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Review

Germplasm resources and secondary metabolism regulation in Reishi mushroom (*Ganoderma lucidum*)

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ABSTRACT

Ganoderma lucidum is a valuable medical macrofungus with a myriad of diverse secondary metabolites, in which triterpenoids are the major constituents. This paper introduced the germplasm resources of genus *Ganoderma* from textual research, its distribution and identification at the molecular level. Also we overviewed *G. lucidum* in the components, the biological activities and biosynthetic pathways of ganoderic acid, aiming to provide scientific evidence for the development and utilization of *G. lucidum* germplasm resources and the biosynthesis of ganoderic acid.

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Contents

1. General situation of genus *Ganoderma* germplasm resources

Ganoderma lucidum (Leyss. ex Fr.) Karst., also known as "Lingzhi", "Reishi" and "Yeongji", mainly grows on rotten roots in tropical and subtropical regions. It has two parts, kidney-shaped semicircular or nearly circular cap and stipe (Chen & Li, 2004). As a medicinal mushroom, *G. lucidum* has anti-HIV and anti-tumor activities, and can also treat coronary heart disease, diabetes, hypertension and other diseases (Lin, 2019; Xie, Zhang, Yu, & Yan, 2021). With the increase of human diseases and the enhancement of health care awareness, *G. lucidum* has attracted more and more attention.

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1.1. Textual research of genus Ganoderma

G. lucidum has a history of more than 6800 years in China, and its culture can be traced back to the Hemudu Period (5000 B.C.) (Yuan et al., 2018). In the historical data of the pre-Qin period, there are many textual researches on the medicinal effects of *G. lucidum*. As the "fairy medicine" sought by the first Emperor of Qin (221 B.C.), *G. lucidum* has a high historical status since ancient times (Zhang, 2022). *Compendium of Materia Medica* records that the medicinal effect of *G. lucidum* is suitable for people of any constitution. Its medicinal properties are warm-natured. It can treat mental diseases, nourish the spleen and stomach, increase wisdom, enhance memory, and is beneficial to longevity. *Shennong's Classic of Materia Medica* divides *G. lucidum* into five categories according to the colours: Qing Zhi, Chi Zhi, Huang Zhi, Bai Zhi, and Hei Zhi. Qing, Chi, Huang, Bai and Hei mean light green, red, yellow, white and black in colour in Chinese, respectively. In addition, there is a type of Zi Zhi, where Zi means purple in colour (Lin, 2019). In 2000, *Chinese Pharmacopoeia* was published for the first time to confirm the medicinal value of genus *Ganoderma*. In 2001, the Ministry of Health issued the *List of Fungal Species that Can Be Used in Health Food* that recognized the medicinal value of three *Ganoderma* species: *G. lucidum*, *G. sinensis* J.D. Zhao, L.W. Hsu & X.Q. Zhang, and *G. tsugae* Murr. (Shi et al., 2012) in China.

1.2. Distribution of genus Ganoderma

Ganoderma was established in 1881 (Karsten, 1881). Donk put forward the concept of *Ganoderma* subfamily in 1933, and promoted the subfamily to family in 1948 (Chen & Li, 2004). At present, according to the statistics of Index Fungorum (http://www.indexfungorum.org/names/Names.asp), 489 records of genus *Ganoderma* can be found (as of October 2022). The genus *Ganoderma* is represented by more than 260 species, which are distributed in northern and southern Europe, Central Africa, South America, East Asia and other regions (Cai, He, & An, 2016). China has abundant germplasm resources with wide distribution. There are over 100 species of *Ganoderma*, distributed in the southwestern, northwestern, and northeastern regions (Wu, Dai & Lin, 2004).

Among the three mentioned species of genus *Ganoderma* with medicinal value, *G. lucidum* mainly distributed in East Asia. *G. lucidum* is mainly distributed in Hubei, Guangxi, Guangdong, Jilin and Jiangxi provinces in China. *G. sinensis* grows mainly in China, Korea and Japan in the world. In China, it grows mainly in Guangxi, Guizhou, Hainan, Taiwan and Yunnan Provinces. *G. tsugae* are mainly distributed in Northwest and Northeast of China and the United States. (Wu, Dai, & Lin, 2004). Table 1 showed the distribution of *G. lucidum*, *G. sinensis* and *G. tsugae* in China.

