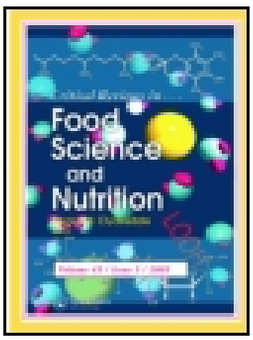

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REVIEW

Phytochemistry of ginsenosides: Recent advancements and emerging roles

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ABSTRACT

Ginsenosides, a group of tetracyclic saponins, accounts for the nutraceutical and pharmaceutical relevance of the ginseng (*Panax sp.*) herb. Owing to the associated therapeutic potential of ginsenosides, their demand has been increased significantly in the last two decades. However, a slow growth cycle, low seed production, and long generation time of ginseng have created a gap between the demand and supply of ginsenosides. The biosynthesis of ginsenosides involves an intricate network of pathways with multiple oxidation and glycosylation reactions. However, the exact functions of some of the associated genes/proteins are still not completely deciphered. Moreover, ginsenoside estimation and extraction using analytical techniques are not feasible with high efficiency. The present review is a step forward in recapitulating the comprehensive aspects of ginsenosides including their distribution, structural diversity, biotransformation, and functional attributes in both plants and animals including humans. Moreover, ginsenoside biosynthesis in the potential plant sources and their metabolism in the human body along with major regulators and stimulators affecting ginsenoside biosynthesis have also been discussed. Furthermore, this review consolidates biotechnological interventions to enhance the biosynthesis of ginsenosides in their potential sources and advancements in the development of synthetic biosystems for efficient ginsenoside biosynthesis to meet their rising industrial demands.

KEYWORDS

Bioactivity; ginseng; ginsenosides; glycosylation; saponins; secondary metabolites

1. Introduction

Plants synthesize an array of secondary metabolites as a line of defence against various external and internal cues (Berini et al. 2018; Arnold, Kruuk, and Nicotra 2019). These secondary metabolites majorly include alkaloids, glycosides, and different types of phenolics such as tannins, lignin, coumarin, and flavonoids, among others. Interestingly, the majority of these secondary metabolites exhibit various nutraceutical and pharmaceutical properties (Clerici and Carvalho-Silva 2011; Leong et al. 2017; Farh et al. 2020; Ying Ying Tang et al. 2020; My et al. 2020). Among a diverse range of high-value specialized metabolites, ginsenosides, a class of triterpene saponins, are of considerable interest because of their unique and wide range of therapeutical and pharmaceutical activities. These ginsenosides are abundantly present in various species of ginseng plants native to Korea, Japan, and China, and contribute to their therapeutic properties for the treatment of several human ailments (Kee et al. 2017; Irfan et al. 2020; My et al. 2020). As of today, 13 species of the *Panax* have been identified including *P. ginseng* (Korean or Asian ginseng), *P. vietnamensis* (Vietnamese ginseng), *P. quinquefolius* (American ginseng), Pseudo ginseng, and *P. japonicus* (Japanese ginseng) (Kim, Yi, et al. 2017). Of different *Panax* species, *P. ginseng* or Korean ginseng contains the highest amount of ginsenosides and is widely used in food supplements due to its adaptogenic properties that enhance resistance to lethal effects of biological stressors (Khan, Tosun, and Kim 2015). Moreover, ginsenosides also possess various therapeutic effects and exhibit anticancerous, anti-inflammatory, antidiabetic, hypolipidemic, anti-allergic, antioxidant, and anti-nephrotoxic properties (Baek et al. 2017; Jeong et al. 2020; Zou et al. 2020).

Ginsenosides are steroid glycosides and based on their aglycone skeletons, these can be classified as dammarane- and oleanane-type that constitute tetracyclic skeleton with attached sugar moieties and pentacyclic skeleton having oleanolic acid (an aglycone) respectively (Christensen et al. 2009; Shin, Kwon, and Park 2015). Based on the position of attachment of sugar moieties, dammarane skeletons are further classified as protopanaxadiols (PPD) and protopanaxatriols (PPT) type of ginsenosides having sugar components attached at 3rd and 6th position of the triterpene structure respectively (Christensen et al. 2009; Lü, Yao, and Chen 2009).

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*Equally contributed.
This article has been corrected with minor changes. These changes do not impact the academic content of the article.

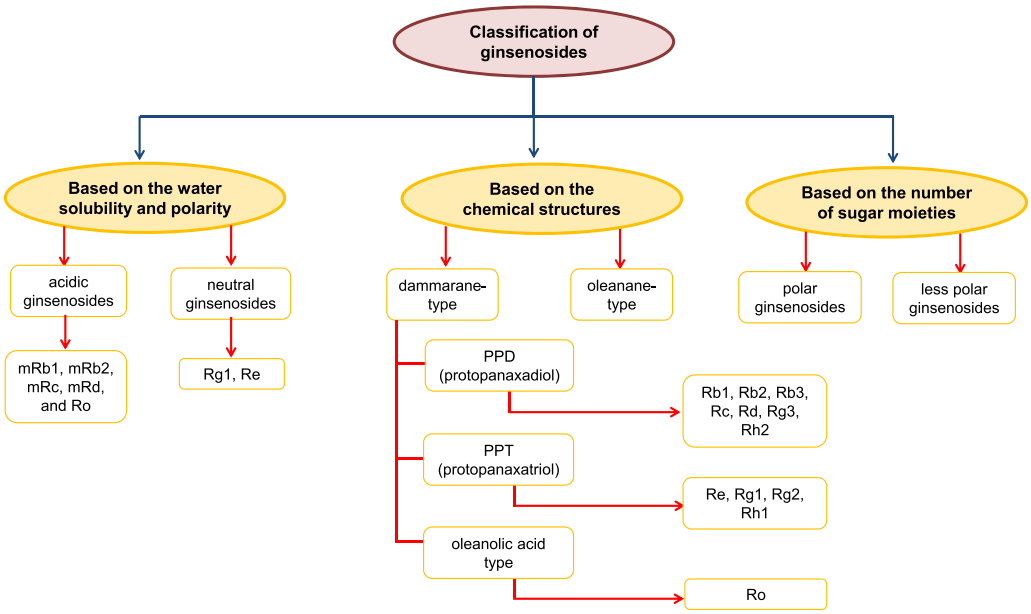

Figure 1. Classification of ginsenosides based on different properties. The parameters used for the classification include their aglycone structures, position, and the number of hydroxyl groups and sugar moieties, accounting for their diversifying structures.

Recent years have witnessed tremendous progress in the field of ginsenosides research that needs to be reviewed to translate the obtained knowledge for developing functional products. An integrated, as well as updated data on recent advancements in researches based on ginsenosides, can help in enhancing their industrial potential like in the case of other major natural compounds specifically in the global food industry (Ying Ying Tang et al. 2020; Rambabu et al. 2019). The present review is a major step in consolidating a wide range of information on ginsenosides which is rarely found. It is a concrete effort in compiling a vast amount of data generated on almost all the aspects of ginsenosides which is not yet done on such a large scale till now up to the best of our knowledge. The present review discusses the basic understanding of ginsenosides including structural and functional diversity, biosynthesis and metabolism, structural-function interaction within ginsenosides, and the transformation of major glycosylated ginsenosides to their aglycosylated forms to increase their biological activity. Moreover, this review also discusses the methods for ginsenoside analysis and the recent advancements in estimating their levels in potential plant sources. Strategies to enhance ginsenoside accumulation including elicitation and transgenic developments have also been presented.

2. Structural classification and distribution of ginsenosides

Ginsenosides are the signatory bioactive compounds found predominantly in Korean ginseng, which is used as an efficient herb for human disease suppression worldwide (Jeon et al. 2020). Ginsenosides are derived mainly from the upper and lower ground portions of ginseng including roots, stems, fruits, and flower buds. However, the amount and types of ginsenosides present in these tissues vary significantly (Christensen 2009). Moreover, the contents of ginsenosides present in different ginseng tissues also depend on the harvest season and geographical conditions (Christensen and Brandt 2006; Chen, Balan, and Popovich 2020).

Approximately, 200 different types of ginsenosides have been identified in Korean ginseng so far and over 100 types have been identified in American ginseng (Jeon et al. 2020; Chen, Balan, and Popovich 2020). Based on the chemical properties, ginsenosides have majorly been grouped as dammarane-type and oleanane-type (Christensen et al. 2009; Kang et al. 2018) (Figure 1). The chemical notation of ginsenosides is “Rx” where R stands for root and x is the chromatographic polarity in the ascending alphabetical order (Gantait, Mitra, and Chen 2020). In Korean ginseng, the majority of the ginsenosides are of dammarane-type that are further classified into PPD (protopanaxadiol) type, PPT (protopanaxatriol) type, and oleanolic acid type (Zheng et al. 2018) (Figure 1). Six major types of ginsenosides including Rb1, Rb2, Rc, Re, Rd, and Rg1 account for more than 90% of the total ginsenoside content in Korean and American ginsengs (Zhou et al. 2014). Based on the water solubility and polarity of ginsenosides, these have also been classified as acidic ginsenosides and neutral ginsenosides (Chuang et al. 1995; Liu et al. 2017) (Figure 1).

Structurally, ginsenosides are thirty carbon-containing tetracyclic-glycosylated triterpenes that consist of a four-ring system of steroid nuclei and diverse varieties of sugar moieties such as xylose, glucose, arabinose, and rhamnose attached to C3, C6, and C20 positions (Shin and Oh 2016; Shi, Zeng, and Wong 2019). Each ginsenoside has two or three hydroxyl (-OH) groups on C3, C20 or C3, C6, C20 that either exist freely or bound to sugar moieties, and the position of these hydroxyl groups on C20 accounts for stereoisomerism in ginsenosides (Lü, Yao, and Chen 2009). Based on the number of sugar moieties, ginsenosides may be categorized as polar ginsenosides and less polar

ginsenosides (Kwon et al. 2001). Less polar ginsenosides exhibit higher biological activities than their polar forms (Le et al. 2015; Zhang et al. 2021). Most of the ginsenosides exist in (S)-configuration, however, some artefactual ones exist as epimers (Shin, Kwon, and Park 2015). The differential orientation of the hydroxyl group at the C20 position exhibits several epimers of ginsenosides in *Panax sp*. that are generally of the 20S-configuration (Guo et al. 2020).

Classification of ginsenosides based on their aglycone structures, position, and the number of hydroxyl groups and sugar moieties account for their diversifying structures that are classified as PPD and PPT. In the PPD type of ginsenoside, sugar moieties are attached at the C3 position of triterpene ring of dammarane-type (Lü, Yao, and Chen 2009). Within the PPT type of ginsenoside, the sugar moieties are attached to the C6 position triterpene ring of dammarane-type and PPT type is formed by the hydroxylation of PPD type of ginsenoside in which OH group on C3 position remains free (Le et al. 2015; Shin, Kwon, and Park 2015). Both the inner and outer sugar moieties attached to C3 are glucose, while at C20 inner moiety is glucose and the outer one can be glucose, xylulose, arabinopyranose, or arabinofuranose (Shin, Kwon, and Park 2015).

More than 80% of the total ginsenosides are glycosylated, however, deglycosylated structures are also found. Interestingly, the deglycosylated ginsenosides exhibit higher biological and pharmacological activities than glycosylated forms (Li and Ji 2017; Shin and Oh 2016). Therefore, hydrolysis of the sugar moieties to produce their deglycosylated forms by heating, microbial and acid transformations, and/or fermentation is highly preferred (Shin and Oh 2016; Quan et al. 2015; E.-J. Yang et al. 2018). All typical PPD- and PPT-type saponins exist in their glycosylated and hydrated forms with no double bond but when they are heated or steamed, they get converted into their artefactual forms i.e., deglycosylated and dehydrated forms at C20 position resulting in the formation of double bond either between C20 and C21 or C20 and C22. Moreover, the products of PPD and PPT obtained by oxidative cleavage that occurs due to various chemical treatments (for example, treatment with an alkali) results in an aldehyde group with few carbons, while dehydration products exhibit a double bond between C21-C22 and the products of acid hydrolysis of ginsenosides results in a six-membered ring formation with the addition of epoxy group. Peroxidation products of PPD and PPT which mostly occurs on or near double bond of C24-C25 lead to the existence of many diverse structures, while some ginsenosides from Korean ginseng flower bud have hydroperoxyl group on C24 and double bond exists between C25-C26, others have a hydroxyl group at C24 that later forms hydroperoxyl group also exist (Shin, Kwon, and Park 2015). Broadly, it can be inferred that the glycosylation products, the peroxidation products, hydration (25-hydroxy-protopanaxadiol) and dehydration products, and cleavage products of PPD and PPT exhibit several structural aberrations (Shin, Kwon, and Park 2015).

