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https://doi.org/10.5109/4493

出版情報：九州大学大学院農学研究院紀要. 47 (2), pp.251-255, 2003-02-01. Faculty of Agriculture, Kyushu University
バージョン：
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J. Fac. Agr., Kyushu Univ., **47** (2), 251–255 (2003)

Variation of Self–incompatibility in *Camellia japonica* L. Cultivars

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(*Received October 22, 2002 and accepted November 7, 2002*)

Self–incompatibility in *Camellia japonica* cultivars was compared. From the self–pollination of ten cultivars, 'Hatsu–arashi', 'Kanto–hatsuwarai', 'Aki–no–yama' and 'Fukumusume' revealed the fruit set from 5.0 to 27.0%, and perfect seeds could be obtained from 'Hatsu–arashi' and 'Fukumusume'. Self– and cross–pollination showed similar values in percentages of perfect seeds and of germination. Two seedlings from self–pollination of 'Hatsu–arashi' were conducted for isozyme analysis, and were found to be derived from self–fertilization.

INTRODUCTION

Camellia japonica is a perennial woody plant, and easily propagated by cutting and grafting. As a long time, at least three or four years, is necessary from seed sowing to flowering in this species, there is little information on the inheritance of phenotypic characteristics. A self–incompatible system plays a role to preserve the genetic heterozygosity and prevents from inbreeding depression. It is another cause of the difficulty of genetic studies in this species.

Self–incompatibility systems are based upon the inherited capacity of the flowers to reject its own pollen, and have been studied in many plant species (De Nettancourt, 1977). *Camellia* spp. are generally known to be self–incompatible (Tanaka, 1985), but detailed investigation of the genetic control of self–incompatibility is restricted. Fuchinoue (1969) reported that *Camellia sinensis* has a gametophytic self–incompatible system by self– and cross–pollination. Hakoda and Matsumoto (1979) described that several cultivars bore fruits by self–pollination in *Camellia sasanqua*. Variation of self–incompatibility was investigated in this study for the basis of the genetic control of self–incompatibility in *C. japonica*.

MATERIALS AND METHODS

All the crosses of the present experiments were conducted using mature *C. japonica* trees cultivated in the field of University Forest of Kyushu University, Fukuoka. Eight diploid cultivars, 'Hatsu–arashi', 'Kanto–hatsuwarai', 'Aki–no–yama', 'Asahi–no–mai',

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'Fuku-musume', 'Kami-kakushi', 'Hana-fuuki' and 'Oniji', two diploid wild strains, Hirado Nos. 2 and 3, and two triploid cultivars, 'Kumagai' and 'Akashigata',were used in this investigation. Ten combinations (Table 1) of self-pollination and eight combinations of cross-pollination were carried out from December 29, 1997 to April 4, 1998, and from January 6 to April 16, 1999. The flowers of seed parents were emasculated one or two days before anthesis, and then pollinated with fresh pollen. All pollinated pistils were covered with paper bags for isolation. Number of normally growing fruits from three to four months after pollination was counted.

Fruits were harvested in the middle September, and the seeds were taken out from the fruits. The seeds were classified into "perfect seed" with normal embryo and "empty seed" without embryo. The perfect seeds were then soaked in 100 mgl^{-1} GA_3 solution for 30 minutes at 25 °C after scarification, and sown in moisted sphagnum moss. After germination, the seedlings were transplanted in pumice in plastic pots, 9 cm in diameter, and supplied for isozyme analysis.

Mature leaves were used for enzyme extraction. Each sample was homogenized in pre-cooled extraction buffer prepared as described by Wendel (1983). Crude enzyme extracts were absorbed onto filter paper wicks (Whatman #3, 11×3 mm) and used for electrophoresis. The detailed procedure of enzyme extraction, horizontal starch gel electrophoresis and gel staining followed the methods of Wendel (1983). Enzymes analyzed were alcohol dehydrogenase (ADH; EC 1.1.1.1), glucosephosphate isomerase (GPI; EC 5.3.1.9), isocitrate dehydrogenase (IDH; EC 1.1.1.41), phosphoglucomutase (PGM; 5.4.2.2) and shikimate dehydrogenase (SKDH; EC 1.1.1.25). Genetic basis and allozyme determination of the loci followed the results of Eguchi *et al.* (1991), and the new allele in ADH-2 region, which was first identified in this study, was named a'.

