Introduction

Jasmonic acid biosynthesis is initiated with the oxidation of polyunsaturated fatty acids like linoleic acid, linolenic acid or arachidonic acid. The oxidation products of polyunsaturated fatty acids are commonly known as oxylipins. Oxylipins are synthesized enzymatically in plants through initial oxidation by lipoxygenases (LOXs) or \( \alpha \)-dioxygenases (\( \alpha \)-DOXs); on the other hand, non-enzymatic autoxidation of polyunsaturated fatty acids (PUFA) also involve in the oxylipin formation in plants (Göbel and Feussner, 2009). Oxylipins represent a highly different group of compounds that are involved in a number of developmental processes and diverse stress responses in plants (Anderson et al., 2006).

These substances are known for their protective activities either as signalling molecules in plants during development, wounding, and insect and pathogen attack, or as direct anti-microbial substance that are toxic to the invader. Role of vast majority of plants oxylipins was not clear. Well-studied plant oxylipins are jasmonates (JAs) including jasmonic acid (JA) and its derivatives such as methyl jasmonate (MeJA), \textit{cis} jasmone jasmonoyl isoleucine (JA-Ile), jasmonoyl ACC (JA-ACC) and several other metabolites.

Second group of plant oxylipins is green leaf volatiles (GLV). GLVs have function in defence responses against herbivores. GLVs trigger local and systemic volatile organic compounds (VOC) emissions upon insect feeding (Farag and Paré, 2002). GLVs are C6 aldehydes, alcohols, and their esters formed through the hydroperoxide lyase (HPL) pathway downstream of LOXs. A number of VOC including monoterpenes, sesquiterpenes and carotenoid-type compounds can be synthesized from plants by the shikimic, lipidic and terpenic pathways (Fons et al., 2010). Most VOCs are not products of the LOX pathway but similar to LOX derivatives serve as signals for insects to choose a suitable host or to lay eggs (Müller 2001). The third group of plant oxylipins is phytoprostanes, which is enzymatically synthesize during lipid Metabolism, which play important roles with OPDA in plant stress responses (Eckardt, 2008).
As lipoxygenase is the first enzyme of the JA and other oxylipins pathway, the role of this enzyme for different product formation has interesting phenomenon to understand how the different pathways can be operated simultaneously.

Lipoxygenase are widely distributed in plants, animals and in fungi (Brash, 1996). The first X-ray structure of a lipoxygenase (Boington et al, 1993) and the structure of the catalytic site (Minor W,1993) of the same lipoxygenase were reported in 1993. Lipoxygenases (LOXs) are non-heme, non-sulfur, iron-containing enzymes that catalyze the stereo- and regio-specific addition of oxygen to the polyunsaturated fatty acids to formed fatty acid hydroperoxides. (Kuhn et al. 1986; Nelson et al.1994; Liavonchanka et al. 2006).

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\text{LOX} \\
\text{Fatty acid} + \text{O}_2 \rightarrow \text{Fatty Acid Hydroperoxide}
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Linoleic acid (18:2) and linolenic acid (18:3) are the substrates for plant lipoxygenase (Hamberg et al. 1967) and arachidonic acid (20:4) is the substrate for animal lipoxygenases (Khanapure et al. 2007). Fungal LOX utilize all three fatty acids (LA, LNA, ARA) as a substrate. LOX converts the poly-unsaturated fatty acid substrates like LA, LNA, ARA into hydroperoxy octadecadienoic acid (HPOD), hydroperoxy octadecatrienoic acid (HPOT) and Hydroperoxy eicosatetraenoic acid (HPETE) respectively. LOX products have various biological functions such as diverse signal molecules, oxidants and modifiers of membrane structures (Gardenar, 1995). In plants, LOX play roles in defence, seed germination, plant growth and development (Porta et al, 2002). In mammals a number of lipoxygenases isoymes are involved in the metabolism of eicosanoids, such as prostaglandins, leukotrienes and nonclassic eicosanoids (Needleman, 1986). The LOXs found in other organisms have been found to be functionally similar to human LOX, catalyzing the dioxygenation of unsaturated fatty acids. In general lipoxygenases are used in food industry such as – bread making, aroma production, fruit ripening, etc.

