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Pathways to Flavor: Decoding the Biosynthesis of Tea Secondary Metabolites

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Abstract Tea (*Camellia sinensis*) is a globally consumed beverage, cherished for its unique flavor and health benefits. The secondary metabolites in tea, such as phenolic compounds, amino acids, alkaloids, and terpenoids, not only contribute to tea's distinctive taste and aroma but also possess significant biological activities, including antioxidant, anti-inflammatory, and anticancer properties. Understanding the biosynthetic pathways of these secondary metabolites is crucial for enhancing tea quality and its health functions. This study systematically analyzes the classification and biosynthetic pathways of tea secondary metabolites, including phenolic compounds (such as flavonoids and catechins), amino acids (such as theanine and glutamic acid), alkaloids (such as caffeine, theobromine, and theophylline), and terpenoids (such as linalool, geraniol, and benzyl alcohol). Additionally, this study discusses the impact of genetic and environmental factors on the synthesis of these tea metabolites and introduces analytical techniques used to study tea metabolites, such as chromatography, mass spectrometry, and nuclear magnetic resonance spectroscopy. Through comprehensive analysis, this research provides a scientific basis for future studies aimed at improving tea quality and market competitiveness through metabolite research and outlines future research directions to promote the development of the tea industry.

Keywords Tea secondary metabolites; Biosynthetic pathways; Phenolic compounds; Amino acids; Alkaloids; Terpenoids

1 Introduction

Tea (*Camellia sinensis*) is one of the most widely consumed beverages worldwide, valued not only for its refreshing taste but also for its numerous health benefits. The unique flavor and health-promoting properties of tea are primarily attributed to its secondary metabolites, including flavonoids, alkaloids, and terpenoids. These compounds play a crucial role in the plant's defense mechanisms and contribute to the sensory qualities of tea, such as astringency, umami, bitterness, and aroma (Zhao et al., 2021). For example, flavonols, a type of flavonoid, are known to impart bitterness to tea, and their levels can be regulated by environmental factors such as UV radiation and shading (Zhao et al., 2021). These secondary metabolites not only have economic importance in enhancing tea quality but also serve as targets for biotechnological production due to their therapeutic properties (Dziggel et al., 2017).

The biosynthesis of secondary metabolites in tea plants involves complex metabolic pathways that are tightly regulated by various genetic and environmental factors. Recent studies have revealed the intricate regulatory networks involved in the production of these compounds. For instance, the synthesis of flavonols in tea leaves is controlled by a network of activating and inhibiting factors, including transcription factors like CsMYB12 and CsbZIP1, which are upregulated by UV-B radiation and downregulated under shading conditions (Zhao et al., 2021). These transcription factors interact with other regulatory proteins to modulate the expression of key biosynthetic genes such as *CsFLS* and *CsUGT78A14*, thereby controlling the flavonol content in tea leaves (Zhao et al., 2021). Advances in next-generation sequencing and gene silencing technologies have further elucidated these pathways, making it possible to reconstruct the metabolic pathways of economically relevant secondary metabolites in microbial hosts (Dziggel et al., 2017).

This study aims to decode the biosynthetic pathways of secondary metabolites in tea leaves, with a particular focus on understanding the regulatory mechanisms involved in their biosynthesis. By synthesizing current research findings, this study provides a comprehensive overview of the genetic and environmental factors influencing the biosynthesis of key secondary metabolites in tea plants. Additionally, the research explores the potential of biotechnological approaches to produce these valuable compounds in microbial systems. Through these analyses, gaps and shortcomings in the current body of knowledge are identified, and future research directions and potential solutions are proposed, aiming to provide a theoretical basis and technical support for the production and quality improvement of tea secondary metabolites. Future research will focus on integrating multi-omics data, systematically analyzing metabolic pathways through systems biology, and developing novel bioreactors to drive innovation and sustainable development in the tea industry.

2 Classification of Major Secondary Metabolites in Tea

2.1 Phenolic compounds in tea

Phenolic compounds are a significant class of secondary metabolites in tea, contributing to its antioxidant properties and overall health benefits. High-performance liquid chromatography-mass spectrometry (HPLC-MS(n)) has been effectively utilized to analyze over 30 phenolic compounds in both green and black tea. These compounds include catechin derivatives, flavonols, theaflavins, and their various gallate derivatives, as well as quercetin and kaempferol glycosides, and quinic acid esters of gallic acid and hydroxycinnamates. Phenolic compounds, particularly flavonoids, are known for their clinical properties such as anti-atherosclerotic, anti-inflammatory, antitumor, and antiviral activities (Saboon et al., 2019). The presence of these compounds in tea underscores its potential as a natural antioxidant source, playing a crucial role in neutralizing free radicals and contributing to the prevention of various diseases.

