Differences in Caffeine, Flavanols and Amino Acids Contents in Leaves of Cultivated Species and Hybrids in the Genus *Camellia*

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Introduction

According to Sealy,[9] the genus *Camellia* includes as many as 82 species which are classified into 12 subgeneric sections. He also noted that it was not easy to set out the interrelationships of the species. *Camellia sinensis* (tea) contains characteristic compounds, such as caffeine, catechins and theanine. The use of tea for beverage is ascribable in large part to these compounds, which are closely related to tea quality and efficiency. Studies on the contents of these compounds in related plants and hybrids of tea are important not only from the angle of tea breeding with interspecific hybrids, but also as means of clarifying the metabolic specificity of tea. In this point of view, this study has been carried out to clarify the differences in the contents of caffeine, theobromine, catechins and amino acids in leaves of camellia plants and hybrids of tea.

Methods of experiments

Most of the plant materials were derived from rooted cutting or seedling plants, because theanine is biosynthesized in roots and translocated to leaves. Young leaves of flush shoots were sampled and steamed, then dried and ground. Caffeine, theobromine, catechins, amino acids in the dried leaf powder were extracted, then assayed by gas chromatography and high performance liquid chromatography. Some compounds in several plants were identified by mass spectra.

Specific differences of camellia plants

The results of the analysis are summarized in Table 1.[4,5,6]

The caffeine content of *C. sinensis* and *C. taliensis* of section *Thea* was identical, whereas *C. irrawadiensis* of the same section contained a trace amount of caffeine. In the plants of the other 5 sections studied, no caffeine was detected except in one clone of *C. kissi*, which has been used for a local beverage in Southeast Asia.

An approximate correlation of the flavanol patterns with subgeneric sections was made. All catechins surveyed were detected in the 3 species of section *Thea*. In all sections except section *Thea*, the galloyled catechins, such as (−)-epicatechin gallate and (−)-epigallocatechin gallate were not detected. In view of the pathway of catechin biosynthesis in plants of the genus *Camellia*, it was suggested that only the member of section *Thea* had some specific enzyme for the formation of galloyled catechins from simple catechins.

In the case of sections *Paracamellia*, *Camellia* and *Heterogenea*, (−)-epicatechin or (+)-catechin or both were detected in all leaves. (−)-epicatechin content of section *Camellia* was high, comparatively. However, the contents of (−)-epicatechin and other catechins were low or negligible in plants of section *Camelliopsis* and *Theopsis*. Furthermore, (−)-epigallocatechin was detected in

Table 1. Caffeine, theobromine, flavanol and amino acids contents in leaves of plants from the genus *Camellia*

Origin of sample	CAF	THB	THE	Flavanol					SQN
				EC	(+)-C	EGC	ECG	EGCG	
Section *Thea*									
C. sinensis var. *sinensis*	⧻	+	⧻	⧻	+	⧻	⧻	⧻	N.D.
C. sinensis var. *assamica*	⧻		⧻	⧻	+	⧺	⧻	⧻	N.D.
C. taliensis	⧻	+	+	⧺	+	⧺	⧺	⧻	N.D.
C. irrawadiensis	±	⧺	+	⧺	+	+	⧺	+	N.D.
Section *Camellia*									
C. japonica	−	−	−	⧻	+	−	−	−	N.D.
C. japonica var. *decumbens*	−		−	⧻	⧻	−	−	−	N.D.
C. japonica subsp. *hozanensis*	−		−	+	⧻	−	−	−	D.
C. saluenensis	−		−	⧺	+	−	−	−	D.
C. pitardii	−		−	⧻	+	⧺	−	−	N.D.
Section *Heterogenea*									
C. furfuracea	−		+	+	⧺	+	−	−	N.D.
C. granthamiana	−		−	+	⧺∼⧻	−	−	−	N.D.
Section *Paracamellia*									
C. sasanqua	−	−	−	+	−	−	−	−	D.
C. oleifera	−		−	−	+	−	−	−	N.D.
C. kissi	±		−	+	−	−	−	−	N.D.
Section *Theopsis* (5 species)	−		−	±	−	−	−	−	N.D.
Section *Camelliopsis* (3 species)	−		−	−	−	−	−	−	N.D.
Hybrids									
C. sinensis × *C. japonica* (3 clones)	+∼⧺		+	⧺∼⧻	+	⧺∼⧻	⧺	⧺∼⧻	N.D.
C. japonica var. *decumbens* × *C. sinensis*	⧺		+	⧻	⧻	⧻	⧻	⧻	N.D.
C. sasanqua × *C. sinensis* (4 clones)	+∼⧻	+	+	+∼⧻	+	+∼⧻	+∼⧺	+∼⧻	D.
Dubiae									
C. wabisuke	−		−	+∼⧻	+∼⧺	−	±	−	N.D.
C. vernalis	−	−	−	+∼⧺	+	−	−	−	D.
C. hiemalis	−		−	+	+	−	−	−	D.
C. tenuiflora	−		−	−	+	−	−	−	N.D.

