

Purine alkaloids in tea plants: component, biosynthetic mechanism and genetic variation

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Abstract

The purine alkaloids, including caffeine, theobromine, and theacrine, are one of the most important quality and functional components of tea plants and commercial teas. In this paper, we review the component, biosynthetic mechanism and genetic variation of purine alkaloids in tea plants. The content of caffeine and theobromine in 403 accessions Chinese tea germplasm core collection preserved in the National Germplasm Hangzhou Tea Repository were analyzed using High Performance Liquid Chromatography (HPLC). The purine alkaloid profiles of different tea varieties, germplasm types, geographical origin, seasons, were highlighted. Some naturally low caffeine or caffeine-free, high theobromine or high theacrine germplasm were identified and the possible biosynthetic mechanism of hypernormal purine alkaloid content in tea plants were partially revealed. Some Cleaved Amplified Polymorphic Sequences (CAPS) DNA markers were developed for the identification of purine alkaloid content. The potential application of genetic engineering and DNA markers developed based on the low caffeine *TCS1* (tea caffeine synthesis gene) alleles for marker assisted selection (MAS), and cocoa tea and kucha in low caffeine/high theacrine tea cultivar breeding programs were also reviewed.

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Introduction

Alkaloids are heterocyclic nitrogen-containing compounds produced by higher plants and some microorganisms. They usually have remarkable biological activity and pharmacological effects, and are widely distributed in more than 2,000 kinds of plants in more than 100 families^[1]. Tea, coffee and cocoa are the three most popular non-alcoholic beverages in the world. They all contain purine alkaloids such as caffeine, and theobromine. Some tea plants also contain theacrine, another purine alkaloid with a different function. Purine alkaloids not only affect the taste and quality of tea, but also have health benefits. The content profiles of main purine alkaloids in Chinese tea germplasm, the molecular mechanism of purine alkaloid metabolism in some hypernormal tea germplasm, the potential of DNA markers in marker-assisted selection and genetic engineering in the low caffeine/high theacrine tea breeding were comprehensively reviewed. In order to better understand the content profile of purine alkaloids in tea germplasm, the function of key genes in the purine alkaloid biosynthesis pathway, as well as providing a roadmap for special tea cultivar's genetic improvement in the future.

Purine alkaloid components in tea plants and their physiological function

Purine alkaloid components in tea plants

The alkaloids in tea plants are mainly purine alkaloids, with a small amount of pyrimidine base. Purine alkaloids are the

secondary metabolite of purine nucleotide^[2], including methylxanthine and methyluric acid. Their chemical structures are based on the skeleton of xanthine and uric acid^[3], differing by the number and position of methyl groups on the purine ring. The three main purine alkaloids in tea plants are caffeine, theobromine and theacrine.

Caffeine

1,3,7-trimethylxanthine, its chemical formula is $C_8H_{10}N_4O_2$ (Fig. 1c). Caffeine is the most abundant purine alkaloid in tea, generally accounting for 2%–5% of the dry matter content^[4]. It is one of the most important quality and functional components in tea. Chen & Zhou^[5] analyzed the caffeine content of 596 accession tea germplasm preserved in China National Germplasm Hangzhou Tea Repository (CNGTR), which basically represent the genetic diversity of Chinese tea plants. The caffeine content varied from 1.2% to 5.9%, averaging 4.2%.

Theobromine

3,7-dimethylxanthine, the chemical formula is $C_7H_8N_4O_2$ (Fig. 1b). Theobromine, a precursor for caffeine synthesis, lacks a methyl group at the N-1 position compared to caffeine^[6]. Theobromine is the main alkaloid of the cocoa plant. Generally, theobromine content is less in tea plants, and the theobromine content in young leaves is 0.05%–0.80%^[7]. Among the tea plants in Sect. *Thea* (L.) Dyer *Camellia* L., *C. ptilophylla* Chang discovered in the 1980s is a new tea plant resource with high theobromine (4.7%) and no caffeine^[8]. Additionally, *C. gymnogyna* Chang^[9], *C. irrawadiensis* P. K. Barua, *C. crassicolumna* Chang, *C. purpurea* Chang et Chen^[10], Hongyacha^[11] are all

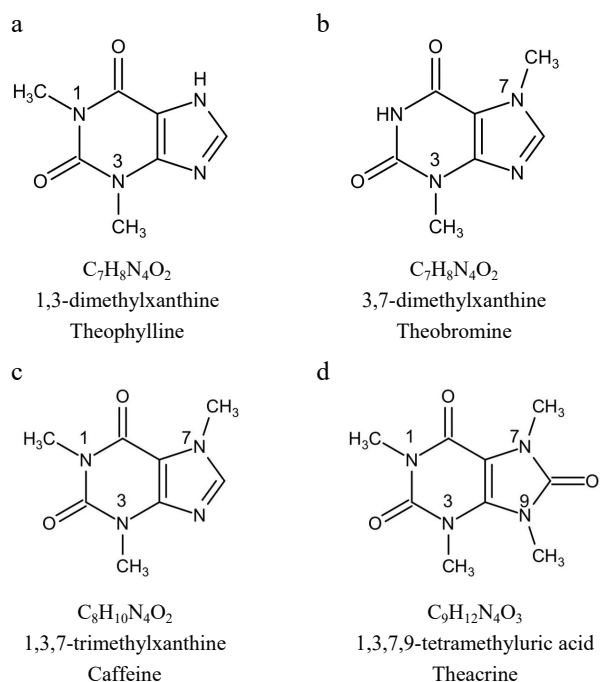


Fig. 1 The chemical structure of the main purine alkaloids in tea plants.

naturally rich in theobromine, no or low caffeine tea genetic resources, respectively.

Theacrine

1,3,7,9-tetramethyluric acid, the chemical formula is $C_9H_{12}N_4O_3$ (Fig. 1d), the structure is similar to caffeine, the difference is that the C8 position is increased by a carbonyl group and the N9 position is increased by a methyl group^[12]. Theacrine is a special purine alkaloid contained in Kucha, a special tasting bitter tea plant. Kucha once was classified into *C. kucha* (Chang et Wang)^[13], *C. sinensis* var. *kucha* and *C. assamica* var. *kucha*, but actually it has no significant morphological difference with *C. sinensis*. Ye et al. isolated and identified a high content of 1,3,7,9-tetramethyluric acid (1.29%) in the young shoots of Kucha for the first time, and they found a new distribution pattern of purine alkaloids in tea plants^[14]. The theacrine content in the tender shoots with 'two and a bud' of Kucha was 1.3%–3.4%. The pure theacrine could be obtained from Kucha leaf by high-speed countercurrent chromatography^[15].

In addition, the purine alkaloids found in tea also include theophylline (1,3-dimethylxanthine) (Fig. 1a), which is the isomer of theobromine, and the content in tea is much lower, generally about 0.05%. It occurs naturally in trace amounts in coffee and cocoa (Table 1).

Table 1. Purine alkaloids in tea, coffee and cacao (%).

