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Review

Strategies on biosynthesis and production of bioactive compounds in medicinal plants

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ABSTRACT

Medicinal plants are a valuable source of essential medicines and herbal products for healthcare and disease therapy. Compared with chemical synthesis and extraction, the biosynthesis of natural products is a very promising alternative for the successful conservation of medicinal plants, and its rapid development will greatly facilitate the conservation and sustainable utilization of medicinal plants. Here, we summarize the advances in strategies and methods concerning the biosynthesis and production of natural products of medicinal plants. The strategies and methods mainly include genetic engineering, plant cell culture engineering, metabolic engineering, and synthetic biology based on multiple "OMICS" technologies, with paradigms for the biosynthesis of terpenoids and alkaloids. We also highlight the biosynthetic approaches and discuss progress in the production of some valuable natural products, exemplifying compounds such as vindoline (alkaloid), artemisinin and paclitaxel (terpenoids), to illustrate the power of biotechnology in medicinal plants.

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1. Introduction

Traditional Chinese medicine (TCM) has a long history of treating human diseases documented in various cultures (Yuan, Ma, Ye, & Piao, 2016). Based on thousands of years of medical practice and experience, TCM has a wealth of "clinical trial" data that guarantees its effectiveness and safety. With the development of modern science and technology, more and more pharmacological effects and therapeutic mechanisms of medicinal plants have been identified, and TCM has become a part of modern medicine. The natural bioactive compounds of medicinal plants are considered to play a leading role in the treatment of diseases, and these compounds will continue to be used in large quantities to meet the urgent need to develop effective drugs. The low content of natural products in medicinal plants and the growing demand for these bioactive compounds have stimulated research into chemical and/or biotechnological synthesis. However, the total chemical synthesis of natural products has proven to be difficult, complex and economically unsuitable for commercial production. While the extraction of natural products from wild plant sources is an alternative. However, wild plants typically accumulate low concentrations of natural products over long growing periods, and over-harvesting of wild plants often leads to negative environmental and ecological impacts. Furthermore, purification of the desired compound requires special separation from a wide variety of compounds in medicinal plants, especially those with similar structures and yields.

Biosynthesis of natural products using biotechnological systems is more forward-looking tools. Advances in plant tissue culture and fermentation processes, as well as improvements in plant and microbial engineering techniques and in synthetic biology of natural products, have opened new avenues for efficient large-scale production of bioactive compounds. These efficient and widely used biosynthetic systems and methods include genetic engineering, plant cell culture engineering, metabolic engineering, and synthetic biology based on multiple "OMICS" technologies (Fig. 1). Herein, we will focus on the challenges and opportunities of current biosynthetic strategies from the four above aspects of comprehensive view and discuss the latest and unprecedented advancements of these valuable natural products in the field of

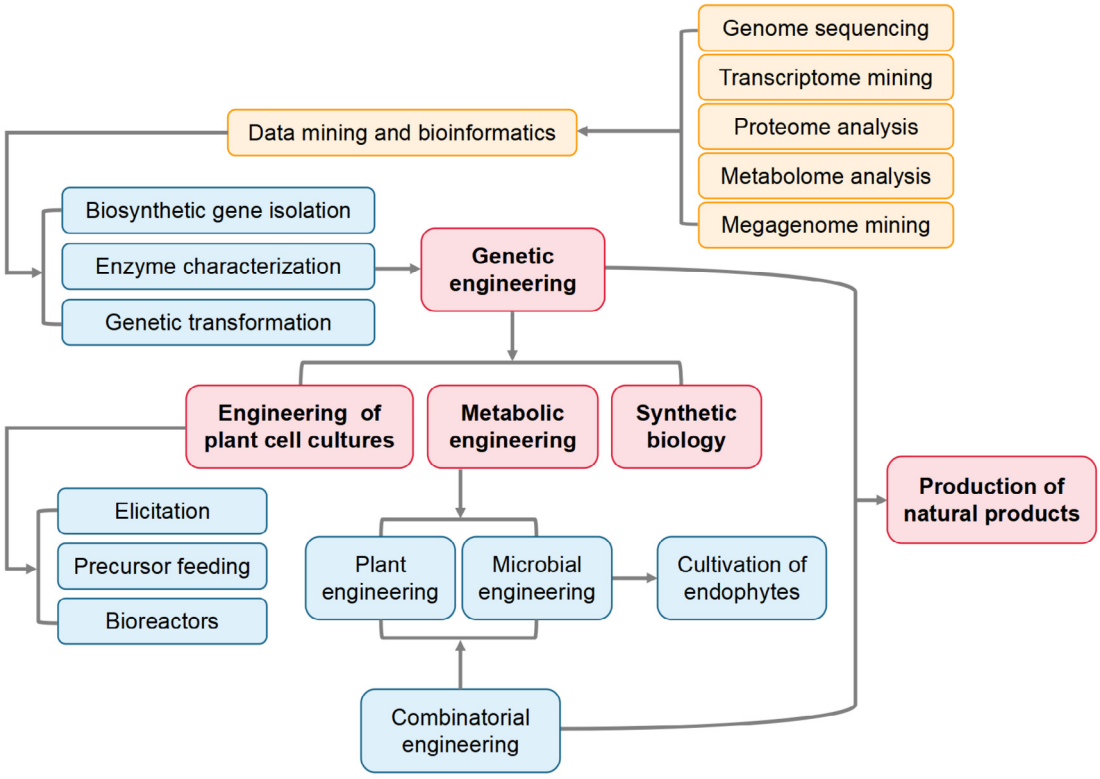

Fig. 1. Strategies for biosynthesis of natural products, including genetic engineering, plant cell culture engineering, metabolic engineering, and synthetic biology based on multiple "OMICS" technologies.

biosynthesis, using the biosynthesis of vindoline (alkaloids), artemisinin and paclitaxel (terpenoids) as examples, to illustrate how biotechnology and biosynthetic strategies could pave the way for a broader manufacturing palette of natural products from medicinal plants.

2. Genetic engineering

The developments and advances in genetic engineering have led to the creation of systems for obtaining valuable natural products on a large scale. Based on genetic engineering approaches, a necessary first step in scaling up natural product production is the identification of genes, enzymes, and metabolites involved in biosynthetic pathways through transcriptomics, genomics, proteomics, and metabolomics technologies.

2.1. Identification of biosynthesis-related genes based on "OMICS"

The strategy of genome-wide expression profiling analysis is a powerful tool for the discovery and identification of genes involved in biosynthetic pathways. In addition to genes encoding enzymes involved in natural product biosynthetic pathways, genes encoding transcription factors and transporters also play roles in the regulation of natural product biosynthesis and compartmentalization (transport) in plant cells. The application of "omics" tools has accelerated the study of transcriptome and metabolite profiles in various tissues under different physiological and pathological conditions. The approaches to genome/transcriptome and metagenomic analysis have led to the discovery of novel genes, gene clusters, and biological processes involved in secondary biosynthetic pathways.