Ganoderma germplasm resources are abundant and have broad industrial prospects. At present, *G. lucidum* is the most species available in the market, so we will mainly focus on *G. lucidum* for the subsequent discussion. It is urgent to breed high-quality *G. lucidum* varieties to meet market demand. *G. lucidum* are mainly classified into two kinds: wild *G. lucidum* and cultivated *G. lucidum*. In order to promote the development of *G. lucidum* industry, it is necessary to collect its germplasm resources, conduct metabolome, transcriptome and proteome sequencing, select and breed high quality *G. lucidum* varieties. By comparing cultivars such as Xianzhi No. 1, Xianzhi No. 2 and Xianzhi No. 3, Xu et al. (2021) found that the genus *Ganoderma* spore and sporocarp of Xianzhi No. 3 had the highest content of polysaccharides, triterpenes and other bioactive substances. Wang and others collected more than 50 wild varieties of *G. lucidum*, adopted systematic breeding method, and finally selected the short growth cycle, good fecundity, stability and adaptability of 'Yu Ze' *G. lucidum* (Xia, Yang, Li & Xia, 2018). The breeding of each exceptional *G. lucidum* cultivar holds great potential for widespread adoption.

Table 1
Distribution of *G. lucidum*, *G. Sinensis* and *G. tsugae* in various provinces of China.

Provinces	Number of species
Beijing	3
Hebei	6
Tianjin	1
Heilongjiang	4
Jilin	8
Liaoning	1
Inner Mongolia Autonomous Region	3
Gansu	1
Shanxi	2
Shandong	3
Henan	3
Shaanxi	1
Jiangsu	6
Zhejiang	8
Fujian	20
Taiwan	6
Jiangxi	9
Anhui	13
Hubei	4
Hunan	8
Guangdong	16
Hong Kong Special Administrative Region	2
Hainan	78
Guangxi Zhuang Autonomous Region	33
Sichuane	26
Tibet Autonomous Region	5
Guizhou	49
Yunnan	41

1.3. Identification of genus Ganoderma germplasm at molecular level

The application and development of molecular biology provided a new method for the identification of genus *Ganoderma* germplasm resources. Based on the whole genome sequencing results of *G. lucidum*, DNA sequence and cluster analysis can eliminate the interference caused by external environmental factors and human factors, and improve the accuracy of *Ganoderma* species identification at the molecular level. As early as 2012, Chen's team has completed the genomic framework map of *G. lucidum* to reveal the molecular mechanism of active ingredient biosynthesis in Chinese medicine, which established a solid foundation for accelerating the selection and breeding of high quality and high yielding *G. lucidum* varieties (Chen et al., 2012). Recently, the following genomes of the genus *Ganoderma* have been published in National Center for Biotechnology Information (NCBI): *G. lucidum*, *G. multipileum*, *G. sinense*, *G. leucocontextum*, *G. tsugae*, *G. meredithae*, *G. boninense*, *Ganoderma* sp. (https://www.ncbi.nlm.nih.gov/genome/?term=ganoderma). The publication of these genomes has laid a solid foundation for the identification of germplasm resources within the *Ganoderma* genus.

Random amplified polymorphic DNA (RAPD) (Huang, Xu, & Zhou., 2013), internal transcribed spacer (ITS) (Schoch et al., 2012), simple sequence repeat (SSR) (He et al., 2022), inter-simple sequence repeat (ISSR) (He et al., 2022), sequence-related amplified polymorphism (SRAP) (Sun et al., 2006), sequence characterized amplified regions (SCAR) (Kwon, Lee, & Park, 2019) and other technologies have been widely used in the identification of genus *Ganoderma*. Zhang used SSR molecular markers to construct molecular IDs for the genus *Ganoderma*, and used the Genetics Statistics software and ID Analysis software to analyze the specificity index and molecular IDs, resulting in the successful clustering of 11 *Ganoderma* strains into four categories. Researchers found that 15 specific ISSR primers can be used for intraspecific

