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メタデータ	言語: eng 出版者: 公開日: 2009-08-25 キーワード (Ja): キーワード (En): 作成者: MA, Biao, OGAWA, Takeshi, TARUMOTO, Isao メールアドレス: 所属:
URL	https://doi.org/10.24729/00009660

Genetic Segregation of Allozymes in Selfed Progenies of Diploid *Lycoris* Species (Amaryllidaceae)

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Abstract

Seven diploid species of *Lycoris* have been considered to be progenitors of other species. In order to clarify the zygotic background of the diploid species, allozyme variations in selfed progenies of five species were investigated. Seven polymorphic loci of APT, GOT and EST were detected. Allozyme segregations were observed in all five species, suggesting that each of the five diploid species is heterozygous. This study demonstrates for the first time that five progenitor species of *Lycoris* are originally heterozygous.

Key Words: allozyme, heterozygosity, *Lycoris*, selfed progeny

Introduction

The genus *Lycoris* is a bulbous flower commonly cultivated in Japan, China, and the United States (Hsu et al., 1994). Twenty *Lycoris* species preserved by clonal propagation have been noted. Each species consists of one cultivar with a specific karyotype except for *L. aurea* (L'Herit) Herb. and *L. sanguinea* Maxim. which contain a few subspecies showing different ploidy or aneuploidy (Hsu et al., 1994). Although the origin and its relationship of *Lycoris* species are not clear, seven diploid species among them were considered to be progenitors of the other species on the basis of cytological studies and hybridization results (Hsu et al., 1994; Kurita and Hsu, 1998). For these diploid progenitor species, although homokaryotypical origins were postulated from intermediate karyotypes and characteristics of F₁ hybrids in *L. sprengeri* Comes ex Bak. × *L. radiata* (L'Herit) var. *pumila*, *L. sanguinea* × *L. sprengeri* and *L. aurea* × *L. radiata* var. *pumila* (Takemura, 1962a,b), they were partly assumed to be heterozygous from genetic segregation of some morphological characters in interspecific hybrids between *L. sprengeri* and *L. radiata* var. *pumila* (Caldwell, 1964). However, direct evi-

dence is required to prove conclusively the heterozygous nature. The knowledge on genetic background such as heterozygous nature and heterozygosity degree of species is important for evolutionary studies and breeding in *Lycoris*. However, such studies are not advancing due to the following difficulties existing in *Lycoris*.

Genetic variation is usually estimated with morphological, physiological and agronomic traits in segregated and/or crossed populations. But preparation of such genetic populations is generally difficult in bulbous plants owing to their poor capability for seed-set (Le Nard and De Hertogh, 1993), and there is much more difficulty for preparing genetic population in *Lycoris* species due to their extremely low capacity for fertilization and seed propagation (Ma, 2001). However, by using ovule culture technique (Ma et al., 2001a), we produced a large number of selfed plants from five diploid *Lycoris* species, *L. aurea*, *L. traubii* Hayw., *L. sprengeri*, *L. sanguinea* and *L. radiata* var. *pumila*, and these selfed progenies were expected to be useful and sufficient material for the genetic studies (Ma et al., 2001b). The second problem in *Lycoris* is the long juvenile period (6-8 years), so it is necessary to grow the

prepared genetic material for more than six years to begin any search for the variation in distinctive traits expressed at flowering time. Isozyme and DNA markers have been commonly applied to detect genetic variation in many plant species (Moore and Collins, 1983; Williams et al., 1990). In *Lycoris*, isozyme markers have been successfully used to detect genetic variations (Chung, 1999a, b; Lee et al., 2001). Therefore, we take advantage of isozyme analysis to do the genetic study of *Lycoris* at seedling and/or juvenile stage (Ma et al., 2001b).

In this study, isozyme analysis of selfed plants was carried out with the five diploid *Lycoris* species. The heterozygous nature of these species was ascertained with allozyme variation in selfed progenies.

Materials and Methods

Plant material

Five diploid species, *L. aurea*, *L. traubii*, *L. sprengeri*, *L. sanguinea* and *L. radiata* var. *pumila*, which we observed as typical karyotypes of *Lycoris*, were used in this study. These species have karyotypes consisting of acrocentric

Table 1. Karyotypes of parental bulbs of the species used in this study.

Species	Karyotype ¹⁾
<i>L. aurea</i>	2n=8M+6T
<i>L. traubii</i>	2n=10M+2T
<i>L. sanguinea</i>	2n=22A
<i>L. sprengeri</i>	2n=22A
<i>L. pumila</i> ²⁾	2n=22A

¹⁾ M, metacentric chromosome; A, acrocentric chromosome; T, telocentric chromosome.

