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Research Progress on NAC Transcription Factors in Regulating Plant Terpenoid Biosynthesis

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Abstract

Terpenoids are among the most abundant compounds produced in plant secondary metabolism and play a crucial role in plant growth and development. They have extensive applications in medicine, biotechnology, and agriculture. As key regulators of gene expression, transcription factors significantly influence the biosynthesis and accumulation of terpenoids by modulating the expression of critical genes involved in their metabolic pathways, thereby enhancing plant stress resistance. This paper focuses on the biosynthetic pathways of terpenoids, emphasizing the role of NAC transcription factors across various plant species and their impact on terpenoid synthesis. Furthermore, it investigates the regulatory mechanisms by which NAC transcription factors govern terpenoid biosynthesis, providing valuable insights into their modes of action. Finally, this review outlines future research directions, aiming to offer new perspectives and strategies to deepen our understanding of how NAC transcription factors regulate terpenoid biosynthesis in plants.

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Introduction

Terpenoids, natural compounds characterized by an isoprene backbone, play essential roles in biological metabolism (Bouvier et al., 2005). They significantly contribute to various aspects of plant life, including growth, development, environmental adaptation, allelopathy, and defense against pests and pathogens. For instance, oleuropein and mannitol enhance olive root resilience under drought conditions (Mechri et al., 2020), while volatile monoterpenes like α -pinene and camphor derived from Salvia leucophylla inhibit germination, DNA synthesis, and meristematic cell elongation in canola seeds (Nishida et al., 2005). In addition to their physiological functions in plants, terpenoids also possess considerable medicinal value. Artemisinin, obtained from Artemisia annua, not only exhibits anti-inflammatory and anticancer properties but remains one of the most effective treatments for malaria (Tu, 2016; Su et al., 2021). Similarly, Tanshinone IIA, a diterpenoid from Salvia miltiorrhiza, has been shown to mitigate Angiotensin II-induced cardiomyocyte hypertrophy (Chen et al., 2023).

Terpenoid biosynthesis is a complex process regulated by various factors, among which transcription factors play a crucial role. These transcription factors modulate the expression of critical genes within the metabolic pathways, thereby influencing both the efficiency and diversity of terpenoid production. For instance, the LaMYC4 transcription factor in *Lavandula* spp. enhances terpenoid biosynthesis by regulating key enzymes like *HMGR*, *FPPS*, and *DXS* (Dong *et al.*, 2022).

Similarly, the overexpression of PfERF106 in *Primula spp.* significantly boosts the synthesis of essential terpenoids like limonene and camphor (Yin *et al.*, 2024). Recently, NAC transcription factors have gained recognition for their critical role in plant secondary metabolism. In *Citrus reticulata*, CrNAC036 interacts with CrMYB68 to suppress CrNCED5 expression, thus inhibiting abscisic acid (ABA) biosynthesis (Zhu *et al.*, 2020). Additionally, OsNAC2 and OsNAP regulate ABA synthesis in *Oryza*

sativa by modulating the expression of OsNCED3, a key gene in the ABA biosynthetic pathway (Kim *et al.*, 2019).

This review aims to provide a comprehensive analysis of the role of NAC transcription factors in regulating terpenoid biosynthesis. By examining the underlying molecular mechanisms, we hope to elucidate how these transcription factors orchestrate the production of these vital compounds. Furthermore, we will explore potential future research directions, particularly the application of advanced genomic tools to enhance our understanding and manipulation of NAC-mediated regulation in plants.

Biosynthetic pathway of plant terpenoids

Terpenoids are classified into several categories based on the number of carbon atoms: monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), tetraterpenes (C40), and polyterpenes (C>40). The biosynthesis of plant terpenoids occurs through four critical stages: the formation of basic building blocks, chain elongation, construction of core structures, and subsequent modification to produce the final compounds. In the initial stage, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are synthesized via either the mevalonic acid (MVA) pathway or the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (Hunter, 2007; Eva et al., 2013) (Figure 1). During the second stage, one DMAPP molecule condenses with one or more IPP molecules, resulting in the formation of geranyl pyrophosphate (GPP), the precursor for monoterpenes, catalyzed by geranyl diphosphate synthase (GPPS) (Croteau, 1987). Similarly, farnesyl pyrophosphate (FPP), the precursor for sesquiterpenes and triterpenes, is synthesized through the action of farnesyl diphosphate synthase (FPPS) (Sallaud et al., 2009; Kim et al., 2014). Geranylgeranyl pyrophosphate (GGPP), which serves as the precursor for diterpenes and tetraterpenes, is produced via geranylgeranyl pyrophosphate synthase (GGPPS) (Srivastava et al., 2022; Thapa et al., 2016). Alternatively, two FPP molecules can condense to form C30 triterpenes (Eva et al., 2013).

