JARQ 28, 117–123 (1994)

Differences in Caffeine and Tannin Contents between Tea Cultivars, and Application to Tea Breeding

Yoshiyuki TAKEDA
Kurume Branch, National Research Institute of Vegetables, Ornamental Plants and Tea (Beppu, Makurazaki, Kagoshima, 898 Japan)

Abstract
Wide variations in the tannin and caffeine contents were observed in the tea cultivars collected from various locations in the world. In three groups of tea cultivars, the Assam group showed the widest variation, followed by the China group, while the Japan group showed a negligible variation. Fifteen cultivars with higher tannin content (>25%) were screened only in the Assam group which belongs to *C. sinensis* var. *assamica*, while no cultivars with a higher tannin content were found in the China or Japan group which both belong to *C. sinensis* var. *sinensis*. Two cultivars with a lower caffeine content (>2%) were screened from the China group and seven from the Japan group. The variant cultivars in tannin or caffeine content are suitable tea breeding materials. On the basis of the tannin and caffeine contents, the Assam group (var. *assamica*) could be discriminated from the China and Japan groups (var. *sinensis*). It was obvious that the Japan group was a very homogeneous group in terms of tannin and caffeine contents. Some characteristics of the Assam cultivars were observed in the Cd cultivars of the China group which were collected in Darjeeling in India.

Discipline: Tea industry
Additional key words: genetic resources, genetic variation

Introduction

Tea (*Camellia sinensis*) generally consists of two variants, *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica*[3]. Tea plants belonging to var. *sinensis* are characterized by a bush type with small leaves, are resistant to the cold and suitable for making green tea and semi-fermented tea. In a broad sense, they are referred to as the China variety. On the other hand, tea plants belonging to var. *assamica* are characterized by a tall tree type with large leaves, are less resistant to the cold and are suitable for making black tea.

Since tea contains many useful components in the leaves, including tannins (mainly catechins), caffeine, amino acids and some kinds of vitamins, etc., it has long been utilized for beverages. Tea contains many tannin substances in the leaves which affect the astringency and bitterness of the taste. A large proportion of tannins in tea leaves consists of catechins, such as (–)-epicatechin, (–)-epicatechin-3-gallate, (−)-epigallocatechin, (−)-epigallocatechin-3-gallate and (+)-catechin. It has been observed that these catechins exhibit many useful properties, including deodorizing and antioxydizing characteristics, ability to attack fungi, tumors and viruses. Thus, a great deal of attention is paid to these components in the food, medicine and chemical industries.

Caffeine, a kind of purine alkaloid, is an important component in the bitterness of the tea taste. Caffeine is a characteristic and representative component of tea because it is only found in limited species of plants including tea, coffee, cacao, mate (*Ilex paraguariensis*), etc. In addition, compared with the other plants, tea contains the largest concentration of caffeine. Due to the pharmacological properties of caffeine as a stimulus to the central nervous system, the demand for low-caffeine tea has increased, just as in the case of coffee consumption.

The purpose of this paper is to describe the quantitative variations of the tannin and caffeine contents and indicate how they can be applied to tea breeding.

Materials and methods

The tannin and caffeine contents in the leaves were analyzed in about 1,500 tea cultivars. The cultivars were introduced as seeds from various locations in Japan and tea-producing countries of the world over a period of 51 years from 1929 to 1979, and preserved as genetic resources at the National Research Institute of Vegetables, Ornamental Plants and Tea. Materials collected from India, Sri Lanka (Ceylon), Bangladesh, Taiwan, Malaysia, Vietnam and Myanmar (Burma) are referred to as the 'Assam' group and belong to *C. sinensis* var. *assamica*. Materials collected from the provinces of Anhuei, Hupei, Chianghsi and Checkiang in mainland China and Darjeeling in India are usually referred to as the 'China' group and belong to *C. sinensis* var. *sinensis*. Materials collected from 23 prefectures in Japan, and which also belong to *C. sinensis* var. *sinensis*, are generally referred to as the 'Japan' group.

In the analysis of the tannin and caffeine contents, young leaves of first flush shoots were steamed and dried immediately after sampling and they were ground. The tannin and caffeine in the dried leaf powder were extracted, thereafter tannins were assayed by spectrophotometry[2], and caffeine was assayed by high performance liquid chromatography[1].