Purine alkaloids	Tea	Coffee		Cacao
		Arabica	Robusta	
Caffeine	2.0–5.0	1–1.5	2–2.7	1.5
Theobromine	0.06–1.0	Trace	Trace	1.8
Theacrine	0.5–3.6 (rare)	/	/	/
Theophylline	0.05	Trace	Trace	Trace

Physiological function

Purine alkaloids not only affect the flavor of tea, but also have important biological activities and physiological functions. Although several major purine alkaloids in tea have similar structures, their functions are quite different.

Caffeine

At present, the role of purine alkaloids in plants has not been fully determined, there are two hypotheses to explain the physiological role of caffeine in plants, the 'chemical defense' and 'allelopathic function' theories^[16]. The 'chemical defense' proposed that high concentrations of caffeine in young tissues and fruits of *Coffea arabica* and *Camellia sinensis* have a defensive effect, protecting young shoots from pathogens and reducing herbivore feeding. And the 'allelopathic theory' proposes that the release of caffeine in the seed coat and fallen leaves into the soil can inhibit the growth of competitive plants and regulate the plant spacing^[17]. Caffeine in plants has long been thought to have a defensive effect, acting as a natural pesticide and antifeedant. But a recent study found that caffeine, which is naturally present in nectar of plants such as *Citrus* and *Coffea*, has no repellent effect on bees. Instead, it may affect the behavior of pollinators to enhance pollination and protect the normal reproduction of plants^[18].

Caffeine occurs naturally in many foods and beverages, and most caffeine is consumed as coffee and tea. In long-term consumption, it has been found that caffeine has significant effects on cognitive, physical and occupational performance^[19]. Current research suggests that low to moderate (approximately 40–300 mg or ~0.5–4.0 mg kg⁻¹) doses of caffeine improve cognition, which is mainly manifested in improving alertness, learning and memory^[19]. Caffeine is structurally similar to adenosine, and exerts central nervous system (CNS) effects by antagonizing adenosine receptors (ARs)^[20], affecting brain function. Epidemiological studies have shown that caffeine, as a non-selective adenosine receptors antagonist^[21], has the potential to treat cognitive disorders, including Alzheimer's disease, Parkinson's disease, depression, and schizophrenia^[20]. It has been confirmed in many studies involving animals and humans^[22]. By studying a transgenic mouse model for Alzheimer's disease (AD), it was found that caffeine can rapidly reduce the level of abnormal protein (amyloid- β ; A β) in the plasma of AD mice and humans (the core of AD pathogenesis)^[23], and reverse AD-induced cognitive impairment in aged mice^[24]. Decreased levels of A β protein may be the mechanism of action of caffeine to improve cognition^[25]. In addition, studies have shown that caffeine also has pharmacological effects such as anti-obesity^[26], analgesia^[27], diuresis^[28], reducing the risk of type II diabetes^[29] and cardiovascular mortality^[30].

Low doses of caffeine had positive effects on cognition, while higher doses improved exercise performance. Doses > 200 mg (~3 mg kg⁻¹) of caffeine showed ergogenic properties on endurance, muscle strength, and high-intensity exercise^[19]. Special occupational groups, especially athletes, should control the time and dosage of caffeine intake.

Theobromine

Compared to caffeine, theobromine's effects are not well studied. Caffeine is a central nervous system stimulant, while theobromine is a smooth muscle stimulant, promoting vasodilation^[31]. The hypotensive effect of cocoa are well

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established^[32], theobromine seems to be partially responsible for this effect. A randomized, double-blind crossover trial demonstrated that high-dose theobromine lowers central systolic blood pressure and is beneficial for cardiovascular health in humans^[33]. In addition, theobromine may have hypoglycemic effects^[34]. In many cases, theobromine is considered a natural compound with therapeutic properties. An *in vitro* study have shown that theobromine inhibits uric acid crystallization and has high clinical potential in the treatment of uric acid nephrolithiasis^[35]. Usmani et al. demonstrated for the first time that theobromine suppresses cough without CNS side effects. It can be used clinically to treat acute and chronic cough^[36]. Similar to caffeine, theobromine has potential obesity therapeutic^[37] and neuroprotective effects^[38]. Notably, theobromine is less stimulatory to the CNS and may have a higher potential than caffeine. In addition, theobromine also has special effects of protecting tooth enamel^[39], immune regulation^[40], and anti-inflammation.

Theacrine

Sheng et al.^[41] comprehensively reviewed the beneficial health effects of theacrine, including antioxidant effect, anti-inflammatory effect, reducing fatigue effects, improving cognitive effect and hypnotic effect. Theacrine has been identified as non-toxic by toxicological tests, and a clinical study in which subjects took theacrine supplementation for 8 weeks also concluded that theacrine is safe for humans^[42]. Its potential medicinal functions have been studied in recent years. A behavioral test showed that oral theacrine significantly reversed central fatigue-induced learning and memory impairments^[43]. There are many studies proving that theacrine can prevent Parkinson's disease by directly activating the Sirt3 signaling pathway, restoring mitochondrial function and inhibiting the apoptosis of dopaminergic neurons^[44]. Unlike caffeine, theacrine does not stimulate central excitation and is now considered a new antidepressant purine alkaloid drug^[45].

Theophylline

Theophylline occurs naturally in tea and cocoa beans. Although the content is extremely low, it has a high medicinal value and is an important alkaloid in tea plants. In 1985, theophylline was first extracted from tea and chemically synthesized. It was found that theophylline has diuretic and

bronchodilating effects. Recent studies have shown that low-dose theophylline helps treat asthma and chronic obstructive pulmonary disease (COPD), with multiple anti-inflammatory effects. Clinically, it can be administered intravenously or orally. It is worth noting that high-dose theophylline has side effects, such as headache, vomiting and arrhythmia, clinical use should pay attention to the recommended dose^[46].

The possible biosynthetic mechanism of purine alkaloids in tea plants**Theobromine and caffeine biosynthesis in tea plants**

Purine alkaloids are secondary metabolites of purine nucleotides. Studies have shown that adenine nucleotide are the most effective presumed precursors in caffeine biosynthesis^[47]. Tea alkaloids are methylxanthine derivatives, xanthine is considered to be the most important precursor in caffeine synthesis, and SAM (S-adenosyl methionine) is an active methyl donor in caffeine biosynthesis^[48]. Koshiishi et al.^[49] proposed the main pathway for SAM to xanthosine (SAM → SAH (S-adenosyl-homocysteine) → adenosine → adenine → AMP → IMP → XMP → xanthosine), and believed that the purine ring in caffeine also came from SAM. In addition to the SAM pathway, xanthine can also be synthesized through the AMP pathway, the GMP pathway and the *de novo* pathway. Currently, it is believed that tea plants in Sect. *Thea* (L.) Dyer *Camellia* L. mainly synthesize xanthine through the SAM pathway and the AMP supplementation pathway (adenosine → AMP), while *Coffea arabica* mainly synthesize xanthine through the AMP pathway^[50] (Fig. 2).

