Distribution, Biosynthesis and Catabolism of Methylxanthines in Plants

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Abstract Methylxanthines and methyluric acids are purine alkaloids that are synthesized in quantity in a limited number of plant species, including tea, coffee and cacao. This review summarizes the pathways, enzymes and related genes of caffeine biosynthesis. The main biosynthetic pathway is a sequence consisting of xanthosine → 7-methylxanthosine → 7-methylxanthine → theobromine → caffeine. Catabolism of caffeine starts with its conversion to theophylline. Typically, this reaction is very slow in caffeine-accumulating plants. Finally, the ecological roles of caffeine and the production of decaffeinated coffee plants are discussed.

Keywords Biosynthesis · Caffeine · Catabolism · Coffee · N-Methyltransferase · Tea · Theobromine

1 Introduction

Methylxanthines and methyluric acids (Fig. 1) are secondary plant metabolites derived from purine nucleotides (Ashihara and Crozier 1999a). The most well known methylxanthines are caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine), which occur in tea, coffee, cacao and a number of other non-alcoholic beverages of plant origin. Caffeine was isolated from tea and coffee in the early 1820s, but the main biosynthetic and catabolic pathways of caffeine were not fully established until recently, when highly purified caffeine synthase was obtained from tea leaves and a gene encoding the enzyme was cloned (Kato et al. 1999; Kato et al. 2000). In this chapter, the distribution, biosynthesis and catabolism of methylxanthines in plants are described. Furthermore, the roles of methylxanthines in planta and production of decaffeinated coffee plants are summarized.

2 Distribution of Methylxanthines in Plants

Methylxanthines have been found in nearly 100 species in 13 orders of the plant kingdom (Ashihara and Suzuki 2004; Ashihara and Crozier 1999a). Compared with other plant alkaloids, such as nicotine, morphine and strychnine, purine alkaloids are distributed widely throughout the plant kingdom although accumulation of high concentrations is restricted to a limited number of species, including Coffea
arabica (coffee), Camellia sinensis (tea) and Theobroma cacao (cacao). All caffeine-containing plants, except Scilla maritima, belong to the Dicotyledoneae. In some species the main methylxanthine is theobromine or methyluric acids, including theacrine (1,3,7,9-tetramethyluric acid), rather than caffeine (Ashihara and Crozier 1999a).

2.1 Coffee and Related Coffea Plants

The caffeine content of seeds of different Coffea species varies from 0.4 to 2.4% dry weight (Mazzafera and Carvalho 1992). Green beans (as opposed to roasted beans, which are used to prepare the beverage) of current commercially cultivated coffee plants contain substantial quantities of caffeine; arabica coffee (Coffea arabica) beans usually contain 1.2–1.4% caffeine (Charrier and Berthaud 1975), while robusta coffee (Coffea canephora) contains 1.2–3.3% caffeine (Charrier and Berthaud 1975; Mazzafera and Carvalho 1992). There are also several wild coffee species where the green beans contain either no caffeine or extremely low levels of caffeine. Such low-caffeine species include Mascarocoffea sp. and Coffea eugenioides (Mazzafera and Carvalho 1992; Rakotomalala et al. 1992; Campa et al. 2005).

Caffeine is distributed mainly in the leaves and cotyledons of Coffea arabica seedlings, at concentrations ranging from 0.8 to 1.9% dry weight. Essentially, there
is no caffeine in roots or in the older brown parts of the shoot (Zheng and Ashihara 2004). Mature leaves of Coffea liberica, Coffea dewevrei and Coffea abeokutae contain the methyluric acids theacrine, liberine \( [(2),1,9\text{-trimethyluric acid}] \) and methylxyluberine \( [(2),1,7,9\text{-tetramethyluric acid}] \) (Fig. 1) (Baumann et al. 1976; Petermann and Baumann 1983). Examples of the purine alkaloid content in the seeds of Coffea species are illustrated in Fig. 2a.