Distinct ginsenoside profiles (ginsenoside Rf) and varying ginsenoside types in different *Panax* species set an easy ground for differentiation and authentication of the desired ginseng for commercial applications (Chan et al. 2000). For instance, PPD and PPT type ginsenosides have been majorly found in Korean-, American-, and Chinese-ginseng while oleanolic acid-type ginsenosides are prominently found in Japanese ginseng, Himalayan ginseng, Vietnamese ginseng, and ginger ginseng. Similarly, ocotillol-type is typically found in pear ginseng, Himalayan ginseng, Vietnamese ginseng, and American ginseng (Christensen 2009).

3. Biosynthesis of ginsenosides

The significance of ginsenosides in their prominent sources including *Panax* sp. necessitates the understanding of insights into their biosynthetic pathway that is crucial to enhance the ginsenosides production in plants. Ginsenosides are commonly biosynthesized from squalene, a linear molecule with 30 C atoms that is formed by head-to-head linking of two molecules of each farnesyl diphosphate (FPP), a derivative of dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) in the presence of enzyme farnesyl diphosphate synthase (FPS) and squalene synthase (SS; Lee et al. 2004; Gantait, Mitra, and Chen 2020). The squalene, thus produced, gets converted into (S)-2,3-oxidosqualene (Han et al. 2010), by cyclization and condensation reactions which involve the formation of an enantioselective primary product called Dammarenediol II (dammarenyl-cation) (Phillips et al. 2006; Kim, Zhang, and Yang 2015; Kang et al. 2018). The major dammarane-type of ginsenosides, PPD, and PPT are synthesized from dammarenediol II via hydroxylation reactions. The reaction cascade from squalene to the production of PPD and PPT ginsenosides involves a set of enzymes including squalene epoxidase, oxidosqualene cyclases, dammarenediol II synthase, protopanaxadiol synthase, and protopanaxatriol synthase (Phillips et al. 2006; Kang et al. 2018). Another important category of enzymes is cytochrome P450s (CYP450s), a group of monooxygenases that catalyze hydroxylation, epoxidation, and oxidation in ginsenoside production (Bernhardt 2006). Once the PPDs and PPTs are synthesized, these are glycosylated (Danieli et al. 2001; Hu et al. 2020), by the addition of sugar moieties using uridine diphosphate (UDP)-activated sugar molecules as an active donor under the controlled activity of regioselective UDP-glycosyltransferases (UGTs) (Kim et al. 2014a; Wei et al. 2015; Cui et al. 2016). A few UGTs responsible for glycosylation of triterpenoid aglycones have been identified in Korean ginseng (Jung et al. 2014), *Medicago truncatula* (Achnine et al. 2005), and *Glycine max* (Shibuya et al. 2010).

The biosynthesis of ginsenosides occurs primarily by two pathways, the mevalonic acid pathway (MVA) operating in the cytosol and the methylerythritol phosphate pathway (MEP) in the plastid (Seemann et al. 2006; Zhao et al. 2014; Kim, Zhang, and Yang 2015) (Figure 2). However, the MVA pathway peculiarly operates in animals, archaebacteria while the MEP pathway operates majorly in yeast, prokaryotes, and Chlorophyta (green algae; Lombard and Moreira 2011; Jo et al. 2017; Xu et al. 2017; N.H. Kim et al. 2018). In

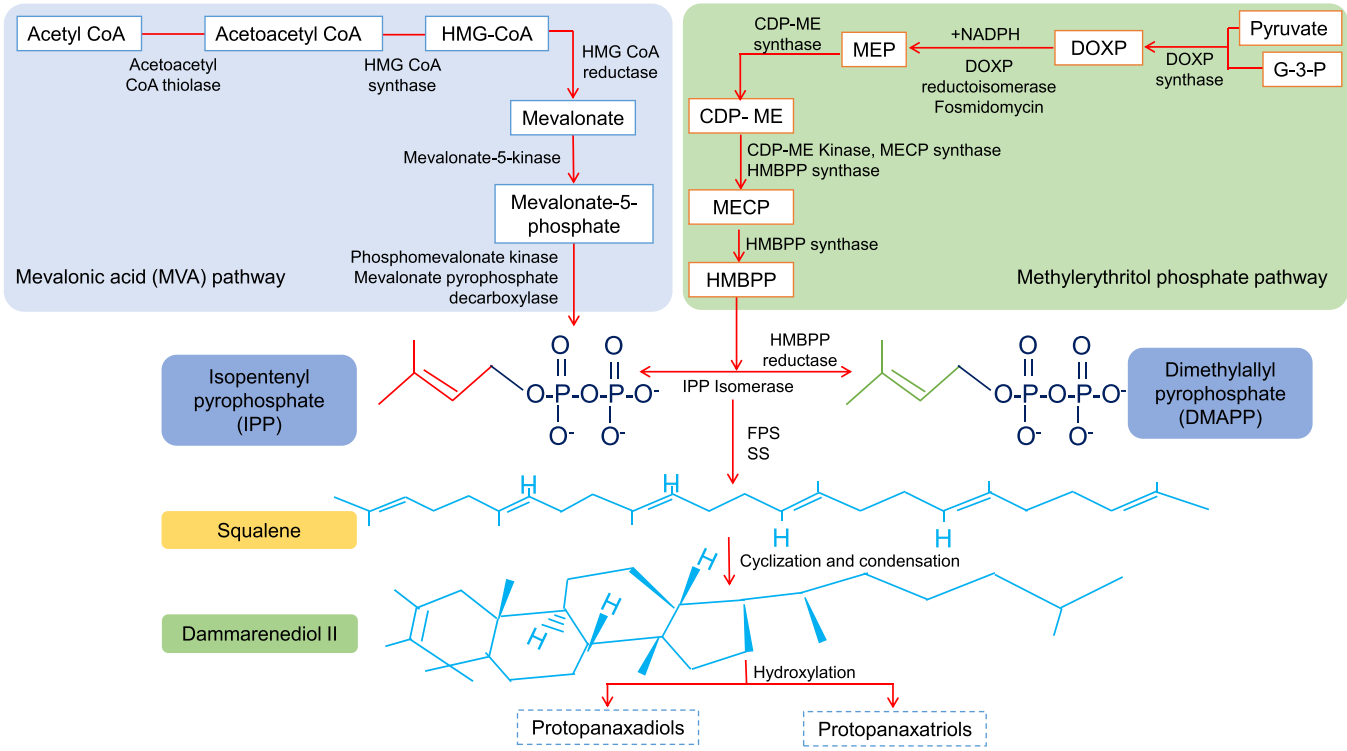

Figure 2. Biosynthesis of ginsenosides in *Panax* and its allied species. Biosynthesis of two major dammarane type of ginsenosides takes place through two different pathways MVA and MEP pathways that occur in cytosol and plastids respectively. These pathways are thus compartmentalized and occur parallelly and finally lead to the formation of universal precursor molecules, Isopentenyl pyrophosphate (IPP) and Dimethylallyl pyrophosphate (DMAPP). IPP and DMAPP further gets converted into squalene and later into dammarenediol II by the activities FPS and SS, and assisted by consecutive condensation and cyclization reactions. The dammarenediol II finally converted into PPD and PPT type of ginsenosides via hydroxylation reactions. Abbreviations: G3P, Glycerladehyde-3-phosphate; MEP, 2-C-methyl-D-erythritol-4-P; CDP-ME, 4-(CDP)-2-C-methyl-D-erythritol; MECP, 2-C-methyl-D-erythritol 2,4 cyclo-PP; MCEP, 1-hydroxy-2-methyl-2-(E)-butenyl-4-PP; HMBPP, 1-hydroxy-2-methyl-2-E-butenyl-4-PP; FPS, Farnesyl diphosphate synthase; S, squalene synthase (SS).

plants, both MVA and MEP pathways operate simultaneously (Hemmerlin et al. 2003) leading to the biosynthesis of isoprenoids by exchanging their intermediates (Rohdich et al. 2003). Enzymatic reaction cascade for MVA pathway is initiated by a condensation reaction between acetyl CoA and acetoacetyl-CoA to ultimately form ginsenoside precursor molecules, IPP and DMAPP via the formation of intermediate molecules such as mevalonic acid and 3-hydroxy-3-methylglutaryl-CoA (Ferguson and Rudney 1959; Durr and Rudney 1960; Miziorko 2011; Zhao et al. 2014; Xu et al. 2017). However, the MVA pathway seems to be a primordial metabolic system in almost all the organisms with the occurrence of conserved enzymes in eukaryotes except archaea which exhibits a divergent pathway with the absence of two components of the MVA pathway (Dellas et al. 2013; Xu et al. 2017). MEP pathway initiates with the condensation of Glyceraldehyde 3-phosphate (a triose phosphate) with C2 unit in pyruvate in a thiamin diphosphate dependent manner that leads to the formation of a pentose phosphate derivative of 1-deoxy-D-xylulose (i.e., 1-deoxy-D-xylulose 5-phosphate or DXP) (Hoeffler et al. 2002; Zhao et al. 2014; Kim, Zhang, and Yang 2015). DXP, thus produced, undergoes reductive isomerization by DXP reductoisomerase to form 2-C-methyl-D-erythritol 4-phosphate (i.e., MEP) that combines with cytidine 5′-triphosphate (CTP) to form methylerythritol cytidyl diphosphate. The C2 carboxyl group of methylerythritol cytidyl diphosphate undergoes phosphorylation, cyclization, and forms 2-C-methyl-D-erythritol-2,4-cyclodiphosphate followed by its reductive dehydration to give rise to (E)-4-hydroxy-3-methylbut-2-enyl diphosphate (HMBPP), finally leading to the formation of IPP and DMAPP (Hoeffler et al. 2002; Eisenreich et al. 2004; Kuzuyama and Seto 2012; Kim, Zhang, and Yang 2015; Gantait, Mitra, and Chen 2020) (Figure 2).

In Korean ginseng, it was assumed that the MVA pathway participates in the biosynthesis of ginsenosides as suggested by transcriptomics, phytochemical analysis, and genome sequencing data (W. Chen et al. 2017; Jo et al. 2017; N.H. Kim et al. 2018). However, later MEP pathway emerged as a major ginsenoside biosynthetic pathway in Korean ginseng as revealed by expression analysis of genes and metabolic regulation of the MEP pathway (Xue et al. 2019). Of 48,165 unigenes identified in *de novo* assembly of gene sets obtained using RNA seq, 380 were related to ginsenoside biosynthesis associated with both MEP and MVA pathways. Moreover, expression analysis of genes associated with ginsenoside biosynthesis in ginseng roots and other tissues revealed that transcript abundances of the genes involved in MEP and MVA pathways were similar in roots while in leaves, the transcripts for MEP pathway were much higher suggesting the extensive participation of MEP pathway in ginsenoside production (Xue et al. 2019). Thus, both MVA and MEP pathways may essentially be important in ginsenoside production within the plants, yet, the molecular and genetic controls to these pathways are still not completely elucidated.

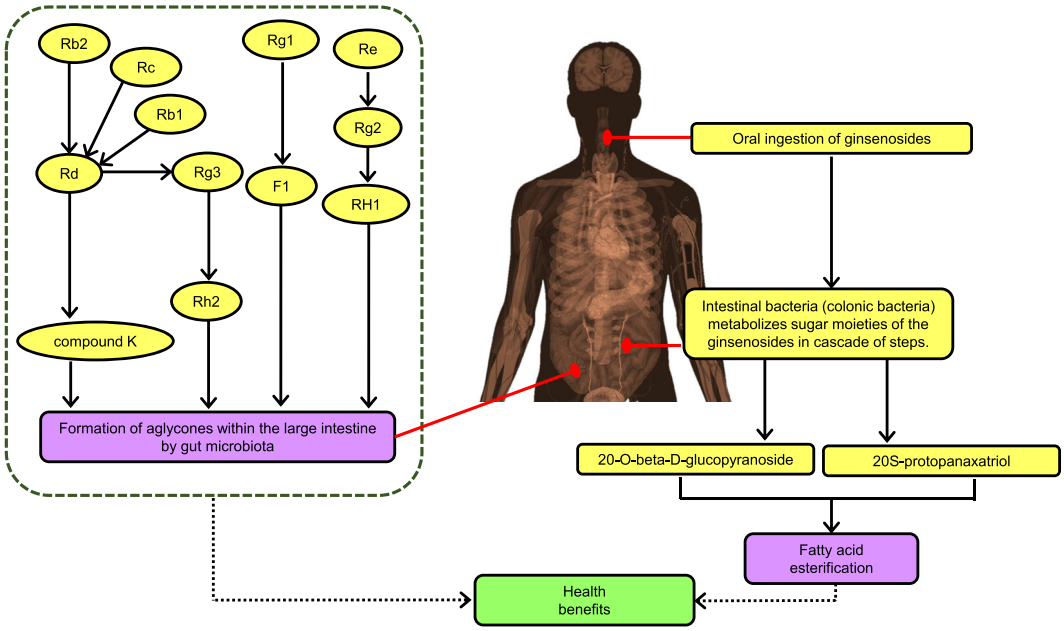

Figure 3. Metabolism of ginsenosides after oral ingestion within human body. After the oral ingestion of ginsenosides, the major glycosylated forms are metabolized into deglycosylated ginsenosides within the large intestine by the gut microbiota/colonic bacteria. As the ginsenoside is taken, it passes the stomach and small intestine without any metabolism as gastric juices and liver enzymes fail to breakdown the sugar moieties. Later, as the ginsenoside reaches large intestine, the sugar moieties are broken down in a step wise manner, and finally lead to the formation of aglycones that further undergoes fatty acid esterification and gets converted into the form which human body can sustain for a longer time.

