Distinctive Traits of *Camellia vernalis*, *C. sasanqua* and *C. japonica* Detected by the Interaction of Pollen Grains in Mixed Pollen Culture

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According to Iwanami (1970), the pollen grains of *Camellia sasanqua* strongly suppress the germination of the pollen grains of other plants in mixed pollen culture. In this study, we examined the effects of the pollen grains of various cultivars of *C. vernalis*, a hybrid of *C. sasanqua* and *C. japonica* (Tanaka 1985), on the germination of pollen grains of wild-type *C. japonica* in mixed pollen culture, to examine the inheritance of this suppressive trait. Among the garden camellias, *C. vernalis* is appreciated for its long flowering period from autumn to spring. This long flowering period has been ascribed to be the incidental trait acquired by wide crosses between *C. sasanqua* (autumn flowering) and *C. japonica* (winter flowering), based on the external morphology of both species (Kirino 1976). We are currently breeding long flowering camellias, using *C. japonica* and *C. vernalis* as mother species, and have obtained the basic information on the genetic background of the cultivars of *C. vernalis* in this study. The interaction of pollen grains in mixed pollen culture was used for the identification of the genetic background.

1. Collection and preservation of pollen grains

Sixteen cultivars of *C. vernalis*, five cultivars of *C. japonica* and one cultivar of *C. sasanqua* were used in the present experiment (Table 1). Pollen grains were collected from fresh flowers and used for the germination test immediately, or stored in a cold and dry box until use.

2. Germination test

Pollen germination was tested on a solidified agar plate (0.1% agar with 1% sucrose) on a slideglass. The pollen grains were aligned in a straight line on the agar plate by stumping using the edge of the cover glass.

The pollen grains of wild-type *C. japonica* were placed in a straight line, and the pollen grains to be tested were placed perpendicularly crossing the line.

After the incubation, the suppressive effect of the pollen grains was judged by the pollen tube growth of the wild-type *C. japonica* near the crossing point, where pollen tube growth was suppressed compared with that at the distal part of the line.

On the same agar plate, and across the same line of the pollen grains to be tested, the pollen grains of *C. sasanqua*, cv. Tachikan, and the cultivars to be tested itself were also placed separately, for comparison. At

Table 1. Type of pollen-interaction of various cultivars of *C. sasanqua*, *C. japonica* and *C. vernalis*, with a wild-type *C. japonica*

Cultivars	Sources ¹⁾	Action type ²⁾
<i>C. sasanqua</i>		
Tachikan	Ehime Univ.	S
<i>C. japonica</i>		
Ikkyu	Kage	N
Tsukinowa	Kage	N
Iwaneshibori	Hisatomi	N
Senbazuru	Matsuyama	N
Asakahime	Matsuyama	N
(Hybrid of <i>C. japonica</i> and <i>C. lutchuensis</i>)		
wild type	Ehime Univ.	N
<i>C. vernalis</i>		
Asahi	Hisatomi	S
Azumabotan	Hisatomi	S
Egao	Hisatomi	S
Egaobeni	Hisatomi	S
Gaisen	Hisatomi	S
Ginryu	Hisatomi	S
Hisatomi	Hisatomi	S
Kamakurashibori	Kage	S
Kokinran	Hisatomi	S
Kyonishiki	Hisatomi	S
Ohmigoromo	Kage	N
Sado	Hisatomi	S
Shiboriegao	Kage	S
Shokkounishiki	Hisatomi	S
Takarazuka	Hisatomi	S
Umegaka	Hisatomi	N

¹⁾ Material source, Matsuyama: Matsuyama Municipal Agricultural Extension Center, Ehime Univ.: Ehime University, Kage: Kage Shinko-en Nursery, Hisatomi: Hisatomi Senkaen Bun-en Nursery

²⁾ Interaction: S: Suppressive type, N: Non-suppressive type

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least three slides were used for each combination, and the test was repeated three times or more.

3. Evaluation of germination

The inoculated plates were placed in an incubator at a constant temperature of 25 C. After 3 to 5 hours, the germination was observed. Based on the results of three replications each with three slideglasses, each cultivar was classified into either one of suppressive type (S type) or non-suppressive type (N type) in the present experiment.

