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Cross Compatibility of Tea (Camellia sinensis) and Its Allied Species in the Genus Camellia

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Abstract
Interspecific cross compatibility between Camellia sinensis and its allied 26 species of 7 subgeneric sections in the genus Camellia was examined. The interspecific crossing abilities varied among the cross combinations, and the fruit-bearing rates were in the range from 0 to 42.6%. The interspecific hybrids obtained from the crossings of C. sinensis with C. japonica, C. pitardii, C. assimilis, C. caudata, C. salicifolia, C. irrawadiensis and C. taliensis showed very low pollen fertilities. Morphological characteristics of those hybrids, including size and shape of leaves, flowers and tree performance, were generally intermediate of their parental species. The hybrids between C. sinensis and C. japonica showed a high level of resistance to such diseases as tea gray bright and tea anthracnose and to cold damage in winter as well. It was presumed that the F1 plants which were obtained from the crossing of C. sinensis with C. sasanqua, C. brevistyla and C. oleifera used as a male parent might be developed through parthenogenesis of a reduced gamete. The cross compatibilities between C. sinensis and subgeneric sections of genus Camellia are: Thea > Camelliopsis > Paracamellia = Camellia = Theopsis > Heterogenea = Corallina.

Discipline: Tea industry
Additional key words: disease resistance, interspecies, interspecific hybrid, parthenogenesis

Tea, *Camellia sinensis*, which is one of the species of the genus *Camellia*, is generally divided into 2 varieties; *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica*. For the last five decades, intraspecific crosses within *C. sinensis* have been undertaken extensively in the breeding program of Japan, through which a number of superior tea cultivars have been released. One of the achievements is a group of cultivars for black tea, which have high cold-tolerance and excellent quality: they were derived from the crosses between Japanese domestic cultivars (var. *sinensis*) and Assam cultivars (var. *assamica*) which had been introduced from India about 100 years ago.

The Assam cultivars are recognized to be very important materials at present in Japan as a gene pool for disease resistance and a source of new aroma of tea as well. It is therefore necessary to take advantages of useful genes through the interspecific and/or intergeneric hybridizations as adopted in many other crops. Such crosses would contribute to providing greater genetic variations in general, and developing breeding materials with high values in particular, such as resistance to pests and diseases, cold tolerance, new aroma of tea and specific characters in chemical components.

Interspecific crosses within the genus *Camellia* have been made with the major purpose of improving the flowers of *Camellia*, thereby a number of *Camellia* cultivars have been released chiefly in Europe, USA and Japan. However, since tea flowers are small and inconspicuous, it has been quite rare that tea plants are used as a parent for breeding of *Camellia*.

The *Camellia* species which have produced interspecific hybrids with *C. sinensis* are *C. japonica*[1,5,7], *C. taliensis*[3], *C. irrawadiensis*[2], *C. sasanqua*[3], *C. kissi*[2] and *C. caudata*[2].

The present paper describes the interspecific cross compatibility between *C. sinensis* and its allied 26 species of 7 subgeneric sections in the genus *Camellia*.

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Table 1. Results of the crosses between *C. sinensis* and its allied species in the genus *Camellia*

