Cytological Studies in Cultivated Species of *Camellia*

IV. Amphidiploid Cultivar-'Fragrant Pink Improved' Appeared from 'Fragrant Pink' Synthesized by the Colchicine Treatment

Katsuhiko KONDO* and William L. ACKERMAN**

* Department of Environmental Sciences, Faculty of Integrated Arts and Sciences,
Hiroshima University, Higashi-Senda-Machi, Hiroshima 730

** United States National Arboretum, United States Department of Agriculture,
Washington, D. C. 20002, U. S. A.

An amphidiploid cultivar of *Camellia*, 'Fragrant Pink Improved', synthesized by colchicine treatment from a diploid intersectional hybrid between a diploid clone of *C. japonica* subsp. *rusticana* (Honda) Kitamura and of *C. lutchuensis* T. Ito named 'Fragrant Pink' was studied to clarify genomic affinity between species of *Camellia*. The majority of the pollen mother cells of 'Fragrant Pink Improved' studied showed the predominant formation of bivalents and high chiasma frequency except for a few univalents and multivalents at metaphase I in meiosis and rather high pollen viability with a mean of 77 percent. The diploid parental clones of the F₁ hybrid 'Fragrant Pink' showed complete and normal 15 bivalent chromosomes at metaphase I in meiosis of pollen mother cells and high pollen viability, while 'Fragrant Pink' showed a single chromosome configuration of 15 bivalents and the chiasma frequency deviated only slightly from those of the parents, but very low pollen viability with a mean of four percent. Thus, each pair of partially homologous chromosomes from *Camellia japonica* subsp. *rusticana* and that from *C. lutchuensis* include different degree of homologous segments. Either cryptic structural differences or differences of the gene constitution without any chromosomal structural differences would be expected between the chromosomes of *C. japonica* subsp. *rusticana* and of *C. lutchuensis*. During the course of investigation, two percent of the pollen mother cells of amphidiploid 'Fragrant Pink Improved' showed complete and normal 15 bivalent chromosomes at metaphase I in meiosis.

The presence of partial pollen sterility shown in the amphidiploid clone of 'Fragrant Pink Improved' might be due to both unstable meiotic chromosome configurations at metaphase I and unusual nuclear divisions in meiosis in pollen mother cells, especially cytomixis.

Introduction

Meiosis in the pollen mother cells of certain *Camellia* taxa has been studied in detail with reference to the pairing behavior of chromosomes at metaphase I (KONDO 1975, 1977 a, 1977 b, 1977 c, 1978). Bivalent formation in most of the cultivated diploid species and cultivars of *Camellia* studied is found to be complete and normal at metaphase I (KONDO 1975, 1977 a). Thirteen F₁ hybrid combinations between diploid species (both intra- and inter-sectional) of *Camellia* showed similar patterns of meiotic chromosome configurations of 15_{II} and high chiasma frequencies which deviated only slightly from those of their parents (KONDO 1975, 1977 a). However, pollen sterility or partial sterility is present in those hybrid clones (ACKERMAN 1969, 1971; KONDO 1975). These results suggest the presence of either changes in gene constitution without chromosomal changes or cryptic structural differences, or both.

Among the diploid F_1 hybrids between diploid *Camellia japonica* subsp. *rusticana* (PI 228187) and diploid *C. lutchuensis* (PI 226756), a horticulturally meritorious clone (PI 315906) has been named and registered as 'Fragrant Pink' by the second author. The two parental species are somewhat distantly related to each other, which apparently is responsible for the low percentage (5%) of normal appearing pollen (ACKERMAN and DERMEN 1972). Fortunately, a fertile amphidiploid clone of this cultivar, namely 'Fragrant Pink Improved' was recently synthesized by colchicine treatment by ACKERMAN and DERMEN (1972). The amphidiploid method is employed to clarify the differential degree of the genomic affinity between *C. japonica* subsp. *rusticana* and *C. lutchuensis*.

