyzed by a crucial enzyme of this pathway, hydroperoxide lyase (HPL). Due to the action of HPL, these hydroperoxides are converted into hemiacetals which are subsequently decomposed to form 6 to 9 carbon-containing volatile aldehydes such as hexanal, (Z)-3-Hexenal, (Z)-3-Nonenal, (E)-2-Nonenal. These aldehydes are converted to alcohol by NAD-containing dehydrogenase (ADH) to confer more stability. All the synthesized aldehydes, their corresponding alcohols, and esters from leaf and vegetative tissues collectively constituted GLVs.

Biological Functions:

To survive in nature, the plant has to deploy its different phyto-artillery systems to prevent different intruders on multiple fronts. Plants are well equipped with multiple defense machinery to neutralize those threats, coming from both biotic and abiotic factors. Jasmonic acid (JA), a product of oxylipin pathway is an extensively investigated phytohormone that confers defense against pathogens, herbivores, and phloem-feeding insects in association with the salicylic acid and ethylene. Green leaf volatiles (GLVs), synthesized from PUFAs through the enzymatic action of HPL have recently been included in the plant defense system and are less understood than jasmonic acids. GLVs serve as defense signals within the same plant as well as in neighbouring plants within the same community.

GLVs in herbivore defense and plant priming:

Herbivorous insects utilize plants for their food and oviposition. Upon herbivory, plants face damage in their tissues and recruit different types of toxins and repellents as direct defense strategies. During herbivory, plants also deploy their indirect defense strategies by releasing GLVs along with other compounds such as nectar to attract the predators of herbivores. Interestingly, the emission of GLVs activates the expressions of several crucial genes related to the indirect defense mechanisms of plants. Z-3-Hexenol, one of the most common GLV has been found to upregulate the expression of defense-related genes in maize (ul Hassan et al., 2015) and therefore perform as a more potent elicitor of defense as compared to jasmonate, salicylate and ethylene (Engelberth et al., 2013). Genes related to the octadecanoid pathway for jasmonic acid formation are found to up-regulate by GLVs (Engelberth et al., 2007). Oral secretions (OSs) of herbivorous insects along with the wounding are found to perform a crucial role in the induced release of GLVs in attacked plants. Maize seedlings treated with two lepidopteran species with wounding increased the emission of Z-3-hexenyl acetate (Yan and Wang, 2006). Application of oral secretions (OSs) of larvae of *Manduca sexta* along with the wounds in *Nicotiana attenuate* leaves, released more amount of different Z-3-hexenyl esters as compared to only wounded leaves (Gaquerel et al., 2009). The verb “to prime” is generally used to describe a situation where someone is already prepared to counter the coming events. In plant “to be primed” means it has already activated its inducible defense mechanism against future threats coming from different biotic factors. Several synthetic and natural chemicals such as salicylic acid, *β*-aminobutyric acid, and benzothiadiazole have been demonstrated for their plant priming properties. Recently, role of GLVs in herbivory induced indirect defense through priming has been demonstrated in several plants. In lima bean plants, herbivore attacks release some volatile compounds that can induce the synthesis of extra-floral nectar in surrounding healthy plants to attract the enemy arthropod to confer the indirect defense against pests (Heil and Kost, 2006). It has also been found that *Spodoptera littoralis* showed a lower growth rate in maize plants, primed by GLVs. Volatile compounds, emitted from these primed maize plants were attracting parasitic wasp *Cotesia marginiventris* to confer indirect defense (Ton et al., 2007).