Cross combination: Female	Cross combination: Male	Chromosome no. (2n)	No. of crosses	No. of fruits	Percent of fruit-bearing	No. of seeds obtained	No. of seedings	No. of seedlings	Cross compatibility*
(Sect. Camellia)					%				
C. sinensis	*C. japonica*	(30)	889	28	3.1	36	22	17	△
″	*C. hongkongensis*	(30)	36	0	0	0	0	0	×
″	*C. pitardii*	(30)	116	7	6.0	9	9	4	△
″	*C. saluenensis*	(30)	92	3	3.3	4	4	0	△
(Sect. Paracamellia)									
C. sinensis	*C. brevistyla*	(30)	164	10	6.1	15	8	1	△**
″	*C. kissi*	(30)	92	19	20.7	24	18	9	○
″	*C. oleifera*	(90)	253	23	9.1	34	20	5	△**
″	*C. sasanqua*	(90,60)	122	2	1.6	2	2	2	△**
(Sect. Camelliopsis)									
C. sinensis	*C. assimilis*	(30)	159	22	13.8	27	23	9	○
″	*C. caudata*	(30)	102	24	23.5	42	42	23	○
″	*C. salicifolia*	(30)	101	14	13.9	18	18	13	○
(Sect. Theopsis)									
C. sinensis	*C. cuspidata*	(30)	50	21	42.0	27	27	2	○
″	*C. fraterna*	(90)	166	3	1.8	3	3	0	△
″	*C. lutchuensis*	(30)	55	0	0	0	0	0	×
″	*C. nokoensis*	(30)	53	8	15.1	14	9	5	○
″	*C. rosaeflora*	(90)	21	0	0	0	0	0	×
″	*C. transarisanensis*	(—)	81	2	2.5	2	2	0	△
″	*C. transnokoensis*	(90)	42	2	4.8	1	0	0	△
(Sect. Thea)									
C. sinensis	*C. irrawadiensis*	(30)	100	29	29.0	50	48	10	○
″	*C. taliensis*	(30)	123	25	20.3	36	34	24	○
(Sect. Heterogenea)									
C. sinensis	*C. furfuracea*	(30)	84	0	0	0	0	0	×
″	*C. granthamiana*	(60)	100	0	0	0	0	0	×
(Sect. Corallina)									
C. sinensis	*C. parviflora*	(—)	26	0	0	0	0	0	?
(Dubiae)									
C. sinensis	*C. drupifera*	(90)	153	2	1.3	2	2	0	△
″	*C. miyagii*	(90)	163	27	16.6	49	49	0	○
″	*C. tenuiflora*	(60)	109	9	8.3	10	10	5	△**

* ○: High, ○: Middle, △: Low, ×: Impossible. ** Possible occurrence of apomixis.

In this experiment, *C. sinensis* was exclusively used as a female parent and the other *Camellia* species for crossing were used as male parents. The cross compatibility of each combination is estimated on the basis of both the crossing results and the growth of hybrids. Cross compatibility and characteristics of the interspecific hybrids are discussed for each subgeneric section of the genus *Camellia* specified by Sealy[6].

All crosses were made with emasculated flowers, using standard procedures. The crossing results between *C. sinensis* and the 26 related species of the genus *Camellia* are shown in Table 1.

Section Camellia

In this section, 4 species including *C. japonica* were crossed with *C. sinensis*. As the flowering time of the species in this subgeneric section is usually spring, pollen used for pollination was stored in a deep freezer at $-80°C$ for about 6 months. Fruit-bearing rate of the cross between *C. sinensis* and *C. japonica* was rather low, or 3.1%. Morphological characteristics of the hybrids such as size and shape of leaves, flowers and tree performance were intermediate of the parents. However, flower color of all the hybrids was reddish pink, which is slightly paler than the red color of *C. japonica* (Table 2).

The pollen fertilities of the hybrids were generally low, ranging approximately from 20 to 50%, and aborted and giant pollen grains were often observed, as usually seen in the interspecific hybrids of other crops. The 9 hybrids obtained were all diploids with 2n = 30, which were the same with their both parents.

The hybrids showed a high resistance to tea gray blight (*Pestalotia longiseta*) and to tea anthracnose (*Gloeosporium theae-sinensis*), as well as cold tolerance in winter. In the chemical components of the hybrids, the caffein content in the new shoots of the first crop was only 10% of the female parent cultivar and the catechin content in mature leaves in winter ranges from 20 to 60% of that of the tea plant (Table 3).

The hybrids obtained in this experiment were named 'Chatsubaki': one of them is presently under processing for registration at the Ministry of Agriculture, Forestry and Fisheries, Japan as a mother plant for crossing between tea and *Camellia* (Plate 1).

In the cross of *C. sinensis* with *C. pitardii*, 7 fruits and 9 seeds were obtained out of 116 crosses and 4 hybrids were produced. All of these hybrids had new leaves with a pale red color and a round shape. These characteristics were different from those in *C. sinensis*, proving that the plants produced were true hybrids between the above species.