2.2 Amino acid compounds in tea

Amino acids are important substances contributing to the flavor of tea. At present, 26 kinds of free amino acids have been identified from tea, including 20 protein source amino acids and 6 non-protein source amino acids, among which the content of non-protein source amino acid theanine can account for more than 50% of the total amino acid content. This was followed by glutamic acid, arginine, serine and aspartic acid (Yu and Yang, 2020). Among them, L-Theanine is the most abundant and representative amino acid in tea, accounting for more than half of the total free amino acids in tea and 1%-4% of dry tea (Deng et al., 2009; Tang et al., 2020). It is one of the characteristic components of tea and the main substance that determines the fresh and refreshing taste of green tea. The content and proportion of amino acids in tea have an important effect on the quality and nutritional value of tea. Because amino acids themselves are taste and aroma substances, the composition and content of amino acids in fresh tea leaves are different, which affects the quality of tea.

2.3 Alkaloids in tea

Alkaloids are another important group of secondary metabolites found in tea, with caffeine and theobromine being the most prominent. These purine alkaloids are identified using HPLC-MS(n) based on their retention time, absorbance spectrum, and MS fragmentation pattern. Alkaloids in tea are primarily known for their stimulant properties, with caffeine being the most well-known for its ability to enhance alertness and reduce fatigue. Additionally, alkaloids possess various pharmacological activities, including anesthetic properties, which contribute to the therapeutic potential of tea. The presence of these compounds not only adds to the flavor profile of tea but also enhances its functional benefits as a beverage.

2.4 Terpenoids in tea

Terpenoids are a diverse class of secondary metabolites also present in tea, contributing to its aroma and potential health benefits. These compounds are known for their antibacterial, anti-inflammatory, anticancer, antimalarial, and antiviral activities. The terpenoids in tea include various compounds that play a role in the unique fragrance and therapeutic properties of tea. Their pharmacological activities make them valuable in both traditional medicine and modern drug development. The presence of terpenoids in tea highlights the multifaceted benefits of this beverage, extending beyond flavor to include significant health-promoting properties.

Secondary metabolites in tea exhibit rich diversity and uniqueness, playing crucial roles in the flavor, health benefits, and disease resistance of tea. These secondary metabolites primarily include phenolic compounds such as catechins, theanine, and caffeine, alkaloids, and terpenoids. Their concentrations vary significantly among different tea varieties and cultivation conditions (Figure 1). By understanding the biosynthesis and roles of these secondary metabolites—phenolic compounds, alkaloids, and terpenoids—in tea, researchers can further explore their potential applications in health and medicine, as well as their contributions to the unique characteristics of tea.