GLVs against pathogen:

Antagonistic effects of oxylipins on several phyto pathogens have been evident in several recent studies (Prost et al., 2005). Several common oxylipins were tested for their efficacy against different microbes and found to be very efficient against eukaryotic pathogens (Prost et al., 2005). In the naturally growing population of *Phaseolus lunatus*, nonanal was found to be the key compound that can activate the PATHOGENESIS RELATED PROTEIN 2 (PR-2) gene for defense purposes (Yi et al., 2009). *Arabidopsis* plants harbouring the HPL gene showed increased resistance against the fungal pathogen *Botrytis cinerea* due to increased accumulation of C6 aldehyde. However, the reverse situation was evident in HPL suppressed *Arabidopsis* plants (Kishimoto et al., 2008). Tomato plant expressing tea HPL gene showed a significant increase in resistance against *Alternaria alternata* f. sp. Lycopersici (Xin et al., 2014). Fungal infections increase GLVs formation in plants but the exact reason behind this phenomenon is unclear and a subject of severe investigations for the last few years. Plants may be producing more amount of GLVs due to fungal invasion or the pathogen itself promoting GLVs formation to increase their virulence or GLVs are producing for the cellular damage caused by invading pathogens. Invading pathogens generally release some effector molecules (toxins) to suppress the plant defense, which imparts oxidative stress on plant cells. This stress leads to membrane disruption and the release of fatty acid from the membrane. These free fatty acids are the main precursor for GLVs formation. It has been found that invading *Fusarium graminearum* produced effector molecule lipase FGL1, which increased the formation of free fatty acids and therefore, increased the substrate for GLVs biosynthesis (Blümke et al., 2014). Fungal infection interferes in the plant hormonal system related to defense and therefore, alters the GLVs and JA formation.

Signal perception and transduction of GLVs in plants:

The role of GLVs in plant defense against herbivory and pathogens has been well documented in several reports. The efficacy of priming of GLVs has already been established by several investigations. However, the mechanisms of perception of GLV signals by plants and the subsequent transduction to the plant cells have not been clearly understood. The perception of volatile compounds through receptor proteins in insects and the subsequent formulation of these olfactory signals has been deciphered at molecular and cellular levels. In plants, receptors related to volatile signal perception except for ethylene have not been well understood (Schaller and Bleecker, 1995). Plant volatiles have mostly been taken up through stomatal pores or by the adsorption process on the leaf surface (Matsui, 2016; Wang and Erb, 2022). GLVs reach the plant membrane by stomata or adsorption process but the mechanism of crossing the cuticle and cell wall layer is still unclear. According to Heil (2014) due to the lipophilic nature of GLVs, they can easily be dissolved into the plasma membrane and reach the cytosol of the plant cell. In the cytosol, GLVs can be metabolized through glutathionylation and glycosylation. The glutathionylation of E-2-Hexenal and glycosylation of Z-3-Hexenol have been reported in plants (Davoine et al., 2006; Sugimoto et al., 2014). GLVs have also been found to depolarize the membrane potential along with the increase in cytosolic Ca^{2+} concentration in different studies (Zebelo et al., 2012). Released Ca^{2+} in the cytosol may act as a crucial secondary messenger to activate different genes related to defense and other physiological processes. Transcriptional activation of *WRKY 40* and *6*, transcription factors crucial for abiotic stress management and defense in plants have been evident due to exposure to GLVs in *Arabidopsis* (Mirabella et al., 2015). Apart from all, GLV-specific receptors have not been identified and many downstream molecular players related to signaling have not been elucidated. Therefore, severe investigations are needed to elucidate the molecular mechanisms of GLVs perceptions and signal transductions in plants.

Conclusions:

The direct and indirect defense mechanisms of plants have been studied extensively and elucidated in the last few decades. The role of volatile jasmonate and salicylate, as crucial players in plant defense has also been established. Recently, GLVs have emerged as another key mediator of plant defense responses. Several experimental outcomes proved its tremendous potential as a defense molecule against herbivores and pathogens infestations. Plants exchange different communication signals among themselves utilizing several volatile molecules to survive and sustain in nature. Plants are found to emit GLVs as alarm signals to their neighbour when are being attacked by different biotic threats. GLVs are also found to develop immunity in different plants through the priming process. However, the molecular mechanisms of GLVs perception and its transduction in plants are not clearly understood. Advanced biochemical strategies and genetic engineering tools may help us to decipher this process which will help us to induce immunity in the agriculturally important crops. Thus, plants will be able to produce their pesticides, antimicrobial compounds, and herbicidal molecules mainly through GLVs. These practices will ultimately reduce the use of toxic effects of pesticides, and the cost of agriculture.

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