In the cross of *C. hongkongensis* with *C. sinensis*, no seed was obtained. Since the crossing between them was small in number, it was not clear whether these species were cross-incompatible or not. However, it is likely that the compatibility of *C. hongkongenesis* with tea is very low or none, judging from the crossing results so far obtained by other breeders.

From the hybridization between *C. sinensis* and *C. saluenensis*, 3 fruits and 4 seeds were produced. However, none of them germinated.

Section Paracamellia

Four species including *C. sasanqua* were crossed with *C. sinensis*. The species used in Paracamellia species bloom generally in autum: they vary in chromosome number (2n), counting 30, 60 and 90.

Fruit-bearing percentages in the crosses between *C. sinensis* and Paracamellia species ranged from 1.6 to 20.7%. The highest rate was obtained in the cross with *C. kissi*, which produced 9 hybrids. All the hybrids had small and elliptical leaves with a slightly

Table 2. Characteristics of mature leaves and flower organs of the hybrids between *C. sinensis* and *C. japonica*

Hybrid (Variety)		Shape	Leaf index (Length /width)	Leaf area	Flower color	Flower size	Hair of ovary	Pollen fertility
				cm^2		cm		%
Chatsubaki*	No.1	Long elliptic	2.38	18.7	Pale red	5.5	Hairly	22.2
"	No.2	Long elliptic	2.23	11.1	Pale red	4.2	Hairly	28.0
"	No.3	Long elliptic	2.16	17.9	Pale red	—	Harily	—
"	No.4	Long elliptic	2.40	16.3	Pale red	5.4	Harily	35.5
"	No.5	Long elliptic	2.22	15.0	Pale red	5.4	Harily	35.1
"	No.6	Long elliptic	2.37	15.6	Pale red	4.4	Harily	47.9
"	No.7	Long elliptic	2.30	18.6	Pale red	4.8	Hairly	—
"	No.8	Long elliptic	1.88	22.8	Pale red	5.8	Harily	28.8
"	No.9	Long elliptic	1.91	25.5	Pale red	6.4	Hairly	26.2
C. sinensis (cv. Sayamakaori)		Long elliptic	2.62	25.2	White	3.9	Hairly	99.2
C. japonica		Elliptic	1.74	29.8	Red	7.0	Glabrous	99.5

* Chatsubaki is the hybrid between *C. sisensis* (cv. Sayamakaori) and *C. japonica*.

Table 3. Physiological characters and contents of caffein, amino acid and catechin of the hybrids between *C. sinensis* and *C. japonica*

Hybrid (Variety)		Disease resistance*		Cold resistance in midwinter	Caffein (%)	Total amino acid (%)	Total catechin (%)
		Tea gray bright	Tea anthracnose				
Chatsubaki	No.1	R	R	Hardy	0.34	0.963	2.10
''	No.2	R	R	Hardy	0.39	0.396	2.63
''	No.3	R	R	Hardy	0.26	0.811	4.28
''	No.4	R	R	Hardy	0.20	0.400	3.91
''	No.5	R	R	Hardy	0.46	0.898	3.56
''	No.6	R	R	Hardy	0.33	0.497	2.35
''	No.7	R	R	Hardy	0.47	1.860	2.00
''	No.8	R	R	Hardy	0.24	0.331	4.57
''	No.9	R	R	Hardy	0.33	1.502	2.07
C. sinensis (cv. Sayamakaori)		R	S	Hardy	3.15	1.290	10.93
C. japonica		R	R	Hardy	0.00	0.162	0.71

* R is resistant and S is susceptible to the diseases, respectively.

