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# Regulation of Saponins Production in Medicinal Plants: Elicitation and Signal Transduction Mechanism

**Sonia Tamta**
Ph.D. Plant physiology, College of Basic sciences and Humanities, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar (Uttarakhand), India

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**Abstract**

Saponins are amphiphilic molecules that are composed of one or more hydrophilic sugar residues and hydrophobic steroidal or triterpenoidal part on the basis of which they are called as steroidal saponin or triterpenoidal saponin. Structural variants of saponin are found in plants due to the presence of different sugar at different position and orientation. Saponins are synthesized from 30-carbon linear precursor molecule 2, 3-oxidosqualene. They are produced in roots, leaves, flowers, stem and fruit. Saponin backbones are synthesized via the isoprenoid pathway. The key enzymes involved in the biosynthesis of saponins in plants are SQS, SE, LS, DS, β-AS, CAS, PDMO and GT. The cellular process and regulatory principle for activation of plant secondary metabolite biosynthesis is that, an extracellular or intracellular signal is perceived by a receptor on the surface of the plasma membrane or endomembrane; the elicitor signal perception initiates a signal transduction network that leads to activation of biosynthesis of transcription factors, which regulate the expression of biosynthetic genes involved in saponin production. MeJA, $Ca^{2+}$, ROS, NO activating the key enzymes of saponin.

## Introduction

Many higher plants are major source of natural products used as Pharmaceuticals, agrochemicals, flavour and fragrance ingredients, food additives and pesticides. Medicinal plants are the rich sources of this organic compounds. These phytochemicals are affected by genotype, physiological, environmental conditions, and pathogens.

**Secondary Metabolites (SMs)/Natural products/Secondary products:** Plant produce a large, diverse array of organic compounds that appear to have no direct function in their growth and development.

SMs are divided into three major groups:

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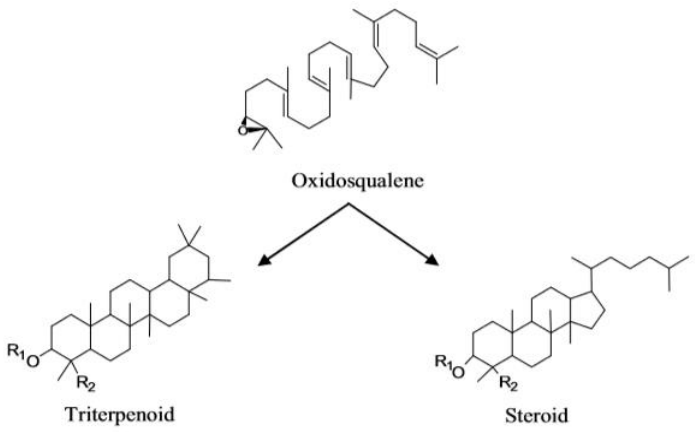
- Terpenes/terpenoids: All terpenes are derived from fusion of five carbon isoprene units
- Phenolic compounds: contain a hydroxyl functional group on an aromatic ring
- Nitrogen containing compounds: contain alkaloids and cyanogenic glycosides

**What are Saponins?**

The word saponin is derived from sapo, i.e., soap, which describes the soapy appearance of saponin when combined with water. Saponins are amphiphilic molecules that are composed of one or more hydrophilic sugar residues and hydrophobic steroidal or triterpenoidal part on the basis of which they are called as steroidal saponin or triterpenoidal saponins. The non-sugar water-insoluble part is called as sapogenin. Due to their amphiphilic properties and tendency to form the foam, they can be used as surfactant or emulsifying agents. Structural variants of saponin are found in plants due to the presence of different sugar at different position and orientation. On the basis of sugar present in saponin molecules:

- Monodesmosidic (contains only one sugar residue)
- Bidesmosidic (contains two sugar residues)
- Polydesmosidic (more than two sugar residues) saponins

Saponins are synthesized from 30-carbon linear precursor molecule 2, 3-oxidosqualene, but during the synthesis of steroidal saponins, there is a loss of three methyl groups that results in the formation of a skeleton having 27 carbon atoms while during synthesis of triterpenoidal saponins all the 30 carbon atoms retain in its backbone.




**(Upadhyay *et al.*, 2018)**

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**Importance of saponins:**

- Pharmaceutical uses
- Pesticidal, insecticidal, molluscicidal and fungicidal activity.
- Food additives
- Industrial applications such as foaming and surface-active agents.

