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Research Progress on Plant Metabolic Pathway Reconstruction Strategies Integrating Gene Editing and Synthetic Biology and Their Application in High-Value Product Synthesis

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Abstract. With the growing demand for green chemicals, biological products and sustainable manufacturing, plant metabolic engineering has gradually become a vital direction for the development of high-value natural products. Plants possess photosynthetic autotrophic capacity, complex organelle structures and abundant endogenous metabolic networks, enabling them to synthesize and accumulate a variety of medicinal natural products, pigments and aromatic compounds. However, plant metabolic pathways are highly complex, characterized by pathway crosstalk, feedback regulation, compartmentalized distribution, isozyme redundancy and tissue specificity, which pose challenges to the targeted synthesis and yield improvement of target products. In recent years, the development of synthetic biology and gene editing technologies has provided new solutions for plant metabolic reconstruction. The combined optimization of modular elements and the CRISPR/Cas gene editing system can regulate the expression of target genes, improve the spatial distribution of enzymes, promote the transport of substrates and products, and reduce the shunting of metabolic flux by competitive pathways. Different chassis platforms such as tobacco, *Arabidopsis thaliana*, tomato, maize and plant cell suspension culture systems also provide diverse options for metabolic pathway analysis, functional verification and high-value product production. In the future, combined with multi-omics analysis, metabolic modeling, AI-aided design and systematic regulation strategies, plant metabolic engineering is expected to further break through limitations such as flux bottlenecks, unstable expression and product toxicity, and play a greater role in green manufacturing, biomedicine and bioeconomic development.

Keywords: Plant metabolic engineering, Synthetic biology, CRISPR/Cas, Metabolic reconstruction, Natural products, Green manufacturing

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1. Introduction

In recent years, with rapid population growth and economic development, a large number of natural resources have been overexploited, and global climate and environmental problems have become increasingly serious. The global demand for green chemicals and biological products continues to rise, and the concepts of environmental protection and sustainable development have increasingly become the core driving force for the development of various industries [1]. Synthesizing target products through chemical pathways causes severe environmental pollution. Finding a method that uses renewable resources, reduces energy consumption, lowers environmental pollution and efficiently produces high-value products has become an important issue for global industrial development.

As natural "chemical factories" that can synthesize various chemical substances through photosynthesis, plants have great potential for producing diverse green chemicals, and metabolic pathways that generate a variety of high-value metabolites exist in the photosynthetic process. Plants inherently possess many key enzymes essential for product synthesis pathways and share metabolic pathways with other natural plants [2]. Their expression systems are also more likely to express genes derived from plants, thereby enabling heterologous expression and pathway reconstruction *in planta* to synthesize various natural products, such as the biosynthesis of artemisinin in tobacco [3]. In addition, the unique adaptability of plants allows them to acquire and improve diverse synthetic pathways at each growth and developmental stage as they adapt to the environment, and highly modified synthetic pathways can form more complex chemical substances. As plants grow, organelle compartmentalization emerges in their cells, enabling them to accumulate toxic natural products and withstand cellular engineering [4]. Most of the required green products and biological products are derived from plant natural products, and many are even plant metabolites. It follows that plants themselves have a strong ability to synthesize, accumulate and store natural products. How to obtain target products and increase yields through plant metabolic engineering has gradually become a research hotspot in plant physiology and molecular biology.

At present, synthetic biology, gene editing technologies and their integrated application have become powerful tools driving plant metabolic engineering. On the one hand, gene editing technologies are not limited to targeted gene knockout, insertion and replacement, but have also expanded into many derivative technical tools, such as zinc-fingers, transcription activator-like effectors and CRISPR [5]. These toolkits can edit genes in a targeted manner, and are used to modify and optimize essential enzymes in plant metabolic pathways [6], and even perform complex biochemical modifications, thereby simplifying metabolic pathways and increasing metabolite yields. In addition, technologies such as CRISPR can also promote the understanding of the molecular regulatory mechanisms of metabolic pathways by regulating the expression of transcription factors [7], which helps address the complexity of plant metabolism and improve metabolic yield. On the other hand, synthetic biology can screen and identify genes in combination with metabolomics or transcriptomics analysis to further clarify the entire metabolic pathway [8]. Furthermore, synthetic biology can also synthesize and optimize functional enzymes involved using genetic elements to develop host platforms for producing complex plant natural products. These strategies provide new ideas for optimizing and reconstructing the biosynthetic pathways of natural products. Although gene editing technologies are becoming increasingly sophisticated, they still face considerable off-target challenges. In this regard, the combination of gene editing tools with emerging fields such as bioinformatics has the potential to overcome the off-target effects encountered in genetic engineering [9].