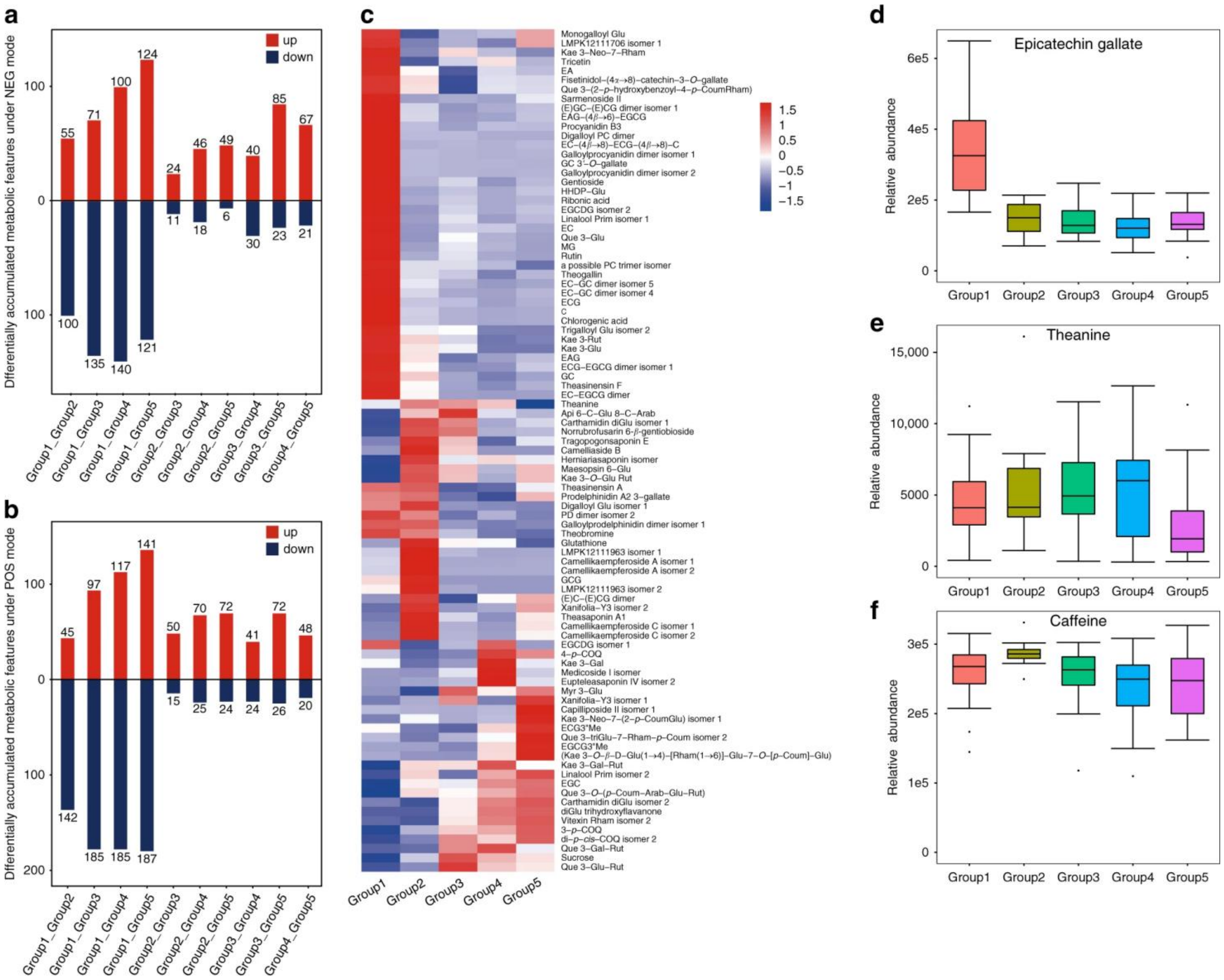

Figure 1 Metabolites that showed significant changes in concentration in pairwise comparisons of tea accessions (Adopted from Yu et al., 2020)

Figure 1 illustrates the differences in metabolite characteristics among five tea plant populations. Figures 1a and 1b show the number of detected metabolic features in negative ion mode (NEG) and positive ion mode (POS), respectively, with red and blue bars indicating increases and decreases in metabolite feature concentrations. The heatmap in Figure 1c displays the abundance patterns of metabolites that showed significant changes in at least one tea plant population, with higher levels of various flavonoids and theobromine accumulating in population 1. Figures 1d to 1f present box plots of the relative abundances of epicatechin gallate (ECG), theanine, and caffeine in different tea plant populations, respectively. The results indicate that the levels of these metabolites are significantly higher in population 1 compared to the other populations, particularly ECG and caffeine, suggesting the uniqueness of population 1 in metabolite accumulation. These findings reveal significant differences in the metabolite profiles of different tea plant populations, potentially related to the upregulation of specific genetic pathways, highlighting the complex relationship between genes and metabolites.

3 Biosynthesis Pathways of Phenolic Compounds

3.1 Flavonoids in tea

Flavonoids are a major class of secondary metabolites in tea plants (*Camellia sinensi*s) that significantly contribute to the quality and flavor of tea. The biosynthesis of flavonoids in tea involves a series of enzymatic reactions that convert phenylalanine into various flavonoid compounds. Key enzymes in this pathway include phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and flavanone 3-hydroxylase (F3H) (Xia et al, 2017; Huang et al., 2018; Zhu et al., 2020). The expression of these enzymes is regulated by various transcription factors, including MYB and bHLH, which modulate the biosynthesis of flavonoids during different growth stages and environmental conditions (Zhu et al., 2020; Zhao et al., 2022).

3.2 Catechins in tea

Catechins are a specific type of flavonoid that are abundant in tea leaves and are known for their health-promoting properties. The biosynthesis of catechins involves the conversion of flavan-3-ols, such as epicatechin and epigallocatechin, through the action of enzymes like anthocyanidin reductase and dihydroflavonol 4-reductase. These enzymes catalyze the reduction of anthocyanidins to produce catechins, which are then accumulated in the tea leaves (Fang et al., 2021). The levels of catechins in tea can be influenced by various factors, including nitrogen availability, which affects the expression of key biosynthetic genes (Huang et al., 2018).

3.3 Theaflavins and thearubigins in tea

Theaflavins and thearubigins are polyphenolic compounds that are formed during the oxidation of catechins in the process of black tea production. Theaflavins are formed through the enzymatic oxidation of catechins, catalyzed by polyphenol oxidase, resulting in the formation of dimeric structures. Thearubigins, on the other hand, are higher molecular weight polymers that contribute to the color and astringency of black tea. The specific pathways and regulatory mechanisms involved in the formation of these compounds are complex and involve multiple enzymatic steps and environmental factors (Zhao et al., 2022).

4 Biosynthesis Pathways of Theanine in Tea

4.1 Theanine as a key amino acid contributing to tea flavor

Theanine, a unique non-proteinogenic amino acid found abundantly in tea plants (*Camellia sinensis*), is a major contributor to the umami taste and relaxation effects of tea infusions. Its presence is directly correlated with the quality and price of green tea (Zhu et al., 2021; Lin et al., 2022). Theanine is synthesized primarily in the roots and then transported to the shoots, where it accumulates in the leaves (Lin et al., 2022). This amino acid not only enhances the flavor profile of tea but also offers various health benefits, making it a critical component in tea production (Lin et al., 2022; She et al., 2022).

4.2 Enzymatic steps involved in theanine biosynthesis

The biosynthesis of theanine involves several key enzymatic steps. Theanine is synthesized from ethylamine and glutamate by the enzyme Theanine Synthetase I (CsTSI) (Zhu et al., 2021; She et al., 2022). Ethylamine itself is produced from alanine through the action of alanine decarboxylase (CsAlaDC) (Zhu et al., 2021). The expression of *CsTSI* is closely correlated with theanine and glutamine levels in various tissues of the tea plant, and its activity is influenced by nitrogen supply (She et al., 2022). Additionally, glutamine synthetase genes (*CsGSs*) have been shown to play a dual role in the synthesis of both glutamine and theanine, particularly in the tender shoots of certain tea cultivars (Yu et al., 2021).