identification and polymorphism analysis of *G. lucidum* (He et al., 2022). Sun et al. (2006) was the first to apply SRAP technology to the systematics of genus *Ganoderma* strains in 2006 and successfully classified these 31 *Ganoderma* strains into five groups. RAPD technology was used to classify eight species of genus *Ganoderma* into two groups (Wang, Su, & Lv, 2011). *G. lucidum* was distinguished from Asia and Europe by ITS sequence analysis (Liao et al., 2015). Using 60 primer pairs, 24 SSR loci with polymorphic bands were screened based on the results of cluster analysis to obtain the affinities among the strains, which is useful for analyzing the genetic diversity of strains (Xu et al., 2020). Therefore, the development of molecular biology provided new methods for *Ganoderma* classification. Researchers can more clearly delineate genus *Ganoderma* by sequencing whole genome and building a phylogenetic tree.

2. Research progress of main chemical constituents in *G. lucidum*

G. lucidum, as a model organism of fungi, has a wide range of chemical composition, including polysaccharides, triterpenoids, proteins, amino acids, sterols, alkaloids, trace elements and other components (Zhang et al., 2021). Polysaccharides and triterpenoids are the main active components of *G. lucidum* (Zhang, Zhou, & Song, 2018). The triterpenoids have complex structures. According to the different functional groups and side chain structures connected to triterpenoids, they are divided into ganoderic acids, ganoderiol, ganoderal and ganolactone.

Ganoderic acids are the main triterpenoids compounds, and their physiological activity is mainly determined by different side chain groups. The content of ganoderic acids (ganoderic acid A and ganoderic acid B) is one of the main bases for evaluating the quality of *G. lucidum*. The higher the content of ganoderic acids is, the better the quality of *G. lucidum* (Yan et al., 2019) is. In 1982, for the first time scientists isolated the two kinds of ganoderic acids (ganoderic acid A and ganoderic acid B) from the genus *Ganoderma*, ganoderic acid A and ganoderic acid B (Kubota, Asaka, Miura, & Mori, 1982). At present, there are more than 380 triterpenoids isolated from genus *Ganoderma*, among which there are about 171 ganoderic acids (Baby, Johnson, & Govindan, 2015). Chemical structures of ganoderic acids were in Fig. 1. The research shows that the composition and content of ganoderic acid vary in, different species, different parts, different growth periods and different producing areas. Yan et al. (2019) compared the contents of ganoderic acid from different origins and found that the total contents of ganoderic acid A and ganoderic acid B were the highest in *G. lucidum* from Anhui Province of China. The triterpene content of wild *G. lucidum* is far lower than that of cultivated *G. lucidum*, and the total triterpene content is only one tenth of that of cultivated *G. lucidum*. Among the 12 kinds of ganoderic acids, ganoderic acid A accounts for the highest proportion (Jia et al., 2017). Ganoderic acid is extracted from the cap and stipe of *G. lucidum* (Zhang et al., 2020). The content of ganoderic acid in the cap is higher than in the stipe, and the main ganoderic acid in the cap is ganoderic acid T, Mk, Me and S. Ganoderic acid in the stipe mainly includes ganoderic acid A, B and C. In addition, at different growth stages of *G. lucidum*, the content of ganoderic acid A and D was higher at the maturity stage, while the content of ganoderic acid B, C2 and G was higher at the bud stage (Ren et al., 2020).

We focus mainly on *G. lucidum*, *G. tsugae*, and *G. sinensis*, the three species with medicinal value to explore the distribution pattern and characteristics of the content of ganoderic acids in genus *Ganoderma*. *G. sinensis* does not contain ganoderic acids A, B, C2, G, E or other ganoderic acid triterpenoids, while *G. lucidum* is rich in ganoderic acid triterpenoids (Ding, Huang, Qiu, Liang, & Wang,