### 2.2 Tea and Related Camellia Plants

The caffeine content of young leaves of first flush shoots of Camellia sinensis, Camellia assamica and Camellia taliensis is 2–3% of dry weight, while the level in Camellia kissi is less than 0.02%. Unusually, theobromine is the predominant purine alkaloid (5.0–6.8%) in young leaves of a Chinese tea, kekecha (cocoa tea) (Camellia ptilophylla) (Ye et al. 1997), and Camellia irrawadiensis (less than 0.8%) (Nagata and Sakai 1985). Theacrine and caffeine are the major purine alkaloids in the leaves of another Chinese tea called “kucha” (Camellia assamica var. kucha). The endogenous levels of theacrine and caffeine in expanding buds and young leaves of kucha are approximately 2.8 and 0.6–2.7%, respectively (Zheng et al. 2002). Some examples of the purine alkaloid content of the leaves of Camellia species are shown in Fig. 2b.

**Fig. 2** The methylxanthine and methyluric acid content of selected plant species. **a** Leaves of Camellia species, **b** seeds of Coffea species and **c** seeds of Theobroma cacao (cacao), Theobroma grandiflorum (cupu), Herrania sp. and Paullinia cupana (guarana), leaves of ilex paraguariensis (mate) and anthers of Citrus maxima (pomelo). Values were obtained from references cited in the text.
2.3 Cacao and Related Theobroma and Herrania Plants

Theobromine is the dominant purine alkaloid in seeds of cacao (*Theobroma cacao*). The cotyledons of mature beans contain 2.2–2.7% on a dry weight basis and 0.6–0.8% caffeine, while shells contain 0.6–0.7% theobromine and 0.5–0.6% caffeine (Senanayake and Wijesekera 1971). Examination of several cacao genotypes representing the three horticultural races Criollo, Forastero and Trinitario revealed considerable variations in the purine alkaloid content of the seed, with slightly higher levels found within the Criollo types (Hammerstone et al. 1994). Roasted seeds of *Theobroma cacao* are used to make cocoa and chocolate products (Duthie and Crozier 2003).

Cupu (*Theobroma grandiflorum*) contains 0.25% liberine in cotyledons and 0.08% in the nut shells (Baumann and Wanner 1980). Hammerstone et al. (1994) reported that theacrine is the principal purine alkaloid in seeds of 11 species of *Theobroma* and nine species of *Herrania*. Quantitative data on purine alkaloid levels in *Theobroma* and *Herrania* species are presented in Fig. 2c.

2.4 Maté, Guarana and Other Species

Maté (*Ilex paraguariensis*) leaves are used to make a beverage that is consumed widely in rural areas of Argentina, Paraguay and Brazil. Young maté leaves contain caffeine (0.8–0.9%), theobromine (0.08–0.16%) and theophylline (less than 0.02%). Methylxanthines have been detected in *Paulinia cupana* (guarana), *Paulinia yoco*, *Paulinia pachycarpa*, *Cola* species and *Citrus* species (Baumann et al. 1995; Kretschmar and Baumann 1999; Weckerle et al. 2003). In seeds of guarana, caffeine is located mainly in the cotyledons (4.3%) and testa (1.6%). Citrus flowers can accumulate up to 0.17% methylxanthines on a fresh weight basis; caffeine is the main methylxanthine, but theophylline is also present. Trace quantities of caffeine have also found in the nectar of citrus flowers (Weckerle et al. 2003). Quantitative data of selected samples are shown in Fig. 2c.

3 Methylxanthine Biosynthesis in Plants

Methylxanthines are formed from purine nucleotides in plants. Historically, there have been a number of proposals on the pathways involved in such conversions (see Ashihara and Crozier 1999a). However, data from studies on in situ metabolism of labelled precursors, as well as enzymes and genes have established that the main caffeine biosynthetic pathway is a four-step sequence consisting of three methylations and one nucleosidase reaction starting with xanthosine acting as the initial substrate (Fig. 3). Although the information has been obtained mainly from coffee
Fig. 3 The biosynthetic pathways of caffeine from xanthosine. The major pathway consists of four steps from I to IV. The enzymes involved are as follows: 7-methylxanthosine synthase (EC 2.1.1.158) (I and II); N-methylnucleosidase (EC 3.2.2.25) (II); theobromine synthase (EC 2.1.1.159) (III); caffeine synthase (EC 2.1.1.160) (III and IV). Minor pathways, shown with dotted arrows, may occur because of the broad substrate specificities of the N-methyltransferases. SAM S-adenosyl-l-methionine, SAH S-adenosyl-l-homocysteine.
(Coffea arabica) and tea (Camellia sinensis), the available evidence suggests that the pathway is essentially the same in other methylxanthine-forming plants (Ashihara et al. 1998; Zheng et al. 2002; Koyama et al. 2003).