In contrast to the biosynthesis of ginsenosides, their metabolism has always received little attention despite their abundant roles in pharmacology. Ginsenosides metabolize extensively within the intestinal tract via gut microbiota (Liu et al. 2009) by a series of deglycosylation reactions that cleaves sugar moieties in a sequential manner (Tawab et al. 2003) (Figure 3). Major ginsenosides such as PPDs including Rd and Rb1 and PPTs such as Re and Rg1 are metabolized to minor deglycosylated ginsenosides such as compound K (L. Yang et al. 2007) as well as F1 and Rh1 respectively (Wang et al. 2000; Yang et al. 2009). The active ginseng metabolites such as compound K shows high chemoprotective activity and induce apoptosis in tumor cells in vivo (Lee et al. 2000). After oral ingestion of the ginsenosides in the form of ginseng, these pass through the stomach and small intestine without getting decomposed by acidic gastric juices or liver enzymes to finally reach the large intestine where ginsenoside metabolism occurs via colonic bacteria (Tawab et al. 2003; Hasegawa 2004; Qi et al. 2011) (Figure 3). The bacteria within the colon decomposes oligosaccharide moieties attached to the ginsenoside aglycones into major metabolites such as 20-O-beta-D-glucopyranoside and 20S-protopanaxatriol that further undergo fatty acid esterification and are strongly sustained in the human body for a longer time than parental ginsenoside metabolites and provides several health benefits (Hasegawa 2004; Qi et al. 2011).

4. Factors affecting ginsenoside biosynthesis

Ginsenosides have extensive and multifarious roles in plant defence and exhibit therapeutic roles in humans, albeit, their accumulation in plants is very slow (Du et al. 2018; Farh et al. 2020; My et al. 2020). Moreover, biosynthesis and accumulation of ginsenosides in plants are critically interfered by various environmental stimuli such as water, light, temperature, and mineral contents (Jiang et al. 2016; J. F. Li et al. 2020; Jung et al. 2020) (Table 1). Abiotic factors, such as water deficiency, not only affect plant growth and development but also alter concentrations of specialized metabolites such as ginsenosides to mediate plant defence responses during unfavorable conditions (Lee and Mudge 2013a). In American ginseng, drought stress-induced accumulation of six ginsenosides including Rb1, Rg1, Rc, Re, Rb2, Rd has been observed in the roots (Lim, Mudge, and Lee 2006). Moreover, drought conditions also elevated individual levels of Rc, Rb1, Rd, Re, and total ginsenoside concentration in roots without affecting the levels of Rb2 and Rg1, suggesting that such minor ginsenosides are less susceptible to stress conditions (Lee and Mudge 2013a).

Irradiance is another critical determinant for ginsenoside biosynthesis and accumulation in plants (Fournier et al. 2003; Zhang et al. 2019a). In American ginseng, intensity and duration of irradiance from sun flecks resulted in significant enhancement of both PPD (Rb1, Rd, Rb2, and Rc) and PPT-type (Re, Rg1) ginsenosides in the roots (Fournier et al. 2003). In the leaves of Korean ginseng, photosynthetically active radiation (PAR) together with soil water potential positively influenced ginsenoside accumulation in roots while temperature and humidity exhibited stronger impacts on ginsenoside accumulation (T. Zhang et al. 2019a). Lee, Park, and Lee (1987) showed that solar radiation (30%)—induced high light levels significantly enhanced ginsenoside content in Korean ginseng (Lee, Park, and Lee 1987), while Park and Lee (1993) revealed that the intensification of solar energy (5, 10, 20, and 30%) was positively correlated with an elevation in ginsenoside levels by 13.6, 16.3, 17.7, and

Table 1. Factors affecting ginsenoside biosynthesis.

External factors	Plant species	Ginsenosides affected	Structural category	Effect on the ginsenoside levels	References
Irradiance/ Light stress	*Panax quinquefolius* L.	Rb_1, Rc, Rb_2, and Rd Rg_1, Re,	PPD-type ginsenosides PPT-type ginsenosides	Root ginsenosides level increases	Fournier et al. 2003
γ-irradiation	*Panax ginseng*	Rb1, Rb2, Rc and Rd Rf, Re, Rg1,	PPD-type ginsenosides PPT-type ginsenosides	increased by 1.8 and 2.3-fold in flask and bioreactor cultures respectively of adventitious roots	Zhang et al. 2011
High temperature and light	*Panax ginseng*	Rg Rb	PPT-type ginsenosides PPD-type ginsenosides	Increases maximum under fluorescent light	Yu et al. 2005
High temperature	*Panax quinquefolius*	Re and Rg_1 Rb_1, Rb_2, Rc, and Rd	PPT-type ginsenosides PPD-type ginsenosides	Increases total storage root ginsenosides	Jochum, Mudge, and Thomas 2007
Chilling treatment	*Panax ginseng* Meyer	Re, and Rg_2 Rb_1 Rg_1 Re Rb_1, Rc, and Rb_2 Rc, Rd and Rb_2 Rb2, and Rd Re	PPT-type PPD-type PPT-type PPT-type PPD-type PPD-type PPD-type PPT-type	Increased levels in roots Increased levels in roots Decreased in root rhizome and epidermis. Increased in epidermis and fine roots Decreased in fine roots Increased levels in upper root Increased levels in lower root	Oh et al. 2014
Cadmium stress	*Panax notoginseng*	Rg_1 Rb_1	PPT-type PPD-type	Ginsenoside level decreases in roots	Zu et al. 2020
Iron (Fe) Ca and Cu	*Panax quinquefolium* L. *Panax quinquefolium* L.	Rb_1, Rb_2, Rc, and Rd Rg_1	PPD-type PPT-type	Ginsenoside level increases in leaf Ginsenoside level decreases in roots	Li and Mazza 1999
Iron (Fe), Zinc (Zn), Manganese (Mn), Copper (Cu)	*Panax ginseng*	Rb1, Rb2, Rb2, Rc and Rd, Rb3 Rg1, Re, Rf	PPD-type PPT-type	Ginsenoside level increases in roots	H. Zhang et al. 2013
Nitrogen (N) and phosphorus (P)	*Panax quinquefolium*	Rb1, Rb2, Rc, Rd Re and Rg1	PPD-type PPT-type	ginsenoside level increases hairy roots	Kochan et al. 2016

19.1 mg g^{-1} dry weight (Park and Lee 1993). However, beyond 50% of solar radiation exposure, the levels of ginsenosides decreased and resulted in the premature death of the plants because of the photobleaching (Parmenter and Littlejohn 2000). Moreover, irradiance from the artificial sources of light such as monochromatic light-emitting diodes (LEDs; source of a specific emission spectrum) not only affected the concentration of ginsenosides but also affected the interconversion of ginsenosides from one form to another (Park et al. 2012). The concentration of ginsenosides (Rg_1, Rb_2, and Rc) increased by approximately 74.1% and 64.9% on exposure to 470 and 450 nm wavelength of light respectively emitted through LEDs along with the increased ratio of PPD and PPT-type ginsenosides (Park et al. 2012). Light quality also influences ginsenoside accumulation within the plant roots as observed in American ginseng where red (R), far-red (FR) elevated ginsenosides (Rb1, Rc, Rb2, Rd, Rg1, Re) to appreciably high amount while blur light did not show any effect on their accumulation (Fournier et al. 2003). The effect of irradiance on ginsenoside biosynthesis was also related to the different growth stages of the plants. Under PAR, the relative levels of Rf, Rg1, and PPT-type ginsenosides increased along with the maximum accumulation in the leaf opened growth stage of ginseng as compared to other growth stages including root growth, green and red fruit stages (Zhang et al. 2018). The plant foliation stage also accounts for ginsenoside accumulation and it is speculated that ginsenosides may get transported from one part to another during the transition of growth stages, thereby leading to altered ginsenoside profiles to sustain the growth and defence machinery of the plant (Kim et al. 2014b). Temperature is another crucial environmental factor that not only mediates plant growth and development but also influences the mass accumulation of ginsenosides (Jochum, Mudge, and Thomas 2007). For instance, hypothermic stimulations significantly increased the total concentration of saponins in adventitious roots of Korean ginseng and promoted the accumulation of several ginsenosides including Rg_1, Rf, Rg_2, Rh_1, Rg_3, Rh_2 by upregulating the transcript levels of ginsenoside biosynthesis genes such as *PgWRKY8, PgWRKY1*, *PgWRKY3*, *PgWRKY5*, *PgWRKY2* and enhancing the activities of associated enzymes such as squalene synthase and geranyl diphosphate synthase (S. Wang et al. 2019).

Besides, soil nutrients are one of the major determinants of plant growth, especially during unfavorable environmental conditions. Soil nutrient levels also affect ginsenoside concentration most significantly in plant leaves (Li and Mazza 1999). Application of gypsum ($CaSO_4.2H_2O$) altered soil properties including pH and electrical conductivity and increased calcium, manganese, copper, aluminum, and sulfur content in the soil, which positively correlated with the

increase in Rb_1, Rc, and Rd ginsenosides with an elevation of total ginsenoside concentration (Lee and Mudge 2013b). However, the fresh weight of root before transplanting was considered as the covariate and contributed to Rb_1 accumulation but did not affect other ginsenosides (Lee and Mudge 2013b).

Heavy metals have emerged as a potential threat to living organisms including both plants and animals due to their toxicity, non-biodegradability, and hazardous health illnesses (Cheng et al. 2019). Heavy metals such as Cd also affect the concentration of ginsenosides, by altering the activities of enzymes associated with their biosynthesis and metabolism, and the growth of the plants (Zu et al. 2020). The concentration of ginsenosides Rg_1, Rb_1, R_1 decreased during cadmium stress, the ratio of Rg_1/Rb_1 negatively correlated with soil Cd levels, and the activities of related enzymes such as SS, mevalonate kinase (MVK) increased while β-amyrin synthase and P450 reductase decreased under Cd stress in Chinese ginseng (Z. Li et al. 2020). Interestingly, the excess of lime treatment reduced soil Cd concentration and alleviated Cd stress toxic effects in the plant by amelioration biomass, root length, surface area, and volume, decreased the ginsenoside concentration and activities of related enzymes (SS, MVK, β-amyrin synthase, and P450 reductase) (Z. Li et al. 2020). Thus, several external and internal factors are responsible for altering ginsenoside concentrations within the plant. Regulating these factors during the cultivation of potential ginsenoside sources may further aid in enhanced ginsenoside production with high efficiency.

5. Methods developed for the quantification of ginsenosides

Analytical analysis of phytochemical contents in their reliable sources is of paramount importance for their therapeutic applications. Moreover, advancements in ginsenoside utilization in pharmacokinetics have necessitated the phytochemical profiling, authentication check, and quality control of such bioactive specialized metabolites. Furthermore, different saponins in *Panax* may have opposing pharmacological activities (Wang et al. 2020) and thus any adulteration within these metabolites may lead to adverse effects in the human body. Therefore, multiway and accurate estimation of ginsenosides are of paramount biological importance to assess the quality and quantity of ginsenosides. A number of methods have been developed for the efficient quantification of ginsenosides based on thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), ultra-high-performance liquid chromatography (U-HPLC), gas chromatography (GC), and gas chromatography-mass spectrometry (GC-MS; Vanhaelen-Fastré, Faes, and Vanhaelen 2000; Kevers et al. 2004; Ha et al. 2013; Lee et al. 2017, 2021; Wang et al. 2021; Chen et al. 2021).

