Methods to Enhance Terpene Production

Introduction

Terpenes are a large and diverse group of natural compounds that constitute the most abundant class of plant secondary metabolites. These volatile organic compounds play critical roles in plant defense against herbivores and pathogens, mediate plant-plant communication, and attract pollinators (1). Beyond their ecological functions, terpenoids have immense commercial value in pharmaceutical, food, cosmetic, and agricultural industries (2). The increasing demand for these compounds in essential oil crops such as mint, citrus, lavender, and other aromatic plants has driven research into methods for enhancing their production. This article reviews scientifically validated approaches to boost terpene biosynthesis in commercially relevant crops.

Mint and orange are two commercially relevant crops where terpene content is strongly related to quality.

Understanding Terpene Biosynthesis

Before discussing enhancement methods, it is important to understand the fundamental pathways of terpene production. Plants synthesize terpenoids through two independent but interconnected pathways: the cytosolic mevalonate (MVA) pathway and the plastidial methylerythritol phosphate (MEP) pathway (3). The MVA pathway primarily produces sesquiterpenes and triterpenes, while the MEP pathway generates monoterpenes and diterpenes. Both pathways produce the universal precursors isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which serve as building blocks for all terpene structures.

Terpene synthases (TPSs) are the key enzymes responsible for converting these precursors into the diverse array of terpene structures found in nature (3). In aromatic plants like mint and citrus, these compounds are produced and stored in specialized structures called glandular trichomes and oil glands, respectively. The expression of TPS genes and the activity of these enzymes are tightly regulated by environmental factors and developmental signals, making them prime targets for enhancement strategies.

Controlled Drought Stress Management

Controlled water deficit represents a powerful tool for enhancing terpene production in many aromatic crops. Plants respond to drought by upregulating the biosynthesis of protective secondary metabolites, including terpenoids (4). This response helps plants cope with oxidative stress and signals other plant tissues to activate defensive mechanisms.

Research on medicinal plants has shown that moderate drought stress significantly increases terpenoid content. A study on

Bupleurum chinense demonstrated that drought stress stimulated the terpenoid backbone and triterpenoid biosynthesis pathways, leading to increased saikosaponin accumulation (5). Similarly, work on cumin plants revealed that drought-stressed plants showed significant increases in terpene levels alongside upregulation of key biosynthetic genes including 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and geranyl diphosphate synthase (GPPS) (6).

In basil and other members of the Lamiaceae family, moderate drought conditions enhanced sesquiterpene production while also improving the overall quality of essential oils (7). The optimal level of drought stress varies by species and growing conditions. Excessive water deficit can inhibit photosynthesis and reduce overall biomass, ultimately decreasing total terpene yield despite higher concentrations per unit mass. Growers should aim for moderate stress that maintains plant health while triggering enhanced secondary metabolism.

Temperature Optimization

Temperature plays a dual role in terpene biosynthesis, affecting both enzyme activity and gene expression. Research on birch and aspen demonstrated that elevated night-time temperatures significantly increased daytime terpenoid emissions. Plants grown with night temperatures of 18 to 22 degrees Celsius showed substantially higher emissions of sesquiterpenes and certain monoterpenes compared to those at lower temperatures (8).

Temperature affects terpene production through multiple mechanisms. Higher temperatures increase the volatility of terpenes, potentially leading to greater emissions from storage structures. More importantly, temperature influences the expression of genes encoding enzymes in both the MEP and MVA pathways (2). However, excessively high temperatures can denature enzymes and degrade already-produced terpenes, so careful monitoring is essential.

For citrus crops, temperature during fruit development significantly affects the terpene profile of essential oils extracted from peels (9). For most aromatic crops, maintaining daytime temperatures between 25 and 30 degrees Celsius with slightly lower night temperatures of 18 to 22 degrees Celsius appears optimal for terpene production. This temperature differential mimics natural conditions and supports robust secondary metabolism without inducing heat stress.