In the third stage, these precursors undergo further modified by terpene synthases, leading to the formation of the core structures of various terpenoids (Pazouki and Niinemets, 2016). Finally, during the last stage, these core structures are subjected to additional modifications, most notably through the activity of cytochrome P450 enzymes (CYPs), which play a key role in generating the structural diversity of terpenoids. It is estimated that approximately 97% of terpenoids undergo CYP-mediated modifications that confer their biological activity (Renault *et al.*, 2014; Banerjee and Sharkey, 2014).

This process highlights the complexity and precision of terpenoid biosynthesis, with each stage finely tuned by various enzymes to produce a diverse wide array of functional compounds essential for plant defense and adaptation.

Structural characteristics of NAC transcription factors

NAC transcription factors represent one of the largest families of transcription factors identified in plants, playing a crucial role in regulating plant growth, development, and responses to environmental stresses. The first NAC gene, NAM (No Apical Meristem), was cloned from *Petunia hybrida* (Souer *et al.*, 1996). Since then, numerous NAC transcription factors have been identified across various species. For examlpe, *Zea mays* contains 129 NAC proteincoding genes have been discovered (Ding *et al.*, 2023), while *Sorghum bicolor* contains 131 members (Sanjari *et al.*, 2019), *Nicotiana tabacum* has 190 members (Yu *et al.*, 2022), and 104 NAC genes have been identified in *Camellia sinensis* (Zhang *et al.*, 2022).

A defining characteristic of NAC transcription factors is their highly conserved N-terminal NAC domain, approximately 150 amino acids in length, along with a variable C-terminal transcription regulatory region (TRR) (Chen *et al.*, 2011). The NAC domain is further subdivided into five subdomains: A, B, C, D, and E (Fig. 2a). Notably, subdomains C and D are rich in positively charged amino acids, which facilitate specific binding to DNA cis-elements (Chen et al., 2011). Subdomain A is thought to mediate dimerization, while subdomains B and E play roles in the diverse functions of NAC genes (Puranik et al., 2012). Most NAC transcription factors are predominantly localized in the nucleus (Zhang et al., 2022), although some are also present in other compartments, such as cellular the plasma membrane, cytoplasm, and endoplasmic reticulum (De et al., 2016; Bhatt et al., 2017). Research indicates that the C-terminal transcriptional regulatory region (TRR) of NAC proteins contains multiple specific motifs that can either activate or repress transcription (Iqbal et al., 2022). These motifs often feature repeating amino acid sequences, such as serine (Ser), threonine (Thr), proline (Pro), and glutamic acid (Glu). While these sequences are conserved within the same NAC subfamily, they exhibit significant variation between different subfamilies (Puranik et al., 2012). In certain cases, the TRR of specific NAC proteins interacts with target proteins (Fig. 2b), thereby contributing to functional diversity (Kleinow et al., 2009). Additionally, some NAC proteins possess a C-terminal transmembrane domain (TMs), referred to as NTL (NAC membranebound transcription factors) proteins (Rong et al., 2020). These membrane-bound NAC proteins are drawn to the nuclear envelope due to their positively charged regions, which interact with the negatively charged biomembranes, such as the plasma membrane or endoplasmic reticulum membrane. Once attracted, they are released and transported into the nucleus to exert transcriptional control (Mohanta et al., 2020).