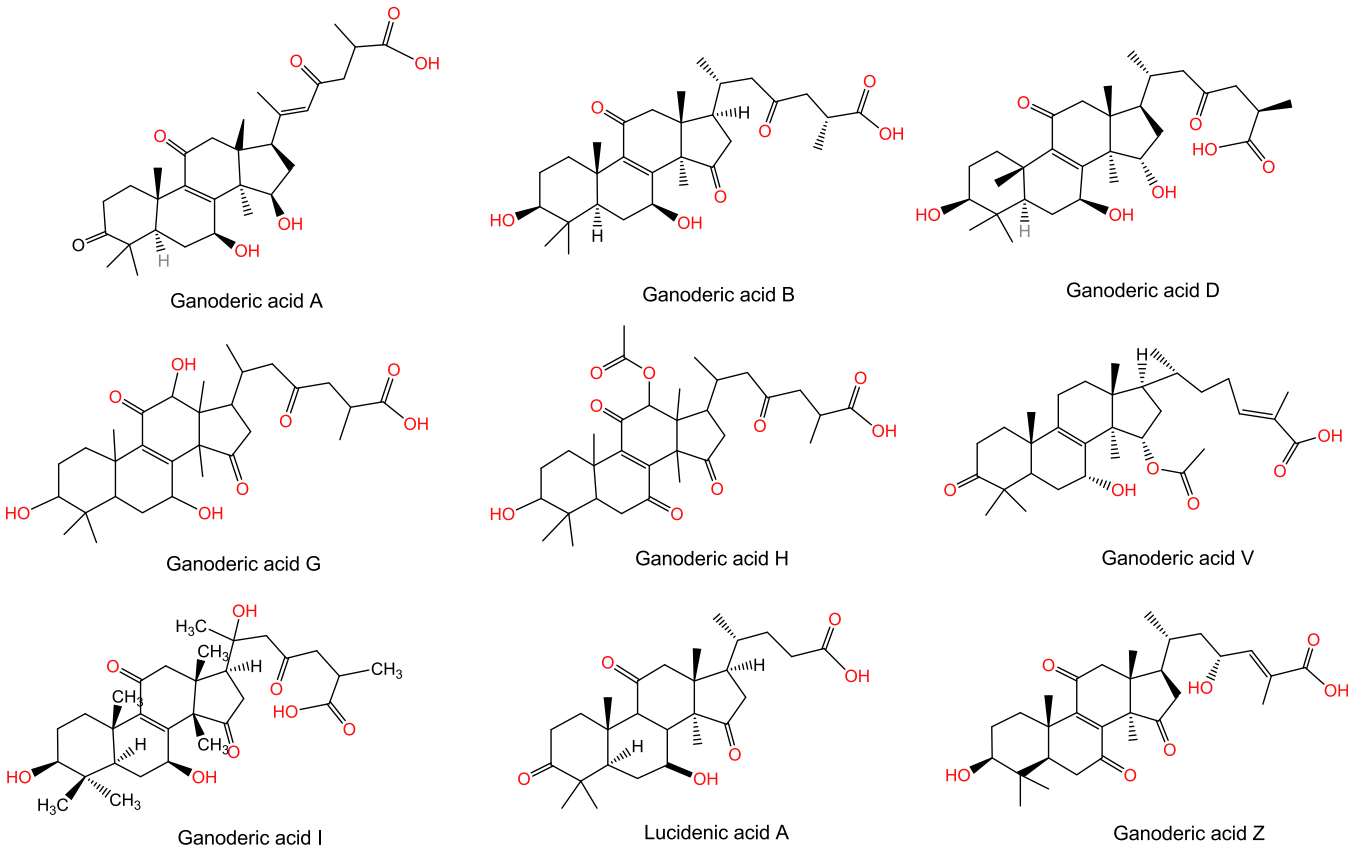

Fig. 1. Chemical structures of ganoderic acids.

2009). *G. tsugae* contains ganoderic acid A, B, C and I, ganodermanontriol, ganoderiol A, ganodermatrio, lucidone A, lucidenic acid C and LM1 (Liu, 2009). Therefore, the triterpenoids in *G. lucidum* and *G. tsugae* are closer to each other, while *G. sinensis* contains fewer triterpenoids, but mainly polysaccharides and sphingolipids. In addition, the content of triterpene acids in the fruiting body is higher than that in the spore powder; The broken-wall spore powder had higher triterpene acids content than the unbroken-wall spore powder; Compared with *G. lucidum* strains not Spaceship-Carried, the triterpene acids content of *G. lucidum* strains Spaceship-Carried increased.

