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Review

Metabolic engineering of caffeine production

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Abstract Among 12,000 alkaloids which are produced in plants, caffeine (1,3,7-trimethylxanthine) is one of the best known due to it uses as an ingredient of pharmaceuticals and beverages. In coffee plants, it is synthesized from xanthosine through three successive methylation and ribose removal steps. We have isolated all genes encoding the corresponding *N*-methyltransferases; xanthosine methyltransferase (XMT), 7-methylxanthine methyltransferase (MXMT) and 3,7-dimethylxanthine methyltransferase (DXMT), as well as for the 7-methylxanthosine nucleosidase. Using these genes, we have engineered caffeine production in two ways. The first is to decrease the caffeine content in coffee plants to cope with occasional health problems caused by caffeine uptake, and the other is to produce caffeine as an insect repellant in crop plants, originally not synthesizing caffeine. The first approach was performed using an RNAi for *MXMT*, yielding a 70% suppression of the caffeine level in leaves of transgenic coffee plants. The other approach was carried out by simultaneous introduction of three genes, *XMT*, *MXMT* and *DXMT*, into tobacco plants, which produced up to 5 μg caffeine per g fresh weight of leaves. This amount of caffeine was enough to repel tobacco cutworms (*Spodoptera litura*), suggesting the method to be practically efficient for construction of herbivore tolerant crops. The significance of the present study is discussed with reference to four topics: practical metabolic engineering; development of a genetic transformation system for tropical trees; generation of genetically modified (GM) plants with a minimal load on the environment; and providing GM foods that bring direct merits to consumers.

Key words: Alkaloid, caffeine, chemical defense, *Coffea*, *Nicotiana*, pest control, public acceptance of GM plants, RNAi, *Spodoptera*.

Plants produce more than 50,000 secondary metabolites, among which over 12,000 are alkaloids (Croteau et al. 2000). The physiological role of alkaloids is not completely understood, but a recent survey points to ecochemical functions, for which two distinct features have been proposed (Ashihara and Crozier 1999). One is chemical defense against pathogens and herbivores, and the other allellopathic effects against competing plant species. In both cases, the effect is due to their toxic nature for many organisms, and a typical and well known example is caffeine, a purine alkaloid.

Caffeine is produced by a variety of plants, including coffee, tea, kola nuts, guarana berries, Yerba mate and cacao beans (Figure 1). While caffeine is toxic for the majority of living organisms, mankind is relatively insensitive, perhaps because of high degrading activity, and has utilized these plants as sources of dietary stimulation. One of caffeine's pharmacological properties is to stimulate the central nervous system, often causing high respiration, high heart rate and diuretic effects, which are not normally serious. However, overconsumption can sometimes cause health problems, including insomnia, palpitation and increase in blood pressure.

Caffeine was shown to be effective as a repellant and pesticide for slugs and snails and also for insects. For example, spraying of tomato leaves with over 1% caffeine solution drastically deters feeding by tobacco hornworms (Nathanson 1984). Caffeine also reduces the reproductive potential of several species of moths (Mathavan et al. 1985) and a recent study showed that slugs fed significantly less on 'Napa' cabbage leaves sprayed with only 0.01% caffeine solution, and topical treatment with over 0.1% caffeine solution was lethal to the snails (Hollingsworth et al. 2002). These results are consistent with the idea that the physiological function of caffeine is to constitute a chemical defense system against pathogen attack and herbivores (Ashihara and Crozier 1999).

Caffeine is a derivative of xanthosine, an intermediate

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Abbreviations: DXMT, 3,7-dimethylxanthine methyltransferase; MXMT, 7-methylxanthine methyltransferase; XMT, xanthosine methyltransferase.

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of purine catabolic pathways, and its biosynthetic pathway in coffee plants is predicted to involve successive use of purine precursors such as AMP and GMP through multiple steps catalyzed by several enzymes (Figure 2). The first step is methylation of xanthosine by xanthosine methyltransferase (XMT), yielding 7-methylxanthosine (Figure 2, step 1), the ribose moiety of which is then removed by 7-methylxanthosine nucleosidase (step 2). The resulting 7-methylxanthine is methylated by 7-methylxanthine methyltransferase (MXMT or theobromine synthase) to produce 3,7-dimethylxanthine (theobromine) (step 3), which is further methylated by 3,7-dimethylxanthine methyltransferase (DXMT or caffeine synthase) to give caffeine itself (1,3,7-trimethylxanthine) (step 4). We have isolated corresponding cDNAs for all these enzymes, designated as *CaXMT1*, *CaMXMT1*, *CaMXMT2* and *CaDXMT1*, respectively, and showed that caffeine can indeed be synthesized *in vitro* by the combination of their gene products (Ogawa et al. 2001; Uefuji et al. 2003). Similar genes to *CaMXMT1* and *CaDXMT1* have also been independently isolated from coffee plants and designated as *CTS1/2* and *CCS1*, respectively (Mizuno et al. 2003).

