Tracing the Parentages of Some Oriental Hybrid Lily Cultivars by PCR-RFLP Analysis

Kazuhisa Haruki¹, Takashi Hosoki¹ and Youji Nako²

¹Faculty of Life and Environmental Science, Shimane University, Nishikawatsu-cho, Matsue, Shimane 690-0823

²Shimane Agricultural Experiment Station, Ashiwata-cho, Izumo, Shimane 693-0035

Summary

PCR-RFLP analysis was applied to some interspecific hybrid cultivars of lily to verify their probable parentage. Six *Lilium* species, *L. auratum* var. *platyphyllum*, *L. japonicum*, *L. speciosum*, *L. nobilissimum*, *L. auratum* and *L. rubellum*, were analyzed with four pairs of primers and 22 restriction enzymes; among them PCR-RFLP markers, characteristic to each species, were detected.

L. ×formolongi, *L. speciosum* and their artificial hybrids derived by embryo culture confirmed that the PCR-RFLP markers in a chloroplast gene of the female parent were inherited by all hybrids, whereas the markers in a nuclear rRNA gene of the hybrids possessed a combined band pattern of the female and pollen parents. Hence, we utilized PCR-RFLP analysis for tracing the parents of interspecific hybrid cultivars.

PCR-RFLP gel patterns were analyzed to trace parents of six lily hybrid cultivars called Oriental hybrids, 'Star Gazer', 'Le reve', 'Casa Blanca', 'Summer Dress', 'Pink Pearl', and 'Marco Polo'. The data indicate that the cytoplasm of 'Casa Blanca', 'Summer Dress', and 'Pink Pearl' was transmitted from *L. speciosum*, whereas that of 'Star Gazer', 'Le reve', and 'Marco Polo', was inherited from *L. auratum* var. *platyphyllum* or *L. japonicum*. The pollen parents of these hybrids were not completely identified because genetic segregation may have occurred at gametogenesis if pollen parents were interspecific hybrids.

Key Words: *Lilium*, Oriental hybrids, PCR, RFLP, DNA marker.

Introduction

Lilies are important ornamental plants. Many cultivars have been created by complicated crossing of wild species and their hybrids; thus, some new cultivars are registered every year under the seed and seedling registration laws in Japan (Takaki, 1994, 1995, 1996). Lily cultivars in Division VII, classified by the Royal Horticultural Society (Leslie, 1982), are called Oriental Lily being hybrids of far eastern species in section *Archelirion* including *L. auratum* Lindl., *L. auratum* var. *platyphyllum* Baker, *L. speciosum* Thunb., *L. nobilissimum* Makino, *L. japonicum* Thunb. and *L. rubellum* Baker. The parents of some Oriental hybrids are unknown; that information would be very important to create new cultivars.

Recently, to identify cultivars, PCR-RFLP analysis or RAPD method was applied to *Diospyros* (Yonemori et al., 1996) and evergreen azalea (Kobayashi et al., 1995). The usefulness of RAPD markers in breeding program was suggested by Kobayashi et al. (1996). In roses, female parents of some hybrid cultivars were traced by PCR-RFLP analysis of chloroplast genes (Takeuchi et al., 1995). In lily, the section specific

RAPD markers of both parental sections were present in some inter-sectional hybrids, and the usefulness of RAPD analysis for identifying the parental section of inter-section hybrids was recognized by Yamagishi (1995).

We have already explored the possibility of classifying some lily species by PCR-RFLP analysis, and did classify some ancestral species of hybrid cultivars in Division VII into three groups with *rbcl* gene in chloroplast and rRNA gene in nucleus (Haruki et al., 1997).

In this study we searched for markers in some chloroplast genes or nuclear rRNA gene by PCR-RFLP analysis to trace the female and pollen parents of some Oriental hybrid cultivars.

Materials and Methods

Lilium species in section *Archelirion* and hybrids in Division VII, used in this experiment, are shown in Table 1. Species from No. 1 to 6 were analyzed for specific markers by PCR-RFLP methods. Species No. 3 and No. 7, and five hybrids in No. 8, obtained by embryo culture between *L. ×formolongi* and *L. speciosum* in Shimane Agricultural Experiment Station, were used to confirm the inheritance of PCR-RFLP markers. Finally, commercial cultivars from No. 9 to 14 were analyzed to trace their parentage.

Received; February 24, 1997. Accepted; August 1, 1997.

Table 1. Species and hybrids examined in this experiment.