Results and discussion

1) Variation in tannin content

A wide variation in the tannin content was observed among the tea cultivars, ranging from 9.37 to 26.82% (Fig. 1). Of the three groups, the cultivars in the Assam group showed the widest variation in tannin content, while the cultivars in the Japan group showed the narrowest variation. The tannin content of the cultivars in the Assam group was distributed over the range of 11.69 to 26.82% and the average amount was 19.39%. The mode of the tannin contents ranged from 17.5 to 20.0% and there was a normal distribution at the center of that range.

The cultivars of the China group showed a normal distribution in the range of 11.32 to 21.61%, and their mean value was 16.27%. Their mode ranged from 15.0 to 17.5%, values lower than those of the Assam group.

The tannin content in the Japan group ranged from 9.37 to 20.0%, values lower than those of both the Assam and the China groups. As the mode of the Japan group ranged from 12.5 to 15.0%, and half of the cultivars were included in this mode, cultivars belonging to the Japan group showed a very narrow variation in tannin content.

Although 15 cultivars with a higher tannin content (more than 25%) were screened out of the Assam group, no cultivars with a higher tannin content were found in the China or

Japan group belonging to var. *sinensis*. The only cultivar with a lower tannin content (less than 10%) was found in the Japan group (Table 1).

Table 1. Cultivars selected from tea genetic resources with higher or lower tannin content

Cultivars with higher tannin content (more than 25%)	Abo2, Abo21 (Malaysia), Ak568, IND8, IND18 (India) PKS97, PKS116, PKS250, PKS349, PKS411, PKS438 (Bangladesh), Taiwan-yamacha23, Taiwan-yamacha71, Taiwan-yamacha81, Taiwan-yamacha95 (Taiwan)
Cultivars with lower tannin content (less than 25%)	Zai88-23 (Japan)