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2. Foundations and challenges of plant metabolic engineering

2.1. Complexity of plant metabolic networks

In plant metabolic engineering, the high complexity of plant metabolic networks is a prominent difficulty. Due to the duplication of reaction sites and catalytic enzymes in plants, or the fact that chemicals required for a certain metabolism are derived from products of other pathways, multi-pathway crosstalk is formed, which further increases the complexity of plant metabolism [10]. Some plant metabolic pathways can also serve as supplementary pathways for others, providing them with precursors or reducing power. For example, the oxidative phase of the pentose phosphate pathway can produce NADPH, which is utilized by other biochemical metabolisms in organisms. Meanwhile, plant metabolic processes often encounter feedback regulation: products of downstream pathways can exert positive or negative regulatory effects on upstream reactions, ultimately regulating the efficiency of the entire pathway and metabolite abundance. In addition to feedback regulation, plant metabolic networks are affected by key enzymes and their activities, and plants also contain complex and diverse isozymes [11]. Furthermore, plant compartmentalization requires transporters and other intermediate mediators to connect metabolic pathways located in different compartments, forming highly complex connectivity and tissue specificity. Differences in the components of different types of plant tissues and cells, which serve their respective physiological functions, lead to differences in required precursor molecules and reaction pathways [12]. At present, the limitations of plant genetic engineering methods make it difficult to elucidate the interrelationships between plant pathways and multiple enzymes.

The metabolic complexity exhibited by plants has hindered their large-scale industrial application to a certain extent. Compared with plants, microbial systems are easy to operate for genetic engineering due to their simple and well-defined genetic background. Their short growth cycles, mature culture systems and fermentation technologies, and highly adaptable endomembrane systems also once made them the most commonly used and dominant metabolite platforms. Some microorganisms can also be used for the semi-synthesis of simplified pathways and to expand the chemical properties of products. However, when using microorganisms to synthesize target products, not only suitable substrates are required, but also a large amount of energy and catalytic mediators. At the same time, problems such as the unstable activity of enzymes from heterologous hosts in microorganisms, the transformation and storage of products in limited spaces, and special microenvironments make plant cells still an irreplaceable platform for target product synthesis [13]. Natural plants have diversity in selection, and some model plants also have short life cycles, which reduce maintenance costs through photosynthetic autotrophy. Plants themselves have subcellular levels and multiple compartments that can provide a stable microenvironment for reactions, specific genetic systems for homologous metabolites, and large capacity for product accumulation. They also carry all or part of the pathways for many products, and their cells are tolerant and effective to genetic engineering modification and optimization. With the vigorous development of genetic engineering technologies, plants will become excellent factories for metabolite synthesis in the future, and may be put into commercial production after overcoming the limitations of plant chassis [14].

2.2. Classification and summary of valuable products

The complexity of plant metabolism connects various pathways, and further extends peripheral metabolic pathways such as secondary metabolism. Primary metabolic pathways, as components of

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plant central metabolism, produce primary metabolites such as amino acids, nucleotides, vitamins and lipids at the genetic level. Part of them are transported and accumulated in plant sink organs to maintain cellular homeostasis and normal life activities; the other part are synthesized into more complex compounds under stress stimulation, and the latter are called secondary metabolites. However, the concentration of secondary metabolites changes with plant and cell types, specific developmental stages and environmental conditions. These metabolites are not only the response of plants to the needs of growth and development cycles and external defense, but can also be eventually developed into green products beneficial to human health and daily life, such as drugs, food additives and aromatic chemicals.

