<Original Article>

STUDIES ON THE INTERSPECIFIC CROSSING OF GENUS FORSYTHIA

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韓昶烈: 개나리屬의 種間交雜에 關한 硏究

ABSTRACT

Harn, Chang Yawl (Chonpuk U., Iri, Korea)—*Studies on the interspecific crossing of* FORSYTHIA Genus. Kor. Jour. Bot. 4(1)1~8 1961: Interspecific crossing of two species, F. saxatilis and F. Koreana, was carried out in order to make clear the segregation ratio of style length, mode of fertility, the fertility of Fl generation, dioecism, and other taxonomic question, the result of which being summarized as follows:

1) Style length is segregated into 1:1 ratio.

2) The behavior of fertility in the legitimate and illegitimate unions between the different species is exactly like that in the two dimorphic forms of the same species.

3) The mode of fertility between the long and short style of the Fl generation also follows that of the heterostyle plants.

4) No difficulties or irregularities are observed in the interspecific crossing and the Fl's fertility.

5) In Fl generation exceedingly high morphological and physiological variations are observed.

6) The short style individual is well fertilized and sets seed when legitimately combined. The insistence that the short style is male, this genus being dioecious, is groundless.

7) Among Fl individuals, are observed a few dwarf-types with tiny and weak vegetative and reproductive organs.

8) The two species used behave in many ways like the different styles of the same species.

INTRODUCTION

It is a well-established fact that Forsythia has the floral form of dimorphism, one form having the long-style short stamened flower, the other, just the opposite one. Recently even the chemical studies concerning the incompatibility in the illegitimate combinations of this genus have been published. MOEWUS reported in his elaborate investigation that the long and short style pollen of F. intermedia contain quercitrin and rutin respectively, the long and short style stigma just yielding certain enzymes inactivating the activities of the above materials. ESSER(1953), LEWIS(1954), ESSER and STRAUB (1954), and TATEEE(1958), using Forsythia and other heterostyle plants, made similar experiments, the result of which were mostly contrary to MOEWUS' hypothesis.

Four species of Forsythia have been reported to grow wild in Korea: F. densiflora, F. koreana, F. ovata, and F. saxatilis. This classification, however, appears to be based on minor external morphological characters.

Present study was designed in order to make clear in the interspecific crossing between the F. saxatilis long style and F. koreana short style, the segregation ratio of style length, the mode of fertility, crossability, fertility of interspecific hybrid (Fl). dioecism, and other taxonomic questions.

MATERIAL AND METHOD

Long style individual (L) of F. saxatilis and short style (S) of F. koreana were used as parental

plants. The exact identification of both parent is somewhat doubtful because Dr. CHUNG, one of the leading taxonomists in Korea, identifies F. saxatilis as having certain similarity to the F. suspensa; and F. koreana to F. densiflora.

The long style of F. saxatilis has been grown in the campus of Chonnam National University. No seed formation was found in nature in 5 years' close observation. The short style of F. koreana was used growing in downtown Kwangju, approximately one mile away from the university campus. It bears abundant seed every year in nature (Fig. 1)

(2) *Interspecific crossing*: The interspecific crossing of L x S was made in 1957 and reciprocal crossing of S x L was carried out in 1958. In 1961 the crossing of both L x S and S x L was repeated in order to obtain clearer figures regarding fertility. Crossing operation was followed by the usual method. The short style of F. koreana bloomed somehow one week earlier than the long style of F. saxatilis.

As the viability of pollen and receptivity of stigma are comparatively stable and long, the difference of blooming date does not seem to give any effect on the fertility or crossing operation. The fertility was judged by the numbr of swollen ovary about 40 days after the crossing.

(2) *Growing the F_1 hybrid*: In March 1958, the interspecific hybrid seed of F. sax. x F. kor. was sown on the seed bed in the green house, transplanted on the open farm after they had grown approximately 10—15 cm. Rate of germination was very high. Out of the 54 individuals grown, 30 individuals had their flowers bloomed in 1960. The ratio of L:S=14:16. In 1961, 43 individuals had their flowers open.