The pollen grains of the five cultivars of *C. japonica* did not exert any effect on the germination of the pollen grains of the wild type (Table 1), which is in agreement with Iwanami's observation (Iwanami 1970). Most of the pollen grains of *C. vernalis* germinated poorly, but suppressed the pollen germination of wild-type *C. japonica* (Fig. 1). Out of 16 cultivars of *C. vernalis* used here, 14 cultivars were of the S type, and the remaining two cultivars, Umegaka and Ohmigoromo, were of the N type. Iwanami (1979) also reported that even the pollen grains that did not germinate displayed the mixture effect on other pollen grains (promoting effect, no effect or suppressive effect), and it was assumed that some water-soluble substances had diffused rapidly after the placement of the pollen grains on the germination medium. Some investigators have stressed the inhibitory effect of the pollen grains of certain species on the pollen grains of others, which was ascribed to pollen allelopathy or a mentor effect (Char 1977, Knox *et al.* 1988, Sukhada and Jayachandra 1980). In the genus *Camellia*, an inhibitory effect as well as promotive effect has been observed in mixed pollen culture (Iwanami 1970).

Among the cultivars of *C. vernalis* examined, Umegaka and Ohmigoromo were of the N type, although all other cultivars were of the S type. These two cultivars showed similar characteristics in their habit and leaf texture and others, to those of the true type of *C. japonica* (Kirino 1976). Assuming that the S-type character of *C. sasanqua* is genetically dominant, the primary hybrids should be of the S type. In the next generation (selfed or backcrossed population), some plants may show different pollen reactions. At present, a large proportion of the cultivars of *C. vernalis* is grown as primary generation from natural hybridization between *C. sasanqua* and *C. japonica*. Therefore, if the pollen grains of *C. sasanqua* are loaded in mixture with those of *C. japonica* on the stigma of *C. japonica* or *C. sasanqua*, the pollen grains of *C. sasanqua* may suppress the germination of the pollen grains of *C. japonica*, and penetrate into the pistil. If many cultivars of *C. vernalis* were of the N type, they must be the secondary or tertiary hybrids. However, *C. vernalis* has only a few N-type cultivars, suggesting that *C. vernalis* is a simple primary hybrid, originating from wide crosses between *C. sasanqua* and *C. japonica*. The suppressive effect of the pollen grains of *C. sasanqua* may account for the

Fig. 1. Typical photographs showing the interaction of the pollen grains of the cultivars of *C. vernalis* and *C. japonica* with the pollen grains of wild-type *C. japonica* (Horizontal lines; stumps of wild-type *C. japonica*: Vertical lines; stumps of cultivars tested). Suppression of the pollen germination and growth of *C. japonica* is detected near the crossing point.

A: Azumabotan, B: Egaobeni, C: Ginryu, D: Kamakura-shibori, E: Kokinran, F: Sado, G: Shokkonishiki, H: Iwane-shibori, I: Umegaka. (H belongs to *C. japonica*, and the others to *C. vernalis*).

Remarks: The photographs were taken after incubation for 3–5 days, and no pollen grains of the cultivars tested at that time germinated, although the pollen tube of the wild-type *C. japonica* elongated well.

successful wide crosses in this combination. Genetic marker analysis should be carried out to confirm this assumption.

At present, the S type is predominant in *C. sasanqua* and *C. vernalis*, and this enables us to distinguish *C. vernalis* from *C. japonica*. Because the pollen germination of other varieties or species is suppressed by the pollen grains of *C. vernalis*, castration, bagging and hand pollination should be performed carefully in the breeding work of camellias. Until now, the species of genus *Camellia* have been identified mainly by paper chromatography (Roberts *et al.* 1958) and chemical analysis (Nagata and Sakai 1984), according to the classification

by Searly (1958). In future, genetic marker analysis will be required to compare such classification with the new classification of *Camellia* proposed by Chang (1981). Pollen interaction in mixed pollen culture may be a convenient method for the classification of *Camellia* genus.

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