1) CAF: caffeine, THB: theobromine, THE: theanine, EC: (−)-epicatechin, (+)-C: (+)-catechin, EGC: (−)-epigallocatechin, ECG: (−)-epicatechin gallate, EGCG: (−)-epigallocatechin gallate, SQN: sasanquin.

2) −: <0.01%, +: 0.01∼0.3%, ⧺: 0.3∼1.0%, ⧻: >1.0%
N.D.: not detected, D.: detected.

2 species in sections other than *Thea*.

In most species studied, (−)-epicatechin content was higher than that of (+)-catechin, but in 4 species *C. furfuracea* and *C. granthamiana* of section *Heterogenea*, and *C. oleifera* of section *Paracamellia* and *C. japonica* subsp. *hozanensis* of section *Camellia*, (+)-catechin was predominant flavanol.

In the view of the flavanol pattern as mentioned above, it is evident that there are considerable intersubgeneric variations in flavanol contents. However, flavanol characteristics alone were not always sufficient to identify a subgeneric section.

As shown in Fig. 1, the gas chromatogram of several plants showed 4 marked peaks. It was consequently confirmed by means of mass spectrometry that these peaks were derived from sasanquin (6-O-(β-D-xyropyranosyl)-β-D-glucopyranosyl eugenol).[7]

Theanine which was the most abundant and characteristic amino acids in *C. sinensis* was detected in all leaves of plants of section *Thea*. Though the theanine content in *C. sinensis*

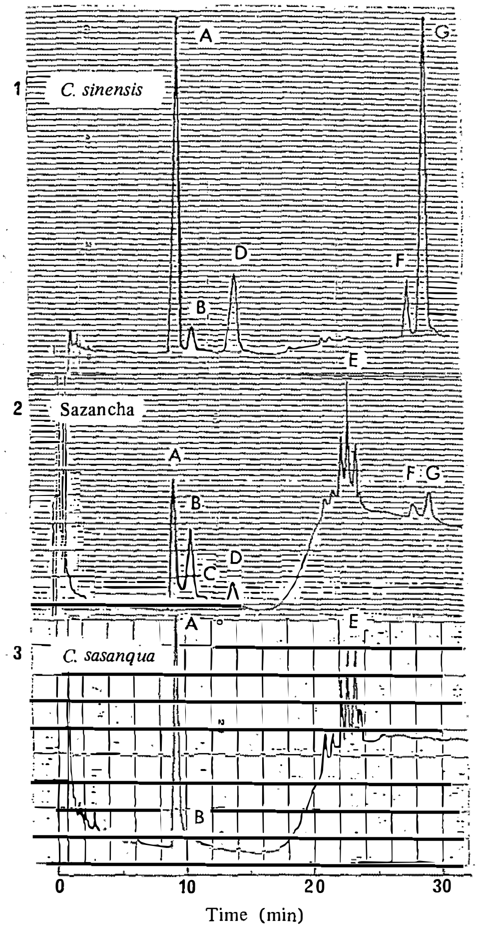

Fig. 1. Chromatograms of flavanols
1: 30 mg *C. sinensis*
2: 90 mg 'Sazancha'
3: 250 mg *C. sasanqua*
Peaks A: internal standard
B: (−)-epicatechin
C: (+)-catechin
D: (−)-epigallocatechin
E: sasanquin
F: (−)-epicatechin gallate
G: (−)-epigallocatechin gallate

was above 1%, that of *C. taliensis* and *C. irrawadiensis* ranged from 0.2 to 0.3%. Theanine was not detected by high performance liquid chromatography in the other 5 sections, except for *C. furfuracea* of section *Heterogenea* which contained 0.02% theanine. However, in several grafted plants of sections other than *Thea,* trace amounts of theanine were detected with the high sensitive method using amino acid analyzer.[3)]

