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RESEARCH ARTICLE

Studies of cocoa tea, a wild tea tree containing theobromine

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Abstract *Camellia ptilophylla* Chang is a wild tea tree containing theobromine and is caffeine-free. Lots of researches have been conducted for its domestication since it was discovered to contain theobromine in its shoots. First, its pharmacological and physiological effects have been studied, demonstrating that it can be used as a new resource of tea as daily and healthy beverage. Cocoa tea differs from traditional tea in that it does not excite the nervous system. Second, various ways of propagation have been investigated, and sexless cutting has been the method adopted currently. Third, through selection and breeding, plantation of cocoa tea can be set up to cultivate new varieties, and cocoa tea of different flavors such as green cocoa tea, oolong cocoa tea, and black cocoa tea can be processed. Thus, cocoa tea will become a choice in the tea market.

Keywords cocoa tea, theobromine, caffeine-free, introduced domestication, healthy beverage

1 Introduction

China is abundant in wild tea trees. According to the *Camellia* taxonomy by Professor Huangta CHANG, 30 of the 32 species of *Camellia* Sect. *Thea* Dyer are distributed in South China and Southwest China. Among them, the *Camellia sinensis* (L.) O. Kuntze and *C. assamica* (Mast.) Chang are traditional cultivated tea trees whose shoots are processed to tea as a daily beverage since at least the Zhou Dynasty of ancient China. Today, tea, together with coffee and cocoa, has become the three most famous beverages in the world. Like coffee, the caffeine in tea can excite nerves. In China, it has been found that in some species of the *Camellia* Sect. *Thea* Dyer, the main component of purine alkaloid is theobromine, with little or even no caffeine. These species include *C. irrawadiensis* P. K. Barua, *C. quinquelocularis* Chang et Liang, *C. crassicolumna* Chang, and *C. ptilophylla* Chang. Among them, the *C. ptilophylla* Chang is a wild tea tree that has been studied most extensively. It contains about 4% to 6.8% of theobromine in shoots and young leaves (flourishing shoots) of dry weight. After years of efforts, it has already been domesticated and introduced as a special tea tree. Another special species is *C. kuch*a (Chang et Wang) Chang with about 2% of theacrine (1,3,7,9-tetramethyl uric acid) in shoots. Compared with caffeine, theobromine, and theaphylline, theacrine is a completely new alkaloid unknown to many people.

In the past, caffeine in tea was known as the primary purine alkaloid, with therbromine and theaphylline as secondary and companionate alkaloids of caffeine. Theacrine in tea was first reported in 1937 by Johnson who extracted the significant component from millions of discarded decaffeinated tea and tested its chemical structure (Johnson, 1937). However, its significant content could not be detected by regular biochemical analysis of tea afterward. A small amount of theacrine has also been found in *Coffea liberica*, but it only exists in some growing periods and catabolizes into other uric acids quickly (Baumann et al., 1976). In 1999, theacrine was discovered in kucha (C. kucha), and its content was 1.3% at dry weight. The theacrine was tested through assay of the elements and chemical structure of kucha by mass spectrography (Ye et al., 1999). Follow-up studies of kucha showed that the content might be higher, and it varied between season and individuals.

There are four kinds of purine alkaloids in tea: caffeine is 1,3,7-trimethylxanthine, theaphylline is

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1,3-dimethylxanthine, theobromine is 1,7-dimethylxanthine, and theacrine is 1,3,7,9-tetramethyluric acid (Fig. 1).

The four kinds of purine alkaloids are all combined with methyl-CH_3 at N-positions of the purine ring. When two methyl-CH_3 are combined, it is named dimethylxanthine, and it has two isomers: one is 1,7-dimethylxanthine, i.e., theaphylline; the other is 1,3-dimethylxanthine, i.e., theobromine.

In order to introduce wild cocoa tea trees into cultivation, we carried out investigations, including its taxonomic position, biochemical components, stability of biochemical components, especially theobromine, ground data as daily and healthy beverage, and the processes of domestication.