3. Pharmacological activities of ganoderic acids

The secondary metabolism process of plants is the result of the plant adaptation to the ecological environment changes in long-term evolution, and it plays an important role in dealing with the relationship between plants and the ecological environment. Ganoderic acid, the main secondary metabolite of *G. lucidum*, has a complex chemical structure and extensive pharmacological activities (Chen, 2020), with anti-toxic and anti-bacterial effects, it prevents cardiovascular diseases, protects the liver and prevents epilepsy (Xing, Liu, He, & Chen, 2017).

Cancer is one of the diseases with the highest mortality. At present, chemotherapy is the main treatment scheme for cancer, and chemotherapy-related fatigue (CRF) is one of the main side effects of chemotherapy on patients, which seriously affects the recovery of patients' physical functions. Relieving chemotherapy-related fatigue is also one of the main medical problems at present. The latest experimental research showed that ganoderic acid can improve mitochondrial activity, promote ATP production, increase sugar content, reduce lactic acid content, and improve the function of muscle fatigue, thus ganoderic acid provides the basis for clinical treatment of CRF (Abulizi et al., 2021). Furthermore, ganoderic acid can specifically bind to tubulin, which provides a direction for microtubulin-targeted anticancer drug design (Kohno et al., 2017). Sorafenib (SRF) has been recognized as the treatment of advanced liver cancer as early as 2007, but its nonspecific intake will cause serious side effects (Zhu, Zheng, Wang, & Chen, 2017). Ganoderic acid can assist SRF in the synergistic treatment of liver cancer, and constructing nano lipidic carriers (NLCs) loaded with SRF and ganoderic acid can effectively restore liver parameters, non-liver parameters and inflammatory indicators, which are close to normal levels (Wang et al., 2021). The normal levels of TNF-α in macrophage can inhibit tumorigenesis and viral replication but its overexpression is the main cause of asthma (Landskron, Marjorie, Thuwajit, Thuwajit, & Hermoso, 2014). Both aqueous extract of three medicinal herbs (*G. lucidum*, *Sophora flavescens* Ait and *Glycyrrhiza uralensis* Fischer) and ganoderic acid in *G. lucidum* can inhibit the production of TNF-α. Ganoderic acid C1 has the strongest inhibitory effect on TNF-α, which is dose-dependent and non-cytotoxic. TNF-α is not completely inhibited, but the level of TNF-α is regulated by signal pathway (Liu et al., 2015). Treatment of cervical cancer cells (Hela cells) with different concentrations of ganoderic acid T, and then irradiated by gamma rays, can induce apoptosis and necrosis of HeLa cells, and with the increase of ganoderic acid T concentration, the necrosis rate also increases (Shao et al., 2021).

Ganoderic acid not only has irreplaceable effect on cancer treatment, but also has high curative effect on the treatment of other diseases; such as multiple sclerosis (MS) (Castillo-Trivino, Braithwaite, Bacchetti, & Waubant, 2013), major depression (MDD) (Bao et al., 2021), cardiovascular disease (Ren, 2019), renal fibrosis and other diseases (Geng et al., 2020). Ganoderic acid A enters the central nervous system through the blood–brain barrier and activates the bile acid receptor FXR to promote the formation of regenerative myelin in the central nervous system for repair and regeneration (Jia et al., 2021). It also regulates the neuroimmune system to achieve antidepressant effect (Bao et al., 2021). Ganoderic acid A can improve renal fibrosis and relieve renal dysfunction by inhibiting the over-activation of TGF-β/Smad signal, the expression of fibronectin and the deposition of extracellular matrix (ECM) in kidney (Geng et al., 2020). Ganoderic acid A activates PI3K/AKT and mTOR by up-regulating miR-153, thereby improves the symptoms of cerebral hypoxia, provides a scheme for the treatment of cardiovascular and cerebrovascular diseases (Li et al., 2020). It also inhibits the release of histamine from cells and promotes the digestion of various organs of the digestive system (Lei, 2019). Ganoderic acid A can inhibit the expression of pro-inflammatory factors and promote the expression of anti-inflammatory factors, which in turn inhibit inflammatory activity. β-Galactosidase is a senescence-related marker. Ganoderic acid D can delay aging in human amniotic mesenchymal stem cell (HAMSC) by activating the PERK/NRF2 signaling pathway, drastically limiting the production of β-galactosidase in a dose-dependent manner, having no cytotoxic side effect, preventing cell cycle arrest, and improving telomerase activity (Xu et al., 2020). The researchers also found that ganoderic acid T induces P53 protein expression and promotes apoptosis (Tang, Wu, Wang, Sun, & Ouyang, 2015). In addition, ganoderal can delay Alzheimer's disease through DNA methylation (Lai et al., 2019).