Ashihara et al.^[16] reviewed the main caffeine biosynthetic pathway, which is the four-step reaction of caffeine synthesis from xanthine, including one nucleosidase step and three methylation steps: xanthosine → 7-methylxanthosine → 7-methylxanthine → theobromine → caffeine (Fig. 3). Among them, the tea caffeine synthase (TCS), which catalyzes N-3 and N-1 methylation, is considered to be the most critical enzyme in the caffeine biosynthetic pathway of tea plants.

Although the caffeine biosynthetic pathway has been extensively studied in tea plants and other caffeine-rich plants, the regulatory mechanism of caffeine synthesis remains unclear. In a recent study, metabolite profiling and transcriptome

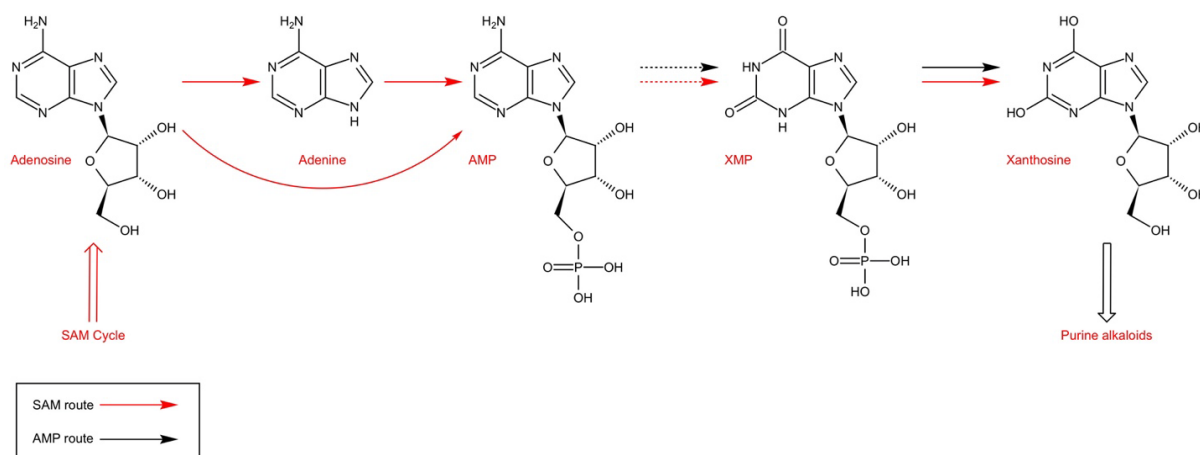


Fig. 2 Purine alkaloids are synthesized from xanthosine which is produced *via* two main routes in tea and coffee: SAM route and AMP route (adapted from Ashihara et al.^[16]).

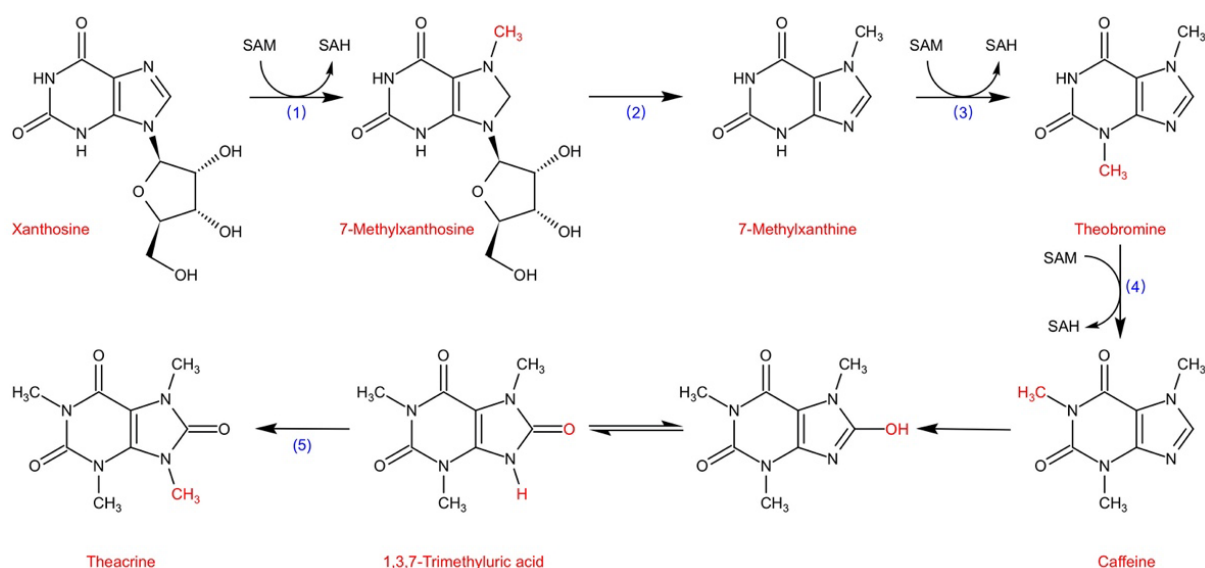


Fig. 3 The main purine alkaloids biosynthesis pathway in tea plants (Adapted from Ashihara et al.^[16]). (1) xanthosine 7-*N*-methyltransferase; (2) *N*-methylnucleosidase; (3) monomethylxanthine *N*-methyltransferase; (4) 3,7-dimethylxanthine *N*-methyltransferase TCS1; (5) 1,3,7-trimethyluric acid *N*-methyltransferase (CkTcS).

profiling provided a comprehensive understanding of the transcriptional regulatory mechanisms of caffeine biosynthesis and metabolism in tea and coffee. The analysis concluded that the regulatory mechanisms of caffeine biosynthesis in tea and coffee plants are partially conserved^[51]. In addition, transcription factors related to the caffeine biosynthesis pathway were identified, and it was determined that CsMYB184 positively regulates caffeine biosynthesis in tea plants^[52].

Screening and identification of *TCS1* alleles

In 2000, the tea caffeine synthase (*TCS*) was first cloned^[53], it belongs to *N*-methyltransferase (*NMT*), and studies have shown that high caffeine biosynthesis in tea plant shoots mainly depends on the expression of *TCS1* gene^[16]. The full-length DNA genome of 6 *NMTs* from 'Baiye 1' (*TCS1-TCS6*) were cloned and found that *TCS1* has the activity of catalyzing *N*-3 and *N*-1 methylation, while *TCS2* does not have *N*-methylation transferase activity; the expression levels of *TCS3-TCS5* are very low in tea shoots, and *TCS6* is a pseudogene. It is believed that in the *NMT* gene family, the function of *TCS1* is the most important for the synthesis and regulation of caffeine^[54]. Further research found that *TCS1* has seven types of allelic variations, namely *TCS1a-TCS1g*, and these genes were all composed by four exons and three introns through analysis of gene structure. Among them, *TCS1a* is the predominant allele, and *TCS1b-g* is the rare allele, which only appears in a few wild germplasms. The natural variation of *TCS1* change the transcription level. *TCS1* alleles with low transcription levels or its encoded proteins with only TS activities (*TCS1b*, *TCS1c*, *TCS1g*), and *TCS1* alleles with high transcription levels or its encoded proteins with both TS and CS activities (*TCS1a*, *TCS1d*, *TCS1e*, *TCS1f*), suggesting that the allelic variation of *TCS1* plays an important role in caffeine biosynthesis^[55]. The *TCS1a* expression protein present in regular tea plant resources converts theobromine accumulated in tea plants into caffeine, so caffeine becomes the purine alkaloid with the highest content in regular tea plants; and the allelic variation with only TS activity is an excellent allelic variation that can be exploited during the breeding of low-caffeine tea cultivars^[55,56].

