Theor Appl Genet (2001) 102:1129–1135

J. Kato · R. Ishikawa · M. Mii

Different genomic combinations in inter-section hybrids obtained from the crosses between *Primula sieboldii* (Section Cortusoides) and *P. obconica* (Section Obconicolisteri) by the embryo rescue technique

Received: 22 August 2000 / Accepted: 22 September 2000

Abstract Inter-section hybrids were successfully obtained by rescuing the immature embryos produced in the cross between *Primula sieboldii* of Sect. Cortusoides and *P. obconica* of Sect. Obconicolisteri. In these hybrid plants two types of triploids with different genome combinations were found in addition to the normal diploid hybrids which each had one genome of the parents. Among the five triploids obtained, four had two genomes of *P. sieboldii* and one genome of *P. obconica*, whereas the remaining one had one genome of *P. sieboldii* and two genomes of *P. obconica*. The possibilities of diploid female gamete formation in *P. sieboldii* and diploid pollen formation in *P. obconica* as the causal factors for these triploid formations were discussed.

Keywords Inter-section hybrid · *Primula sieboldii* · *Primula obconica* · Unreduced gamete formation

Introduction

Primula is one of the largest and most widespread genera in the moist and cool regions of the northern hemisphere, and approximately 425 species classified into 37 sections are included (Richards 1993). Most *Primula* species have beautiful attractive flowers and some species such as *Primula malacoides* Franch., *Primula* × *polyantha* and *Primula obconica* Hance are now ranked as some of the most important ornamental crops.

Among the commercially important primulas, *P. obconica* Hance, which is classified in the Sect. Obconicolisteri, is relatively tolerant to low light intensity and considered to be a good germplasm for the breeding of primulas suitable for indoor cultivation. However, it is sensitive to several diseases and one of the main aims of its breeding is considered to be the introduction of a disease-resistant character from other species by inter-specific hybridization.

In Japan, *P. sieboldii* Morr., which is classified in the Sect. Cortusoides (Richards 1993) is also an important ornamental species, and has approximately 300 cultivars produced since the Edo era (about 300 years ago). This species is perennial with summer dormancy, produces one flower stem per shoot in spring, and has no serious disease problem (Torii 1985), suggesting that it can be used as a good source for the breeding of disease-resistant primulas.

In the genus *Primula*, inter-specific hybrids have been produced between species belonging to the same section; i.e., *Primula* × *kewensis* W. Wats., a natural hybrid between *Primula verticillata* Forssk. and *Primula floribunda* Wall. in the Sect. Sphondylia, *P.* × *polyantha*, a complex hybrid among *Primula veris* L., *Primula vulgaris* Huds. and *Primula elatior* Hill in the Sect. Primula, and hybrids between *Primula japonica* A. Gr. and *Primula burmanica* Balf. f. & Ward in the Sect. Proliferae (Richards 1993). Very recently, we have succeeded in producing hybrids between *Primula sieboldii* and *Primula kisoana* Miq. in the Sect. Cortusoides by the embryo-rescue technique (Kato and Mii 2000).

In the present paper, we demonstrate the production of inter-section hybrids between *P. sieboldii* and *P. obconica* and show that three genome-combination hybrids were produced.

Communicated by H.F. Linskens

J. Kato (✉) · R. Ishikawa
Department of Life Science, Aichi University of Education, 1, Hirosawa, Igaya-cho, Kariya city, Aichi Prefecture 448-8542, Japan
e-mail: jkatoh@auecc.aichi-edu.ac.jp,
Tel. and Fax: +81-(0)566-26-2645

M. Mii
Laboratory of Plant Cell Technology,
Faculty of Horticulture, Chiba University, 648 Matsudo,
Matsudo City, Chiba Prefecture 271-8510, Japan

Materials and methods

Plant materials

Eight strains of *P. obconica* and 54 cultivars/strains of *P. sieboldii* were used in the present study. Eight strains of *P. obconica* were purchased from a local flower market in late February and cultivated in the glasshouse throughout the experimental period. The cultivars/strains of *P. sieboldii* used had been maintained outside

Table 1 List of cultivars/strains classified with the types of heterostyly used for the inter-section crosses between *P. sieboldii* and *P. obconica*

Species name	Type of heterostyly	Number of cultivars/ strains	Names of cultivars/strains			
P. sieboldii	Pin	32	B-5	Asagiri	Asahi	Asahizuru
			Ayanami	Beppu (wild strain)	Fukiagezakura	Gyokukoubai
			Harunoyuki	Hien	Hinotukasa	Jindaikan
			Kazakuruma	Kotobuki	Kourohou	Kyouganoko
			Mihonokoji	Miyuki	Musumesakari	Ooasahi
			Shinnyonotuki	Shishinden	Siboritatuta	Tagonoura
			Tajima-Shiro (wild strain)	Tamabotan	Tamafuyou	Tokinohina
			Uchu	Yatugatake-Shiro (wild strain)	Youdainoyume	Yuubinosugata
	Thrum	22	B-6	Akatonbo	Gifu (wild strain)	Hakutaka
			Hanaguruma	Hatuzakura	Hinohakama	Hinomaru
			Karakoasobi	Nankinsibori	Niouume	Okegawa (wild strain)
			Oosuma	Ruriden	Sakuragenji	Sangokukou
			Senyuu	Sinjisyuu	Siragajisi	Syunsyou
			Tajima-Aka (wild strain)	Zendaimimon		
P. obconica	Pin	4	MP	OP	PP	P-1
	Thrum	4	MT	OT	PT	T-1

the glasshouse as potted plants at the Laboratory of Plant Cell Technology, Faculty of Horticulture, Chiba University. The plants were transferred for crossing at the bolting stage into the glasshouse. The types of heterostyly (pin or thrum) of these cultivars/strains are shown in Table 1.