(3) *Sister crossing between the F_1 individuals*. Three individuals of long and short style forms of Fl hybrid grown on the same farm plot were selected and designated as L_1, L_2, L_3, S_1, S_2, and S_3. Both legitimate and illegitimate pollinations were made for the purpose of clarifying the Fl's fertility and mode of fertilization. (Fig. 11)

RESULTS AND DISCUSSION

1. *Ratio of style length:* In the heterostylous plants of other families, long and short style individuals occur ordinarily just equal in number, i.e., L : S=1 : 1; long style being homo recessive and short style, heterozygous. Although Forsythia has been known as the plant with dimorphic flowers, no report has been published yet on the segregation of style length. Out of the 54 offspring, 43 individuals had their flowers open with the segregation of style length being 1 : 1. (Table 1)

Table 1: Segregation of style length in interspecific hybrid

	Long	Short
O	23	20
C	21,5	21,5
	P=0,7-0,5	

In the present experiment despite the two parents belonging to the different species, the segregation ratio is exactly identical to that between the different forms of same species. The two species used in the present experiment behave just as if they were of the same species as long as the segregation of style length is concerned.

2. *Fertility:* (a) Interspecific crossing

Interspecific crossings between the long style of F. saxatilis and the short style of F. koreana were made consecutively in 1957, 1958, and 1961, the results of which are given in the tables 2, 3, and 4. The symbols L and S of tables 2—4 are long style of F. saxatilis and short style of F. koreana, respectively.

Table 2: Fertility (1) 1957

Combination	Flowers pollinated	Ovary swollen	Fruit matured
L selfed	85	0	0
L x L	24	0	0
L x S	38	34	34

Table 3: Fertility (2) 1958

Combination	Flowers pollinated	Ovary swollen
S selfed	12	_*
S x L	71	Abundant*

*Failed to obtain exact figures owing to an unexpected accident. A Similar experiment was repeated in 1961.

Table 4: Fertility (3) 1961

Combination	Flowers pollinated	Ovary swollen	Remarks
L wrapped without pollination	19	0	April 7
L selfed	20	0	//
L x S	28	22	Old S pollen used*
S wrapped without pollination	48	0	March 26
S selfed	24	0	//
S x L	48	35	April 7 Pollinated onto old S stigma

* As the short style of F. koreana is early bloomer, the pollen and stigma of short style are old ones, but no perceptible abnormality was observed with regard to the pollen viability and stigma receptivity.

Heterostylous plants in general tend to have poor fertility in the illegitimate combinations, such as L selfed, L x L, S selfed, S x S; and good fertility and seed setting in the legitimate pollination, L×S and S x L. The present experiment reveals the fact that despite the parental plants used being of different species the behavior in fertility and seed-setting is just as if the two parents were the dimorphic forms of the same species. MOEWUS (1950), using two forms of F. intermedia, made

legitimate and illegitimate combinations and found that the mode of fertility of Forsythia follows the pattern of ordinary heterostyle plants. The present experiment bears specific interest in that even in the interspecific crossing the pattern of fertility follows exactly that of common heterostyle plants.

Although the unions of L x S and S x L are interspecific, in the formation of the hybrid seed no difficulty arises as if the combinations were of intraspecific. The hybrid seeds are normal in their germination and fertility, and other irregularities commonly found in the interspecific hybrids are not observed.

(b) Legitimate and illegitimate unions in F_1 generation.

Several F_1 hybrids bloomed in 1960 were observed bearing fruit through natural pollination. In 1961 various kinds of pollination were made using the F_1 hybrid. The purposes of these extensive experiments were to make clear the following questions: (1) Mode of fertilization between the long and short style individuals of F_1 generation. (2) Clarification of the claim by some of Korean taxonomists that Forsythia is dioecious and the short style individuals are self- and cross-sterile.

Table 5: Fertility in the F_1 generation 1961

Combination	Flowers pollinated	Flowers fertilized	Combination	Flowers pollinated	Flowers fertilized
L_1 } wrapped without	22	0	S_1 x S_2	24	0
L_2 } pollination	4	0	S_2 x S_1	22	0
L_3 }	10	0	S_1 x S_3	24	0
L_1 }	13	0	S_3 x S_1	22	0
L_2 } selfed	10	0	S_2 x S_3	13	0
L_3 }	6	0	S_3 x S_2	16	0
L_1 x L_2	6	0	L_1 x S_1	29	3
L_2 x L_1	6	0	L_2 x S_2	23	0
L_1 x L_3	9	0	L_3 x S_3	36	0
L_3 x L_1	12	0	L_1 x S_3	14	0
L_2 x L_3	14	0			
L_3 x L_2	11	0	S_1 x L_1	30	14
S_1 } wrapped without	42	0	S_2 x L_2	52	13
S_2 } pollination	39	0	S_3 x L_3	20	6
S_3 }	35	3	S_1 x L_3	36	18
S_1 }	25	0			
S_2 } selfed	27	0			
S_3 }	15	6			