The ginsenosides must be isolated well for their efficient quantification. Several extraction methods (traditional and advanced) with different solvents have been developed for the isolation of ginsenosides. The traditional solvent technique for the extraction includes heat-reflux, shaking, and ultrasound-assisted methods, each having its own merits and demerits (Jegal, Jeong, and Yang 2019). On the other hand, some of the modern extractions techniques use microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), pulsed electric field extraction (PEFE). The modern extraction techniques based on ultra-pressure are relatively less time-consuming and more efficient as compared to the conventional heat-reflux methods (Luque de Castro and Garcıá-Ayuso 1998). At first, a two-dimensional thin-layer chromatography and spectrophotometry were used to analyze the isolated ginsenosides in Asian and American ginseng (Lui and Staba 1980). Among the Ginsenosides F11, Rg1, Rg2, Rf, Rc, Rd, Rc Rb2, Rb1, and Ro that were separated on TLC plate and the presence of ginsenoside Rf was found to be characteristic of Asian ginseng. HPTLC densitometry after detection with thionyl chloride was shown to be an attractive method for the simultaneous determination of the six major ginsenosides in Korean ginseng roots concerning the accuracy, selectivity, and reproducibility in comparison to the other known methods and can be performed without any derivatization step (Vanhaelen-Fastré, Faes, and Vanhaelen 2000). As of today, HPLC is the most commonly used method for the analysis of ginsenosides, due to its speed, sensitivity, adaptability to polar compounds, and availability of several techniques for the detection of ginsenosides such as ultraviolet (UV), evaporative light scattering detection (ELSD), fluorescence, and MS (Christensen 2009; Fuzzati 2004). A comparative analysis of ginsenoside content among 21 ginseng products, including Korean ginseng and North American ginseng in several forms were performed using HPLC with ultraviolet detection at 203 nm (Li and Fitzloff 2002). As UV detection of ginsenosides is usually performed at a short wavelength of 198 to 205 nm, it has some drawbacks such as a noisy baseline, reduced sensitivity, and limited choice of solvents (Baek et al. 2012). Another detection, termed as evaporative light scattering detector (ELSD) was first developed by Park et al. 1996 and has been used to analyze ginsenosides in Korean ginseng. This ELSD-based detection is claimed to be superior to the UV-based detection method with respect to both sensitivity and separation with minimum detectable concentration to be more than 35 ng of ginsenosides on the column. However, a comparative analysis of in-line connected UV and ELSD methods in the quantification of ginsenosides by reversed-phase HPLC showed that the UV detection has good linearity in the range of 100–2000 ng of ginsenoside with a minimum detectable concentration of 10 ng of ginsenosides on the column. On the other hand, the sensitivity of the ELSD was found to be 5 times lower than that of UV with a minimum detectable concentration of 50 ng of ginsenosides on the column (Li and Fitzloff 2002). Although quantitative evaluation in terms of ginsenosides showed similar results using both methods of detection, UV is still a recommended method for the routine analysis of ginseng samples. Shangguan et al. (2001) developed a new HPLC method with fluorescence-based detection in which pre-column derivatization based on the double bond in the C_{24}-C_{25}

position of all the ginsenosides was explored using reference ginsenoside Rg_1 and ginsenoside Rb_1. The derivatized products, thus produced, were then separated and characterized using HPLC and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS. This HPLC fluorescence method was found to be the most sensitive method for detection of ginsenosides as compared to other known methods up to then having limits of detection, 2 ng (about 2.5 pmol) and 1 ng (about 0.9 pmol) with a signal-to-noise ratio of 3 for ginsenosides Rg_1 and Rb_1 respectively. However, because of the lack of a suitable fluorescence chromophore in ginsenosides, the samples must be derivatized prior to the analysis (Shangguan et al. 2001). Recently, six non-polar ginsenosides (that are known to be specifically found in red ginseng) have been detected and quantified using reverse phase HPLC technique coupled to an integrated pulsed amperometric detection in the rhizome head, main roots, lateral roots as well as hairy roots (HR) of white ginseng (Song, Song, and Hong 2020).

A liquid chromatography-tandem mass spectrometry (LC – MS – MS) method was developed for unambiguous on-line identification and discriminating Korean ginseng and North American ginseng. The method is based on the baseline chromatographic separation of two potential chemical markers present in ginseng root methanolic extracts, ginsenoside Rf and 24(*R*)-pseudoginsenoside F_{11}, and their using tandem mass spectrometry (Li et al. 2000). Using GC and GC-MS methods in ginsenoside estimation, Cui et al. (1993) conducted a study in which alkaline and acidic cleavage products of ginsenosides Rb_1, Rc, Rd, Re, and Rg_1 were separated, identified, and characterized by GC, GC-MS, and HPLC and an optimized alkaline hydrolysis method was also developed for production of aglycone components of 20*S*-protopanaxadiol and 20*S*-protopanaxatriol from ginsenosides in 80% yield and alkaline treatment caused no epimerization, no hydroxylation, and no cyclization of the side chain as compared to the acidic hydrolysis treatment for ginsenosides. Despite good sensitivity and resolution, GC requires complex sample preparations and can only be applied for limited analysis (Cui et al. 1993). Corthout et al. (1999) validated a new TLC-densitometric assay for the quantitative determination of ginsenosides for ginseng roots and a ginseng dry extract and the method was found to be linear and accurate with a precision of 2.73% for Rb1-group, 4.59% for Rg1-group, and 2.88% for the determination of the total ginsenosides in ginseng roots. For dry extract, the reported values were 2.41, 3.45, and 2.36% respectively. This method is relatively quick and straightforward as compared to GC and is more sensitive than the existing HPLC-based methods.

Moreover, near-infrared spectroscopy (NIRS) has also been used for simultaneous quantification of ginsenosides in American ginseng roots (Ren and Chen 1999). This spectroscopic technique does not require any sample preparation and the accuracy and precision of the method for the ginsenosides quantification are comparable with those obtained with the HPLC method (Ren and Chen 1999). However, the only drawback of this method is that the instrument has to be calibrated for quantitative analysis by using a set of samples with known analyte concentrations which are obtained by suitable reference methods (Fuzzati 2004).

UHPLC is a relatively recently evolved liquid chromatography that provides better separation in a short period (Nováková, Matysová, and Solich 2006). It has also found its applications in ginsenoside analysis along with other pharmaceutical analyses. A UPLC-PDA method was developed for simultaneously quantitative and qualitative determinations of 30 ginsenosides in various *P. ginseng* preparations (Nováková, Matysová, and Solich 2006; Park et al. 2013; Chen et al. 2021).

Tian et al. (2009) developed the capillary electrophoresis (CE) method for the determination of ginsenoside Rg1, Re, and Rb1 in ginseng samples with limits of quantification (S/N = 10) at about 5.2–7.3 μg ml^{-1} for Rg1, Re, and Rb1 under the detection at 203 nm, whereas the overall recoveries were greater than 83.0% spectrometry (Li et al. 2000). In addition, recently developed enzyme immunoassays are considered to be the recent biochemical advancement in ginseng analysis for the determination of ginsenosides. These include enzyme-linked immunosorbent assay (ELISA), immunoblotting, and immunofluorescence. Sritularak et al. (2009) employed a monoclonal antibody for ELISA-based determination of ginsenoside Rb_1, Rg_1, and Re in American ginseng berries and flowers. Meanwhile, an immunodetection technique called "Eastern blotting" was developed to determine ginsenoside Rb_1, Rg_1, and Re with monoclonal antibodies targeting each ginsenoside (Tanaka, Fukuda, and Shoyama 2007). Besides, eastern blotting and ELISA also find their application in the analysis of the distribution of ginsenosides in Araliaceous plants using Rb_1 antibody (Tung et al. 2013). Further, Yokota, Onohara, and Shoyama (2011) performed immunofluorescence to monitor the localization of ginsenoside Rb_1 in the various parts of *P. ginseng*. Also, despite having the highest sensitivity, the enzyme immunoassay is suitable for only one ginsenoside and the other ginsenosides, if co-existing, may interfere with the determination. Altogether, a number of methods have been developed for quantitative analysis of ginsenosides each having its own merits and demerits, however, the choice of analysis method depends on experimental conditions and requirements of the planned study.

6. Functional attributes of ginsenosides: Nutraceutical, pharmaceutical, and plant defence

Ginseng (*Panax ginseng* and *Panax notoginseng)* is one of the most popular herbal plants having roles both as a dietary supplement and herbal medicine (Chan and Fu 2007). The nutraceutical roles of ginseng are mainly because of the bioactive ginsenosides that are not only effective as a functional food and in the treatment of human ailments but also play important roles in plant defence against several biotic and abiotic stress conditions (Huang et al. 2019; Farh et al. 2020; My et al. 2020).

6.1. Significance of ginsenosides in pharmaceutical biology and its toxicity issues

The branches of phytochemistry and pharmacology are continuously evolving to facilitate the promotion of human health and the suppression of diseases. Identification, isolation, and functional characterization of plant-derived specialized metabolites with pharmaceutical properties are primarily being used in drug discovery and pharmacology to assist the treatment of severe ailments (Velu, Palanichamy, and Rajan 2018). Several plant bioactive compounds or phytochemicals have been identified with anti-diabetic (Aba and Asuzu 2018), anti-inflammatory (Zhu, Du, and Xu 2018), adaptogenic (Kamal, Arif, and Jawaid 2017), antioxidant, antifungal (Prakash et al. 2020), antibacterial (Barbieri et al. 2017), hepato- (Pereira, Barros, and Ferreira 2016) and neuroprotective properties (Huang et al. 2019). Of these, ginsenosides, in particular, show anti-carcinogenic (Zou et al. 2020; My et al. 2020), anti-hyperglycemic (Jeong et al. 2020), immunomodulatory (F. Chen et al. 2017), adaptogenic (Fernández-Moriano et al. 2017), and anti-inflammatory (J. Jiang et al. 2020) effects and thus are of great value to pharmacological sciences (Table 2). For many years, ginsenosides are being used in treating diabetes mellitus due to their ability to control blood glucose levels by improving β-cell functions and reducing oxidative stress, inflammatory effects, autophagy, and ultimately apoptosis (B. Zhang et al. 2019b; Shao et al. 2020). Interestingly, clinical trials using ginsenoside extracts for controlling diabetes have been successful with no adverse effects reported so far (Shao et al. 2020). Ginsenosides such as Rb1, Rg1 show anti-carcinogenic effects against tumor cell cytotoxicity and help in curing other cancer-induced abnormalities such as inflammation and angiogenesis (Lahiani et al. 2017; Mohanan et al. 2018). For instance, ginsenoside Rg3 effectively reduced chemosensitivity in hypoxic lung cancer therapy (Wang et al. 2018 Wang et al., 2014) while Rh2 effectively inhibited prostate cancer cells by suppression of microRNA-4295, which activates translation of CDKN1A, a gene that plays a key role in cell cycle arrest (Gao and Zheng 2018). Similarly, a rare, dammarane type ginsenoside with PPT sapogenin and D-glucopyranose, Rk3 has been shown to reduce oesophagal tumor growth by repressing various signaling pathways such as mTOR, Akt, and PI3K that are associated with cell proliferation and cancer (H. Liu et al. 2019). Moreover, ginsenosides Rk3 and Rh4 have been shown to exhibit anti-nephrotoxic effects, reduction in kidney dysfunctions, inhibition of kidney damage conditions such as renal edema, and reduction of oxidative stress damages by increasing activities of antioxidant enzymes in renal cells (Baek et al. 2017). Ginsenosides such as Re and Rb1 possess antioxidant efficacy to treat severe cardiovascular ailments such as myocardial infarction, diabetic cardiovascular complications, and atherosclerosis by protecting cardiomyocytes against oxidative stress due to overaccumulation of oxidants (Kurian et al. 2016). The effect of ginsenosides on the central nervous system (CNS) is also indicated by the presynaptic facilitatory action of Rb1and Rg1 on glutamate release by activating A protein kinase from cortical synaptosomes (Chang et al. 2008).

Apart from the pharmaceutical relevance of ginsenosides, some short-term and long-term toxic effects have also been observed in some patients provided with different doses of ginsenosides. Dosage of ginsenosides taken, may influence absorption, metabolism, and excretion of the ginsenosides and lead to their toxic effects (Gao et al. 2020). Ginsenosides intake is mainly through the consumption of ginseng either directly as the root or consumed in the form of energy drinks or beverages (Ku 2016). However, oral intake of ginsenosides is a bit ineffective as the absorption rate of major ginsenosides is very low due to poor cell membrane permeability, instability in the gastrointestinal tract, less solubility in saliva, different cleavage efficiency of gastric acids, and microbiota diversity in different individuals (Bae, Park, and Kim 2000; Yu, Chen, and Li 2012, Xia et al. 2013; H. Kim et al. 2018). Moreover, other properties of ginsenosides also confer to their low bioavailability in the human body including lesser membrane permeability to the large size dammarane skeletons (Li et al. 2011), functioning of a glycoprotein efflux system which results in the efflux of glycosylated ginsenosides (X. Zhang et al. 2013) and lack of sugar moieties in aglycone structures that reduce ginsenoside solubility (Leung and Wong 2010). Contrarily, unwanted delivery of ginsenosides may lead to their hyperaccumulation in non-targeted sites of the body that can further lead to ginsenoside toxicity (Vazquez and Aguera-Ortiz 2002). Henceforth, depending on the type of ailment, appropriate dosage values and delivery systems such as microemulsions, polymeric nano-, or microparticles, have been designed for ensuring optimal bioavailability of ginsenosides that may modify ginsenosides at the target sites in the body without any lethality (Han et al. 2009; Beak et al. 2015; H. Kim et al. 2018).