No.	species and hybrids	Source
1.	<i>L. auratum</i> var. <i>platyphyllum</i> Baker #1	Yamato-noen Co., Ltd.
	<i>L. auratum</i> var. <i>platyphyllum</i> Baker #2	Takii Seed Co., Ltd.
2.	<i>L. japonicum</i> Thunb. #1	Takii Seed Co., Ltd.
	<i>L. japonicum</i> Thunb. #2	Wild (Matsue Shimane)
	<i>L. japonicum</i> Thunb. #3	Wild (Matsue Shimane)
	<i>L. japonicum</i> Thunb. #4	Wild (Yoshida Shimane)
	<i>L. japonicum</i> Thunb. #5	Nara Agr. Exp. Stn.
3.	<i>L. speciosum</i> Thunb. 'Uchida-Kanoko'	Sakata Seed Corp.
	<i>L. speciosum</i> Thunb. 'Beni-Kanoko'	Takii Seed Co., Ltd.
4.	<i>L. nobilissimum</i> Makino	Sakata Seed Corp.
5.	<i>L. auratum</i> Lindl. #1	Yamato-noen Co., Ltd.
	<i>L. auratum</i> Lindl. #2	Shunso-en Nursery
6.	<i>L. rubellum</i> Baker. #1	Shunso-en Nursery
	<i>L. rubellum</i> Baker. #2	Takii Seed Co., Ltd.
7.	<i>L. × formolomgi</i> 'No.22'	Shimane Agr. Exp. Stn.
8.	'HB-1' (<i>L. × formolomgi</i> × <i>L. speciosum</i>)	Shimane Agr. Exp. Stn.
	'HB-2' (<i>L. × formolomgi</i> × <i>L. speciosum</i>)	Shimane Agr. Exp. Stn.
	'HB-3' (<i>L. × formolomgi</i> × <i>L. speciosum</i>)	Shimane Agr. Exp. Stn.
	'HB-4' (<i>L. × formolomgi</i> × <i>L. speciosum</i>)	Shimane Agr. Exp. Stn.
	'HB-5' (<i>L. × formolomgi</i> × <i>L. speciosum</i>)	Shimane Agr. Exp. Stn.
9.	'Star Gazer' (hybrid, Division V II)	Takii Seed Co., Ltd.
10.	'Le reve' (hybrid, Division V II)	Dai-ichi Seed Co., Ltd.
11.	'Casa Blanca' (hybrid, Division V II)	Dai-ichi Seed Co., Ltd.
12.	'Summer Dress' (hybrid, Division V II)	Sakata Seed Corp.
13.	'Pink Pearl' (hybrid, Division V II)	Dai-ichi Seed Co., Ltd.
14.	'Marco Polo' (hybrid, Division V II)	Dai-ichi Seed Co., Ltd.

Table 2. PCR primers used for this experiment.

Target gene	Primer	Sequence
Chloroplast		
	<i>rbcl</i>	
	SH020 ^z	5' -CGCGGTGGACTTGATTTTACC-3'
	SH021 ^z	GGCATATGCCAAACATGAATACC
<i>rbcl-atpB</i>	SH019 ^y	GGTAAATCAAGTCCACCGCG
	SH022 ^y	GCACTCATAGCTACAGCTCTAAC
<i>matK</i>	matK-AF ^x	CTATATCCACTTATCTTTCAGGAGT
	matK-MR ^x	CCAGAAGAGGTTAATCGTAAATGA
Nucleus		
	<i>rRNA</i>	
	SH013 ^z	ACACACCGCCCGTCGCTCCTA
	SH015 ^z	ACTCGATGGTTCACGGGATTCTG

^z Referred to Haruki et al. (1997)^y The primers were designed based on the nucleotide sequences of lily *rbcl* and rice *atpB* gene.^x Referred to Ooi et al. (1995) and Endo (1995).

Extraction of DNA and PCR-RFLP analysis were previously reported (Haruki et al., 1997); the nucleotide sequences of primers (Table 2) and their positions are indicated in Fig. 1. The primers in the maturase (*matK*) gene (Johnson and Soltis, 1994) designed by Ooi et al. (1995) and Endo (1995) were used. Other primers were designed, based on the nucleotide sequences of ribulose-1, 5-bisphosphate carboxylase large subunit (*rbcl*) gene (Nishizawa and Hirai, 1987), ATPase subunit B (*atpB*) gene (Moon et al., 1987), and nuclear ribosomal RNA (*rRNA*) gene (Baldwin, 1993) searched by MAC/CD (ver. 29.0.0) and GENETYX-MAC (ver. 7.0) software package (Software Develop-