3.1 Formation of 7-Methylxanthine

The formation of monomethylxanthine in the main caffeine biosynthetic pathway is initiated by the conversion of xanthosine to 7-methylxanthosine (Fig. 3). This reaction is catalysed by 7-methylxanthosine synthase (xanthosine 7N-methyltransferase, EC 2.1.1.158). The genes encoding 7-methylxanthosine synthase, CmXRS1 (AB034699) and CaXMT (AB048793), were isolated from Coffea arabica (Mizuno et al. 2003a; Uefuji et al. 2003). The second step involves a nucleosidase which catalyses the hydrolysis of 7-methylxanthosine. It was thought that N-methylnucleosidase (EC 3.2.2.25), which occurs in tea leaves, participates in this reaction (Negishi et al. 1988), but structural studies on coffee 7-methylxanthosine synthase suggested that the methyl transfer and nucleoside cleavage may be coupled and catalysed by a single enzyme (McCarthy and McCarthy 2007).

3.2 Formation of Theobromine

The third step in the caffeine biosynthesis pathways is also catalysed by S-adenosyl-L-methionine (SAM)-dependent N-methyltransferase(s). Highly purified caffeine synthase (EC 2.1.1.160) obtained from young tea leaves has broad substrate specificity and catalyses the two-step conversion of 7-methylxanthine to caffeine via theobromine (Kato et al. 1999). This enzyme is distinct from the N-methyltransferase that catalyses the first methylation step in the caffeine pathway. The isolated complementary DNA from young tea leaves, termed TCS1 (AB031280), consists of 1,438 base pairs and encodes a protein of 369 amino acids (Kato et al. 2000). The function of TCS2 (AB031281), which occurs as a paralogous gene to TCS1 in the tea genome, has not yet been determined (Yoneyama et al. 2006). Plural genes encoding N-methyltransferases which have different substrate specificities have been isolated from coffee plants. CCS1 (AB086414), CcCS7 (AB086415) and CaDXMT1 (AB084125) are caffeine synthase genes (Mizuno et al. 2003a; Uefuji et al. 2003). The recombinant caffeine synthases (EC 2.1.1.160) can utilize paraxanthine, theobromine and 7-methylxanthine as substrates. CTS1 (AB034700), CTS2 (AB054841), CaMXMT1 (AB048794) and CaMXMT2 (AB084126) were identified as genes encoding theobromine synthase (Mizuno et al. 2001; Ogawa et al. 2001). The activity of the recombinant theobromine synthase (EC 2.1.1.159) is specific for the conversion of 7-methylxanthine to theobromine.
Theobromine synthase, but not the dual-functional caffeine synthase, appears to participate principally in theobromine synthesis in theobromine-accumulating plants, such as *Theobroma cacao*, *Camellia ptilophylla* and *Camellia irrawadiensis* (Yoneyama et al. 2006).

### 3.3 Conversion of Theobromine to Caffeine

Conversion of theobromine to caffeine is performed by the dual-functional caffeine synthase discussed already. The methylation of N1 of 7-methylxanthine by caffeine synthase is much slower than that of N3, and as a consequence, theobromine is temporally accumulated in caffeine-synthesizing tissues. This is the final step in the main caffeine biosynthesis pathway, i.e., xanthosine → 7-methylxanthosine → 7-methylxanthine → theobromine → caffeine.

To date, three caffeine synthase genes have been identified in coffee plants (Mizuno et al. 2003b; Uefuji et al. 2003). Expression profiles of these genes in different organs are variable and the kinetic properties of each recombinant enzyme, such as $k_m$ values, are different. Therefore, the enzymes participating in caffeine biosynthesis in organs and at different stages of growth may vary.

In addition to the main caffeine biosynthesis pathway, various minor routes may also operate (Fig. 3) which are mainly dependent upon the broad specificities of the N-methyltransferases, especially caffeine synthase. For example, caffeine synthase catalyses the synthesis of 3-methylxanthine from xanthine. Paraxanthine is synthesized from 7-methylxanthine. However, little accumulation of these compounds occurs in plant tissues. 3-Methylxanthine may be catabolized to xanthine, and paraxanthine appears to be immediately converted to caffeine. Paraxanthine is the most active substrate of caffeine synthase, but only limited amounts of paraxanthine accumulate in plant tissues, because the N1-methylation of 7-methylxanthine is very slow (Ashihara et al. 2008).