As for kucha, the current studies focus on its biochemical components and pharmacological and physiological effects. The study of its cultivation has not begun yet.

2 Taxonomic position and physiecological characteristics

2.1 Taxonomic position of cocoa tea

C. ptilophylla Chang was first published in 1981 (Chang, 1981). It is also called cocoa tea since its buds and young leaves are rich in theobromine (Ma and Zhang, 1982, 1984; Chang et al., 1988). *C. ptilophylla* belongs to *Camellia* Series *Sinensis* Chang in the *Camellia* Section *Thea* Dyer congeneric with *C. sinensis* and *C. assamica*. Its leaves and young shoots are covered with grey-yellowish hair, and its flowers are axillary, with hair on pedicel, two facies of bracteoles, sepals, and back of outer petals.

2.2 Anatomic characteristics of leaves

2.2.1 Epidermis of leaves

The epidemic cells are closely arranged and distributed with cuticle and distribute stomata. The stomata are mainly on the lower surface of leaves and at the same level with other epidemic cells. The most distinctive character is that much hair is developed from the epidemic cells on the lower surface.

2.2.2 Mesophyll tissue

C. ptilophylla is a typical plant of bifacial leaves in mesophilous circumstance, and its mesophyll tissue consists of spongy tissue and one layer of palisade tissue on the upper surface of leaves. Some parenchyma and phloem cells in the mesophyll tissue and ground tissue contain starch grains or rhombohedral or spherical cluster crystals. Stone cells scatter in mesophyll tissue and vary in size and form like bone, claver, multiangular, and evident ramose.

2.2.3 Vascular tissue

Retinerved venation spreads over the whole lamina, with grosser vein continuously branching into slender veins that are covered in the mesophyll tissue; whereas middle veins are covered in the ground tissue. Slender veins are surrounded by 1–2 layer cells of vascular bundle sheath. In slender veins, especially in veinlets of phloem, there are parenchymal cells with dense protoplast. The diameter of main veins exceeds the thickness of lamina, and toward the upper surface of the main veins is the xylem of spiral vessels; while toward the lower surface is the phloem, and around them are mechanical tissues.

2.3 Physiecological characteristics of *C. ptilophylla*

Under different ecological conditions, comparison of *C. ptilophylla*'s water content, photosynthesis, respiration, and the content of chlorophyll and observation of its growth and development showed that the extent of transpiration intensity, wilting ratio, and water deficit of *C. ptilophylla* grown in shady conditions did not vary greatly, and the leaves had a strong water-retention capacity. The largest transpiration coefficient of *C. ptilophylla* is observed at two o'clock in the afternoon. The average transpiration coefficient of *C. ptilophylla* in open fields in July is 494.7 mg/g·h at fresh weight with a large amount of water consumption. However, the transpiration coefficient of *C. ptilophylla*, flourishing with dark green leaves, in shady and wild fields is 368.2 mg/g·h at fresh weight and 314.7 mg/g·h at dry weight, respectively, with less amount of water consumption. Research of its photosynthesis showed that the photosynthesis ratio increased as the degree of light efficiency increased in weak light condition (under 25 kLux), and the