Fig. 1. Positions of pair primers : SH020 and SH021, SH019 and SH022, *matK*-AF and *matK*-MR, SH013 and SH015 represented by short solid lines. The nucleotide sequences of SH019 and SH020 are complementary. The internal transcribed spacers region (ITS) of nuclear *rRNA* gene is included between SH013 and SH015.

ment Co., Ltd.). The PCR reaction mixtures were incubated 94°C for 1 min for initial denaturation and were subjected to amplification in a thermal cycler for 35 cycles which consisted of 1 min at 94°C, 1 min at 55°C, 2 min at 72°C, and followed by 3 min at 72°C. When the primers in *matK* gene were used, the temperature for annealing was lowered from 55°C to 50°C. All the reactions were repeated at least twice in different experiments.

The PCR products in species No. 1 to 6 were digested by 5–10 units of 22 restriction enzymes (*Cfo* I, *Msp* I, *Rsa* I, *Hae* III, *Mvn* I, *Hin* d II, *Alu* I, *Hin* f I, *Sau* 3A I, *Sau* 96 I, *Sma* I, *Ban* I, *Scr* F I, *Taq* I, *Hsp* 92 II, *Dde* I, *Tru* 9 I, *Sty* I, *Bsp* 1286 I, *Eco* R I, *Vsp* I, *Mbo* II). The PCR products in No. 9 to 14 were digested by the restriction enzymes which generated the marker fragments in No. 1 to 6. The PCR products in No. 3, 7, and 8 were digested by *Cfo* I and *Hae* III to confirm the inheritance of PCR-RFLP markers. The digestion products were separated by agarose gel electrophoresis with 3.0% Nusieve GTG agarose (FMC Bio Products) or 1.5% Agarose S (Nippon gene Co., Ltd.) in TAE buffer (40 mM Tris-acetate pH 8.0, 1 mM EDTA), containing 0.5 mg·liter⁻¹ ethidium bromide and observed under UV light.

Results

1. PCR-RFLP in section *Archelirion*

The length of the fragments amplified with SH020+SH021 primers was about 570bp of *rbcl* gene, and

polymorphisms were observed when the fragments were digested with three out of 22 restriction enzymes tested (Table 3). The length of the fragments with SH019 + SH022 was about 1500bp of the non-coding region between *atpB* and *rbcl* genes, and that of *matK*-AF + *matK*-MR primers was about 650bp within *matK* gene. Their polymorphisms were observed when digested with four out of 22 restriction enzymes. The length of the fragments with SH013 + SH015 was about 520bp of rRNA genes, including an ITS region; polymorphisms appeared when digested with two out of 22 restriction enzymes. Sixteen polymorphic bands out of 159 bands were generated when 88 combinations of four pair-primers and 22 restriction enzymes were tested. Therefore, the six species tested had high genetic similarity.

Examples of PCR-RFLP analysis are shown in Fig. 2, and the detected markers are shown in Table 3. Some markers were present in chloroplast genes. *L. speciosum* (No. 3) was distinguished with *Vsp* I₈₅₀ in *rbcl*-*atpB* from the other species, and *L. nobilissimum* (No. 4) was separated with *Mbo* II₁₇₀ in *matK*. *L. rubellum* (No. 6) was characterized with *Hin* f I₁₃₀ or *Sau* 3A I₃₀₀ in *matK*. *L. auratum* (No. 5) was set apart by the presence of *Bsp* 1286 I_{350,170} in *rbcl* and absence of *Hin* f I₁₃₀ or *Sau* 3A I₃₀₀ in *matK*. *L. auratum* var. *platyphyllum* (No. 1) and *L. japonicum* (No. 2) could not be differentiated by these markers. On the other hand, the markers in nuclear rRNA gene distinguished *L. speciosum* and *L. nobilissimum* from other species.

Individual plants in the same species were labelled with different markers (Table 1) by PCR-RFLP techniques as a check; the results are shown in Fig. 3. Two individuals of *L. auratum* var. *platyphyllum* (No. 1), five of *L. japonicum* (No. 2), two of *L. auratum* (No. 5), two of *L. rubellum* (No. 6), or two cultivars of *L. speciosum* (No. 3) had the same PCR-RFLP patterns in each species (*L. auratum* not shown in Fig. 3).

Table 3. Polymorphic band patterns obtained by restriction enzymes in *rbcl*, *matK* and rRNA gene amplified with the some pairs of primers.

