Cytogenetical studies on *Camellia* species

III. An interspecific hybrid between *Camellia japonica* L. and *C. sinensis* (L.) O.Kunze.

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For the breeding of cold resistant varieties of the tea plant, *Camellia japonica* L. var. *spontanea* MAKINO (Yabu-tsubaki) and *C. sinensis* (L.) O.Kunze var. *sinensis* (Yabukita) were crossed. The gross morphological forms and chromosome pairing in PMCs of F₁ plant were observed. In this F₁ the forms of leaves and flowers were intermediate between both parents. The formation of flower buds on shoots was resemble to that of *C. sinensis*, and the flower buds bore on both the sides of the terminal bud. The length of the pedicels was intermediate between both parents. The univalent chromosomes were often observed at MI in PMCs. The type of the sporads in the pollen tetrad stage were various, there were 1-7 sporads. The PMCs sometimes contained micronuclei. From these phenomena it was concluded that the F₁ plant was a true hybrid between *C. japonica* and *C. sinensis*.

Introduction

In the previous papers, for the breeding of cold resistant varieties of the tea plant, cytological observations of *Camellia* species have been reported (KATO and SIMURA 1970, 1971). Therefore, in order to make the genetical respects of these species more clear, the crosses between *C. japonica* and *C. sinensis* were tried. The hybrids between *C. sinensis* and other *Camellia* species have so far been reported (FUCHINOUE 1970, H.P.BEZBARUH and S.C.GOGOI 1972). In these cases the crossing-abilities seemed to increase with polyploidy. ACKERMAN (1973) and KONDO (1977) reported the interspecific hybrids in *Camellia* genus, and they used diploid varieties in *C. japonica* and *C. sinensis*. In the present paper also the diploid varieties were used for the investigation of the genetical relationship between *C. sinensis* and *C. japonica*. Since the tea plant flowers in autumn and *C. japonica* in spring, the crosses between *C. sinensis* and *C. japonica* are usually difficult. In the present studies *C. japonica* was used as maternal plants and with the special technique the crosses were made with *C. sinensis*. The gross morphological form of flowers and leaves and meiosis in PMCs of the F₁ plant between *C. japonica* and *C. sinensis* were observed.

Materials and Methods

C. sinensis (L.) O.Kunze var. *sinensis* (Yabukita 2n=30) and *C. japonica* L. var. *spontanea* MAKINO (Yabu-tsubaki 2n=30) were used. These materials were cultured in the field of the Faculty of Agriculture, Nagoya University, Nagoya. As the flowering time of *C. sinensis* was different from *C. japonica*, the pollen grains of *C. sinensis* were stocked in the deep freezer at -20°C until spring, when *C. japonica* flowered. The buds were collected at various stage, fixed in 70% isopropyl alcohol, and then the PMCs were squashed in 1% aceto-orcein.

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Results

The percentage of germination of the stocked pollen grains was shown in Table 1 compared with the fresh ones. The germination of stocked pollen grains on the agar medium was the same as its fresh pollen grains. When *C. japonica* as the femal plant was pollinated with the stocked pollen grains of *C. sinensis*, only one F_1 plant was obtained from the crosses (Table 2).

1. Gross morphological form of F_1 plant

Leaf: The leaf margin was serrated and leaf form, size and thickness were intermediate between both parents.

Flower: The flower-colour was more pale-red than *C. japonica*. The pedicels were long, and its length was intermediate between both parents. The flower size was intermediate between both parents. The inflorescence was resemble to *C. sinensis*, being markedly different from that of *C. japonica*. The flower buds bore on both the sides of the terminal bud. They flowered in autumn like *C. sinensis* (Fig. 1).

2. Meiosis in PMCs of the F_1 plant

Many abnormal divisions were observed in MI. The F_1 plant had a high frequency of univalent chromosomes at MI (Table 3). In AI, univalent chromosomes and laggards were also observed (Fig. 2 A). There were various types of the sporads in pollen tetrad stage, and 1-7 sporads were observed (Fig. 2 B; Table 4). The micronuclei were observed in 41.4% of the PMCs examined (Table 4). Pollen grains at flowering stage were empty, and very small pollen grains were often observed. The pollen grains were sterile (Table 5; Fig. 2 C). According to the histological observation, the microsporogenesis was normal,