**Locations for synthesizing saponins:**

The distribution of saponins mainly in oil canals in the periderm and outer cortex regions of the root. The phloem and resin ducts are both metabolically active sites for sterol and saponin biosynthesis. In leaves, organelles such as plastids, peroxisome, and vacuoles have been shown to be involved in synthesizing saponins.

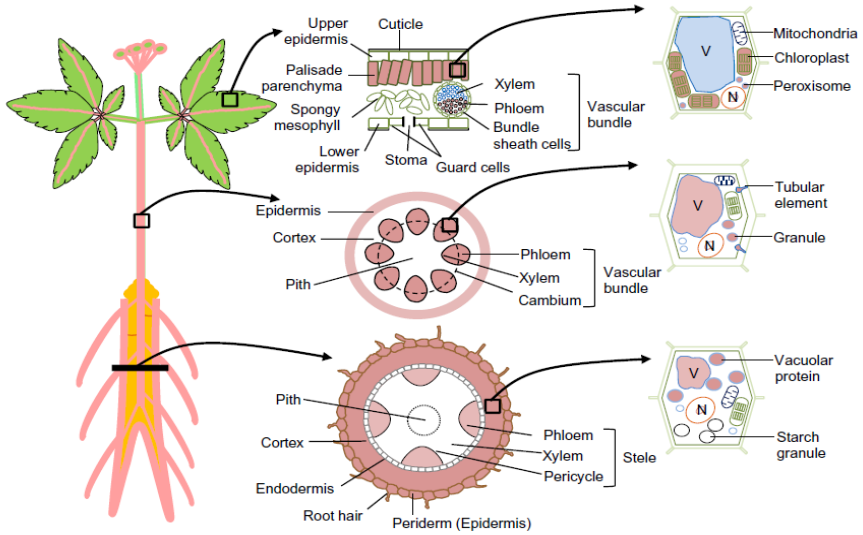


**Fig. 1.** Distribution and cellular localization of Ginsenoside (saponins) in Ginseng plants. Pink color indicates high accumulation of ginsenosides in cells. Ginsenosides are highly accumulated in epidermis in roots and vascular bundles. Ginsenosides are located in chloroplasts, peroxisomes, and cytoplasm of parenchymal cells in leaves, vacuoles and granules in vascular bundles in stem, vacuoles and vacuolar protein of parenchymal cells in roots. V = vacuole; N = nucleus. (**Kim *et al.* 2015)**

**Biosynthesis of saponins**

Saponin backbones are synthesized via the isoprenoid pathway. Squalene (30-carbon intermediate), which is a precursor for both triterpenoid and steroidal saponins is produced from the mevalonate and non-mevalonate [2-Cmethyl-D-erythritol 4-phosphate (MEP)]

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pathways through a series of enzymatic reactions involving geranyl diphosphate synthase (GPS), farnesyl diphosphate synthase (FPS) and squalene synthase.

**Key Enzymes of Saponin Biosynthesis**

- Squalene synthase (SS or SQS)
- Squalene epoxidase (SE or SQE)
- Cycloartenol synthase (CAS)
- Lupeol synthase (LS)
- Dammarenediol synthase (i.e., dammarenediol-II synthase, DS or DDS)
- β-amyrin synthase (β-AS)
- Cytochrome P450-dependent monooxygenase (PDMO)
- Glycosyltransferase (GT)

**Squalene synthase (SS or SQS)**

SS catalyzes two molecules of farnesyl-pyrophosphate (FPP) producing one molecule of squalene, which is the common precursor of the biosynthesis of terpenes, for example, triterpene, sterols.

**Squalene epoxidase (SE or SQE)**

SE catalyzes the transformation from squalene to 2, 3-oxidosqualene (OS), which is the first oxygenation step in triterpenoid saponin biosynthesis**.**

**Cycloartenol synthase (CAS)**

Cycloartenol synthase (CAS) are members of the Oxidosqualene cyclase (OSC) gene family, and catalyse the cyclization of 2,3-oxidosqualene to cycloartenol. OS undergoes protonation, cyclization, rearrangement and deprotonation to form the diverse triterpene structures

**Five kinds of 2, 3-oxidosqualene cyclases (OSCs) have been identified from plants:** LS, DS, β-AS, cycloartenol synthase (CAS) and lanosterol synthase (LAS).