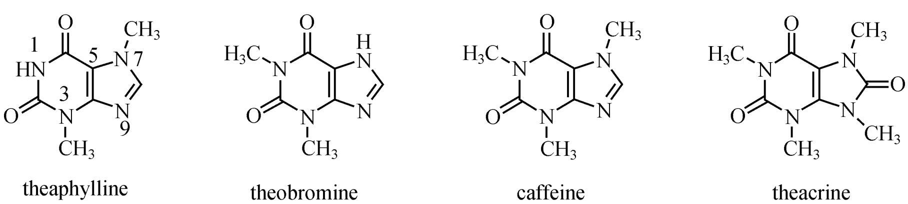

Fig. 1 Chemical structures of the four kinds of purine alkaloids in tea

photosynthesis ability in shady conditions was higher than that in open fields, while the photosynthesis ability was lower than that in open fields in strong light (28–35 kLux). When the light efficiency exceeded 25 kLux under shady conditions, the photosynthesis ratio began to descend dramatically. This descending trend suggested that when the light efficiency increased to a certain level, the photosynthesis ratio did not increase, and there were few or even no photosynthesis product. The photosynthetic reaction reached saturation at this point. The light saturation point of *C. ptilophylla* is 25 kLux in shady conditions and 37 kLux in open fields. *C. ptilophylla* is a kind of shade plant, which adapts to weak light and grows in good condition, with rational contracture of populations and a large individual crown area ranging from 0.6–1.0 m^2. The branch number of individuals varies from 192–274, leaf area 12 000–26 000 cm^2, and bud density 15–20/100 cm^2. The quantity of fresh buds and young leaves is 320–400 g per individual. In open fields, the individual crown area is 0.4–0.8 m^2 and the number of branches varies from 188–282, leaf area 6000–18 000 cm^2, less than that in shady conditions, and bud density 10–15/100 cm^2. The quantity of fresh buds and young leaves is 150–280 g per individual (Wang et al., 1993a, 1993b).

3 Chemical components of cocoa tea

3.1 Purine alkaloids of wild cocoa tea

Mixed samples were assayed to detect alkaloids, including vegetative mass and reproductive mass of *C. ptilophylla* collected from the population in wild fields. The test results showed that they all contained theobromine and the content in the plant of one bud and one leaf reached the highest amount of 4.04%, while the content in the plant of one bud and two leaves was less, followed by that of one bud and three leaves…one bud and six leaves. The theobromine content in aged leaves was lowered to 0.59%. Besides, theobromine could be detected in the hairs of buds and leaves, roots, stems, fruit stalks, peels, and seeds of cocoa tea (Ma and Zhang, 1984).

Theobromine content varied in different season, being 4.4% in spring, 3.57% in summer, and 3.62% in autumn. Light and altitude influenced the content of theobromine in cocoa tea, which was positively correlated with altitude (Ma and Zhang, 1984).

According to their colors, buds of cocoa tea trees could be divided into white buds, red buds, and dark purple buds. The theobromine content in white buds was 6.17% at dry weight, red buds 5.00%, and dark purple buds 4.50% (Ma and Zhang, 1984).

3.2 Purine alkaloids of cocoa tea after cultivation

Through chemical analyses of the wild *C. ptilophylla*, the theobromine-containing and caffeine-free plants were selected for vegetative propagation and cultivation. Plants of one bud and two leaves were selected for complete chemical analysis, and the results showed that theobromine was the main chemical component consistently; however, the content of amino acid was raised due to artificial fertilization from 1.07% at dry weight in wild fields to 2% or higher in plantation (Ye et al., 1996).

4 Pharmacological and physiological effects of cocoa tea

In the place of origin, people drink cocoa tea as herbal medicine to prevent and treat cold and throat inflammation. Studies of pharmacological and physiological effects of cocoa tea have proven that cocoa tea might be a healthy beverage.

4.1 Acute toxicity of cocoa tea

By standard mice and standard test method, the aqueous extract and acetone extract of cocoa tea were tested for its acute toxicity, using Longjing Cha as a control. The result showed that for the test samples of the aqueous extract of the stomach perfusion and abdominal cavity injection, the 50% lethal dose (LD_{50}) was 3.7025 ± 0.0326 g/kg and 0.1375 ± 0.0408 g/kg, respectively; for the test samples of the acetone extract of stomach perfusion and abdominal cavity injection, the $LD_{50} = 4.67 \pm 0.039$ g/kg and $LD_{50} = 0.483 \pm 0.0432$ g/kg, respectively (Xu et al., 1990a). Data of the median lethal dose ($LD_{50} \pm 95\%$) by intravenous injection of the aqueous extract and acetone extract showed that the confidence interval ($LD_{50} \pm 95\%$) and slope of cocoa tea was 237 ± 23 mg/kg (b = 10.9) in the control and 236.4 ± 12 mg/kg (b = 10.5) for Longjing Cha. The difference in LD_{50} between them was very slight, and the toxicity in both types of tea was very low (Xu et al., 1990b).