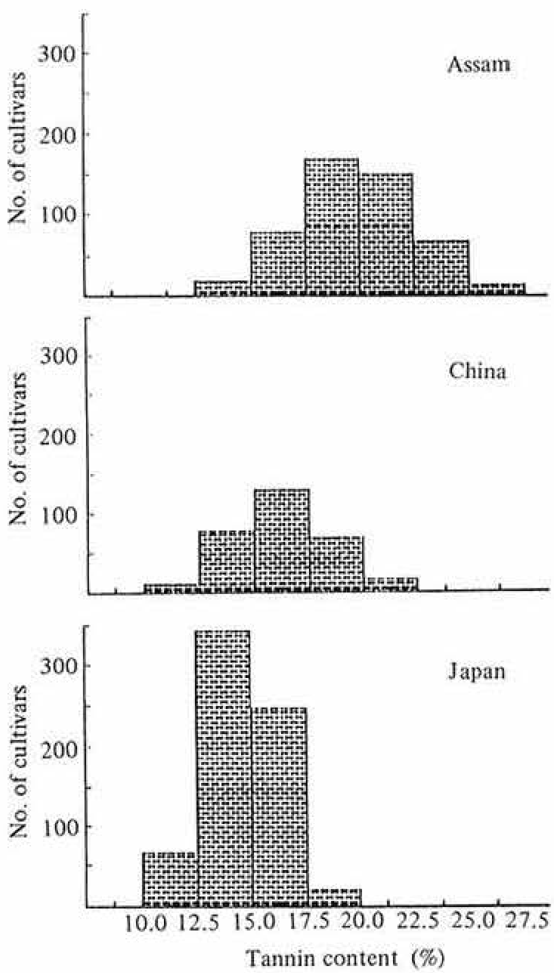

Fig. 1. Variation in tannin content

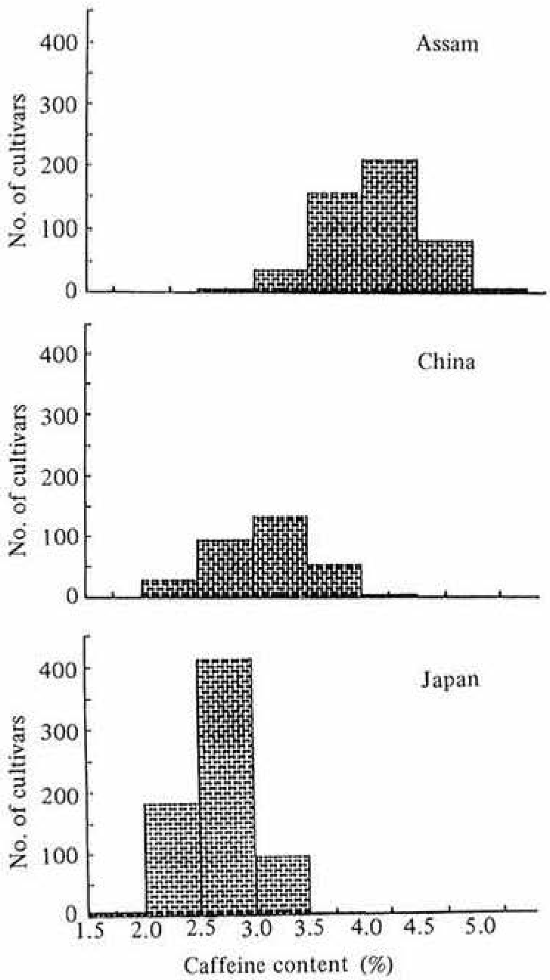

Fig. 2. Variation in caffeine content

2) Variation in caffeine content

In addition to the wide variation in the tannin content, a wide variation was also found in the caffeine content. The contents ranged from 1.64 to 5.46% (Fig. 2). The caffeine content of the Assam cultivars varied from 2.67 to 5.46%, a wider variation than that of either the China or the Japan group cultivars. The mean value of the Assam cultivars was 4.09%, and the mode ranged from 4.0 to 4.5%.

In the China group, the caffeine content ranged between 1.64 and 4.60%, and the mean value was 3.11%, next to the Assam group.

The caffeine content of the Japan group was the lowest among the three groups, the mean value being 2.66%. The mode ranged from 2.5 to 3.0% and the values were distributed in the range of 1.85 to 3.87%. The variation was very limited as in the case of the tannin content because 57% of the cultivars were included in this mode.

Nine cultivars with a higher content of caffeine (more than 5%) were screened out of the Assam group. On the other hand, two cultivars with a lower caffeine content (less than 2%) were selected from the China group and seven from the Japan group (Table 2).

3) Classification of tea cultivars collected from various locations in the world

Among the tea cultivars collected from various locations in the world, the mean values of the collection sites were calculated for the tannin and caffeine contents (Table 3) and plotted in Fig. 3. As shown in Fig. 3, two groups, such as the Assam group and the China – Japan group, could be clearly classified on the basis of the tannin and caffeine contents. Collection sites of the Assam group were included in the 16 to 21% tannin range and in the 4.0 to 4.3% caffeine range. The cultivars belonging to the China and Japan groups exhibited lower ranges than the Assam group. Collection sites of the Assam group with high tannin and high caffeine contents were placed in the upper right-hand corner of Fig. 3, and sites of the China and Japan groups which belong to the var. *sinensis* were placed in the lower left-

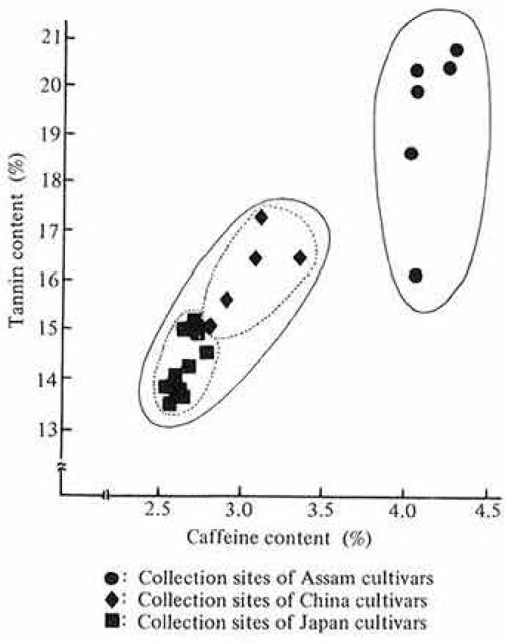

Fig. 3. Classification of tea cultivars collected from various locations in the world