Table 1. Percentage of germination of stocked pollen grains compared with fresh pollen grains in *C. sinensis* and *C. japonica*

	Fresh pollen grains	Stocked pollen grains (-20°C for 6 months)
<i>C. sinensis</i>	93.2%	52.8%
<i>C. japonica</i>	35.9%	7.3%

Table 2. Results of crosses between *C. sinensis* and *C. japonica*

Combinations	No. of flowers pollinated	No. of fruits	No. of growing plants
♀ <i>C. japonica</i> ♂ <i>C. sinensis</i>	226	11	1
♀ <i>C. sinensis</i> ♂ <i>C. japonica</i>	1579	0	0

Table 3. Chromosome pairing at MI in PMCs of F_1 plant

Type of pairing	15II	14II	13II	12II	11II	8II	Total
		+	+	+	+	+	
		2I	4I	6I	8I	14I	
No. of PMCs observed	3	5	6	4	1	1	20

Table 4. Various types of sporads in pollen tetrad stage of F_1 plant

Number of sporads	1	2	3	4	5	6	7	Total	With micronuclei
No. of PMCs	9	42	37	96	69	22	6	281	
%	3.2	14.9	13.2	34.2	24.6	7.8	2.1	100	41.4

Table 5. Percentage of the pollen fertility

Species	Pollen fertility
<i>C. sinensis</i>	90.1%
F_1	0

The pollen fertility was counted by staining with 1% aceto-carmin

the embryos were normal in 40% of the ovules observed at flowering stage. This F_1 plant had flowered three times in the past, but those flowers had not fructified.

Discussion

In the present F_1 Plant, the gross morphological form of the tree was more resemble to *C. japonica* but the form of leaves and flowers were intermediate between both parents. The pedicel was long, the inflorescence was resemble to *C. sinensis*, and the ovaries were hairy, though they were grabrous in *C. japonica*. In PMCs of the F_1 plant, univalents at MI and AI and micronuclei at pollen tetrad stage were observed, and these were possibly derived from the abnormal chromosome pairings in PMCs. From these appearances it was concluded that the present F_1 plant was a true hybrid between *C. japonica* and *C. sinensis*. In the karyotype analysis of *C. sinensis* and *C. japonica* there were partial differences (FUKUSHIMA *et al* 1966, KATO and SIMURA 1971); therefore it was presumed that in the PMCs of the present F_1 plant the abnormal chromosome pairings were observed. But KONDO (1977) reported that F_1 hybrid combinations between diploid species in *Camellia* showed similar patterns of meiosis and chiasma frequencies at MI in PMCs. The genetical relationships between *C. sinensis* and *C. japonica* should be further studied through the detailed observations of chromosome aberrations in the present hybrid. Since 40% of the embryos in the present hybrid are normal at the flowering stage, it can be used as a maternal parent for the back cross.

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Camellia 属植物における細胞遺伝学的研究

III. チャとツバキの雑種について

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チャの耐凍性育種のため, ツバキとチャの交雑を行ない, その F_1 の外部形態や花粉母細胞の染色体対合について調べた。ツバキにはヤブツバキ, チャにはヤブキタを用いた,

F_1 の外部形態については, 木全体はヤブツバキに似ていたが, 葉や花の形態は両親の中間を示した。とくに花のつぼみの着き方はチャに似ていて, 頂芽をはさんで2個づつ着く場合が多く, 大体3~4個のつぼみが一ヶ所についていた。花柄はチャと同様に長く, 花の大きさは両親の中間を示した。子房の表面はツバキでは毛がなく, 滑かであったが, F_1 の子房にはチャと同様に毛が密生していた。開花期はチャと同様に秋で, 花の色はヤブツバキが濃い赤, チャが白であるに対し, F_1 の花はうすい赤であった。

花粉母細胞の染色体対合では, 1価染色体がしばしば観察され, 花粉四分子期には1~7分子の小胞子が見られ, 小核をもつものもあった。これらのことから, この F_1 は確かにヤブツバキとチャの雑種であると推察された。

Fig. 1. The gross morphological forms of the flower of F_1 plant.
A. Flower buds. B. Flowers of *C. sinensis* (left) and of F_1 (right).

Fig. 2. Meiosis in PMCs of F_1 plant.
A. Anaphase I. Univalent was shown by arrow.
B. Various types of sporads at tetrad stage.
C. Pollen grains. Small pollen grains were observed.