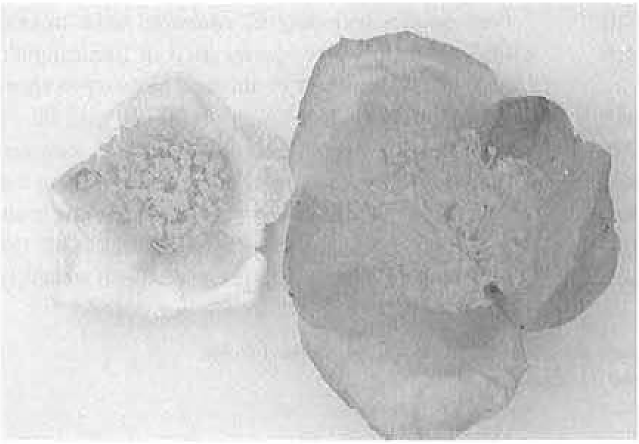

Plate 1. The flowers of *C. sisnensis* and Chatsubaki No. 1
Left: *C. sinensis* (cv. Sayamakaori).
Right: Chatsubaki (Hybrid of *C. sinensis* × *C. japonica*).

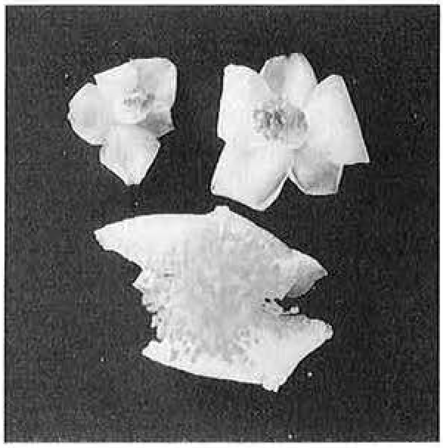

Plate 2. The flowers of *C. sinensis*, *C. caudata* and their hybrid
Upper line: *C. caudata* (left), Hybrid (right).
Lower line: *C. sinensis* (cv. Yabukita).

sharp tip at their matured stage. They were identified as true hybrids because these morphorogical characteristics were the same with those of *C. kissi* used as a male parent.

In each cross combination of *C. sinensis* with *C. brevistyla*, *C. oleifera* and *C. sasanqua*, all the F_1 plants produced beared a close resemblance to *C. sinensis* used as a female parent. Their pollen fertilities were more than 95%, which were of the same level of the tea cultivars used as a female parent. One of those hybrids obtained from the cross with 'Yabukita', a cultivar of *C. sinensis*, and *C. oleifera* showed a 'Koro' type. The typical character of the Koro type of tea plant is a large and undulating leaf. It is reported that this character is genetically controlled by a complete recessive gene of '*k*', and the relevant phenotype takes place when the gene *k* is in a homozygote[8]. Since Yabukita has this Koro gene in heterozygote, it is suggested that the F_1 plants which were obtained from the crossings of *C. sinensis* with *C. brevistyla*, *C. oleifera* and *C. sasanqua* used as male parents be developed through parthenogenesis of a reduced gamete.

Section Camelliopsis

Three species of Camelliopsis were used as a male parent for crossings with *C. sinensis*. The species in this section showed a relatively high cross compatibility with *C. sinensis*, and the fruit-bearing rate was 13.8% with *C. assimilis*, 23.5% with *C. caudata* and 13.9% with *C. salicifolia*, respectively. The growth of the hybrids were generally good, though they showed a variety of morphorogical characteristics, each of them corresponded to those of the respective male parents. For example, the hybrids of *C. sinensis* with *C. assimilis* had reddish new leaves covered by plenty of hairs and the leaf shape was stenophyllous like *C. assimilis*.

In the cross of *C. sinensis* with *C. caudata*, 42 seeds were obtained out of 24 fruits: the seeds per fruit were 1.75 on an average. The hybrids resembled *C. caudata* in various morphorogical characters: their mature leaves were thin and stenophyllous, their new shoots were reddish and covered by plenty of short hairs, and their flowers were white and small like *C. caudata* (Plate 2).

In the cross of *C. sinensis* with *C. salicifolia*, 13 hybrids were produced from 18 seeds and their morphorogical characteristics were different from those of *C. sinensis*. Some features of *C. saliciforia*, such as reddish and hairly new shoots and stenophyllous in leaf shape, were clearly observed in these hybrids.