The origin of primary metabolites indicates that they generally exist stably in various plants for a long time, perform overall regulatory functions, and are indispensable life components of plants. Secondary metabolites, by contrast, play an indirect role in plants, assisting plants in reproduction and interactions with insects and microorganisms, and in turn exert effects on primary metabolic pathways. According to their biosynthetic pathways, secondary metabolites are mainly divided into phenols, terpenes, and nitrogen- and sulfur-containing compounds, which are further classified into alkaloids, flavonoids and so on. Based on primary metabolites, secondary metabolites acquire diverse functional groups and stereochemical properties by activating and reacting through peripheral metabolic pathways. They often have wider pharmacological properties than primary metabolites, thus exerting therapeutic and preventive functions. Some secondary metabolites are accompanied by toxicity and other biological activities, and can be made into medical or agricultural products for antibacterial, disinfection and insecticidal purposes, such as toxic alkaloid compounds. In addition, primary and secondary metabolites can also be used as chemical raw materials, biomedical precursors and nutrition-enhancing molecules [15].

3. Application of synthetic biology in plant metabolic reconstruction

3.1. Modular elements

In plant metabolic reconstruction, the role of synthetic biology is not limited to introducing a single exogenous gene, but to achieve systematic regulation of target metabolic pathways through the standardized combination and optimization of different functional elements. These elements usually include promoters, UTRs, terminators, coding sequences, subcellular localization signals, transporters, transcription factors and so on. Through the reasonable combination of these modules, the expression intensity of target genes can be adjusted, the spatial distribution of key enzymes in cells can be optimized, the transport of substrates and products can be promoted, and the shunting effect of competitive pathways on metabolic flux can be weakened, thereby improving the synthesis efficiency of target metabolites.

Commonly used constitutive promoters include CaMV 35S and Act; promoters such as rbcS and Lhcb are mostly used for specific expression in green tissues; promoters such as Oleosin and Glutenin are often used for seed-specific expression. In addition, inducible promoters such as HSP18.2, GBSS, SR2 and Rd29 can initiate target gene expression under induced conditions, and are suitable for regulating metabolic pathways with growth burden or potential toxicity [16]. UTR and terminator elements also affect gene expression efficiency. For example, 5′UTR elements such as AMV leader and TEV 5′ leader can improve translation efficiency, while 3′-end elements such as NOS 3′UTR and OCS 3′UTR are related to mRNA stability and transcription termination [17]. Subcellular localization elements are also an important component of plant metabolic reconstruction. The RbcS transit peptide can direct proteins to chloroplasts, and the BiP signal peptide can guide

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proteins into the secretory pathway or endoplasmic reticulum. Through these localization elements, target enzymes can be brought closer to their substrates, cofactors or suitable reaction environments. In addition, transcription factors and transporters can be quickly combined with the above elements into testable, replaceable and further optimizable expression units, thereby improving the controllability and stability of target metabolite synthesis and accumulation in plant chassis.

3.2. Construction of chassis cells and expression platforms

In plant metabolic reconstruction, the selection of chassis cells and expression platforms directly affects the expression efficiency of exogenous pathways, the location of metabolite accumulation, and the feasibility of subsequent scale-up production. Compared with microbial chassis, plant chassis inherently have a complete organelle system, complex post-translational modification capabilities, and an endogenous metabolic background related to plant natural product synthesis. Therefore, they are more suitable for reconstructing complex metabolic pathways that depend on plant-specific enzymes, compartmentalized environments or multi-step modification reactions. At present, commonly used plant expression platforms mainly include whole-plant systems such as tobacco, *Arabidopsis thaliana*, tomato and maize, as well as plant cell suspension culture systems.