Table 2. Cultivars selected from tea genetic resources with higher or lower caffeine content

Cultivars with higher caffeine content (more than 5%)	IND113 (India), PKS96, PKS224, PKS274, PKS283, PKS423 (Bangladesh), SRL17, SRL19, SRL85 (Sri Lanka)
Cultivars with lower caffeine content (less than 2%)	Cm22, Cn108 (China), Zai17-1, Zai81-8, Zai116-1, Zai133-2, Zai133-3, Zai138-14, Zai146-23 (Japan)

Table 3. Mean values of tannin and caffeine contents in some collections of tea cultivars

Collection name	Country	Mean value of tannin content (%)	Mean value of caffeine content (%)
Assam group			
Ai	India	16.29±2.42	4.05±0.55
Ak	India	18.69±2.66	4.00±0.45
IND	India	20.42±2.19	4.02±0.50
PKS	Bangladesh	20.40±2.41	4.24±0.20
SRL	Sri Lanka	20.84±2.72	4.29±0.30
Taiwan-yamacha	Taiwan	19.89±2.45	4.02±0.34
China group			
Cm	China	15.69±3.72	2.90±0.39
Cn	China	15.18±3.48	2.81±0.43
Ck	China	17.35±1.96	3.11±0.38
Cp	China	16.50±3.50	3.09±0.25
Cd	India	16.49±2.22	3.35±0.39
Japan group			
Fukuoka	Japan	15.02±1.37	2.62±0.24
Ibaraki	Japan	13.83±1.34	2.62±0.33
Kagoshima	Japan	15.08±1.60	2.72±0.25
Kochi	Japan	13.86±1.88	2.51±0.24
Kyoto	Japan	15.23±1.47	2.72±0.27
Mie	Japan	14.12±1.49	2.62±0.30
Miyazaki	Japan	14.56±1.50	2.77±0.34
Nara	Japan	14.21±1.30	2.64±0.26
Shiga	Japan	13.70±1.77	2.61±0.26
Tokushima	Japan	13.50±1.15	2.56±0.32

hand corner, near the origin. It can be observed in Fig. 3 that the caffeine content is a suitable criterion for differentiating var. *sinensis* from var. *assamica*.

In the Assam group, there was a significant variation in the tannin contents among the collection sites, with a maximum difference of 4% between the SRL cultivar with the highest content and the Ai cultivar with the lowest content. However, the mean values of the caffeine content of the collection sites only ranged from 4.00 to 4.29%, showing less variation than the tannin content values.

In the collection sites of var. *sinensis*, the Japan group was near the origin and deviated from the China group. Mean values of some of the collection sites for the China group were higher both in the tannin and caffeine contents than those of the Japan group and were intermediate between the values of the Japan group and the Assam group.

It was observed that the Japan group was very homogeneous when compared with the Assam and the China groups, because the variation and standard deviations of both tannin and caffeine components were very narrow.

In the China group, the Ck cultivars collected from Keeman in mainland China showed a relatively high tannin content, and the Cd cultivars collected from Darjeeling in India showed a high content of caffeine. As these two sites are renowned black tea-producing regions in the world using tea leaves of var. *sinensis*, it was assumed that cultivars with a high content of tannins or caffeine have been unconsciously selected for making black tea with high quality for a long time.

The presence of a high caffeine content is one of the typical characteristics of the Assam cultivars. It is very interesting to note that this property also found in the Cd cultivars could be detected by the pubescence on the surface of young leaves[6], the color of mature leaves[7] and the fermentation ability[8].

4) Utilization of cultivars with various tannin and caffeine contents for tea breeding

Among the cultivars with varying tannin and caffeine contents, cultivars with high tannin and/or low caffeine contents are useful tea breeding materials. Cultivars with a high tannin content were selected only from the Assam group. Since the Assam cultivars display a low resistance to cold temperatures, they can only be cultivated in a very limited zone in the southern part of Kyushu in Japan. On the basis of this resistance, the individuals with a high tannin content in the progenies, resulting from crosses between cultivars with a high tannin content (Assam group) and cold-resistant cultivars of var. *sinensis* should be selected. As a relatively high tannin content was found both in the Ck and Cd cultivars belonging to the var. *sinensis*, they could be used as breeding materials.