Figure 1. Coffee plantation in Brazil. (A) Harvesting of coffee beans. (B) Ripening beans of *Coffea canephora*. (C) Collecting dried beans (Photograph kindly provided by Dr. Luis Carlos S. Ramos, Agronomic Institute, Brazil).

It is therefore now possible to engineer caffeine biosynthesis not only in coffee plants but also in many agriculturally important plant species. Two approaches are conceivable: one is construction of transgenic coffee plants, in which caffeine production is suppressed; and the other is production of caffeine in normally non-caffeine producing plants. The former was practically performed by introducing an RNAi construct for *MXMT1* into *Coffea canephora* and *C. arabica*, thereby creating decaffeinated coffee beans (Ogita et al. 2003, 2004). The latter was realized using a multi-gene transfer system, involving cDNAs for all three methyltransferases. Resulting transgenic tobacco plants indeed produced caffeine in leaves, and exhibited resistance to herbivorous insects (Uefuji et al. 2005). In this article, we briefly summarize the current status of caffeine metabolic engineering mainly based on our results.

Suppression of caffeine biosynthesis in coffee plants

A cup of coffee contains 150 mg of caffeine on average, which can cause health problems for many people. Consequently, decaffeinated coffee has high popularity,

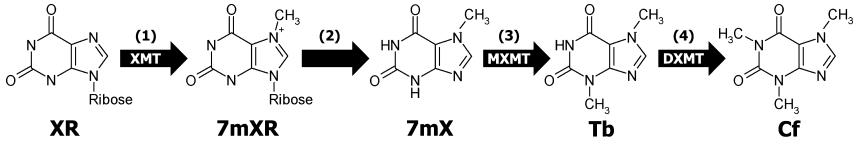

Figure 2. Caffeine biosynthetic pathway in coffee plants. The first (1), third (3), and fourth (4) steps feature methyl group transfer, and the second (2) step involves ribose (Rib) removal. XR, xanthosine; 7mXR, 7-methylxanthosine; 7mX, 7-methylxanthine; Tb, theobromine; Cf, caffeine; XMT, xanthosine methyltransferase; MXMT, 7-methylxanthine methyltransferase; DXMT, 3,7-dimethylxanthine methyltransferase.

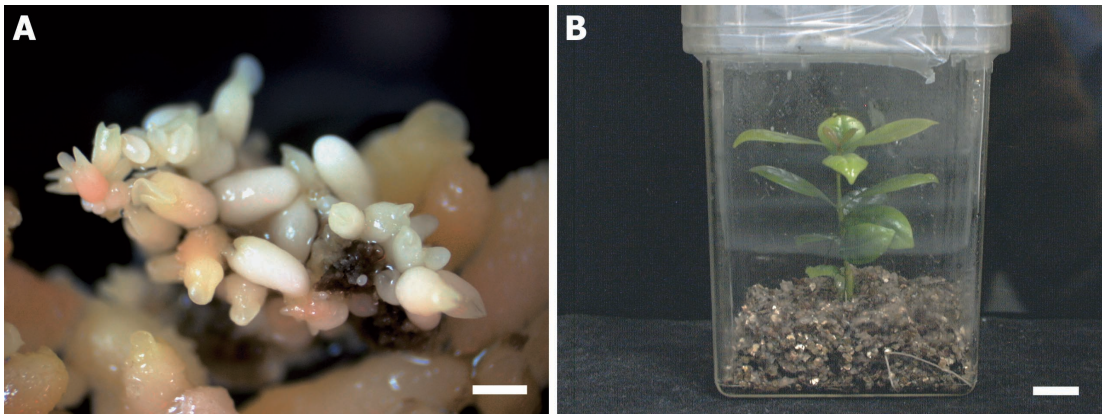

Figure 3. Somatic embryogenesis for transformation of coffee species. (A) Secondary somatic embryos directly proliferating from the initially formed embryos. (B) Regenerated small plantlets in soil. Scales in (A) and (B) indicate 1mm and 1cm, respectively.

accounting for about 10% of the world coffee market. It is mainly manufactured by supercritical extraction, but ingredients relating to fragrance and taste are also simultaneously removed, resulting in 'tasteless' coffee. A molecular approach for genetic modification of caffeine biosynthesis in coffee plants can overcome this demerit, and might be of help to produce a cup of aromatic coffee, in which only the caffeine content is reduced.