Materials and Methods

All the original materials are cultivated and deposited with the accession numbers of the United States Department of Agriculture Plant Introduction System (PI numbers) in U.S. Plant Introduction Station, Science and Education Administration, Federal Research, United States Department of Agriculture, Glenn Dale, Maryland, U.S.A. The female parent, PI 228187 *C. japonica* subsp. *rusticana* (Fig. 1-a), has peony form flowers, 8.8 cm across by 3.8 cm high, medium red but no fragrance. The male parent, PI 226756 *C. lutchuensis* (Fig. 1-b), has single white flowers, 3.1 cm across, inconspicuous, but highly fragrant. *Camellia japonica* subsp. *rusticana* is placed taxonomically in Sect. *Camellia* and *C. lutchuensis* in Sect. *Theopsis* (SEALY 1958). 'Fragrant Pink', thus is an intersectional hybrid clone. 'Fragrant Pink' (Fig. 1-c) has loose, peony form flowers 5.6 cm across by 3.1 cm high, medium pink, with ten petals and 12 petaloids and is highly fragrant. This cultivar has an average of only 5% normal-appearing pollen. Some individuals of 'Fragrant Pink' were treated with colchicine in attempts to double the chromosome number, which led to the synthesis of the amphidiploid clone named 'Fragrant Pink Improved' (B 60345, Fig. 1-d) (ACKERMAN and DERMEN 1972). 'Fragrant Pink Improved' has flowers 6.7 cm across by 3.8 cm high, and compared to 'Fragrant Pink', they have darker pink petals of heavier substance, longer and stouter filaments and styles, and an average of 77% normal-appearing pollen (ACKERMAN and DERMEN 1972).

Root tips were prefixed in 0.2% aqueous solution of colchicine for two hours at room temperature (20°C) and then fixed in 95% ethanol : glacial acetic acid in a ratio of 3 to 1. Smear preparation of root tips were made in aceto-carmine for mitotic chromosome observations by the second author.

Anthers were fixed in regular Carnoy's solution (95% ethanol : chloroform : glacial acetic acid in a ratio of 6 : 3 : 1) by the second author. Smear preparations of anthers were made in aceto-orcin for meiotic chromosome observation by the first author. The figures on chromosome configurations were based on an analysis of 30 complete pollen mother cells at the first metaphase of meiosis for each hybrid. Chiasma frequency, expressed as the average number per cell and per bivalent chromosome for the cells, was scored.

Results and Discussion

Mitotic divisions are presented of the parental species, 'Fragrant Pink', and 'Fragrant Pink Improved' (Fig. 2-a thru d), as background pedigree information for the data presented

Fig. 1. Flowers (X 0.6) of: a. *Camellia japonica* subsp. *rusticana* (PI 228187); b. *C. lutchuensis* (PI 226756); c. *C. cv.* 'Fragrant Pink' (PI 315906=*C. japonica* subsp. *rusticana* × PI 226753 *C. lutchuensis*) d. *C. cv.* 'Fragrant Pink Improved' (B 60345=amphidiploid clone appeared from PI 315906 'Fragrant Pink').

Fig. 2. Mitotic chromosomes (X 1525) of: a. *Camellia japonica* subsp. *rusticana* (PI 228187), $2n=30$; b. *C. lutchuensis* (PI 226750), $2n=30$; c. *C. cv.* 'Fragrant Pink' (PI 315905=PI 228187 *C. japonica* subsp. *rusticana* $\times$ PI 226756 *C. lutchuensis*), $2n=30$; d. *C. cv.* 'Fragrant Pink Improved' (B 60345=amphidiploid clone appeared from PI 315906 'Fragrant Pink'), $2n=60$.

Table 1. Chromosome configurations and number of chiasmata at metaphase I in pollen mother cells of the amphidiploid clone ('Fragrant Pink Improved') and its parental strains

Taxon and clone identification	Configurations				No. of cells	Xta range	Mean no. of Xta per PMC	Mean no. of Xta per bivalent	Total no. of PMC's observed
	I	II	III	IV					
<i>C. japonica</i> subsp. <i>rusticana</i> (PI 228187)		15			30	30	30	2.0	30
<i>C. lutchuensis</i> (PI 226756)		15			30	30	30	2.0	30
<i>C. cv.</i> 'Fragrant Pink' (PI 315906=PI 228187 <i>C. japonica</i> subsp. <i>rusticana</i> $\times$ PI 226756 <i>C. lutchuensis</i>)		15			30	27~30	29	1.9	30
<i>C. cv.</i> 'Fragrant Pink Improved' (B 60345=amphidiploid clone appeared from PI 315906 'Fragrant Pink')		20		5	12	44~53	54	1.8	30
		24		3	4				
		26		2	3				
	1	26	1	1	2				
		28		1	7				
	1	28	1		2				

Xta=chiasmata; PMC=pollen mother cell

Configurations identified: I=univalent; II=bivalent; III=trivalent; IV=quadrivalent

on the meiotic behavior of these same clones. Observations of photomicrographs did not disclose any numerical or fragmentary aberrations of chromosomes. Aneuploidy has been observed among *Camellia* interspecific hybrids (ACKERMAN 1973), but was not evident in these studies.