In addition, the 225th and 269th amino acids of *TCS1* were identified as key active sites by site-directed mutagenesis. The 225th amino acid residue was arginine or histidine, which determined whether *TCS1* had caffeine synthase activity^[55]. By resequencing the *TCS1* locus of 44 accessions tea genetic resources, 87 SNPs were identified. And two polymorphic sites, SNP4318 and SNP6252, were selected to develop CAPS markers^[57]. Through association analysis, SNP4318 was determined to be associated with caffeine content in four environments, explaining 4.0%–7.7% of the phenotypic variance^[57].

QTL mapping for caffeine and theobromine

A F1 pseudo-testcross population which was generated by crossing between *C. sinensis* var. *sinensis* 'Yingshuang' and *C. sinensis* var. *pubilimba* 'Beiyue Danzhu' was used for purine alkaloids QTL (quantitative trait locus) analysis^[58]. Ten QTLs were identified for four measurement years in total: 1 controlled caffeine content (CAF), 4 controlled theobromine content (TBR), 2 controlled sum of caffeine and theobromine (SCT), 3 controlled caffeine-to-theobromine ratio (CTR)^[58]. The major QTL that controls caffeine content (*qCAF1*) was mapped onto Linkage Group (LG) 01, explaining an average of 20.1% of the phenotypic variance, helping to elucidate the molecular mechanisms of theobromine and caffeine accumulation for genetic improvement of tea plants.

Theacrine biosynthesis in tea plants

Theacrine has special health-care effects, and it has, in recent years, gradually become well known to the public, but the molecular mechanism of its metabolism is still unclear. Zheng et al.^[59] used the ¹⁴C isotope tracer method to demonstrate for the first time that theacrine in Kucha leaves is synthesized from caffeine, which may produce the intermediate 1,3,7-trimethyluric acid and is further methylated to generate 1,3,7,9-tetramethyluric acid. Wang et al.^[60] analyzed the transcriptome of Kucha and found that the *NMT* encoded by TEA024443 may catalyze *N*-9 methylation. Zhang et al.^[61] identified the theacrine synthase (CkTcS) from Kucha, which has *N*9-methyltransferase activity and can only catalyze the methylation of 1,3,7-

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trimethyluric acid to form theacrine, confirming that C8 oxidation takes place prior to N9-methylation, but currently the mechanism of C8 oxidation is unclear (Fig. 2). The above findings are helpful to analyze the synthesis mechanism of theacrine and provide new approaches for the production of decaffeinated beverages.

Catabolism

Kalberer^[62] first reported the catabolism of caffeine in *Coffea arabica* using ¹⁴C-labeled tracer experiment, and believed that caffeine could be completely degraded in coffee leaves. Ashihara et al.^[63] reported the catabolism of purine alkaloids in *Camellia sinensis*, and indicated that the catabolism of caffeine in tea leaves is very slow, which is the main reason for the accumulation of caffeine in tea plants. The conversion of caffeine to theophylline is the major rate-limiting step of caffeine catabolism. At present, it is believed that caffeine catabolism begins with its conversion to theophylline catalyzed by N7-demethylase, and demonstrate that the major catabolic pathway is caffeine → theophylline → 3-methylxanthine → xanthine, and xanthine is further degraded by the conventional purine catabolism pathway to CO₂ and NH₃^[16]. Theophylline and 3-methylxanthine produced during metabolism can be reused to synthesize caffeine in young leaves^[16].

Some *Camellia* species accumulate other purine alkaloid instead of caffeine. Theobromine is the major purine alkaloid in *C. ptilophylla*, and studies have shown that theobromine is a precursor of caffeine, as opposed to a catabolite, of caffeine. Theobromine is finally degraded to CO₂ and NH₃ via 3-methylxanthine, xanthine^[64]. In *C. sinensis*, caffeine can also be converted to theobromine to xanthine, but it is not the main catabolic pathway of caffeine (Fig. 4).

Content profiling of typical tea germplasm collections

Chinese core collection

The China National Germplasm Tea Repository (CNGTR) holds more than 3,000 accessions of tea germplasm from tea growing provinces of China and around the world. It is the most abundant field genebank for preserving tea germplasms in the world. Until 2019, 2,296 accessions were preserved in the China National Germplasm Hangzhou Tea Repository (CNGHTR)^[65]. The rich and diverse tea genetic resources

provide the material basis for tea plant genetic improvement. However, it is still difficult to identify, evaluate and utilize all resources. Therefore, it is necessary to establish the core collections of tea plants in China, hoping to represent the diversity of the entire genetic resources to the greatest extent with the minimum number of resources and the minimum genetic repeatability^[66]. The identification and evaluation of the core collections will help to discover excellent genes of tea plants, and screen out a batch of tea germplasms with specific purine alkaloid components, and promote tea plant improvement and the breeding of new varieties.

According to the Chinese Agriculture Industry Standard 'Evaluating standards for elite and rare germplasm resources-Tea plant (*Camellia sinensis* (L.) O. Kuntze) (NY/T 2031–2011)', accessions with caffeine content ≥ 5% or ≤ 1.5% are cataloged as rare germplasms. Chen & Zhou tested the caffeine content of 596 accessions tea germplasm collected from tea producing provinces in China and found that more than 60% of the resources were higher than 4.1%^[5]. Among them, 30 accessions were high caffeine (≥ 5.0%) rare germplasms, and most of the valuable hypernormal germplasms were found to come from Yunnan and Fujian.

The profiling of caffeine and theobromine content for selected 403 accessions Chinese tea germplasm core collection were identified using High Performance Liquid Chromatography (HPLC). The results showed that caffeine content was generally stable between years and seasons, ranging from 0.23% to 5.34% in spring and 0.11% to 6.0% in autumn, and more than 93% of the resources varied from 2.5% to 4.5%^[67]. Theobromine content varied significantly in spring, and about 80% of the resources varied from 0.2% to 0.6%^[67]. The representative 403 accessions genetic resources were collected from 18 tea producing provinces in China, from which three high caffeine, two low caffeine and high theobromine, and one low caffeine and high theacrine accessions, a total of six specific tea accessions were screened, all from Yunnan and Guangdong. It is believed that Yunnan and Guangdong had higher coefficient of variation and diversity indices.