LOX catalysis includes the following (Nelson et al. 1994; Schneider 2007) (Figure 1)

i) the stereospecific hydrogen removal from a pentadiene group

ii) Delocalization of the electrons.

iii) stereospecific insertion of molecular oxygen.
Lipoxygenases have been studied from many fungal species like *Fusarium, Aspergillus, Penicillium, Geotrichum, Mortierella;* oomycetes *Lagenidium, Saprolegnia, Acylia;* mushroom *Morchella, Pleurotus;* ascomycetes *Saccharomyces, Gaeumannomyces;* thermophilic actinomycete *Thermoactinomyces vulgaris* etc [Filippovich et al, 2001; Kuribayashi *et al,* 2002; Perez *et al,* 2005], etc. Soyabean LOX is one of the most studied enzymes. In plants, LOX involve in the various physiological process (Kolomiets *et al,* 2000; Verinosi *et al,* 1996). It is a monomeric protein of about 95 to 100 kD that made two domains amino terminal 25-30 kD and carboxyl terminal domain of about 55 - 65 kD. The exact biological function of LOX in plants is unknown (Andreou and Feussner, 2009). Seven different metabolic pathways of LOX –hydroperoxides are shown in Figure 2. Among this AOS, HPL, POX, and DES are well studied. Compounds which are generated by the LOX pathway like aldehydes, jasmonic acid, etc. have roles in various physiological processes such as seed development, germination, vegetative growth, generation of fatty acid derived flavoured compound, wounding, stress response, senescence and cell signalling. Among this, we have focus on jasmonic acid biosynthesis pathway. Various functions of LOX metabolites are shown in table 1 (Porta *et al,* 2002)
Figure 2:-The lipoxygenase pathway. Production of various metabolites from 9 & 13 hydroperoxides. AOS, allene oxide synthase; DES, divinyl ether synthase; ®-DOX, ®-dioxygenase; EAS, epoxy alcohol synthase; HPL, hydroperoxide lyase; LOX, lipoxygenase; POX, peroxygenase; PUFAs, polyunsaturated fatty acids (figure is taken from Feussner, 2002).
Secondary metabolites from the oils of jasmine flowers (JA) (Demole et al., 1962), which was discovered in 1960s, have important biological roles and received increased attention of researchers in the past decades. Jasmonates were progressively become realized as a defence and fertility hormone, and as such alter numerable processes relating to development and stress responses.

JA biosynthesis and signalling JA has an important role against biotic and abiotic stress. Jasmonates mediate resistance responses to insect attack, to certain necrotrophic fungal pathogens and non-pathogenic fungi, and to the bacterium *Erwinia carotovora* (Turnar et al.2002, Norman-Setterblad et al.2000). Jasmonic acids and its derivatives like methyl jasmonates have important role as signalling molecule in plants. In animals, steroid and prostaglandins have structural similarity to jasmonates also act as a signalling molecule. Jasmonates induce higher molecular weight proteinase inhibitor, a defence related compound, and jasmonate induce some
low molecular weight compounds like phytoalexins (Farmer, 1990; Gundlach 1992). It is known that JA control environmental stresses and developmental stages in flowering plants. Wasternack has, carefully studied JA biosynthesis and its regulatory mechanism. (Wasternack, 2007). JA was first isolated from culture filtrates of the fungus Lasiodiplodia theobromae in 1971 by Aldridge, 1971 and MeJA was described as a constituent of the essential oils of Jasminum grandiforum L. and Rosmanum officinalis L. (Demole, 1962). There are also many reports supporting the role of JA and its precursor 12-oxo-phytodienoic acid in signal transduction of defence responses (Kachroo and Kachroo, 2009; Park et al. 2013). Although L. theobromae is one of the very well known sources of LOX, producing jasmonic acid (JA) in culture, it is not yet studied and characterized; also, other enzymes of the pathway were not studied from this fungus.

**Jasmonic acid biosynthesis pathway:**

The pathway for biosynthesis of Jasmonic Acid has been elucidated by Vick and Zimmerman, in 1983. Four steps involved in JA biosynthesis are:-

1) Release of precursor, linolenic acid from cellular membranes by phospholipase.

2) Free Linolenic acid is oxidized at position of 9c and 13c which is converted into 9,13-hydroperoxide by lipoxygenase (9-LOX, 13-LOX respectively).

