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Interspecific Hybridization with *Camellia chrysantha*

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To breed new yellow flowered camellias, Camellia ***chrysantha*** var. *chrysantha* and var. *microcarpa* were adopted to cross with 9 other ***Camellia*** species. Thirteen embryos obtained from 247 pollinations were cultured on Anderson's medium. Six of them developed to plantlets, but only 3 survived after habituation. Two plants were from C. *crapnelliana* and 1 from C. ***granthamiana,*** both crossed with C. ***chrysantha*** var. *microcarpa*. Morphological and isozyme studies confirmed them truly interspecific.

INTRODUCTION

The yellow flowered camellia, *Camellia* ***chrysanthu,*** was introduced to Japan and the western nations from the People's Republic of China in 1979 (Hagiya, 1982). Although the yellow but small flowers are usually nodding and hidden under the large leaves, it is still considered to be the most promising parent to breed new camellias of large yellow or orange flowers. Due to its cross-incompatibility with other camellias (Hagiya, 1982), only combination of C. *japonica* x ***C. chrysanthu*** (Yamaguchi et al., 1987 ; Uemoto et al., 1988), C. *reticulata* x ***C. chrysanthu*** and C. *pitardii* var. *yunnanensis* x ***C. chrysanthu*** (Xia, 1984) and C. ***vietnamensis*** x ***C. chrysanthu*** (Nadamitsu et al., 1986) have been successful. Since flowers of desirable colors and shapes have not yet been obtained, other interspecific hybrids of C. ***chrysantha*** are required.

Embryo culture has been a great aid in obtaining F_1 hybrids in a wide range of genera such as *Camellia* (Yamaguchi et al., 1987), *Lilium* (Asano and Myodo, 1977, 1978), *Helianthus* (Chandler and Beard, 1983), *Cucurbita* (Wall, 1954), etc., in all of which the interspecific F_1 plants are hardly obtainable by conventional methods of crossing. Liang et al. (1986) reported that hybrid embryos of C. ***pitardii*** var. ***yunnanensis x C. chrysanthu*** aborted during plumule differentiation. To avoid abortion of hybrid embryo and to shorten germination time, we adopted embryo culture.

Comparisons of morphological characteristics and isozyme banding patterns of leaf tissue of the hybrids were executed to confirm them interspecific.

MATERIALS AND METHODS

Camellia species used in this experiment are listed in Table 1. They were grown in a greenhouse except for 3 cultivars of C. *japonica* which were planted in the open field. The flowers of pistilate parents were emasculated and covered with paper bags

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Table 1. Listing of subgeneric sections, species and varieties, and their 2n chromosome number of camellias used for hydridization.

Subgeneric sections	Species	2n chromosome number*
Archecamellia	C. ***granthamiana*** Sealy	60
Camellia	C. *japonica* Linn.	30
	C. ***chekiangoleosa*** Hu	30
Chrysantha	C. ***chrysan*** *tha* var. ***chrysantha*** Tuyama	30
	C. *chrysantha* var. *microcarpa* Tuyama	30
Furfuracea	C. *crapnelliana* Tutcher	30
	C. *furfuracea* Cohen Stuart	30
	C. *gigantocarpa* **Hu**	30
Luteoflora	C. *meiocarpa* Hu	60
Oleifera	*C.* ***miyagii*** Makino	90
	C. *oleifera* Abel	90

*Hakoda, N. and N. Adachi, 1985

one day before anthesis, and then pollinated on the next day with fresh pollen from C. ***chrysantha*** var. ***chrysanthu*** or C. ***chrysanthu*** var. *microcarpa* from November 15, 1986 to February 10, 1987. All pollinated pistils were covered again with paper bags for isolation.

Seeds were taken out from capsules after harvest from June 27 to August 3, 1987, and number of capsules set and fruiting percentage (number of capsules set/number of flowers pollinated) were recorded. Embryo development and the number were examined on a clean-bench. Immature embryos of various size, ranging from 1 to 8 mm in diameter, were excised and placed on Anderson's medium (Anderson, 1984), supplemented with 30 g/Z sucrose and 2 g/Z gellan gum but not with any plant hormone. The medium was adjusted to pH 5.5 and distributed 10 ml to each tube before autoclaving (121°C, 15 min). The cultures were incubated at 25°C under continuous light supplied by cool white fluorescent lamps, giving an intensity about 800 lux, for approximately six months. They were then subcultured on 20 ml fresh medium of the same constitution for additional three months. Plantlets obtained in test tubes were taken out and rinsed with tap water to remove gellan gum. They were transplanted in sterile vermiculite in 9 cm pots in diameter and grown in a shaded greenhouse.

Phosphoglucose isomerase (PGI) of the mature, fully expanded leaves of habituated F_1 progenies and of their parents were resolved by starch gel electrophoresis. Extraction and staining procedures, and gel and buffer compositions followed the methods of Wendel and Parks (1982).

