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Cytological Studies on Selfed Plants and Interspecific Crosses Produced in Four Species of Genus *Lycoris* (Amaryllidaceae)

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Abstract

Self-pollination and interspecific hybridization were carried out in *Lycoris sanguinea, L. sprengeri, L. squamigera* and *L. radiata*. Through embryo rescue for obtaining more progenies, selfed plants were obtained in three species and hybrid plants were obtained from five interspecific crosses. Most selfed plants raised from diploid species were diploid with the same karyotype as the parent, but three S_1 plants obtained from *L. sprengeri* (2n=22A) were found to be two triploids with 2n=33A and one aneuploid with 2n=32 (31A+1M). All selfed plants raised from triploid species, *L. radiata*, were aneuploid with chromosome number near to the diploid taxon, *L. radiata* var. *pumila*.

Hybrid plants derived from interspecific hybridization between diploid species were all diploids. However, various aneuploids were frequently occurred in the hybrids between triploid and diploid species. Those results suggested that interspecific hybridization between triploid and diploid species would become an effective breeding method for increasing the character variation in the genus *Lycoris*.

Key Words: *Lycoris*, self-pollination, interspecific hybridization, cytological study, aneuploid, triploid

Introduction

In the genus *Lycoris*, interspecific hybridization is one of the important breeding methods for improving flower shape, flower color and extending flowering period. However, the breeding efficiency in interspecific hybridization is low mainly due to the difficulty of obtaining F_1 plants caused by incongruity (Coertze, 1990), inefficient embryo rescue technique (Van Tuyl, 1997) and so on. Moreover, little knowledge of the genetic background of species as well as the inheritance of characters is available. For elucidating the genetic relationship among species and karyotype evaluation, cytological studies on selfed plants and interspecific hybrids have been made by some authors, such as Kihara (1954), Koyama (1953, 1955, 1959), Takemura (1961, 1962a, 1962b) and Shii(1997). However, more information is needed to clarify the genetic background of species. In the present study, self-pollination and interspecific hybridization were carried out, and embryo rescue technique was applied for obtaining more progeny plants. The chromosome observations of S_1 and F_1 plants were carried out.

Materials and Methods

Plant materials:

Diploid species, *L. sanguinea* (2n=22) and *L. spengeri* (2n=22), and triploid species, *L. radiata* (2n=33) and *L. squamigera* (2n=27), were used. All the parental plants were grown in the field of Osaka prefecture university, Sakai.

Pollination:

Scapes were collected 1—2 days before anthesis and were kept in flasks filled with tap water (Koyama, 1959). For interspecific hybridization, flowers were emasculated before anthesis by removing anthers using tweezers. Four self and five interspecific pollinations shown in Table 1 were conducted by using fresh pollens or pollens stored in refrigerator (Mori, 1993). The frequency of fruit set was investigated 30 days after

pollination.

Embryo rescue:

The ovaries were collected 30—35 days after self- and cross-pollination. They were surface-sterilized in 70% ethanol for 60 seconds, 2% sodium hypochlorite for 30 minutes, and rinsed three times with sterilized water, successively. Ovules or young seeds were excised and inoculated on 1/2 MS medium supplemented with 3%(w/v) sucrose and 0.2%(w/v) Gellan Gum (pH 5.8) at 25℃ in dark. After germination, the cultures were incubated under 16 h photoperiod of light. When leaves and roots emerged, the bulblets were counted and transplanted to MS medium containing 6%(w/v) sucrose for bulb development.

Cytological observation:

Root tips of parents, S_1 and F_1 plants were pretreated with cold water (0℃) for 24 hours, fixed in Farmer's fluid for 2 hours, hydrolyzed in 1N HCl for 6 minutes at 60℃, stained with Feulgen solution for 15 minutes at room temperature, squashed in 45% acetic acid. Chromosome number was counted in more than five cells per plant, and chromosomes were classified into three categories, M type (metacentric chromosome), A type (acrocentric chromosome) and T type (telocentric chromosome) according to Kurita (1986).

Results

Production of selfed plants and interspecific hybrids

Enlarged fruits were observed after the self- and cross-pollination. The percentage of fruit set were varied from 61.0—90.0% in self and 52.9—91.7% in cross, respectively (Table 1).

Through embryo rescue, S_1 plants were obtained in three species of *L. sanguinea, L. sprengeri* and *L. radiata,* and F_1 plants were obtained in all the five interspecific crosses. The number of plantlets regenerated was shown in Table 1. The efficiency of plantlet formation through embryo rescue was 0—1.54 plantlets per floret (Table 1), and was estimated about 3—166 times higher than that of previous reports (Koyama, 1953; Takemura, 1962a), in which progeny plants were produced without using embryo rescue procedures.