Research indicates that genetic differences among tea plant varieties and growth conditions significantly affect the theanine content (Yang et al., 2021). For example, shading cultivation can significantly increase theanine content in tea leaves due to the upregulation of theanine synthetase gene expression under low light conditions.

4.3 Genetic and environmental factors affecting theanine levels

Theanine levels in tea plants are influenced by a combination of genetic and environmental factors. Transcription factors such as MYB have been identified as critical regulators of theanine biosynthesis. For instance, CsMYB9 and CsMYB49 are involved in the regulation of theanine biosynthesis, while CsMYB73 acts as a repressor (Wen et al., 2020; Li et al., 2022). Environmental factors such as nitrogen availability also play a significant role. Nitrogen promotes theanine biosynthesis while repressing flavonoid biosynthesis in tea plant roots (Wang et al., 2021). Additionally, temperature variations can affect theanine levels through hormone signal transduction pathways involving jasmonic acid and its derivatives (Zhu et al., 2023). These findings highlight the complex interplay between genetic regulation and environmental conditions in determining theanine content in tea plants.

5 Biosynthesis Pathways of Alkaloids

Alkaloids are a significant class of secondary metabolites in tea, influencing both the flavor and aroma of tea, as well as having notable physiological activities. The primary alkaloids in tea include caffeine, theobromine, and theanine. The biosynthesis pathways of these compounds involve a series of complex enzymatic reactions and are regulated by genetic factors and environmental conditions.

5.1 Caffeine in tea

Caffeine is one of the most well-known alkaloids in tea, widely recognized for its stimulating effects and ability to enhance alertness. Researchers have identified 13 N-methyltransferase (*NMT*) genes responsible for the three key methylation steps in caffeine biosynthesis (Figure 2). Comparative analysis indicates that tea plants have fewer *NMT* genes than cocoa and coffee, and the biosynthesis of caffeine begins with the synthesis of purine alkaloid compounds (Xia et al., 2017). Initially, theanine produced during the theanine synthesis process is converted into the precursor of caffeine, xanthine nucleotide. Subsequently, xanthine nucleotide is gradually converted into caffeine through a series of enzymatic actions, including xanthine nucleotide synthase and xanthine nucleotide methyltransferase (Ashihara and Crozier, 2001). In this process, S-adenosylmethionine (SAM) serves as a methyl donor to participate in the methylation reactions, ultimately leading to the production of caffeine. Studies have shown that the key enzyme for caffeine synthesis, xanthine nucleotide methyltransferase (XMT), exhibits different expression levels among various tea cultivars, resulting in differences in caffeine content across different types of tea.

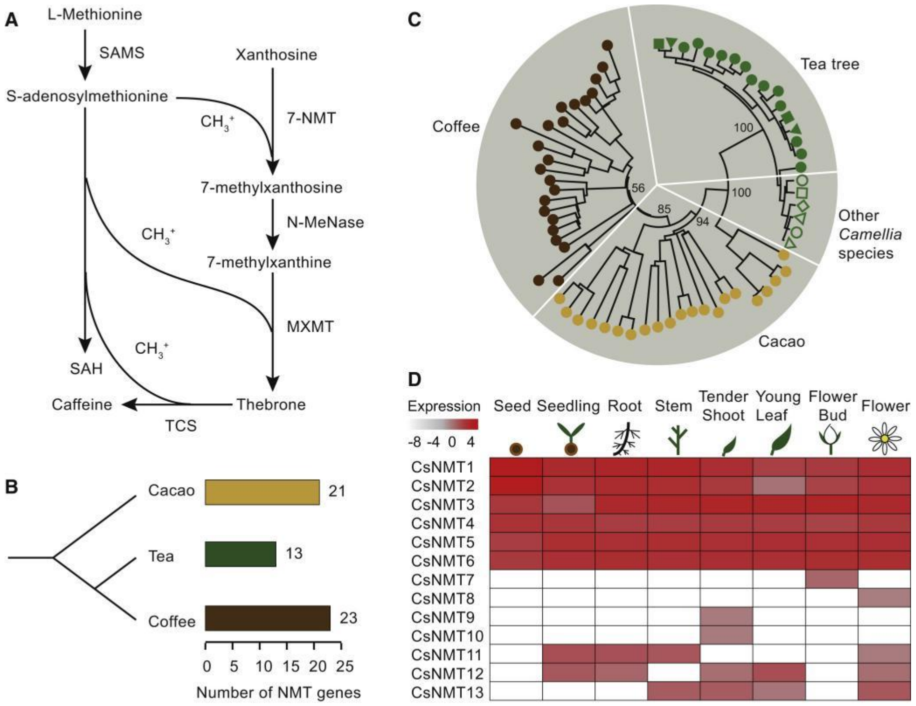

Figure 2 Evolution of Caffeine Biosynthesis (Adopted from Xia et al., 2017)

Figure 2 in Xia et al. (2017) illustrates the caffeine biosynthesis pathway and the expression distribution of *NMT* genes in different tissues. Figure A shows the three key methylation steps catalyzed by *NMT* that convert xanthine into caffeine. Figure B compares the number of *NMT* genes in tea, coffee, and cocoa, highlighting that tea plants have the fewest. Figure C displays the expression levels of *NMT* genes across various tissues, indicating higher expression in leaves and flowers. This figure underscores the independent evolution of caffeine biosynthesis in tea plants and the tissue-specific expression of key biosynthetic genes, providing a comprehensive overview of caffeine synthesis and accumulation in tea plants.