Primers	Restriction enzyme	Restriction fragment length (bp)	Species ^z					
			1	2	3	4	5	6
Chloroplast								
SH020+SH021 (<i>rbcl</i>)	<i>Alu</i> I	270	+ ^y	+	+	+	- ^y	-
	<i>Alu</i> I	210	-	-	-	-	+	+
	<i>Cfo</i> I	100	-	-	-	-	+	+
	<i>Cfo</i> I	80	+	+	+	+	-	-
	<i>Bsp</i> 1286 I	350	-	-	-	-	+	+
	<i>Bsp</i> 1286 I	170	-	-	-	-	+	+
SH019+SH022 (<i>rbcl</i> - <i>atpB</i>)	<i>Vsp</i> I	950	+	+	-	+	+	+
	<i>Vsp</i> I	850	-	-	+	-	-	-
<i>matK</i> -AF+ <i>matK</i> -MR (<i>matK</i>)	<i>Hin</i> f I	150	+	+	+	+	+	-
	<i>Hin</i> f I	130	-	-	-	-	-	+
	<i>Sau</i> 3A I	300	-	-	-	-	-	+
	<i>Mbo</i> II	320	+	+	+	-	+	+
	<i>Mbo</i> II	170	-	-	-	+	-	-
Nucleus								
SH013+SH015 (<i>rRNA</i>)	<i>Sau</i> 96 I	180	+	+	-	-	+	+
	<i>Hsp</i> 92 II	300	-	-	+	+	-	-
	<i>Hsp</i> 92 II	220	-	-	+	+	-	-

¹ 1: *L. auratum* var. *platyphyllum* 2: *L. japonicum*

² 3: *L. speciosum* 4: *L. nobilissimum* 5: *L. auratum* 6: *L. rubellum*

^y +: present, -: absent

2. Inheritance of PCR-RFLP bands

PCR-RFLP analysis was applied to *L. × formolongi* (female parent), *L. speciosum* (pollen parent) and their hybrids by embryo culture to confirm the inheritance of the PCR-RFLP markers (Fig. 4). The band pattern characterized by *rbcl* gene of female parent, *L. × formolongi*, was detected in all the hybrids. The band patterns of nuclear rRNA gene in the hybrids possess a combined band pattern of their female and pollen

Fig. 2. A gel plate for PCR-RFLP analysis. PCR products with primer SH020 and SH021 were digested by *Cfo* I and *Bsp* 1286 I. Lanes: 1: *L. auratum* var. *platyphyllum*. 2: *L. japonicum*. 3: *L. speciosum*. 4: *L. nobilissimum*. 5: *L. auratum*. 6: *L. rubellum*. M: 50bp ladder marker.

Fig. 3. Gel plates for PCR-RFLP analysis of individual cultivars in some species. A : PCR products with primer SH020 and SH021 were digested by *Cfo* I. B : PCR products with primer SH020 and SH021 were digested by *Bsp* 1286 I. C : PCR products with primer SH013 and SH015 were digested by *Sau* 96 I. Lanes : 1a-1b : *L. auratum* var. *platyphyllum* individuals # 1- # 2. 2a-2e : *L. japonicum* individual # 1- # 5. 3a : *L. speciosum* 'Uchida-Kanoko' 3b : *L. speciosum* 'Beni-Kanoko' 6a-6b : *L. rubellum* individual # 1- # 2. M : 50bp ladder marker.

Fig. 4. Comparison of gel plates to confirm the inheritance of the PCR-RFLP markers. A : PCR products with primer SH020 and SH021 in chloroplast gene digested by *Cfo* I. B : PCR products with primer SH013 and SH015 in nucleus rRNA gene digested by *Hae* III. Lanes 5 : *L. speciosum* 'Beni-Kanoko'. 8a-8e : 'HB-1'-'HB-5'. 7 : *L. ×formolongi*.

parents.

3. Inference of the parental species of Oriental hybrid cultivars

Table 4 shows the result of PCR-RFLP analysis obtained from six commercial interspecific hybrid cultivars, 'Star Gazer', 'Le reve', 'Casa Blanca', 'Summer Dress', 'Pink Pearl', and 'Marco Polo'. Fig. 5 shows examples of PCR-RFLP analysis. 'Casa Blanca' (No. 11),

'Summer Dress' (No. 12) and 'Pink Pearl' (No. 13) have a unique marker, *Vsp* I₈₅₀ in *rbcL-atpB*, found only in the chloroplast gene of *L. speciosum*. 'Star Gazer' (No. 9), 'Le reve' (No. 10), and 'Marco Polo' (No. 14) have the same marker in the chloroplast gene as *L. auratum* var. *platyphyllum* or *L. japonicum*. The PCR-RFLP pattern in nuclear rRNA gene, 'Star Gazer' (No. 9), 'Le reve' (No. 10), 'Casa Blanca' (No. 11), and 'Pink Pearl' (No. 13) have the same pattern of