Section Theopsis

In this section, 7 species were crossed with *C. sinensis*. Results from the crossings varied greatly among the male parent species used: the rates of the fruit-bearing ranged from 0 to 42%. The highest rate was obtained in the cross with *C. cuspidata*: 21 fruits and 27 seeds were produced from 50 crossings. The germinated hybrids had small and elliptic leaves with a sharp tip, which were diagnostic features of *C. cuspidata*.

In the cross between *C. sinensis* and *C. nokoensis*, fruit-bearing rate was relatively high within this section, or approximately 15%. The germinated F_1 plantes had small and round leaves with a light green color, which resembled those of *C. nokoensis*.

In regard to the other species of Theopsis, no fruits were produced in the crosses of *C. sinensis* with *C. lutchuensis* and *C. rosaeflora*. In some crosses with *C. fraterna*, *C. transarisanensis* and *C. transnokoensis*, a few seeds were obtained with a failure of germination.

It was indicated that the species having $2n = 90$ chromosomes tended to have a lower cross compatibility with *C. sinensis*.

Section Thea

Two species of Thea including *C. irrawadiensis* and *C. taliensis* were subjected to crossing with *C. sinensis*. They showed high cross compatibilities with *C. sinensis* which also belongs to the subgeneric section Thea. Their fruit-bearing rates of the hybrids were over 20%.

The hybrids between *C. sinensis* and *C. irrawadiensis* showed a distinguished character in new shoots, the color of which was dark purplish red, although the presence of antocian was hardly identified in the parents used in this cross. The new leaves of *C. irrawadiensis* contain approximately 0.8% of theobromine, which is a precursor of caffein, while they contain few caffein. On the contrary, tea (*C. sinensis*) contains approximately 3% of caffein and few theobromine. The F_1 hybrids between them were intermediate of the 2 parent species in both terms of caffein and theobromine contents. This result confirmed that the F_1 plants were true hybrids not only in morphological characters but also in chemical components.

The intrasectional crossing of *C. sinensis* with *C. taliensis* was relatively easy in producing the hybrids, and its fruit-bearing rate was approximately 20%. The growth of their hybrids was vigorous. They showed an intermediate type in various morphological characters. Their mature and new leaves were both light green in color and elliptic in shape.

Section Heterogenea

Each of the species of *C. furfracea* and *C. granthamiana* was crossed with *C. sinensis*. No fruits, however, could be produced in these 2 cross combinations. It is concluded therefore that the cross compatibility between tea and these 2 species was extremely low or none; in fact, all the flowers pollinated fell at the early stage after pollination.

Section Corallina

C. parviflora was subjected to crossing with *C. sinensis*, but no fruits were obtained. However, since the species used in this section and the flowers crossed in the experiment were limited in number, the cross compatibility of *C. sinensis* with Corallina species was not clearly identified.

Others

Three species including *C. tenuiflora*, *C. drupifera* and *C. miyagii* were used as male parents for corssing with *C. sinensis*. The section to which these 3 species belong was not specified by Sealy[6]. It is very likely that they would be put in the section Paracamellia. In the cross of *C. sinensis* with *C. miyagii*, the fruit-bearing rate was 16.6% and 49 seeds were obtained. The seeds produced were as large as tea seeds, while none of them germinated.

In the hybridization between *C. sinensis* and *C. tenuiflora*, the fruit-bearing rate was 8.3% and 5 plants were grown. All these plants closely resembled *C. sinensis* used as a female parent in morphological characters, and their pollen fertilities were over 95%, which was also of the same level as the tea cultivars used. It is suggested that those F_1 plants be also developed from parthenogenesis of the reduced gamate of *C. sinensis* used as a female parent, as in the case of crossings with *C. sasanqua*, *C. oleifera* and *C. brevistyla*.

From the results as stated above, it is concluded that the cross compatibility of *C. sinensis* with other species of the genus *Camellia* is relatively high with a few exceptions. The cross compatibilities between *C. sinensis* and subgeneric sections are summarized as follows:

Thea > Camelliopsis > Paracamellia = Camellia = Theopsis > Heterogenea = Corallina.

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