4. Biosynthetic pathways of ganoderic acid

Ganoderic acid which is structurally similar to highly oxidized lanosterol-derived compounds of triterpenoids containing a carboxyl structure (Zhao, Xu, & Zhong, 2011), and is an important secondary metabolite of *G. lucidum*. It was found that the synthetic pathway of ganoderic acid follows the mevalonate pathway (Shiao, 2013). The biosynthesis of ganoderic acid has three stages: the generation of mevalonate precursors, the synthesis of the lanosterol skeleton, and the biosynthesis of ganoderic acid. However, the specific synthetic pathway from lanosterol to ganoderic acid is very complex, and in recent decades, there has been no specific pathway, that represents a difficult problem in the research field. It is speculated that there may be a series of redox reactions of lanosterol. The synthetic pathway of lanosterol has been studied in detail, but the biosynthetic process from lanosterol to ganoderic acid has not been studied in detail so far. The ganoderic acid biosynthesis pathway was shown in Fig. 2.

Lanosterol synthase (LS), farnesyl pyrophosphate synthase (FPS), 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR) and squalene synthase (SQS) are the key enzymes in the ganoderic acid synthesis pathway (Shi, Ren, Mu, & Zhao, 2010). The synthesis of lanosterol from 2,3-epoxysqualene is catalyzed by LS, and the expression of lanosterol synthase gene and the content of ganoderic acid are positively correlated (Luo, Li, & Xu, 2022). *LaeA* is a global regulatory gene, and the production of ganoderic acid is positively correlated with the transcription level of *LaeA* gene (Luo et al., 2022). HMGR is the rate-limiting enzyme which is responsible for catalyzing the reduction of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to mevalonate (Zhao, Zhong, Liang, Wang, & Jong, 2004). The synthesis of farnesyl pyrophosphate (FPP) into squalene (SQ) is at a metabolic branch point, this chemical reaction is catalyzed by SQS. Therefore, LS, FPS, HMGR and SQS play a decisive role in the synthesis of ganoderic acid.

Many regulatory factors influence the biosynthesis of ganoderic acid. The newly sequenced genome of *G. lucidum* in Changbai Mountain, Jilin Province, China, 360 genes are identified to be involved in triterpenoid biosynthesis, 300 genes belong to cyto-