Crossing method

A total of 140 cross combinations, mostly consisting of legitimate combinations between pin and thrum or between thrum and pin, were conducted between *P. sieboldii* as maternal parent and *P. obconica* as pollen parent. In the legitimate crosses, the pin type of *P. sieboldii* was used as the maternal parent in 49 cross combinations and the thrum type in 33 combinations (see Table 2). In the illegitimate crosses (pin/pin or thrum/thrum), the pin type of *P. sieboldii* was used as maternal parents in 44 combinations and the thrum type in 14 (Table 2). Two to three days before blooming, flowers of *P. sieboldii* were emasculated after excising petals and covered by paraffin paper bags. Two to three days after emasculation, flowers were pollinated with pollen of *P. obconica*. The pollinated flowers were then covered again with the bags.

Establishment of seedlings in vitro

Immature ovaries were collected 3–4 weeks after pollination and the number of seeds, including those developing partially, was counted for each pollinated flower. The ovaries collected were surface-sterilized for 15 min with sodium hypochlorite solution (1% available chlorine) containing a drop of Tween 20, and then rinsed twice with sterile distilled water. After surface-sterilization, the placenta with ovules was excised from an ovary and put onto 0.25% (w/v) gellan gum-solidified half-strength MS medium (Murashige and Skoog 1962) (1/2 MS) supplemented with 5% (w/v) sucrose, 20 mg/l of gibberellic acid (GA_3), 0.1 mg/l of 1-naphtaleneacetic acid (NAA) and 0.1 mg/l of 6-benzylaminopurine (BA). Seedlings germinated normally were transferred to 0.25% (w/v) gellan gum-solidified half-strength MS medium with 3% (w/v) sucrose. After two or three transplants on the same medium, plants with more than five leaves and well-developed roots were acclimatized. The seedlings showing non-vigorous growth were transferred onto 0.2% (w/v) gellan gum-solidified MS medium supplemented with 3% (w/v) sucrose, 1 mg/l of NAA and 1 mg/l of BA to induce calli and regenerate plants. The regenerated plantlets were also acclimatized and transferred to the glasshouse.

Flow-cytometry analysis

Genome combinations of the plants that were obtained by inter-section crosses were analyzed by flow-cytometry using PA (Partec, Münster, Germany) according to the methods of Mishiba et al. (2000). Leaf segments of approximately 0.2 cm^2 or calli of about 20 mg were chopped with a razor blade in a 200-µl solution A of High Resolution DNA-staining kit type P (Partec) and 1 ml of DAPI staining solution [10 mM Tris- HCl, pH 7.5, containing 50 mM sodium citrate, 2 mM $MgCl_2$, 1%(w/v), PVP K-30 (Polyvinylpyrrolidone K-30) (Wako Chemicals), 0.1%(v/v) Triton X-100, 2 mg/l DAPI (4´,6-diamidino-2-phenylindole dihydrochloride)] was added. Then the solution mixture was filtered by a 45-µm mesh and analyzed. The DNA contents of the parental species and the hybrids obtained were estimated by comparing with that of *Hordeum vulgare* L. (10.4 pg) which was used as the internal standard.

Extraction of DNA and random amplified polymorphic DNA (RAPD) analysis

DNA extraction from the parental plants and the plants derived from inter-specific crosses were performed using the plant DNA extraction kit, Nucleon Phyto Pure (Amersham Pharmacia Biotech Co. Ltd.), for confirming the hybridity by RAPD analysis. The rTth DNA polymerase (Toyobo Co.) was employed for the RAPD analysis, in which a 25-µl reaction solution containing 1 ng of total plant DNA was subjected to analysis according to the manufacture's pro-

tocol. The primers used were three kits of Operon 10-mer primers, KIT K, KIT R and KIT I (Operon Co.). DNA fragments were amplified by repeating 40 cycles of the following thermal treatments; 94°C for 1 min, 41°C for 1 min and 72°C 1 min, in a Takara PCR Personal Thermal Cycler (Takara Co.). Electrophoresis of the amplified DNAs was conducted on a 5% acrylamide gel in a TBE buffer system. Digested ϕx174/*Hae*III was used as the size marker.