5.2 Theobromine in Tea

Theobromine is another important alkaloid in tea, structurally similar to caffeine but with different physiological effects. Theobromine has diuretic, smooth muscle relaxant, and cardiac stimulant properties. The biosynthesis of theobromine is similar to that of caffeine, starting from the metabolism of theanine. Theanine undergoes a series of reactions to produce xanthosine nucleotide, which is then partially converted into theobromine instead of caffeine under the catalysis of xanthosine nucleotide methyltransferase (Ashihara and Crozier, 2001). Studies have found that environmental conditions, such as light intensity and temperature, can significantly affect theobromine content in tea, indicating the influence of environmental factors on its biosynthesis.

The biosynthesis pathways of alkaloids in tea are diverse and complex, involving various enzymes and metabolic steps. The synthesis of caffeine and theobromine depends not only on the genetic background of the tea plant but is also significantly influenced by environmental conditions. By deeply studying these synthesis pathways and regulatory mechanisms, we can better understand and optimize the flavor and functional properties of tea, thereby improving its quality and market competitiveness. Moreover, utilizing modern biotechnological approaches such as gene editing and metabolic engineering can further enhance the synthesis capabilities of these secondary metabolites in tea plants, meeting the growing market demand.

6 Biosynthesis Pathways of Terpenoids

Terpenoids are a diverse class of naturally occurring organic chemicals derived from five-carbon isoprene units. They play a significant role in the flavor profile of tea. This section delves into the biosynthesis pathways of three key terpenoids: linalool, geraniol, and nerolidol, which contribute to the unique aroma and taste of tea.

6.1 Linalool in tea

Linalool is a monoterpenoid alcohol that imparts a floral scent to tea. The biosynthesis of linalool in tea involves the mevalonate (MVA) pathway and the methylerythritol phosphate (MEP) pathway. Studies have shown that the levels of linalool and its oxides are significantly higher in certain tea cultivars, such as 'Bai-Sang Cha' (BAS), compared to others like 'Fuding-Dabai Cha' (FUD) (Han et al., 2016). The enhanced transcription of terpenoid biosynthetic genes in 'BAS' suggests a genetic basis for the increased production of linalool. Additionally, the withering process during tea manufacturing has been found to enhance the metabolism of terpenoids, including linalool, thereby increasing its content in the final product (Wang et al., 2019).

6.2 Geraniol in tea

Geraniol is another monoterpenoid alcohol that contributes to the floral aroma of tea. Similar to linalool, geraniol is synthesized via the MVA and MEP pathways. The levels of geraniol in tea can be influenced by both genetic factors and manufacturing processes. For instance, 'Bai-Sang Cha' (BAS) contains higher levels of geraniol compared to 'Fuding-Dabai Cha' (FUD), which can be attributed to the enhanced transcription of terpenoid biosynthetic genes in 'BAS' (Han et al., 2016). However, the levels of geraniol tend to decrease during certain manufacturing processes, such as pan-firing, which reduces the floral scent of the tea (Han et al., 2016). Moreover, the expression of specific microRNAs (miRNAs) has been found to be positively correlated with the content of geraniol, indicating a regulatory role of miRNAs in its biosynthesis (Li et al., 2021).

6.3 Nerolidol in tea

Nerolidol is a sesquiterpenoid alcohol that adds a woody and floral note to tea. The biosynthesis of nerolidol involves the MVA pathway, which is responsible for the production of sesquiterpenoids. Although specific studies on nerolidol biosynthesis in tea are limited, it is known that the withering process can enhance the metabolism of terpenoids, potentially increasing the content of nerolidol in tea leaves (Wang et al., 2019). Additionally, the regulatory relationship between miRNAs and the biosynthesis of volatile compounds, including nerolidol, suggests that miRNAs may play a crucial role in modulating its levels in different tissues of the tea plant (Li et al., 2021).

The biosynthesis of linalool, geraniol, and nerolidol in tea is influenced by both genetic factors and manufacturing processes. The MVA and MEP pathways play a central role in the production of these terpenoids, while miRNAs and specific manufacturing steps can modulate their levels, thereby affecting the flavor profile of tea.