The mode of fertilization is just identical to that found in ordinary heterostylous plant, i.e., good seed-set in legitimate combination and poor in illegitimate unions. S_3 individual has set seed both in "selfed" and "wrapped without pollination". The exact cause would be made clear by further researches, but at present it may be assumed that either contamination, parthenogenesis, parthenocarpy, or self-fertile nature might be one of the reasons. Many cases of self-fertile individuals have been reported in self-incompatible plants and heterostyle species. Unexpected low fertility in the combination of L x S appears to be brought about due partly to the old pollen of short style and partly to pollen abnormality. As the duration of pollen viability and stigma receptivity were not investigated before-hand, the bagged period after pollination was unduly long for fear of contamination, during which a long spell of rainfall continued. As the long and short style individuals alike are fertilized well by the pollen from the flower of opposite forms, the Korean taxonomists' claim that the short-style individual is a male and accord-

ingly Forsythia is dioecious plant is an apparent misunderstanding. Actually the author has observed frequently short style individuals with their fruits set in nature. The short style individual used as parent in the interspecific crossing in the present experiment bears fruit well in nature and the short style individuals grown on the campus of Songgyunkwan University, Seoul, have been found to occasionally set fruit. (Fig. 3) This genus, however, in general sets few fruit as compared with the other heterostylous plants. Easy vegetative propagation seems to compensate for the degenerated seed production. Though pollen sterility and few opportunities in nature for pollen transfer are also assumed to be some causes for scanty seed formation, these questions would be solved by thorough cytological investigation.

3. *Variability of F_1 generation :* The morphological observation reveals that the 54 individuals of Fl generation are extremely variable, some of the individuals being so much different from the parental plants that they are hardly recognized as Forsythia. In general, the leaf shape, leaf size, internode, stem colour, serrate, abnormality of floral organ, dwarf type, vigour, blooming season, years needed for blooming, were conspicuous characters observed variable among the F1 generation.

This genus is a naturally cross-pollinated plant and mostly propagated vegetatively which leads to extreme heterozygosity. When interspecific crossings are conducted between heterozygous plants usually variable F1 plants are expected to result through new gene combinations and gain or loss of vigour. The most remarkable feature, among other things, in the F1 plants is the emergence of the dwarf type. (Fig. 4) This type bears slender and tiny leaves and stems, grows slowly, and seems extremely weak in general appearance. No floral buds developed in 1960. Only a handful of flowers bloomed in 1961. (Fig. 5) The difference between the F1 dwarf and the parents is given in the tables 6—10.

Table 6: Floral bud (mm)

Parent (L)		F1 dwarf		Parent (S)	
Lengthwise	Crosswise	Lengthwise	Crosswise	Lengthwise	Crosswise
5, 01	2, 01	2, 60	1. 54	7, 17	2, 56

Table 7: Anther size (mm)

Parent (L)	F1 dwarf (S)	Parent (S)
Length 2, 93	2, 20	3, 00
Width 1, 90	1, 70	2, 00

Table: 8 Floral size (cm)

	Parent (L)	F1 dwarf (S)	Parent (S)
Floral diameter	3, 1	1, 9	3, 5
Petal length	2, 0	1, 3	2, 2
Petal width	0, 6	0, 5	0, 9

Table 9: Pollen size (μ)

	Parent (L)	F1 dwarf (S)	Parent (S)
Length	33, 25	35, 81	39, 31
Width	20, 63	23, 93	26, 35

Floral bud of short style is far larger and rougher than that of long style. Floral bud of F1 generation varies in different individuals. Dwarf type F1 individuals have extremely smaller ones. (Fig. 6) Anther and flower of dwarf F1 are smaller than those of both parent. (Fig 7) Long style pollen size is definitely smaller than that of short style both in parental species and among long and short of F1 generation. In heterostyle plants the pollen size is mostly smaller in long style than in short style. In this experiment as the long and short style of parent belong to different species, comparing the pollen size beteen the two styles seems in a sense meaningless. But the fact that the pollen size of long style species is smaller than the short style species as in the cases of the same species is of great significance. The pollen size of dwarf short style is relatively large compared to the degenerated vegetative and floral organs, even larger than those of the long style parent.