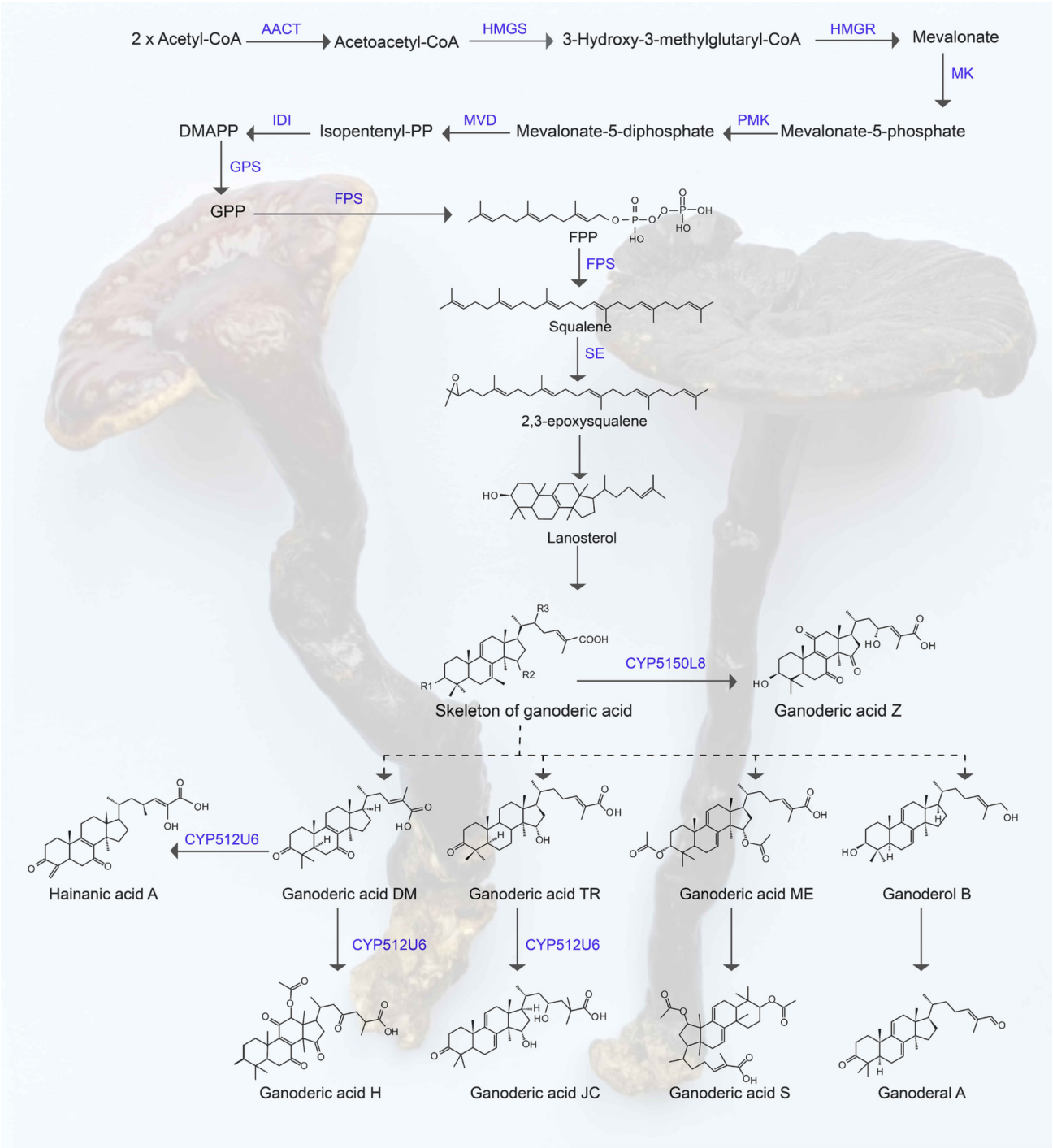

Fig. 2. Biosynthetic pathway of ganoderic acid (Shiao, 2013; Ren et al., 2013).

chrome P450 (CYP450), and 18 genes are the main genes of triterpenoid biosynthesis pathway, which is the genome with the largest number of triterpenoid biosynthesis in *G. lucidum* genome report at present. It is speculated that the increase of triterpenoid genes is one of the main reasons for the increase of triterpenoid content (Tian et al., 2021). It was found that when HMGR, FPS and SQS transcripts reached their highest levels, so did ganoderic acid B, C2 and G (Ren et al., 2020). Studies have shown that multiple action factors regulate the levels of ganoderic acid by regulating the levels of reactive oxygen species (ROS). For example, Cu^{2+} stress can regulate three key enzyme genes in the ganoderic acid biosynthesis pathway by regulating ROS levels. Under Cu^{2+} stress, ROS were produced excessively, and the expression of key enzymes (SQS, oxidosqualene cyclase, HMGR) genes is up-regulated, which in turn regulating ganoderic acid biosynthesis and mycelial growth (Gao et al., 2019). Increased ROS content accelerates methyl jasmonate (MeJA)-induced ganoderic acid biosynthesis (Jiang et al., 2019). AreA is a GATA-type transcription factor that inhibits ganoderic acid biosynthesis through the induction of intracellular NO production (Zhu, Sun, Shi, & Song, 2019). Under heat stress (HS) conditions, ROS content raised, regulatory heat shock protein (HSP) expression enriched, and eventually gan-