²⁾ *L. radiata* var. *pumila*.

chromosomes (A-type) or metacentric and telocentric chromosomes (M+T type) as shown in Table 1, which were considered to be original karyotypes of *Lycoris* species (Kurita, 1988). The bulbs of these species were obtained from Germplasm Conservation Society of *Lycoris* (Japan) or Chiba University. The parental plants were grown in the field of Osaka Prefecture University, Sakai, Osaka.

In order to obtain selfed progenies more efficiently, scapes were cut just before anthesis and kept in flasks filled with tap water (Koyama, 1959); artificial self-pollination was done using the pollen grains collected from the same plant,

Table 2. Allozyme variation in selfed progenies of several *Lycoris* species.

Locus	Band pattern ¹⁾	<i>L. aurea</i>	<i>L. traubii</i>	<i>L. sprengeri</i>	<i>L. sanguinea</i>	<i>L. pumila</i> ³⁾
<i>Apt-1</i>	P	47	25	30	0	21
	A	0	4	0	64	0
<i>Apt-2</i>	P	47	24	18	33	17
	A	0	5	12	31	4
<i>Got-2</i>	F	35	0	0	64	0
	FS	12	29	0	0	0
	S	0	0	30	0	21
<i>Got-3</i>	F	- ²⁾	-	0	-	6
	FS	-	-	0	-	10
	S	-	-	30	-	5
<i>Got-4</i>	F	0	0	0	2	-
	FS	0	0	0	42	-
	S	47	29	30	20	-
<i>Est-1</i>	F	-	-	12	13	-
	FS	-	-	10	34	-
	S	-	-	8	17	-
<i>Est-3</i>	P	0	0	0	24	15
	A	47	29	30	40	6
No. of S1		47	29	30	64	21
No. of parental plant		6	3	8	16	5

¹⁾ P, presence; A, absence; F, fast migration; S, slow migration.

²⁾ -, no band

³⁾ *L. radiata* var. *pumila*

and immature seeds were collected 30 to 35 days after pollination. The selfed seeds were then cultured on 1/2 MS medium for germination, and the seedlings obtained were transferred onto MS medium for bulb development and maintenance (Ma et al., 2000, 2001a).

After observing the chromosome number of these selfed plants, diploid individuals with the same chromosome complements as that of their corresponding parents were used for isozyme analysis, because rare production of triploid and tetraploid was reported in the same kind of *in vitro* culture (Ma and Tarumoto, 2002).

Electrophoresis

Fresh leaves collected from *in vitro* plants were used for enzyme extraction. About 0.5 g (3–5 cm in length) of leaf tissue per plant was crushed in 3 to 4 drops of extraction buffer (1 M Tris-HCl with 0.1% (v/v) 2-mercaptoethanol, pH 7.2) at room temperature (Ma, 2001). Paper wicks (10 mm × 5 mm, Advantec No. 526) were used to absorb the crude extracts and placed into the gel. Twenty one to sixty four selfed plants per each of five species were used to analyze (Table 2).

Horizontal starch gel electrophoresis was used to examine variation in three enzyme systems: acid phosphatase (APT, EC 3.1.3.2.), glutamate-oxaloacetate transaminase (GOT, EC 2.6.1.1.) and esterase (EST, EC 3.1.1.1.). For all systems 11% starch (Sigma) gel was used. The gel buffer for APT and GOT consisted of nine parts of 0.05 M Tris-citrate (pH 8.3 with 1M citric acid·H₂O) and one part of 0.2 M boric acid (pH 8.3 with 1M LiOH·H₂O), and the gel buffer for EST consisted of nine parts of 0.066 M Tris-citrate (pH 8.3) and one part of 0.3 M boric acid (pH 8.3). The electrode buffer was 0.2 M boric acid (pH 8.3) for all the systems. Electrophoresis was conducted in a refrigerator (4 °C) at 150 V constant voltage for 20 min., after that the wicks were removed, and the electrophoresis was continued at 40 mA constant amperage for 3 to 4 h until the front had migrated 8 cm from the origin. The gels were sliced into 3 pieces and stained with the recipes described by Vallejos (1983).

Data analysis

Since there are no variant band patterns within parental plants used in each species, allozyme loci were tentatively determined by variation patterns different from the corresponding parental ones. The putative loci were numbered sequentially from the cathode side, and alleles were designated as “F” (fast) or “S” (slow) according to their migration rate; in loci with a single band, the presence and absence of a band were denoted by “P” and “A”, respectively (Figs. 1,2). A locus was considered to be polymorphic if two or more alleles were detected. Segregations of alleles were tested against monogenic segregation of 1:2:1 or 3:1. The Chi-square analysis of parental plants in each species was not performed because of their clonal origin.