4. *Pollen fertility:* In the investigation of pollen fertility, micropollen and empty or lightly stained pollen are counted as sterile pollen. Pollen fertility is far higher in long style as is given in table 10. (Fig. 8)

Table 10: Pollen fertility (%)

Parent (L)	F1 dwarf (S)	F1 normal (L)	F1 normal (S)	Parent (S)
90, 9	74, 2	85, 5	64, 4	44, 6

In Persicaria japonica of Polygonaceae the long style pollen were observed almost degenerated while short style pollen were highly fertile. Forsythia has just the opposite feature from the Persicaria. Further studies on this trend should be conducted to make sure whether this is true in other Forsythia species. F1 dwarf type has comparatively good pollen fertility while its floral and vegetative organs are extremaly degenerated. The details on the fact that the short styl individual has abundant sterile pllen may be made clear by cytological observation, together with the caryological researches of the F1 dwarf type. It is observed that the F1 generation, though interspecific hybrid, does not seem to have more pollen irregularity than the parental plants.

5. *Difference between long and short style individvals:* Although the individuals of F1 generation are highly variable each other a clearcut difference exists between long and short style individuals. (Table. 11)

Table 11: Difference between long and short*

	Long	Short
Pollen size	small	large
Pollen fertility	+++	+
Floral size	small	large
Blooming season	late	early

*Parental species included.