Transcriptome profiling is a promising method to rapidly elucidate biological processes in medicinal plants, which can provide genetic information related to biosynthesis, growth, development, and genetic diversity of biologically active compounds. Expressed sequence tag (EST), DNA microarrays, and next-generation sequencing (NGS) technologies can simultaneously analyze the expression levels of thousands of genes and provide reliable transcriptomic data for comparing the relative expression level of the same transcript under different conditions. The analysis of differential gene expression using transcriptome datasets in certain samples can reveal up- or down-regulated transcripts encoding proteins responsible for the biosynthesis of desired compounds. Currently, genome (transcriptome) sequences based on NGS technology provide a large amount of data for the identification of biosynthetic genes (Luo & Chen, 2019; Morozova & Marra, 2008). Whole genome sequences contain genetic information about the origin, evolution, growth and development, and biosynthesis of medicinal plant active ingredients. The genes involved in biosynthesis and transport of bioactive compounds have been screened and identified by genome sequencing of *Ganoderma lucidum* (Curtis) P. Karst. (Tian, Wang, Liu, Zhang, & Li, 2021), *Wolfiporia cocos* (F. A. Wolf) Ryvarden & Gilb. (Luo et al., 2020), *Dendrobium officinale* Kimura et Migo (Niu et al., 2021), *Salvia miltiorrhiza* Bunge (Song et al., 2020; Xu, Ji, Zhang, Song, & Chen, 2016), *Panax ginseng* C. A. Meyer (Kim et al., 2018), *Artemisia annua* L. (Shen et al., 2018), *Papaver somniferum* L. (Guo et al., 2018; Yang et al., 2021), *Glycyrrhiza uralensis* Fisch. (Mochida et al., 2017) and *Siraitia grosvenorii* (Swingle) C. Jeffrey (Itkin et al., 2016).

Metagenomics approaches for studying natural products have been used to identify novel biosynthetic gene clusters involved in biosynthesis of natural products (Uchiyama & Miyazaki, 2009). This metagenomic approach begins by isolating mixed genomic DNA from pools of multi-organism origin and then cloning the DNA into appropriate vectors (e.g. bacterial artificial chromosomes and cosmid shuttle vectors). The vector is then transformed into a suitable host strain and grown under proper conditions. The heterologous expression of this environment-derived DNA (metagenomics) has been explored to identify microbial biosynthetic pathways. This method has also been used to assess the potential production of secondary metabolites and drugs in marine and terrestrial bacterial symbionts. The field of metagenomic-based gene discovery offers enormous opportunities and potential to advance the development of microbiology and biotechnology. The advantage of this approach is that most bacterial secondary metabolic pathways are tightly clustered on chromosomes. Gene clusters make it possible and feasible to clone and heterologously express biosynthetic genes from fragments of genomic DNA. However, experimental verification of the predicted products produced by these biosynthetic gene clusters and expansion of the resulting heterologous hosts to different genera remains a formidable challenge.

2.2. Characterization of enzymes

Proteomics has emerged as a potential method to identify key enzymes or proteins involved in natural product biosynthesis. In contrast to transcriptome analysis or genome mining, proteomics allows the direct identification of enzymes or novel proteins involved in biosynthetic pathways. Transcriptome/genome analysis can only provide information about the biosynthetic potential of an organism, but cannot reveal which biosynthetic pathways are expressed. Proteomic analysis of *A. annua* elucidated the biosynthetic pathway of the antimalarial pro-drug artemisinin, and identified multiple proteins related to artemisinin biosynthesis, such as 3-hydroxy-3-methyl glutaryl coenzyme A reductase, cytochrome P450, artemisinin aldehyde A reductase (Bryant, Flatley, Patole, Brown, & Cramer, 2015). Jacobs, Gaspari, van der Greef, van der Heijden, & Verpoorte, 2005 used a proteomic approach to identify novel proteins involved in alkaloid biosynthesis from *Catharanthus roseus* cell cultures. Desgagné-Penix et al. (2010) identified the enzymes involved in sanguinarine biosynthesis in poppy cell cultures by integrating next-generation 454 pyrosequencing and advanced LC-MS/MS analysis.

Metabolomics is defined as the comprehensive analysis of the global metabolite profile in a biological system (such as a cell, tissue or organism) under given conditions, where all metabolites can be identified and quantified. In contrast to transcriptomics and proteomics, metabolomics represents the ultimate biochemical phenotypes associated with the physiological, developmental and pathological states of biological systems. Metabolites can represent functional entities at the functional genomic level. The isolation of transcripts encoding opium poppy salutaridine reductase based on analysis of macroarray-based expression and metabolic profiling data of morphinan-producing and non-morphinan-producing *Papaver* species (Ziegler et al., 2006).

2.3. Genetic transformation of biosynthesis-related genes

Genetic transformation techniques for transforming biosynthetic genes into cultured plant cells, tissues or microorganisms for heterologous expression have proven to be powerful tools for the production of compounds. To increase the yields of compounds, genetic transformation methods have been employed to overexpress biosynthetic genes encoding enzymes in specific rate-limiting steps, or to modulate transcription factors that control multiple biosynthetic genes to block competing pathways/-catabolism or generate new compounds in alternative plant species. It is attractive to produce transgenic plants (or microorganisms) to obtain consistently high yields of compounds. For example, the transformation of the taxadiene synthase gene into

A. thaliana and tomato resulted in the accumulation of taxadiene in *Arabidopsis* cells and tomato freeze-dried fruit, respectively (Kovacs et al., 2007; Besumbes et al., 2004). Uefuji et al. (2005) reported that tobacco plants were transformed with genes such as xanthosine methyltransferase, 7-methylxanthine methyltransferase and 3,7-dimethylxanthine methyltransferase to produce caffeine.

3. Engineering of plant cell cultures

Plant cells are biosynthetic totipotent, a property that results in plant cells, organs and tissues producing various chemicals found in the parent plant *in vitro*. Compared to the time-consuming, costly, and environmental damage inherent in isolating natural products from wild plants, plant cell culture provides a renewable, large-scale, and easily scalable source of natural products. Plant cell culture facilitates the production of compounds free of microbial and insect contamination in a controlled environment, unaffected by climate change and various environmental factors. However, slow growth of plant cells, genetic instability, low productivity, and inability to maintain photoautotrophic growth in culture still present challenges for their application in natural product production. Therefore, screening high-yielding cell lines and manipulating nutrients to optimize culture conditions are prerequisites for increasing yield. In addition, plant cell cultures can also be used to study biosynthetic pathways and molecular mechanisms of bioactive compounds. Numerous strategies have been developed and utilized to enhance the production of natural products using plant cell cultures, such as precursor feeding and elicitation.

3.1. Precursor feeding

Precursor feeding has been used to increase the production of secondary metabolites in plant cell cultures. Precursors or intermediates that are present in abundance at the beginning of a biosynthetic pathway often provide a good opportunity to increase the yield of the final product in the biosynthetic pathway. The addition of precursors, such as 1-deoxy-*D*-xylulose, loganin, and tryptamine, resulted in increased alkaloid production in *C. roseus* hairy root cultures and cell suspensions, respectively (Peebles, Hong, Gibson, Shanks, & San, 2006). The addition of mevalonic acid lactone to cell cultures of the Indian variety of *A. annua* stimulated the production of artemisinin (Baldi & Dixit, 2008). These studies illustrate complex aspects of secondary biosynthetic pathways in plant cell cultures.