Fig. 5. Examples for comparisons of PCR-RFLP gel analysis of commercial interspecific lily hybrids. A : PCR products with primer matK-AF and matK-MR in chloroplast gene digested by *Mbo* II. B : PCR products with primer SH019 and SH022 in chloroplast gene digested by *Vsp* I. C : PCR products with primer SH013 and SH015 in nucleus rRNA gene digested by *Sau* 96 I. Lanes : 9 : 'Star Gazer'. 10 : 'Le reve'. 11 : 'Casa Blanca'. 12 : 'Summer Dress'. 13 : 'Pink Pearl'. 14 : 'Marco Polo'. 4 : *L. nobilissimum*. 2 : *L. japonicum*. 3a : *L. rubellum*. 3b : *L. speciosum* Thunb. 'Uchida-Kanoko'. 3b : *L. speciosum* 'Beni-Kanoko'.

Table 4. PCR-RFLP markers observed in six hybrid cultivars.

Primers	Restriction enzyme	Restriction fragment length (bp)	Hybrid cultivars ^z					
			9	10	11	12	13	14
Chloroplast								
SH020+SH021 (rbcL)	<i>Alu</i> I	270	+ ^y	+	+	+	+	+
	<i>Alu</i> I	210	- ^y	-	-	-	-	-
	<i>Cfo</i> I	100	-	-	-	-	-	-
	<i>Cfo</i> I	80	+	+	+	+	+	+
	<i>Bsp</i> 1286 I	350	-	-	-	-	-	-
	<i>Bsp</i> 1286 I	170	-	-	-	-	-	-
SH019+SH022 (rbcL-atpB)	<i>Vsp</i> I	950	+	+	-	-	-	+
	<i>Vsp</i> I	850	-	-	+	+	+	-
matK-AF+matK-MR (matK)	<i>Mbo</i> II	320	+	+	+	+	+	+
	<i>Mbo</i> II	170	-	-	-	-	-	-
Nucleus								
SH013+SH015 (rRNA)	<i>Sau</i> 96 I	180	+	+	+	-	+	+
	<i>Hsp</i> 92 II	300	+	+	+	+	+	-
	<i>Hsp</i> 92 II	220	+	+	+	+	+	-

^a 9 : 'Star Gazer' 10 : 'Le reve' 11 : 'Casa Blanca'

12 : 'Summer Dress' 13 : 'Pink Pearl' 14 : 'Marco Polo'

^y + : present, - : absent

Discussion

The presence of the positive markers with which a parent species is distinguished from other species is necessary to trace the parents of interspecific hybrid cultivars. The markers must be inherited and be similar among individuals in a species. Based on this hypothesis, Haruki et al. (1997) detected six markers in *rbcL* gene using three restriction enzymes, *Alu* I, *Cfo* I and *Bsp* 1286 I (Table 3). With these markers *L. auratum* and *L. rubellum* were distinguishable from other four species, *L. auratum* var. *platyphyllum*, *L. nobilissimum*, *L. speciosum*, and *L. japonicum*. In this study we detected seven more markers in *matK* or *rbcL-atpB* gene with four restriction enzymes, *Vsp* I, *Hin* f I, *Sau* 3A I, and *Mbo* II. With these markers, four species except *L. auratum* var. *platyphyllum* and *L. japonicum* were differentiated. For tracing the pollen parent, we used the markers in nuclear rRNA gene found in the previous report (Haruki et al., 1997).

Shimizu (1987a) reported that *L. auratum* var. *platyphyllum* is a taxonomic variety of *L. auratum*. Asano (1986) showed that *L. rubellum* is closely related to *L. japonicum*, according to cluster analysis with 53 morphological characters. However, our PCR-RFLP data in the previous report (Haruki et al., 1997) and in the present study with *rbcL*, *rbcL-atpB* and *matK* in

rRNA gene digested with *Sau* 96 I and *Hsp* 92 II. Whereas 'Summer Dress' (No. 12) and 'Marco Polo' (No. 14) have a different pattern.

chloroplast indicate that *L. auratum* var. *platyphyllum* is closer to *L. japonicum* than it is to *L. auratum*, and *L. rubellum* is closer to *L. auratum* than it is to *L. japonicum* (Table 3). More investigations are necessary to clarify their relationship.