### 3.4 Formation of Methyluric Acids

Formation of methyluric acids occurs in a limited number of plant species. As noted in Sect. 2.2, theacrine is found in kucha leaves in high concentrations (Zheng et al. 2002). Radiolabelled feeding experiments, indicate that theacrine is synthesized from caffeine. Conversion of caffeine to theacrine probably occurs by successive oxidation and methylation steps with 1,3,7-trimethyluric acid acting as the intermediate. Leaves of *Coffea dewevrei*, *Coffea liberica* and *Coffea abeokutae* convert caffeine to liberine probably via theacrine and methyl liberine (Petermann and Baumann 1983).
3.5 Supply of Xanthosine for Caffeine Biosynthesis

Xanthosine, the initial substrate of purine alkaloid synthesis, is supplied by at least four different pathways: de novo purine biosynthesis (de novo route), degradation of adenine nucleotides (AMP route), the SAM cycle (SAM route) and guanine nucleotides (GMP route) (Fig. 4).

3.5.1 De Novo Route

Like mammals, plants synthesize purine nucleotides by de novo and salvage pathways (Ashihara and Crozier 1999a; Moffatt and Ashihara 2002; Stasolla et al. 2003), although some sections of the pathways are unique to plants. Utilization of IMP, formed by the de novo purine biosynthetic pathway, for caffeine biosynthesis was demonstrated in young tea leaves using $^{15}$N-glycine and $^{14}$C-labelled precursors and inhibitors of de novo purine biosynthesis (Ito and Ashihara 1999). Xanthosine is produced by an IMP $\rightarrow$ XMP $\rightarrow$ xanthosine pathway. IMP dehydrogenase (EC 1.1.1.205) and 5'-nucleotidase (EC 3.1.3.5) catalyse these reactions. Ribavirin, an inhibitor of IMP dehydrogenase, reduces the rate of caffeine biosynthesis in tea and coffee plants (Keya et al. 2003).

![Diagram](image-url)
3.5.2 AMP Route

A portion of the xanthosine used for caffeine biosynthesis is derived from the adenine and guanine nucleotide pools which are produced by the de novo and salvage pathways. There are several potential pathways for xanthosine synthesis from AMP, although the AMP → IMP → XMP → xanthosine route is likely to predominate. All three enzymes involved in the conversion have been detected in tea leaves (Koshiishi et al. 2001).

3.5.3 SAM Route

The SAM route is a variation of the AMP route. SAM is the methyl donor for various methylation reactions in the caffeine biosynthetic pathway. In the process, SAM is converted to S-adenosyl-L-homocysteine (SAH), which is then hydrolysed to homocysteine and adenosine. Homocysteine is recycled via the SAM cycle to replenish SAM levels, and adenosine released from the cycle is converted to AMP and utilized for caffeine biosynthesis by the AMP route. Since 3 moles of SAH are produced via the SAM cycle for each mole of caffeine that is synthesized, in theory this pathway has the capacity to be the sole source of both the purine skeleton and the methyl groups required for caffeine biosynthesis in young tea leaves (Koshiishi et al. 2001).

3.5.4 GMP Route

Xanthosine utilized for caffeine biosynthesis is also produced from guanine nucleotides by a GMP → guanosine → xanthosine pathway. 5'-Nucleotidase (EC 3.1.3.5) and guanosine deaminase (EC 3.5.4.15) participate in this conversion (Negish et al. 1994).