RESULTS AND DISCUSSION

Among 3 cultivars of C. ***japonica*** pollinated by C. ***chrysanthu*** var. ***chrysantha,*** only 'Hatsuarashi' showed 12% fruit set, while the others bore nothing (Table 2). There seems to be some cultivar difference in cross compatibility within the species, and more cultivars should be tried for obtaining hybrids with this species. ***Camellia***

Table 2. Fruits, seeds and embryos obtained from 7 ***Camellia*** species pollinated by C. ***chrysantha*** var. ***chrysantha.***

Species and cultivars	No. of pollinated flowers	Capsule set No. (%*)	No. of seeds obtained	No. of embryos obtained
C. japonica				
'Muruishibori'	27	0 (0%)	0	0
'Shiragiku'	32	0 (0%)	0	0
'Hatsuarashi'	34	4 (12%)	6	0
C. ***chekiangoleosa***	11	7 (64%)	42	
C. crapnelliana	3	0 (0%)	0	0
C. *furfuracea*	3	1 (33%)		0
C. *gigantocarpa*		2 (29%)	5	0
C. *meiocarpa*	9	2 (22%)	3	0
C. *oleifera*	2	1 (50%)	1	1

*No. of capsules set / No. of pollinated flowers

Table 3. Fruits, seeds and embryos obtained from 6 ***Camellia*** species pollinated by C. *chrysantha* var. *microcarpa.*

Species	**No.** of pollinated flowers	Capsule set ***No.*** (%*)	No. of seeds obtained	No. of embryos obtained
C. ***crapnelliana***	***7***	***2 (29%)***	***7***	***2***
C. furfuracea	***22***	11 **(50%)**	**22**	**0**
C. gigantocarpa	***24***	***8 (33%)***	***27***	***0***
C. *granthamiana*	10	2 (20%)	3	3
C. ***miyagii***	***33***	18 (55%)	14	6
C. oleifera	23	1 (4%)	1	0

'No. of capsules set / No. of pollinated flowers

chekiangoleosa gave the highest percentage of fruit set and 42 seeds were obtained, whereas the other species-except C. ***crapnelliana,*** which bore nothing-set only 1 to 5 seeds. Unfortunately, most of the seeds were empty or endosperm without embryo, indicating that some of the embryos degenerated and disappeared at a very early stage. Two embryos were obtained from the total of 58 seeds, one of which was the progeny of C. ***chekiangoleosa*** and the other was the offspring of C. *oleifera* both of which had been crossed with C. ***chrysantha*** var. ***chrysantha.***

Six species of *Camellia* were selected to be crossed with C. ***chrysantha*** var. *microcarpa*. The combinations of C. *furfuracea* X ***C. chrysanthu*** var. *microcarpa* and C. ***miyagii*** x ***C. chrysanthu*** var. *microcarpa* gave the best results with more than 50% of fruit set, while moderate or poor percentage of fruit set was obtained in other crossings, among which C. *oleifera* x ***C. chrysanthu*** var. *microcarpa* showed the lowest fruit set. Eleven embryos were obtained from 74 seeds. Two of them were the offspring of C. ***crapnelliana*** x ***C. chrysantha*** var. *microcarpa*, ***3*** obtained from the combination of C. *granthamiana* x ***C. chrysanthu*** var. *microcarpa*, and the other 6 were progenies of C. ***miyagii*** x ***C. chrysanthu*** var. *microcarpa* (Table 3). It seems that C. ***chrysantha*** var. *microcarpa* is more compatible with other camellias than the variety of

chrysantha. Even though this bears smaller flowers than the var. ***chrysantha,*** it may be more useful in cross breeding.

Totally, 13 embryos, some were malformed, from the crossings with either var. ***chrysanthu*** or *microcarpa* were cultured. Some of them died very soon during the embryo culture, but six plantlets survived (Table 4).

Hilsman (1966) and Savige (1967) reported that the most successful crosses in ***Camellia*** breeding were found within the same section, particularly the species with the same ploidy. Hagiya (1982, 1986) also found that the ***Camellia*** species belonging to the same section are more compatible than the species of different sections, and in cases of intersectional crosses, those with the same ploidy are more compatible than those with different chromosome numbers. Ackerman (1973) showed that species of certain sections are more closely related to each other than to species of other section based on the cross compatibility between their representative species. Compatibility of C. ***chrysanthu*** and other ***Camellia*** species was very low in our experiments. This could be attributed to the distantly intersectional crosses. However, the compatibility of C. ***chrysanthu*** crossing with the species in different sections with different ploidies was not always lower than that in different sections with the same ploidies in our crossing tests.

In order to bring embryos up into healthy seedlings, Liang ***et al. (1986)*** mentioned that it was necessary to isolate hybrid embryos of C. *pitardii* var. ***yunnanensis x C. chrysanthu*** before plumule differentiation to culture ***in vitro.*** In our study, the hybrid embryos obtained were not uniform in size, and we observed that the size of hybrid embryos was one of the important factors affecting their survival. All the embryos of which the size was less than 2 mm in diameter died during the culture. To ensure a hybrid embryo of camellia to both survive and develop as a plant, the embryo must be at least larger than 3 mm, which is about 1/4 of a normal size. Progressed culture technique should be studied to rescue these small and incompletely developed embryos.