7 Genetic and Environmental Factors

7.1 Influence of genetic variability on tea metabolites

Genetic variability plays a crucial role in the biosynthesis of secondary metabolites in tea plants. Different tea cultivars exhibit distinct metabolic profiles, which are influenced by their genetic makeup. For instance, a study on 100 tea plant cultivars revealed that genetic factors significantly affect the composition of microbiomes in different plant compartments, such as the rhizosphere, root endosphere, and phyllosphere. Specific genes related to cell wall and carbon catabolism were linked to root endosphere microbial composition, while genes associated with metal ion metabolism influenced rhizosphere microbiomes (Tan et al., 2022). Additionally, MYB transcription factors have been identified as key regulators of secondary metabolite biosynthesis, including flavonoids, caffeine, and theanine, in tea plants. These transcription factors also play roles in shoot development and stress responses, further highlighting the genetic control over metabolite production (Li et al., 2022). Furthermore, the age of tea plants can influence metabolite profiles, with older plants showing upregulated flavonoid biosynthesis due to differential gene expression (Yue et al., 2022). These findings underscore the importance of genetic variability in determining the quality and composition of tea metabolites.

7.2 Environmental impacts on tea metabolite production

Environmental factors significantly impact the production of secondary metabolites in tea plants. Climate change, including shifts in seasonality, water stress, and temperature variations, can lead to substantial changes in metabolite concentrations. For example, a systematic review found that seasonality and water stress consistently affected phenolic compound levels, with seasonal shifts often resulting in decreased concentrations and drought stress leading to increased levels. Geographic factors also play a role, as the metabolome of tea plants varies with location, climate, and cultural practices. Multivariate statistical analysis has shown strong correlations between environmental factors and the levels of theanine and catechin derivatives in teas from different regions (Lee et al., 2018). Additionally, stress conditions such as cold, drought, and intense light can alter the profiles of primary and secondary metabolites, affecting tea quality. Controlled stress exposure during tea manufacturing has been proposed as a strategy to enhance tea quality by inducing the production of specific volatile compounds (Shao et al., 2021). These studies highlight the complex interplay between environmental conditions and tea metabolite production.

7.3 Interaction between genetics and environment in tea plants

The interaction between genetic factors and environmental conditions is crucial in shaping the metabolite profile of tea plants. Genetic determinants can influence the tea plant's response to environmental stresses, thereby affecting the accumulation of metabolites. Fang et al. (2021) through genome-wide association analysis (GWAS), studied molecular markers of important secondary metabolites related to tea flavor (such as theanine, caffeine, and catechins) in tea plants. These secondary metabolites play a key role in the formation of tea flavor and have high heritability. The study results showed that the content of these metabolites varied significantly across different seasons and exhibited considerable genetic diversity among different tea plant varieties (Table 1). These findings not only reveal the genetic mechanisms of secondary metabolites in tea plants but also provide theoretical basis and technical support for future tea plant improvement. Additionally, the organ-dependent changes in metabolite profiles indicate that the interaction between genetics and environment is crucial for understanding tea quality. The study demonstrated that the metabolite profiles related to tea quality in young leaves could distinguish genetic populations of tea, while the profiles in stems could not, indicating a complex network of genetic and environmental interactions (Yamashita et al., 2021). The interaction between the age of tea plants and environmental factors affects metabolite production, with older tea plants exhibiting specific genotype responses under different environmental conditions (Yue et al., 2022). These findings underscore the need for integrated approaches that consider both genetic and environmental factors to optimize tea quality and metabolite production.

Table 1 Variations in tea flavor-related metabolite levels in Population (Adopted from Fang et al., 2021)

Metabolite	Season	Range (%)	Mean (%)	^{a}SD	^{b}CV	^{C}H'	Heritability
Theanine (TN)	Spring	0–4.03	1.56	0.84	0.54	2.03	0.6
	Summer	0–2.58	0.94	0.51	0.54	2.06	
	Autumn	0–6.01	1.92	1.03	0.54	2.01	
	Mean	–	1.47	0.79	0.54	2.03	
Caffeine (CAF)	Spring	0.09–5.36	2.72	0.81	0.3	1.9	0.7
	Summer	0–3.48	2.29	0.64	0.28	1.85	
	Autumn	0.03–5.0	2.84	0.81	0.29	1.83	
	Mean	–	2.62	0.75	0.29	1.86	
(+)-Gallocatechin (GC)	Spring	1.08–3.50	2.19	0.47	0.21	2.04	0.55
	Summer	1.38–4.21	2.63	0.6	0.23	2.05	
	Autumn	1.40–5.31	2.84	0.81	0.29	1.79	
	Mean	–	2.55	0.63	0.24	1.96	
(−)-Epigallocatechin (EGC)	Spring	0.40–5.11	2.11	0.81	0.39	2.02	0.59
	Summer	0.40–9.93	2.37	1.14	0.48	1.96	
	Autumn	0.40–5.18	2.48	0.93	0.38	2.07	
	Mean	–	2.32	0.96	0.42	2.02	
(+)-Catechin (C)	Spring	0.83–4.01	1.22	0.45	0.37	1.49	0.9
	Summer	0.87–3.14	1.3	0.44	0.34	1.51	
	Autumn	0.88–3.95	1.36	0.44	0.32	1.58	
	Mean	–	1.29	0.44	0.34	1.53	
(−)-Epicatechin (EC)	Spring	0.08–0.37	0.16	0.05	0.31	1.88	0.7
	Summer	0.08–0.46	0.18	0.06	0.33	1.92	
	Autumn	0.09–0.34	0.17	0.05	0.29	1.99	
	Mean	–	0.17	0.05	0.31	1.93	
(−)-Epigallocatechin-3-gallate (EGCG)	Spring	3.55–16.24	8.85	2.28	0.26	2.09	0.67
	Summer	3.42–12.40	7.47	1.87	0.25	2.08	
	Autumn	3.68–13.76	8.28	1.96	0.24	2.09	
	Mean	–	8.2	2.04	0.25	2.09	
(−)-Gallocatechin (GCG)	Spring	3.10–12.22	5.27	1.16	0.22	1.87	0.75
	Summer	3.19–7.59	4.93	0.83	0.17	2.03	
	Autumn	3.05–9.08	5.35	0.83	0.17	2.02	
	Mean	–	5.18	0.94	0.19	1.97	
(−)-Epicatechin-3-gallate (ECG)	Spring	1.96–8.35	3.7	0.93	0.25	1.92	0.57
	Summer	2.02–6.70	3.62	0.73	0.2	2	
	Autumn	2.06–6.79	3.75	0.86	0.23	1.93	
	Mean	–	3.69	0.84	0.23	1.95	
Catechin gallate (CG)	Spring	3.00–5.74	3.7	0.42	0.11	1.93	0.56
	Summer	3.08–4.94	3.73	0.35	0.09	2.04	
	Autumn	3.06–6.51	3.82	0.43	0.11	1.89	
	Mean	–	3.75	0.4	0.1	1.95	