Meiosis in the pollen mother cells of *Camellia japonica* subsp. *rusticana* (PI 228187), *C. lutchuensis* (PI 226756), *C.* 'Fragrant Pink' (PI 315906=PI 228187 *C. japonica* subsp. *rusticana* × PI 226756 *C. lutchuensis*) and the amphidiploid clone of 'Fragrant Pink', 'Fragrant Pink Improved' (B 60345) was studied in detail with reference to the chromosome configurations at metaphase I. In the diploid taxa the comparative study of meiosis did not reveal any particular marked differences in chromosome pairing (Table 1). They showed 15 bivalent chromosomes at metaphase I of meiosis in pollen mother cells which were mostly ring-shaped, strongly conjugated at both equal end. In 'Fragrant Pink' chiasma frequency did not deviate significantly from those of its parents (Table 1). Thus, these results were quite similar to the previous report (KONDO 1977a). The result of chromosome associations in the amphidiploid clone, 'Fragrant Pink Improved' is given in Table 1. Among various chromosome configurations shown in 30 pollen mother cells, the commonest

Fig. 3. Photomicrographs (X 822) of: a. Meiotic chromosomes in pollen mother cell of the amphidiploid 'Fragrant Pink Improved', $5_{IV}+20_{II}$. b. Haploid set of meiotic chromosomes in pollen mother cell found in a few anthers of the amphidiploid 'Fragrant Pink Improved', 15_{II} . c. Trinuclear condition in pollen mother cell found in the amphidiploid 'Fragrant Pink Improved'. d. Cytomixis in pollen mother cells found in the amphidiploid 'Fragrant Pink Improved'.

chromosome configuration at metaphase I was $5_{IV}+20_{II}$ (Fig.3-a). The mean number of chiasmata per bivalent was 1.8. Although the diploid hybrid of *C. japonica* subsp. *rusticana* $\times$ *C. lutchuensis* showed a fairly regular meiotic pairing and the chiasma frequency deviated only slightly from those of the parents, its amphidiloid clone showed still a lower number of multivalents and lower chiasma frequency than expected. This fact implies that the degree of genome homology between these two diploid species could not be sufficiently close to show high number of multivalents and high chiasma frequency in their amphidiploid as it would in an autotetraploid. In contrast, no meiotic chromosome configuration of 30_{II} was shown in the amphidiploid clone, that was quite different from the common appearance of 30_{II} in the naturally found tetraploid species, *Camellia granthamiana* Sealy and certain tetraploid hybrids between certain diploid and hexaploid species (KONDO 1978). Thus, *Camellia japonica* subsp. *rusticana* and *C. lutchuensis* could have some degree of mutually common homologous segments on each matched chromosome between the two species and their genomes are not completely distinct, and thus merge into one another within some categories. Between the chromosomes of *Camellia japonica* subsp. *rusticana* and *C. lutchuensis* either cryptic structural difference or difference of multiple gene substitution of the gene constitution without any chromosomal structural difference might be present.

During the course of investigation, two percent of pollen mother cells had the meiotic chromosome configuration of 15_{II} (the haploid set of chromosomes) (Fig.3-b). This percentage appears to support the ACKERMAN and DERMEN's observation of the presence of up to three percent small normal-appearing pollen grains (ACKERMAN and DERMEN 1978). Thus, it appears that 'Fragrant Pink Improved' produces some viable pollen at the haploid level as well as the diploid pollen normally expected of a tetraploid. This occurrence might be due to the colchiploid branches of 2-4-4 cytochimera composition of 'Fragrant Pink Improved'.

Some unusual nuclear divisions were observed in the pollen mother cells of the amphidiploid 'Fragrant Pink Improved' studied, such as cytomixis (Fig.3-d), multinuclear division (Fig.3-c) and laggards in pollen mother cells. Among them, cytomixis commonly appeared; some anthers densely packed cytomictic pollen mother cells. Pollen grains, which appeared after these unusual nuclear divisions were produced, could contain different amounts of chromosomes and could be genetically unbalanced and thus would not usually be viable. Even if they were viable they would likely fail to complete fertilization (CLAUSEN 1926~27; KHAN 1951; NAKAJIMA 1953).