Regulation of terpenoid biosynthesis in plants by NAC transcription factors

The diversity and extensive biological functions of plant terpenoids make them essential in various application fields. NAC transcription factors play a critical role in regulating terpenoid biosynthetic pathways, significantly influencing plants' adaptability to environmental changes and their physiological processes. By precisely modulating key metabolic pathways, these transcription factors not

only enhance plant stress resistance but also directly impact terpenoid synthesis. Recent studies have shown that NAC transcription factors are involved in the biosynthesis of a broad spectrum of terpenoids, including monoterpenes, sesquiterpenes, diterpenes, triterpenes, and tetraterpenes (Table 1).

| Regulatory Functions | Plants | NAC transcription factors | Regulatory mechanism | Reference |
|-------------------------|------------------------------------|----------------------------------|--|-------------------------------|
| Terpene Biosynthesis | Citrus reticulata Blanco | CrNAC036 | Co-regulates ABA biosynthesis with other transcription factors like MYB. | Zhu <i>et al.</i> ,2020 |
| | Oryza sativa L. | OsNAC2 OsNAP | Directly regulates genes involved in abscisic acid (ABA) synthesis. | Kim <i>et al.</i> , 2019 |
| | Artemisia annua L. | AaNAC1 | Boosts artemisinin biosynthesis gene expression, increasing yield and resistance. | Lv et al.,2016 |
| | Zea mays | NAC | Modulates sesquiterpene synthase, enhancing resistance to aphids. | Pingault <i>et al</i> ., 2021 |
| | Sindora glabra | NAC | Co-regulates terpene biosynthesis with MYB and WRKY transcription factors. | Niu <i>et al.</i> , 2020 |
| | Aquilaria sinensis | AsNAC019 AsNAC098 | Activates sesquiterpene synthase genes, promoting synthesis. | Yang <i>et al.</i> , 2023 |
| Diterpene Biosynthesis | Actinidia | AaNAC2 / 3 / 4 | Activates specific terpene synthase (TPS) genes, affecting terpene yield. | Nie <i>et al.</i> ,2015 |
| | C.medica var. sarcodactylis | Cm303080 | Interacts with MYB to coregulate TPS gene expression. | Ya et al., 2019 |
| | Pinus sylvestris var. mongolica | PsNAC1 | Regulates monoterpene biosynthesis genes, improving drought resistance. | Zhou <i>et al.</i> , 2024 |
| | Lavandula spp. | NAC | Modulates monoterpene-related pathways, affecting plant defense. | Habán <i>et al</i> ., 2023 |
| Terpene Biosynthesis | Gossypium spp | VND1-7, NST1-3, XND1 and VNI2 | Regulates ethylene synthesis, impacting fruit ripening. | Wang <i>et al.</i> ,2021 |
| | Oryza sativa L. | OsNAC2 | Inhibits gibberellin synthesis genes, modulating hormone balance. | Chen <i>et al.</i> ,2015 |
| | Stevia rebaudiana | NAC | Influences the MVA and MEP pathways, regulating stevioside biosynthesis. | Singh <i>et al.</i> , 2017 |
| | Salvia miltiorrhiza Bge. | SmNAC2 | SmNAC2 acts as a negative regulator of tanshinone biosynthesis and is induced by methyl jasmonate. | Zhang <i>et al.</i> , 2021; |
| | Andrographis paniculata | ApNAC83 ApNAC21_22 ApNAC02 | Regulates sesquiterpene biosynthesis enzymes. | Kumar <i>et al</i> ., 2024 |
| Triterpene Biosynthesis | Panax ginseng C.A.Meyer | PgNAC41-2 | Participates in stress response and directly or indirectly regulates ginsenoside biosynthesis. | Liu, 2020 |
| | Panax notoginseng | PnNAC2 | Activates PnSS, PnSE, and PnDS genes, regulating saponin biosynthesis. | Huang <i>te al.</i> , 2024 |
| | Akebia trifoliata | AktNAC095 | Linked to various metabolites during Akebia trifoliata development, possibly regulating triterpene synthesis. | Liu <i>et al.</i> , 2023 |
| | Camellia sinensis var. jinxuan | CsNAC41 | Activates specific biosynthesis pathways through promoter regulation, increasing triterpene synthesis. | Gao <i>et al.</i> , 2023 |
| | Solanum lycopersicum L. | SNAC4 | Regulates ethylene synthesis pathways, indirectly affecting carotenoid biosynthesis and delaying fruit ripening. | Zhu <i>et al</i> .,2014 |
| Triterpene Biosynthesis | Carica papaya L. | CpNAC1 | Activates key enzyme genes CpPDS2/4 in papaya, promoting fruit coloration and ripening. | Fu <i>et al.</i> ,2016 |
| | Cucumis melo var. makuwa Makino | CmNAC34 | Binds to the CmLCYB promoter in oriental melon, promoting β-carotene accumulation. | Zhao <i>et al.</i> ,2022 |
| | Fortunella crassifolia Swingle | FcrNAC22 | Regulates carotenoid accumulation under red light, enhancing fruit coloration. | Gong <i>et al.</i> ,2021 |