Note: ^{a}SD: standard deviation; ^{b}CV: Coefficient of variation; cH': Shannon-Wiener diversity index

Table 1 shows the variation in the content of ten flavor-related secondary metabolites in tea plants across the spring, summer, and autumn seasons. The content of these metabolites varied significantly across different seasons, with high diversity indices and heritability. Specifically, the coefficient of variation for theanine (TN) was the highest, indicating that its content is greatly influenced by environmental factors, while catechins (C) had the highest heritability, indicating that its content is mainly controlled by genetic factors. These data provide important basis for genetic improvement of tea plants, emphasizing the necessity of selecting and cultivating high-quality tea plant varieties under different environmental conditions.

8 Analytical Techniques for Studying Tea Secondary Metabolites

8.1 Chromatography in tea metabolite analysis

Chromatography is a pivotal analytical technique in the study of tea secondary metabolites. It allows for the separation, identification, and quantification of complex mixtures of compounds. Various forms of chromatography, such as gas chromatography (GC) and liquid chromatography (LC), are extensively used to analyze the flavor compounds in tea. These techniques are essential for isolating individual metabolites from the complex tea matrix, enabling detailed studies of their chemical properties and contributions to tea flavor (Zhai et al., 2022).

8.2 Mass spectrometry in tea research

Mass spectrometry (MS) is another critical tool in tea metabolite research. It is often coupled with chromatographic techniques to enhance the identification and quantification of tea metabolites. MS provides detailed information on the molecular weight and structure of compounds, which is invaluable for characterizing the diverse array of secondary metabolites present in tea. The combination of MS with chromatographic techniques, such as GC-MS and LC-MS, has been particularly effective in identifying and quantifying flavor compounds in tea, including those present in trace amounts (Zhai et al., 2022).

8.3 NMR spectroscopy in tea metabolite identification

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful technique for the structural elucidation of tea metabolites. NMR provides detailed information on the molecular structure and dynamics of compounds, making it an indispensable tool for identifying unknown metabolites in tea. This technique complements chromatography and mass spectrometry by providing additional structural insights that are crucial for a comprehensive understanding of tea secondary metabolites. NMR spectroscopy has been used to identify various flavor compounds in tea, contributing significantly to the knowledge of tea chemistry and flavor formation (Zhai et al., 2022).

By integrating these analytical techniques, researchers can achieve a more comprehensive understanding of the biosynthesis and functional roles of secondary metabolites in tea. This multi-faceted approach is essential for advancing the field of tea research and improving the quality and flavor of tea products.

9 Case Studies

9.1 Case study 1: Catechin biosynthesis in green tea

Catechins are a group of flavonoids that significantly contribute to the astringency and health benefits of green tea. The biosynthesis of catechins in green tea involves a series of enzymatic reactions that convert phenylalanine into various catechin derivatives. In a study examining the flavor determinants in green tea, it was found that the cultivar 'Bai-Sang Cha' ('BAS') contained higher levels of catechins compared to 'Fuding-Dabai Cha' ('FUD') (Han et al., 2015). This difference was attributed to the enhanced transcription of catechin biosynthetic genes in 'BAS', suggesting a genetic basis for the increased catechin production.

During the manufacturing process, the levels of catechins can be influenced by the method of tea processing. For instance, steam treatment was shown to reduce the levels of catechins compared to pan-fire treatment, which consequently reduced the astringency associated with catechins (Han et al., 2015). This indicates that both genetic factors and manufacturing processes play crucial roles in determining the catechin content and, thereby, the flavor profile of green tea.