LS, DS and β-AS form a family are responsible for the precursor synthesis of triterpenoids.

CAS and LAS are responsible for the precursor synthesis of sterols.

LS catalyzes the cyclization of OS to lupeol.

DS catalyzes the cyclization of OS to dammarenediol.

β-AS catalyzes the cyclization of OS into β-amyrin

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**Functions of PDMO and GT:**

PDMO and GT are both involved in the late stage of Saponin biosynthesis. The triterpene structures produced from OS subsequently undergo various modifications (oxidation, substitution and glycosylation), mediated by PDMO, GT and other enzymes. So far, little is known about the oxidation and glycosylation reactions.

**CYP450**

The cyclic skeleton synthesized by OSC undergoes site-specific oxidization by cytochrome P450s (P450s). P450s are monooxygenases that introduce an oxygen atom into their substrates from molecular oxygen.

**UGT**

Plant triterpenoids are further diversified through glycosylations at hydroxyl and/or carboxyl groups by UGTs. UGTs catalyze glycosylation using a UDP-sugar donor such as UDP-glucose, UDP-galactose, UDP-arabinose, UDP-rhamnose, UDP xylose or UDP-glucuronic acid.

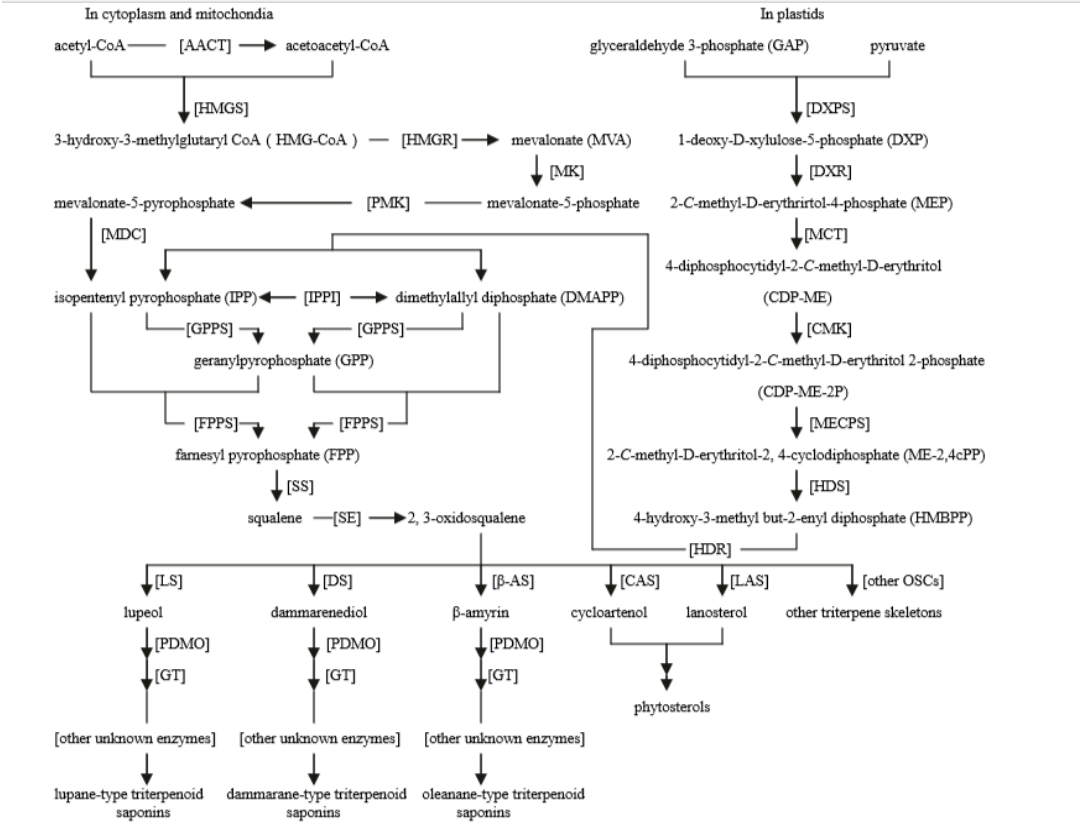


**Fig 2.** Triterpenoid Saponin Biosynthesis **(Zhao *et al.*, 2010)**

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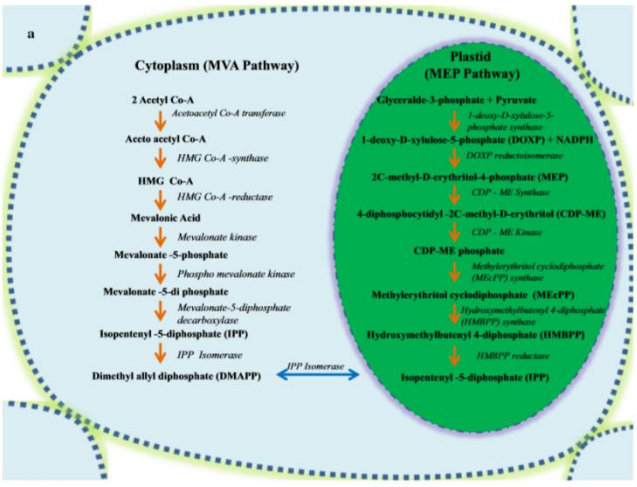