RESULTS

The results of the self- and cross-pollination are represented in Table 1. Four combinations of the cross-pollination, 'Hana-fuuki'×'Kanto-hatsuwarai', 'Hatsu-arashi'× 'Kanto-hatsuwarai', 'Kanto-hatsuwarai'× 'Fuku-musume' and 'Kanto-hatsuwarai'× 'Hatsu-arashi' showed 27.6 to 58.8% fruit set, while the rest combinations brought no fruit set. Most of the self-pollinated crosses resulted no fruit set, whereas 'Aki-no-yama', 'Fuku-musume', 'Hatsu-arashi' and 'Kanto-hatsuwarai' showed 5.0 to 27.0% fruit set. All the fruit from self-pollination of 'Aki-no-yama', however, dropped before maturity, and there were no perfect seeds in the fruit from 'Kanto-hatsuwarai' self-pollination. Six and eight perfect seeds were obtained from self-pollination in 'Fuku-musume' and 'Hatsu-arashi', respectively. Percentages of perfect seeds in the harvested seeds from self- and cross-pollination were the same (51.9%), and the similar values of percentage of germination in the perfect seeds were also recognized in self- (57.1%) and cross-pollination (57.9%).

Most of the seedlings from self-pollination showed depressed growth, and only two seedlings from 'Hatsu-arashi' self-pollination were supplied for isozyme analysis. Among the five enzyme systems analyzed, five regions, ADH-2, GPI-2, IDH, PGM-2 and SKDH, were resolved in this study as shown in Fig. 1. Genotypes of Hatsu-arashi self-1 were the same as those of the parent 'Hatsu-arashi' in *Adh-2*, *Gpi-2*, *Idh* and *Skdh* loci, and

Table 1. Results of self- and cross-pollination in *C. japonica*

Cross combination		No. of flowers pollinated	No. of fruits set	(%)	No. of fruits harvested	No. of seeds			No. of seeds germinated	(%)
Seed parents	Pollen parents					Total	Perfect	Empty		
Self-pollination										
Aki-no-yama	Self	37	10	(27.0)	0	–	–	–	–	–
Asahi-no-mai	Self	25	0	(0)	–	–	–	–	–	–
Fuku-musume	Self	33	2	(6.1)	2	8	6	2	4	(66.7)
Hana-fuuki	Self	20	0	(0)	–	–	–	–	–	–
Hatsu-arashi	Self	16	3	(18.8)	2	18	8	10	4	(50.0)
Hirado No. 2	Self	5	0	(0)	–	–	–	–	–	–
Kami-kakushi	Self	8	0	(0)	–	–	–	–	–	–
Kanto-hatsuwarai	Self	20	1	(5.0)	1	1	0	1	–	–
Akashigata (3x)	Self	20	0	(0)	–	–	–	–	–	–
Kumagai (3x)	Self	27	0	(0)	–	–	–	–	–	–
Total (Mean)		211	16	(7.6)	5	27	14	13	8	(57.1)
Cross-pollination										
Fuku-musume	Kanto-hatsuwarai	4	0	(0)	–	–	–	–	–	–
Hana-fuuki	Kanto-hatsuwarai	17	10	(58.8)	9	83	35	48	2	(5.7)
Hatsu-arashi	Kanto-hatsuwarai	36	20	(55.6)	19	151	78	73	58	(74.4)
Hirado No. 2	Hirado No. 3	12	0	(0)	–	–	–	–	–	–
Hirado No. 3	Hirado No. 2	19	0	(0)	–	–	–	–	–	–
Kanto-hatsuwarai	Fuku-musume	6	3	(50.0)	3	10	9	1	6	(66.7)
Kanto-hatsuwarai	Hatsu-arashi	29	8	(27.6)	7	26	18	8	15	(83.3)
Ooniji	Kanto-hatsuwarai	18	0	(0)	–	–	–	–	–	–
Total (Mean)		141	41	(29.1)	38	270	140	130	81	(57.9)

