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AN EASY TECHNIQUE TO ISOLATE TRUE F_1 HYBRIDS FROM THE SEEDLING POOLS OBTAINED FROM ARTIFICIAL CROSS-POLLINATION DIRECTLY INVOLVED YELLOW-FLOWERED CAMELLIA CHRYSANTHA

Xia Lifang, Gu Zhijian, Na Haiyan

(Kunming Institute of Botany, Academia Sinica, China)

Kondo Katsuhiko*

(Faculty of Integrated Arts and Sciences, Hiroshima University, Japan)

Abstract Among 56 seedlings with red-colored cotyledons and hypocotyls obtained after hand pollination betweed *Camellia pitardii* var. *yunnanica* (hexaploid) and *C. chrysantha* (diploid), 55(98.2%) were of true tetraploid-hybrids ($X^2 = 0.0128$, D. f. = 1, $0.95 > p > 0.90$), while among 50 seedlings with yellowish-white-colored cotyledons and hypocotyls, 13(26%) were of true tetraploid-hybrids ($X^2 = 27.38$, D. f. = 13, $0.02 > p > 0.01$). In contrast, among 78 seedlings with red-colored cotyledons and hypocotyls obtained after hand pollination between *C. reticulata* (hexaploid) and *C. chrysantha*, 77(98.7%) were of true tetraploid-hybrids ($X^2 = 0.0128$, D. f. = 1, $0.95 > p > 0.90$), while among 78 seedlings with yellowish-white-colored cotyledons and hypocotyls, 21 (26.9%) were of true tetraploid-hybrids ($X^2 = 41.65$, D. f. = 24, $0.02 > p > 0.01$). This red-colored pigmentation in the majority of the hybrid seedlings could be a hereditary character of the paternal parent *C. chrysantha* caused by a phenomenon of xenia. Another characteristic of multi-cotyledons (more than three cotyledons) in the majority of the seeds or seedlings in *C. chrysantha* was exhibited imperfectly by xenia in some hybrid seeds or seedlings. Thus, isolating seedlings with red-colored cotyledons and hypocotyls is a promising method to screen readily true F_1 hybrids involved *C. chrysantha* at their germination stage.

Key words Artificial hybrids; *Camellia chrysantha*; Cotyledons; Hypocotyls

Since a yellow-flowered camellia, *Camellia chrysantha* endemic to Yongning-Dongxin area, Guangxi Province, the People's Republic of China, was introduced to cultivation approximately ten years ago, various camellia breeders

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* Exchange Professor of Japan Society for Promotion of Science to Academia Sinica during the fiscal year of 1985.

in the world have become interested in producing new, large, yellow-flowered camellias by artificial hybridization. However, most of all interspecific cross-combinations involved *C. chrysantha* produced aborted seeds or seeds with partially developed embryos and only a few combinations produced viable seeds, most of which either failed to germinate or grew abnormally and subsequently died [2, 6, 7]. Hybrid combinations involved *C. chrysantha* and leading to the development of plants which have not yet been identified as true hybrids up to the present are listed as follows: *C. japonica* × *C. chrysantha* [3, 5] (Yamaguchi, unpublished), *C. pitardii* var. *yunnanica* × *C. chrysantha* and its reciprocal cross-combination [7], *C. reticulata* × *C. chrysantha* [6, 7] and its reciprocal cross-combination [7], *C. vietnamensis* × *C. chrysantha* [4], and *C. yunnanensis* × *C. chrysantha* (Xia *et al.*, unpublished). Among them, only the plants obtained in the *in vitro* culture of the cotyledons of *C. vietnamensis* × *C. chrysantha* were determined karyomorphologically as the true hybrids [4]. Since the camellia plants are woody, relatively slow growers, need large spaces for cultivation, and take 3 — 4 years to get the first flowers after germination (human labor, time and space consuming ornamental-plants), it is necessary for camellia breeders to identify and isolate true hybrid plants in the offspring pools at their seedling or juvenile stages. Possible methods for identifying true hybrids in general are of karyomorphological comparisons, isozyme and other chemical comparisons and phytoserological comparisons, if both parents are distinctly different in those characters from each other.

In the course of our interspecific hybridization program, an easy technique found to isolate F_1 hybrids in the seedling collection involved *C. chrysantha* is here reported as a part of breeding technique.

Materials and methods

The pink to pinkish-white flowered, hexaploid *Camelli pitardii* var. *yunnanica* and *C. reticulata* were used as the female parents and the yellow-flowered *C. chrysantha* was used as the male parent. The interspecific crosses of these combinations were relatively easy in comparison with other cross combination involving *C. chrysantha* and resulted producing numerous seeds in 1985.