Somatic embryogenesis of coffee plants

Breeding of coffee species has been performed by seeding or grafting after selecting superior varieties. However, this takes several decades due to the life cycle of this species (Spiral et al. 1999). In order to shorten the breeding period, which is a prerequisite for molecular engineering, tissue culture methods and somatic embryogenesis technology have been developed (Berthouly and Etienne 1999). Indirect somatic embryogenesis, i.e. embryogenic callus induction that allows large amounts of somatic embryo formation is generally recommended for micropropagation of interesting plants. However, intricacy of plant regeneration from somatic embryos derived from embryogenic calli still remains and sometimes prevents efficient genetic transformation. To overcome the difficulties, we have developed a methodology for direct formation of somatic embryos.

Yellow-whitish somatic embryos were thus directly induced from cut edges of young leaves of both *Coffea* arabica and *C. canephora* on modified 1/2MS medium (m1/2MS) containing 20 μM of 2-isopentenyladenine (2ip). Secondary somatic embryos directly proliferated (Figure 3A) in the same m1/2MS medium for approximately 3-week-periods. In order to induce further development, somatic embryos were transferred to m1/2MS media, hormone-free or containing 5 μM of N^6-benzyladenine (BA). They then vigorously germinated and developed into small plantlets on the modified 1/2MS medium. These plantlets could further be maintained healthy in soil (Figure 3B).

Construction of decaffeinated coffee plants

Several attempts at transformation of coffee plants, including introduction of a reporter GUS gene (Hatanaka et al, 1999), or an insect-resistant gene (Leroy et al, 2000), have been reported. Based on the successes obtained, the potential for molecular breeding for genetically modified (GM) decaffeinated coffee production was stressed although relevant genes were not available at that time (Ashihara and Crozier 2001). Subsequently, we have identified genes encoding key enzymes of caffeine biosynthesis in coffee plants (Ogawa et al. 2001, Uefuji et al, 2003), making it possible to suppress caffeine biosynthesis by the double stranded RNA interference (RNAi) method (Ogita et al. 2003; 2004).

The 3′-untranslated region (UTR) and the coding region of *CaMXMT1* cDNA were selected to design the RNAi constructs. Two particular RNAi constructs, RNAi-S and RNAi-L (Figure 4A), were inserted into the pBIH1-IG vector (Ohta et al. 1990), which was introduced into the EHA101 strain of *Agrobacterium*

