

Advances in the Regulation of Andrographolide Accumulation and Biosynthesis: Postprint

Authors: Zhong Chu, Jian Shaofen, Chen Dongliang, Miao Jianhua

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Abstract

Andrographis paniculata is one of the important southern traditional Chinese medicines in China. Its main active components, andrographolide compounds, possess heat-clearing, detoxifying, antibacterial, and anti-inflammatory effects, and also exhibit significant efficacy in anti-HIV, antithrombotic, and hepatoprotective applications. Due to the difficulty of chemical synthesis, enhancing the biosynthesis of andrographolide compounds in *A. paniculata* through cultivation regulation or breeding methods is of great significance for improving the quality of *A. paniculata* medicinal materials. To provide reference for related research, this article reviews the relationship between andrographolide accumulation and plant development, the effects of cultivation measures and environmental factors on andrographolide accumulation, and the research progress achieved domestically and internationally in the past decade regarding the biosynthesis and molecular regulatory mechanisms of andrographolides. Furthermore, it proposes three key aspects requiring focused attention in future research aimed at enhancing andrographolide biosynthesis and accumulation: (1) deeply analyzing the biosynthetic pathway of andrographolides and the functions of key genes to elucidate the mechanism of andrographolide accumulation at the molecular level; (2) combining molecular biology methods and crop cultivation theory to deeply investigate the patterns and interrelationships between *A. paniculata* growth/development and yield/quality formation; and (3) revealing the signal regulatory network of andrographolide biosynthesis.

Full Text

Advances in Regulation Studies on Accumulation and Biosynthesis of Andrographolide Components in *Andrographis paniculata*

Zhong Chu^{1,2}, Jian Shaofen^{1,2}, Chen Dongliang^{1,2}, Miao Jianhua^{1,2}

¹Guangxi Key Laboratory of Medicinal Resource Protection and Genetic Improvement, Guangxi Botanical Garden of Medicinal Plants, Nanning 530023, China

²Guangxi Engineering Research Centre of TCM Intelligent Creation, Guangxi Botanical Garden of Medicinal Plants, Nanning 530023, China

Abstract: *Andrographis paniculata*, known as ‘Chuanxinlian’ in China, is an important southern medicinal herb. Its main active ingredients, andrographolide components, possess heat-clearing, antibacterial, and anti-inflammatory properties, and have demonstrated significant efficacy against HIV, thrombosis, and liver disease. Due to the difficulty of artificial synthesis, enhancing the biosynthesis of andrographolide components in *A. paniculata* through cultivation regulation and breeding methods is crucial for improving medicinal material quality. This review synthesizes research on the relationship between andrographolide accumulation and plant development, the effects of cultivation measures and environmental factors on andrographolide accumulation, and advances in biosynthesis and molecular regulatory mechanisms over the past decade. We propose three priority areas for future research aimed at enhancing andrographolide biosynthesis and accumulation: (1) elucidating the complete biosynthetic pathway and key gene functions at the molecular level; (2) integrating molecular biology with crop cultivation theory to investigate growth patterns and yield-quality relationships; and (3) revealing the signal transduction networks regulating andrographolide biosynthesis.

Keywords: *Andrographis paniculata*, environmental factors, andrographolide, biosynthesis, phytohormone

Andrographis paniculata is an annual herbaceous medicinal plant in the Acanthaceae family that thrives in bright light and hot, humid climates. Originating from South Asia (India and Sri Lanka), it holds significant importance in traditional medicine systems across India, Southeast Asia, China, and Japan, where it is used to treat jaundice, skin diseases, indigestion, fever, and mental disorders. Recent studies have revealed that its primary active compound, andrographolide—a labdane diterpenoid lactone with a complex structure—also exhibits potent anti-HIV, anti-inflammatory, anti-thrombotic, and hepatoprotective effects. Since chemical synthesis remains challenging, andrographolide is primarily obtained through plant extraction, making the enhancement of its content in raw materials a key objective for cultivation and breeding programs.

This review summarizes advances over the past decade in cultivation and environmental regulation of andrographolide accumulation, and discusses biosynthetic pathways and molecular regulatory mechanisms, providing a valuable reference for quality production and future molecular and genetic research.