The Role of NAC transcription factor in Regulating sesquiterpene biosynthesis

Sesquiterpenes are key secondary metabolites in plants that play a vital role in plant defense and have extensive applications in the pharmaceutical industry. Research has shown that NAC transcription factors serve as crucial regulators of sesquiterpene biosynthesis. For instance, in *Artemisia carvifolia*, overexpression of the NAC transcription factor AaNAC1 significantly enhances artemisinin production by precisely modulating the expression of key genes within its biosynthetic pathway, thereby increasing both artemisinin yield and the plant's stress resistance (Lv *et al.*, 2016; Franco *et al.*, 2014). In *Zea mays*, NAC transcription factors regulate the expression of enzymes involved in sesquiterpene synthesis, which activates defense mechanisms and improves resistance to aphids (Pingault *et al.*, 2021).

Similarly, in Sindora glabra, NAC transcription factors regulate sesquiterpene biosynthesis in conjunction with other regulators such as MYB and ARF (Niu *et al.*, 2020). Furthermore, in Aquilaria sinensis, the transcription factors AsNAC019 and AsNAC098 bind to the promoter of the polyketide synthase gene AsPKS07, initiating its transcription and facilitating sesquiterpene biosynthesis (Yang *et al.*, 2023). By controlling the expression of key genes in sesquiterpene biosynthetic pathways, NAC transcription factors significantly influence the production and accumulation of these essential compounds.

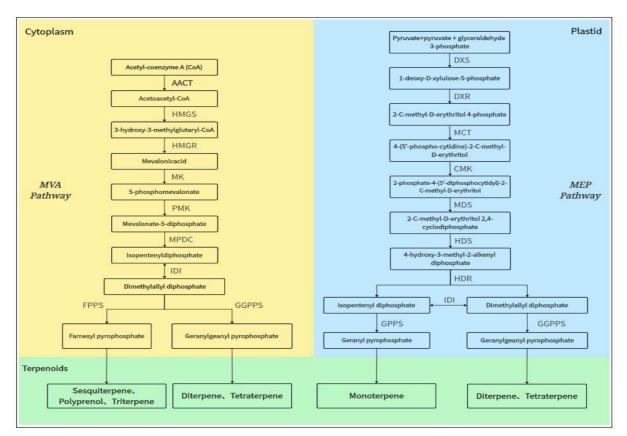


Fig. 1. Plant Terpenoid Biosynthesis Pathway.

Key enzymes involved in the enzymatic reactions of the MVA pathway: AACT: Acetyl-CoA acyltransferase; HMGS: 3-hydroxy-3-methylglutaryl-CoA synthase; HMGR: 3-hydroxy-3-methylglutaryl-CoA reductase; MK: Mevalonate kinase; PMK: Phosphomevalonate kinase; MPDC: Mevalonate-5-diphosphate decarboxylase; IDI: Isopentenyl diphosphate isomerase; FPPS: Farnesyl diphosphate synthase; GGPPS: Geranylgeranyl diphosphate synthase.

The Role of NAC transcription factor in Regulating monoterpene biosynthesis

Monoterpenes, integral components of plant essential oils, play a pivotal role in mediating plantenvironment interactions. NAC transcription factors regulate the quantity and composition of monoterpenes by modulating the expression of key enzymes in the monoterpene biosynthesis pathway. In Actinidia, NAC transcription factors activate the transcription of terpene synthase (TPS) genes, which are essential for monoterpene production. Variations in NAC expression levels significantly influence terpene yields (Niels *et al.*, 2015). Xu *et al.* (2019) demonstrated the critical role of NAC transcription

factors in regulating monoterpene biosynthesis through metabolomic and transcriptomic analyses of C. medicavar. sarcodactylis. Their study showed that the NAC gene Cm303080 interacts with the MYB family member Cm258540 to co-regulate TPS gene expression, thereby affecting monoterpene synthesis. Similarly, PsNAC1 regulates drought resistance in Pinus sylvestris var. mongolica by influencing the expression of genes related to monoterpene biosynthesis (Zhou et al., 2024). Further studies on Lavandula spp. explore how NAC transcription factors modulate metabolic pathways linked to monoterpene biosynthesis, enhancing the plant's environmental adaptability and defense mechanisms (Habán et al., 2023). Beyond the direct activation of TPS genes, NAC transcription factors collaborate with other transcriptional regulators, establishing complex networks that further enhance the efficiency of monoterpene biosynthesis.