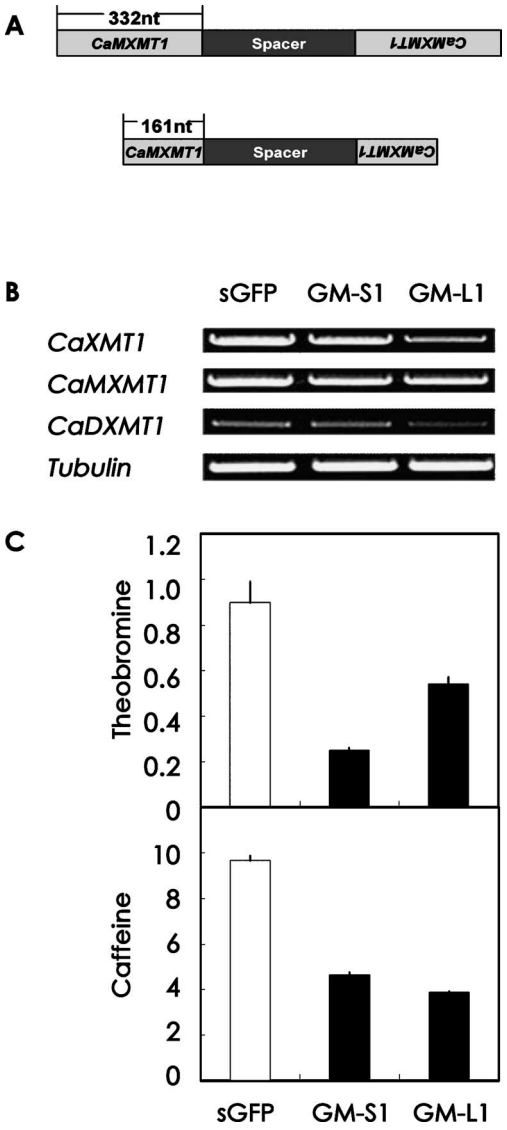

Figure 4. Genetic transformation of coffee plants. (A) Design of RNAi constructs. An RNAi-S (short) was constructed using 139-bp (positions 1139–1277 of the *CaMXMT*1 cDNA) and 161-bp (positions 1117–1277) nucleotides. As the spacer, a 517-bp DNA fragment derived from the GUS gene at positions 3436–3952 was inserted between the two. The RNAi-L (long) was constructed with two identical sequences of 332-bp (positions 946–1277) separated by the same spacer. (B) Transcript levels of methyltransferase genes in young leaves of regenerated plantlets of *C. canephora*. Total RNA was isolated and analyzed for transcript levels of *CaXMT1* and *CaMXMT1*, and *CaDXMT1* by 32 and 35 cycles of RT-PCR, respectively. As an internal control, transcripts for α-tubulin were simultaneously measured by 32-cycle PCR. (C) Purine alkaloid contents in trangenic plantlets. Endogenous theobromine and caffeine levels (mg g^{-1} fresh weight) were estimated in young leaves of transgenic sGFP (control), and indicated transgenic RNAi plantlets of *C. canephora*.

tumefaciens to transform *C. arabica* and *C. canephora* on m1/2MS media containing 100 mg l^{-1} hygromycin. Resulting transformed lines were assayed for expression of methyltransferase genes by RT-PCR, and it was found that transcripts for not only *CaMXMT1* but also *CaXMT1* and *CaDXMT1* were obviously suppressed with the *CaMXMT1*-RNAi construct (Figure 4B). These results are consistent with a finding that RNAi effects spread from the initiator region into the adjacent regions of the target gene, as well as other genes whose sequences are closely related (Vaistiji et al. 2002). The similarity among *CaMXMT1*, *CaXMT1* and *CaDXMT1* is over 90% in the coding region (Uefuji et al., 2003), suggesting that the primary small double-stranded *CaMXMT1*-RNA progressively produces many secondary small double-stranded RNAs spanning its coding region to the adjacent sequence of the initiator region, which in turn destroys mRNAs for *CaXMT1* and *CaDXMT1*. The reduced level of transcripts suggested decreased activities of the corresponding enzymes, and this was confirmed by directly measuring their products, theobromine and caffeine, by HPLC. The caffeine content in the controls was approximately 8.4 mg per g fresh leaf tissue, while that in both RNAi-S and RNAi-L was 4.0 mg, showing an average 50% reduction (Figure 4C). However, the amount was variable depending on the line, with a notable example showing up to 70% reduction (Ogita et al. 2003). At maturity, these plants may produce essentially normal coffee beans except for low caffeine content. In order to practically cultivate the decaffeinated plants, some technical improvements are desirable. For example, the RNAi should preferably be expressed only in beans so that other parts of plant bodies retain wild-type caffeine production. Targeting of multiple genes is also practical to achieve 100% decaffeinated beans. In this context, construction of a limited expression system might be useful, employing, for example, the promoter of the 11S seed storage protein gene of *C. arabica* (Marraccini et al. 1999). Additional RNAi constructs covering common regions among the three methyltransferase genes may also be practical.

Finally it should be mentioned that the initial RNAi plants are now four-years old, and flowered for the first time in 2005 (Figure 5). They were manually pollinated and will concievably make beans within a period of months. If their caffeine content is indeed lower than in wild type controls, then the beans might be practically used after appropriate safety evaluation for public acceptance.

Production of caffeine in tobacco plants

The physiological function of caffeine has been proposed to constitute a part of chemical defense systems against pathogens and herbivores (Ashihara and Crozier 1999). Indeed, exogenously applied caffeine has been found to markedly increased the resistance of food plants of several pests, affecting their growth and survival (Nathanson 1984). These observations led us to construct caffeine-producing transgenic plants, which originally did not synthesize the compound (Uefuji et al. 2005).