Tobacco is one of the most widely used expression chassis in plant synthetic biology and natural product metabolic engineering. This system has the characteristics of short growth cycle, simple genetic operation and high expression efficiency, and is suitable for rapidly verifying candidate gene functions, testing multi-gene combinations and reconstructing complex natural product pathways. In recent years, tobacco has been widely used in the analysis of plant natural product pathways and the construction of heterologous expression platforms. The improvement of its genomic information and gene editing tools has further enhanced its application value as a chassis strain for plant metabolic engineering [18]. Compared with tobacco, *Arabidopsis thaliana* is more used as a platform for mechanistic research and proof of concept. With a clear genetic background, abundant mutant resources and a mature transformation system, *Arabidopsis thaliana* is suitable for verifying regulatory elements, analyzing metabolic networks and establishing basic models of plant metabolic reconstruction. However, due to its small biomass, it is generally not suitable as a large-scale production platform [19]. Tomato is often used as a chassis for fruit metabolic engineering, especially for studying and modifying metabolites closely related to fruit quality such as carotenoids, flavonoids and anthocyanins. Tomato fruits can accumulate high levels of nutritional or functional metabolites, so they have certain advantages in nutrition fortification and high-value metabolite production [20]. Maize is more suitable for metabolic engineering oriented to agricultural applications and seed trait improvement, such as the modification of oils, starch, proteins, vitamins or some stress-related metabolites. Maize has high agricultural production value and clear grain storage organs, but its genetic transformation and regeneration systems are relatively complex, and still rely on mature tissue culture, genetic transformation and screening systems, which is quite difficult [21].

Plant cell suspension culture systems provide another idea for metabolite production. Compared with whole plants, cell suspension culture involves placing callus cells induced from plant tissues in liquid medium and culturing them in a shaker or bioreactor, so that these cells grow in suspension in the form of single cells or small cell clusters. This culture method is not limited by seasons, climate and planting areas, the culture conditions are easier to control, and it is more suitable for combination with bioreactor scale-up production. Therefore, this system is often used for the production of secondary metabolites, pigments, spices and other high-value natural products from medicinal plants. However, this system also has problems such as insufficient genetic stability of

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cell lines, fluctuating product levels, long culture cycles, sensitivity to shear force during scale-up, and high difficulty in pollution control. Therefore, in practical applications, strategies such as cell screening, precursor addition, induction treatment, medium optimization and metabolic engineering modification are usually combined to improve the yield and stability of target metabolites [22].

Different plant chassis and expression platforms have their own applicable scenarios. This is also an advantage of plants over microorganisms. Through reasonable selection of chassis systems, combined with organelle-targeted expression, tissue-specific expression and multi-gene assembly technologies, the efficiency and application potential of plant metabolic reconstruction can be further improved.

3.3. Application of CRISPR/Cas systems in plants

The discovery of the CRISPR/Cas system has greatly improved the efficiency of plant genetic manipulation. Compared with traditional transgenic methods, CRISPR/Cas can not only be used to introduce exogenous genes, but also directly perform targeted knockout, site-specific insertion, sequence replacement, base editing, promoter editing and multi-target editing on plant's own metabolic pathways, thereby changing metabolic flux distribution, reducing the activity of competitive pathways or enhancing target product accumulation, as shown in Table 1. Early studies have demonstrated that sgRNA can guide Cas9 to achieve site-specific mutations in crops such as rice and wheat, laying the foundation for site-specific editing of plant genomes.

Targeted knockout by the CRISPR/Cas system is usually used to weaken competitive branches, remove negative regulators or alter product degradation pathways. For example, editing γ-aminobutyric acid (GABA) metabolism-related genes in tomato via CRISPR/Cas9 can alter the activity of key enzymes in the GABA shunt and increase GABA accumulation in fruits. Some studies have also used a multiplex CRISPR/Cas9 system to simultaneously target multiple key genes in the tomato GABA metabolic pathway, obtaining edited materials ranging from single to multiple mutations. CRISPR/Cas can also be used for site-specific insertion and precise replacement. Through homologous recombination or donor template-mediated repair, exogenous sequences can be inserted or specific fragments replaced at target sites, thereby achieving promoter replacement, functional domain modification or site-specific integration of exogenous genes. For example, studies in maize have used Cas9 and guide RNA to achieve targeted mutation, precise editing and site-specific insertion, indicating that CRISPR/Cas can be used for site-specific modification of crop genomes. However, the efficiency of large-fragment site-specific insertion in plants is still generally lower than that of knockout editing, and donor DNA delivery and homologous recombination efficiency remain limiting factors. In plants, base editors and multi-target editing can also be used for fine gene modification to regulate enzyme activity. In plant metabolic reconstruction, this system can be used to analyze pathway functions, regulate metabolic flux, increase target metabolite accumulation, and combine with strategies such as synthetic promoters, multi-gene assembly, transporter engineering and organelle-targeted expression to further enhance the designability and controllability of plant chassis [9].