The Role of NAC transcription factor in Regulating diterpene biosynthesis

The NAC transcription factor plays a pivotal role in regulating diterpene accumulation by either activating or suppressing key genes in the biosynthesis pathways of diterpenes. Studies in cotton have demonstrated that NAC transcription factors interact with DELLA proteins to mediate gibberellin (GA) signaling, influencing the formation of the secondary cell wall (SCW) and regulating the biosynthesis of GA and other diterpene plant hormones (Wang et al., 2021; Karel et al., 2017). In rice, the OsNAC2 transcription factor has been shown to suppress GA synthesis by downregulating key genes such as GA200x and GA30x, thereby affecting terpenoid metabolism and overall plant development (Chen et al., 2015). In Stevia rebaudiana, NAC transcription factors modulate secondary metabolite production by regulating key enzymes such as DXS and HMGR involved in both GA and steviol glycosides (SG) biosynthetic pathways (Singh et al., 2017). In Salvia miltiorrhiza, the SmNAC2 protein, identified among 84 NAC proteins, serves as a negative regulator of tanshinone biosynthesis, thus influencing specific diterpene production (Zhang et al., 2021). Moreover, in Andrographis paniculata, NAC transcription factors, including ApNAC83, ApNAC21_22, and ApNAC02, regulate the synthesis of the diterpene andrographolide by modulating the expression of key genes such as terpene synthase (TPS). Notably, ApNACo2 is upregulated under ABA treatment, suggesting its involvement in stressinduced sesquiterpene biosynthesis (Kumar et al., 2024). In summary, NAC transcription factors are central to diterpene biosynthesis and hormone signaling in plants. Through their interactions with other proteins and the regulation of critical genes, NACs orchestrate the complex biosynthesis of diterpenoid compounds.

The Role of NAC transcription factor in Regulating triterpene biosynthesis

Triterpenoids play a crucial role in plant growth and development, and also exhibiting significant pharmacological properties. NAC transcription factors can regulate triterpenoid biosynthesis by activating or repressing key metabolic pathways involved in their production. Ginsenosides, the primary active components of Panax ginseng are well-known for their anticancer and immuneboosting effects. Research has demonstrated that the gene PgNAC41-2, which is involved in ginsenoside biosynthesis, not only responds to stress but also directly or indirectly modulates ginsenoside production (Liu et al., 2023). In Panax notoginseng, it was found that NAC transcription factor PnNAC2 positively regulate the biosynthesis of triterpenoid saponins by binding to the promoters of key biosynthetic genes (PnSS, PnSE, and PnDS) (Huang et al., 2024). Similarly, during early seed development in Akebia trifoliata, the transcription factor AktNAC095 has been linked to the synthesis of metabolites, including triterpenoids, multiple highlighting its essential role in specific triterpenoid biosynthetic pathways (Liu et al., 2023). In Camellia sinensis var. jinxuan, it has been shown that CsNAC41 regulate the accumulation of triterpenoid by modulating enzyme activity within key metabolic pathways and directly affecting gene promoters involved in biosynthesis (Gao et al., 2023).

Collectively, these studies illustrate that NAC transcription factors regulate triterpenoid accumulation by binding to the promoter regions of

specific metabolic genes, thereby either activating or repressing the expression of triterpene synthase genes.

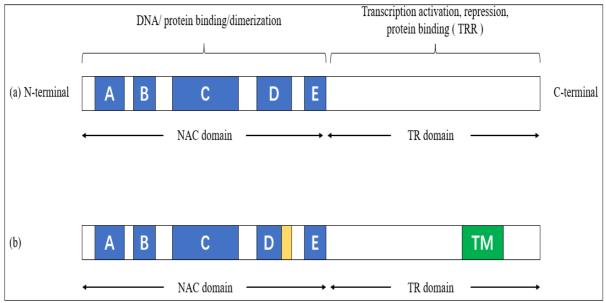


Fig. 2. Structure of NAC Proteins.