Palmer et al. (1988) reported that genes in the chloroplast were considerably conserved at the nucleotide sequence level among higher green plants and *rbcL* gene in the chloroplast can be utilized to trace the evolutionary relationships among genera in a family. Also, the internal transcribed spacers of nuclear rRNA gene, within which nucleotide sequence was conserved among species, were clarified, and based on their results, evolutionary relationships among 33 species in *Paeonia* were analyzed (Sang et al., 1995). In our experiment, the individuals in the same species have the same PCR-RFLP markers in chloroplast genes and in the nuclear rRNA gene, whereas the different species have different PCR-RFLP markers.

The results of our PCR-RFLP analysis on hybrids of *L. × formolongi* and *L. speciosum* disclosed that the PCR-RFLP markers were inherited according to the theory of nuclear or extranuclear (chloroplast) gene inheritance. Grun (1976) reported that although chloroplasts were inherited from the female parent in most plants, they were inherited from both female and pollen parents in the genera, *Phaseolus*, *Medicago* and *Oenothera*. Our PCR-RFLP analysis showed that the chloroplasts of *L. × formolongi* were inherited from the female parent only. Therefore, it is possible to utilize PCR-RFLP analysis for tracing the parents of interspecific hybrid cultivars in lily, namely, the female parent could be related back to the chloroplast genes, whereas the pollen parent could be traced to their nuclear rRNA gene.

The parentage of lily cultivars examined in this study is known only for 'Summer Dress'; the female parent is *L. speciosum*, and the pollen parent is a hybrid of *L. speciosum* × *L. auratum* and a variant of *L. auratum* (Shimizu, 1987b). In our experiment, we found that 'Summer Dress' has a unique marker in chloroplast gene of *L. speciosum* (Table 3 and Table 4) so that our data on female parent of 'Summer Dress' supported Shimizu's record (1987b). But the PCR-RFLP pattern of the nuclear rRNA gene of 'Summer Dress' is "—, +, +" (Table 4), the same as *L. speciosum* and not "+, +, +". It appears that PCR-RFLP markers which characterized *L. auratum* segregated when the pollen parent of 'Summer Dress' was crossed with *L. speciosum*. Thus, our result on pollen parent agrees with Shimizu's record (1987b).

The maternal parent of 'Casa Blanca' is 'Jamboree', (*L. speciosum* × *L. auratum*) × *L. speciosum*, whereas the pollen parent is unknown (Shimizu, 1987b). The PCR-RFLP markers in chloroplast (Tables 3 and 4) indicate that the cytoplasm of 'Casa Blanca' was derived from *L. speciosum* as reported by Shimizu. The

PCR-RFLP pattern with nuclear rRNA gene of 'Casa Blanca' and *L. speciosum* was "+, +, +" and "—, +, +", respectively (Table 3 and Table 4). Therefore, the pollen parent or ancestry of 'Casa Blanca' may be *L. auratum* var. *platyphyllum*, *L. japonicum*, *L. auratum*, or *L. rubellum* in which PCR-RFLP pattern was "+, —, —" (Table 3).

'Pink Pearl' has the same chloroplast and nuclear rRNA gene marker patterns as 'Casa Blanca' (Table 4). Thus, the cytoplasm of 'Pink Pearl' was inherited from *L. speciosum*, whereas its pollen parent or ancestry was *L. auratum* var. *platyphyllum*, *L. japonicum*, *L. auratum*, or *L. rubellum*.

The parentage of 'Star Gazer' and 'Le reve' are unknown (Okazaki, 1993). The PCR-RFLP pattern of chloroplasts from 'Star Gazer' and 'Le reve' (Table 4) indicate that their cytoplasm was transmitted from *L. auratum* var. *platyphyllum* or *L. japonicum*; whereas their rRNA gene pattern was "+, +, +" (Table 4), but that of *L. auratum* var. *platyphyllum* and *L. japonicum* was "+, —, —" (Table 3). Therefore, the nucleus gene of these hybrids may have been inherited from *L. speciosum* or *L. nobilissimum* for which the PCR-RFLP pattern was "—, +, +" (Table 3). Shimizu (1987b) believes that 'Star Gazer' is a hybrid of *L. speciosum* and *L. nobilissimum*. But, our results of the PCR-RFLP analysis reveal that either *L. speciosum* or *L. nobilissimum* is the pollen parent or an ancestor of 'Star Gazer'.