Crop Type	Optimal Day Temperature	Optimal Night Temperature	Effect on Terpenes
Mint species	25-30°C	18-22°C	Enhanced monoterpene production
Citrus species	24-28°C	16-20°C	Improved essential oil quality
Basil and herbs	26-30°C	18-22°C	Increased sesquiterpene content
Lavender	22-28°C	15-18°C	Enhanced linalool production

Nutrient Management Strategies

Soil nutrient availability profoundly impacts terpene biosynthesis through its effects on carbon and nitrogen allocation. The carbon-nutrient balance hypothesis suggests that when nitrogen is limiting, plants allocate more carbon to secondary metabolites like terpenes rather than to nitrogen-rich primary compounds such as proteins (10).

Phosphorus and potassium play particularly important roles in terpene production. Phosphorus is essential for the production of the phosphorylated precursors DMAPP and IPP, while potassium affects enzyme activation and osmotic regulation under stress conditions. Moderate nitrogen limitation during the reproductive phase can enhance terpene production by shifting metabolism toward secondary compound synthesis (10).

In mint cultivation, nutrient management significantly affects essential oil yield and composition. Studies have shown that excessive nitrogen application can reduce menthol content while promoting vegetative growth at the expense of oil production (11). Sulfur supplementation deserves special attention as this element is incorporated into certain terpenes and affects the overall terpenoid profile. Research has shown that sulfur-containing amendments can enhance the production of sulfur-bearing terpenes while supporting general secondary metabolism.

Elicitor Application

Plant hormones and signaling molecules can act as powerful elicitors of terpene biosynthesis. Methyl jasmonate (MeJA) is the most extensively studied elicitor, with numerous studies demonstrating its ability to dramatically increase terpenoid production (2).

MeJA treatment induces the expression of TPS genes and upregulates the entire terpenoid biosynthetic pathway. In Norway spruce, MeJA application increased terpene emissions by more than 100-fold for linalool and over 30-fold for sesquiterpenes (12). The hormone mimics the plant's natural defense response to herbivore damage, triggering a cascade of gene expression changes that result in enhanced secondary metabolism.

Salicylic acid represents another important elicitor that can promote terpenoid biosynthesis. Research has shown that salicylic acid upregulates key enzymes in the terpenoid pathway, including farnesyl pyrophosphate synthase (FPPS) in various species (12). In mint species, jasmonate application has been shown to enhance both the quantity and quality of essential oils, particularly increasing the production of oxygenated monoterpenes like menthol and menthone. The optimal concentration and timing of elicitor application depend on the target species and desired terpene profile.

Transcription Factor Regulation

Understanding the transcriptional regulation of terpene biosynthesis opens possibilities for targeted enhancement. Several families of transcription factors (TFs) play crucial roles in controlling terpenoid production, including WRKY, MYB, AP2 or ERF, bHLH, and NAC families (13).

These transcription factors respond to environmental signals and developmental cues by binding to specific promoter regions of genes involved in terpene biosynthesis. For example, WRKY transcription factors regulate sesquiterpene artemisinin synthesis in Artemisia annua and diterpene biosynthesis in rice (13). In citrus, the transcription factor MYC5 has been identified as crucial for oil gland development and the biosynthesis of essential oils (14). While direct genetic manipulation of transcription factors requires advanced techniques, understanding their role helps in timing environmental interventions to coincide with periods of high TF activity.

Metabolic Engineering in Mint Production

Mint species, particularly peppermint and spearmint, represent important commercial sources of monoterpene essential oils. The monoterpenoid biosynthesis pathway in mint is well characterized, making these crops attractive targets for metabolic engineering approaches to enhance oil production (15).

Research has demonstrated that overexpressing genes encoding enzymes in the MEP pathway, particularly 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), can increase essential oil yields in peppermint. The most encouraging results were obtained when multiple genes were manipulated simultaneously. Plants where DXR was overexpressed and menthofuran synthase was down-regulated showed oil yield increases of up to 61% over wild-type controls while reducing undesirable by-products (16).