3) 13-hydroperoxy octadecatrienoic acid (HPOTri) is converted into allene oxide, a highly unstable compound which is transformed in to 12-oxophytodienoic acid (OPDA) by the action of allene oxide synthase (AOS) and allene oxide cyclase (AOC).

4) OPDA is converted in to OPC (8:0) by the action of 12-oxophytodienoic acid reductase (OPR: 3) & subsequently three cycles of β-oxidation to produce (+)-iso-JA (6) [(3R, 7R)-configuration], which is further epimerized at C-7 to provide (-)-JA (7) [(3R, 7S) configuration]. Due to keto-enol interconversion, the cis-isomer, (+)-iso-JA (6), is readily converted into the more stable trans-isomer, (-)-JA (7), for steric reasons.
Figure 3: The Vick and Zimmerman pathway for JA biosynthesis. Pathway intermediates are abbreviated as 13-HPOT for (9Z11E15Z13S)-13-hydroperoxy-9,11,15-octadecatrienoic acid (that is, 13(S)-hydroperoxy linolenic acid), allene oxide for (12,13(S)-epoxy-9(Z), 11,15(Z)-octadecatrienoic acid, cis-(+)-OPDA for cis-(+)-12-oxophytodienoic acid, and OPC 8:0 for 3-oxo-2(2’(Z)-pentenyl)-cyclopentane-1-octanoic acid. The enzymes are indicated as 13-LOX for 13-lipoxygenase, AOS for allene oxide synthase, AOC for allene oxide cyclase, and OPR3 for 12-oxophytodienoate reductase 3. (Figure taken from Florian Schaller 2005)
Lasiodiplodia theobromae

It is a plant pathogenic fungus, which is found in tropical and subtropical regions of the world, causing considerable damage to crops during storage. In Brazil, this phytopathogen is considered a serious problem in agriculture sector (Barros-Filho et al, 2010). Previous literature reports that use of growing cells of L. theobromae in the biotransformation of natural products, such as steroids (Despreaux et al, 1886), terpenes, ionones, and a sesquiterpene lactone (Barros-Filho et al, 2010). The kinetics of a racemic epoxide by this microorganism has also been reported.

Theobroxide 1 \{(1S, 2R, 5S, 6R)-3-methyl-7-oxa-bicyclo [4.1.0] hept-3-en-2,5-diol\}, a compact epoxy cyclohexanoid, is a metabolite of the Lasiodiplodia theobromae (Nakamori et al, 1994), which robustly induces flower bud formation in morning glory (Pharbitis nil), and tuber formation in potato (Solanum tuberosum) under non-inducing conditions (Yoshihara et al, 2000). Another metabolite, 2 \{(1S, 4R, 5S, 6R)-7, 9-dioxa-3-methyl-8-oxobicyclo [4.3.0]-2-nonene-4, 5-diol\} was identified while systematic survey on potato-tuber inducing substances from a culture of L. theobromae. Theobroxide 1 was confirmed to inhibit stem elongation in morning glory and spinach throughout the suppression of gibberellin biosynthesis and stimulation of jasmonic acid biosynthesis. Immunoblot analysis of major enzymes in JA biosynthesis showed that lipoxygenase, allene oxide synthase and allene oxide cyclase activity, and endogenous levels of JA in Pharbitis nil were induced by theobroxide1. Administration of $^{13}$C labeled acetates ($[1^{-13}C]$, $[2^{-13}C]$ and $[1,2^{-13}C_2]$ to Lasiodiplodia theobromae showed the tetraketide origins of both theobroxide, a potato-tuber inducing substance \{1, (1S, 2R, 5S, 6R)-3-methyl-7-oxa-bicyclo[4.1.0]hept-3-en-2,5-diol\} and its carbonyldioxy derivative \{2, (1S, 4R, 5S, 6R)-7,9-dioxa-3-methyl-8-oxobicyclo [4.3.0]-2-nonene-4,5-diol\} (Li et al, 2007). This fungus is known to produce a variety of bioactive compounds like jasmonic acid (Aldridge et al., 1971). Recently it is found that application of culture filtrate obtained during the fermentation of Botryodiplodia theobromae in different crop such as strawberry, pineapple, sugar cane, banana, grapefruit, potatoes and rice increases yield crops, induces root development and has favourable effects in response to biotic and abiotic stress. Jasmonic acid is one of the important secondary metabolite, which is used in perfume industry, and in the preparation of toilet soap, cigarette, etc.