About 500 accessions of tea germplasm preserved in the China National Germplasm Menghai Tea Repository Branch (CNGMTRB) were comprehensively identified and evaluated. Nine accessions with super caffeine content were screened out, including seven high caffeine and two low caffeine accessions^[68]. Among the tea plant in Sect. *Thea* (L.) Dyer

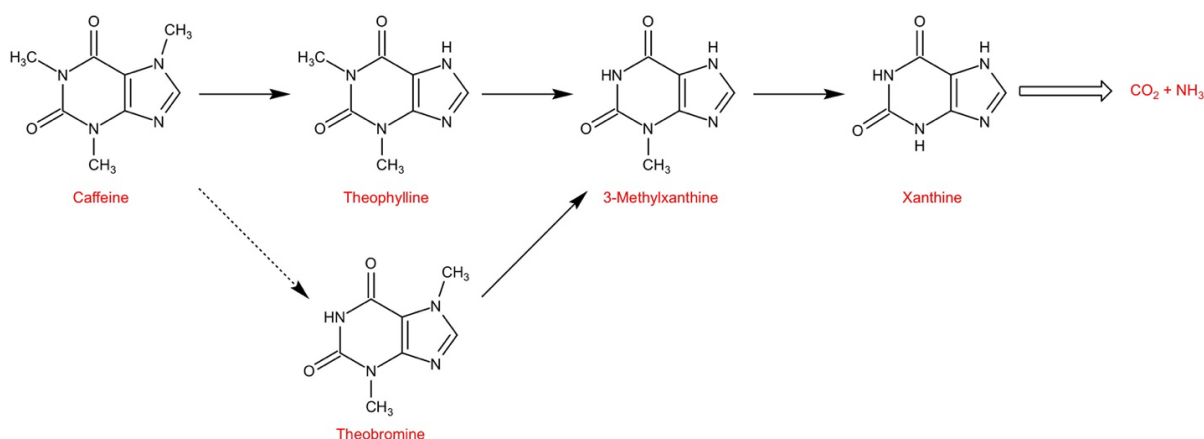


Fig. 4 The main purine alkaloids catabolic pathway in tea plants (Adapted from Ashihara et al.^[16]).

Camellia L., natural low-caffeine resources are scarce, which is of great significance for the breeding of low-caffeine tea plant cultivars. In addition, the biochemical components of 66 Yunnan tea genetic resources were analyzed, and the results showed that coefficient of variation of purine alkaloids was the total amount of purine alkaloids < caffeine < theobromine^[69], which was the same as the results of Jin et al.^[67]. Moreover, four rare germplasms of high theacrine were screened out.

Japanese and South Korea collections

In addition to China, Japan, India, Kenya and other tea producing countries have also preserved a large number of tea plant germplasms. According to reports, Japan has been planning to introduce a large number of tea plant seeds from China, South Korea, India, Indonesia, Sri Lanka and other tea growing countries, and has accumulated more than 6,800 tea genetic resource accessions^[70]. Takeda^[71] analyzed the tannin and caffeine content of 1,500 accessions of tea germplasm, the caffeine content ranged from 1.64% to 5.46%, similar to Chinese tea germplasms^[67]. The caffeine content of the two varieties of *C. sinensis* (L.) O. Kuntze was significantly different. Compared with *C. sinensis* var. *sinensis*, the variation in caffeine content of the *C. sinensis* var. *assamica* was larger, and the average content was higher (4.1%). And then, nine high caffeine content accessions were screened out from the *assamica* variety. The results of the Chinese core collection also showed that the caffeine content of the three varieties of *C. sinensis* (L.) O. Kuntze was significantly different, while the caffeine content of the *assamica* variety increased in autumn, and the content changed significantly between seasons^[67].

Ogino et al.^[72] first reported the detection of caffeine-less plants in the offspring of cultivated tea. In the natural hybrid progeny of the hybrid 'Cha Chuukanbohon Nou 6' (*C. taliensis* × *C. sinensis*), two low-caffeine and high-theobromine tea resources were found, named 'CafLess1' and 'CafLess2', respectively^[72]. According to reports, the National Institute of Vegetable and Tea Science in Japan has preserved about 3,600 accessions of tea germplasms^[72]. Before that, no caffeine-less (≤ 0.2%) germplasm was found in any resource. The discovery of the two rare germplasms will be applied to the breeding of caffeine-less tea varieties. Analysis using simple sequence repeat (SSR) marker suggested that caffeine-less traits may be recessively inherited. This not only facilitates the analysis of the mechanism of caffeine synthesis, but also obtains DNA markers closely related to caffeine-free trait for molecular marker-assisted selection (MAS) in tea breeding programs.

In South Korea, a set of 462 tea accessions was used for the core collection construction^[73]. The average caffeine content in two years was 1.67% (0.04–3.25). It is low compared with the Chinese and Japanese collections.

Some newly discovered hypernormal tea germplasms

Since the discovery of cocoa tea (*C. pilophylla* Chang) by Professor Chang Hong-ta in Guangdong, China in the 1980s, people have realized that the tea germplasm with specific purine alkaloids may have special pharmacological effects, and thus began to pay attention to the collection, screening and utilization of natural low-caffeine tea germplasms. Wild tea genetic resources are abundant in Southwest China. The wild tea plants were screened with caffeine content as an indicator, and a number of high-caffeine tea germplasms were screened in Yunnan, but only two natural low-caffeine accessions were

found: 'Jinchang Dashucha' (0.06%) and 'Daba Dashucha' (0.07%)^[68]. Taxonomically, both belong to *C. crassicaolumna* Chang. Compared with the main cultivars in Yunnan, their economic characters and sensory qualities are poor^[74]. Two high-caffeine accessions were screened in Sichuan-Chongqing region: 'Nanchuan 1' (6.25%) and 'Nanchuan 2' (5.87%), and one natural low-caffeine accession: 'Huangshan Kucha' (0.323%) and its progeny plant (0.36%). It is believed that the caffeine content of tea plants may be determined by the characteristics of the variety, and 'Huangshan Kucha' can be used as breeding material for low-caffeine tea plants^[75]. In Guangxi, a germplasm (*C. gymnogyna* Chang) containing theobromine, caffeine and theacrine was screened, which provided new materials for the development of functional components of tea plants^[76]. Recently, two new tea germplasms with specific purine alkaloid components were discovered in Fujian, which have high research value and will be introduced separately below.

'Hongyacha' (HYC) is a naturally caffeine-less/free wild tea plant^[77]. It was first reported in Fujian Province, China in 2018, which is significantly different from cocoa tea in morphological characteristics and biochemical components. The young leaves are purple or light purple, contain abundant theobromine, trans-catechins, gallicocatechin-(4→8)-gallicocatechin gallate (GC-(4→8)-GCG), 1,3,4,6-tetra-O-galloyl-β-D-glucopyranose(1,3,4,6-GA-glc), and (-)-gallicocatechin-3,5-di-O-gallate (GC-3,5-diGA), which are not detected in regular tea. In addition, the study found that the recombinant protein of caffeine synthase gene (*TCS1*) in HYC only has theobromine synthase activity, and it is considered that 'Hongyacha' is a new type of caffeine-less tea plant, which can be used for scientific research and efficient utilization in the future.