**Abbreviations:**

AACT: acetyl CoA: acetyl CoA *C*-acetyltransferase or acetoacetyl-CoA thiolase; CAS: cycloartenol synthase; CMK: 4-(cytidine 5′-diphospho)-2-*C*-methyl-D erythritol (i.e. 4-diphosphocytidyl-2-*C*-methyl-D-erythritol) kinase; DS i.e. DDS: dammarenediol synthase (i.e. dammarenediol-II synthase); DXPS, i.e. DXS: 1-deoxy-D-xylulose-5-phosphate synthase; DXR: 1-deoxy-D-xylulose-5-phosphate reductoisomerase; FPPS i.e. FPS: farnesyl diphosphate (i.e. farnesyl pyrophosphate) synthase; GPPS i.e. GPS: geranyl diphosphate (i.e. geranyl pyrophosphate) synthase; GT: glycosyltransferases; HDR: 4-hydroxy-3-methyl but-2-(*E*)-enyl diphosphate (i.e. 1-hydroxy-2- methyl-2-(*E*)-butenyl 4-diphosphate) reductase or isopentenyl pyrophosphate (IPP)/3, 3-dimethylallyl pyrophosphate (DMAPP) synthase; HDS: 4-hydroxy-3-methyl but-2-(*E*)-enyl diphosphate (i.e. 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate) synthase; HMGR: 3-hydroxy-3-methylglutaryl CoA reductase; HMGS: 3-hydroxy-3-methylglutaryl CoA synthase; IPPI, i.e. IDI: isopentenyl diphosphate (i.e. isopentenyl pyrophosphate) isomerase; LAS: lanosterol synthase; LS i.e. LUS: lupeol synthase; MCT: 2-*C*-methyl-D-erythritol 4-phosphate cytidylyl transferase or 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol (i.e. 4- diphosphocytidyl-2-*C*-methyl-D-erythritol) synthase; MDC, i.e. PMD: mevalonate-5-pyrophosphate (i.e. mevalonate-5-diphosphate) decarboxylase; MECPS: 2-C-methyl-D-erythritol-2, 4-cyclodiphosphate synthase; MK i.e. MVK: mevalonate kinase; OSCs: 2, 3-oxidosqualene cyclases; PDMO: cytochrome P450-dependent monooxygenases; PMK, i.e. MPK: Phosphomevalonate (i.e. mevalonate-5-phosphate) kinase; SE: squalene epoxidase; SS i.e. SQS: squalene synthase; β-AS: β-amyrin synthase

**Steroidal saponins classified as:**

• Spirostanol saponins

• Furostanol saponins.