4.2 Acute hypotensive effect

The experimental results of acute hypotensive effect in healthy cats by 50 mg/kg aqueous extract of cocoa tea showed that the decrease rate of blood pressure was $38.6\% \pm 2.32\%$ ($P < 0.001$), duration of hypotension was (2.24 ± 0.4) min ($P < 0.001$), and the proportion of hypotension area was $18.3\% \pm 2.1\%$. Compared with 20 mg/kg aqueous extract of Longjing Cha, the decrease rate of blood pressure was $37.0\% \pm 2.8\%$ ($P < 0.001$), duration of hypotension was (1.8 ± 0.5) min ($P < 0.001$), and the proportion of hypotension area was $19.8\% \pm 1.98\%$. Both had an evident hypotensive effect, but the dosage of Longjing Cha was lower than that of cocoa tea. It suggested that with the same dose, the hypotensive effect of cocoa tea was more moderate. The results also showed that the mechanism of hypotension by cocoa tea and traditional tea was peripheral without fast endurablity and was not related to central or the vagus nerve system (Xu et al., 1990b).

4.3 Increasing contraction amplitude of heart muscle

Experimental results of the perfusion flow of isolated rabbit heart showed that the aqueous extract of cocoa tea could increase the contraction amplitude of the heart muscle by 7.9% ± 3% and increased the peak value by 12.0% ± 4%. Compared with the physiological saline, the difference was significant ($P < 0.05$). However, Longjing Cha did not have obvious effect on the contraction amplitude of heart muscle (Xu et al., 1990b).

4.4 Protective effect on anoxia mice under normal pressure

The experimental result of anoxia mice under normal pressure showed that both of the aqueous extract of cocoa tea and acetone extract of Longjing Cha could prominently protect the mice and prolong their survival time. The former prolonged the survival duration of mice by 46.7% in contrast to physiological saline, while the latter prolonged only by 17.9% ($P < 0.001$). Cocoa tea could evidently prolong the survival duration of mice, and the effect was 1.6 times more than that of Longjing Cha. In addition, it exhibited protective effects on heart muscle of anoxia mice under normal pressure in static state (Xu et al., 1990c).

4.5 Influence on time of sleep

The effect of the extract of cocoa tea and Longjing Cha on sleep time of mice tested with sodium pentobarbital showed that they had significant influence on mouse's time of falling asleep and time of sleep. Large, medium, and small doses of the aqueous extract of cocoa tea prolonged the time of falling asleep by −11.6%, 22.6%, and 275.2%, respectively, and reduced the time of sleep by 35.6%, 25.2%, and 13.1%, respectively, as against physiological saline. The acetone extract of Longjing Cha prolonged the time of falling asleep by 58.1% and reduced the time of sleep by 16.7% as against physiological saline. It showed that cocoa tea did not influence the time of falling asleep but enhanced sleep quality and reduced sleep time. Moreover, it proved that cocoa tea did not excite the nervous system, whereas Longjing Cha could excite the nervous system and prolong the time of falling asleep (Xu et al., 1990c).

4.6 Effect on motive endurance of mice

The burden experiment of healthy mice weighing from 18 to 20 g showed that the extract of cocoa tea and Longjing Cha could effectively strengthen their motive endurance using the stomach perfusion, and the effect was related to the amount of the extract. In contrast to physiological saline, higher and middle dose of cocoa tea prolonged survival duration of mice in burden swimming by 61.1% ($P < 0.001$) and 31.1% ($P < 0.01$), and the acetone extract of Longjing Cha prolonged survival duration of mice in burden swimming by 27.7% ($P < 0.01$), with a significant difference between the two ($P < 0.01$). The effect on the motive endurance of mice of smaller doses of cocoa tea was not evident ($P > 0.05$). The experimental result showed that higher concentrations of cocoa tea could strengthen the motive endurance of mice, and the effect was stronger than Longjing Cha (Xu et al., 1990c).