Construction of transgenic tobacco

In order to accomplish this project, we first established a system for multiple transfer of the three coffee *N*-methyltransferase genes, *CaXMT1* (Uefuji et al. 2003), *CaMXMT1/2* (Ogawa et al. 2001) and *CaDXMT1* (Uefuji et al. 2003). Practically, each gene was initially independently introduced into pBI221, and then individual expression cassettes containing the CaMV *35S* promoter, cDNA clone and *NOS* terminator were removed and successively inserted into the multiple cloning site of pBluescript II SK (−). The three connected cassettes were finally replaced with the GUS coding sequence and *NOS* terminator of pIG121Hm

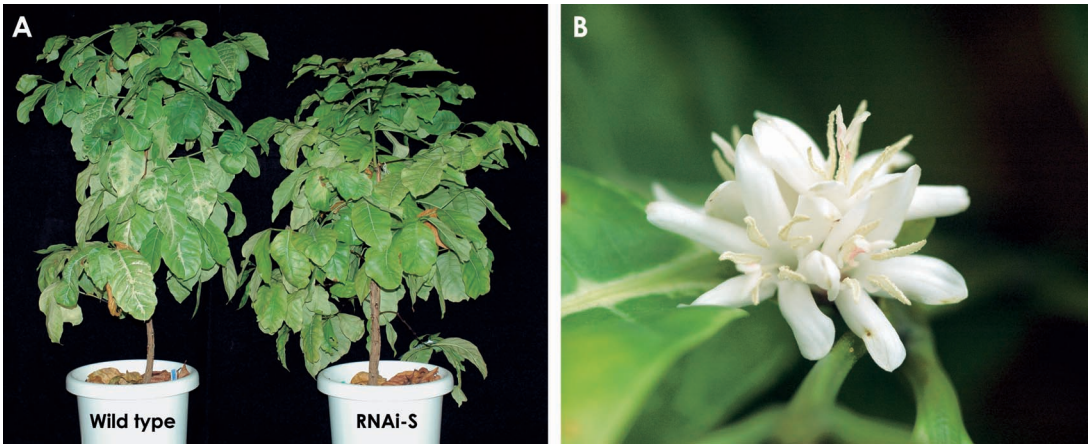

Figure 5. GM decaffeinated coffee plants growing in a greenhouse. (A) 4-year old transgenic coffee trees. Samples are RNAi (right side) and wild type (left side). (B) Flowering of RNAi transgenic coffee plants.

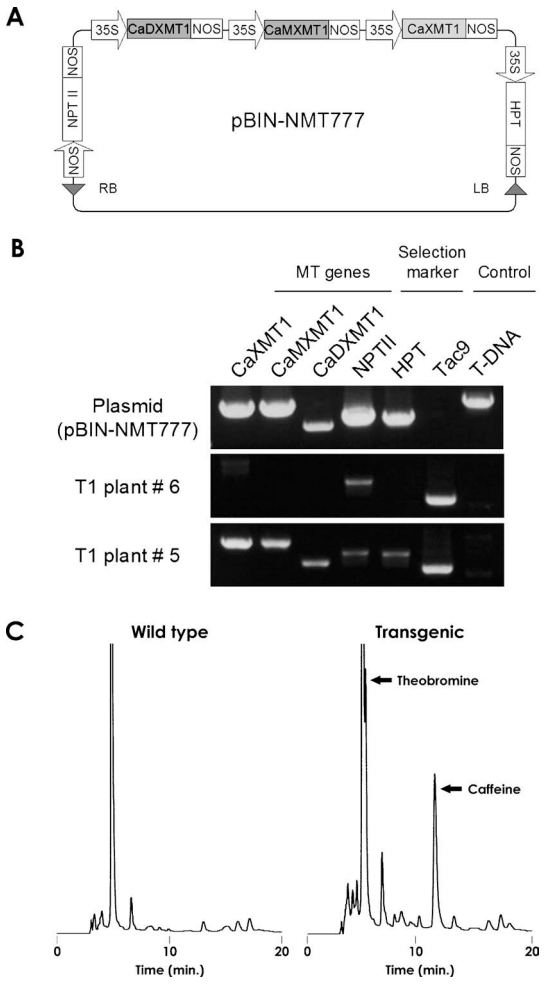

Figure 6. Construction of transgenic tobacco plants producing caffeine. (A) Schematic illustration of the vector used for tobacco transformation. LB, left border; RB, right border; p, promoter; t, terminator; *NOS*, nopaline synthase; *35S*, CaMV 35S RNA; *NPT II*, neomycin phosphotransferase; *HPT*, hygromycin phosphotransferase. Gene products of *CaXMT1*, *CaMXMT1* and *CaDXMT1* catalyze the first, third, and fourth steps of the caffeine biosynthetic pathway, respectively. (B) RT-PCR analysis of transgene expression by RT-PCR. Tobacco actin (*Tac9*) and *NOS* promoter (*NOS p*) sequences were tested as an endogenous gene and for the untranscriptional T-DNA region, respectively. Transgenic line #5 expressed all introduced genes, while #6 was inactive in their expression. The vector pBIN-NMT777 was used for the control. (C) HPLC analysis. Purine alkaloids were purified from mature leaves from wild type control (left) and transgenic line #5 (right). Arrows indicate peak positions of caffeine and theobromine. The major peak in both samples is an impurity.