1 Accumulation Patterns of Andrographolide Components

The accumulation of active compounds in medicinal plants is closely linked to developmental stages. Andrographolide components show distinct patterns across growth phases, understanding which is fundamental for cultivation and biosynthetic regulation studies. Andrographolides primarily accumulate in leaves, with content increasing rapidly during the vegetative growth stage, slowing during reproductive growth, peaking at flowering onset, and declining post-flowering as plants senesce. Therefore, harvesting before flowering yields higher andrographolide content. Later flowering correlates with longer growth duration and higher andrographolide accumulation. Dehydroandrographolide, however, shows no clear pattern and negatively correlates with flowering time, suggesting differential regulatory mechanisms for various andrographolide components. This relationship provides valuable guidance for breeding late-flowering cultivars with enhanced biomass and andrographolide content.

Metabolomic analyses reveal that older leaves contain lower levels of andrographolide, dehydroandrographolide, and neoandrographolide compared to younger leaves, paralleling changes in glucose and choline content. This suggests that andrographolide metabolism may be influenced by primary metabolism. Since flowering represents a critical transition in resource allocation, further investigation into the relationship between andrographolide dynamics and primary metabolism across growth stages could elucidate intrinsic regulatory mechanisms.

Comparative studies between greenhouse and field conditions show that maximum leaf andrographolide content occurs during seed formation in greenhouse plants $[(24.72 \pm 1.89) \text{ mg} \cdot \text{g}^{-1}]$, but during vegetative growth in field-grown plants $[(43.16 \pm 0.92) \text{ mg} \cdot \text{g}^{-1}]$, demonstrating that growing environment modulates developmental patterns of andrographolide accumulation.

2.1 Effects of Sowing Date and Planting Methods on Andrographolide Components

Andrographis paniculata is predominantly cultivated in subtropical regions south of the Yangtze River, including Guangxi, Guangdong, and Fujian. With increasing demand, cultivation has expanded northward to Yunnan, Sichuan, Zhejiang, Jiangsu, Jiangxi, Shandong, and Beijing. Sowing dates vary widely across these regions, spanning early April to early May due to temperature differences. In southern China, early April sowing yields optimal biomass and andrographolide content. Although sowing date affects yield and andrographolide content, it does not significantly influence flowering time. Since flowering is a critical period for andrographolide accumulation, early sowing can be used to extend the vegetative growth period, increasing dry matter accumulation and consequently improving both yield and andrographolide content.

Planting methods include direct seeding and transplanting, while cropping sys-

tems involve monoculture and intercropping. No systematic comparisons of these methods exist, but direct seeding typically creates higher plant densities that may inhibit growth and nutrient utilization, potentially reducing andrographolide accumulation. Monoculture maximizes light utilization and andrographolide content, though appropriate intercropping with crops like spring maize does not compromise mid-to-late season growth or andrographolide accumulation.

2.2 Effects of Planting Density on Andrographolide Components

Andrographis paniculata tolerates relatively dense planting. In Guangxi, maximum yield occurs at densities of 165,000–180,000 plants \cdot ha⁻¹, while studies in northern India recommend 30 cm \times 15 cm spacing (approximately 222,000 plants \cdot ha⁻¹). These regional differences likely reflect variations in light, temperature, and nutrient conditions. At 222,000 plants \cdot ha⁻¹, biomass and contents of andrographolide and neoandrographolide reach their maxima, presumably because denser stands improve resource utilization efficiency while higher biomass supports greater andrographolide accumulation.

Planting density influences andrographolide content by altering plant architecture and dry matter accumulation. Under dense planting, individual plants show suppressed growth—increased height, reduced crown width, fewer branches, and smaller branch angles—while maintaining higher dry matter yield per unit area. Correlation analyses reveal significant negative relationships between andrographolide content and leaf number/tiller count per plant, and between deoxyandrographolide and leaf number/dry weight indicators, but positive correlations with leaf length. These findings indicate a trade-off between growth and andrographolide accumulation, where moderate growth inhibition enhances andrographolide content, providing valuable phenotypic references for breeding high-andrographolide cultivars.

2.3 Effects of Nutrient Management on Andrographolide Components

As the whole plant is used medicinally, high biomass is the primary cultivation goal, with fertilization being essential for achieving this. Balanced fertilization improves both yield and quality, with adequate nutrients promoting growth and andrographolide accumulation. Integrated application of nitrogen, phosphorus, potassium, and organic fertilizers is recommended, with organic fertilizer combined with NPK and biofertilizer producing optimal growth and andrographolide yield.