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Table 1. Application of CRISPR/Cas systems in plant metabolic pathway reconstruction strategies and high-value product synthesis

Plant/System	Target Gene/Region	High-Value Product/Metabolic Pathway	Specific Application	Reference
Atropa belladonna	**AbH6H**，	hyoscyamine	Disrupts AbH6H via CRISPR/Cas9 to prevent the further conversion of hyoscyamine into anisodamine and scopolamine	[23]
Papaver somniferum	4′OMT2	Morphine	Knocks out 4′OMT2 using SpCas9	[24]
Solanum lycopersicum	**SlGAD2**、**SlGAD3**	γ-aminobutyric acid (GABA)	Introduces mutations or premature stop codons before the autoinhibitory domains of SlGAD2/SlGAD3 via CRISPR/Cas9 to keep GAD enzymes in a higher activity state	[25]
Rice (*Oryza sativa*)	Genomic safe harbors (GSHs)	Carotenoids	Precisely inserts a 5.2 kb carotenoid biosynthetic expression cassette into rice genomic safe harbors via CRISPR-Cas9	[26]
Nicotiana benthamiana	Multiple endogenous enzyme genes in the flavonoid pathway	Flavonoids	Selectively activates endogenous genes related to the flavonoid pathway using the dCas9 transcriptional activation system dCasEV2.1	[27]

4. Typical cases of high-value product synthesis

Medicinal natural products are the most representative type of high-value products in plant metabolic reconstruction, including paclitaxel, artemisinin, morphine alkaloids and flavonoids. Such compounds usually have complex multi-step synthetic pathways involving terpene synthases, cytochrome P450s, glycosyltransferases, methyltransferases, reductases and transcription factors, so

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they are also important objects for testing the construction capacity of plant synthetic biology platforms.

Paclitaxel is an anticancer diterpenoid compound derived from *Taxus* plants. Its biosynthetic pathway is long and includes multiple P450-mediated oxidative modification steps. Due to the high difficulty of reconstructing the complete paclitaxel pathway, current studies mostly focus on the synthesis of key intermediates and pathway analysis. For example, in *Nicotiana benthamiana*, researchers achieved the synthesis of key paclitaxel intermediates taxadiene and taxadiene-5α-ol through chloroplast compartmentalization engineering and isoprene precursor pool enhancement, demonstrating that plant chassis have advantages in expressing membrane-bound P450s and reconstructing complex terpene pathways. Researchers have also used CRISPR-guided DNA methylation in *Taxus* cells to regulate the phenylpropanoid supporting pathway and control the metabolic flux of the supporting pathway, providing an auxiliary optimization strategy for paclitaxel synthesis [28, 29].

Artemisinin is an antimalarial sesquiterpene lactone produced by *Artemisia annua*. Its synthetic pathway takes FPP as a precursor and is gradually formed through intermediates such as amorpha-4,11-diene, artemisinic alcohol, artemisinic aldehyde and artemisinic acid. Researchers have achieved rapid production of precursors such as artemisinic acid in *N. benthamiana* through *Agrobacterium*-mediated transient expression of combined artemisinin precursor synthesis genes. Meanwhile, engineered yeast has also been used to produce artemisinic acid, providing an important basis for the semi-synthesis of artemisinin.

Flavonoids include flavonols, anthocyanins, isoflavones and proanthocyanidins, which have antioxidant, coloring and health functional values. Their pathways are relatively clear, and enhanced accumulation is often achieved through transcription factor regulation and multi-gene engineering. For example, expressing the Delila and Rosea1 transcription factors from snapdragon in tomato can significantly increase fruit anthocyanin content; expressing *Arabidopsis thaliana* AtMYB12 can enhance the accumulation of metabolites related to flavonol and phenylpropanoid pathways.