Generally, a camellia is two-cotyledoned, but C. ***chrysanthu*** has 3 to 6 cotylendons, in which 3 to 4 are more commonly found. The hybrid embryos had 2, 3 or 4 cotyledons (Fig. 1). Their initial color was cream and gradually became reddish-purple or dark greenish-purple during the culture in test tubes. The shoots were also dark purple like

Table 4. Results of embryo culture of F, hybrids.

Parentage		Number of embryos cultured	Number of plantlets obtained	Survival* rate
C. chekiangoleosa	x ***C. chrysanthu*** var. ***chrysantha***	1	1	1.00
C. *oleifera*	× ***C. chrysantha*** var. ***chrysanthu***	1	0	0.00
C. *crapnelliana*	x ***C. chrysantha*** var. *microcarpa*	***2***	***2***	1.00
C. gran ***thumiana***	× ***C. chrysantha*** var. *microcarpa*	***3***	1	***0.33***
C. miyagii	×*C.* ***chrysanthu*** var. *microcarpa*	***6***	***2***	***0.33***

*Number of plantlets obtained / number of embryos cultured

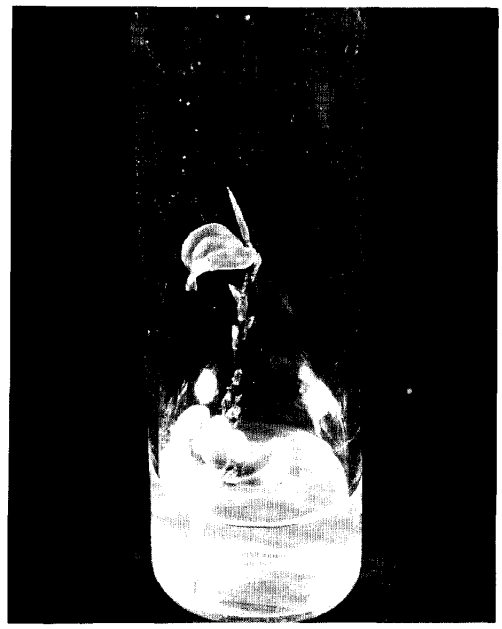

Fig. 1. A plantlet from a four cotylendon embryo of C. *granthamiana* x ***C. chrysanthu*** var. *microcarpa.*

the color of C. *chrysantha* shoot.

Because of distant hybridization, some hybrid seedlings were very weak. After being planted out, half of the plantlets obtained from embryo culture gradually died. The survivors, however, including two F_1's of C. *crapnelliana* x ***C. chrysanthu*** var. *microcarpa* and one F_1 of C. *granthamiana* x ***C. chrysanthu*** var. *microcarpa* grew vigorously in the greenhouse.

Results of isozyme analysis of their leaves are given in Fig. 2. One band of PGI isozyme migrated anodally at 36 mm in F_1's and their pollen parent C. ***chrysanthu*** var. *microcarpa*, but not in any mother parent (Fig. 2). The isozyme banding pattern and the morphological characteristics prove that these offspring plants are truly interspecific hybrids.

Our results suggest that there are possibilities to obtain more interspecific hybrids than those already obtained with C. ***chrysanthu*** by crossing and embryo culture. In this experiment, the hybridization was carried out in only one direction, the reversed direction and other combinations with C. ***chrysanthu*** should be tried.

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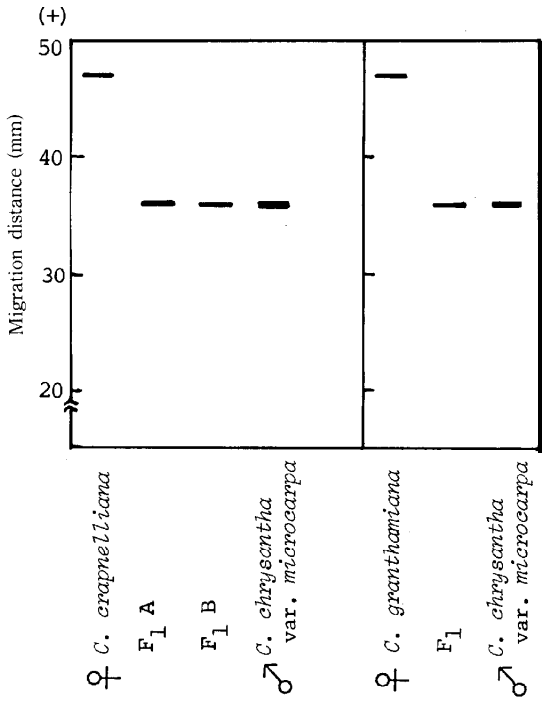

Fig. 2. Schematic presentation of the zymogram of phosphoglucose isomerase in 3 F_1 progenies and their parents.

Fig. 3. Plants derived from embryos of C. *crapnelliana* x ***C. chrysantha*** var. *microcarpa* (left 2) and C. ***granthamiana*** x ***C.*** *chrysantha* var. *microcarpa* (right).

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