'Marco Polo' has the same chloroplast PCR-RFLP pattern as that of 'Star Gazer' and 'Le reve' (Table 4), and the same PCR-RFLP pattern of nuclear rRNA as that of *L. auratum* var. *platyphyllum*, *L. japonicum*, *L. auratum* and *L. rubellum* (Table 3). Therefore, we think that the cytoplasm of 'Marco Polo' is derived from *L. auratum* var. *platyphyllum* or *L. japonicum*, and that its pollen parent or recent ancestor is *L. auratum* var. *platyphyllum*, *L. japonicum*, *L. auratum* or *L. rubellum*.

Although species in *Archelirion* hybridize readily with other section species, such as *L. regale* Wilson, *L. henryi* Baker, *L. longiflorum* Thunb. or *L. formosanum* Wallace (Asano, 1987), hybrid cultivars in this study have specific PCR-RFLP markers in *Archelirion* as previously reported (Haruki et al., 1997). Therefore, we consider these cultivars to be intra-section hybrids of *Archelirion*. However, *L. alexandrae* hort. Wallace, which was not tested here, is included in *Archelirion*, and therefore the possibility that *L. alexandrae* is one of the parental species or ancestor of the tested hybrid cultivars can not be ruled out.

Thus PCR-RFLP analysis with chloroplast gene was useful for tracing the maternal parent of interspecific hybrids. However, the PCR-RFLP analysis of rRNA gene in the nucleus gave some information about the pollen parent or its ancestry. We were not able to determine the exact pollen parent because of genetic seg-

regation. That the PCR-RFLP markers at gametogenesis of the pollen parent may have segregated in 'Summer Dress' (Table 4 and Fig. 5). However, it is impossible to test this possibility because the pollen parent of 'Summer Dress' is no longer available. Therefore, to trace pollen parents of interspecific hybrids, more kinds of nucleus genes whose nucleotide sequences are conserved in a species but variable among species need to be rediscovered.

Literature Cited

- Asano, Y. 1986. A numerical taxonomic study of the genus *Lilium* in Japan. J. Fac. Agr. Hokkaido Univ. 62 : 333-341.
- Asano, Y. 1987. Breeding technique of lilies. p. 112-125. In : M. Shimizu (ed.). The lilies of Japan. Seibundoshinkosha, Tokyo. (In Japanese).
- Baldwin, B. G. 1993. Molecular phylogenetics of Calycadenia (Compositae) based on ITS sequences of nuclear ribosomal DNA : chromosomal and morphological evolution reexamined. Amer. J. Bot. 80 : 222-238.
- Endo, Y. 1995. Availability of matK nucleotide sequence to phylogeny. Analysis of Prunoideae in Rosaceae. Study of Species Biology 19 : 11-17. (In Japanese).
- Grun, P. 1976. Cytoplasmic Genetics and Evolution. p. 130-133. Columbia Univ. Press. New York.
- Haruki, K., T. Hosoki, Y. Nako and K. Ohta. 1997. Possibility of classification in some species of *Lilium* by PCR-RFLP of ribulose-1,5-bisphosphate carboxylase large subunit (rbcL) gene and ribosomal RNA gene. J. Japan. Soc. Hort. Sci. 66 : 189-192.
- Johnson, L. A. and D. E. Soltis. 1994. matK DNA sequences and phylogenetic reconstruction in Saxifragaceae s. str. Syst. Bot. 19 : 143-156.
- Kobayashi, N., R. Takeuchi, T. Handa and K. Takayanagi. 1995. Cultivar identification of evergreen azalea with RAPD method. J. Japan. Soc. Hort. Sci. 64 : 611-616.
- Kobayashi, N., M. Akabane, T. Handa and K. Takayanagi. 1996. Inheritance of morphological characters and RAPD markers in intersubgeneric hybrids of azalea, (*Rhododendron kiusianum* Makino $\times$ *R. indicum* (L.) Sweet) $\times$ *R. japonicum* (A. Gray) Suringer f. *flavum* Nakai. J. Japan. Soc. Hort. Sci. 65 : 145-153.
- Leslie, A. C. 1982. The international lily register, 3rd edit. p. 8-9. The Royal Horticultural Society, London.
- Moon, E., T. Kao and R. Wu. 1987. Sequence of the chloroplast-encoded atpB-atpE-trnM gene clusters from rice. Nucleic Acids Res. 15 : 4358-4359.
- Nishizawa, Y. and A. Hirai. 1987. Nucleotide sequence and expression of the gene for the large subunit of rice ribulose 1,5-bisphosphate carboxylase. Jpn. J. Genet. 62 : 389-395.
- Okazaki, K. 1993. History of cultivar breeding. p. 17-21. In : M. Kunishige (ed.). Lilium : Breeding and culture. Seibundoshinkosha, Tokyo. (In Japanese).
- Ooi, K., Y. Endo, J. Yokoyama and N. Murakami. 1995. Useful primer designs to amplify DNA fragments of the plastid gene matK from angiosperm plants. J. Jpn. Bot. 70 : 328-331.
- Palmer, J. D., R. K. Jansen, H. J. Michaels, M. W. Chase and J. R. Manhart. 1988. Chloroplast and variation and plant phylogeny. Ann. Missouri Bot. Gard. 75 : 1180-1206.
- Sang, T., D. J. Crawford and T. F. Stuessy. 1995. Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA : Implications for biogeography and concerted evolution. Proc. Natl. Acad. Sci. USA 92 : 6813-6817.
- Shimizu, M. 1987a. Species, varieties and cultivars of lilies. p. 47-97. In : M. Shimizu (eds.). The lilies of Japan. Seibundoshinkosha, Tokyo. (In Japanese).
- Shimizu, M. 1987b. Ornamental species and cultivars of lilies. p. 148-165. In : M. Shimizu (ed.). The lilies of Japan. Seibundoshinkosha, Tokyo. (In Japanese).
- Takaki, T. 1994. New crop cultivars registered under seed and seedling law (12). Breeding Science 44 : 439-444.
- Takaki, T. 1995. New crop cultivars registered under seed and seedling law (14). Breeding Science 45 : 251-256.
- Takaki, T. 1996. New crop cultivars registered under seed and seedling law (20). Breeding Science 46 : 397-402.
- Takeuchi, S., H. Uchiyama, K. Yoneda, and S. Suzuki. 1995. Identification and phylogeny of wild and cultivated roses using DNA markers. 3. Maternal inheritance of chloroplast DNA and inference of mother line in some hybrids. J. Japan. Soc. Hort. Sci. 64 (Suppl.1) : 400-401. (In Japanese).
- Yamagishi, M. 1995. Detection of section-specific random amplified polymorphic DNA (RAPD) markers in *Lilium*. Theor. Appl. Genet. 91 : 830-835.
- Yonemori, K., D. E. Parfitt, S. Kanzaki, A. Sugiura, N. Utsunomiya and S. Subhadrabandhu. 1996. RFLP analysis of an amplified region of cpDNA for phylogeny of the genus *Diospyros*. J. Japan. Soc. Hort. Sci. 64 : 771-777.