6.2. Role of ginsenosides in plant defence

Specialized metabolites are the bioactive compounds that are synthesized within the plant in response to unfavorable environmental conditions to enhance the survivability of the plant during stress conditions (Hiruma 2019). Ginsenosides are one such metabolite that plays a major role in plant defence responses against several biotic and abiotic stress conditions (Yang et al. 2015; Farh et al. 2020). Ginsenosides also exert potential allelopathic effects during plant-microbe interaction that may protect the plant from microbial diseases (Bernards, Yousef, and Nicol 2006). Besides, ginsenosides also function as fungitoxic compounds in plant defence and are thus considered as phytoanticipins (Favel et al. 1994; Nicol, Traquair, and Bernards 2002). Natural ginsenosides protect the ginseng plant from pests and pathogens, while the bitter taste of the plants makes it an antifeedant (H. Yang et al. 2018). A ginsenoside extract consisting of 9 ginsenosides including Rg1, Rf, Re, Rb1, Rc, Rg2, Rb2, Rd, and Rb3, prepared from the aerial parts of Korean ginseng showed significant antifeedant activity against *Plutella xylostella,* the most dangerous insect

Table 2. Functional attributes of ginsenosides in treatment of human ailments.

Type of study	Source plant	Ginsenoside characterized	Structural characterization	Method used for detection	Medicinal property	Biological effect of identified ginsenoside	References
In vitro	*Panax notoginseng*	Rb1	PPD ginsenoside	HPLC	Anticancer effect	Cytotoxic activity against breast carcinoma (MCF-7) cell lines and human lung carcinoma (Lu) cell lines	My et al. 2020
In vitro	*Panax ginseng*	Rg3	PPD ginsenoside	–	Anticancer effect	Supresses the cell growth of chemo-resistant pancreatic cancer.	Zou et al. 2020
In vivo	Black ginseng processed from *Panax ginseng* by steaming	Rh4, Rg5, and Rk1	PPD ginsenoside	HPLC	Anti-hyperglycemic and hypolipidemic effects	Decrease the levels of fasting blood glucose, HbA1c, insulin levels and thiobarbituric acid reactive substances values. Improves hepatic steatosis in the liver and the size of adipocytes in muscle tissue.	Jeong et al. 2020
In vitro	*Panax ginseng*	Rb1 Rg1	PPD ginsenoside PPT ginsenoside	HPLC	Adaptogenic effect	Amelioration in redox status within the cells; they reduced ROS and TBARS levels and improved the glutathione system, as well as they enhanced SOD activity and Nrf2 pathway activation. Protects neuronal cells against MMP loss, calcium homeostasis disruption and aconitase inhibition	Fernández-Moriano et al. 2017
In vitro	*Panax ginseng*	Rk3 Rh4	Consisting of PPT sapogenin and D-glucopyranose –	Reverse phase-HPLC, Chromatography-electrospray ionization-mass spectrometry and HPLC-ELSD.	Anti-nephrotoxic effect	kidney dysfunctions, inhibits kidney damage conditions such as renal edema	Baek et al. 2017
In vivo	*Panax notoginseng*	Rd	PPD ginsenoside	Spectroscopic methods (IR, MS, and NMR) and HPLC	Immunostimulatory effect	Promote production of Th1 or Th2 cytokines to induce immune responses in mice against ovalbumin (OVA)	Z. Yang et al. 2007
In vitro *and* in vivo	*Panax notoginseng,*	Rf	PPT ginsenoside	–	Neuroprotective and anti-inflammatory effect	Suppresses neuroinflammation and alleviates A-induced neuronal death in N2A cells and inhibition of neuronal apoptosis.	Du et al. 2018
In vitro	*Panax ginseng*	Rb1	PPD ginsenoside	–	Anti-angiogenic effect	Mediates peroxisome proliferator-activated receptor-γ signaling. By upregulating pigment epithelial-derived factor (PEDF) protein expression that inhibits endothelial tube formation, further inhibiting inhibition of angiogenesis.	Lu et al. 2017

"–" means "not found."

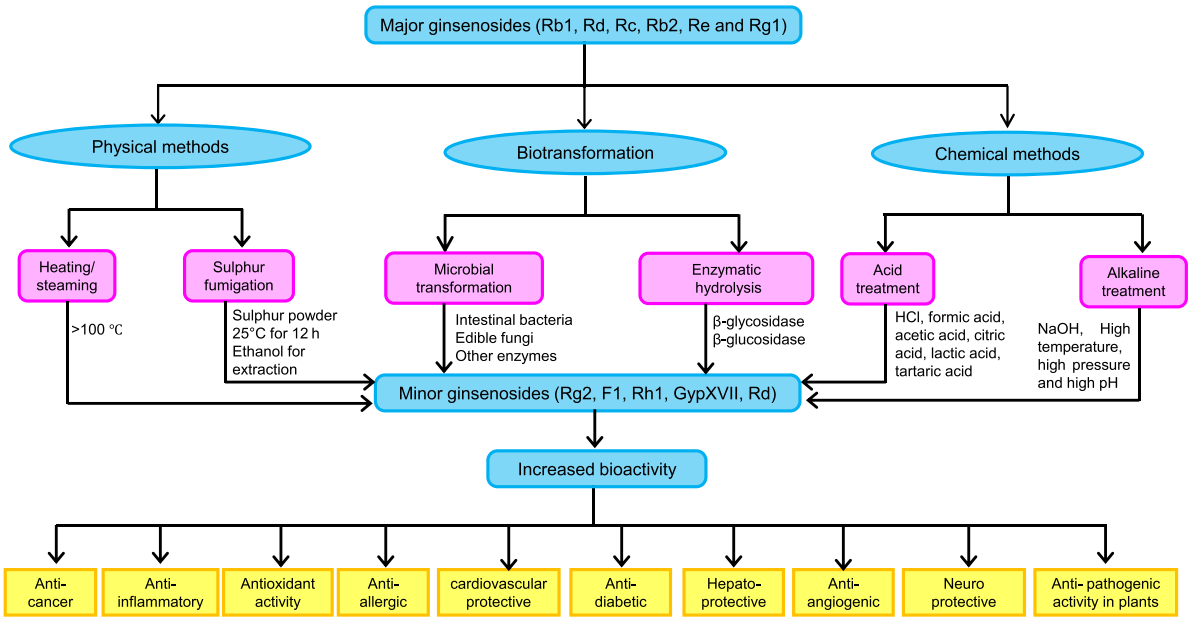

Figure 4. Biotransformation of major ginsenosides into minor deglycosylated forms. Major ginsenosides such as Rb1, Rd, Rc, Rb2, Re and Rg1 are glycosylated forms of ginsenosides with less biological activities as compared to the minor aglycone forms. Thus, there are several methods to transform major ginsenosides into minor ginsenosides such as Rg2, F1, Rh1, GypXVII, Rd that show more biological activity as compared to major glycosylated forms. Majorly, transformation can be carried out either by physical methods that involved heating, steaming and Sulfur fumigation or by chemical methods that involve treatment of ginsenosides with acids or alkali. Most crucial method among these is biotransformation that involved utilization of several microbes (bacteria, fungi, etc.) that can carry out transformation with efficiency. Further, minor ginsenosides are of great pharmacological significance and show several therapeutic effects such as antidiabetic, anti-cancer, and anti-inflammatory neuroprotective activities.

affecting cruciferous crops (H. Yang et al. 2018). American ginseng also showed resistance toward common ginseng pathogens as evident by the secretion of ginsenosidases by *Pythium irregulare* to degrade ginsenosides for increased disease progression in the plant (Ivanov and Bernards 2012). This ginsenoside metabolism-induced disease progression further supported the studies for the antipathogenic activity of the ginsenosides.

7. Biotransformation of ginsenosides

Ginsenosides are the most promising bioactive compounds that account for the pharmacological relevance of ginseng. However, among all the naturally occurring ginsenosides identified so far, Rb1, Rd, Rc, Rb2, Re, and Rg1 are considered as major ginsenosides as they constitute more than 80% of the total ginsenoside content in different *Panax* species (Park et al. 2017; Yu et al. 2017). Interestingly, there is a specific structure-function interrelation in ginsenosides suggesting that the structural alterations in ginsenosides modulate their biological activities and thus change their pharmaceutical properties (Quan et al. 2015; Ying et al. 2018). Ginsenosides can be polar or less polar depending upon the number of sugar moieties present that majorly account for their biological activity (Kwon et al. 2001; Chen et al. 2009; Xue et al. 2020). It has been observed that the less polar or rare ginsenosides, containing a lesser number of sugar moieties exhibit more biological activity than polar or major ginsenosides (Chen et al. 2009; Xue et al. 2020). In addition, advancing evidence has demonstrated that minor ginsenosides are biologically more active than major ginsenosides (Kim et al. 2013; M.-S. Kim et al. 2019; Hong et al. 2020). Therefore, to ensure high biological activity for efficient assistance in disease suppression and other health benefits, consistent efforts are being made to convert major ginsenosides into minor ginsenosides by employing various physical, biological, and chemical transformation methods (Peng et al. 2017; Jin et al. 2012; Park et al. 2017; Chen, Nose, and Ogihara 1987) (Figure 4).

Biotransformation of major glycosylated ginsenosides into more active deglycosylated forms is carried out using enzymatic and/or microbial methods. Major ginsenoside Rb1, isolated from ginseng roots, has been transformed to minor ginsenoside C-K using β-glycosidase derived from endophytic fungus *Arthrinium*, stain GE 17-18 (Fu et al. 2016). Similarly, Rg1 and Re ginsenosides were transformed to Rh1 and Rg2 using a recombinant Bgp1 (β-glucosidase) from *Microbacterium esteraromaticum* that not only transformed major ginsenosides to minor but also enhanced the ginsenoside productivity (Quan et al. 2012). However, most of these microbial-based transformations of ginsenosides do not meet the grade scale for utilization as food, therefore, food-compatible microbes such as lactic acid bacteria are being used nowadays for biotransformation of major ginsenosides into deglycosylated forms (Park et al. 2017). Recently, it was reported that major ginsenosides Re, Rb1, Rg1 can be converted to deglycosylated and rare ginsenosides GypXVII, Rd, PPT, and Rg2 with improved anti-inflammatory activity at the cellular level using *Cellulosimicrobium* sp. TH-20 (Yu et al. 2017).

Enzymatic hydrolysis is another most prominent biotransformation method in which the sugar moieties of major ginsenosides are hydrolyzed using microbial glycosidases such as β-D-glycosidases. These microbial enzymes are

highly substrate-specific and are utilized on an industrial scale to ensures high productivity (Park et al. 2010; Geraldi et al. 2020). Moreover, exogenous treatment with other hydrolytic enzymes such as rapidase derived from *Aspergillus niger* and *Trichoderma longibrachiatum* particularly increases aglycones such as Rg3 within the plant (Choi et al. 2014). Some important minor ginsenosides such as Rg2, F1, Rh1 were obtained from a PPT saponin mixture using *β*-galactosidases from *Aspergillus oryzae*, naringinase from *Penicillium decumbens,* and lactase from *Penicillium* respectively (Ko et al. 2003). Another minor ginsenoside, Rg1 was obtained from Re selectively hydrolyzed by hesperidinase derived from *Penicillium sp.* (Ko et al. 2003). Bacterial strains such as *E. coli* strain BL21 also aided in the production of rare and biologically active ginsenosides F2 and gypenoside XVII (that possess neuroprotective and cardiovascular protective properties) by enzymatic hydrolysis of major ginsenoside Rb1 using recombinant *β*-glucosidase (Geraldi et al. 2020).

In addition to the above methods, biotransformation of major ginsenosides to minor ones has also been carried out using chemical treatments such as acids and alkalis along with physical methods such as heating, steaming, sulfur fumigation, or by heat from microwave (Peng et al. 2017; Zheng et al. 2017). In Chinese ginseng, 16 dammarane glycosides on treatment with artifact gastric juice were converted into deglycosylated, dehydrated, and hydrated secondary products with ameliorated cytotoxic effects to cancer cells (Wang et al. 2014). At 37 °C, treatment of 0.1 N HCl (a strong acid) for two hours led to the conversion of major ginsenosides Rg1 and Re to pro-sapogenin Re and Rg1 (Han et al. 1982). However, the pro-sapogenin formed complicated mixtures and hence were difficult to trace. In addition to the HCl, organic acids such as formic acid, acetic acid, lactic acid, citric acid, tartaric acid have also been used for the conversion of major ginsenoside to minor ones (Zheng et al. 2017). Similarly, alkaline hydrolysis (presence of an alkali such as NaOH) decomposes major ginsenosides into minor ginsenosides under extreme conditions such as high temperature, high pressure, and high pH (Zhu et al. 2014).