'Baiyacha' (BYC) is a wild tea plant naturally rich in theacrine^[78]. Most recently, Jin et al.^[78] reported that 'Baiyacha' was also found in Fujian Province, China, which has similar morphological characteristics to *C. gymnogyna* Chang. Some individual plants rich in catechins, 3"-methyl-epigallocatechin gallate (EGCG3"Me) and theacrine. The study found that with increasing leaf maturity, the content of caffeine decreased significantly, and the content of theacrine and EGCG3"Me increased. EGCG3"Me is an O-methylated EGCG, which has obvious pharmacological effects like theacrine. 'Baiyacha' is the first discovered tea plant resource rich in both theacrine and EGCG3"Me, which is of great value for breeding new varieties with functional components and conducting scientific research in the future.

Conclusions and future perspectives

Genetic engineering for low caffeine tea

Purine alkaloids are a class of substances that exist in large quantities in non-alcoholic beverages, of which caffeine is the most important component. Beverages containing caffeine, such as coffee, tea, and functional drinks, have a large global consumption. Appropriate intake of caffeine is beneficial to human health, but excessive intake of caffeine or special populations' intake of caffeine may cause negative effects of anxiety and insomnia. Some consumers are naturally sensitive to caffeine intake. At present, the market demand for low-caffeine or decaffeinated products is increasing rapidly. To decaffeinate tea, manufacturers often treat it with supercritical

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carbon dioxide or hot water treatments. However, these methods can affect the flavor of the brew and destroy compounds in the tea associated with health benefits. Since Kato et al. successfully cloned the caffeine synthase gene in 2000, scientists began to explore the possibility of obtaining natural low-caffeine coffee or tea through genetic engineering. Using the identified gene encoding caffeine synthase, transgenic plants with reduced caffeine content can be produced by RNA interference. It has been reported that coffee seedlings with a 70% reduction in caffeine content have been obtained through genetic engineering^[64], but there has been no relevant reports on tea.

Mohanpuria et al.^[79] tried to use RNAi technology to produce low-caffeine content tea plants. Transgenic plants showed a significant suppression of Caffeine Synthesis transcript expression and also showed a reduction of 44%–61% in caffeine and 46%–67% in theobromine content as compared to controls^[79]. These results suggest that the RNAi construct developed using a single partial fragment of CS gene reduced the expression of the targeted endogenous gene significantly^[79]. It might be a potential molecular technique for generating low-caffeine tea plants.

Application of low caffeine TCS1 alleles

Allelic variations of *TCS1* play an important role in caffeine biosynthesis of tea plants. There are two molecular mechanisms controlling the caffeine biosynthesis in low-caffeine-accumulating tea germplasms. *TCS1* natural variant reduces transcript level or its encoded protein has only theobromine synthase activity. Allelic variations of *TCS1* can be used for the selection of low-caffeine tea plant varieties. Low caffeine individuals might be selected from progenies resulting from crosses between specific accessions containing *TCS1b*, *TCS1c*, or *TCS1f*, *TCS1g* with low caffeine biosynthetic activity^[55]. Ogino et al. believed that the caffeine-free phenotype was closely related to the *TCS1*, and used a three-nucleotide insertion TTC in *TCS1*, developed a DNA molecular marker 'CafLess-TCS1', and confirmed that 'CafLess-TCS1' is an effective selection marker for breeding of caffeine-less tea cultivars^[80].

Application of cocoa tea and Kucha

Among hypernormal tea germplasms, although the health benefits of cocoa tea and Kucha have long been known, relevant scientific study is still very limited. In recent years, scientists have paid more attention to the discovery and utilization of natural tea germplasm rich in theobromine or theacrine. Hypernormal germplasms not only have different morphological and biochemical characteristics, but may also have unique regulatory mechanisms and candidate genes. Ye et al. screened out a batch of low-caffeine, high-theobromine tea germplasms from the wild population, and established the first natural caffeine-free tea germplasm base in China. In the later stage, the screening of excellent individuals of wild cocoa tea will be strengthened to further determine the pharmacological and physiological functions of cocoa tea. Several recent studies on Kucha have identified highly expressed *TCS* genes (TEA010054, TEA022559) at the transcriptional level^[81], which are helpful to reveal the metabolic mechanism of purine alkaloids at the molecular level. This will help in identification and tea plant breeding rich in theobromine or theacrine.

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Conflict of interest

The authors declare that they have no conflict of interest.

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REFERENCES

- Committee AD. 1998. *An Agricultural Dictionary*. 1458 pp. Beijing: China Agricultural Press
- Xu J. 2006. *Studies on comparison of chemical constituents of four species of Camellia Sect. Thea and the central nervous system activity of theacrine, a special purine alkaloid in C. assamica var. kucha*. Thesis. Shenyang Pharmaceutical University, China
- Ashihara H, Mizuno K, Yokota T, Crozier A. 2017. Xanthine alkaloids: occurrence, biosynthesis, and function in plants. *Progress in the Chemistry of Organic Natural Products* 105:1653
- Wan XC. 2003. *Tea Biochemistry*. Beijing: China Agricultural Press
- Chen L, Zhou ZX. 2005. Variations of main quality components of tea genetic resources [*Camellia sinensis* (L.) O. Kuntze] preserved in the China National Germplasm Tea Repository. *Plant Foods for Human Nutrition* 60:31–5
- Li J. 2013. *A correlation study of caffeine contents with theobromine contents transcriptional expression and cSNP of key enzyme genes in tea plants*. Thesis. Anhui Agricultural University, China
- Zhou C, Jin J, Yao M, Chen L. 2011. Progress on purine alkaloids metabolism in tea and other plants. *Journal of Tea Science* 31:87–94
- Zhang H, Ye C, Zhang R, Ma Y, Zeng P. 1988. A discovery of new tea resource — cocoa tea tree containing theobromine from China. *Acta Scientiarum Naturalium Universitatis Sunyatseni* 27:131–33
- Teng J. 2018. *The biochemical characteristics and N-methyltransferase of purine alkaloid pathway in Camellia gymnogyna Chang from Dayao Mountain*. Thesis. South China Agricultural University, China
- Ye C, Zheng X, Yuan C, Gao K, Shi X, Zhang H. 2001. A review of research on cocoa tea, a new resource of caffeine-free tea plant. *Guangdong Agricultural Science* 2001:12–15
- Yan Z, Huang J, Wang D. 2020. Research progress in natural theobromine-enriched tea plants. *Guangdong Agricultural Sciences* 47:37–44
- Qin D, Wang Q, Li H, Fang K, Jiang X, et al. 2021. Progress in research on *Camellia kucha* (Chang et Wang) Chang and its special constituent theacrine. *Food Science* 42:353–59
- Shi X, Zheng X, Song X, Wang Y, Ye C. 2008. A new combination of Kucha. *Acta Scientiarum Naturalium Universitatis Sunyatseni* 47:129–30
- Ye C, Lin Y, Su J, Song X, Zhang H. 1999. Purine alkaloids in *Camellia assamica* var. *kucha* Chang et Wang. *Acta Scientiarum Naturalium Universitatis Sunyatseni* 38:82–86