4 N-Methyltransferases Involved in Methylxanthine Biosynthesis

4.1 Gene Expression in Coffee and Tea Plants

Expression of genes involved in caffeine biosynthesis has been demonstrated in young leaves, flower buds and developing endosperm of Coffea arabica (Mizuno et al. 2003a, b). The expression of CmXRS1, CTS2 and CCS1, which encode 7-methylxanthosine synthase, theobromine synthase and caffeine synthase, respectively, was examined. Transcripts of CmXRS1 and CCS1 were observed in all organs, but the
highest level was found in developing endosperm. Significant expression of CTS2 was found only in flower buds. The patterns of expression of CmXRS1 and CCS1 were synchronized. During development of Coffea arabica fruits, the transcripts of CmXRS1 and CCS1 are present in every stage of growth except in fully ripened tissues. The pattern of expression of these genes during growth is roughly related to the in situ synthesis of caffeine from adenine nucleotides, although exceptions were found in the very early and the later stages of fruit growth. Since the level of CTS2 transcripts encoding theobromine synthase is very low in fruits, the alternative CCS1 gene encoding the dual-functional caffeine synthase may be operative for the last two steps of caffeine biosynthesis. In developing Coffea arabica fruits, the levels of transcripts of CmXRS1 and CCS1 are higher in seeds than in pericarp. Native caffeine synthase (3N-methyltransferase) activity is distributed in both organs in a similar manner. Therefore, caffeine accumulating in ripened coffee seeds appears to be synthesized within the developing seeds and is not transported from pericarp (Koshiro et al. 2006).

In Camellia sinensis, expression of TCS1 encoding caffeine synthase is higher in young leaves than in mature leaves, stems or roots (Li et al. 2008). This is consistent with the fact that biosynthetic activity of caffeine occurs mainly in young leaves (Ashihara and Kubota 1986). Recent studies using Camellia sinensis tissue culture indicate that the expression of TCS1, and possibly the unidentified gene encoding 7-methylxanthosine synthase, represents the principal control mechanism for caffeine biosynthesis. Although increased caffeine content was observed when cultures were grown in media containing paraxanthine, addition of adenosine, guanosine or hypoxanthine did not have a similar impact. Thus, neither the supply of non-methylated purine precursors nor the availability of SAM appears to be an important factor in the regulation of caffeine biosynthesis (Li et al. 2008; Deng et al. 2008).

4.2 Evolutionary Relationship of Caffeine Synthase and Related Enzymes

Figure 5 shows the amino acid sequences of caffeine synthase and related enzymes. There are four highly conserved regions: motif A, motif B’, motif C and the YFFF region in the amino acid sequence of the caffeine synthase family (Kato and Mizuno 2004). Three conserved motifs, A, B and C, of the binding site of the methyl donor of SAM have been reported in the majority of plant SAM-dependent O-methyltransferases (Joshi and Chiang 1998). The motif B’ and YFFF region contains many hydrophobic amino acids which are specific to the motif B’ methyltransferase family. Most members of this newly characterized motif B’ methyltransferase family catalyse the formation of small and volatile methyl esters by using SAM as a methyl donor and substrates with a carboxyl group as the methyl acceptor. Members of this family include salicylic acid carboxyl methyltransferase (SAMT) (Ross et al. 1999),
benzoic acid carboxyl methyltransferase (BAMT) (Dudareva et al. 2000), jasmonic acid carboxyl methyltransferase (JAMT) (Seo et al. 2001), farnesoic acid carboxyl methyltransferase (FAMT) (Yang et al. 2006), indole-3-acetic acid methyltransferase (IAMT) (Zhao et al. 2008), gibberellic acid methyltransferase (GAMT) (Varbanova et al. 2007) and loganic acid carboxyl methyltransferase (LAMT) (Murata et al. 2008). The motif B\textsuperscript{0} methyltransferase family is also referred to as the SABATH family, based on the initial letters of the names of the substrates (D’Auria et al. 2003). Crystallographic data on SAMT from Clarkia breweri suggest that members of this family exist as dimers in solution (Zubieta et al. 2003). Further structural analysis of 7-methylxanthosine synthase and caffeine synthase from Coffea canephora also revealed a dimeric structure (McCarthy and McCarthy 2007). Amino acid sequences of the caffeine synthase family derived from coffee are more than 80% homologous but share only 40% homology with caffeine synthase.
from tea. There is a similar homology between SAMT from *Clarkia breweri* and caffeine synthase from tea and coffee plants. That is to say, the amino acid sequences share a high degree of sequence identity within the same genus.

Figure 6 shows the phylogenetic tree analysis of the motif B’ methyltransferase family. This implies that the caffeine biosynthetic pathways in coffee, tea and cacao might have evolved in parallel with one another, consistent with different catalytic properties of the enzymes involved. Recently, Ishida et al. (2009) reported the occurrence of theobromine synthase genes in purine alkaloid-free species of *Camellia*. This represents additional evidence that monophyletic genes occur in *Camellia* plants.