PCR-RFLP 分析によるオリエンタルハイブリッド系のユリ数品種の交配親推定の可能性

春木和久¹・細木高志¹・名古洋治²¹島根大学生物資源科学部 690-0823 島根県松江市西川津町²島根県農業試験場 693-0035 島根県出雲市芦渡町

摘 要

ユリの種間交雑系統・品種を用いて PCR-RFLP 分析を行い、交配親推定の可能性を検討した。6 種のユリ、サユリ (*L. auratum* var. *platyphyllum*)、ササユリ (*L. japonicum*)、カノコユリ (*L. speciosum*)、タモトユリ (*L. nobilissimum*)、ヤマユリ (*L. auratum*) およびヒメサユリ (*L. rubellum*) を 4 組のプライマーと 22 種の制限酵素を用いて分析し、16 本の PCR-RFLP マーカーを検出した。

PCR-RFLP マーカーが遺伝することを確認するために、シンテッポウユリ、カノコユリおよびその交雑個体を比較検討した。母親の葉緑体遺伝子にみられた PCR-RFLP マーカーは全ての交雑個体にみられ、交雑個体の核の rRNA 遺伝子にあるマーカーは、母親と花粉親のバンドパターンを合わ

せたものになった。従って、PCR-RFLP 分析は、交雑品種の親の推定に利用できるものと考えられた。

PCR-RFLP 分析をオリエンタルハイブリッドと呼ばれるユリの 6 品種、‘スターゲザー’、‘ル・レーブ’、‘カサブランカ’、‘サマードレス’、‘ピンクパール’および‘マルコポーロ’を用いて行い、その交配親を推定した。その結果、‘カサブランカ’、‘サマードレス’および‘ピンクパール’の細胞質はカノコユリ由来であり、‘スターゲザー’、‘ル・レーブ’および‘マルコポーロ’の細胞質はサユリあるいはササユリからきているものと考えられた。一方、これらの品種の花粉親が交雑種の場合には交配時にマーカーが分離する可能性を考慮する必要があるので、花粉親の特定は困難であった。