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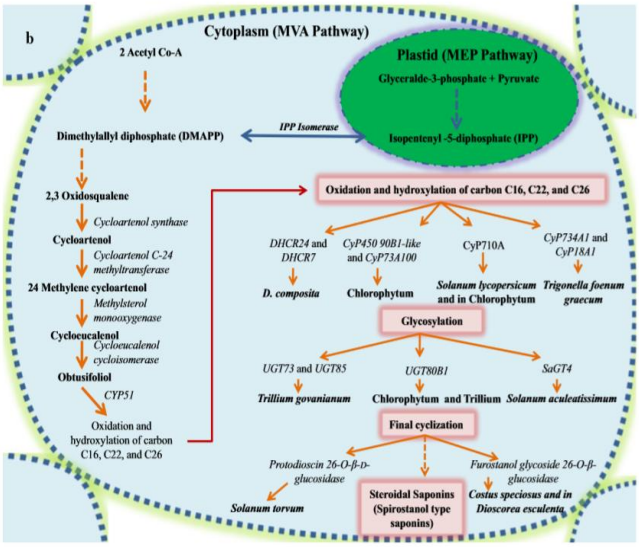


**Fig.3.** Steroidal saponin biosynthetic pathway **(Upadhyay *et al.*, 2018)**

a. Overview of steroidal saponin biosynthesis in plants that involve both (MVA) and (MEP) pathways.

b. It represents known P450s, UGTs and glucosidases involved in hydroxylation, oxidation, glycosylation and final cyclization of saponin backbone. Enzyme and species names are italicized. Dashed arrows imply multiple steps in the pathway. *DHCR24* delta 24-sterol reductase, *DHCR7* 7-dehydrocholesterol reductase, *CyP51* sterol 14α-demethylase.

**Signalling pathway-mediated saponin production:**

**JA signalling**

Polyunsaturated fatty acids can produce signals, such as oxylipins, which include JA, JA methyl ester, JA amino acid conjugates, and further JA metabolites. This pathway is started by usage of α-linolenic acid (α-LNA) as the substrate and catalyzed by several chloroplastic enzymes of lipoxygenase (LOX), allene oxide synthase (13-AOS), and allene oxide cyclase which finally end up by the peroxisomal oxophytodienoate reductase (OPR).

**ROS and RNS signalling**

The accumulation of reactive oxygen species (ROS), free radical substances such as hydrogen peroxide ($H_2O_2$), superoxide radical ($O^{2-}$), and hydroxyl radical causes significant cellular damage to cell by protein degradation, enzyme inactivation, gene alterations, and various important metabolic pathway interference.

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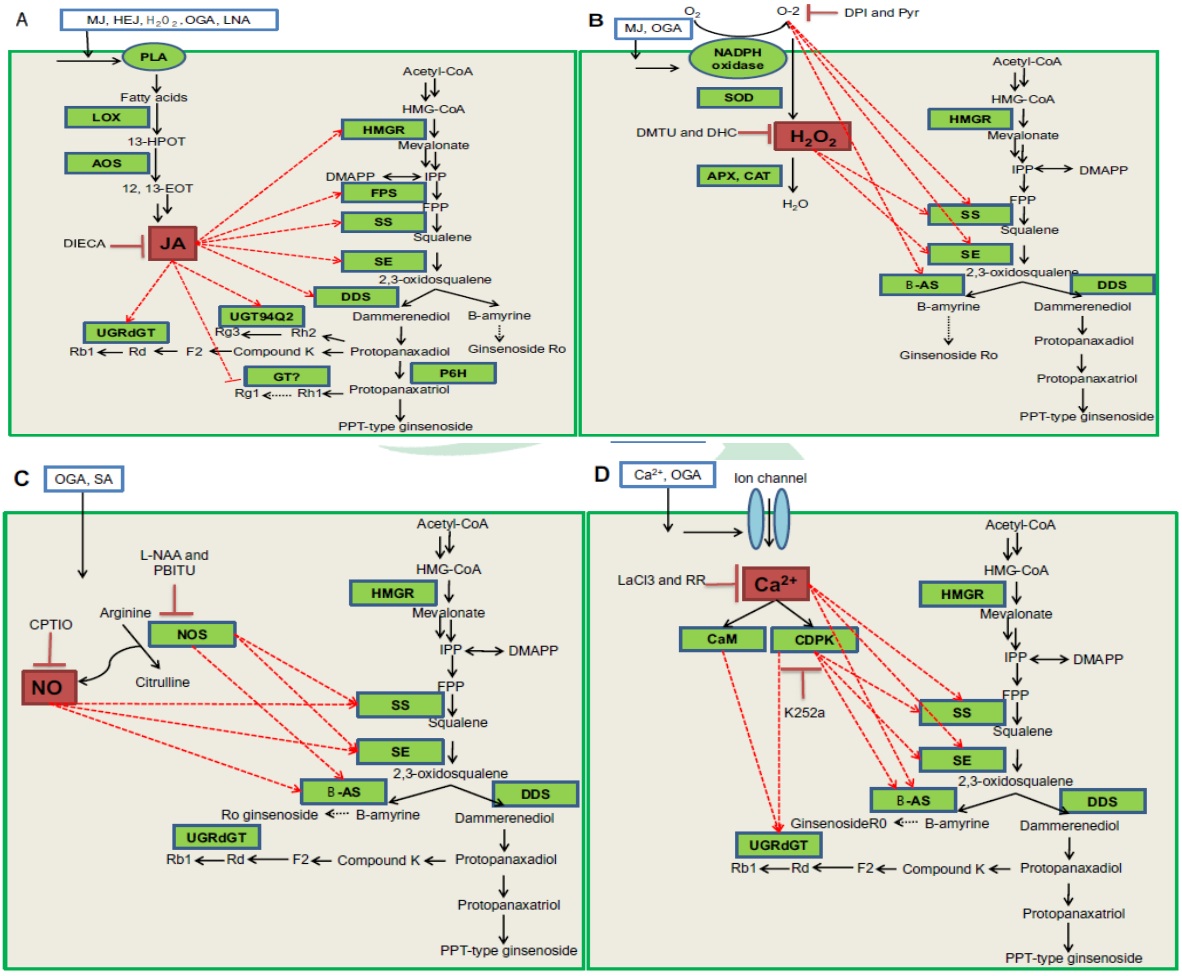
**$Ca^{2+}$ signalling**

