Phytochemistry, Vol. 24, No. 10, pp. 2271–2272, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

PURINE BASE PATTERN OF *CAMELLIA IRRAWADIENSIS*

TADAHIRO NAGATA and SHINSUKE SAKAI

Division of Agronomy, National Research Institute of Tea, Kanaya, Shizuoka 428, Japan

(*Received* 17 *December* 1984)

Key Word Index—*Camellia irrawadiensis*; *C. sinensis*; Theaceae; tea; theobromine; caffeine; leaf.

Abstract—Theobromine and caffeine in the flush shoot leaves of hybrids of tea and other camellia plants were assayed by HPLC. In *C. sinensis* and *C. taliensis* of section *Thea* the caffeine content exceeded 2% and the theobromine content was below 0.2%. However, in *C. irrawadiensis* of the same section, the theobromine content was more than 0.5% while the caffeine content was below 0.02%. The theobromine content of tea hybrids was also below 0.2%. In sections other than *Thea*, *C. sasanqua*, *C. japonica* and *C. vernalis* did not contain detectable amounts of theobromine or caffeine.

INTRODUCTION

According to Sealy, section *Thea* in the genus *Camellia* includes *C. sinensis* (tea), *C. taliensis* and *C. irrawadiensis* [1]. On the basis of morphological characteristics, *C. irrawadiensis* can be regarded as intermediate between *C. sinensis* var. *assamica* and *C. taliensis*. Recently Kondo 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 [2]. In previous studies, caffeine (1,3,7-trimethylxanthine) could not be detected by paper chromatography [3] or by gas chromatography [4] in *C. irrawadiensis*. Chemically, therefore, this species is distinct from the other species of section *Thea*.

RESULTS AND DISCUSSION

We have previously reported that *C. taliensis* shows phytochemical similarities with *C. sinensis* on the basis of caffeine, catechins and amino acid contents [4]. The theobromine (3,7-dimethylxanthine) content of the two species was also identical. *Camellia sasanqua* of section *Paracamellia*, *C. japonica* of section *Camellia*, and *C. vernalis* of section *Dubiae* did not contain theobromine or caffeine. We showed that hybrids of tea contained the characteristic components derived from tea such as caffeine, theanine, epicatechin gallate and epigallocatechin gallate, which were not detected in most species of sections other than *Thea* [5]. Similarly, theobromine was detected in hybrids of tea studied.

In this study, we obtained two plants as *C. irrawadiensis*. The two plants were given designations as *C. irrawadiensis* No. 1 and No. 2. The former, which had been examined in a previous study [4], was spread throughout Japan. On the basis of morphological characteristics such as flower size and colour, however, No. 1 was different from the description given by Sealy and similar to 'Burma Baby', a hybrid between *C. irrawadiensis* and *C. japonica* [6]. On the other hand, the morphology of No. 2 was in good agreement with Sealy's description.

The purine base patterns of both plants were different from those of the other camellia plants studied (Table 1): the theobromine content exceeded 0.5% while the caffeine content was below 0.02%. The presence of both compounds in *C. irrawadiensis* No. 2 was confirmed by their mass spectra. Since theobromine is a precursor of caffeine [7], *C. irrawadiensis* may have negligible ability to form caffeine from theobromine.

The new leaves of No. 2 were sampled in September. The caffeine content of *C. sinensis* in the spring was almost equal to that in the autumn. On the basis of data reported by Annan and Nakagawa [8], there was little correlation between caffeine content and the season, although the former was affected by leaf age. The purine base pattern of *C. irrawadiensis* No. 2 in the spring may therefore be similar to that in the autumn. In tea and coffee, caffeine is the predominant purine base and the theobromine content is below 0.2% [9]. The mate examined in this study also showed a similar purine base pattern. However, the pattern for cocoa is quite different: theobromine predominates. According to Zoumas *et al.*, commercial cocoas contain, on average, 1.89% theobromine and 0.21% caffeine [10].