15. Cheng Y, Yan Z, Lu J, Ye C, Wang D. 2010. Isolation and preparation of theacrine by high-speed counter-current chromatography from *Camellia assamica* var. Kucha. *Acta Scientiarum Naturalium Universitatis Sunyatseni* 49:65–69
16. Ashihara H, Sano H, Crozier A. 2008. Caffeine and related purine alkaloids: biosynthesis, catabolism, function and genetic engineering. *Phytochemistry* 69:841–56
17. Anaya AL, Cruz-Ortega R, Waller GR. 2006. Metabolism and ecology of purine alkaloids. *Frontiers in Bioscience-Landmark* 11:2354–70
18. Arnold SEJ, Dudenhöffer JH, Fountain MT, James KL, Hall DR, et al. 2021. Bumble bees show an induced preference for flowers when primed with caffeinated nectar and a target floral odor. *Current Biology* 31:4127–4131.E4
19. McLellan TM, Caldwell JA, Lieberman HR. 2016. A review of caffeine's effects on cognitive, physical and occupational performance. *Neuroscience & Biobehavioral Reviews* 71:294–312
20. Ribeiro JA, Sebastião AM. 2010. Caffeine and adenosine. *Journal of Alzheimers Disease* 20:S3–S15
21. Cellai L, Carvalho K, Faivre E, Deleau A, Vieau D, et al. 2018. The adenosinergic signaling: a complex but promising therapeutic target for Alzheimer's disease. *Frontiers in Neuroscience* 12:520
22. Fiani B, Zhu L, Musch BL, Briceno S, Andel R, et al. 2021. The neurophysiology of caffeine as a central nervous system stimulant and the resultant effects on cognitive function. *Cureus* 13:e15032
23. Arendash GW, Cao C. 2010. Caffeine and coffee as therapeutics against Alzheimer's disease. *Journal of Alzheimers Disease* 20:S117–S126
24. Arendash GW, Mori T, Cao CH, Mamcarz M, Runfeldt M, et al. 2009. Caffeine reverses cognitive impairment and decreases brain amyloid- β levels in aged Alzheimer's disease mice. *Journal of Alzheimers Disease* 17:661–80
25. Arendash GW, Schleif W, Rezai-Zadeh K, Jackson EK, Zacharia LC, et al. 2006. Caffeine protects Alzheimer's mice against cognitive impairment and reduces brain β -amyloid production. *Neuroscience* 142:941–52
26. Huang Y, Liu Y, Dushenkov S, Ho CT, Huang M. 2009. Anti-obesity effects of epigallocatechin-3-gallate, orange peel extract, black tea extract, caffeine and their combinations in a mouse model. *Journal of Functional Foods* 1:304–10
27. Scott JR, Hassett AL, Brummett CM, Harris RE, Clauw DJ, et al. 2017. Caffeine as an opioid analgesic adjuvant in fibromyalgia. *Journal of Pain Research* 10:1801–9
28. Sharangi AB. 2009. Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.) – A review. *Food Research International* 42:529–35
29. van Dam RM. 2006. Coffee and type 2 diabetes: from beans to beta-cells. *Nutrition, Metabolism & Cardiovascular Diseases* 16:69–77
30. Guessous I, Pruijm M, Ponte B, Ackermann D, Ehret G, et al. 2015. Associations of ambulatory blood pressure with urinary caffeine and caffeine metabolite excretions. *Hypertension* 65:691–96
31. Coleman WF. 2001. Chocolate: theobromine and caffeine. *Journal of Chemical Education* 81:1232
32. Ried K, Sullivan TR, Fakler P, Frank OR, Stocks NP. 2017. Effect of cocoa on blood pressure. *Cochrane Database of Systematic Reviews*
33. van den Bogaard B, Draijer R, Westerhof BE, van den Meiracker AH, van Montfrans GA, et al. 2010. Effects on peripheral and central blood pressure of cocoa with natural or high-dose theobromine: a randomized, double-blind crossover trial. *Hypertension* 56:839–46
34. Sarriá B, Martínez-López S, Sierra-Cinos JL, García-Diz L, Goya L, et al. 2015. Effects of bioactive constituents in functional cocoa products on cardiovascular health in humans. *Food Chemistry* 174:214–18
35. Grases F, Rodriguez A, Costa-Bauza A. 2014. Theobromine inhibits uric acid crystallization. A potential application in the treatment of uric acid nephrolithiasis. *PLoS One* 9:e111184
36. Usmani OS, Belvisi MG, Patel HJ, Crispino N, Birrell MA, et al. 2005. Theobromine inhibits sensory nerve activation and cough. *FASEB Journal* 19:231–33
37. Tanaka E, Mitani T, Nakashima M, Yonemoto E, Fujii H, et al. 2022. Theobromine enhances the conversion of white adipocytes into beige adipocytes in a PPAR γ activation-dependent manner. *Journal of Nutritional Biochemistry* 100:108898
38. Cova I, Leta V, Mariani C, Pantoni L, Pomati S. 2019. Exploring cocoa properties: is theobromine a cognitive modulator. *Psychopharmacology* 236:561–72
39. Kargul B, Özcan M, Peker S, Nakamoto T, Simmons WB, et al. 2012. Evaluation of human enamel surfaces treated with theobromine: a pilot study. *Oral Health & Preventive Dentistry* 10:275–82
40. Camps-Bossacoma M, Pérez-Cano FJ, Franch A, Castell M. 2018. Theobromine is responsible for the effects of cocoa on the antibody immune status of rats. *Journal of Nutrition* 148:464–71
41. Sheng Y, Xiang J, Wang Z, Jin J, Wang Y, et al. 2020. Theacrine from *Camellia kucha* and its health beneficial effects. *Frontiers in Nutrition* 7:596823
42. Taylor L, Mumford P, Roberts M, Hayward S, Mullins J, et al. 2016. Safety of TeaCrine[®], a non-habituating, naturally-occurring purine alkaloid over eight weeks of continuous use. *Journal of the International Society of Sports Nutrition* 13:2
43. Li Y, Chen M, Wang C, Li X, Ouyang S, et al. 2015. Theacrine, a purine alkaloid derived from *Camellia assamica* var. *kucha*, ameliorates impairments in learning and memory caused by restraint-induced central fatigue. *Journal of Functional Foods* 16:472–83
44. Duan W, Liang L, Pan M, Lu D, Wang T, et al. 2020. Theacrine, a purine alkaloid from *kucha*, protects against Parkinson's disease through Sirt3 activation. *Phytomedicine* 77:153281
45. Ouyang S, Zhai Y, Wu Y, Xie G, Wang G, et al. 2021. Theacrine, a potent antidepressant purine alkaloid from a special Chinese tea, promotes adult hippocampal neurogenesis in stressed mice. *Journal of Agricultural and Food Chemistry* 69:7016–27
46. Barnes PJ. 2013. Theophylline. *American Journal of Respiratory and Critical Care Medicine* 188:901–6
47. Suzuki T, Takahashi E. 1976. Caffeine biosynthesis in *Camellia sinensis*. *Phytochemistry* 15:1235–39
48. Suzuki T. 1972. The participation of S-adenosylmethionine in the biosynthesis of caffeine in the tea plant. *FEBS Letters* 24:18–20
49. Koshiishi C, Kato A, Yama S, Crozier A, Ashihara H. 2001. A new caffeine biosynthetic pathway in tea leaves: utilisation of adenosine released from the S-adenosyl-L-methionine cycle. *FEBS Letters* 499:50–54
50. Xie G, He R, Kurihara H. 2010. Research progress on biosynthesis and catabolism of tea alkaloids. *Chinese Journal of Natural Medicines* 8:153–60
51. Zhang Y, Fu J, Zhou Q, Li F, Shen Y, et al. 2022. Metabolite profiling and transcriptome analysis revealed the conserved transcriptional regulation mechanism of caffeine biosynthesis in tea and coffee plants. *Journal of Agricultural and Food Chemistry* 70:3239–51
52. Li P, Ye Z, Fu J, Xu Y, Shen Y, et al. 2022. CsMYB184 regulates caffeine biosynthesis in tea plants. *Plant Biotechnology Journal* 20:1012–14
53. Kato M, Mizuno K, Crozier A, Fujimura T, Ashihara H. 2000. Caffeine synthase gene from tea leaves. *Nature* 406:956–7
54. Jin J, Yao M, Ma C, Ma J, Chen L. 2014. Cloning and sequence analysis of the N-methyltransferase gene family involving in caffeine biosynthesis of tea plant. *Journal of Tea Science* 34:188–94
55. Jin J, Yao M, Ma C, Ma J, Chen L. 2016. Natural allelic variations of *TCS1* play a crucial role in caffeine biosynthesis of tea plant and its related species. *Plant Physiology and Biochemistry* 100:18–26
56. Liu Y, Jin J, Yao M, Chen L. 2019. Screening, cloning and functional research of the rare allelic variation of caffeine synthase gene (*TCS1g*) in tea plants. *Scientia Agricultura Sinica* 52:1772–83