Nitrogen, the most demanded nutrient, participates in all physiological processes. In Guangxi, optimal nitrogen application reaches 172.5 kg N \cdot ha⁻². However, Indian studies show that andrographolide increases with nitrogen up to 80 kg N \cdot ha⁻² but declines at 100 kg N \cdot ha⁻², though these results may

be unreliable as the maximum andrographolide content reported (0.178%) falls far below the 1.5% minimum required by the *Pharmacopoeia of the People's Republic of China* (2020 Edition). While nitrogen deficiency typically increases non-nitrogenous secondary metabolites like terpenoids (consistent with the carbon/nutrient balance hypothesis), the existence of an optimal nitrogen rate suggests more complex regulation of the growth-secondary metabolism trade-off in *A. paniculata*, warranting further investigation.

3 Environmental Regulation of Andrographolide Accumulation

Analysis of samples from different producing regions reveals substantial variation in andrographolide content, with environmental factors considered primary drivers. Current research focuses on light intensity, soil microorganisms, and salt stress.

3.1 Effects of Light Intensity and Quality on Andrographolide Components

Solar radiation is crucial for photosynthesis and biomass accumulation. Light response analyses show that during vigorous growth, maximum net photosynthetic rate is approximately $4 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, with light saturation and compensation points around $800 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and $100 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, respectively, with photoinhibition occurring above $1,000 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. However, other measurements indicate maximum net photosynthetic rates exceeding $20 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and light saturation points above $2,000 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ [Figure 1: see original paper], characteristic of sun-loving plants. These findings guide planting mode selection. Intercropping with spring maize is common: maize provides shade during early growth to prevent seedling scorching, while post-harvest light availability in mid-to-late stages promotes andrographolide accumulation. In contrast, understory planting in eucalyptus or banana plantations yields lower andrographolide due to persistent low light.

Research on light intensity effects remains inconclusive. Some studies report increased andrographolide content with decreasing light intensity, while others find that moderate shading promotes growth without affecting andrographolide content, but severe shading dramatically reduces both growth and andrographolide. Light influences morphogenesis and likely modulates andrographolide accumulation through effects on carbon assimilation and allocation.

Light quality also affects plant development and medicinal component accumulation. Red film increases plant height and total andrographolide content, while blue and yellow films are detrimental. Similar red light promotion has been reported in *Sophora tonkinensis* and *Salvia miltiorrhiza*, though mechanisms remain unclear. Since phytochrome expression responds to light quality, photoreceptor genes may regulate active component synthesis. In tobacco, altered

NtphyB expression affects morphology and secondary metabolites, with alkaloid, polyphenol, chlorogenic acid, and rutin contents correlating with *NtphyB* expression levels, offering insights into molecular mechanisms of light quality effects on andrographolide accumulation.

3.2 Effects of Soil Microorganisms on Andrographolide Components

Medicinal plants interact intimately with soil microorganisms, with microbe-plant interactions representing a hot topic in secondary metabolism regulation. Inoculation with *Bacillus* sp. increases andrographolide yield by 28–61%. Arbuscular mycorrhizal fungi (AMF), ancient and widespread root symbionts colonizing 90% of terrestrial plants, enhance mineral nutrient absorption, growth, yield, and product quality. AMF inoculation significantly improves *A. paniculata* growth and andrographolide content, with *Glomus leptotichum* and *G. intraradices* being most effective. AMF also increases phosphorus content by 31.68–127.57% in shoots and 19.58–43.55% in roots, which is considered a primary mechanism for promoting growth and active component accumulation, though other physiological processes may also be involved.

3.3 Effects of Salt Stress on Andrographolide Components

Andrographis paniculata exhibits moderate salt tolerance, though genetic variation exists. Pot experiments show normal growth at 0.4% NaCl, while hydroponic studies indicate that 41.1 mM NaCl ($4 \text{ dS} \cdot \text{m}^{-1}$) does not affect plant height or leaf area, but 92.4 mM NaCl ($8 \text{ dS} \cdot \text{m}^{-1}$) significantly inhibits growth. Using relative growth rate and salt tolerance index, the threshold was identified as $12 \text{ dS} \cdot \text{m}^{-1}$ for 15 days. Salt-responsive proteins related to antioxidant defense and photosynthesis have been identified, including 17 kDa and 45 kDa proteins affecting photosynthesis and andrographolide, respectively.

Although andrographolide content increases with salt concentration, biomass reduction often exceeds 50% while content increases by only ~30%, suggesting that the apparent increase reflects a concentration effect rather than enhanced secondary metabolism. Proper assessment requires analysis of andrographolide biosynthetic gene expression.