Recently, Tsushida and Takeo[10)] reported that theanine in *C. japonica* and *C. sasanqua* was identified exclusively in the period of seed germination and subsequent growth. They also noted that theanine was not detectable in the derived shoots and roots of the actively growing 1-year old cutting of *C. japonica* and *C. sasanqua* nor also in the leaves of naturally grown plants.

On the other hand, the contents of main amino acids other than theanine such as arginine, glutamine, aspartic acid, glutamic acid and serine varied among the leaves examined. There was no clear correlation between the contents of these 5 amino acids and the taxonomical classification of the species or the subgeneric sections.

As mentioned above, a remarkable difference was revealed between the leaf components of section *Thea* and those of the other 5 sections of the genus *Camellia.* Chemically, *C. taliensis* was closely related to *C. sinensis.*

Patterns of tea hybrids

As shown in Figs. 1 and 2, the chromatogram of 'Sazancha' (*C. sasanqua*×*C. sinensis*) gave an intermediate pattern between that of *C. sinensis* and that of *C. sasanqua.* In other words, 'Sazancha' contained both galloyled catechins and 2 purine bases which are characteristic of section *Thea* as well as sasanquin characteristic of *C. sasanqua.*

Similarly, all the clones of hybrids between *C. sinensis* and other species such as *C. sasanqua* and *C. japonica* contained caffeine, theobromine, galloyled catechins and theanine. Wood and Barua[11)] reported similar results and demonstrated that in the paper chromatograms of F_1 hybrids between *C. irrawadiensis* and *C. sinensis* var. *assamica* tea compounds undetected in the former species could be detected.

Thus it becomes clear that the ability to biosynthesize these compounds was hereditary to hybrids of tea. Since these compounds were not detected in *C. sasanqua* and *C. japonica,*

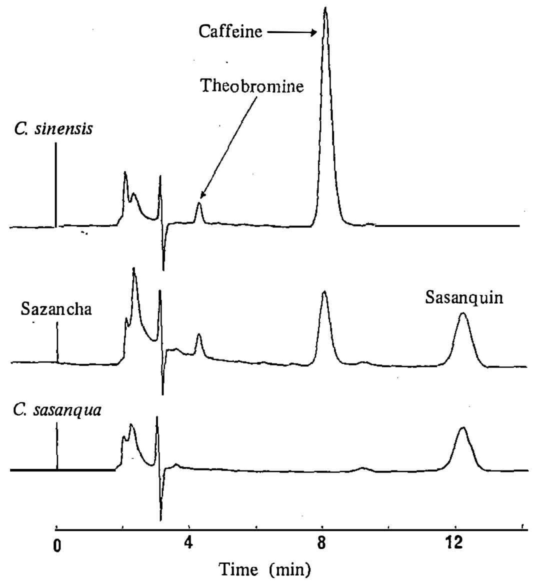

Fig. 2. HPLC analysis of the purine bases Absorbance (272 nm) of *C. sinensis*, 'Sazancha' and *C. sasanqua*

they were considered to be suitable chemical markers for hybrids between a species of section *Thea* and a species of the other sections.

In this study, the wide variation in the contents of these compounds might be explained to some extent by inherent characters. Thus, these findings suggest the possibility of interspecific hybridization.

Pattern of *Dubiae*

Dubiae for which Sealy[9] was unable to devise a proper classification includes *C. wabisuke*, *C. vernalis* and *C. hiemalis*. On the basis of the morphological characteristics and karyotype studies, it has so far been reported that *C. wabisuke* was related to both *C. japonica* and *C. sinensis*. However, the flavanol pattern of *C. wabisuke* resembled that of species of section *Camellia* studied. Caffeine, theanine and (−)-epigallocatechin gallate were not detected in all clones of *C. wabisuke*.