3.2. Elicitation of plant cell cultures

Elicitation of plant cell cultures has been cited as an effective strategy to increase the productivity of bioactive secondary metabolites. Various biotic and abiotic elicitors are efficient in stimulating the production of many valuable natural products in plant cell cultures (Thanh, Murthy, Yu, Hahn, & Paek, 2005). The methyl jasmonate (MeJA) elicitation for taxoid biosynthesis stimulation was shown to be efficient because a number of important genes in the terpene pathway (e.g., geranylgeranyl diphosphate synthase and taxadiene synthase) were MeJA inducible. Wang & Zhong, (2002) reported that multiple jasmonic acid (JA) inducers in a bioreactor can increase paclitaxel production in cell suspensions to 612 mg/L. Vázquez-Flota et al. (2009) reported differential responses to chemical elicitors such as MeJA, salicylic acid, and ethylene in *C. roseus in vitro* cultures, including cell suspensions, hairy roots, and rootless shoot cultures, and they suggested that different elicitors induced the accumulation of products in different cultures.

3.3. Plant cell cultures in bioreactors

Bioreactors have been widely used for the production of biomass (e.g., cells, shoots, roots, organs, or embryogenic propagules), metabolites and enzymes, and biotransformation of exogenously added metabolites. Bioreactor-based biotransformation offers great potential for the production of a variety of bioactive compounds from plant cell cultures. Plant cell cultures in bioreactors designed to produce important pharmaceutical compounds through metabolic engineering have become more competitive due to their higher productivity than whole plants. The production of vinblastine, vindoline (Zhao & Verpoorte, 2007), paclitaxel (Frense, 2007), artemisinin (Liu, Zhao, & Wang, 2006), and ginsenoside (Palazón et al., 2003) have been successfully carried out in bioreactors. A number of strategies have been investigated to increase the yield of plant cell cultures in bioreactors, such as reactor design, cell fixation, enzyme induction, shear sensitivity, and characterization of cell heterogeneity and variability in plant cell suspension cultures (Kolewe, Gaurav, & Roberts, 2008). The optimization of media ingredients, concentration, and environmental factors, combined with the use of appropriate culture strategies, paves the way for successful bioprocessing of natural products. Furthermore, fermentation technology based on plant cell culture in bioreactors can not only be applied to produce natural products, but can also be optimized by cellular and molecular breeding techniques to consistently provide high and stable yields.

4. Metabolic engineering

Metabolic engineering is a platform technology for optimizing industrial fermentation processes by introducing targeted genetic changes to produce compounds using recombinant DNA technology. Production optimization of natural products is often accomplished by reconstituting biosynthetic pathways in heterologous microorganisms to construct engineered, genetically tractable microbial strains or in plants. Successful metabolic engineering relies on improved strains designed and constructed through genetic engineering based on a comprehensive analysis of cellular function. Metabolic engineering provides a powerful approach to achieve yield enhancement of natural products in a short period of time in a large-scale mode in microorganisms or plants.

4.1. Strategies of metabolic engineering

Recombinant microorganisms or transgenic plant tissue cultures can be used to produce important natural compounds for industrial production only if the metabolites of interest need to be overproduced. In addition to the selection of high-yielding cell lines and other growth condition parameters, a comprehensive understanding of the molecular mechanisms of metabolic pathways is necessary to overexpress key genes involved in rate-limiting steps. After obtaining and characterizing biosynthetic genes, the following challenges for metabolic engineering are efficient genetic manipulation of microbial or plant cells/tissues, including fine-tuning of regulatory elements, and expression of native and heterologous pathways. Previous studies related to the importance of microbial or plant cell/tissue culture through metabolic engineering have successfully increased yields of natural products such as paclitaxel, artemisinin, flavonoids and alkaloids (Engels, Dahm, & Jennewein, 2008). Table 1 summarizes molecular strategies for metabolic engineering overproduction of natural products. The ultimate aim is to develop more general methods

Table 1
Strategies of metabolic engineering for production of natural products.

Explanation of strategies	References
Elucidation of biosynthetic pathways	
Combination of "omics" technology with all known pathway intermediates and enzymes/genes	Oksman-Caldentey, Inzé, & Orešič, 2004
Selection of genes encoding the pathway enzymes in the case of multi-gene families	
Genetic manipulation of biosynthetic pathways	
Regulation of transcription factors whose products may function as transcriptional activators or repressors	Broun, 2004
Overexpression of rate-limiting genes encoding key enzymes that limit specific pathways	Verpoorte & Memelink, 2002
Selection of appropriate promoters (e.g., 35S CaMV promoter or tissue/cell-specific, or -independent promoters)	
Utilization of inter- and intra-cellular transport mechanisms for compartmentation of intermediates and end-products	Allen et al., 2004
Down-regulation of competitive pathways or existing reactions	Guo, Chen, Dong, Zhang, & Luo, 2022
Optimization of codons for heterologous proteins	
Overproduction of precursors of desired compounds	Leonard et al., 2010
Introduction of genes encoding secreted proteins that convert the substrates to compounds which can be directly metabolized	
Engineering approaches	
Engineering of cytochrome P450 enzymes	Chemler & Koffas, 2008
Using the combinatorial biosynthesis approaches	Julsing, Koulman, Woerdenbag, Quax, & Kayser, 2006

that allow efficient increases in yield or productivity through metabolic engineering techniques.

4.2. Combinatorial biosynthesis

Combinatorial biosynthesis is based on the application of genetic engineering to modify biosynthetic pathways in microbes to produce novel or altered-structure natural products. Specifically, the approach of combinatorial biosynthesis is to combine biosynthetic genes from different microorganisms to produce libraries of hybrid structure. Hopwood et al. (1985) described the formation of novel isochromanequinone metabolites through genetic engineering and demonstrated the feasibility of this approach for the first time. The application of combinatorial biosynthesis for natural products led to the production of polyketides, oligopeptides, and erythromycin analogs (Julsing, Koulman, Woerdenbag, Quax, & Kayser, 2006). The combinatorial biosynthesis of natural products, in the broader sense of the term, falls into the category of metabolic engineering. Combinatorial biosynthesis must be defined not only on the metabolic level but also on the molecular level, so as to combine genes (also the corresponding enzymes) and products of different organisms to produce bioactive compounds. The transfer of the biosynthetic genes (or gene clusters) of desired compounds into heterologous hosts, based on the combinatorial biosynthesis concept, is an attractive alternative to produce the original products and even to generate novel analogs with modified and complex structures. However, several problems hinder the application of this technique, such as inefficiency of regulatory elements in the regulation of foreign gene expression, limitation of substrate specificity of the biosynthetic enzymes, lower yields of engineered new metabolites and so on. With the increasing identification of biosynthesis-related genes and the comprehensive understanding of biosynthetic pathways from different organisms, the approach of combinatorial biosynthesis continues to be viable and can be a most exciting area for biotechnology.