(Hiei et al. 1994) and designated as pBIN-NMT777 (Figure 6A). Tobacco (*Nicotiana tabacum* cv Xanthi) leaf discs were transformed with pBIN-NMT777 by the *Agrobacterium*-transformation method. After appropriate culture and selection, twenty-three kanamycin-resistant transgenic plantlets were obtained, among which fifteen were confirmed by RT-PCR to express all three *N*-methyltransferase genes (Figure 6B). The selected lines were grown to maturity, and accumulation of purine alkaloids in leaves was examined by HPLC. Initial

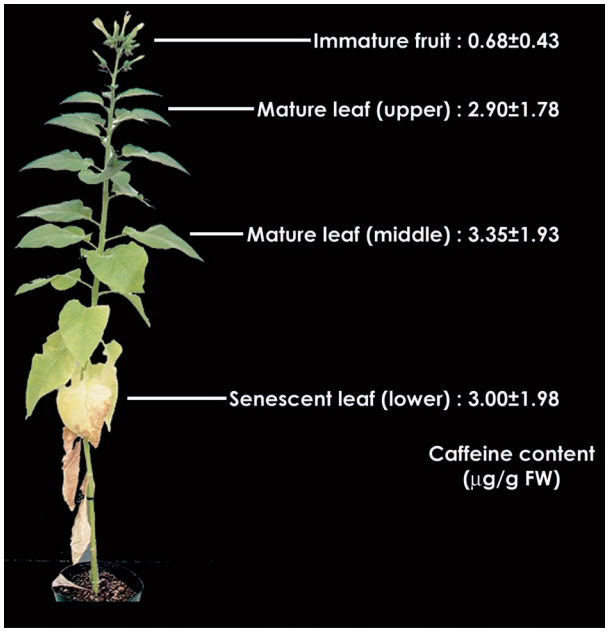

Figure 7. Caffeine contents of mature tobacco plants. Transgenic line #5 was grown to maturity, and caffeine was extracted from indicated organs and quantified by HPLC. Caffeine content is expressed in μg/g fresh weight (FW) with standard deviation.

analysis using mature leaf samples showed the transgenic plants to efficiently accumulate caffeine and theobromine, in contrast to control plants containing an empty vector (Figure 6C). Subsequently caffeine content was determined in individual leaves at different developmental stages (Figure 7). Plants in the vegetative growth phase contained limited amounts of caffeine, and in immature leaves the alkaloid was often undetectable. In mature leaves, however, the average caffeine content was 0.2 μg per g fresh weight and when plants aged and entered the reproductive stage to form flower buds, caffeine content increased to over 5 μg per g fresh weight. Immature fruits contained caffeine at a rather low level, up to 1.3 μg per g fresh weight. No caffeine was detected in any parts of control plants. The results thus indicated that caffeine was indeed synthesized in transgenic tobacco leaves, and that its content was higher in the older leaves of plants in the reproductive growth phase.

Repelling effects on tobacco cutworms

Since tobacco cutworms (*Spodoptera litura*) damage a wide range of crops, including tobacco plants, we tested feeding behavior of caterpillars using transgenic lines which produced different levels of caffeine. Third-instar larvae (Figure 8A) were starved for several hours and then allowed to select and feed on leaf discs prepared from transgenic or control plants. Results showed that insects selectively fed on the control materials, or positively avoided the transgenic material (Figure 8B). A quantitative estimation indicated that eaten areas were up

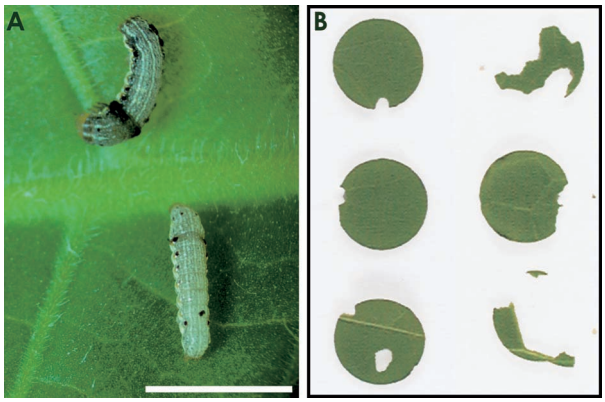

Figure 8. Effects on tobacco cutworms. (A) Tobacco cutworm (*S. litura*) larvae at the third instar. The bar indicates 5 mm. (B) Leaf disc choice test. Larvae were allowed to feed on six leaf discs, three from caffeine producing, and three from control plants. After feeding for 3 h in the dark, each disc was collected and photographed. The tested transgenic plant contained caffeine at average 5 μg/g fresh weight. Disc samples are from caffeine-containing (left) and control leaves (right).

to 1.1 cm^2 for the control, while the values was less than 0.02 cm^2 for the transgenic discs containing caffeine at 5 μg per g fresh weight. Transgenic discs accumulating as little as 0.4 μg caffeine per g fresh weight were also effective in repelling caterpillars.