4.7 Effect of anti-lipid-peroxidation

The aqueous extract of cocoa tea and acetone extract of Longjing Cha had similar effect of anti-lipid-peroxidation on the hearts, livers, and kidneys of experimental mice. Compared with the effect of physiological saline, the difference was significant ($P < 0.01$) (Xu et al., 1990c).

4.8 Effect of decreasing cholesterol (CH) and haematolipid

The experiments showed that in contrast to physiological saline, the aqueous extract and acetone extract of cocoa tea and acetone extract of Longjing Cha could effectively decrease CH, triglyceride (TG), high-density lipoprotein (HDL)-CH and β-limit dextrin (β-LD) in serum in the experimental hyperlipoidemic mice, and reduce their muscle fat content. Thus, they also had a weight-reducing effect (Xu et al., 1990a).

4.9 Promotion of growth of human fetal lung fibrocyte and human fetal kidney cells

The aqueous extract could promote the growth of human fetal lung fibrocyte (HFLF) and human fetal kidney cells (HFK) (Liu et al., 1996).

4.10 Anticancer effect

4.10.1 Inhibitory effect on Ehrlich ascites cancer

Large and medium doses of cocoa tea extract had inhibitory effects on culture of Ehrlich ascites cancer (EAC) *in vitro* compared with physiological saline (Xu et al, 1990c). *In vivo* experiment showed that cocoa tea extract could obviously inhibit Ehrlich ascites solid cancer (Xie et al., 1992c).

4.10.2 Inhibitory effect on HeLa, CNE2, and MGC-803 cell lines

The extract of cocoa tea and Longjing Cha showed significant cytotoxic effects on HeLa cell line, poorly differentiated nasopharyngeal carcinoma cell line (CNE2), and gastric cancer cell line (MGC-803) *in vitro*. The IC_{50} of cocoa tea was 495.6, 243.4, and 268.6 μg/mL, respectively, and the IC_{50} of Longjing Cha was 421.1, 311.4, and 274.6 μg/mL, respectively (Xie et al., 1992). The unknotting activity of DNA topoisomerase II was inhibited completely by the extract of cocoa tea and Longjing Cha both at the concentration of 50 μg/mL. Therefore, DNA topoisomerase II may be a target enzyme of these extracts (Liu et al., 1996).

4.10.3 Inhibitory effect on human hepatic cancer cell line (BEL-7402)

The IC_{50} of cocoa tea extract on human hepatic cancer cell line (BEL-7402) was 351.1 μg/mL by the 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide (MTT) assay and 115.2 μg/mL by the trypan blue exclusion method (TBE) assay against BEL-7402 cells. The experiment proved that cocoa tea had evident cytotoxic action (Liu et al., 1996).

4.10.4 Inhibitory effect on human erythroleukemia cell line (K_{562})

Experimental results showed that its IC_{50} on human erythroleukemia cell line (K_{562}) was 1000 μg/mL by the MTT assay and 81.4 by the TBE assay (Liu et al., 1996).

4.10.5 Influence on DNA of human nasopharyngeal cancer cells (CNE2)

Human CNE2 was treated with cocoa tea extract at 125–500 μg/mL concentration to test its effect on DNA breakage. The degree of DNA breakage increased with the increase of the concentration of cocoa tea extract (Liu et al., 1996).

4.11 *In vivo* synergism of antitumor effect

The inhibitory rates (IR) of the mixture of cocoa tea extract with Ara-C, CTX, or ADM against L_2 in mice were higher than that of the drugs used separately. The average inhibitory rates of Ara-C mixed with the extract of cocoa tea was increased from 38.2% to 64%. The IR of CTX mixed with the extract of cocoa tea was increased from 31.3% to 48.9%, and the IR for ADM increased from 15.2% to 45.5%, all $q>1$. It suggests that the combination of the drugs could produce synergistic antitumor effect (Liu et al., 1996).