5 Catabolism of Methylxanthines in Plants

5.1 Conversion of Caffeine to Theophylline

Limited amounts of caffeine are very slowly degraded with the removal of the three methyl groups, resulting in the formation of xanthine in almost all caffeine-forming plant species (Fig. 7). Catabolism of caffeine has been studied using $^{14}$C-labelled
Fig. 7 Catabolic pathways of caffeine and theobromine. Caffeine is catabolized mainly to xanthine via theophylline and 3-methylxanthine. Theobromine is catabolized to xanthine via 3-methylxanthine. Xanthine is further degraded to CO₂ and NH₃ by the conventional oxidative purine catabolic pathway. *Dotted arrows* indicate minor routes.
caffeine (Ashihara et al. 1997; Ashihara et al. 1996; Mazzafera 2004; Suzuki and Waller 1984). Caffeine catabolism usually begins with its conversion to theophylline catalysed by N7-demethylase. This conversion is the rate-limiting step in purine alkaloid catabolism and provides a ready explanation for the high concentration of endogenous caffeine in species such as *Coffea arabica* and *Camellia sinensis*. The involvement of the P450-dependent monooxygenase activity for this reaction has been proposed (Huber and Baumann 1998; Mazzafera 2004), although the activity of this enzyme has not yet been demonstrated. In leaves of *Coffea eugenioides*, which contain low levels of caffeine, [8-14C]caffeine is catabolized rapidly primarily by the main caffeine catabolic pathway via theophylline. This suggests that the low caffeine accumulation in *Coffea eugenioides* is a consequence of rapid degradation of caffeine, perhaps accompanied by a slow rate of caffeine biosynthesis (Ashihara and Crozier 1999b).

### 5.2 Metabolism of Theophylline

In caffeine-producing plants such as tea, coffee and maté, [8-14C]theophylline is catabolized rapidly (Ito et al. 1997). The main route of theophylline degradation in higher plants involves a theophylline → 3-methylxanthine → xanthine → uric acid → allantoin → allantoic acid → CO₂ + NH₃ pathway (Fig. 7). In contrast, theophylline is catabolized at extremely low levels in non-methylxanthine-forming plants. Higher plants do not convert [8-14C]theophylline to either 1-methyluric acid or 1,3-dimethyluric acid, which are the main catabolites of theophylline in mammals (Scheline 1991). In tea and maté, large amounts of [8-14C]theophylline are also converted to theobromine and caffeine via a theophylline → 3-methylxanthine → theobromine → caffeine salvage pathway (Ito et al. 1997).

### 5.3 Catabolism of Theobromine

In contrast to theophylline, theobromine is a precursor, as opposed to a catabolite, of caffeine. However, degradation of theobromine has been observed in mature leaves (Koyama et al. 2003) and pericarp of the theobromine-accumulating plant *Theobroma cacao* (Zheng et al. 2004). Theobromine was degraded to CO₂ via 3-methylxanthine, xanthine and allantoic acid (Fig. 7). Although conversion of caffeine to theobromine was detected in *Theobroma cacao*, caffeine was catabolized principally to CO₂ via theophylline, which is the same degradation pathway that operates in *Coffea arabica* and *Camellia sinensis*.
6 Ecological Roles of Purine Alkaloids

The physiological role of purine alkaloids in planta is largely unknown. It appears not to act as a nitrogen reserve since considerable amounts remain in leaves after abscission. There are two hypotheses concerning the ecological roles of caffeine in plants.

6.1 Chemical Defence Theory

The chemical defence theory proposes that the high concentrations of caffeine in young leaves, fruits and flower buds of species such as tea and coffee act as a defence to protect young soft tissues from pathogens and herbivores. It has been shown that spraying tomato leaves with caffeine deters feeding by tobacco horn-worms, while treatment of cabbage leaves and orchids with caffeine acts as a neurotoxin and kills or repels slugs and snails (Hollingsworth et al. 2003). This work has now been extended and convincing evidence for the chemical defence theory has recently been obtained with transgenic caffeine-producing tobacco plants (Kim et al. 2006; Uefuji et al. 2005).