These observations indicate caffeine to have a clear repelling effect on pest insects. However, the amount produced in transgenic plants (up to 5 μg/g fresh weight) was not high enough to confer a lethal effect, as caterpillars apparently grew normally with these materials up to the pupating stage. In order to obtain an idea how much caffeine was required to kill caterpillars of this moth, we preliminarily examined the dose/lethal relationship, and found that over 10 mg of caffeine per g fresh weight of artificial food was necessary. This indicates that, although caterpillars positively avoid leaves containing low amount of caffeine, they are able to feed on such materials if there is no choice, and therefore that caffeine might not be a powerful pest controller in agriculture. However, it was later found that, even when caterpillars fed apparently normally on caffeine containing diets, their reproductive potential has greatly reduced (Mathavan et al. 1985). Experimentally, mature moths were shown to lay fewer eggs with lower protein content in comparison with the controls fed on untreated diets. It was consequently concluded that producing caffeine is indeed a practical approach for overall pest suppression, acting as a repellant even at a low concentration, and as a toxicant at higher concentrations.

In order to practically use caffeine in *planta*, however, its production should hopefully be increased up to 2- to 3-fold the order in the present tobacco plants. Several approaches are conceivable: increasing the pool of the starting material, xanthosine; application of other genes encoding enzymes with efficient catalytic properties; and manipulation of caffeine transportation and assimilation in vacuoles. Despite problems that should be clarified, our present results suggest the possibility of producing relatively large amounts of caffeine in useful crops, thereby simultaneously conferring herbivore resistance and also supplying pharmaceutical materials.

Perspectives

The significance of the current work is as follows: (1) it provides a practical example of metabolic engineering of alkaloids; (2) transgenic methodology has been established suitable for tropical trees; (3) generation of GM plants with a low burden to the environment is concievable; and (4) this is an example of a GM plant, which gives direct merits to consumers.

Metabolic engineering

The biosynthetic pathway of 12,000 alkaloids is not thoroughly understood yet, but it is conceivable that approximately the same number of enzymes, and encoding genes, are involved. A complicating feature is that, to synthesize a given compound, multiple steps are required, in which sometimes over ten different enzymes successively catalyze the step-wise reactions. For example, scopolamine is synthesized through more than 10 steps, and berberine through 13 steps (Sato et al. 2001). In contrast, caffeine biosynthesis takes only four steps when the direct precursor, xanthosine, is available, encompassing three methylation and one ribose removal reactions. This relative simplicity made it possible to isolate all genes encoding the corresponding enzymes, and to manipulate the pathway positively or negatively, thus achieving metabolic engineering.

Metabolic engineering has been suggested to be a promising technology for production of pharmaceuticals (Croteau et al. 2000). To date, more than 30 genes for alkaloid metabolic pathways have been cloned (Hashimoto and Yamada 2003), and some are actually used for overproduction. One example is scopolamine, which is produced in *Atropa belladonna* (Yun et al. 1992). Manipulation of caffeine synthesis is another practical application of the technology, by which decaffeinated coffee plants and caffeine over-producing plants could be constructed. Particularly, the success of the latter case indicates that plants are able to produce compounds which are not originally part of their make-up, and therefore offers a general strategy to produce a desired metabolite in a given plant.

Transgenic methodology for tropical trees

The unique feature of woody plant species is long-span life cycle and large biomass. Many morphological and physiological events occurring during their growth phase are consequently much more complicated in comparison

with those of herbaceous plants. These specific traits of trees make it difficult to carry out genetic engineering for molecular breeding. In the case of herbaceous plant species, new tissue culture techniques, which were intended for genetic transformation, have been developed in, for example, a model plant *A. thaliana* of which all the genomic information has been decoded. The developed methods can readily be applied to other plant species. In contrast, there are no versatile techniques for tissue culture and transformation for woody plants. Furthermore, knowledge and techniques for economically important tropical trees are limited. In order to overcome this problem, a well organized scheme with high-standard techniques must be developed such as, for example, high frequency tissue culture systems, efficient vector systems to suppress or express the target gene(s), and techniques for selection and modification of transformation.