4.3. Engineering of cytochrome P450s

Cytochrome P450 enzymes play key roles in different reactions (e.g., oxidation and hydroxylation) involved in the biosynthesis of a wide range of compounds, such as alkaloids, terpenoids, sterols, phenylpropanoids, hormones, lignins, and fatty acids. P450s generally catalyze the reactions of C-hydroxylation, heteroatom oxygenation, heteroatom release and epoxide formation. Some of the P450s functionalize the core structure of the molecule in a regio- and stereo-selective manner to increase the structural diversity of plant secondary metabolites. However, P450s, belonging to diverse gene families and possessing a variety of conserved domains, are difficult to isolate, and it is difficult to identify the exact enzyme involved in a specific biosynthesis pathway. In addition, P450s are membrane-bound enzymes and are suitable to heterologous expression only in eukaryotes like yeast. By comparison with eukaryotic heterologous systems, the prokaryotic expression systems, like *Escherichia coli* for P450s, are limited, due to several reasons, including improper protein folding or membrane translation, a lack of the energy production and post-translational modification of the heterologous expression of proteins, and a general toxicity to the host cells. Many studies and efforts have demonstrated that the optimization of the heterologous expression of P450s facilitates the biosynthetic production of natural products. Membrane translation by altering a P450 membrane anchor is a promising alternative to successful heterologous expression of P450s in *E. coli*. The functional expression of some P450s in *E. coli* with the proper membrane translation has led to the production of terpenoids (Chang, Eachus, Trieu, Ro, & Keasling, 2007), flavonoids (Leonard, Yan, & Koffas, 2006), and isoflavones (Leonard & Koffas, 2007). Gillam (2008) also reported the strategies for engineering P450 enzymes in biological applications. The creation of P450 chimeras, which mimic protein complexes that exist in native plants, led to the fusion of P450 to the enzyme providing the P450 substrate (Tian & Dixon, 2006).

5. Natural products of medicinal plants by synthetic biology

Based on the research of herbal genomics, synthetic biology of medicinal plants can realize the orientable and efficient heterologous synthesis of medicinal active ingredients, through understanding the biosynthetic pathway of pharmacological products and reconstructing the biosynthetic pathways and metabolic networks in heterologous expression system. The research strategies of TCM synthetic biology include the following aspects: exploring the biological elements that participate in metabolic pathways of pharmacological products; designing and standardizing the biological elements; selection and modification of the heterologous expression cells; assembly and integration of the metabolic pathways, synthesis of active ingredients and structural identification. In this section, we will discuss the significant progress of vindoline, taxol and artemisinin synthetic biology, which greatly relieved the

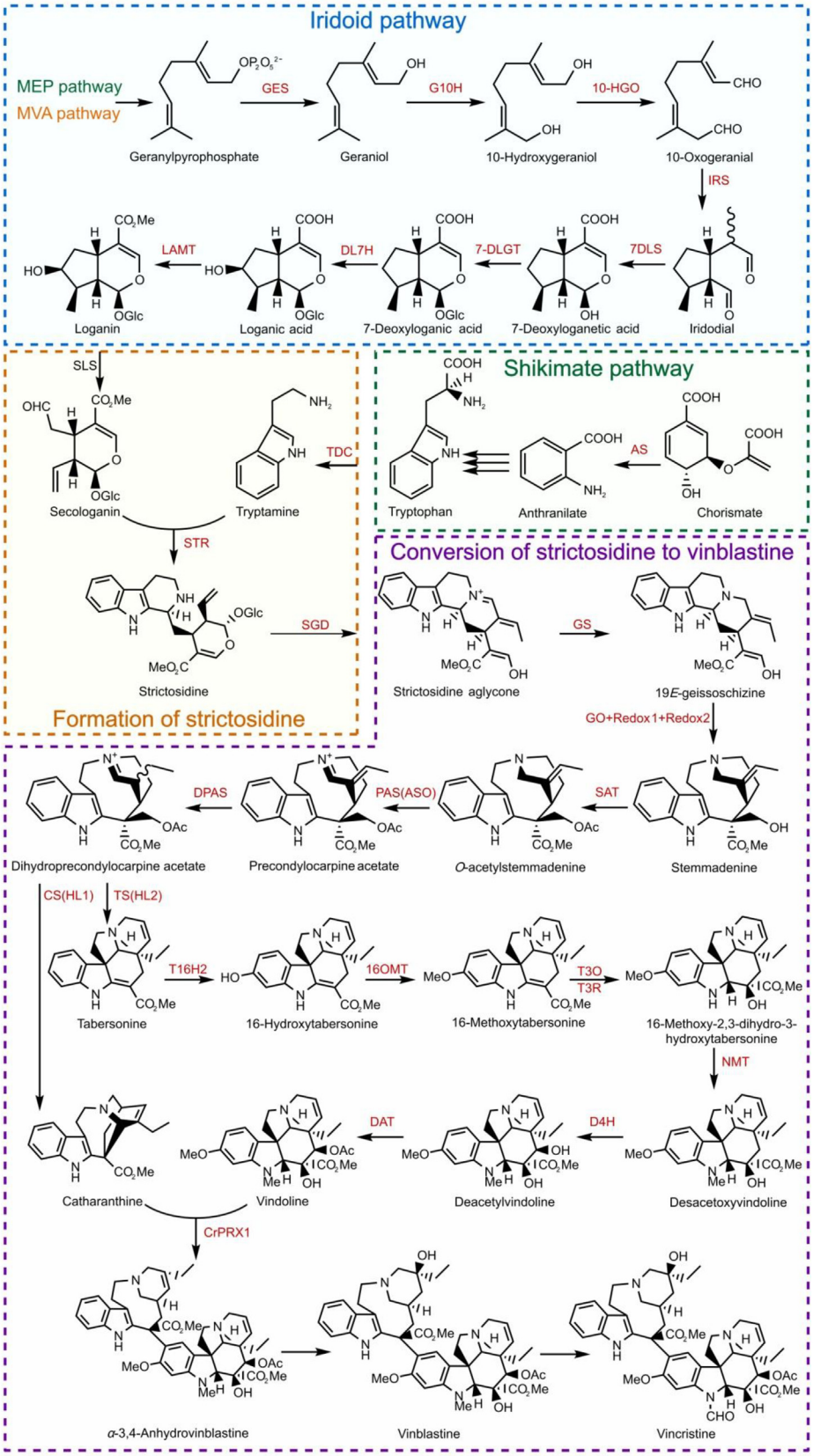

Fig. 2. Biosynthetic pathways of vindoline and vinblastine in *C. roseus*. The enzyme-coding genes represented on the solid lines in the pathways were summarized from the references listed in Table 2.

problem of the ever-growing demand for above bioactive natural products and the low content in relevant medicinal plants. Specially, these compounds possess the significant anticancer activity and antimalarial resistance. The researches on the biosynthesis and production of these compounds *in vivo* and *in vitro* have important reference value for exploiting new resource of other medicinal active ingredients using TCM synthetic biology.

5.1. Engineering of vindoline biosynthesis

Vincristine and vinblastine, the terpenoid indole alkaloids (TIAs) produced in *C. roseus*, are used as important anticancer drugs. Biosynthesis of TIAs starts with the production of strictosidine, which is formed by the condensation of the terpenoid precursor secologanin and the indole precursor tryptamine (Fig. 2). And then tabersonine is sequentially converted to vindoline by a seven-step enzymatic process. In the last, vincristine and vinblastine are derived from the coupling of vindoline and catharanthine. The genes of TIA biosynthetic pathway encoding the enzymes have been characterized. In addition, several studies have demonstrated that several transcription factors regulate the biosynthesis of vinblastine and vindoline (Table 2).