In the breeding of cultivars with a low caffeine content, the caffeine content of selected cultivars should be less than 1%. Two cultivars with a caffeine content less than 2% were screened from the China group and seven from the Japan group, and a cultivar with the lowest content (1.64%) was found in the Cn cultivars collected from the province of Chianghsi in mainland China. They could be suitable parents for the breeding of cultivars with a low caffeine content.

The use of allied species belonging to the genus *Camellia* may also enable to breed tea cultivars with a low caffeine content. Hybrids between tea and some species of the genus *Camellia* which have been bred always showed a low caffeine content. For example, caffeine contents in hybrids between tea and *C. japonica* ranged from 0.2 to 0.5%, which is equivalent

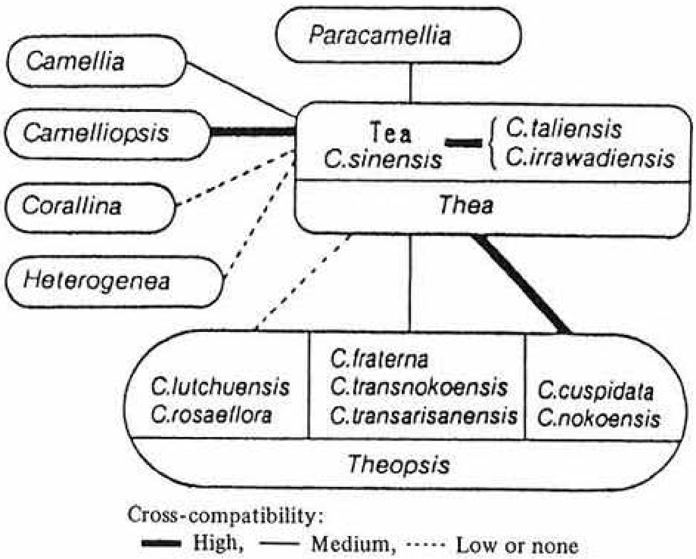

Fig. 4. Cross-compatibility between *C. sinensis* and subgeneric sections of the genus *Camellia*

to 10% of the tea caffeine concentration. These hybrids are promising materials for the breeding of cultivars with a low caffeine content[4]. However, it is generally difficult to obtain interspecific crosses between tea and the allied species[5]. Cross-compatibility of tea and its allied species is shown in Fig. 4. It may be possible to utilize the allied species if the difficulties in obtaining crossed seeds could be overcome. The use of biotechnology procedures such as embryo culture and protoplast fusion in tea plants may enable to solve this problem.

In the near future breeding of cultivars with a low caffeine content less than 1% using the above methods may become possible.

References

1) Ikegaya, K. (1985): Determination of caffeine in tea by high-performance liquid chromatography. *Nippon Shokuhin Kogyo Gakkaishi*, **32**, 61-66 [In Japanese with English summary].
2) Iwasa, K. et al. (1970): Improvement of official chemical analysis of tea. 3. Examination of tannin determination. *Study of Tea*, **40**, 69-73 [In Japanese with English summary].
3) Sealy, J. R. (1958): A revision of the genus *Camellia*. Roy. Hort. Soc., London, 34-35.
4) Takeda, Y. et al. (1987): Breeding of interspecific hybrids between *Camellia sinensis* (L.) O. Kuntze and *C. japonica* L. and their characteristics. *Bull. Natl. Res. Inst. Veg. Ornam. Plants & Tea*, **B1**, 11-22 [In Japanese with English summary].
5) Takeda, Y. (1990): Cross compatibility of tea (*Camellia sinensis*) and its allied species in the genus *Camellia*. *JARQ*, **24**, 111-116.
6) Takeda, Y. et al. (1991): Classification of tea plants by the pubescence on the young tea leaf. *Tea Res. J.*, **74** (suppl.), 18-19 [In Japanese].
7) Takeda, Y. et al. (1992): Classification of tea genetic resources by means of the mature leaf color. *Tea Res. J.*, **76** (suppl.), 8-9 [In Japanese].
8) Toyao, T. et al. (1982): Varietal difference of fermentation ability (polyphenol oxidase activity) in tea plant. *Study of Tea*, **63**, 14-18 [In Japanese with English summary].

(Received for publication, July 27, 1993)