EXPERIMENTAL

Plant materials. C. irrawadiensis No. 1 and *C. taliensis* were received from Saitama Prefectural Tea Experiment Station, and the tea hybrids were supplied by Shizuoka Tea Experiment Station and the Faculty of Agriculture at Niigata University. *C. irrawadiensis* No. 2 was obtained from a garden shop in Mitaka. The other materials were collected at the National Research Institute of Tea.

Theobromine and caffeine determination. A modification of the procedure described in ref. [11] was used for analysis. Young leaves of flush shoots were sampled and steamed, then dried and ground. The dried leaf powder was then stored at 4° until analysis. Samples of leaf powder, 250 mg each, were extracted with 40 ml of H_2O and 80° for 30 min. After cooling, extracts were filtered and the vol. adjusted to 50 ml by the addition of H_2O. Extract (10 ml) was then passed through a column of PVP powder (0.5 g, 1 cm i.d.), eluted with H_2O and made up to 50 ml. Samples were then injected for HPLC with a 10 cm × 8 mm i.d. Radial-Pack C_{18} used in a Radial Compression Separation

Table 1. Theobromine and caffeine contents in leaves of plants studied (% of dry weight)

Origin of sample	Sampling time	Theobromine	Caffeine
Genus *Camellia*			
Section *Thea*			
C. sinensis var. *sinensis*	May	0.11	3.02
	Sep.	0.10	2.93
C. taliensis	Apr.	0.14	2.28
C. irrawadiensis No. 1*	May	0.53	tr†
C. irrawadiensis No. 2*	Sep.	0.81	0.02
Section *Paracamellia*			
C. sasanqua	May	—	—
Section *Camellia*			
C. japonica	May	—	—
Section *Dubiae*			
C. vernalis	May	—	—
Hybrid			
C. sasanqua × *C. sinensis*	May	0.15	1.03
C. vernalis × *C. sinensis*	Sep.	0.09	0.62
Processed Mate (*Irex paraguayensis*)	?	0.08	0.88

*Refer to the text.
†tr: trace: indicates a content of less than 0.01%.

System (Z-Module). The mobile phase was 5 mM NaH_2PO_4–MeOH (11:9) and the flow rate was 10 ml/min.

The limit of detection was *ca* 0.01% for each compound based on the dry wt of the leaves. The quantitative value obtained for each sample was estimated by duplicate measurement.

Identification of theobromine and caffeine in C. irrawadiensis. The extract of *C. irrawadiensis* No. 2 was concentrated, and then subjected to prep HPLC as described above. Both theobromine and caffeine were identified by e.i. mass spectrometry.

Acknowledgements—We would like to thank Professor K. Hagiya of Niigata University, Dr. Y. Fuchinoue of Saitama Prefectural Tea Experiment Station and Mr. T. Hidaka of Shizuoka Tea Experiment Station providing research facilities and plant materials for this work.

REFERENCES

1. Sealy, J. R. (1958) *A Revision of the Genus Camellia*, p. 239. Royal Horticultural Society, London.
2. Kondo, K. (1979) *Japan. J. Breed.* **29**, 205.
3. Robert, E. A. H., Wight, W. and Wood, D. J. (1958) *New Phytol.* **57**, 211.
4. Nagata, T. and Sakai, S. (1984) *Japan. J. Breed.* **34**, 459.
5. Nagata, T. and Sakai, S. (1985) *Japan. J. Breed.* **35**, 1.
6. Kirino, S. (1975) *Gendai Tubaki Meikan (The Modern Camellia Directory)*, p. 19. Bunka Shuppan-kyoku, Tokyo.
7. Suzuki, T. and Takahashi, E. (1976) *Phytochemistry* **15**, 1235.
8. Annan, T. and Nakagawa, M. (1974) *Nippon Nogeikagaku Kaishi* **48**, 91.
9. Jalal, M. A. F. and Collin, H. A. (1976) *New Phytol.* **76**, 277.
10. Zoumas, B. L., Kreiser, W. R. and Martin, R. A. (1980) *J. Food Sci.* **45**, 314.
11. Tsushida, T. and Takeo, T. (1984) *Nippon Nogeikagaku Kaishi* **58**, 277.