Chromosome number and karyotype

Most of the plantlets derived from both self- and cross-pollinations grew vigorously after subculturing onto MS medium containing 6% sucrose and developed bulbs with the diameter of 1—1.5 cm after almost one years culture. The plants with normal roots were examined for chromosome number and karyotype.

The chromosome number and karyotypes of four species used as parents in this study were shown in Table 2, where they were recognized as standard chromosome number and karyotypes in each of the species (Hsu *et al.*, 1994).

In S_1 plants of *L. sanguinea* (2n=22A), all of the progenies were diploids with 2n=22A same as the parent *L. sanguinea.* In S_1 plants of *L. sprengeri* (2n=22A), seventeen were diploids with karyotype 2n=22A, two were triploids with 2n=33A and one was aneuploid with 2n=32 (31A+1M) as illustrated in Fig. 1. In three S_1 plants of *L. radiata* (2n=33A), one with 2n=24A and two with 2n=25A were observed as in Fig. 2.

Table 1. Fruit set and plantlet formation in self-pollination and interspecific hybridization

Cross combinations		No. of florets pollinated	Fruit set		No. of ovules or young seeds inoculated	No. of plantlets regenerated (per floret)
♀	♂		No.	%		
L. sanguinea	self	100	90	90.0	135	76 (0.76)
	× *L. squamigera*	101	57	56.4	59	9 (0.09)
L. sprengeri	self	13	11	84.6	39	20 (1.54)
	× *L. sanguinea*	12	11	91.7	34	13 (1.08)
L. squamigera	self	59	36	61.0	51	0 (0)
	× *L. sanguinea*	34	18	52.9	30	5 (0.15)
L. radiata	self	15	10	66.7	39	3 (0.20)
	× *L. sanguinea*	66	56	84.8	102	29 (0.44)
	× *L. sprengeri*	41	28	68.3	46	10 (0.24)

They were the aneuploids, nearly alike the fertile diploid taxon *L. radiata* var. *pumila* (2n=22A).

In F_1 plants of *L. sprengeri*×*L. sanguinea*, all of them were diploids with 2n=22A. In F_1 plants of *L. sanguinea*×*L. squamigera*, although one was aneuploid with 2n=25 (2M+4T+19A), all of the other F_1 were diploids with 2n=22A same as the female parent *L. sanguinea*. In the reciprocal cross, *L. squamigera*×*L. sanguinea*, aneuploids with 2n=24 (4M+3T+17A) and 2n=25 (4M+3T+18A) were observed as in Fig. 3. In the F_1 plants of *L. radiata*×*L. sanguinea* and *L. radiata*×*L. sprengeri*, all progenies were aneuploids with a series chromosome number of

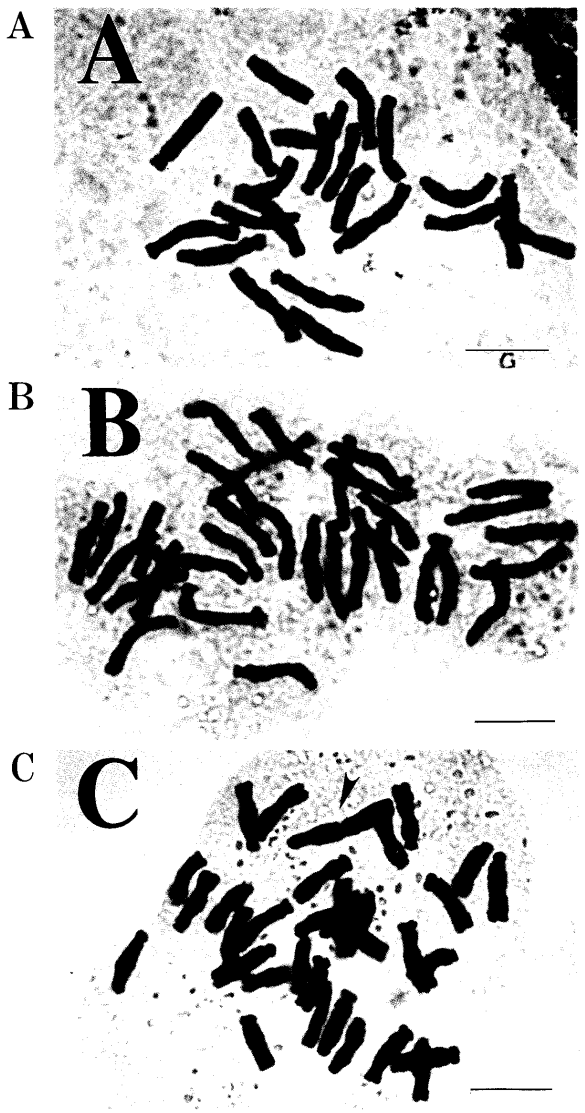

Fig. 1. Metaphase plate of chromosomes in the root tips of *L. sprengeri* and its selfed progeny. A: a metaphase cell of *L. sprengeri*, 2n=22A; B: a metaphase cell of one of the triploid selfed plants, 2n=33A; C: a metaphase cell of the aneuploid selfed plant, 2n=31A+1M. Arrow indicates the M type chromosome. Bar=10μ m.