Heating at a high temperature >100 °C may also transform major ginsenosides into their minor forms. Kim et al. (2013) demonstrated that the thermal deglycosylation of major ginsenosides including Rb1, Rb2, Rc, Rd, and Re generated less polar ginsenosides 20(S, R)-Rg3, Rk1, and Rg5. Furthermore, diol type ginsenosides Rb1, Rb2, Rc, Rd indicated high proliferative activity as compared to Re with Rd exhibiting prominent anticancerous properties amongst all other ginsenosides (Kim et al. 2013). Since the transformation of ginsenosides by heating is a slow process, microwaves have also been used for the efficient and rapid transformation of ginsenosides (Zheng et al. 2017). Sulfur fumigation is also considered as an efficient method for transforming major ginsenosides into minor forms by treating ginseng with sulfur powder extract at 25 °C for 12 h, followed by the ethanol-mediated extraction of sulfur fumigated ginseng (Jin et al. 2012). However, sulfur fumigation can affect the contents of existing ginsenosides also and thus is not well adapted.

In nutshell, the broad-spectrum pharmacological efficiencies of minor ginsenosides and their large-scale industrial applications have persuaded researchers to transform major ginsenosides into minor deglycosylated forms, and the application of biotransformation, chemical, and physical methods have largely implicated in the feasibility of enhancing the biological activity of ginsenosides.

8. Elicitation based enhancement in ginsenoside accumulation

A consistent increase in utilization has put undue pressure on ginseng biologists for finding multifarious ways to enhance ginsenoside production for meeting up with high industrial demands. Elicitation-based approaches have emerged as an asset to upraise the biogenetic pathway of ginsenosides for their enhanced production (Kochan et al. 2017; Gantait, Mitra, and Chen 2020). Elicitors are the biotic or abiotic factors that act as the signals to enhance the accumulation of ginsenosides by triggering the activity of genes or enzymes associated with their biosynthesis (Halder, Sarkar, and Jha 2019). Based on nature and origin, various hormonal, chemical, and biological elicitors have been identified that enhance the production of ginsenosides in plants (Table 3).

8.1. Hormonal and signaling molecules based elicitors

The most common types of elicitors are the signaling molecules or phytohormones that intricately affects plant growth during adverse environmental conditions (Jamwal, Bhattacharya, and Puri 2018). The accumulation of specialized metabolites simultaneously increases within the plants to alleviate stress toxicity (Berini et al. 2018; Arnold, Kruuk, and Nicotra 2019). Growing evidence is detailing the effects of different phytohormones on the biosynthesis and accumulation of ginsenosides in plants (Bae et al. 2006; Kim et al. 2009; Gantait, Mitra, and Chen 2020).

JA and its derivatives such as methyl jasmonate (MeJA) have emerged as strong hormonal elicitors that enhance ginsenoside production in plants (Kim et al. 2009). Exogenous supplementation of JA (10 mg l^{-1}) promoted the accumulation of Rg and Rb ginsenosides in adventitious root cultures of Korean ginseng, however, the accumulation of Rb increased more significantly than Rg (Yu et al. 2002). Similarly, the application of 300 mg l^{-1} JA also increased total ginsenoside concentration and enhanced ginsenoside production in the hairy root cultures of Korean ginseng with the most significant increase in Rb ginsenosides (PPD-ginsenosides) and deleteriously affected adventitious root growth (Yu et al. 2000), suggesting JA might stimulate the activity of enzymes associated with PPD-type ginsenosides. Besides, MeJA also affects the production of ginsenosides in plants. For instance, exogenous supplementation of MeJA (250 μM l^{-1}) to the hairy root culture of *P. quinquefolium* for 7 days altered the expression of the squalene synthase gene and increased the concentration of Rb1 by 5.6-fold and

Table 3. Several types of elicitors including chemical, biological and hormonal elicitors that enhances the production of ginsenosides in *Panax* and its allied species.

Plant	Name of the elicitor	Concentration	Duration of exposure	Culture type	Ginsenoside enhanced	Other plant traits affected	References
Hormonal elicitors							
Panax ginseng	Ethephon + Methyl jasmonate	50 μM + 100 μM	8 days	Adventitious root cultures	Rg group (Re, Rf, and Rg1)	Dry weight of the culture enhanced along with the ginsenoside productivity.	Bae et al. 2006
Panax ginseng	Methyl jasmonate	150 μM	40 days	Adventitious root cultures	Rb (Rb1, Rb2, Rc and Rd) > Rg (Re, Rf and Rg)	Inhibition of root growth	Kim et al. 2004
Panax ginseng	Methyl jasmonate	200 μM	8 days	Callus suspension culture	Rb group (Rb1 content increased 4-times but the contents of Rb2, Rc and Rd increased slightly). Rg group (Rg1 and Re increased 2.3-fold and 3.0-fold respectively) while Rf increased slightly	Fresh weight, dry weight and growth ratio of the cells, decreased	Thanh et al. 2005
Hybrid ginseng (*Panax ginseng*×*P. quinquefolium)*	Auxin (3-indole butyric acid (IBA), 1-naphtaleneacetic acid (NAA), 3-indoleacetic acid (IAA)) IBA + NAA	2.5 μM 0.5 μM +1.0 mM	8 weeks 8 weeks	Hairy root culture Hairy root culture	Rb1 and Re increased most significantly approximately by 5-folds and 3-folds respectively. Besides, total ginsenoside content (Rb2 + Rc, Rd + Rb1+ Re + Rg) increased up to 1.63% in NAA 1.58% in IBA 1.49% in IAA and 1.42% in hormone-free media Yield of ginsenosides increased by 1.7-folds	Lateral root growth formation and fresh weight of hairy roots increased	Washida et al. 2004
Panax quinquefolium	Methyl jasmonate	250 μM l^{-1}	7 days	Hairy root culture	Rb1 increased by 5.6-fold and Re increased by 1.8-fold	Altered expression of the squalene synthase gene	Kochan et al. 2018
Panax ginseng	Methyl jasmonate	22.4 mg l^{-1}	28 days	Hairy root culture	Total concentration of Rb and Rg group ginsenosides increased by 2-fold, 1.8-fold and 4-fold greater in root lines C-M, (callus-like morphology); HR-M (hairy root morphology) T-M roots (thin without branching morphology) respectively	Reduced biomass formation	Palazón et al. 2003
Biotic elicitors							
Panax ginseng	*Mesorhizobium amorphae* (GS3037) *Mesorhizobium amorphae* (GS336)		5 days	Adventitious roots culture	Rb2 and Rb3 increased (19.4- and 4.4-fold, and 18.8- and 4.8-fold) by *Mesorhizobium amorphae* (GS3037) and *Mesorhizobium amorphae* (GS336), respectively. Increase in total concentration of ginsenosides PPD-group (Rb1, Rb2, Rb3, Rc, Rd., Rg3, Rh2, and CK) and PPT-group (Re, Rf, Rg1, and Rh1) by 1.3-fold.	Root growth was reduced after treatment with all bacteria examined which led to dramatically reduced root biomass compared with untreated adventitious roots	Le et al. 2018
Panax quinquefolius	*Alternaria panax Cylindrocarpon destructans*	4 mg L^{-1} 20 mg L^{-1}	8 days 8 days	Adventitious roots culture Adventitious roots culture	Production of Rg3, Rh2, and Re increased with enhanced productivity for about 2.3-folds Production of Rg3, Rh2, and Rf increased with total ginsenoside content 4.0-fold higher that untreated root culture	Biomass, NO and Putrescine levels increased,	Yu et al. 2016

(*continued*)

Table 3. Continued.

Plant	Name of the elicitor	Concentration	Duration of exposure	Culture type	Ginsenoside enhanced	Other plant traits affected	References
Panax quinquefolius	*Trichoderma atroviridae*	2.5 % v/v	5 days 15 days	Cell suspension culture	Highest cumulative yield of ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rg1, Rg2, and Rh1) increased by 3.2-fold of control Ginsenoside Rg1 was 7-8-fold higher		Biswas et al. 2016
Panax quinquefolius	*Trichoderma harzianum*	1.25 % v/v	15 days	Cell suspension culture	Enhanced production of ginsenosides Rb1, Rg3, Rh2		Biswas et al. 2016
Panax quinquefolius	*Pseudomonas monteilii*	2.5 % v/v	5 days	Cell suspension culture	Total concentration of ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rg1, Rg2, and Rh1) increased by 2.4 folds		Biswas et al. 2016
Chemical elicitors							
Panax ginseng Suspension cell cultures	N, N′- The maximal content of total ginsenosides				dicyclohexylcarbodiimide (DCCD) (Rg1 + Re + Rb1 + Rc + Rd) increased to 3.0-fold that of untreated control. Rg1 and Re ginsenosides increased by 2.5-folds and Rb1 increased by 8.9-fold respectively	10 μM DCCD inhibited cell growth. The genes and enzyme activity associated with ginsenoside biosynthesis significantly increased.	4 Days Huang, Qian, and Zhong 2013
Panax ginseng	Vanadate	50 μM	4 days	Suspension cell cultures	Total ginsenoside content (Rg1 + Re + Rb1 +Rc + Rd) by vanadate elicitation increased by 3.6-folds. Rb1 increased by 10.1 folds, and Rg1 and Re was increased by 1.5 and 5.6 folds respectively	Endogenous JA biosynthesis was induced and transcript levels of sqs, se and ds genes were upregulated	Huang and Zhong 2013
Panax ginseng	Colchicine Mutagenesis	100, 200, and 300 mg·L^{-1}	1, 2, and 3 days	Adventitious roots culture	Enhanced ginsenoside productivity (Rb1, b2, Rb3, Rc, Rd, Rg3, Rh2, Re, Rf, Rg1, Rg2) by 4.8-fold. Mutant 100–1-18 produced the highest amounts of total PPD and PPT ginsenosides (2.5-, 2.12-, and 2.68-fold higher than the control, respectively)	Increased root biomass, lateral root number and length	Le et al. 2020

Re by 1.8-fold (Kochan et al. 2018). The application of two or more hormonal elicitors in combinations has also been used to enhance ginsenoside levels in plants to an appreciable extent (Washida et al. 2004). The application of 25 μM indole-3-butyric acid and 100 μM MeJA elevated ginsenoside accumulation in the adventitious roots of Korean ginseng (Kim et al. 2007). Similarly, the application of ethylene precursor molecule ethephon in Korean ginseng enhanced ginsenoside content in roots and also contributed to root growth, however, the productivity of ginsenosides also increased more significantly by the synergistic effect of the combined application of ethephon and MeJA in the cell culture of adventitious roots (Bae et al. 2006).

MeJA enhanced total ginsenoside accumulation (Rg1 + Rf + Re + Rc + Rb1 + Rb2+ Rg3+ Rd + Rh2) in the adventitious root culture of Korean ginseng along with the increment in Putrescine (Put) Hydrogen peroxide (H_2O_2) and Nitric oxide (NO) contents (Wu et al. 2020). However, when the MeJA induced cultures were treated with the inhibitors such as D-(-)-arginine (an inhibitor of polyamine putrescine), catalase (CAT; a ROS scavenging enzyme), and inhibitors of nitric oxide synthases (enzymes catalyzing NO production) such as NG-nitro-L-arginine methyl ester and S, S′-1,3-phenylene bis (1,2-ethanediyl)-bis-iso thiourea ($C_{12}H_{18}N_4S_2$), significantly reduced ginsenoside accumulation was observed in root cultures (Wu et al. 2020). Application of sodium nitroprusside (SNP), a source of NO led to an increase in Rc concentration by 2.6-folds and Rb2 by 2 folds when supplemented individually. SNP in combination with MeJA elicited the concentration of ginsenosides Rb1by 1.3-folds, Rc by 1.3-folds, and Re by 1.2-folds, suggesting the efficiency of combinational application of signaling molecules in ginsenoside production (Rahimi et al. 2015). In addition to JA and ethylene, SA also mediates ginsenoside elicitation in close association with NO production by inducing the formation of superoxide anion in adventitious roots of ginseng (Tewari and Paek 2011). Treatment of adventitious root suspension culture of Korean ginseng with 100 μM SA induced ginsenoside accumulation enhanced NADPH oxidase activity and mediated ROS accumulation by increasing activities of antioxidant enzymes such as ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD; Tewari and Paek 2011). The production of ginsenosides also increased in Korean ginseng on treatment with 0.1 to 0.5 mM of SA and 0.1 to 1.0 mM of acetylsalicylic acid (ASA; Jeong et al. 2005). Elicitation of ginsenosides within the plant has also been reported to occur by exposure to heavy metals such as Copper and Vanadium (Ali, Hahn, and Paek 2006; Huang and Zhong 2013). Recently, a mutagenesis-based elicitation of ginsenoside content in adventitious root cultures of ginseng using a mutagenic agent such as colchicine has also emerged as an efficient approach that enhances ginsenoside content, increases biomass with lateral root number and length (Le et al. 2020). However, among all these, JA and especially its derivative, MeJA are considered as the most commonly used and highly efficient elicitors for the improvement of ginsenoside biosynthesis (Marsik et al. 2014; Kochan et al. 2017) as they regulate the transcriptional activity of candidate enzymes of ginsenoside biosynthesis such as FPP, SS, squalene epoxidase (Kim et al. 2009). Thus, there are ample elicitors that are recognized by their specific receptors present on the plant cell membrane and act as signals to trigger an increase in biosynthesis and accumulation of ginsenosides within their potential sources.