Counting chromosome numbers

The method for counting chromosome numbers was basically the same as that reported previously (Kato and Mii 2000). Briefly, root tips were pre-treated with 0.001% (w/v) colchicine solution for 4 h at room temperature, then fixed with ethanol-acetic acid (3:1 v/v). After washing with distilled water, the fixed root tips were macerated with an enzyme solution consisting of 4.0% (w/v) Cellulase Onozuka RS (Yakult Honsha Co. Ltd., Japan), 1% (w/v) Pectolyase Y-23 (Seishin, Japan), 1% (w/v) Hemicellulase (Sigma) and 1 mM of EDTA, which was adjusted to pH 4.2. The dispersed root-tip cells were stained with a drop of 1% (w/v) aceto-carmine (Merck).

Results

Crossability

The mean numbers of seeds obtained per placenta in the illegitimate crosses between *P. sieboldii* and *P. obconica* were 10.4 in the pin (female) /pin (pollen) crosses and 9.0 in the thrum/thrum ones, respectively (Table 2). By contrast, the mean numbers of seeds obtained per placenta in the legitimate crosses were 30.3 in the pin/thrum crosses and 22.1 in the thrum/pin ones, respectively (Table 2).

Three germinable seeds were obtained from two illegitimate cross combinations between *P. sieboldii* 'Kourohou' (pin) and *P. obconica* 'PP' (pin) and between *P. sieboldii* 'Asahi' (pin) and *P. obconica* 'MP' (pin). In the legitimate pin/thrum crosses, 12 germinable seeds were obtained from nine cross combinations, i.e. 'Asahi' and 'MT', 'Asahi' and 'PT', 'Beppu' and 'MT', 'Beppu' and 'OT', 'Fukiagezakura' and 'MT', 'Fukiagezakura' and 'PT', 'Miyuki' and 'MT', 'Musumesakari' and 'MT', and 'Tamabotan' and 'PT'. In the legitimate thrum/pin crosses, nine germinable seeds were obtained from three cross combinations, i.e. 'Gifu' and 'PP', 'Tajima-Aka' and 'MP', and 'Tajima-Aka' and 'OP'. No germinable seed was obtained from the illegitimate thrum/thrum crosses.

Six out of twenty four germinable seeds did not grow and died after germination. Most of the seedlings thus obtained showed poor growth due to inhibited root growth at the initial stage. One seedling obtained by the cross between 'Fukiagezakura' and 'MT' died 6 months after germination (Table 3). Three seedlings obtained from the crosses between 'Asahi' and 'MP', between 'Kourohou' and 'PP', and between 'Miyuki' and 'MT' showed very poor growth and eventually callused. Recovery of vigorous plants from these poor-growing seed-

Table 2 Effect of heterostyly type on seed production and germination in inter-section crosses between *P. sieboldii* and *P. obconica*

Heterostyly type of *P. sieboldii* (♀)	Hetrostyly type of *P. obconica* (♂)	No. of cross combinations	No. of flowers pollinated	No. of flowers producing seeds	Total no. of seeds	No. of germinated seeds	No. of plants obtained
Pin	Pin	44	111	54	542	3	2
Pin	Thrum	49	154	129	3,911	12	8
Thrum	Pin	33	151	118	2,612	9	8
Thrum	Thrum	14	31	12	108	0	0

Table 3 Flow-cytometric analysis of the ploidy level and genome combination of progenies in an inter-section cross between *P. sieboldii* (SS genome) and *P. obconica* (OO genome)

Cross		No. of progeny	Ploidy level and genome combination				
P. sieboldii	*P. obconica*		*P. sieboldii* (2x) (SS)	Diploid hybrid (SO)	Triploid hybrid (SOO)	Triploid hybrid (SSO)	*P. sieboldii* (4x) (SSSS)
Asahi (pin)	MP (pin)	1	1[a,b]				
Asahi (pin)	PT (thrum)	2		2			
Gifu (thrum)	OP (pin)	1				1	
Kourohou (pin)	PP (pin)	1					1[a,b]
Tajima-Aka (thrum)	MP (pin)	3		2	1		
Tajima-Aka (thrum)	OP (pin)	4		3		1	
Tamabotan (pin)	PT (thrum)	1		1			
Fukiagezakura (pin)	MT (thrum)	1		1[c]			
Fukiagezakura (pin)	PT (thrum)	1		1			
Beppu (pin)	MT (thrum)	1		1			
Beppu (pin)	OT (thrum)	1				1	
Miyuki (pin)	MT (thrum)	1				1[b]	