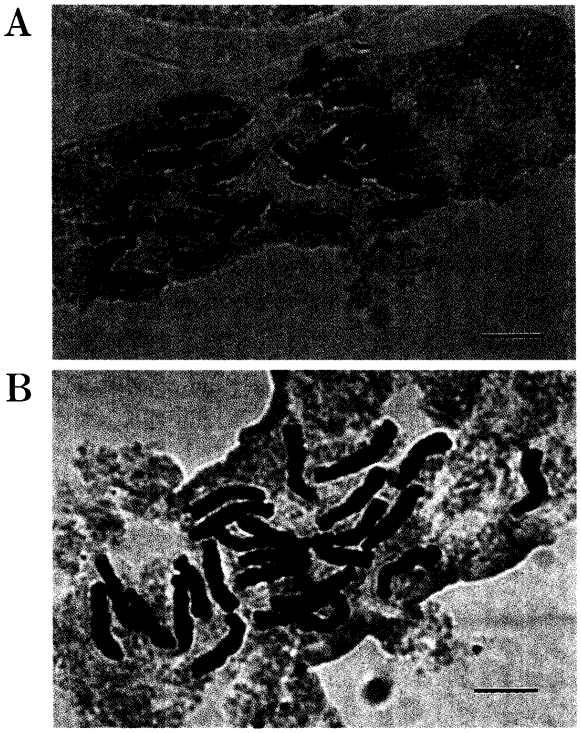

Fig. 2. Metaphase chromosomes in root tip cells of S_1 plants from *L. radiata var. radiata*. A: 2n=24A; B: 2n=25A. Bar=10μ m.

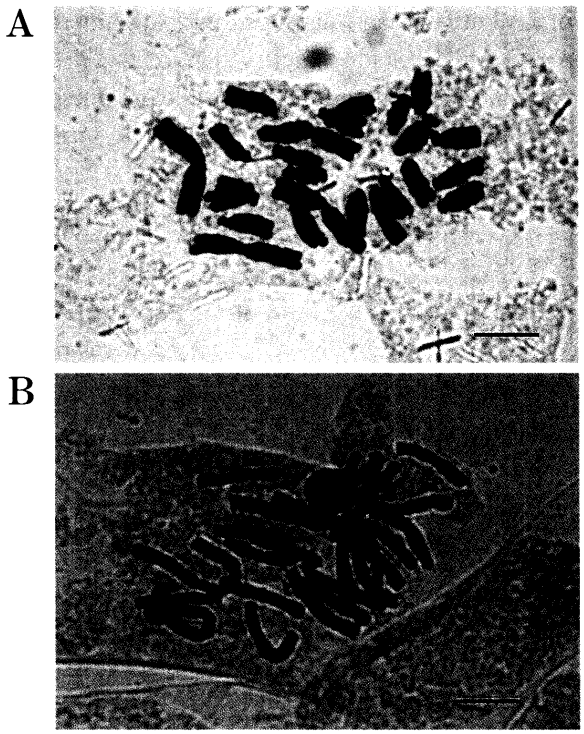

Fig. 3. Metaphase chromosomes in root tip cells of interspecific hybrids between *L. squamigera* and *L. sanguinea*. A: 2n=24(4M+3T+17A); B: 2n=25(4M+3T+18A). Bar=10μ m.

2n=23A−31A and 2n=23A−28A, respectively (Table 2).

Discussion

A few triploid S_1 plants were induced in *L. spengeri,* although most of S_1 plants were diploids in self-pollination of diploid species. The triploid plants (2n=3x=33) of *L. sprengeri* were first discovered in the wild population collected at Anhui, China by Zhang *et al.,* (1999). The artificial triploids of *L. sprengeri* were induced firstly in this study. With regard to the cytological origin of the artificial triploid plants, some possible mechanisms should be taken into account: 1) a union of an unreduced diploid gamete and a reduced haploid gamete (Grant, 1981); 2) embryogenesis from 3n endosperm cell (Muniyamma, 1977); 3) the fertilization of an egg by two generative nuclei (Lapidot et al, 1994). In *Lycoris,* there are some natural triploid species such as *L. radiata* (2n=3x=33) and *L. squamigera* (2n=3x=27), and the triploid plants (2n=3x=33) were also found in *L. sanguinea var. kiushiana* (Kurita, 1988a). The origin of those natural triploids was still under discussion, although it was hypothesized generally as the result of the union between an unreduced diploid gamete and a reduced haploid gamete (Inariyama, 1951; Liu and Hsu, 1989). The fact that triploid plants were induced through self-pollination in *L. sprengeri* would provide a practical approach for elucidating the mechanisms of the origin of those natural triploid species or taxa in the genus *Lycoris.*