$Ca^{2+}$ can activate calmodulin (CaM) and calcium-dependent protein kinase (CDPK), a superfamily of protein kinases. Inhibition of calcium fluxes and protein phosphorylation also significantly inhibited OGA-induced ginsenoside accumulation and SS, SE, and β- AS mRNA level which approve the requirement of calcium fluxes for ginsenoside production.

**Ethylene signalling**

S-adenosylmethionine is converted to 1-aminocyclopropane-1- carboxylic acid by 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, and then, ACC oxidase catalyzes the production of ethylene. It was found that ACC oxidase activity was increased upon fungal elicitation and, subsequently, the released ethylene-mediated accumulation of SS, SE, and β-AS, and, finally, ginsenoside accumulation in *P. ginseng*. When ethylene production was blocked, saponin synthesis was inhibited; reversely, ethylene treatment of ginseng cells promotes saponin synthesis.



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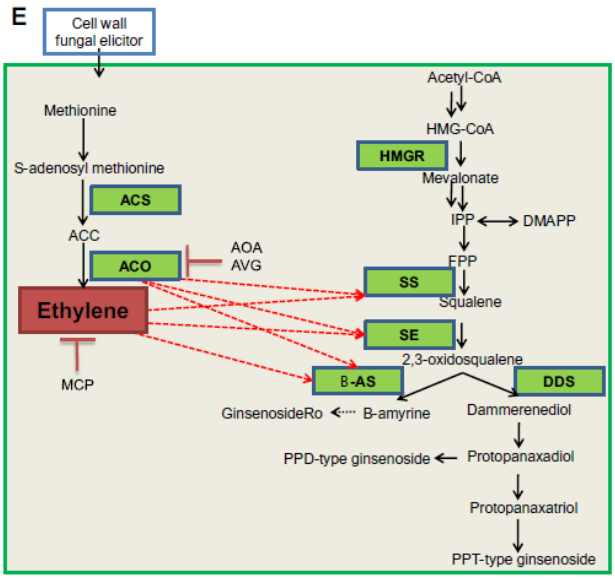


**Fig. 6.** Saponin (Ginsenoside) gene activation by signalling molecules. A) Jasmonic acid, b) hydrogen peroxide, c) nitric oxide, d) calcium signalling and e) ethylene signalling. **(Rahimi *et al.*, 2015)**