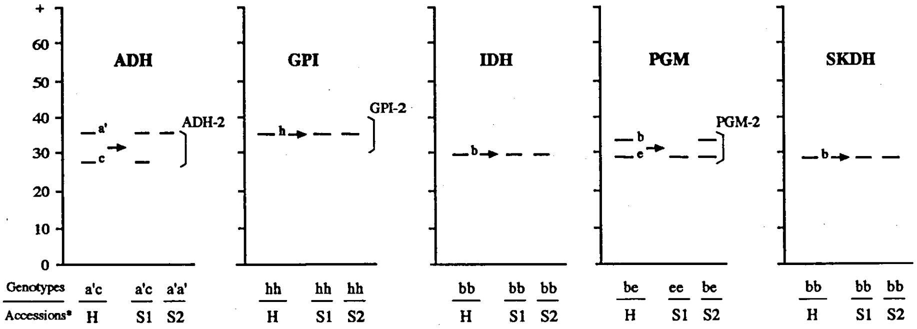

Fig. 1. Allozyme banding patterns and the genotypes in 'Hatsu-arashi' and its selfed progenies. *H: 'Hatsu-arashi', S1: Hatsu-arashi self-1, S2: Hatsu-arashi self-2.

Hatsu–arashi self–1 had only allele "e" that was derived from 'Hatsu–arashi' in *Pgm–2* locus. The genotypes of the other progeny Hatsu–arashi self–2 showed the similar results.

DISCUSSION

Perfect seeds were obtained from self–pollination of 'Hatsu–arashi' and 'Fuku–musume'. Fruit set was recognized in the self–pollination of 'Kanto–hatsuwarai' and 'Aki–no–yama', although no perfect seeds were obtained. Hagiya (1978) described that the fruit whose ovule was not fertilized dropped before one month after pollination in *Camellia*. Fertilization might, therefore, occur in the self–pollination of 'Kanto–hatsuwarai' and 'Aki–no–yama', and no perfect seeds were obtained in consequence of zygote degeneration during embryogenesis.

Most of the self–pollination and several cross–pollination were incompatible. Fuchinoue (1969) reported that there was a gametophytic self–incomopatibility system in tea (*C. sinensis*), and the incompatibility was governed by a single locus. If the similar self–incompatibility system exists in *C. japonica*, cross–pollination between different parents with the same self–incompatible genotypes might bring no fruit set. The selfed progenies of 'Hatsu–arashi' represented expected allozyme genotypes in each locus. It was reported that gynogenetic dihaploid plants were obtained as a result of pseudogamy in interspecific crosses between *C. sasanqua* and *C. japonica* (Fuchinoue, 1969). If the selfed progenies were gynogenetic dihaploids, the genotypes must be homogenic in all allozyme loci. In this study, the selfed progenies were not gynogenetic dihaploids when judged from the results of isozyme analysis, so that these may be derived from self–fertilization.

Camellia japonica is known as a protogyny species, and pistil is acceptable from a few days before anthesis (Hagiya, 1978; Hakoda and Matsumoto, 1979). The self–incompatible rejection phenomenon involves the recognition of identical gene products in pollen and style, and the time of gene action coincides with the opening of the flower (De Nettancourt, 1977). Bud pollination is known to be effective for overcoming self–incompatibility in *Brassica* (Brown *et al.*, 1991) and *Petunia* (Cruzan, 1993).

Here in this investigation, bud pollination was carried out to examine the self–incompatibility. As Hakoda and Matsumoto (1979) indicated that the percentages of fruit set by the self–pollination on and one day before anthesis were 21.8 and 7.9%, respectively, in *C. sasanqua*, bud pollination may not be effective to rise the rates of fruit set. This study proved that there are self–compatible or semi–self–compatible cultivars in *C. japonica*. These cultivars will play an important role to resolve the self–incompatible system and to study the inheritance of phenotypic characters for efficient cross breeding program in this species.

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