The extremely low yield of vindoline from *C. roseus* plants (3 mg/kg) triggered the efforts to explore the production of these alkaloids by engineering approaches. The early availability of genes of *TDC* and *STR*, both of which have been studied extensively in *C. roseus* cell cultures, facilitated several metabolic engineering studies on the TIA production. The constitutive over-expression of *STR* or *TDC* led to the efficient accumulation of TIAs in the transgenic *C. roseus* cell lines (Whitmer, van der Heijden, & Verpoorte, 2002a, 2002b), and hairy root cultures (Morgan & Shanks, 2000). Geerlings et al. (2001) reported that the co-overexpression of *SGD* in the recombinant host *Saccharomyces cerevisiae*, which contained *TDC* and *STR*, resulted in the formation of a large amount of strictosidine. Besides the above-mentioned engineering manipulations, *TDC* and *STR* isolated from *C. roseus* have also been transformed into suspension cell cultures of *Nicotiana tabacum*, which has led to the production of strictosidine upon feeding with secologanin (Hallard et al., 1997). In addition, a promising alternative in the expression engineering of a single or a few genes is to regulate

Table 2
Genes or transcription factors involved in vindoline and vinblastine biosynthesis.

Genes or transcription factors	Abbreviations	References
Iridoid pathway		
Geraniol synthase	*GES*	Simkin et al., 2013
Geraniol 10-hydroxylase	*G10H*	Collu et al., 2001
10-Hydroxygeraniol oxidoreductase	*10-HGO*	Krithika et al., 2015
Iridodial synthase	*IRS*	Geu-Flores et al., 2012
7-Deoxyloganetic acid synthase	*7DLS*	Salim, Wiens, Masada-Atsumi, Yu, & De Luca, 2014
7-Deoxyloganetic acid glucosyltransferase	*7-DLGT*	Asada et al., 2013
7-Deoxyloganic acid 7-hydroxylase	*DL7H*	Salim, Yu, Altarejos, & De Luca, 2013
Loganic acid *O*-methyltransferase	*LAMT*	Murata, Roepke, Gordon, & De Luca, 2008
Secologanin synthase	*SLS*	Irmler et al., 2000
Shikimate pathway		
Anthranilate synthase	*AS*	Sun, Manmathan, Sun, & Peebles, 2016
Trytophan decarboxylase	*TDC*	Sharma, Verma, Mathur, & Mathur, 2018
Formation of strictosidine		
Strictosidine synthase	*STR*	Sharma, Verma, Mathur, & Mathur, 2018
Conversion of strictosidine to vindoline		
Strictosidine *β*–*D*-glucosidase	*SGD*	Geerlings, Ibañez, Memelink, van der Heijden, & Verpoorte, 2000
Geissoschizine synthase	*GS*	Qu et al., 2018a
Geissoschizine oxidase	*GO*	Qu et al., 2018b
Redox 1	*Redox1*	
Redox 2	*Redox2*	
Stemmadenine-*O*-acetyltransferase	*SAT*	
Precondylocarpine acetate synthase (*O*-acetylstemmadenine oxidase)	*PAS(ASO)*	Caputi et al., 2018
Dihydroprecondylocarpine synthase	*DPAS*	
Hydrolase 1	*TS(HL1)*	Qu et al., 2018b
Hydrolase 2	*CS(HL2)*	
Tabersonine-16-hydroxylase 2	*T16H2*	Qu et al., 2015
16-Hydroxytabersonine-*O*-methyltransferase	*16OMT*	Levac, Murata, Kim, & De Luca, 2008
Tabersonine 3-oxygenase	*T3O*	Qu et al., 2015
Tabersonine 3-reductase	*T3R*	
N-methyltransferase	*NMT*	Deluca, Balsevich, Tyler, & Kurz, 1987
Deacetylvindoline-4- hydroxylase	*D4H*	Qu et al., 2015
Deacetylvindoline-4-*O*-acetyltransferase	*DAT*	Wang et al., 2010
Peroxidase 1	*CrPRX1*	Costa et al., 2008
Transcription factors		
Apetala2/ethylene response factors	ORCA2	Li et al., 2013
	ORCA3	Pan et al., 2012
	ORCA4/ORCA5	Paul et al., 2017
	CR1	Liu et al., 2017
Basic helix-loop-helix factors	MYC2	Zhang et al., 2011
	BIS1/BIS2	Van Moerkercke et al., 2016
	RMT1	Patra, Pattanaik, Schluttenhofer, & Yuan, 2018
Cys2/His2-type zinc finger proteins	ZCT1/ZCT2/ZCT3	Pauw et al., 2004
MYB-like factor	BPF1	van der Fits, Zhang, Menke, Deneka, & Memelink, 2000
G-box-binding factors	GBF1/GBF2	Sui et al., 2018
Jasmonate ZIM domain	JAZ	Patra, Pattanaik, Schluttenhofer, & Yuan, 2018

transcription factors, which generally control multiple steps of genes involved in a biosynthetic pathway. Because the ORCA3 transcription factor controls several steps of TIA biosynthesis, overexpression of ORCA3 in *C. roseus* cell cultures was sufficient to elevate the level of several intermediates, but it did not result in increased alkaloid production due to the fact that it did not regulate *G10H* (Pan et al., 2012). A three-fold increase in the alkaloid production in the ORCA3 transgenic *C. roseus* cell cultures was detected, as compared with control cells, only when the cultures were supplemented with loganin, the secologanin precursor (van der Fits & Memelink, 2000). As the full-step TIAs biosynthetic pathway from *C. roseus* was identified, significant progress has been made in *de novo* biosynthesis for producing vindoline using engineered microbial strain. Liu et al. (2021) used multiple metabolic engineering strategies via the CRISPR/Cas9 mediated multiplex genome integration technology, and the production of vindoline was increased to a final titer as high as 16.5 mg/L. Zhang et al. (2022) accomplished the *de novo* microbial biosynthesis of vindoline and catharanthine using a highly engineered yeast, and *in vitro* chemical coupling to vinblastine.

5.2. Engineering of artemisnin biosynthesis

Artemisinin, a sesquiterpene lactone, are famous for their use in the treatment of malaria. So far, the artemisinin biosynthetic pathway has been well elucidated. Briefly, the two independent pathways of the cytosol-localized mevalonate acid (MVA) pathway and the plastid-localized 2-*C*-methyl-*D*-erythritol 4-phosphate (MEP) pathway exist in higher plants leading to the formation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). IPP and DMAPP were converted to FPP via Farnesyl diphosphate synthase (FPS), followed by cyclization of amorpha-4,11-diene synthase (ADS) and formation of the main intermediate of artemisinin, amorpha-4,11-diene (AD). AD can be cyclized to form the direct precursor of artemisinin under the catalysis of a series of enzyme. The direct precursor of artemisinin was transferred into artemisinin in the end (Fig. 3, Table 3).