**Abbreviations:**

ACC: 1-aminocyclopropane-1-carboxylic acid; ACS: 1- aminocyclopropane-1-carboxylic acid synthase; ACO: 1- aminocyclopropane-1-carboxylic acid; AOA: aminooxyacetic acid; AVG: aminovinylglycine; CPTIO: 2-4-carboxyphenyl- 4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; DDS: dammarenediolSynthase; DHC: 2,5-dihydroxycinnamic acid methyl ester; DIECA: diethyldithiocarbamic acid; DMAPP: dimethylallyl pyrophosphate; DMTU: dimethlthiourea; DPI: diphenyleniodonium; 12,13-EOT: 12,13(S)-epoxyoctadecatrienoic acid FPP: farnesyl pyrophosphate; FPS: farnesyl pyrophosphate synthase; HEJ: 2- hydroxyethyl jasmonate; HMG-CoA: 3-hydroxy-3-methyl-glutaryl-CoA; HMGR: 3-hydroxy-3-methylglutaryl CoA reductase; 13-HPOT; (13S) hydroperoxyoctadecatrienoic acid; IPP: isopentenyl diphosphate; MCP: 1-metylcyclopropene; $O^{2\ -}$:superoxide radical; OGA: oligogalacturonide acid; P6H: protopanaxadiol 6-hydroxylase; PPD: protopanaxadiol; PPT: protopanaxatriol; RR: ruthenium red; UGRdGT: UDPG/ginsenoside Rd glucosyltransferase.

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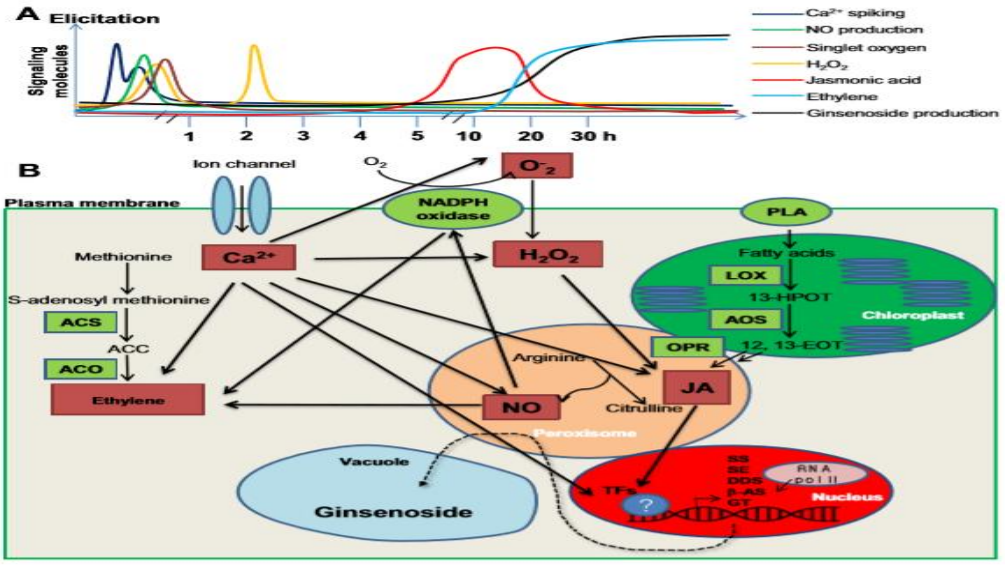


**Fig 7.** (a) Schematic illustration of the sequential signalling pathways activated in elicited *P. ginseng*. (b) Model of cross talk between different signal transductions. **(Rahimi *et al.*, 2015)**

**Abbreviations:**

ACC 1-aminocyclopropane-1-carboxylic acid; ACS 1-aminocyclopropane-1-carboxylic acid synthase; ACO 1aminocyclopropane-1-carboxylic acid; AOS allene oxide synthase;; β-AS beta-amyrin synthase; DDS dammarenediol synthase; 12,13-EOT 12,13(S)-epoxyoctadecatrienoic acid; $H_2O_2$ Hydrogen peroxide; 13HPOT (13S)-hydroperoxyoctadecatrienoic acid; JA Jasmonic acid; LOX lipoxygenase; NO nitricoxide; NOS nitricoxide synthase; $O^{2-}$ superoxide radical; OPR oxophytodienoate reductase; PLA phospholipase; SS squalene synthase; SE squalene epoxidase; TFs transcription factors; UGRdGT UDPG/ginsenoside Rd glucosyltransferase.

**Conclusion and future perspective:**

The evidences of these key enzymes of saponins have potential for the synthesis of drugs, and their manipulation leads to the generation of new drugs. The diversity of saponins offers the opportunity for further research on phytochemistry and biological activity of saponins.

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