8.2. Biotic elicitors

Biotic elicitors are the microbial or pathogenic stimulators that trigger the accumulation of secondary metabolites to enhance plant defence responses in unfavorable environments (Le et al. 2018). There are ample studies that have reported the elicitation of ginsenoside production via biotic elicitors such as fungal elicitors either individually (Kochan et al. 2017; Le et al. 2018) or in combination with hormonal elicitors such as JA (Xu et al. 2005; Rahimi et al. 2014). For example, different concentrations of yeast extract elicit the accumulation of PPD-type (Rb1, Rb2, Rc, Rd) and PPT-type (Re Rg1) ginsenosides by 1.57 folds in hairy root cultures of American ginseng (Kochan et al. 2017). The fungal elicitors not only affect ginsenoside production but also influences other signal transducers such as JA and NO. Among three fungal elicitors prepared from fermentation and mycelium broth (*Aspergillus flavus, Aspergillus niger*, *Aspergillus oryzae*, *A. niger* elicited the maximum accumulation (3.52-folds) of ginsenosides in the adventitious roots of Korean ginseng along with the significant enhancement in the concentration of salicylic acid (SA), NO, JA and upregulation of genes associated with ginsenoside biosynthesis including FPS, SS, dammarenediol synthase, squalene epoxidase, cytochrome P450 genes (*CYP716A53v2*, *CYP716A47*) and UGT *(UGTpg100*, *UGT94Q2,* and *UGTAE2*) (Li et al. 2016). A comparative analysis using two antagonistic fungal elicitors, *Fusarium oxysporum,* and *Penicillium* sp. YJM-2013 revealed that *Fusarium oxysporum* enhances Rb, Ro, and Rg group ginsenosides with the maximum increase in Rb by 4.57-folds while *Penicillium* sp. YJM-2013 elicited the concentration of Ro, and the Ro ginsenosides by 4.44-folds suggesting that although both the fungal elicitors promoted ginsenoside production in adventitious roots of ginseng, the extent of their elicitation and efficiency differed (Wang et al. 2020).

Another important category of biotic elicitors consists of bacterial stimulators such as endophytic and nitrogen-fixing bacteria that enhance ginsenoside accumulation in plants (Song et al. 2017; Le et al. 2018). Ample of reports suggest the role of endophytes in ameliorating the metabolite content of the plants (Saraf, Pandya, and Thakkar 2014; Khare, Mishra, and Arora 2018). Endophytic bacteria LB 5-3 (10.0 mL), a close relative of *Bacillus altitudinis* (KX230132.1), the enhanced total concentration of ginsenosides (Rh1, Rb1, F1, Rd, Rg3, CK, Rh2) by four times in adventitious root culture of Korean ginseng, however, the combinational effect of MeJA with LB 5-3 negatively affected production of ginsenosides and adventitious root growth (Song et al. 2017). Foliar application of endophytic

elicitors may also enhance ginsenoside content within the plants. For instance, the field experiments suggested that the inoculation of *Paenibacillus polymyxa*, by foliar application or irritation supplementation by irrigation results in significant enhancement of ginsenosides of both PPD and PPT type (Re, Rg1, Rf, Rg2, Rb1, Rb2, Rd, and Rb3) along with the increased yield and plant quality (Gao et al. 2015). These studies pave new ways for advanced researches on ginsenosides enhancement within the plant by using potential biotic elicitors.

9. Transgenics advancements for improving ginsenoside concentration in potential sources

Consistent efforts for ameliorating the production of ginsenosides in their potential sources are being made. Biotechnological tools, employing genetic manipulation of genes and enzymatic activities associated with ginsenoside biosynthesis, are constantly evolving to reduce the pressure of the global ginsenoside demands. JA and its derivatives are the major elicitors that have shown effective results in ginsenoside production during in vitro cultures (Yu et al. 2002; Kochan et al. 2018). However, the molecular link between JA and ginsenoside biosynthesis has been established by the *PgLOX6* gene (JA biosynthetic 13-lipoxygenase gene; Rahimi et al. 2016) that shows a positive correlation with the expression of genes associated with ginsenoside biosynthesis. Ectopic expression of *P. ginseng PgLOX6* in Arabidopsis enhanced the concentration of JA and MeJA, upregulated the expression of triterpene biosynthesis genes such as *AtSS1* and *AtSE1*, and increased squalene content in the transgenic lines (Rahimi et al. 2016). Moreover, overexpression of *PgLOX6* resulted in increased transcripts for *PgSS1* (4.1-times), *PgSE1* (14-times), and *PgDDS* (8.4-times) genes in ginseng roots accompanied with an effective enhancement in ginsenoside levels (2.3-folds) most prominently in Rb2, Rb1, Rc, and Rd that were further enhanced up to 2.8-folds on MeJA treatment as compared to MeJA treatment to non-transgenic lines (Rahimi et al. 2016). Squalene synthase catalyzes the first step in the synthesis of phytosterols and triterpenoids and the overexpression of a squalene synthase gene (*PgSS1*) in Korean ginseng enhanced biosynthesis of triterpene glycosides (ginsenosides) and phytosterols (Lee et al. 2004). Furthermore, transgenic ginseng lines overexpressing squalene synthase gene, *PgSQS1* increased Rb1 (2-folds), Rc (3-folds), and Rd (17-folds) in vitro, whereas further treatment of transgenic lines with synthetic auxins (indole butyric acid or 1-naphtalene acetic acid) resulted in proliferated root growth, and enhanced dry weight of transgenic roots but inhibited relative concentration of ginsenosides in ginseng adventitious root cultures (Shim et al. 2010). Interestingly, a complete reversal in patterns of growth rate and ginsenoside concentration was observed on the treatment of transgenic lines with elicitors such as chitosan and JA that lead to ginsenoside accumulation and root growth retardation in ginseng plants indicating a negative regulation of phytohormones and a positive regulation of elicitors in ginsenoside accumulation. These molecular studies of genes associated with ginsenoside biosynthesis have revealed that transcript levels of such genes are stimulated or mediated by the presence of phytohormones such as MeJA (Han et al. 2010). Overexpression of a JA carboxyl methyltransferase gene (*AtJMT*) in adventitious roots of ginseng affected root growth as well as ginsenoside biosynthesis by upregulating genes involved in ginsenoside biosynthesis including *PgSE*, *PgSS1*, *PgDDS* along with the expression of JA-responsive genes such as *PgPR10-2*, increased ginsenoside levels (Rb1, Rc, and Rb2) by 2-folds and also altered heterogeneity of ginsenosides by modulating PPD/PPT ratio (Kim et al. 2012). Transgenic ginseng plants with the enhanced concentration of ginsenosides Rb1 (2-folds), Rc (3-folds), and Rd (17-folds) have been generated by overexpressing *PgSQS1* gene encoding squalene synthase enzyme, a prerequisite for ginsenoside biosynthetic pathway (Shim et al. 2010).

The genes involved in ginsenoside biosynthesis are also regulated by various trans-regulatory elements or transcription factors that bind to the candidate genes and regulate their expression to promote ginsenoside production more efficiently (Rahimi et al. 2016; T. Liu et al. 2019). For instance, several transcription factors such as *WRKY* (Yao et al. 2020), *bHLH* (Zhang et al. 2017), and *MYB* (T. Liu et al. 2019) have been identified that regulate ginsenoside biosynthesis. *PgWRKY4X* showed binding to the W-box of the promoter region of *SQUALENE EPOXIDASE* (*PgSE*) and increased the concentrations of Rb2, Rb1, Re, Rd, Rg1 by 1.38, 2.06, 2.68, 1.62, 2.34-folds respectively (Yao et al. 2020). Furthermore, several transcription factors have been identified that regulate ginsenoside accumulation in *Panax sp.* (Table 4).

10. Omics tools elucidating the pathways for ginsenosides biosynthesis and accumulation in plants

Although ample studies regarding pharmaceutical and health-promoting roles of ginsenosides are known, there are still fewer reports on operant of ginsenoside biosynthesis pathways along with the genes involved in it. In efforts to delineate the biosynthetic pathway of ginsenosides and their accumulation in plants, omics-based strategies are widely being used (Luo et al. 2011; Xue et al. 2019; X. Li et al. 2020). Omics tools such as genomics, proteomics, and metabolomics have aided in the identification of candidate genes and their expression profiles, proteins, and metabolites respectively associated with ginsenoside biosynthesis in plants and have successively backed up their applications in the clinical sector.

10.1. Genomics

Genomic information of intricate biosynthetic pathway of ginsenosides and the information on expression analysis of the related genes further aim to provide more clear insights into ginsenoside production (Xue et al. 2019). Although efforts are being made to unravel genes and their expression control networks that underlie ginsenoside biosynthesis,

Table 4. TFs regulating ginsenoside production in *Panax* and its allied species.

Plant	TF family	TF	Target gene	Promoter Sequence	Effect on ginsenoside concentration	Reference
Panax ginseng	WRKY	*PgWRKY4X*	*PgSE*	HMGR (Forward, 5′-TTTCTGA CTATATCCATC-3′, Reverse, 5′-AAAGACTGATATAGGTAG-3′) and PgSE (Forward, 5′-GAACGAATTTGACATAAATGTC-3′, Reverse, 5′-CTTGCT TAA ACTGTATTTACAG-3′)	Concentration of Rb1, Rb2, Rd, Re and Rg1 significantly improves as 2.06, 1.38, 1.62, 2.68, 2.34 folds respectively.	Yao et al. 2020
Panax quinquefolius	WRKY	*PqWRKY1*	*HMGR, FPS2, SQS1* and *SQE2* genes	CaMV35S	Enhances production of triterpenoid ginsenosides	Sun et al. 2013
Panax ginseng	MYB	*PgMYB2*	*dammarenediol synthase (DDS)*	CaMV35S	Increases *PgDDS* under different conditions treated by MeJA that may ultimately increase ginsenoside production	Liu et al. 2019
Panax notoginseng	bHLH	*PnbHLH1*	*PnDS, PnSS, PnSE, and PnFPS*	CaMV35S	Enhances biosynthesis of triterpenoid saponins (Rg1, Re, Rb1, and Rd)	Zhang et al. 2017
Panax ginseng	LOX	*PgLOX6*	*PgSS1, PgSE1 and PgDDS*	CaMV35S	Biosynthesis JA and increases ginsenoside content (Rg1, Re, Rf, Rb1, Rb2, Rc, and Rd) by1.4-fold	Rahimi et al. 2016
Panax notoginseng	AP2/ ERF	*PnERF1*	*PnERF1, DS and SS*	–	Enhances ginsenoside (Rg3, Rh1, Rd, Rg1, F1 and Re) levels	Deng et al. 2017

adequate information on the genetic basis for ginsenoside biosynthesis in *Panax* sp. is still scanty. High-quality chromosome level genomic and transcriptomic analysis of *P. notoginseng* was used to explore the saponin biosynthesis pathway spatially and temporally and the results revealed that genes related to saponin biosynthesis were differentially expressed in plant tissues at different plant ages (Z. Jiang et al. 2020). Spatially, most of the saponin biosynthesis-related genes including *PnUGT2*, *PnUGT3*, and *PnUGT4* (genes encoding post-modification enzymes) were found to express in flower, roots, and rhizomes, and during expression profiling, 20 UGTs were identified with increased expression levels and expression patterns consistent to pathway genes (Z. Jiang et al. 2020). Transcriptome analysis of roots of *P. notoginseng* by next-generation sequencing technology revealed the candidate genes encoding enzymes that are some of the major players in triterpene saponin biosynthetic pathways such as dammarenediol synthase and genes encoding cytochrome P450 (*Pn02132* and *Pn00158*) and UGTs (*Pn00082*) that are likely to be involved in hydroxylation or glycosylation of aglycones (Luo et al. 2011). Transcriptome profiling of Korean ginseng showed that transcript abundances of genes related to ginsenoside biosynthesis relevant to different growth ages and different tissues of ginseng plants get altered (Xue et al. 2019). In the below-ground tissues, the transcript levels of genes related to ginsenoside biosynthesis were found to be higher in root barks, lateral roots, and rhizomes than in root cores while in above-ground parts, the genes related to the MEP pathway (such as DXR, DXS, IspD, IspF, IspE, IspG, and IspH) and metabolic pathway module of ginsenoside biosynthesis were transcribed more in leaves than in stems indicating that the origin of MEP pathway is primarily from the plastids. Additionally, 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (IspD) was also predicted as a key enzyme of the MEP pathway for ginsenoside production (Xue et al. 2019). Thus, it can be elucidated that the studies based on ginsenoside biosynthesis-related genes and their expression profiling aids in better understanding the genetic basis that mediates ginsenoside biosynthesis in *Panax* sp.