Furthermore camellidins, which have an antifungal activity characterized by causing abnormal germination, were found in *C. japonica*. The detection of their activity was restricted to *C. japonica*, *C. granthamiana* and *C. wabisuke* by spore germination tests. Their activity was not detected from *C. pitardii* and *C. saluenensis* of section *Camellia*, and 9 clones of hybrids between *C. sinensis* and *C. japonica*.[1]

These results suggested that *C. wabisuke* was closely related to *C. japonica*. But it was difficult to consider that *C. wabisuke* was related to *C. sinensis*.

C. vernalis and *C. hiemalis* were found to contain a large amount of (−)-epicatechin as well as sasanquin. Therefore, both species could be considered as hybrids resulting from crosses between *C. sasanqua* and a species of section *Camellia*, which is in agreement with the assumption of several researchers on the basis of various methods.

Purine base pattern of *C. irrawadiensis*

On the basis of morphological characteristics *C. irrawadiensis* can be regarded as intermediate between *C. sinensis* var. *assamica* and *C. taliensis*.[9] Recently Kondo[2] reported that *C. irrawadiensis* and *C. sinensis* showed some degree of interspecific variations in their karyotypes, even though each species had its own characteristic karyotype. Robert et al.[8] initially investigated the chemotaxonomy of the genus *Camellia* and reported that the presence of caffeine (1, 3, 7-trimethylxanthine) in *C. irrawadiensis* could not be confirmed by paper chromatography. From gas chromatographic survey, caffeine was not also detected in *C. irrawadiensis*. Chemically, this species is clearly distinct from other species of section *Thea*. Its specific metabolism and distribution of purine bases deserved further investigation.

As shown in Fig. 3 of high performance liquid chromatogram, the purine base patterns of *C. irrawadiensis* was remarkably different from the other species of section *Thea*: the theobromine (3,7-dimethylxanthine) content exceeded 0.5% while the caffeine content was below 0.02%. Furthermore, the presence of both compounds in *C. irrawadiensis* was con-

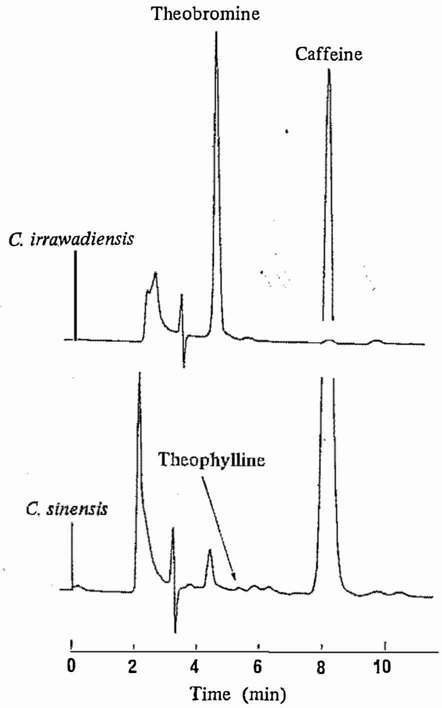

Fig. 3. HPLC analysis of the purine bases
Absorbance (272 nm) of *C. irrawadiensis* and *C. sinensis*

firmed in mass spectra of 2 fractions obtained with preparative high performance liquid chromatography. Since theobromine is a precursor of caffeine, it is possible to assume that *C. irrawadiensis* has a negligible ability for formation of caffeine from theobromine so that an accumulation of the latter occurs.

The distribution of caffeine and theobromine has been extensively studied in stimulant crops. In the case of tea, caffee and maté, caffeine is the predominant purine base and theobromine content is below 0.2%. However, the pattern of cocoa is quite different: theobromine predominates.

Both compounds share in common several pharmacological actions, however, differ in the intensity of each action. For example, caffeine is a powerful central nervous system stimulant whereas theobromine is virtually inactive. Theobromine is more active diuretic than caffeine.

From the angle of tea breeding with interspecific hybrids, *C. irrawadiensis* may be important due to its characteristic purine base pattern. Previously Wood and Barua[11] reported the phenolic constituents of F_1 hybrids between *C. irrawadiensis* and *C. sinensis*, but purine base patterns of these hybrids have not yet been investigated and a study of hybridization between *C. sinensis* and *C. irrawadiensis* is currently in progress at National Research Institute of Tea.

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