The reconstruction of synthetic pathways for medicinal natural products usually requires optimization at three levels: first, enhancing precursor supply, such as increasing the levels of precursors such as FPP and GGPP in terpene pathways; second, optimizing the expression and subcellular localization of key enzymes, especially solving the problems of functional expression and spatial coupling of P450 enzymes; third, coordinating multiple reaction steps through transcription factors, transporters or multi-gene assembly systems. Different hosts such as tobacco, tomato and yeast each have their own advantages. Among them, plant chassis are more suitable for reconstructing pathways that depend on plant organelles, membrane systems and complex modification reactions, while microbial chassis have advantages in rapid engineering and fermentation scale-up.

5. Challenges and countermeasures

Synthetic biology has provided many new tools and ideas for plant metabolic reconstruction, but there are still many limitations in practical applications. Among them, insufficient metabolic flux is a prominent difficulty. The metabolic network in plants is very complex, and target synthetic pathways often need to compete with other primary or secondary metabolic pathways for precursors, energy and cofactors. If precursor supply is insufficient, or the catalytic efficiency of some key enzymes is low, the accumulation of target products may fail to reach ideal levels even if related pathways have been introduced. The expression of multi-gene pathways in plants also has uncertainty. Factors such as promoter strength, exogenous gene insertion position, copy number,

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post-transcriptional regulation and gene silencing may affect the expression effect of target pathways. For complex pathways containing multiple enzymatic reaction steps, uncoordinated expression ratios between different genes may also lead to blocked metabolic flux and even affect normal plant growth. Some medicinal natural products or active secondary metabolites themselves have strong biological toxicity, and excessive accumulation may cause stress to cells and interfere with plant growth, development and basic metabolism. Therefore, while improving the synthesis level of target products, it is also necessary to consider how to reduce the impact of intermediates or final products on plants themselves.

To address these problems, future plant metabolic reconstruction cannot rely on a single gene or a single strategy, but needs to combine multi-omics analysis, metabolic modeling and systematic regulation methods. Genomics, transcriptomics, proteomics and metabolomics can help us identify key rate-limiting steps, competitive branches and potential regulators; metabolic flux analysis and computational models can be used to predict metabolite distribution patterns and optimize modification targets. On this basis, combined with technologies such as CRISPR/Cas editing, multi-gene assembly and organelle localization, the controllability of plant chassis and the accumulation stability of target products can be more effectively improved.

6. Prospects

With the development of artificial intelligence, systems biology and multi-omics technologies, plant metabolic engineering is gradually shifting from traditional empirical modification to more precise design-based modification. AI technology can be used to predict candidate enzyme functions, screen suitable regulatory elements, design possible synthetic pathways, and assist in optimizing multi-gene expression combinations. At the same time, multi-omics data can reflect changes in plant metabolic networks at the overall level, providing a basis for subsequent targeted regulation.

The industrial application of plant chassis is also an important direction for future development. Tobacco, tomato, maize, medicinal plant cell culture systems and chloroplast engineering platforms all have different application potentials. With the improvement of culture systems, genetic transformation technologies and bioreactor processes, plant platforms are expected to play a greater role in the production of drug precursors, natural pigments, nutrition-enhancing molecules and green chemicals.

However, the promotion and application of plant metabolic engineering must also attach importance to safety and standardized management. For genetically modified plants, gene-edited plants and plant platforms capable of synthesizing highly active metabolites, a more comprehensive risk assessment system needs to be established, focusing on issues such as exogenous gene spread, ecological impacts, product safety and environmental release risks. Only on the basis of simultaneous advancement of technological development and safety supervision can plant synthetic biology truly achieve stable, controllable and sustainable development.

7. Conclusion

Plant metabolic engineering is gradually being deeply integrated with synthetic biology, CRISPR/Cas technology, multi-omics analysis, AI design and cell culture technologies, and the designability and application value of plant chassis are constantly improving. Through the systematic optimization of metabolic pathways, regulatory elements, organelle localization and host platforms, plants are not only important objects for natural product research, but also expected to become important biofactories for the production of drugs, nutrients and green chemicals. In the

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future, with the continuous maturity of related technologies, plant metabolic engineering will play a more important role in green manufacturing, biomedicine, agricultural improvement and bioeconomic development.

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