oderic acid content increased (Zhang et al., 2016). Nitrogen oxides also play an irreplaceable role in the regulation of ganoderic acid biosynthesis. Nitric oxide (NO) can reduce the content of mitROS by inhibiting the activity of aconitase, which in turn affects the biosynthesis of ganoderic acid. APSES family transcription factors are unique to fungi (Doedt et al., 2004). GlSwi6, an APSES family transcription factor, was identified from *G. lucidum* and found to be involved in *G. lucidum* substratum development and regulation of ganoderic acid biosynthesis (Zhang et al., 2018). Calcium signal plays a key role in calcium-regulated phosphatase-mediated regulation of ganoderic C acid biosynthesis, and Ca^{2+} can enhance ganoderic acid biosynthesis (Han & Zhong, 2020).

Previous studies have shown that many *CYP450* genes are involved in *Ganoderma* triterpene synthesis. *CYP512U6* is an important gene responsible for *Ganoderma* triterpene synthesis, and the *CYP512U6* gene was highly related to the content of eight *Ganoderma* triterpene components. Therefore, the researchers speculated that the contribution of the *CYP512U6* gene may not have been limited to the synthesis of hainanic acid H, ganoderic acid Jc, and ganoderic acid ZXYL (Jiang et al., 2022). *CYP5150L8* is also a key gene that regulates lanosterol into ganoderic acid biosynthesis. *In vitro* enzyme experiments showed that CYP5150L8 catalyzes the three-step oxidation of lanosterol at c-26 to synthesize 3-hydroxy-lanosta-8,24-dien-26-oic acid (HLDOA): First the methyl group is oxidized to hydroxyl group, followed by the conversion of hydroxyl group to formyl group, and finally the conversion of formyl group to carboxyl group (Lu et al., 2020). Glutamine synthase (GS) is a central nitrogen metabolizing enzyme that plays an important role in the nitrogen regulatory network and secondary metabolism of fungi. GS activity is inhibited under nitrate condition, which adversely affects the biosynthesis of ganoderic acid (Zhu et al., 2021). Histone acetylation plays an important role in regulating the growth and secondary metabolic synthesis of fungi. It was found that histone acetylation can regulate the growth and development of *G. lucidum* through its global regulatory factors, and then can affect the biosynthesis of polysaccharides (Wang et al., 2020). By cloning *PacC* gene in response to environmental changes, it was found that *PacC* plaied an important role in regulating the transcription level of key enzyme genes, intermediate metabolites and ganoderic acid content in the ganoderic acid biosynthesis pathway (Wu et al., 2016). The analysis of three ceramide synthases from *G. lucidum* (lag1, lag2 and lag3) revealed that RNA interference inhibition of lag1 reduced the synthesis of ganoderic acid in the mutant, while dual inhibition of lag2/lag3 increased the synthesis of ganoderic acid in the mutant (Tao et al., 2021).

5. Prospects

In recent years, *G. lucidum* has been a hot spot for researchers. The genus *Ganoderma* and its relatives, its species and their morphologically diversity are delineated at the molecular level, that provides conditions for improving *Ganoderma* breeding and germplasm resources. Ganoderic acids are some of the main substances in *G. lucidum* that exerts pharmacological activity. Different ganoderic acids can coordinate with each other and play a role together. Due to the influence of a variety of exogenous and endogenous substances on ganoderic acids biosynthesis, the biosynthesis pathway from lanosterol to ganoderic acid is unclear. How to improve the content of ganoderic acid in *G. lucidum* has been an important research direction so far. In order to investigate the key compounds that play a regulatory function in its secondary metabolism, mine critical genes, and enhance ganoderic acid production, we analyzed the different kinds of ganoderic acids, their pharmacological activity and biosynthetic pathways.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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