Another successful strategy involved overexpression of lipid transfer proteins, which increased trichome size and enhanced monoterpenoid production. Plants expressing tobacco lipid transfer protein showed increases in limonene levels of 1.6-fold and dramatic increases in other monoterpenes (15). While metabolic engineering requires sophisticated molecular biology techniques, these advances demonstrate the substantial potential for enhancing terpene production through targeted genetic modifications.

Agronomic Practices for Enhanced Production

Beyond molecular and environmental approaches, specific agronomic practices can significantly impact terpene yields in essential oil crops. For mint cultivation, planting method, timing, and plant density all influence essential oil production (11).

Ridge planting systems have been shown to provide superior results compared to flat-bed cultivation. Studies on menthol mint demonstrated that plants grown on ridges with optimal spacing of 166,666 plants per hectare yielded maximum essential oil content while reducing water requirements and accelerating crop maturity by approximately 30 days (11). The timing of planting also significantly affects oil yield, with early season planting generally producing higher essential oil content and better quality profiles.

For citrus crops, proper handling during harvest and post-harvest processing critically affects terpene retention. Cold-pressing methods preserve more volatile terpenes compared to heat-based extraction, and storage conditions must be carefully controlled to prevent oxidation and degradation of essential oils (17).

Harvest Timing Considerations

The timing of harvest critically affects the final terpene content of plant material. Terpene concentrations fluctuate throughout plant development and can vary substantially even over the course of a single day due to circadian regulation (12).

For many aromatic plants, terpene content peaks during specific developmental stages. In mint, essential oil content typically reaches maximum levels just before full flowering. For citrus, the maturity stage of fruit significantly influences both the quantity and composition of peel oils, with different terpene profiles characterizing immature versus fully mature fruit (9).

Harvesting during the morning hours, after dew has evaporated but before peak temperatures, often captures plants at their maximum terpene content before heat-induced volatilization occurs. Post-harvest handling also significantly impacts terpene retention. Rapid drying at moderate temperatures (below 30 degrees Celsius) and protection from light help preserve volatile terpenes. Proper curing in controlled environments allows for the gradual breakdown of chlorophyll while maintaining terpene content.

Crop	Optimal Harvest Stage	Time of Day	Post-Harvest Consideration

Peppermint	Just before full bloom	Mid-morning	Rapid drying at 25-30°C
Spearmint	Early flowering	Morning hours	Shade drying preferred
Citrus peels	Fully mature fruit	Any time	Cold-press immediately
Basil	Before flowering	Early morning	Quick drying essential

Integrated Enhancement Strategies

The most effective approach to enhancing terpene production often involves combining multiple strategies rather than relying on a single method. Environmental factors interact in complex ways, and their effects on terpene biosynthesis can be synergistic (10).

A practical integrated approach for mint cultivation might include selecting cultivars with naturally high terpene production as the foundation, implementing controlled drought stress in the final weeks before harvest, optimizing the nutrient regime to favor secondary metabolism with moderate nitrogen restriction during flowering, applying elicitors such as methyl jasmonate at strategic developmental stages, using appropriate planting methods and densities, and timing harvest to coincide with peak terpene accumulation.

For citrus production, an integrated strategy would focus on temperature management during fruit development, appropriate irrigation scheduling to avoid excessive vegetative growth, balanced fertilization that does not over-supply nitrogen, and optimization of harvest maturity and processing methods to preserve volatile compounds.

Challenges and Future Directions

While significant progress has been made in understanding and manipulating terpene biosynthesis, several challenges remain. The genetic regulation of terpenoid production is extremely complex, involving hundreds of genes that respond to multiple environmental signals (3). Predicting how plants will respond to combined stresses or elicitor treatments remains difficult.