5 Stability of cocoa tea containing theobromine

5.1 Migrated transplant

In 1988, some plants of cocoa tea were migrated and planted in the campus of Sun Yat-sen University at 14 m alt compared with 700–900 m alt in their place of origin. The assay of biochemical components in those plants showed that most of the plants contained theobromine predominantly, and only a few plants contained caffeine mainly. After three years of planting, the assay result, which is consistent with that in 1995, showed that they retained such characteristics, i.e., they still contained theobromine in flourishing shoots predominantly. The content of theobromine varied from the highest 5.62% to the lowest 4.24% (Ye et al., 1996). From 1995–1997, the buds and young leaves from 23 trees were respectively tested for purine alkaloids by high performance liquid chromatography (HPLC) from March to September, one sample per month in three years. The results showed that all trees contained theobromine with little or no caffeine, and the average value in three years showed that the content of theobromine was 3.21% at dry weight in spring, highest to 5.59% in midsummer, and 4.42% in autumn (Hunag et al., 1999).

Most individuals of cocoa tea population in place of origin contained theobromine with little or no caffeine, and a few individuals contain caffeine predominantly. However, these two types look similar in form.

In 2005, five plants cultivated on the Sun Yat-sen University campus were tested for purine alkaloids, and the result showed that two of the five trees predominantly contained caffeine and the other three trees predominantly contained theobromine. This is consistent with the analysis results in 1995 (Table 1).

Table 1 Assay result of cocoa tea by HPLC — % of dry weight

sample time	biochemical composition	biochemical content						
		1#	4#	5#	7#	10#	17#	22#
1995–1997	theobromine		2.32±0.0309	2.30±0.0599	4.28±0.0585			
	caffeine		4.00±0.0117	4.62±0.00252	5.57±0.171			
2005	theobromine	4.24			2.3	4.28	0.372	4.32
	caffeine	nd			3.644	nd	2.86	nd

1#, 4#, 5#, …, 22#: serial number of cocoa tea trees. nd: not detected. HPLC: high performance liquid chromatography.

5.2 Cutting propagation

Based on the detection result of chemical composition in the individuals of cocoa tea population in place of origin, the trees that were ramose with middle-sized leaves and contained theobromine without caffeine were selected as cutting materials to propagate seedlings of cocoa tea. Buds and leaves from both the maternal trees and their seedlings of cutting propagation were collected for the test, and the result showed they were the same in chemical composition.

5.3 Grafting propagation

Cocoa tea was grafted to cultivated tea trees. Samples from grown plants were tested, showing that grafted cocoa tea plants contained the same amount of theobromine with the maternal plants of cocoa tea.

In 2002, the grafted plants of cocoa tea were detected; it showed that theobromine remained the main component of purine alkaloids, and the caffeine in stocks did not influence the composition of purine alkaloids in scions.

By 2007, through the grafting method, 40 acres of cocoa tea plantation have been set up. Four varieties of cocoa tea all contained theobromine as the main component of purine alkaloids after scions of cocoa tea were grafted to stocks of cultivated tea.

5.4 Metabolic pathway of purine alkaloids in leaves of cocoa tea

By the use of radioisotope trace element, the metabolic pathway of purine alkaloids in the leaves of cocoa tea was studied. The result showed that theobromine was synthesized from [8-^{14}C] adenine, and the rate of its biosynthesis in the segments from young and mature leaves from flushing shoots was approximately 10 times higher than those from aged leaves from 1-year old shoots. Neither cell-free extracts nor segments from cocoa tea leaves could convert theobromine to caffeine. A large number of [2-^{14}C] xanthine taken up by the leaf segments was degraded to $^{14}CO_2$ *via* the conventional purine catabolic pathway that included allantoin as an intermediate. However, small amounts of [2-^{14}C] xanthine were also converted to theobromine. A considerable amount of [8-^{14}C] caffeine exogenously supplied to the leaf segments of cocoa tea was changed to theobromine. These results indicated that the leaves of cocoa tea exhibited unusual purine alkaloid metabolism as they had the capacity to synthesize theobromine from adenine nucleotides, but they lacked adequate methyltransferase activity to convert theobromine to caffeine in detectable quantities, and the leaves had a capacity to convert xanthine to theobromine, probably *via* 3-methylxanthine. This showed that theobromine, as a special metabolite, relied on hereditary gene, and environmental change had not altered the chemical composition, i.e., containing theobromine. The following diagram shows the biosynthesis and metabolic pathways of purine alkaloids in leaves of cocoa tea (Hiroshi et al., 1998).