Several weeks after they were germinated in fall-winter, their seed-coats were removed and determined coloration on their cotyledons and hypocotyls (Fig. 1A, B) and counted cotyledon number. Fifty-six main-root tips from

seedlings with red-colored cotyledons and hypocotyls and 50 from seedlings with yellowish-white-colored cotyledons and hypocotyls in the seedling pool of *C. pitardii* var. *yunnanica* × *C. chrysantha* and 78 from seedlings with red-colored cotyledons and hypocotyls and 78 from seedlings with yellowish-white-colored cotyledons and hypocotyls in the seedling pool of *C. reticulata* × *C. chrysantha* were randomly chosen before they were treated in 0.003 *M* hydroxyquinoline at 20°C for four hours. Then, they were fixed, hydrolyzed, stained and squashed by standard methods for observation of metaphase chromosomes.

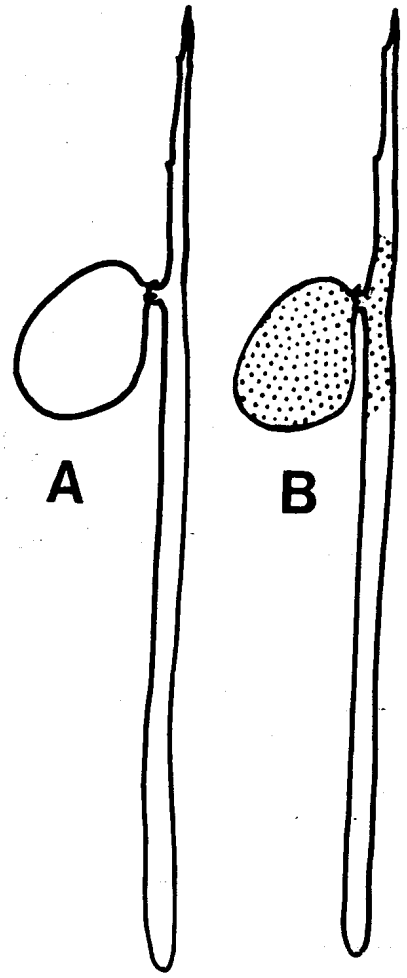

Fig. 1 Diagrams of seedlings obtained by hand pollination between *Camellia pitardii* var. *yunnanica* and *C. chrysantha* and between *C. reticulata* and *C. chrysantha* and within respective parental species. A. Representative seedling figuring yellowish-white-colored cotyledons and hypocotyl of two maternal parents and mixture of hybrids and non-hybrids. B. Representative seedling figuring red-colored cotyledons and hypocotyl of the paternal *C. chrysantha* and mostly hybrids. The seedlings of *C. chrysantha* and some hybrids exhibit actually deep red to purplish-red-colored cotyledons, hypocotyls, and even shoots.

Results and discussion

The results were tabulated in Table 1. The seedlings of the diploid *Camellia chrysantha* showed deep red to purplish-red colored cotyledons and hypocotyls. Yellow-color pigments in flowers of *C. chrysantha* are of characterization of quercetin, kaempferol and so on of the flavonoids [1] and thus, deep red to purplish-red color pigments in cotyledons and hypocotyls of the seedlings could be of characterization of chalcone or other constituents of the flavonoids. The seedlings of the hexaploid *C. pitardii* var. *yunnanica* and the hexaploid *C.*

reticulata showed yellowish-white colored cotyledons and hypocotyls. Pink to pinkish-white flower pigments in the hexaploid *C. pitardii* var. *yunnanica* and *C. reticulata* are of characterization of anthocyan or other constituents of the flavonoids. However, the yellowish-white-colored cotyledons and hypocotyls of the two species could not be caused by any of the flavonoids produced.

The red or purplish-red color pigment in the cotyledons and hypocotyls of *C. chrysantha* could be hereditable into F_1 hybrid seedlings by a phenomenon of xenia by double fertilization, which imprints color characteristics in embryos or embryo-origined organs of F_1 hybrid seeds and seedlings (Fig. 1; Table 1). Among 56 seedlings with red-colored cotyledons and hypocotyls obtained after hand pollination between *C. pitardii* var. *yunnanica* and *C. chrysantha*, 55 (98.2%) were of true tetraploid-hybrids ($X^2=0.0128$, D. f. $=1$, $0.95>p>0.90$). In contrast, among 78 seedlings with red-colored cotyledons and hypocotyls obtained after hand pollination between *C. reticulata* and *C. chrysantha*, 77 (98.7%) were true tetraploid-hybrids ($X^2=0.0128$, D. f. $=1$, $0.95>p>0.90$). However, the seedling collection of *C. pitardii* var. *yunnanica* × *C. chrysantha* which had always yellowish-white cotyledons and hypocotyls included a few true tetraploid-hybrids (13/50 individuals or 26%; $X^2=27.38$, D. f.$=13$, $0.02>p>0.01$), and that of *C. reticulata* × *C. chrysantha* included a few true tetraploid-hybrids (21/78 or 26.9%; $X^2=41.65$, D. f.$=24$, $0.02>p>0.01$).