Future research should focus on developing more precise tools for monitoring terpene production in real-time, allowing for adaptive management strategies. Advanced metabolic engineering approaches, including CRISPR-based gene editing of regulatory elements, may eventually allow for the creation of plants with constitutively elevated terpene production without the need for environmental manipulation (18). Understanding the molecular mechanisms controlling oil gland and trichome development will also be crucial for maximizing the sites of terpene biosynthesis and storage (14).

Conclusion

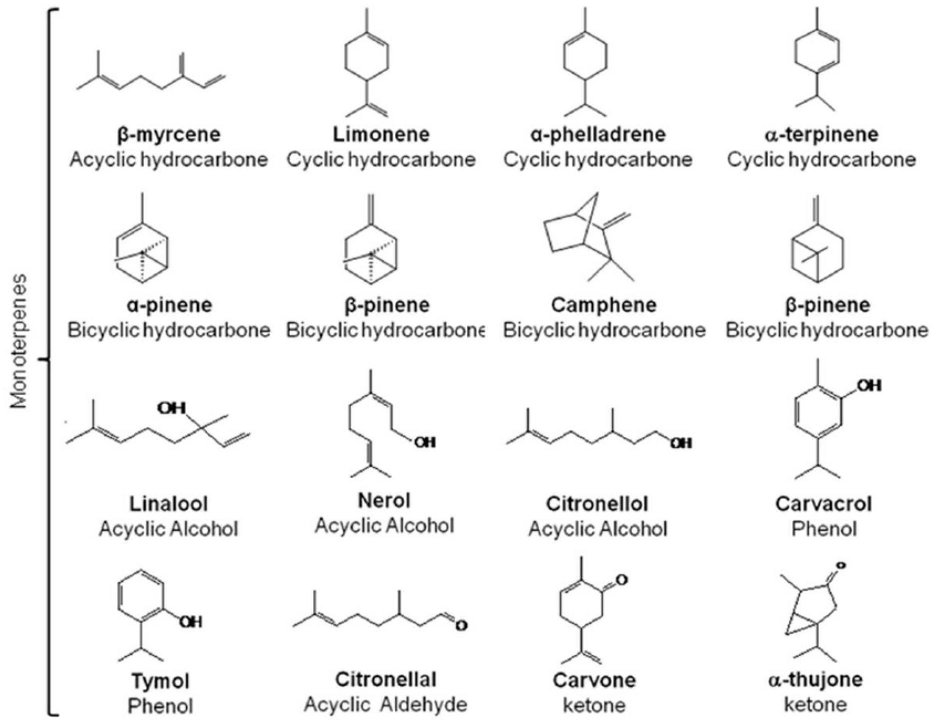
Enhancing terpene production in commercially important plants requires a multifaceted approach based on sound scientific principles. Controlled drought stress, when carefully managed, can significantly increase terpenoid concentrations through activation of stress response pathways. Temperature optimization, particularly elevated night-time temperatures, enhances terpene biosynthesis and emission. Strategic nutrient management, including moderate nitrogen limitation coupled with adequate phosphorus and potassium, shifts plant metabolism toward secondary compound production.

The application of elicitors such as methyl jasmonate provides a powerful tool for rapidly inducing terpene biosynthesis. Understanding the role of transcription factors in regulating these pathways helps in timing interventions for maximum effectiveness. In crops like mint where the biosynthetic pathways are well characterized, metabolic engineering offers promising opportunities for substantial yield improvements. Appropriate agronomic practices, including planting methods, spacing, and timing, significantly influence essential oil production. Finally, optimizing harvest timing and post-harvest handling ensures that enhanced terpene production translates into improved final product quality.

As our understanding of terpene biosynthesis continues to grow, new enhancement strategies will undoubtedly emerge. Growers who stay informed about the latest research and are willing to experiment with different approaches will be best positioned to maximize the terpene content of their crops. However, success requires careful attention to plant health, as excessive stress can be counterproductive. The goal is to find the optimal balance that stimulates terpene production while maintaining overall plant vigor and yield. The commercial value of essential oils continues to drive innovation in this field, promising continued advances in our ability to enhance these valuable compounds in aromatic and medicinal plants.