6 Breeding of cocoa tea

Although in the population of cocoa tea, most individuals contain predominantly theobromine, a few individuals do predominantly contain caffeine. Therefore, before sexless propagation, those individuals should be tested to confirm that they predominantly contain theobromine in their buds and young leaves. Then, their cuttings will be used for transplanting, from which the cocoa tea sexless line could be established. After the setting up of sexless lines, their performance traits are measured against cultivated tea. If the cocoa tea sexless line is better in production, quality, and resistance, it could be regarded as a new variety.

7 Seedling propagation

Sexual reproduction usually cannot be adopted in seedling propagation in that the descendents might be either the plants containing theobromine or the plants containing caffeine. Therefore, sexless propagation is the main method to breed cocoa tea seedlings. It includes cutting, grafting between two species, and tissue culture, etc. Tissue culture is processed on the Schenk and Hildebrandt (SH) cultivate base by young embryo or terminal buds or axillary buds cut from young seedlings (Zhu and Chen, 1990). In agriculture, cuttage and graft are the principal propagation methods.

7.1 Cutting

Adventitious roots of cuttings of cocoa tea were developed from calluses in the parenchyma cells of xylem ray and phloem (Zhu et al., 1992). As cocoa tea is a wild tea tree, its sexless propagation capacity is weaker, and it is difficult for its cuttings to develop roots. The varieties selected and bred were quite different in sexless capacity. Therefore, one of the important criteria was to choose those having excellent sexless propagation capacity. It could produce a better effect to use hormones 1-naphthlcetic acid (NAA) and indole butyric acid (IBA) at suitable ratio (Ye, 1991; Ye et al., 1992).

7.2 Graft

Based on the theory of alkaloids synthesized in leaves, grafting between species were processed several times in which the stocks were traditional cultivated tea trees and were grafted by cocoa tea so that the stocks contained caffeine and the scions contained theobromine. After grafting, grown plants contained theobromine and retained the features of cocoa tea. It is necessary to pay attention to