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57. Jin J, Yao M, Ma C, Ma J, Chen L. 2016. Association mapping of caffeine content with *TCS1* in tea plant and its related species. *Plant Physiology and Biochemistry* 105:251–59
58. Ma J, Jin J, Yao M, Ma C, Xu Y, et al. 2018. Quantitative trait loci mapping for theobromine and caffeine contents in tea plant (*Camellia sinensis*). *Journal of Agricultural Food Chemistry* 66:13321–27
59. Zheng X, Ye C, Kato M, Crozier A, Ashihara H. 2002. Theacrine (1,3,7,9-tetramethyluric acid) synthesis in leaves of a Chinese tea, kucha (*Camellia assamica* var. *kucha*). *Phytochemistry* 60:129–34
60. Wang S, Chen J, Ma J, Jin J, Chen L, Yao M. 2020. Novel insight into theacrine metabolism revealed by transcriptome analysis in bitter tea (Kucha, *Camellia sinensis*). *Scientific Reports* 10:6286
61. Zhang Y, Li Y, Wang Y, Tan L, Cao Z, et al. 2020. Identification and characterization of *N9*-methyltransferase involved in converting caffeine into non-stimulatory theacrine in tea. *Nature Communications* 11:1473
62. Kalberer P. 1965. Breakdown of caffeine in the leaves of *Coffea arabica* L. *Nature* 205:597–98
63. Ashihara H, Gillies FM, Crozier A. 1997. Metabolism of caffeine and related purine alkaloids in leaves of tea (*Camellia sinensis* L.). *Plant and Cell Physiology* 38:413–19
64. Ashihara H, Kato M, Crozier A. 2011. Distribution, biosynthesis and catabolism of methylxanthines in plants. In *Methylxanthines*, ed. Fredholm BB. vol 200. Springer, Berlin, Heidelberg. pp. 11–31 https://doi.org/10.1007/978-3-642-13443-2_2
65. Chen J, Ma C, Chen L. 2019. 40 years of research on tea germplasm resources in China. *China Tea* 41:1–5+46
66. Wang X, Chen L, Yang Y. 2011. Establishment of core collection for Chinese tea germplasm based on cultivated region grouping and phenotypic data. *Frontiers of Agriculture in China* 5:344
67. Jin J, Zhou C, Ma C, Yao M, Ma J, et al. 2014. Identification on purine alkaloids of representative tea germplasms in China. *Journal of Plant Genetic Resources* 15:279–85
68. Song W, Liu B, Yi B, Jiang H, Ma L, et al. 2011. Identification, evaluation and screening on elite and rare tea germplasms from Yunnan Province. *Journal of Tea Science* 31:45–52
69. Liu Y, Pang D, Jiang H, Tian Y, Li Y, et al. 2021. Biochemical component analysis and specific resource selection of 66 accessions of tea germplasms in Yunnan. *Journal of Southern Agriculture* 52:693–99
70. Wang Z, Chen L. 2011. Japanese tea cultivars and breeding techniques. *China Tea* 33:10–13
71. Takeda Y. 1994. Differences in caffeine and tannin contents between tea cultivars, and application to tea breeding. *Japan Agricultural Research Quarterly* 28:117–23
72. Ogino A, Tanaka J, Taniguchi F, Yamamoto MP, Yamada K. 2009. Detection and characterization of caffeine-less tea plants originated from interspecific hybridization. *Breeding Science* 59:277–83
73. Hyun DY, Gi GY, Sebastrin R, Cho GT, Kim SH, et al. 2020. Utilization of phytochemical and molecular diversity to develop a target-oriented core collection in tea germplasm. *Agronomy-Basel* 10:1667
74. Tang Y, Song W, Yi B, Ji P, Wang P, et al. 2010. Identification and evaluation on low caffeine content in tea germplasm. *Southwest China Journal of Agricultural Sciences* 23:1051–54
75. Zhang K, Ding Y, Yang J. 2013. Comparative analysis of catechin and caffeine content of wild tea plant in Sichuan-Chongqing region. *Chinese Journal of Applied Environmental Biology* 19:379–82
76. Teng J, Zeng Z, Huang Y. 2018. Composition characteristics of purine alkaloids and biochemical components of *Camellia gymnogyna*. *Guihaia* 38: 568–76
77. Jin J, Chai Y, Liu Y, Zhang J, Yao M, et al. 2018. Hongyacha, a naturally caffeine-free tea plant from Fujian, China. *Journal of Agricultural Food Chemistry* 66:11311–19
78. Jin J, Jiang C, Yao M, Chen L. 2020. Baiyacha, a wild tea plant naturally occurring high contents of theacrine and 3"-methyl-epigallocatechin gallate from Fujian, China. *Scientific Reports* 10:9715
79. Mohanpuria P, Kumar V, Ahuja PS, Yadav SK. 2011. Producing low-caffeine tea through post-transcriptional silencing of caffeine synthase mRNA. *Plant Molecular Biology* 76:523–34
80. Ogino A, Taniguchi F, Yoshida K, Matsumoto S, Fukuoka H, et al. 2019. A new DNA marker *CafLess-TCS1* for selection of caffeine-less tea plants. *Breeding Science* 69:393–400
81. Li H, Fang K, Qin D, Jiang X, Wu H. 2021. Comparative transcriptome analysis reveals putative genes responsible for high theacrine content in Kucha (*Camellia kucha* (Chang et Wang) Chang). *Tropical Plant Biology* 14:82–92



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