Exogenous Terpenes in Agriculture: Can External Application Improve Crop Performance?

Terpenes are among the most diverse and abundant secondary metabolites produced by plants. While these compounds are well known for their roles in plant defense and stress responses, recent research has explored whether applying terpenes externally to plants can provide practical benefits in commercial agriculture. This post examines the current scientific understanding of exogenous terpene applications through both foliar sprays and root zone treatments.



Models for a collection of commonly found monoterpenes

What Are Terpenes and Why Do Plants Produce Them?

Terpenes represent the largest class of plant secondary metabolites, with approximately 55,000 known members across the plant kingdom (1). Plants synthesize these compounds through the methylerythritol phosphate pathway in plastids and the mevalonate pathway in the cytosol. The resulting molecules range from simple monoterpenes containing 10 carbon atoms to complex diterpenes with 20 carbons and beyond.

The primary ecological function of terpenes involves plant protection. These volatile organic compounds help plants defend against pathogens and herbivores, attract beneficial insects and pollinators, and provide protection against environmental stresses such as heat and drought (2). Given these natural protective functions, researchers have investigated whether externally applied terpenes might confer similar benefits to crops.

Foliar Application of Monoterpenes

The most comprehensive study on foliar terpene application comes from research on tomato plants under water deficit stress. When a mixture of nine monoterpenes was applied as a foliar spray at concentrations ranging from 1.25 to 5 mM, the treated plants showed significant improvements in oxidative stress management (3).

The foliar-applied monoterpenes were readily absorbed by tomato leaves, increasing total foliar monoterpene content by up to 2.5-fold compared to untreated controls. Most importantly, the treatment substantially decreased hydrogen peroxide accumulation and lipid peroxidation in plants exposed to drought stress. At the optimal concentration of 1.25 mM, plants showed a 50% reduction in oxidative damage compared to controls, though this protective effect did not extend to preventing photosynthetic decline (3).

The mechanism appears to involve direct quenching of reactive oxygen species by the terpenes themselves. Interestingly, higher concentrations of 2.5 and 5 mM increased activity of antioxidant enzymes like superoxide dismutase and ascorbate peroxidase, but also induced some oxidative stress, suggesting a threshold effect where lower concentrations may be more beneficial than higher ones.

Monoterpene Concentration	**H_2O_2 Reduction (%)**	**Lipid Peroxidation Reduction (%)**	**Enzyme Activity Change**
1.25 mM	~50%	~45%	No change

2.5 mM	~35%	~30%	Increased
5.0 mM	~25%	~20%	Increased

Root Zone Applications and Belowground Signaling

While foliar applications have shown promise for stress mitigation, root zone applications of terpenes have been explored primarily for pest management through biological control. The sesquiterpene E-β-caryophyllene serves as a particularly well-studied example of how terpenes function in the rhizosphere.

When maize roots are damaged by western corn rootworm larvae, they naturally emit E-β-caryophyllene, which attracts entomopathogenic nematodes that parasitize and kill the pest insects (4). Field experiments demonstrated that when synthetic E-β-caryophyllene was applied to soil near maize varieties that do not naturally produce this signal, adult beetle emergence decreased by more than 50%, demonstrating the practical potential of this approach.

The effectiveness of soil-applied E-β-caryophyllene depends heavily on soil properties. Research has shown that this sesquiterpene diffuses primarily through the gaseous phase of soil rather than the aqueous phase. In clay soils at 10% water content, diffusion was significantly limited, but increasing moisture to 20% substantially improved signal propagation in clay loam and sandy loam soils (5).