Table 1. Coloration and numbers of cotyledons of F_1 hybrid-seedlings between Camellia pitardii var. yunnanica and C. chrysantha and between C. reticulata and C. chrysantha and of seedlings of the parental species

Seedlings	Coloration of cotyledons and hypocotyls	Seedling numbers observed	True tetraploid hybrids (2n = 60)	Hexaploid non-hybrids failure (2n = 90)	Seedling numbers observed	Cotyledon numbers per seedling		
						2	3	4 — 6
C. chrysantha (diploid; 2n = 30)	deep red to purplish-red	10	—	—	10	3	4	3
C. pitardii var. *yunnanica* (hexaploid; 2n = 90)	white to yellowish-white	10	—	—	10	10		
C. reticulata (hexaploid; 2n = 90)	white to yellowish-white	10	—	—	10	10		
C. pitardii var.	red	78	77	1	65	61	4	
yunnanica × *C. chrysantha*	white to yellowish-white	50	13	37	52	51	1	
C. reticulata ×	red	78	77	1	29	23	6	
C. chrysantha	white to yellowish-white	78	21	56	46	41	5	

The seeds of *C. chrysantha* contained two to six cotyledons, while those of *C. pitardii* var. *yunnanica* and *C. reticulata* contained only two cotyledons as the standard members of the dicot. Thus, the seeds of F_1 hybrids of *C. pitardii* var. *yunnanica* × *C. chrysantha* and of *C. reticulata* × *C. chrysantha* contained mostly two cotyledons but sometimes three cotyledons (Table 1). The F_1 hybrid seeds exhibited incomplete xenia in cotyledon number.

The reason why the xenia causes imperfectly color pigment in cotyledon and hypocotyl and cotyledon numbers in F_1 hybrids involved *C. chrysantha* could be due to hexaploidy of the maternal parents. In other words, if the maternal parents were diploids, they would perform perfectly xenia in their seeds and seedlings after hybridized with *C. chrysantha* pollen.

These results conclude that if young seedlings with red-colored cotyledons and hypocotyls are selected from the seedling pool obtained after interspecific cross-pollination involving *C. chrysantha*, probability of F_1 hybrids must be drastically increased at seedling stage. This understanding of the xenia of genetic relationships of a group of species is one of the best foundations to improve readily and quickly breeding program of yellow-flowered cultivars.

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一种鉴定金花茶人工杂交F_1代杂种苗的简易方法

夏丽芳 顾志建 纳海燕

（中国科学院昆明植物研究所）

近藤胜彦

（日本广岛大学综合科学部）

摘要 本试验选用了两个以金花茶作父本的人工杂交组合F_1代实生苗，经根尖体细胞染色体观察鉴定四倍体杂种。一、云南野山茶（*Camellia pitardii* var. *yunnanica* 六倍体）×金花茶（*C. Chrysantha*二倍体）。结果为：所获56株子叶和下胚轴为红色的F_1代杂种苗中，有55株（98.2%）是真正的四倍体杂种（$X^2=0.0128$，D. f.=1，$0.95>p>0.90$）；所获50株子叶和下胚轴为黄白色的杂种苗中，有13株（26%）为真正四倍体杂种（$X^2=27.38$，D. f.=13，$0.02>p>0.01$）。二、云南山茶花(*C. reticulata*六倍体）×金花茶。结果为：在78株子叶和下胚轴为红色的杂交苗中，有77株（98.7%）为真正四倍体杂种（$X^2=0.0128$，D. f.=1，$0.95>p>0.90$）；而在78株子叶及下胚轴为黄白色的杂种苗中，只有21株（26.9%）是真正四倍体杂种（$X^2=41.65$，D. f.=24，$0.02>p>0.01$）

在多数杂种实生苗中的这种红色素，是因种子直感现象而发生的父本金花茶的一种遗传性状。金花茶的另一特征——多子叶现象（3枚以上），则在一些F_1代杂种苗或杂种种子中表现得不明显。因此，利用F_1代杂种苗子叶和下胚轴所具有的红色特征，在杂种种子萌发期用来鉴别以金花茶为亲本的F_1代杂种的真伪，是一种简便、快速的、有发展前途的方法。

关键词 人工杂交苗；金花茶；子叶；下胚轴

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中国科学院与日本学术振兴会1985年度学者交换合作课题。