[a] No evidence of hybridity by RAPD analysis
[b] Callusing
[c] Death

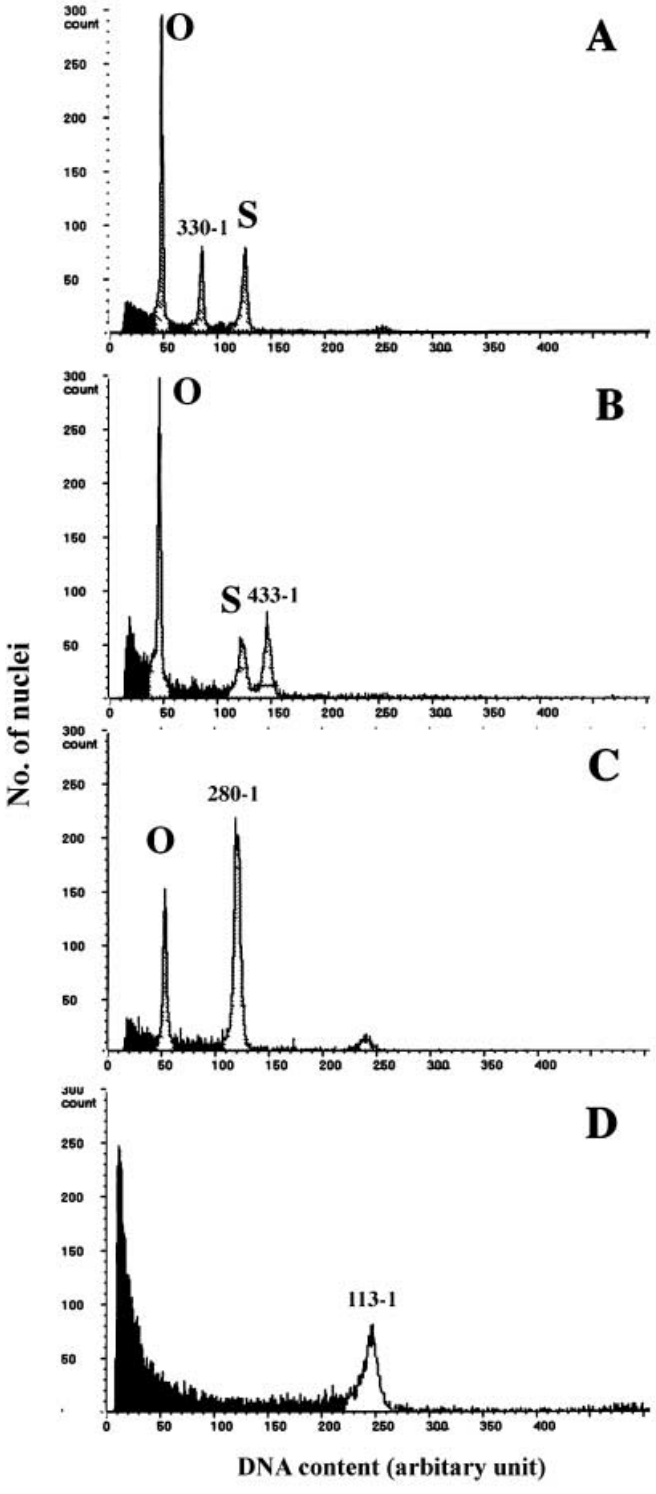

Fig. 1A–D Flow-cytometric profiles of the plants obtained from the inter-section cross between *P. sieboldii* used as maternal parent and *P. obconica* used as pollen parent. **A** Diploid hybrid '330–1' with an SO genome obtained from the cross between *P. sieboldii* 'Fukiagezakura' (SS genome) and *P. obconica* 'PT' (OO genome). **B** Triploid hybrid '433–1' with an SSO genome obtained from the cross between *P. sieboldii* 'Gifu' and *P. obconica* 'OP'. **C** Triploid hybrid '280–1' with an SOO genome obtained from the cross between *P. sieboldii* 'Tajima-Aka' and *P. obconica* 'MP'. **D** '113–1' obtained from the crosses between *P. sieboldii* 'Kourohou' and *P. obconica* 'PP'. **O** *P. obconica*, S *P. sieboldii*