Fig. 2

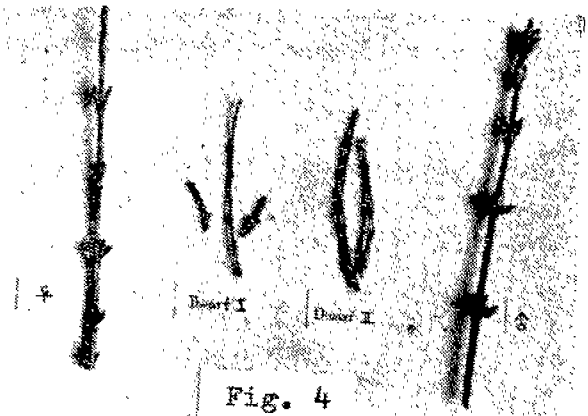

Fig. 4

Fig. 1

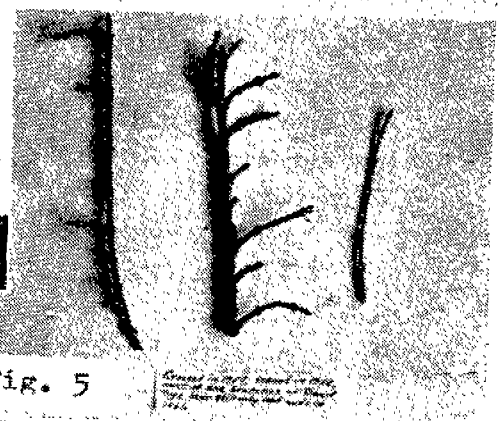

Fig. 5

Fig. 3

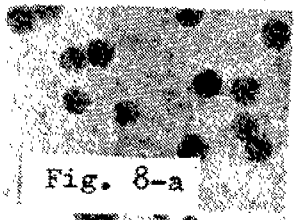

Fig. 8-a

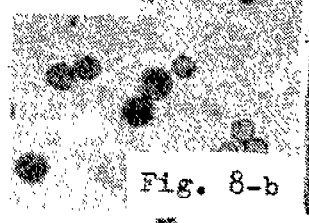

Fig. 8-b

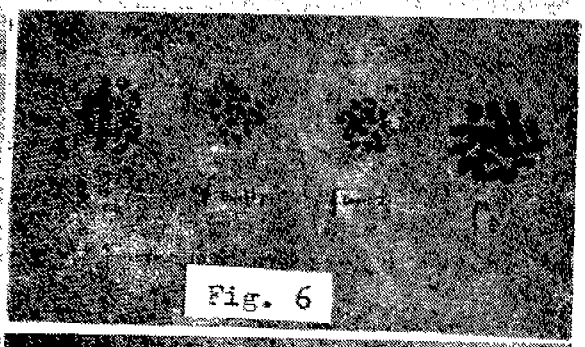

Fig. 6

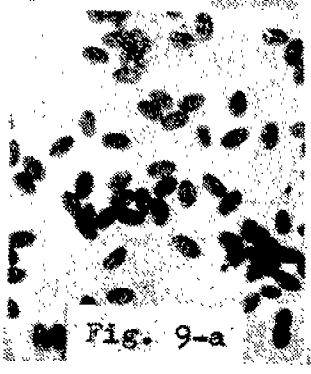

Fig. 9-a

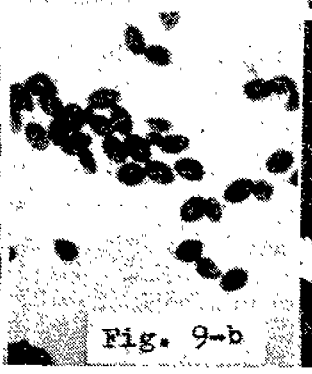

Fig. 9-b

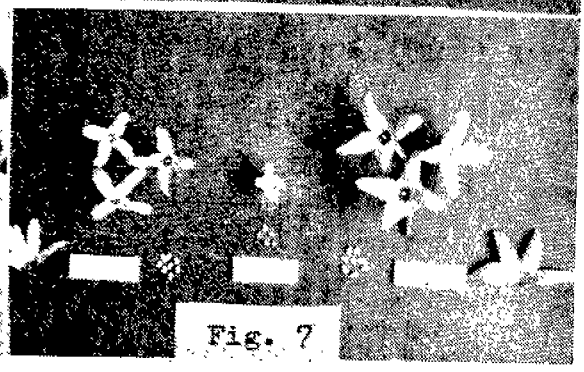

Fig. 7

Both in parental and F1 generation it was observed that floral and pollen size are smaller in long style with more fertile pollen than in short style. (Fig. 9) Long style tends to bloom late.

CONCLUSION

Korean Forsythia has been classified into four species, mostly based on minor morphological characteristics. The two species used as parental plants in the present experiment behave in its style length segregation, mode of fertilization, and in many other ways exactly like the different forms of the same species in heterostyle plants. In addition, the crossability, good germination rate of F1 generation, the good fertility of the F1 individual, and the regularity of the F1 pollen clearly indicate that the two parental species are in reality of same species. Although this genus is heterostyle, and has lost its self-fertile character, these kinds of heterozygous and vegetatively propagated plants usually produce extremely variable individuals in their progeny if selfing is forced. To split the Korean Forsythia into four species based on the minor characters is supposed unreasonable when the results of the present experiment are taken into consideration, although only two species are used in this crossing. Korean taxonomists generally regard the Forsythia as dioecious plant, the short style individuals being male. The present experiment reveals that the short style individuals bear seed well even in the so-called "interspecific crossing."

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摘 要

分類學에서 異種으로 取扱되고 있는 Forsythia屬의 種間交雜을 實施하여 花柱長의 分離比, 兩花柱間의 受精樣式, F_1의 稔性, ♀♂性 其他 分類學上의 諸問題를 究明한바 그 要旨는 다음과 같다.

(1) 花柱長은 L:S=1:1로 分離한다. (2) 異種間의 適, 不適法授粉인데도 兩親 L, S의 受精樣式은 一般異型蕊植物의 同一種內의 2型花와 全혀 同一하다. (3) F_1의 L, S間의 受精樣式도 一般異型蕊植物과 同一하다. (4) 種間交雜은 잘되고 F_1의 稔性도 異常없다. (5) 交雜第一代의 形態, 生理的變異는 甚하다. (6) S株도 適法授粉에서는 稔性이 良好하고 Forsythia가 ♀, ♂ 異株라는 것은 잘못이다. (7) F_1에는 榮養生長 및 生殖器官이 貧弱한 矮型이 나타난다. (8) 本實驗에 使用한 두種은 異種이라기보다 同一種內의 異花型個體라고 하는 것이 妥當하다.

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Fig. 1. F. koreana, short style. Fig. 2. Legitimate and illegitimate combinations in F_1 generation. Fig. 3. Buds and preceding year's fruit on the short-style individual. Fig. 4. Outside : parent, Inside: Dwarf types. Fig. 5. Dwarf types bear few floral buds. Fig. 6. Buds inside are of Dwarf types. Fig. 7. Left: long style, middle: Dwarf. Right: short style. Fig. 8-a. long-style. Fig. 8-b. short-style. Fig. 9-a. Long-style. Fig. 9-b. Short-style.