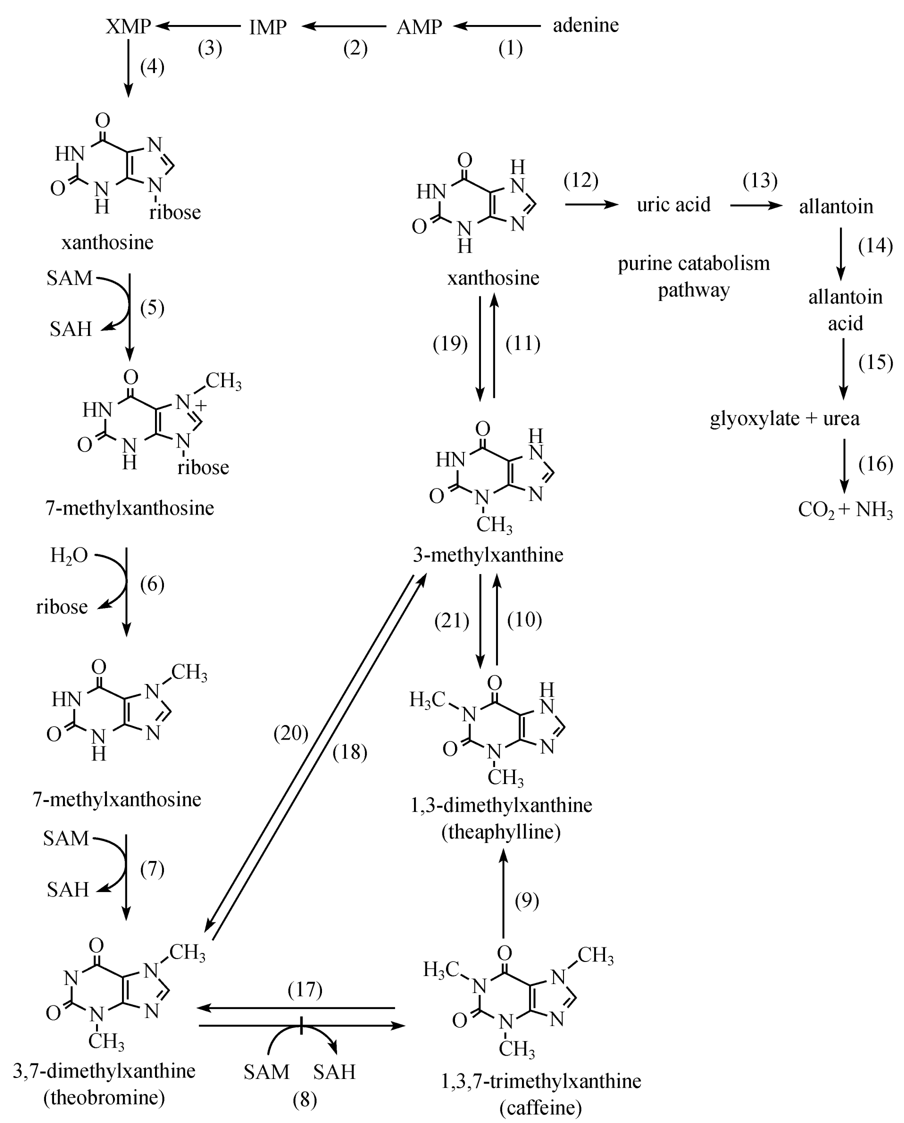

Fig. 2 Possible purine alkaloid metabolic pathways operating in the leaves of cocoa tea. Arrow with a vertical bar represents a blocked conversion. Enzymes: (1) Adenine phosphoribosyltransfersae; (2) amp deaminase; (3) IMP dehydrogenase; (4) 5’-nucleotidase; (5) SAM, xanthosine 7N-methyltransferase; (6) 7-methylxanthosine nucleosidase; (7) SAM, 7-methyxanthosine 3N-methyltransferase; (8) SAM, theobromine 1N-methyltransferase; (9) 7N-demethylase; (10) 1N-demethylase; (11) 3N-demethylase; (12) xanthine dehydrogenase; (13) uricase; (14) allantoinase; (15) allantoicase; (16) urease; (17) 1N-demethylase; (18) 7N-demethylase; (19) SAM: xanthine 3N-methyltransferase; (20) SAM, 3-methylxanthine 7N-methyltransferase; (21) SAM, 3-methylxanthine 1N-methyltransferase. Several enzymes shown above have not yet been demonstrated in higher plants. IMP: inosine 5’-monophosphate; SAH: S-adenosylhomocysteine; XMP: xanthosine 5’-monophosphate.

that tilled shoots that emerged from stocks should have been exploited since the tilled shoots of stocks would influence purine alkaloids of scions.

8 Processing of cocoa tea

With the progress in breeding and selection of cocoa tea, plantations have been set up to cultivate new varieties. In the light of traditional tea production methods, novel processing technology of cocoa tea has been invented to manufacture green cocoa tea, black cocoa tea, and oolong cocoa tea.

Studies showed that varieties of different characters are suitable for different processing methods. For instance, some varieties are more suitable to make oolong tea and green tea, whereas some are only suitable to make black tea.

The pleasant flavors of black cocoa tea and oolong cocoa tea are similar to the high-quality black tea and oolong tea sold in the market. However, cocoa tea is caffeine-free compared with traditional tea. It is more likely to be favored by some people who cannot drink beverages containing caffeine since it will not excite the nervous system. Cocoa tea will become a choice for some people in the tea market.

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