Literature Cited

- ACKERMAN, W. L. 1969. Interspecific and intergeneric hybridization of *Camellia*. Ph. D. Dissertation, Univ. Maryland, pp.128.
- 1971. Genetic and cytological studies with *Camellia* and related genera. Tech. Bull. 1427, USDA, U. S. Gov. Print. Office, Washington, D. C., pp.115.
- 1973. Aneuploidy in the *Camellia*. J. Hered. **64** : 197~202.
- and H. DERMEN 1972. A fertile colchiploid from a sterile interspecific *Camellia* hybrid. J. Hered. **63** : 54~59.
- and ——— 1978. Doubling the chromosomes of sterile interspecific hybrid 'Fragrant Pink' to make it fertile: In Japanese Camellia Society Ed., Encyclopedia of *Camellia*, Kodansha Publ. Co., Tokyo, pp.456.

- CLAUSEN, J. 1926-27. Genetical and cytological investigations on *Viola tricolor* L. and *V. arvensis* Murr. *Hereditas* 8: 1~156.
- KHAN, S. I. 1951. Pollen sterility in *Solanum tuberosum* L. *Cytologia* 16: 124~130.
- KONDO, K. 1975. Cytological studies in cultivated species of *Camellia*. Ph.D. Dissertation, Univ. N. C., Chapel Hill, pp. 260+x.
- 1977 a. Cytological studies in cultivated species of *Camellia*. I. Diploid species and their hybrids. *Japan. J. Breed.* 27: 28~38.
- 1977 b. Cytological studies in cultivated species of *Camellia*. II. Hexaploid species and their hybrids. *Japan. J. Breed.* 27: 333~344.
- 1977 c. Chromosome numbers in the genus *Camellia*. *Biotropica* 9: 86~94.
- 1978. Cytological studies in cultivated species of *Camellia*. III. Tetraploid species and hybrids between diploid species and hexaploid species. *Japan. J. Breed.* 28: 197~204.
- NAKAJIMA, G. 1953. A cytological study on the intergeneric F_1 hybrid between *Triticum sphaerococcum* and *Secale cereale*. *Cytologia* 18: 43~49.
- SEALY, J. R. 1958. A revision of the genus *Camellia*. *Roy. Hort. Soc., London*, pp. 239.

ツバキ属栽培種における細胞学的研究

VI. 二倍体種間交雑およびコルヒチン処理により作出された
複二倍体栽培品種 'Fragrant Pink Improved'

近藤勝彦*・ウィリアム L. アッカマン**

(*広島大学総合科学部)

(**アメリカ合衆国農務省樹木園)

1960 年より始められた合衆国農務省のツバキ属育種計画シリーズの一部として、二倍体種ユキツバキ (PI 228187 *C. japonica* subsp. *rusticana*) × ヒメサザンカ (PI 226756 *C. lutchuensis*) の二倍体種間 (節間) 雑 'Fragrant Pink' を作出し、その後この栽培品種のコルヒチン処理により、複二倍体植物 ($2n=60$) 'Fragrant Pink Improved' (B 60345) を育成した。本報告は、'Fragrant Pink Improved' とその栽培品種作出に関与したすべての系統の花粉母細胞減数分裂における染色体対合に関する観察結果である。

二倍体ユキツバキ (PI 228187) とヒメサザンカ (PI 226756) において、減数分裂 第一中期で検鏡したすべての花粉母細胞に 15 個の二価染色体がみられた。それぞれの二価染色体で、キアズマは常に 2 個ずつみられた。これら 2 種の花粉稔性はほぼ 100% と高い。それら 2 種間にできた二倍体雑種 'Fragrant Pink' においてもまた 15 個の二価染色体がすべての花粉母細胞減数分裂中期で観察された。それぞれの二価染色体で、キアズマの頻度が平均 1.9 と、両親の頻度に比べてほんのわずかな落ちることが確認された。しかしこの雑種は平均 4% という非常に低い花粉稔性を示した。

'Fragrant Pink' のコルヒチン処理により得た複二倍体栽培品種 'Fragrant Pink Improved' の花粉母細胞減数分裂中期の染色体対合を調べると、二価染色体形成が常に優位を占めて、最も普通にみられた対合式は $5_{IV} + 20_{II}$ であった。これ以外にもさまざまな対合式がみられた。これにともない、キアズマの頻度も幅の広い変化を示した。この複二倍体は、平均 77% のかなり高い花粉稔性をもつ。

以上の結果から、ユキツバキとヒメサザンカは、分類学上の節の区分を越えて、互いに比較的高い相同関係にあり、同一カテゴリーに入れるべきであろうが、それらの染色体の多くには種々の程度の微細な構造的差異が存在すると思われる。