A. annua is the only natural source of artemisinin in plants, and the artemisinin content in this plant is very low (0.01 to 1.0% by dry weight). As the low artemisinin content, unstable supply of plant-derived artemisinin and unfeasible total synthesis of artemisinin, research efforts aimed at developing alternative production processes, using advanced breeding (Graham et al., 2010) and metabolic engineering (Covello, 2008), were initiated in the early 2000s. Considerable progress has been made with expressing parts of the artemisinin biosynthetic pathway in microbial hosts, followed by semi-synthetic conversion to the end product.

An attractive approach for high-yielding biological producing artemisinin is using synthetic biology to develop strains of *S. cerevisiae*. Artemisinic acid, the precursor of artemisinin, can be used for the semi-synthesis of artemisinin, which ensures that the further conversion of artemisinic acid to artemisinin is not complex (Zeng, Qiu, & Yuan, 2008). In 2003, Keasling laboratory introduced codon-optimized *ADS* gene from *A. annua* into *E. coli*, combining

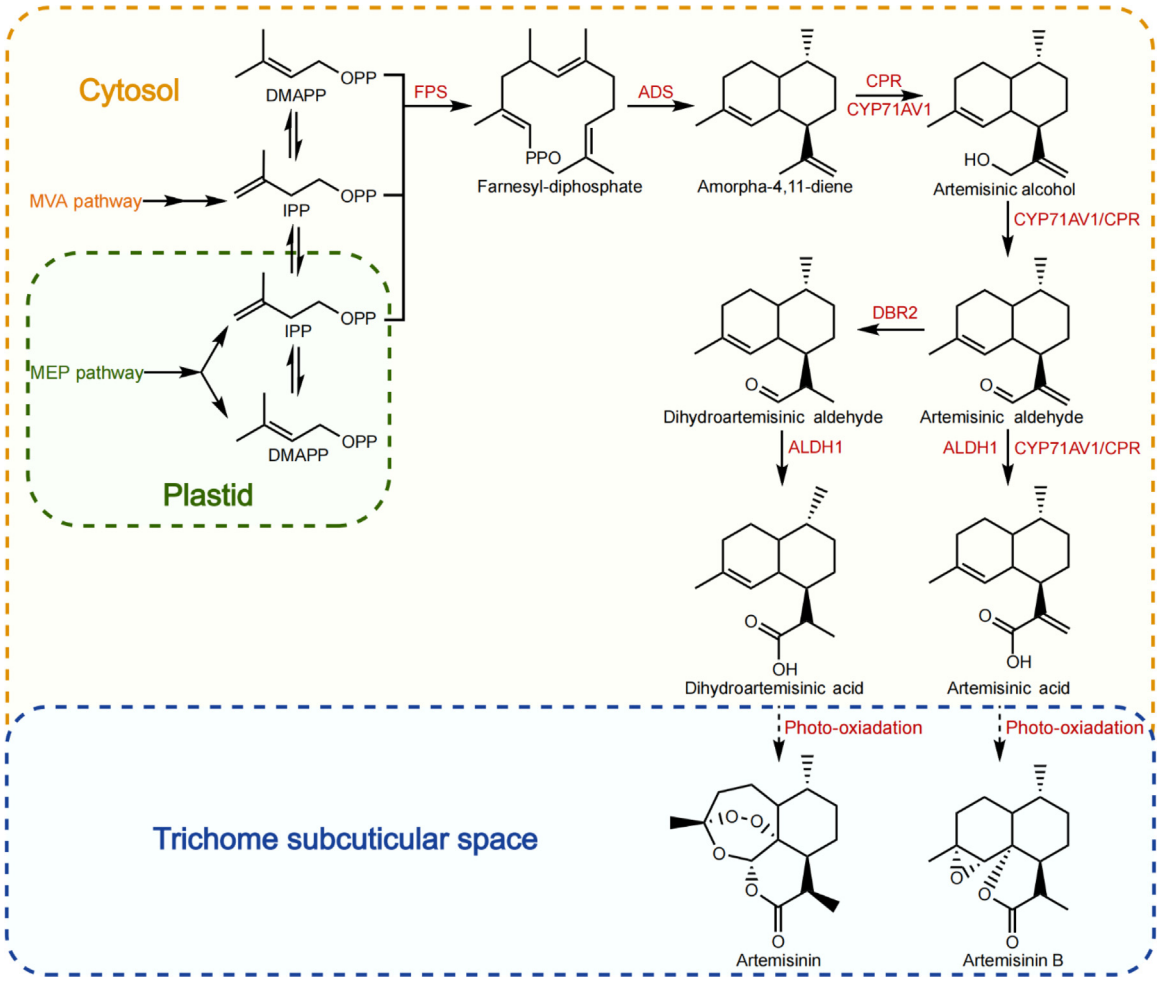

Fig. 3. Biosynthetic pathways of artemisinin in *A. annua*. The enzyme-coding genes represented on the solid lines in the pathways were summarized from the references listed in Table 3. The dashed line indicates the enzymes for the proposed catalytic reactions were not identified.

Table 3
Genes or transcription factors involved in artemisinin biosynthesis.

Genes or transcription factors	Abbreviations	References
Gene names		
3-Hydroxy-3-methylglutaryl-CoA reductase	*HMGR*	Aquil, Husaini, Abdin, & Rather, 2009
1-deoxy-*D*-xylulose 5-phosphate synthase	*DXS*	Olsson et al., 2009
1-deoxy-*D*-xylulose 5-phosphate reductoisomerase	*DXR*	Takahashi, Kuzuyama, Watanabe, & Seto, 1998
Farnesyl diphosphate synthase	*FPS*	Sharma & Agrawal, 2013
Amorpha-4,11-diene synthase	*ADS*	Nguyen, Towler, & Weathers, 2013
Cytochrome P450 monooxygenase	*CYP71AV1*	Wang, Han, Kanagarajan, Lundgren, & Brodelius, 2013
Cytochrome P450 reductase	*CPR*	Simtchouk, Eng, Meints, Makins, & Wolthers, 2013
Alcohol dehydrogenase 1	*ADH1*	Sarker, Galata, Demissie, & Mahmoud, 2012
Artemisinic aldehyde Δ11(13) reductase	*DBR2*	Wu, Wang & Guo, 2012
Aldehyde dehydrogenase 1	*ALDH1*	Wang et al., 2017
Transcription factors		
WRKY transcription factor	WRKY1	Han, Wang, Lundgren, & Brodelius, 2014
	GSW1	Chen et al., 2017
Basic leucine zipper factor	bZIP1	Zhang et al., 2015
Apetala2/ethylene response factors	ERF1/ERF2	Yu et al., 2012
	TAR1	Tan et al., 2015
	ORA	Lu et al., 2013
MYB transcription factor	MYB1	Matías-Hernández et al., 2017
	MYB3	Li, Qiu, Huang, Yin, & Yang, 2019
	MIXTA1	Shi et al., 2018
Basic helix-loop-helix factors	MYC2	Shen et al., 2016
	bHLH1	Ji et al., 2014
	HD1	Yan et al., 2017
	HD8	Yan et al., 2018