4.1 Biosynthetic Pathways of Andrographolide Components

Plant terpenoids are synthesized from C5 units—*isopentenyl diphosphate* (IPP) and *dimethylallyl diphosphate* (DMAPP)—via two compartmentalized pathways: the *methylerythritol-4-phosphate* (MEP) pathway in plastids and the *mevalonate* (MVA) pathway in the cytosol. Radioisotope tracing confirmed that both pathways contribute to andrographolide biosynthesis, with MEP being the primary route. *Geranylgeranyl diphosphate* (GGPP) is the main precursor, and its cyclization to *ent-copalyl diphosphate* (ent-CPP) by *ent-copalyl diphosphate synthase* (CPS) represents the key committed step. The *ApCPS*

gene (GenBank: JN216843.1) was cloned in 2012, and subsequent studies using virus-induced gene silencing, polyploidy analysis, and developmental expression profiling confirmed its strong correlation with andrographolide accumulation, establishing it as a key rate-limiting enzyme. Five *ApCPS* genes (*ApCPS1-5*) were later identified through genome sequencing, with functions described for *ApCPS1*, *ApCPS3*, and *ApCPS2*.

While the ent-CPP biosynthetic pathway is well characterized, downstream steps to andrographolide remain poorly understood. Genome sequencing and methyl jasmonate (MeJA)-treated transcriptome analysis identified candidate enzymes including cytochrome P450 monooxygenases, 2-oxoglutarate-dependent dioxygenases, and UDP-dependent glycosyltransferases. One glycosyltransferase was found to catalyze O-linked glucosylation of andrographolide to produce neoandrographolide [Figure 2: see original paper].

4.2 Molecular Regulatory Mechanisms of Andrographolide Component Synthesis

Beyond *ApCPS*, other terpenoid metabolism genes significantly regulate andrographolide biosynthesis. *ApCPS* silencing reduces andrographolide accumulation and downregulates upstream geranylgeranyl diphosphate synthase (*GGPS*) expression, while 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGR*) and 1-deoxy-D-xylulose-5-phosphate synthase (*DXS*) remain unaffected, indicating feedback regulation and suggesting that control is concentrated at *GGPS* and *ApCPS*.

Phytohormones regulate plant development and metabolism, participating in signal transduction networks for secondary metabolite synthesis. Abscisic acid (ABA), gibberellin (GA₃), and jasmonic acid (JA) induce andrographolide accumulation by upregulating terpenoid metabolism genes. MeJA, a key defense signaling hormone, induces differential expression of GGPP synthase isoforms and increases andrographolide content 5.25-fold at 5 μM after 24 h, correlating with upregulation of MVA and MEP pathway genes (*ISPH*, *GGPS*, *HMGR*). MeJA also induces *HMGR*, *DXS*, and *GGPS* expression earlier than *ApCPS*, suggesting upstream regulation of *ApCPS*.

While signal transduction mechanisms are crucial for understanding medicinal material quality, current research focuses primarily on correlations between a few key genes and andrographolide content, with limited investigation of upstream gene interactions and environmental signal transduction pathways.

5 Problems and Prospects

Despite significant progress in andrographolide biosynthesis and regulation over the past decade, research on *A. paniculata* lags behind major crops and model plants, lacking systematic investigation. Advanced findings from conventional crops offer valuable references. Future research should prioritize three areas:

First, comprehensive elucidation of the andrographolide biosynthetic pathway and key gene functions is essential for understanding molecular responses to environmental stimuli. While upstream enzymes are partially characterized, the complete pathway and gene interactions remain unclear. Since GGPP is also a precursor for gibberellins, carotenoids, and chlorophyll, pathway analysis will illuminate metabolic flux partitioning and relationships among different andrographolide components.

Second, deeper investigation of cultivation theory and technology is needed. Medicinal plant cultivation research generally trails behind conventional crops, which have developed sophisticated theories including leaf age models, source-sink theory, population quality theory, and chemical regulation. Integrating molecular biology with these crop cultivation theories will provide scientific support for growth regulation, population structure optimization, and yield-quality balance in *A. paniculata*.

Third, enhanced research on signal transduction networks is critical. Secondary metabolism represents a key physiological response to environmental changes, regulated by complex signaling networks that form the theoretical basis for cultivation interventions. Elucidating signal transduction and gene regulatory networks in andrographolide biosynthesis will enable precise genetic manipulation to promote accumulation.

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