The present study with coffee plants offers pointers to how most problems described above can be overcome. First, we established a direct somatic embryogenesis method. The advantage of this method is that, since somatic embryos are directly induced from somatic tissues, the frequency of abnormities and somaclonal variations caused by callus formation could be suppressed to a minimum. The method also has advantages over published methods (Hatanaka et al. 1991; van Boxtel and Berthouly 1996) in terms of efficiency and reproducibility. Second, we employed the RNAi technique, which was found to be highly efficient to suppress expression of target gene(s) in comparison with the anti-sense method. Third, using the *Agrobacterium* EHA101 strain, we established an efficient transformation procedure for somatic embryos of two varieties, *C. arabica* and *C. canephora*. Overall, our procedure proved generally effective to create homogeneous healthy regenerated GM coffee plants. We believe that the methodology developed here will become a high-standard technique to transform not only coffee plants but also many other tropical tree species.

Reduction of environmental load

Up to 37% of world agricultural production is reported to be lost by herbivore damage, among which 13% is due to insects (Jouanin et al. 1998). Pest control is therefore one of the most important and urgent measures for a stable supply of foodstuffs. In addition to the development of a variety of chemical pesticides, transgenic technology has proven to be efficient in this context. The most used and perhaps the only practice is utilization of genes encoding toxins, which kill larvae of many pest insects. The so-called Bt toxins, and corresponding genes are widely transformed into maize, soybean, potato, cotton and oilseed rape (Shah et al. 1995). Although these crops are excellent in terms of pest tolerance, some problems have currently become evident. For example, disturbance of ecosystem is repeatedly suggested, such as evolution of resistant pests (Gould et al. 1997), and *Bt* gene introgression into wild plants, thereby possibly killing non-pest insects which feed on such plants (Wolfenbarger and Phifer 2000). There are also some difficulties in acquiring consumers' agreement in utilization of 'toxic' proteins. In comparison with those Bt crops, which give a certain load to environment, caffeine transgenic crops might be less risky. First, they do not kill pests. Second, they might save much use of pesticides in field, since caffeine repels not only insects but also snails and slugs, the latter causing serious damage to fruits and horticultural plants. Third, consumers might be more tolerant of caffeine than of Bt toxins, facilitating public acceptance of GM plants. Overall, they may exemplify the second generation of herbivore resistant transgenic crops, which save labor and agricultural costs, and also might mitigate the environmental load of pesticides in future.

Public acceptance of GM plants

The first generation of GM crops including maize, tomato and soybean with insect or herbicide resistant traits was commercially developed in the early 1990s. Since then the number and quantity of GM crops, or those directly referred to as GM foods, have increased year after year. Indeed, nearly 60% of soybeans produced in the world today are reported to be transgenic. After long debates pro and con GM plants, recent arguments have focused on potential benefits of new GM technology, which may confer certain merits to society. However, we will accept the benefits only when we understand that the technology and resulting plants are safe, functional and effective in our life. In this context, "the way of thinking" that underlies distrust of GM plants, still forms an invisible barrier for general acceptance among consumers, manufacturers, suppliers, administrators, and even among researchers. In order to get over this barrier, it is practical to raise the common knowledge of GM plants (foods) beyond social and cultural differences. Regarding public acceptance, the most important is to clearly point out what is the benefit of an individual GM plant.

In the case of GM decaffeinated coffee (Ogita et al. 2003), the worldwide mass media promptly responded with requests for further information, perhaps because coffee is one of the most popular and testable beverages, and because nearly 20% of coffee-lovers in the world are advised not to consume coffee containing caffeine due to health problems. Diverse views were aired, such as appreciation from researchers, negative comments from manufacturers of (decaf) coffee, and many doubts or demands from general consumers. All the contributions, however, directly or indirectly referred to the "merit" of

GM decaf, converging on the health aspects. "Since this GM coffee directly brings out merits on health, careful and separate arguments from other GMOs are necessary", the BBC reported. It can be learned from the present case that, for public acceptance of GM plants, presentation of direct merits for consumers is extremely important, in addition to other general merits such as agricultural and environmental benefits.

GM decaffeinated coffee plants are now growing in a greenhouse (Figure 5A) and bloomed in 2005 (Figure 5B). When aromatic and highly economical GM decaf is developed and accepted by consumers, it will play an important role in formulating new directions for GM technology.

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