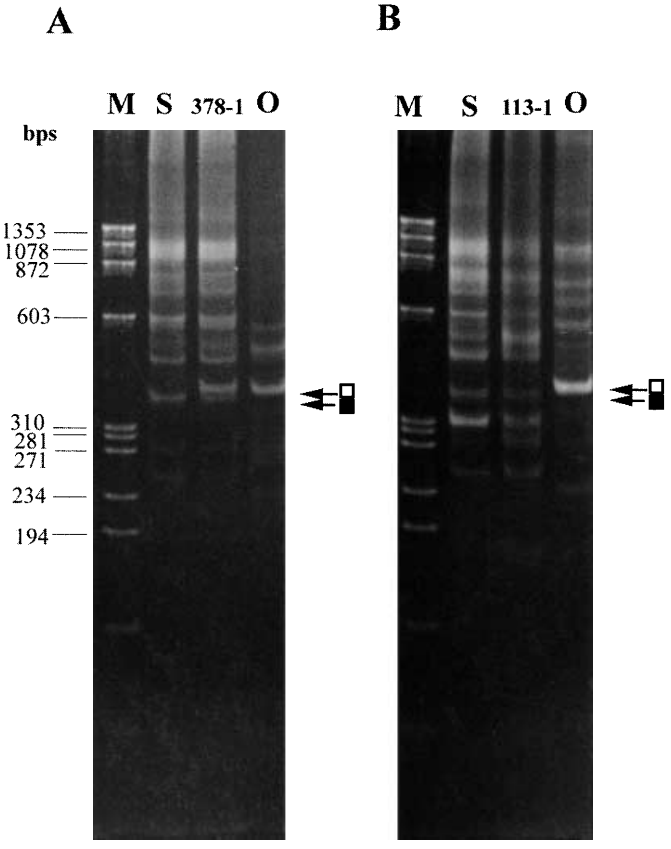

Fig. 2A, B PCR analysis using the OPI-3 primer on the hybridity of the progeny in inter-section crosses between *P. sieboldii* and *P. obconica*. **A** Hybrid '378–1' with an SO genome obtained from the cross between *P. sieboldii* 'Asahi' and *P. obconica* 'PT'. **B** Non-hybrid '113–1' with a tetraploid genome of *P. sieboldii* obtained from the cross between *P. sieboldii* 'Kourohou' and *P. obconica* 'PP'. **M** φX174/*Hae*III-digestion size marker, **S** maternal parent, **O** pollen parent, ■ the specific band of *P. sieboldii*, □ the specific band of *P. obconica*

lings with callus formation could not be achieved despite the addition of a higher concentration of NAA and BA. The other plants grew normally till flowering.

Analysis of genome combination by flow-cytometry

The DNA contents of *P. obconica* and *P. sieboldii* were estimated to be 2.6 pg and 6.5 pg, respectively, by flow-cytometric analysis. Most of the plants obtained by the inter-section crosses showed an intermediate peak location, which was estimated as approximately 4.5 pg, between the two parental species (Fig. 1A and Table 3). By postulating that the genomes of *P. sieboldii* and *P. obconica* were SS and OO, respectively, the plants that had an intermediate peak between both of the parents can be considered to have an SO genome. However, the DNA contents of the three plants and the one callus obtained from the crosses between 'Gifu' and 'OP' (433–1), between 'Tajima-Aka' and 'OP' (436–1), between 'Beppu' and 'OT' (418–1), and between 'Miyuki' and 'MT' (711–1) were estimated to be 7.6, 8.1, 7.5, and