the relevant genes in MVA pathway from yeast to synthesize AD for the first time (Martin, Pitera, Withers, Newman, & Keasling, 2003). In 2006, with the identification of the key P450 gene *CYP71AV1* which catalyzed AD to artemisinic acid, Keasling team successfully constructed the first yeast strain producing artemisinic acid by expressing the *ADS* together with the identified *CYP71AV1* and *CPR* in *S. cerevisiae* (Ro et al., 2006). Another case for the production of artemisinic acid from *S. cerevisiae* in a bioreactor led to a significant increase of 25-fold and up to 2.5 g/L (Lenihan, Tsuruta, Diola, Renninger, & Regentin, 2008). Ro et al. investigated the use of plant-derived ABC transporters, and the optimization of ADS activity led to the improvement of artemisinic acid production in the engineered yeast (Ro et al., 2008). In 2012, Keasling laboratory doubled artemisinic acid production and produced AD up to 40 g/L through overexpressing every enzyme of the MVA pathway to *ERG20* in *S. cerevisiae* and development of fermentation processes for the reengineered strain (Westfall et al., 2012). In addition, a chemical process was developed to convert AD to dihydroartemisinic acid, which could subsequently be converted to artemisinin. In 2013, Paddon et al. (2013) identified *ADH1* and *ALDH1* genes, which catalyzed artemisinol to artemsinic aldehyde and artemsinic aldehyde to artemisinin respectively. Meanwhile, combining overexpressing all genes in the upstream of MVA pathway and reducing the expression of gene in competing pathway, the team realized the production of artemisinic acid up to 25 g/L, initially reached the industrialization level. The research achievement greatly shortens the production cycle and eases the problem of growing shortage of artemisinin. In addition, the research on the regulation of key enzymes of artemisinin biosynthesis pathway enables gene engineering methods to effectively increase the artemisinin content in *A. annua*. The promising candidate transcription factors AabZIP1 (Shu et al., 2022) and AaWRKY9 (Fu et al., 2021) have been identified for the development of *A. annua* plants with high artemisinin content in bioengineering breeding.

5.3. Engineering of paclitaxel biosynthesis

Paclitaxel, first isolated from *Taxus breviflia* with a low yield (500 mg/kg), is a diterpenoid that accumulates in the bark and needles of different *Taxus* trees. The efficiency of paclitaxel against several types of cancer, due to its unique mode of action on the microtubular system, renders it one of the most promising anticancer drugs. Paclitaxel biosynthesis starts with the cyclization of geranylgeranyl diphosphate, the universal progenitor of diterpenoids. Most of the enzymatic steps in paclitaxel biosynthesis are related to hydroxylation and other oxygenation reactions for the modification of the taxadiene skeleton yielding the key intermediate, baccatin III. These reactions include a series of oxygenations catalyzed by cytochrome P450s, acyl/aroyl transfers that occur in a CoA-dependent manner, the formation of expoxide and oxetane, the oxidation at C9, and the ensuing side chain attachment. The elucidation of the biosynthetic mechanisms of paclitaxel by the previous studies has facilitated the bioprocess for paclitaxel production. (Fig. 4, Table 4).

Exciting progress has been achieved in the biosynthesis of paclitaxel based on current biotechnological engineering approaches. Recent studies have been focused on the heterologous expression of the paclitaxel biosynthetic genes and optimization of the intermediates in microbial hosts for combinatorial biosynthesis. As a case for the combinatorial biosynthesis, the taxane intermediates of paclitaxel have been produced by engineering in *E. coli* (Huang, Roessner, Croteau, & Scott, 2001). The engineered *E. coli*, co-expressing four genes isolated from different organisms, led to a taxadiene production of 1.3 mg/L (Huang, Roessner, Croteau, & Scott, 2001). Expression of *DBAT* gene in *E. coli* produced baccatin III (Walker & Croteau, 2000a). Moreover, Loncaric, Merriweather, & Walker, 2006 demonstrated that *E. coli* producing endogenous acetyl-CoA combined with overexpression of acetyltransferase can convert exogenously supplied 10-deacetylbaccatin III to baccatin III. As compared to *E. coli*, that does not have an efficient iso-

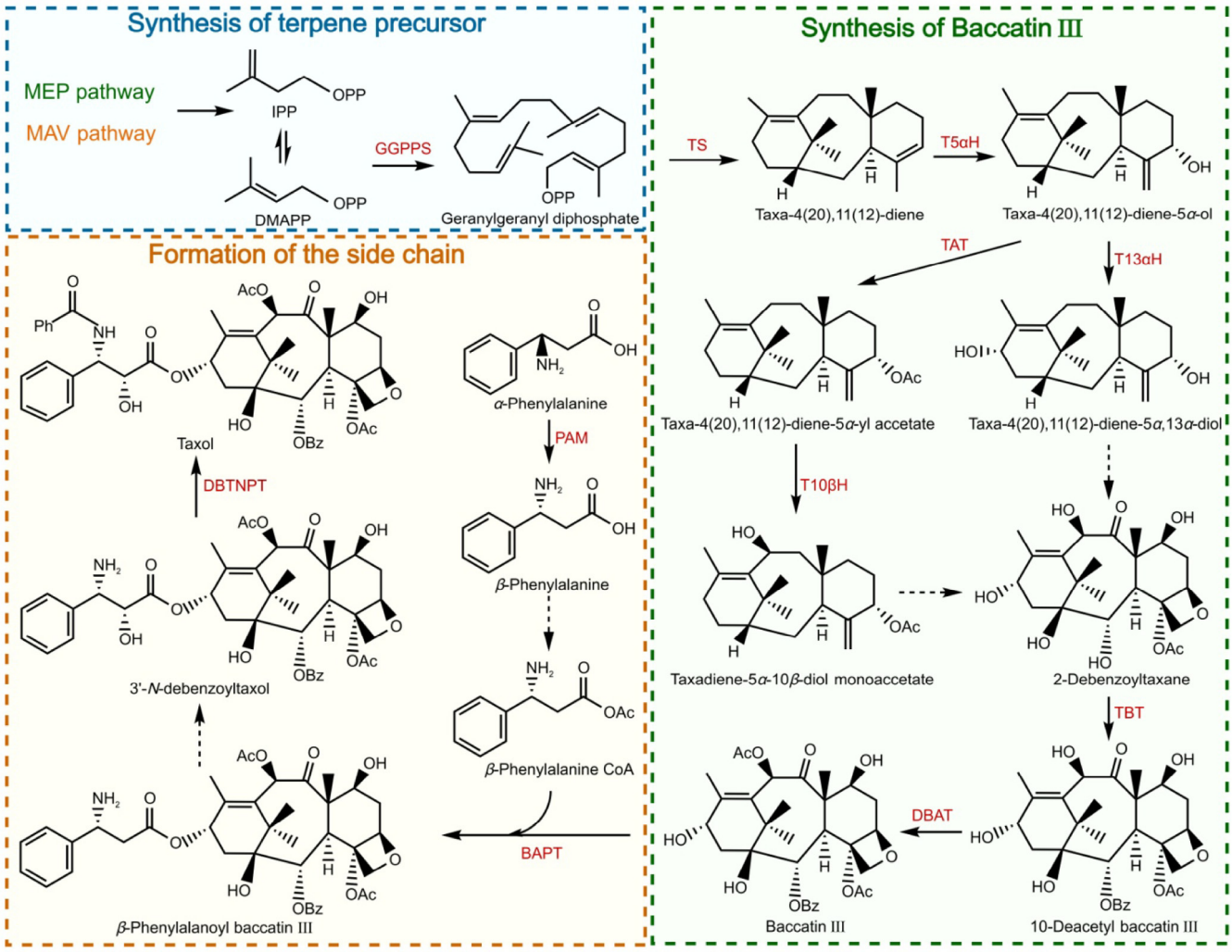

Fig. 4. Biosynthetic pathways of taxol in *T. breviflia*. The enzyme-coding genes represented on the solid lines in the pathways were summarized from the references listed in Table 4. The dashed line indicates the enzymes for the proposed catalytic reactions were not identified.