Results and Discussion

Fourteen allozyme loci were resolved from the three enzyme systems. Among them, seven loci were well stained, and isozyme polymorphism was detected. The seven clear polymorphic loci were used for evaluation of population.

Examples of allozyme segregation are given in Fig. 1, and the putative loci of each isozyme are shown in Fig. 2. The variation of band patterns observed in selfed progenies of the five species are summarized in Table 2. As shown in Figs. 1 and 2, for Apt, polymorphism was detected in two loci (*Apt-1* and *Apt-2*), and a single-banded phenotype was observed in each of the loci; for Got, polymorphism was detected in three loci (*Got-2*, *Got-3* and *Got-4*), and two kinds of single band (F and S) and a triple-banded one (FS) were observed in each of the loci; for Est, polymorphism was detected in two loci (*Est-1* and *Est-3*), two kinds of single band (F and S) and a double-banded one was observed in *Est-1*, and a single-banded phenotype was observed in *Est-3*. The results suggest that Apt and Est are monomeric and Got is dimeric in structure. In addition, an invariant triple-banded phenotype for *Got-2* locus was observed in all selfed progenies of *L. traubii*. This band pattern was interpreted as fixed heterozygote (FS) which may result from gene duplication.

As shown in Table 2, allozyme segregations

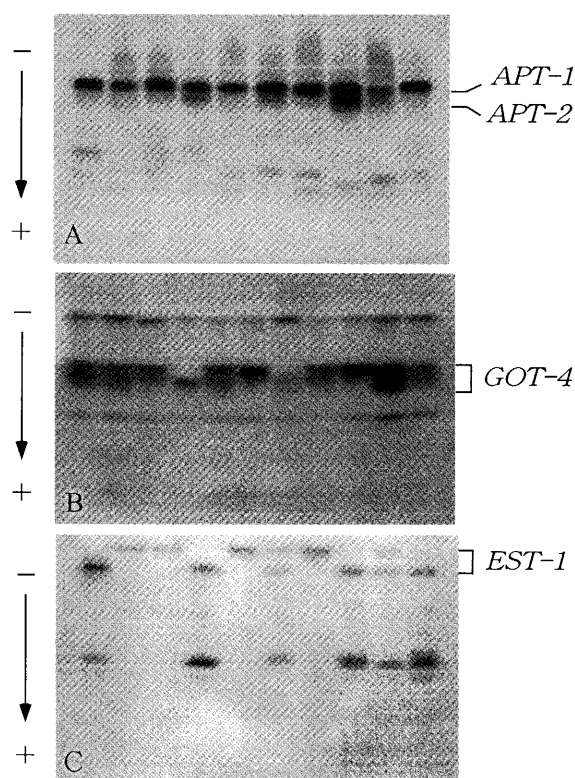


Fig. 1. Samples of allozyme segregation detected in selfed progenies of *Lycoris* species.

A: zymogram of APT in *L. sprengeri*; zymogram of GOT in *L. sanguinea*; C: zymogram of EST in *L. sprengeri*.

were observed in all of the five species. For *L. aurea*, all the loci are homozygous except for *Got-2* in which segregation was observed; for *L. traubii*, segregations were observed in *Apt-1* and *Apt-2*, and the other loci are homozygous; for *L. sprengeri*, segregations were observed in *Apt-2* and *Est-1*; for *L. sanguinea*, segregations were observed in *Apt-2*, *Got-4*, *Est-1* and *Est-3*; and for *L. radiata* var. *pumila*, segregations were observed in *Apt-2*, *Got-3* and *Est-3*.

Chi-square analysis showed that most of the segregation patterns fit the monogenic segregation of 3:1 or 1:2:1 except for the locus *Got-2* from *L. aurea* and the loci *Apt-2*, *Est-1* and *Est-3* from *L. sanguinea* (Table 3). The skewed segregations may be caused by an incompatible reaction or lethal reaction of the corresponding gametes (Kahler and Lay, 1985).