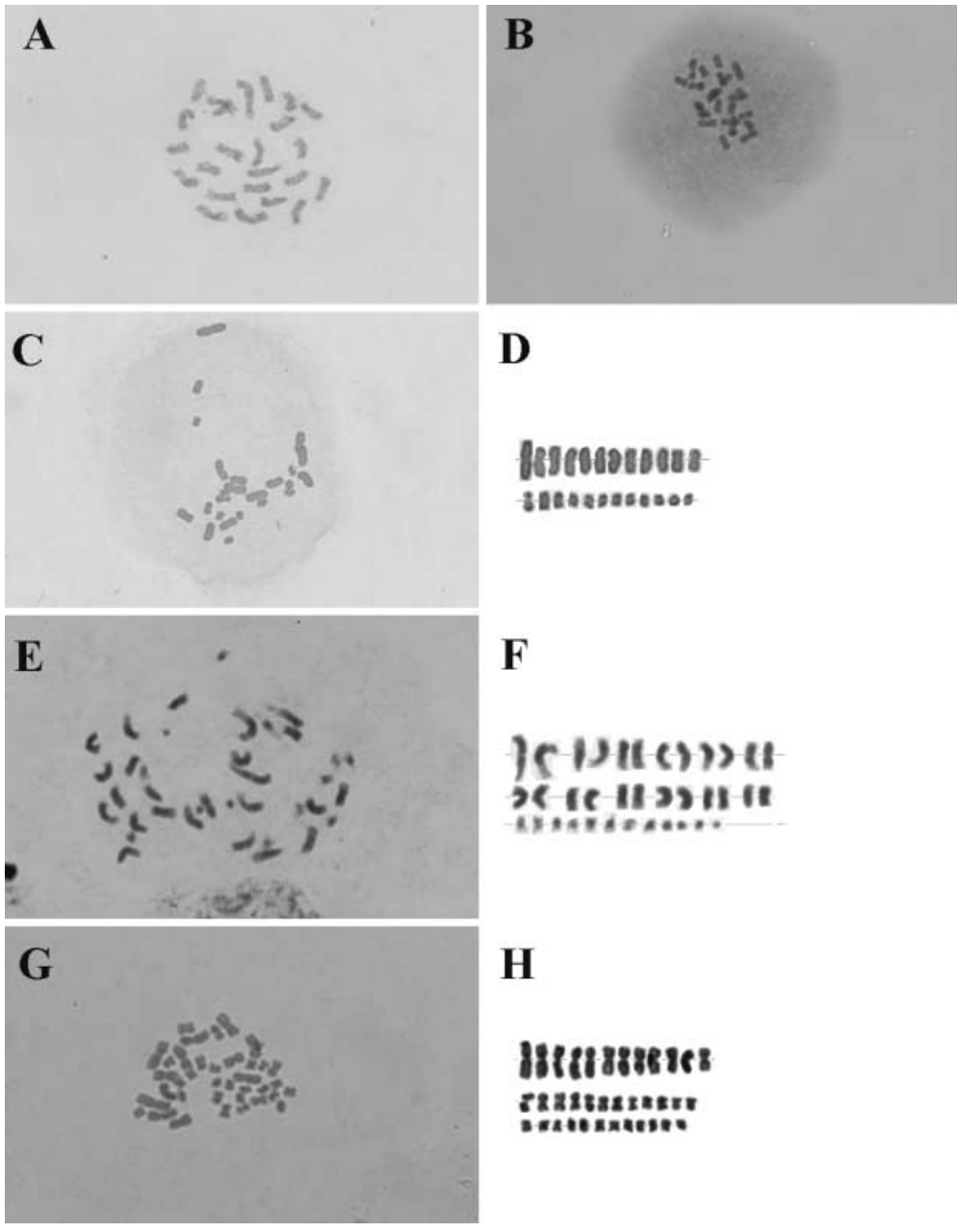

Fig. 3A–H Somatic chromosomes and karyotype of the inter-section hybrid between *P. sieboldii* and *P. obconica.* **A** *P. sieboldii* 2n=2*x*=24, **B** *P. obconica* 2n=2*x*=24, **C** and **D** a diploid hybrid with an SO genome, **E** and **F** a triploid hybrid with an SSO genome, **G** and **H** a triploid hybrid with an SOO genome

8.0 pg, respectively. These values were approximately the same as the expected value of the triploid SSO genome calculated as 7.8 pg (Fig. 1B and Table 3). The DNA content of the plant obtained from the cross between 'Tajima-Aka' and 'MP' (280–1) was estimated to be 6.0 pg, which was close to the expected value of the triploid SOO genome calculated at 5.9 pg (Fig. 1C and Table 3). In the illegitimate crosses between pin and pin, the DNA content of the calli obtained from the cross between 'Asahi' and 'MP' (261–1) was estimated to be 6.5 pg, which was the same as that of *P. sieboldii* (Table 3). The DNA content of calli obtained from the cross between 'Kourohou' and 'PP' (113–1) was 13.3 pg, which coincided with the tetraploid genome content of *P. sieboldii* (Fig. 1D and Table 3).

Hybridity analysis

RAPD analysis was performed to detect specific markers for *P. obconica* as pollen parent in the putative hybrids. The plants that were estimated to involve the O genome by flow-cytometric analysis had less than two specific markers of the pollen parent among the 60 primers used. The fragment of approximately 400 bp obtained using the OPI-3 primer (5´-CAGAAGCCCA-3´) was inherited from each *P. obconica* strain used as the pollen parent to putative hybrids with the O genome (Fig. 2A). This fragment was not detected in the two calli, 261–1 and 113–1, which gave no evidence as having a genome of *P. obconica* by flow-cytometric analysis (Fig. 2B). Consequently, this marker was considered to be utilized as a