9.2 Case study 2: Linalool biosynthesis in oolong tea

Linalool is a monoterpene alcohol that contributes to the floral and fruity aroma of oolong tea. The biosynthesis of linalool in oolong tea involves the conversion of geranyl pyrophosphate into linalool through the action of linalool synthase. A comprehensive metabolomics study on oolong tea revealed that the ZuoQing process, which includes alternating YaoQing and TanQing steps, is critical for the formation of linalool and other volatile terpenes (Chen et al., 2020).

During the ZuoQing process, the accumulation of terpene precursors such as farnesyl pyrophosphate and geranyl pyrophosphate was observed, providing substrates for the synthesis of downstream volatile terpenes, including linalool (Chen et al., 2020). Additionally, the fixation step in oolong tea processing was found to facilitate the conversion of amino acids into aromatic compounds, further enhancing the floral and fruity fragrances of the final tea product (Chen et al., 2020). This study highlights the importance of specific processing steps in modulating the biosynthesis of linalool and other flavor-related metabolites in oolong tea.

10 Applications and Implications

10.1 Health benefits of tea secondary metabolites

Tea secondary metabolites, such as flavonoids, catechins, and polyphenols, have been extensively studied for their health benefits. These compounds exhibit a variety of biological activities, including antioxidant, anti-inflammatory, and anti-carcinogenic properties. For instance, catechins in green tea have been shown to reduce the risk of cardiovascular diseases and improve metabolic health by modulating lipid metabolism and reducing oxidative stress (Dziggel et al., 2017). Additionally, polyphenols in tea have been linked to improved cognitive function and a lower risk of neurodegenerative diseases, such as Alzheimer's and Parkinson's (Dziggel et al., 2017). The health-promoting effects of these metabolites underscore the importance of understanding their biosynthesis pathways to enhance their presence in tea products.

10.2 Industrial applications of tea metabolites

The industrial applications of tea metabolites extend beyond their health benefits. These compounds are also valuable as flavoring agents and preservatives in the food and beverage industry. The ability to control and enhance the flavor profile of tea through the manipulation of its secondary metabolites can significantly impact product quality and consumer preference. For example, the novel approach of Reverse Pathway Engineering (RPE) has been used to predict and replicate flavor-forming pathways in lactic acid bacteria, which can be applied to improve aroma formation in fermented food products (Liu et al., 2014). Furthermore, the biotechnological production of tea metabolites in microbial hosts offers a sustainable and scalable method to meet market demands, as demonstrated by the successful de novo production of natural products like resveratrol and taxol® in engineered microorganisms (Dziggel et al., 2017).

10.3 Potential for improving tea quality through metabolite research

Research into the biosynthesis of tea secondary metabolites holds significant potential for improving tea quality. By elucidating the metabolic pathways involved in the production of key flavor and health-promoting compounds, scientists can develop strategies to enhance these attributes in tea plants. Advances in gene silencing and next-generation sequencing have already facilitated the detailed reconstruction of complex biosynthetic pathways, enabling the targeted manipulation of specific genes to increase the yield of desirable metabolites (Dziggel et al., 2017). This knowledge can be applied to breed tea varieties with superior flavor profiles and enhanced health benefits, ultimately leading to higher quality tea products that cater to consumer preferences and health trends.

11 Concluding Remarks

The biosynthesis of tea secondary metabolites, which are crucial for the flavor and aroma of tea, involves complex metabolic pathways. Recent studies have employed advanced metabolomics and biotechnological approaches to elucidate these pathways. For instance, pathway-based metabolomics has been used to explore the biosynthetic mechanisms of key flavor compounds in mango, revealing core metabolic pathways such as butanoate metabolism, phenylalanine biosynthesis, and terpenoid backbone biosynthesis. Similarly, the reconstruction of metabolic pathways in microbial systems has enabled the production of economically relevant plant secondary metabolites, highlighting the potential for biotechnological applications in tea metabolite biosynthesis.

The insights gained from these studies have significant implications for the tea industry and research. Understanding the biosynthetic pathways of tea secondary metabolites can lead to the development of strategies to enhance the flavor and aroma of tea, thereby improving its marketability and consumer appeal. Additionally, the ability to produce these metabolites in microbial hosts offers a sustainable and scalable alternative to traditional cultivation methods, potentially reducing production costs and environmental impact. This could also provide reference for the production of novel tea flavors and the enhancement of specific health benefits associated with tea consumption.

Future research in tea metabolite biosynthesis should focus on several key areas. There is a need for comprehensive pathway-based metabolomics studies to identify and characterize the full spectrum of secondary metabolites in different tea cultivars. This will require the integration of untargeted and targeted metabolomics approaches, as demonstrated in mango. Advances in next-generation sequencing and gene silencing techniques should be leveraged to elucidate the genetic and regulatory mechanisms underlying these pathways. Finally, the development of biotechnological methods for the production of tea metabolites in microbial systems should be further explored, with an emphasis on optimizing yield and scalability.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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