Taken together, genetic segregation for several allozyme loci in selfed progenies were observed in all of the five diploid progenitor species. From the results, it would be concluded that *L. aurea*, *L. traubii*, *L. sprengeri*, *L. sanguinea* and *L. radiata* var. *pumila* are of a heterozygous nature. A similar heterozygous nature revealed by molecular markers has been reported in a number of vegetatively propagated plants such as potato (Hosaka and Hanne-

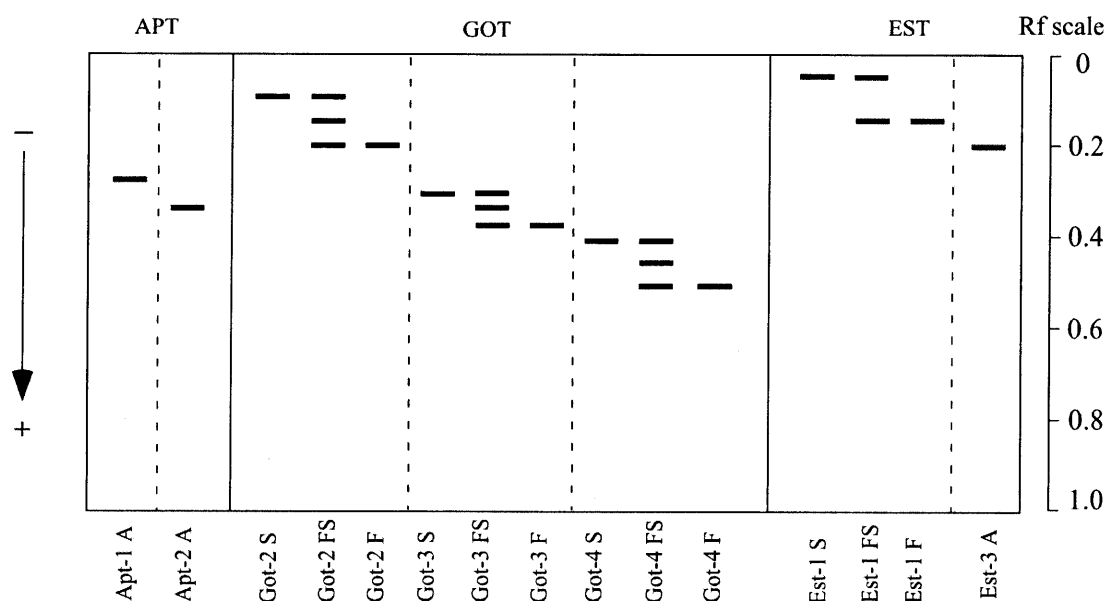


Fig. 2. Isozyme band patterns and putative APT, GOT and EST loci assignments for selfed progenies of *Lycoris* species.

Table 3. Chi-square test for allozyme segregation in selfed progenies of *Lycoris* species

Species	Locus	Expected ratio	χ^2	P
<i>L. aurea</i>	<i>Got-2</i>	1:2:1	63.383	0.00
<i>L. traubii</i>	<i>Apt-1</i>	3:1	1.942	0.10-0.20
	<i>Apt-2</i>	3:1	0.931	0.30-0.50
<i>L. sprengeri</i>	<i>Apt-2</i>	3:1	3.600	0.05-0.10
	<i>Est-1</i>	1:2:1	4.400	0.10-0.20
<i>L. sanguinea</i>	<i>Apt-2</i>	3:1	18.750	0.00
	<i>Got-4</i>	1:2:1	16.375	0.00
	<i>Est-1</i>	1:2:1	0.750	0.70-0.80
	<i>Est-3</i>	3:1	48.000	0.00
<i>L. pumila</i> ¹⁾	<i>Apt-2</i>	3:1	0.397	0.50-0.60
	<i>Got-3</i>	1:2:1	0.143	0.90-0.95
	<i>Est-3</i>	3:1	0.143	0.70-0.80

¹⁾*L. radiata* var. *pumila*

man, 1988) and black currant (Lanham, 1996).

In *Lycoris*, hybridization has been proved to be one of important modes of speciation (Bose and Flory, 1963; Kurita, 1988; Kurita and Hsu, 1996). From the result in our study, it is thus supposed that the five diploid species may have hybrid origins, and their vegetative propagation habit should be responsible for maintaining the heterozygosity under natural conditions.

Acknowledgements

We would like to thank Prof. Siro Kurita, Chiba Univ., and Prof. Genjiro Mori, Osaka Pref. Univ., for their kind offer of some plant materials as well as valuable advice. We are grateful to assistant Prof. Toshinobu Morikawa, Osaka Pref. Univ. for his kind assistance in statistic analysis, and to Dr. Jonathan Seely, University of Arizona, for his critical reading of the manuscript.

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(Received Dec. 22, 2003; Accepted Mar. 15, 2004)