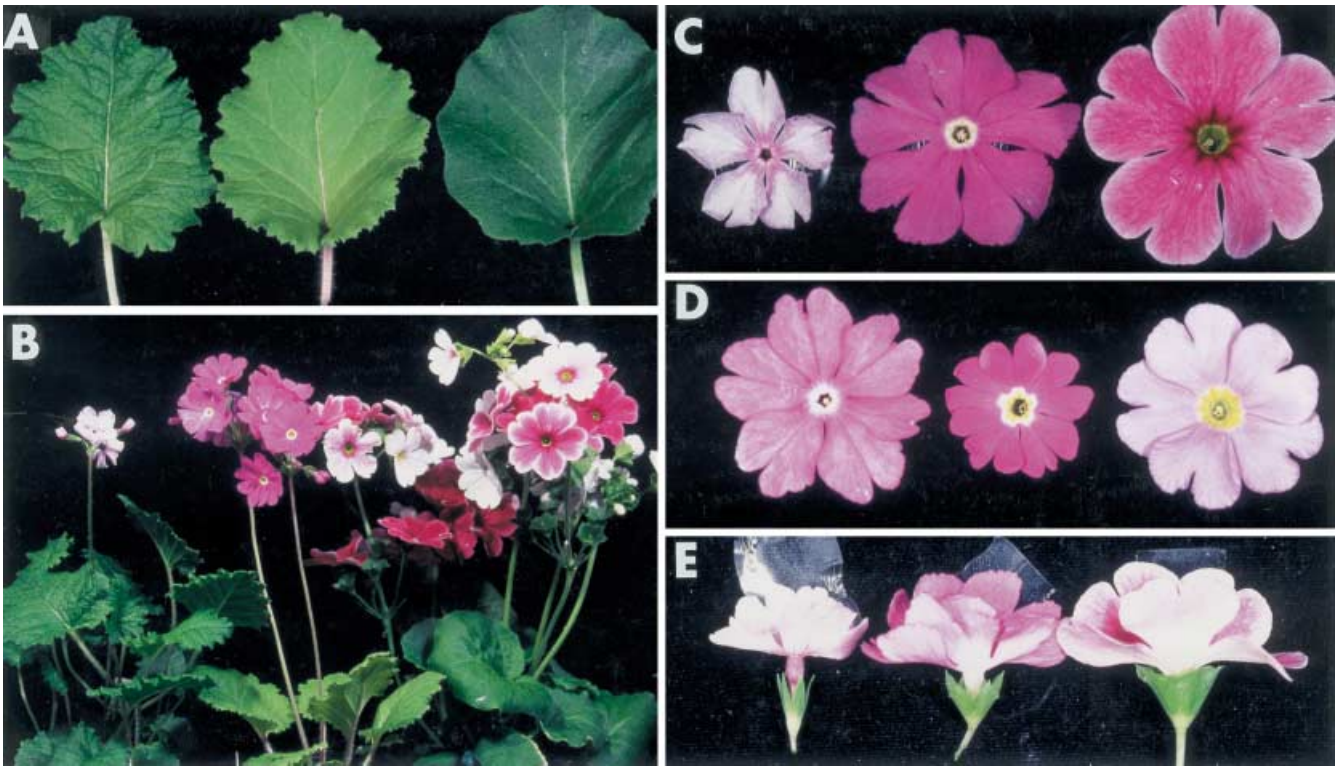

Fig. 4A–E Characteristics of the hybrids obtained from the cross between *P. sieboldii* and *P. obconica*. **A** Leaf shape of *P. sieboldii* 'Fukiagezakura' (left), hybrid '330–1' (center) and *P. obconica* 'PT' (right), **B** Flowering plant of *P. sieboldii* 'Fukiagezakura' (left), hybrid '330–1'(center) and *P. obconica* 'PT' (right), **C** Petal shape of *P. sieboldii* 'Fukiagezakura' (left), hybrid '330–1' (center) and *P. obconica* 'PT' (right), **D** Petal shape of *P. sieboldii* 'Tajima-Aka' (left), hybrid '280–2' with an SO genome (center) and *P. obconica* 'MP' (right), **E** Sepal shape of *P. sieboldii* 'Fukiagezakura' (left), hybrid '330–1' (center) and *P. obconica* 'PT' (right)

species-specific marker of *P. obconica* in inter-section hybrids between *P. sieboldii* and *P. obconica*.

Chromosomal analysis

The results of the flow-cytometric analysis were also confirmed by counting the chromosome numbers in root-tip cells of the three types of hybrids and the parental plants. Both of the parental species showed the same diploid chromosome number (2n=24), as expected (Fig. 3A and B). The set of chromosomes in *P. sieboldii* was much larger than that in *P. obconica*. The plant '280–2' which was determined to be a diploid hybrid with an SO genome possessed 24 chromosomes consisting of 12 large chromosomes inherited from *P. sieboldii* and 12 small chromosomes inherited from *P. obconica* (Fig. 3C and D). The plant '436–1' which was determined to be a triploid hybrid with an SSO genome possessed 36 chromosomes consisting of 24 large chromosomes of *P. sieboldii* and 12 small chromosomes of *P. obconica* (Fig. 3E and F). The plant '280–1', which was a rare, and the only triploid hybrid with an SOO genome, possessed 36 chromosomes consisting of 12 large chromosomes and 24 small ones (Fig. 3G and H).

Plant trait in hybrid plants

Some of the hybrid plants obtained were successfully acclimatized and grown in the glasshouse. The leaf shape of the SO and SSO genome hybrids was intermediate between both parents (Fig. 4A), whereas leaves of the SOO genome were abnormally folded. Two SO genome hybrids, 330–1 obtained from the cross between 'Fukiagezakura' and 'PT', and 280–2 obtained from the cross between 'Tajima-Aka' and 'MP', bloomed 5 months after transfer to the glasshouse (Fig. 4B). Both of the bloomed plants had two flower stems, which has never been observed in *P. sieboldii* These two hybrids produced thrum-type flowers with the same purple-red color, which was different from that of either parent. Both the petal and sepal shapes of the hybrids were intermediate between those of the parents (Fig. 4C, D and E). The flowers had doubled eyes in which the inside was yellow like that of *P. obconica* while the outside was white like that of *P. sieboldii* (Fig. 4C and D). Both hybrids had non-dehiscent anthers without normal pollen.

Discussion

In the present study, 10 out of 140 cross combinations in the inter-section crosses, using seven cultivars/strains of *P. sieboldii* as the maternal parent with pollen of *P. obconica*, successfully yielded 14 hybrid plants. Three

out of the seven maternal *P. sieboldii* plants were wild strains with different origins, and 10 of the 14 hybrids were obtained from these wild maternal strains (Table 3), suggesting that the genetic background of these wild *P. sieboldii* strains might favor the formation of hybrid plants. In inter-specific crosses between *P. sieboldii* and *Primula kisoana*, *P. sieboldii* 'Miyuki' used as the maternal parent produced many hybrids (Kato and Mii 2000). However, in the present study, where 'Miyuki' was used as the maternal parent, only one callus which had no regeneration ability was obtained, suggesting that 'Miyuki' had a less-favorable genetic background for hybrid production in the cross with *P. obconica* than in the cross with *P. kisoana*.