6.2 Allelopathy Theory

The allelopathic or autotoxic function theory proposes that caffeine in seed coats and falling leaves is released into the soil to inhibit germination of seeds around the parent plants (Anaya et al. 2006). In caffeine-synthesizing cells, caffeine accumulates in vacuoles, so caffeine does not impact on cellular metabolism. Exogenously applied caffeine does, however, inhibit various aspects of metabolism in the cells. Although there is experimental evidence from laboratory studies to support this proposal, it is unclear to what extent caffeine is involved in allelopathy in natural ecosystems, especially as soil bacteria such as *Pseudomonas putida* can degrade methylxanthines (Hohnloser et al. 1980; Gluck and Lingens 1988).

7 Production of Decaffeinated Coffee

Demand for decaffeinated coffee has increased gradually since the early 1970s. Worldwide sales of “decaf” have achieved a 12% share of the market, estimated to be worth more than US $4 billion (Heilmann 2001). Modern methods of decaffeination, such as supercritical fluid extraction with carbon dioxide, may have minimal effect on the organoleptic quality of the beverage if carried out
correctly (Vitzthum 2005). Nevertheless, coffee cultivars combining high cup quality with a low caffeine content may provide a superior, less expensive and ecofriendly alternative to meet the demand for decaffeinated coffee.

7.1 Production by Breeding

Silvarolla et al. (2004) discovered naturally decaffeinated mutant plants in the progeny of Coffea arabica accessions from Ethiopia. Three of these Ethiopian mutant plants were almost completely free of caffeine. The seeds of those plants had low caffeine content (mean caffeine content 0.076% dry weight), but significant amounts of theobromine (about 0.61%), another methylxanthine which is capable of causing physiological effects similar to those of caffeine (Eteng et al. 1997). It would, therefore, appear to be worth searching for mutant plants with a low theobromine and caffeine content.

Recently, Nagai et al. (2008) produced a new low-caffeine hybrid coffee which is a tetraploid interspecific hybrid developed in Madagascar from Coffea eugenioides, Coffea canephora and Coffea arabica. Green beans of selected hybrids contain 0.37% caffeine and no detectable theobromine. Low caffeine accumulation is due mainly to the low biosynthetic activity of purine alkaloids, possibly the extremely weak N-methyltransferase reactions in caffeine biosynthesis.

7.2 Production by Genetic Engineering

Attempts to use genetic engineering to produce transgenic caffeine-deficient coffee have to date had only limited success. Low-caffeine-containing transgenic Coffea canephora plants have been produced but the caffeine content of the leaves was variable, depending on the line; the most notable example yielded a reduction of up to 70% (Ogita et al. 2003; Ogita et al. 2004). Coffee produced from beans of Coffea arabica has a flavour superior to that of robusta coffee but as yet caffeine-deficient transgenic arabica beans have not been produced. When this is achieved, because of the substantial market for decaffeinated coffee, it is likely to have major commercial implications.

8 Summary and Perspectives

The major route to caffeine in higher plants is a xanthosine → 7-methylxanthosine → 7-methylxanthine → theobromine → caffeine pathway. The precursors of caffeine are derived from purine nucleotides. The rate of caffeine biosynthesis appears to be regulated primarily by the induction and repression of N-methyltransferases,
especially 7-methylxanthosine synthase. The first paper on the cloning of caffeine synthase from tea appeared in 2000. Since then there has been a veritable explosion of research that has led to the successful cloning of a number of $N$-methyltransferase-encoding genes from coffee. The rate-limiting step in the caffeine biosynthetic pathway, the initial conversion of xanthosine to 7-methylxanthosine, is catalysed by 7-methylxanthosine synthase, and the encoding gene for this $N$-methyltransferase has been isolated from coffee. Although funding from industry has been very limited to non-existent, much of the extensive interest in this research has been fuelled by the possibilities of using genetic engineering to obtain transgenic, low-caffeine-containing coffee and tea that could be used to produce “natural” decaffeinated beverages. Although transgenic *Coffea canephora* seedlings with a 70% reduced caffeine content have been obtained, there is as yet no information on the caffeine content of beans produced by these plants. The real breakthrough in commercial terms will come with the production of transgenic caffeine-deficient *Coffea arabica* beans.

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