Table 4
Genes involved in taxol biosynthesis.

Gene names	Abbreviations	References
Synthesis of terpene precursor		
Geranylgeranyl diphosphate synthase	*GGPPS*	Koepp et al., 1995.
Synthesis of Baccatin III		
Taxadiene synthase	*TS*	Wildung & Croteau, 1996
Taxoid 2α-hydroxylase	*T2αH*	Chau & Croteau, 2004
Taxadiene 5α-hydroxylase	*T5αH*	Jennewein, Long, Williams, & Croteau, 2004
Taxoid 7β-hydroxylase	*T7βH*	Chau & Croteau, 2004
Taxoid 10β-hydroxylase	*T10βH*	Schoendorf, Rithner, Williams, & Croteau, 2001
Taxoid 13α-hydroxylase	*T13αH*	Jennewein, Rithner, Williams, & Croteau, 2001
Taxoid 14β-hydroxylase	*T14βH*	Jennewein, Rithner, Williams, & Croteau, 2003
Taxadienol 5α-*O*-acetyl transferase	*TAT*	Walker, Schoendorf, & Croteau, 2000
Taxane-2α-*O*-benzoyltransferase	*TBT*	Walker & Croteau, 2000b
10-Deacetylbaccatin III-10-*O*-acetyltransferase	*DBAT*	Walker & Croteau, 2000a
Formation of side chain		
Baccatin III:3-amino-3-phenylpropanoyltransferase	*BAPT*	Walker, Fujisaki, Long, & Croteau, 2002
3′-*N*-debenzoyl-2′-deoxytaxol *N*-benzoyltransferase	*DBTNBT*	Walker, Long, & Croteau, 2002
Phenylalanine aminomutase	*PAM*	Walker, Klettke, Akiyama, & Croteau, 2004

prenoid biosynthesis pathway and has a limited supply of complementary NADPH: cytochrome P450 reductase, yeast is a more likely candidate for the production of paclitaxel. Co-expression of *Taxus* cytochrome P450 reductase with cytochrome P450 oxygenase in yeast suggests that the transgenic *Taxus* reductase, coupled with a homologous cytochrome P450 hydroxylase, plays an important role in the initial step of paclitaxel biosynthesis in microbial hosts (Jennewein et al., 2005). Dejong et al. (2006) described that reconstitution of the first five committed steps in the production of taxdien-5α-acetoxyl-10β-ol in *S. cerevisiae* resulted in approximately 1 mg/L production of taxadiene. The metabolic engineering of taxadiene biosynthesis successfully enhanced the production of 40-fold taxadiene and significant amount of geranylgeranoil in *S. cerevisiae* (Engels, Dahm, & Jennewein, 2008). The strategy for the above study included the introduction of biosynthetic genes and regulatory elements to inhibit competitive pathways for combinatorial biosynthesis, and the use of codon optimization of the *T. chinensis* taxadiene synthase gene to enhance its expression level (Engels, Dahm, & Jennewein, 2008).

5.4. Challenges and perspectives

During the past decades, synthetic biology of natural products has become an attractive alternative for chemical synthesis and plant-origin extraction. Commercial production has been successfully implemented using microbial biosynthesis on certain natural products, such as artemisinin (Paddon et al., 2013). However, the development of complex biosynthetic processes for other natural products remains time-consuming due to the unidentified functional genes/enzymes, unknown intermediates, and unclear biosynthetic pathways.

With continued advancements in high-throughput genome sequencing, bioinformatics, genetics, molecular biology, and strain engineering, the potential for total biosynthesis of natural products awaits to be realized. The improvement of genome sequencing technology provided rich genetic resources for research related to synthetic biology. Decreasing costs of high-throughput sequencing continue to allow comprehensive profiling of plant genomes and transcriptomes, providing plentiful putative enzyme targets that can be mined via comparison with existing databases of enzymes with known function. Inexpensive DNA synthesis enables wholesale synthesis of dozens of predicted enzymes for any given step in a pathway, which will help to produce platform strains for a given intermediate, and the discovery of downstream pathways are greatly facilitated. Furthermore, the development of machine learning facilitated in the elucidation of complicated biosynthetic pathways. The construction of a novel model based on machine learning algorithms (Mukherjee, Blair, & Wang, 2022) by comprehensive analysis of multiple omics data from medicinal plants is probably an effective method to promote the accuracy of gene function and metabolic pathways prediction.

6. Conclusion

Traditional Chinese medicine resources are the basis for the inheritance and development of Chinese medicine industry. The active components of TCM are the material basis for the efficacy of TCM, and also the source of innovative drugs. More than 85% of traditional Chinese medicine comes from medicinal plants, and most of the active ingredients in TCM are secondary metabolites of cultivated or wild medicinal plants, which are accumulated in specific tissue parts and specific growth stages of plants. With the frequent occurrence of global natural disasters, the growth of medicinal plants is seriously threatened by drought, salinity, low temperature, high temperature, diseases and insect pests, leading to the yield of active components is not stable. In addition, harvesting from the wild, the main source of natural active products, is causing loss of genetic diversity and habitat destruction. Therefore, the modern medicine urgently seeks advanced technologies and strategies to ensure medicinal plants quality security, while increasing natural products yields to meet the needs of sustainable green development.

Genetic engineering, engineering of plant cell cultures, metabolic engineering, and synthetic biology based on the multiple "OMICS" technologies provide a cost-effective, sustainable and well-controlled means for mass production of the active principles of medicinal plants. With the development of herbal genomics and synthetic biology, the past decades have witnessed notable progress in the development and application of medicinal plant biosynthesis for production of pharmaceuticals (Chen et al., 2015). The commercial application of biosynthesis for production of vindoline, artemisnin and paclitaxel has proved the feasibility of synthetic biology for large-scale production of plant pharmaceuticals. The rapid advances in plant genomics, transcriptomics and proteomics, plus the recent emergence of metabolomics and experimental techniques for molecular biology and analytical chemistry, will greatly facilitate and enhance the biosynthesis engineering of medicinal plants. Moreover, synthetic biology, along with gene editing technology (Guo, Chen, Dong, Zhang, & Luo, 2022), is valuable, versatile, and efficient tools for research, development, and commercialization of natural products of medicinal plants.

CRediT authorship contribution statement

Miaoxian Guo: Writing - original draft. **Haizhou Lv:** Data curation. **Hongyu Chen:** Data curation. **Shuting Dong:** Data curation. **Jianhong Zhang:** Visualization. **Wanjing Liu:** Visualization. **Liu He:** Writing – review & editing. **Yimian Ma:** Writing – review & editing. **Hua Yu:** Writing – review & editing. **Shilin Chen:** Writing – review & editing. **Hongmei Luo:** Writing – review & editing.

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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