The mean seed production of the legitimate cross (thrum/pin and pin/thrum) was 26.4 and that of the illegitimate cross (pin/pin and thrum/thrum) was 9.9 (Table 2). The mode of pollen-tube growth in the legitimate crosses also differed from that in the illegitimate crosses. Pollen tubes in the legitimate crosses grew very well but pollen germination or tube growth was inhibited on the stigma in the illegitimate crosses (data not shown). Therefore, it is likely that the heteromorphic self-incompatibility system was commonly preserved in these two species belonging to the different Sections and that the intensity of the incompatibility reaction may depend on the genetic background of each cultivar/strain.

Both flow-cytometric and RAPD analyses could not confirm the hybridity of the two calli, 261–1 and 711–1, obtained from the illegitimate crosses (Fig. 1, Fig. 2 and Table 3). In inter-specific crosses between *P. sieboldii* and *P. kisoana*, the illegitimate cross using 'Kourohou' as the maternal parent produced some progeny without hybridity, and one of which bloomed had a similar flower to that of the maternal parent (unpublished results). These results may suggest that some *P. sieboldii* cultivars/strains had a capacity for parthenogenesis or for pollen parent chromosome elimination, as previously reported in inter-specific crosses between *H. vulgare* and *Hordeum bulbosum* (Kasha and Kao 1970), with chromosome doubling in the illegitimate inter-specific pin/pin crosses. It is interesting to note that the legitimate inter-section cross produced a hybrid and that the illegitimate inter-section cross yielded plants without any effect of the pollen parent in the cross of 'Asahi' used as the maternal parent.

Three genome types were found in the hybrids obtained in this study based on the difference in the DNA content of the parents (Table 3). In our previous study (Kato and Mii 2000) we suggested that diploid female gametes were partially formed in some cultivars of *P. sieboldii* and that the triploid cultivars observed in *P. sieboldii* could have originated from the fertilization between a haploid male gamete and a diploid female gamete produced by the diploid parents. In the present study, we found that at least four cultivars/strains, 'Gifu', 'Tajima-Aka', 'Beppu' and 'Miyuki', had a capacity to partially produce unreduced egg cells because of the formation of hybrids with an SSO genome by crossing with *P. obconica*.

In Cyclamen (Primulaceae), reciprocal crosses between diploid and tetraploid plants yielded some tetraploid plants, which was considered to relate to diploid egg and pollen formation (Takamura and Miyajima 1996). The production of a triploid hybrid with an SOO genome in the present study suggests that *P. obconica* occasionally formed diploid pollen. The possibility of fertilization by diploid pollen depended on the percentage of diploid pollen formation and the competition for fertilization between haploid and diploid pollen. Consequently, plants with SOO were casually formed.

Hermsen (1984) described diploid gamete formation, which occurs as a result of first-division restitution (FDR) or second-division restitution (SDR) at meiosis. The genotypes of gametes resulting from FDR should be identical to the parent genotype, but those resulting from SDR should segregate into nearly fixed diploid homo genotypes. The presence of the callus with no regeneration ability, which had an SSO genome obtained from 'Miyuki', might suggest that weak or lethal recessive genes accumulated in 'Miyuki' became homologous by SDR, which resulted in the loss of regeneration ability.

This is the first report on the production of an intersection hybrid between Sect. Cortusoides and Sect. Obconicolisteri. Although the hybrids with an SO genome can not be directly used for further breeding, due to hybrid sterility, amphi-diploid production by artificial chromosome doubling may be useful for recovering the fertility. The hybrid with an SSO genome may be used to develop the breeding of *P. sieboldii*, and the one with SOO genome may be used to introduce disease-resistant genes from *P. sieboldii* to *P. obconica*.

Acknowledgements The authors wish to thank Dr. K.Mishiba, Faculty of Horticulture, Chiba University, for his technical advice on flow-cytometry and Mr. Matsumoto, Center of Flower and Garden Plant in Saitama prefecture, for his advice on the cross between *P. sieboldii* and *P. obconica*.

References

Hermsen JG (1984) Mechanisms and genetic implications of 2n-gamete formation. Iowa State J Res 58:421–434

Kasha KJ, Kao KN (1970) High-frequency haploid production in barley (*Hordeum vulgare* L.). Nature 225: 874–876

Kato J, Mii M (2000) Differences in ploidy level of inter-specific hybrids obtained by reciprocal crosses between *Primula sieboldii* and *P. kisoana*. Theor Appl Genet 101:690–696

Mishiba K, Ando T, Mii M, Watanabe H, Kokubun H, Hashimoto G, Marchesi E (2000) Nuclear DNA content as an index character discriminating taxa in the genus *Petunia sensu* Jussieu (Solanaceae). Ann Bot 85:665–673

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15: 473–497

Richards J (1993) Primula. Timber Press, Portland, Oregon, USA

Takamura T, Miyajima I (1996) Cross-combination and the ploidy of progenies in crosses between diploid and tetraploid *Cyclamen* (*Cyclamen persicum* Mill.). J Jpn Soc Hort Sci 64: 883–889

Torii T (1985) Sakurasou (in Japanese). Nihon-terebi, Akatuki – Insatu Press, Tokyo