10.2. Proteomics

Proteome analysis usually relies on the information of genome sequence from the database and thus the information of ginsenoside biosynthesis-related genes obtained from genomics studies serves as an excellent target for proteomics research (Kim et al. 2014; Gupta et al. 2015). During ginsenoside biosynthesis, major variations in protein abundances are observed in plants such as *P. ginseng* (Ma et al. 2016). Proteomics can be used for the isolation and identification of proteins in different parts of the plants to explore the role of abundant proteins as markers and in relative metabolic pathways associated with ginsenoside biosynthesis (Kim et al. 2016). Proteome analysis of forest cultivated ginseng and wild-growing ginseng using 2DE and iTRAQ coupled with mass spectrometry provided insights into the accumulation of ginsenosides by identifying differential expression of enzymes (such as FPS, cycloartenol synthase, squalene epoxidases, and squalene synthase) involved in ginsenoside biosynthesis during different stages of plant development (Ma et al. 2016; Kim, Yi, et al. 2017). X. Li et al. (2020) identified 2,732 and 3,608 proteins from roots and cauline leaves of *P. ginseng* respectively by proteomic analysis and comprehensively studied UGT, cytochromes P450, and other related proteins involved in ginsenoside biosynthesis in *P. ginseng*. Interestingly, the UGT and cytochrome P450 were extensively expressed in cauline leaves but not in ginseng roots suggesting that post glucoside synthesis of ginsenosides may be conducted in cauline leaves while growth and then carried to roots at withering (X. Li et al. 2020). A total of 83 differentially modulated protein spots during different growth years were identified in forest cultivated ginseng out of which 62 spots and 21 spots showed increased and decreased abundances during differential growth period using 2DE and iTRAQ analyses (Ma et al. 2013). Further, a label-free quantitative proteome analysis showed that abiotic stress conditions such as heat stress also change the abundance of proteins associated with ginsenoside biosynthesis (S.W. Kim et al. 2019). Out of 3,332 proteins identified by proteomics analysis, 847 proteins including ginsenoside biosynthesis-related proteins encoded by isopentenyl diphosphate isomerase 1 (*IPP1*), lupeol synthase 2 (*LUP2*), and several glycosyltransferases showed differential modulations in response to heat stress (S. W. Kim et al. 2019). The proteomic analysis in the red-skin disorder of *P. ginseng* using 2DE and iTRAQ approaches showed that 137 proteins were differentially modulated including farnesyl diphosphate synthase, cycloartenol synthase, squalene epoxidases, squalene synthase involved in ginsenoside biosynthesis that were downregulated in red-skin disorder ginseng (Ma et al. 2019). Thus, the aforementioned studies highlight proteomics as a major tool for analyzing changes in the abundance of protein patterns associated with ginsenoside production and accumulation in plants.

10.3. Metabolomics

Ginsenosides are the chief constituents that are present in its potential sources such as *P. ginseng* and *P. quinquefolius* and during ginsenoside biosynthesis, tissue-specific metabolite profile changes (Liu et al. 2017). Understanding the changes in growth and tissue metabolic fluxes is a critical determinant of the quality and quantity of ginsenosides in plants. Lately, metabolomics is one of the latest tools added in the wave of omics approaches to witness the changes in primary and secondary metabolite profiles in the plant to get clearer insights into metabolite network flux associated with various plant tissues (Chen et al. 2018). Substantial advancements in metabolomics have assisted the studies of metabolic profiles that change in response to ginsenoside accumulation in their potential sources (Liu et al. 2017). Tissue-specific metabolome analysis of two different ginseng species, *P. ginseng* and *P. quinquefolius* employing GC-MS and LC-MS methods resulted in the identification of 149 primary metabolites along with 10 ginsenosides suggesting

an interesting mutuality between ginsenosides and primary metabolites. Additionally, it was also found that the tissue-specific metabolite profiles get altered during ginsenoside biosynthesis and ginsenoside accumulation was dependent on energy metabolism (C assimilation and C accumulation) in main and lateral roots of *P. ginseng* and *P. quinquefolius* respectively (J. Liu et al. 2017a). Metabolomics studies combined with multivariate statistical analysis have not only shown the differences in metabolite contents in plants during ginsenoside biosynthesis (J. Liu et al. 2017b) but has also assisted in mitigating adulteration by authenticating species based on age discrimination (Kim et al. 2011), discrimination based on cultivation area (Song et al. 2013), and differences in raw and processes ginseng samples (Park, Choi, and Kim 2014). Further, a comparative metabolome analysis of mountain ginseng (also called Lin-Xia-Shan-Shen) and garden ginseng using UPLC Q-TOF-MS showed that the increased levels of 16 metabolites involved in the biosynthesis of rare ginsenosides (such as Rg3, Rh1, Rh2), galactose metabolism, the citric cycle, GABA shunt, and amino acid metabolism in mountain ginseng than in garden ginseng (Chen et al. 2018). The contents of ginsenosides (Rd, Ra3, 20(S)Rg3, Rh2, Rh1, 20(S)F1 and R1) were higher in mountain ginseng while Rb1, Rc, Re, F3, Rf, and F11 (a pseudoginsenoside) were lower in mountain ginseng than in garden ginseng (Chen et al. 2018). This study counteracted the limitation of the inability to discriminate mountain and garden ginseng based on a change in ginsenoside contents. In order to describe discriminating marker components in main and fine roots of red ginseng, a UPLC-Q-TOF-MS based metabolome analysis was carried out which showed that both main and fine roots had 4 and 5 discriminating markers respectively that included ginsenosides Rc, Rd, Rb2, Rb1,20(S) Rg2 (In et al. 2017).

Thus, metabolomics is a promising analytical approach that has been used to unravel the metabolite fluctuations and metabolic fluxes associated with ginsenoside accumulation in plants. Metabolome analysis is not only helping in understanding biochemical processes and plant tissues associated with ginsenoside accumulation but has also aided in the development of markers discriminating various ginseng species.

11. Cell factories for effective ginsenoside production: A synthetic biology approach

With the exhausting supplies of *Panax* and its allied species, the potential sources for ginsenosides are still limited as compared to the over-utilization of ginsenosides. Thus, constructing new potential donors of ginsenosides may further help in meeting global ginsenoside demand for medicinal uses. Synthetic biology has emerged as a new hope for the generation of potent ginsenoside donors. The candidate genes associated with ginsenoside biosynthesis from the potential sources have been introduced into plants that are unable to synthesize ginsenosides efficiently to enhance their nutritional quality (Huang et al. 2015; Han et al. 2019). A transgenic 'ginseng rice' was produced by introducing a β-amyrin synthase gene (*βAS*) from Korean ginseng into *Oryza sativa* that resulted in the synthesis of oleanane-type sapogenin (Huang et al. 2015).

Metabolic engineering of microbes may also aid in the production of valuable ginsenosides. Among all the microbes, yeast has low harmful effects and has a high tolerance to heterologous systems of plants, making it a potentially strong host for metabolic engineering-induced ginsenoside production (Chu, Montecillo, and Bae 2020). Metabolic engineering of yeast for synthesizing ginsenosides can be conducted by the expression of heterologous genes from potential plant sources into yeast or by fine-tuning the expression of genes and optimizing the related metabolic pathways (Zhang et al. 2015; Dai et al. 2014; Chu, Montecillo, and Bae 2020). For example, for the production of PPD-type ginsenosides, dammarenediol-II synthase and protopanaxadiol synthase genes from ginseng, and NADPH-cytochrome P450 reductase (producer of PPD-type ginsenosides) from Arabidopsis were introduced in *Saccharomyces cerevisiae* that resulted in an enhanced PPD-type ginsenoside production for up to 262-folds. Further analysis suggested that the enhanced ginsenoside production in transgenic yeast was due to increased content of PPD-precursor molecules such as squalene, 2,3-oxidosqualene through overexpression of genes *ERG20, ERG9, ERG1* encoding enzymes FPS, SS, and squalene epoxidase respectively (Dai et al. 2013). Furthermore, the introduction of Korean ginseng-derived *PPT*-synthase into bioengineered *S. cerevisiae* having PPD-biosynthetic pathway aided in the production of both the ubiquitous aglycons PPDs and PPTs (Dai et al. 2014). In addition, a 'Ginseng yeast', synthesizing PPD-type of ginsenosides was produced by ectopic gene expression of a CYP enzyme involved in the formation of PPD-type ginsenosides *(CYP716A47)* and co-expression of *CYP716A47* (protopanaxadiol synthase and *PgDDS* (dammarenediol synthase) genes in recombinant WAT21 yeasts (Han et al. 2011). The biosynthetic pathway for the production of dammarane type of ginsenosides in *S. cerevisiae* strain GIL77 deficient in lanosterol synthase, an enzyme involved in the cyclization of (S)-2,3 oxidosqualene to lanosterol has been constructed by heterologous expression of PNA (an OSC gene; Tansakul et al. 2006). Optimizations in genetic cassette and metabolic pathways involved in ginsenoside production have also been done by fine-tuning the genetic elements such as promotor and terminators strength, and trans-regulatory factors mediating gene expression (Sun et al. 2012; P. Wang et al. 2019). For example, the yield of ginsenoside Rh2 has been enhanced by up to 3-folds by fine-tuning the glycosylation efficiency using an artificial promotor with the ability to increase transcript levels of *UGTPg45-HV* that improves glycosylation efficiency (P. Wang et al. 2019). Other optimization strategies include maintaining the metabolic flux and the pools of precursor molecules for ginsenoside biosynthesis such as acetyl CoA, a precursor for IPP/DMAPP in the MVA pathway (Huang, Qian, and Zhong 2013; Wu et al. 2019), engineering the availability of cofactors such as NADPH and NAD^+ (Minard and McAlister-Henn 2005; J. E. Kim et al. 2018), and modulating the substrate

specificities and activity of enzymes associated with ginsenoside biosynthesis such as UGTs (Liang et al. 2017; P. Wang et al. 2019). Thus, synthetic biology has constructed a new platform for enhancing ginsenoside biosynthesis by metabolic engineering of the potential cell factories such as yeast to fulfill the large-scale industrial demands of ginsenosides.

12. Conclusion and future prospective

Ginsenosides, a class of triterpene saponins, are multi-functional secondary metabolites produced primarily in the members of the genus *Panax*. The increasing demand for ginsenosides in the pharmaceutical industry has put pressure on the ginseng industry to look for alternate sources of ginsenosides production. A growing body of evidence suggests different phytohormones, especially JA and its derivatives, exert a positive effect on ginsenosides production in plants. Moreover, transgenic plants overexpressing one or more JA biosynthetic or signaling proteins have shown promising results in terms of enhanced ginsenosides production. Since the biological activities of different ginsenosides vary with deglycosylated ginsenosides being higher effective, efforts have also been made to transform the lesser efficient glycosylated ginsenosides into highly efficient deglycosylated ginsenosides using a variety of chemical and biological methods. In nutshell, ginsenosides are the most promising specialized metabolites with high efficiency as a nutraceutical as well as in plant defence mechanisms. Further investigation on hidden genes and their regulatory networks that underlies biosynthetic and metabolic pathways of ginsenosides can help in the generation of plants with high ginsenoside contents. Thus, ginsenoside accumulation and acquisition from plants can be ameliorated further by enhancing its production in the potential sources to meet the global demand for ginsenosides-based nutraceuticals and drugs. It is also imperative and crucial to study and understand the genomics, transcriptomics, and interactomics of ginsenosides in more detail to provide a better platform to manipulate the expression of genes known to accumulate ginsenosides to significantly appreciable levels in *Panax* spp. This would certainly help in unleashing the unexplored health benefits of ginsenosides.

Disclosure statement

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