Key enzymes involved in the enzymatic reactions of the MEP pathway: DXS: 1-deoxy-D-xylulose 5-phosphate synthase; DXR: 1-deoxy-D-xylulose 5-phosphate reductoisomerase; MCT: 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase; CMK: 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase; MDS: 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase; HDS: 4-hydroxy-3-methylbut-2-enyl diphosphate synthase; HDR: 4-hydroxy-3-methylbut-2-enyl diphosphate synthase.

(a) A typical NAC protein possesses a highly conserved NAC domain at the N-terminus, which harbors DNAbinding (DB) capability and is responsible for protein binding and dimerization. The C-terminal region is highly variable and serves as a potential transcription regulatory domain, carrying activating or inhibitory functions and, at times, may exhibit protein bindingactivity.

(b) In certain specific NAC proteins, under certain circumstances, the NAC domain may include a negative regulatory domain (highlighted in yellow), and the C-terminus may contain a transmembrane motif (TM).

The Role of NAC transcription factor in Regulating tetraterpene biosynthesis

Tetraterpenoids, which are primary components of carotenoids, play a crucial role in determining plant coloration and antioxidant properties. NAC transcription factors significantly regulate the biosynthesis of these compounds, thereby influencing fruit ripening and stress resilience in plants. For instance, in Solanum lycopersicum, the NAC transcription factor SNAC4 has been shown to modulate both carotenoid and ethylene biosynthesis pathways, resulting in reduced ethylene production and delayed fruit ripening (Zhu et al., 2014). Specifically, silencing SNAC4 downregulates ACC

synthase genes, leading to decreased levels of the ethylene precursor ACC (1-aminocyclopropane-1carboxylic acid), indicating that NAC factors indirectly regulate carotenoid synthesis through ethylene modulation. In *Carica papaya*, CpNAC1 activates key enzymes in the carotenoid biosynthesis pathway, such as CpPDS2/4, thereby enhancing fruit pigmentation and maturation (Fu *et al.*, 2016). Similarly, in *Cucumis melo* var. *makuwa*, CmNAC34 binds to the promoter of the CmLCYB gene, promoting the accumulation of β -carotene (Zhao *et al.*, 2022). In *Fortunella crassifolia*, FcrNAC22 responds to red light, selectively regulating carotenoid accumulation and improving fruit color

(Gong *et al.*, 2021). Therefore, NAC transcription factors influence the production of tetraterpenoids by controlling key genes in the carotenoid biosynthesis pathway, which modulates fruit ripening and pigmentation, and enhances stress resistance, antioxidant capacity, and overall plant adaptability.

Future prospects

The role of NAC transcription factors in regulating plant terpenoid biosynthesis has been extensively studied across various metabolic pathways, including sesquiterpenes, monoterpenes, diterpenes, triterpenes, and tetraterpenes. NAC transcription factors modulate gene expression by directly binding to the promoter regions of key enzyme genes, thereby controlling both the efficiency and composition of terpenoid synthesis. Additionally, they interact with other regulators, such as MYB and ARF, forming intricate networks that optimize secondary metabolic pathways in plants. Their influence ranges from enhancing artemisinin production in Artemisia to improving sesquiterpene-related insect resistance in Zea mays, and even regulating gibberellin synthesis through the modulation of GA200x and GA30x genes. In medicinal plants, NAC transcription factors play a crucial role in regulating diterpene and triterpene synthesis via the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways, particularly in species like Panax ginseng and Panax notoginseng. Furthermore, they also govern carotenoid and tetraterpene biosynthesis, impacting pigmentation and plant defense responses. Despite substantial research on NAC's role in terpenoid biosynthesis, key mechanisms remain unclear, especially regarding their variability across different plant species, interactions with environmental signals, co-regulation with other transcription factors, and involvement in non-coding RNA regulation.

Future studies should employ high-throughput sequencing and CRISPR/Cas9 technologies to elucidate the precise regulatory mechanisms of NAC. Multi-omics approaches and single-cell sequencing can offer insights into the spatiotemporal dynamics of NAC-mediated regulation. Moreover, synthetic biology provides promising avenues for optimizing terpenoid biosynthetic pathways, enhancing their applications in medicine and agriculture by improving terpenoid content and stress resilience, ultimately boosting crop yield and quality.

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