In controlled field trials, maize plants engineered to constitutively emit E-β-caryophyllene and treated with entomopathogenic nematodes suffered 60% less root damage and had significantly fewer adult beetles emerge compared to non-emitting lines (6). This demonstrates that strategic application of specific terpenes to the root zone can enhance biological control efficacy.

Disease Resistance Through Diterpene Application

Diterpenes have shown particularly strong antimicrobial properties when applied to plants. Two labdane-type diterpenes isolated from tobacco, sclareol and cis-abienol, were tested as exogenous treatments on tobacco, tomato, and Arabidopsis plants. These compounds effectively inhibited bacterial wilt diseases, with microarray analysis revealing that they activated genes encoding components of plant immune responses, including MAP kinase cascades and defense-related biosynthetic pathways (1).

In maize, the diterpene epoxydolabranol demonstrated simultaneous effectiveness against two major fungal pathogens, Fusarium graminearum and Fusarium verticillioides. The diterpene momilactone B showed allelopathic properties, completely inhibiting germination of several weed species at concentrations of 4 to 20 ppm when applied to soil (1).

Terpene Type	Application Method	Target Organism	Effective Concentration
Monoterpenes (mixed)	Foliar spray	Drought stress	1.25 mM
E-β-caryophyllene	Soil drench	Root pests	200-20,000 ng
Sclareol/cis-abienol	Root application	Bacterial wilt	Not specified
Momilactone B	Soil application	Weeds	4-20 ppm

Practical Considerations for

Application

Several factors influence the effectiveness of exogenous terpene applications. For foliar treatments, the physiochemical properties of individual terpenes significantly affect uptake and translocation. Compounds like α-terpinene and terpinolene show greater solubility and cellular accumulation compared to more volatile molecules like α-pinene and limonene (3).

Timing and application frequency also matter. Foliar sprays applied twice daily showed better results than single applications, suggesting that maintaining adequate concentrations on leaf surfaces requires repeated treatments. For soil applications, the water content and texture of the growing medium critically influence how well terpene signals diffuse through the root zone.

Cost remains a significant consideration. Production of terpenes for agricultural use requires either chemical synthesis or bio-production in heterologous hosts. For structurally complex terpenes, chemical synthesis may be economically prohibitive, making microbial production platforms like engineered Escherichia coli or Saccharomyces cerevisiae more practical options (7).

Limitations and Future Directions

Current research reveals important limitations. While exogenous monoterpenes effectively reduced oxidative stress in tomato plants, they did not prevent the photosynthetic decline associated with stomatal closure during drought. This suggests that terpene applications may be most useful as supplementary treatments rather than standalone solutions for stress management (3).

The dose-response relationship appears complex, with higher concentrations sometimes producing counterproductive effects. In the tomato study, 5 mM monoterpene applications induced oxidative stress while attempting to protect against it, highlighting the importance of careful concentration optimization for each crop and application method.

Much of the existing research has been conducted under controlled laboratory or greenhouse conditions. Large-scale field trials examining the agronomic and economic viability of exogenous terpene applications remain limited. Questions about the persistence of applied terpenes under field conditions, their environmental fate, and potential non-target effects require further investigation.

Conclusions

Exogenous terpene applications represent an emerging area of agricultural research with demonstrated benefits in specific scenarios. Foliar monoterpene sprays can mitigate oxidative stress from drought at appropriate concentrations. Soil-applied sesquiterpenes like E-β-caryophyllene enhance biological pest control by attracting beneficial nematodes. Diterpenes show promise as antimicrobial agents when applied to roots.

However, practical adoption requires further development. Growers interested in this technology should recognize that terpene applications are most likely to succeed as part of integrated management strategies rather than as standalone interventions. The variable responses across different terpene types, concentrations, and application methods mean that each crop system will require careful optimization.

As production costs decrease and application protocols become more refined, exogenous terpenes may find their place in the grower’s toolkit, particularly for organic production systems seeking alternatives to synthetic pesticides. Until then, this remains a promising but still developing technology that warrants continued research attention.