From the self-pollination of *L. sprengeri,* an aneuploid (2n=32=31A+1M) with chromosome number near to triploid was obtained. Similar karyotype was also found in *L. sanguinea* (Kurita, 1989) and *L. radiata var. radiata* (Kurita, 1987), in which the M type chromosome was considered to be a production of Robertsonian change between two A type chromosomes. The aneuploid is considered to be induced from the combination of an unreduced gamete with 2n=22A and a reduced one with n=9A+1M. However the origin of this M type chromosome was not understood yet in the present investigation, and so further studies are needed to clear the above assumption.

Although only a few S_1 plants were induced from *L. radiata,* all the S_1 from triploid species were aneuploids with chromosome number near to its diploid taxon. Since *L. radiata* is an autotriploid species as proved by Inariyama (1951), and the gametic chromosome number ought to vary from 11 to 22 theoretically (Inariyama, 1951), the S_1 plants of *L. radiata* are expected to vary in chromosome number between 2n=22 and 44. However, actually the aneuploids with chromosome number near to

Table 2. Karyotype of parents, selfed plants and interspecific crosses in *Lycoris*

Species (Karyotype observed)	Combinations	Karyotype	No. of plants observed
L. sanguinea (2n=2x=22A)	Self	2n=22A	66
	×*L. squamigera*	2n=22A	8
		2n=25(2M+40T+19A)	1
L. sprengeri (2n=2x=22A)	Self	2n=22A	17
		2n=33(33A)	2
		2n=32(31A+1M)	1
	×*L. sanguinea*	2n=22A	13
L. squamigera (2n=3x=27(6M+10T+11A))	×*L. sanguinea*	2n=24(4M+3T+17A)	1
		2n=25(4M+3T+18A)	1
L. radiata (2n=3x=33A)	Self	2n=24A	1
		2n=25A	2
	×*L. sanguinea*	2n=23A	2
		2n=24A	1
		2n=26A	3
		2n=27A	5
		2n=28A	5
		2n=29A	2
		2n=31A	1
	×*L. sprengeri*	2n=23A	1
		2n=25A	2
		2n=26A	1
		2n=27A	2
		2n=28A	1

diploid were only obtained in our study. Kihara et al (1954) also reported that they obtained offsprings with chromosome number varied from 2n=22 to 25 by self-pollination in *L. radiata*. From the results of the chromosome behavior in self-pollination of *L. sprengeri* and *L. radiata*, it is considered that the autotriploids (3x=33A) of *L. radiata*, *L. sanguinea var. kiushiana* and *L. sprengeri* would be originated in self-pollination of their diploid species. At present, the authors are studying for inducing S_1 plants in *L. radiata var. pumila* (2x=22A) and *L. sanguinea var. kiushiana* (2x=22A).

In the interspecific hybridization between diploid species (2x×2x), all of the F_1 plants of *L. sprengeri*×*L. sanguinea* were diploids. The result was similar to that obtained by Takemura (1962a). However, it is necessary to do further studies such as isozyme and morphological analysis to conform the hybridity, because the chromosomes came from two parents could not be distinguished visually in the hybrids.

Most of F_1 plants obtained from 2x×3x combination of *L. sanguinea*×*L. squamigera* were diploids. The same phenomenon was reported in the genus *Crinum* (Lehmiller, 1992). Although the phenomena would be due to false hybrid like pseudogamy, it would be needed to clarify the cause by further studies.

In interspecific hybridization of 3x×2x, hybrids obtained from *L. radiata*×*L. sanguinea* varied in chromosome number from 2n=23 to 31. This result accords fundamentally with the previous report (Koyama, 1955). However, the frequency of F_1 plants with different chromosome number in the present study was more accord with the expected frequency of gametes of *L. radiata*. This difference between our study and the previous was attributed probably to the using of embryo rescue technique.

As shown in the Table 2, many and various aneuploids were induced from 3x×2x as in *L. squamigera*×*L. sanguinea*, *L. radiata*×*L. sanguinea* and *L. radiata*×*L. sprengeri*. In *Nerine* and *Narcissus*, the other members of Amaryllidaceae, many cultivars were found to be aneuploids (Javaki-Ammal, 1951; Wylie, 1952). Moreover, aneuploid is widespread in many crops such as *Claytonia virginica* (Rothwell, 1959) and *Crocus* (Brighton, 1978). Though aneuploid is sterile generally, it can be maintained by means of vegetative propagation. Therefore, it could be concluded that interspecific hybridization between triploid and diploid species would become an effective breeding approach for increasing the character variation in the genus *Lycoris*.

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