Cytogenetical studies in *Primula sieboldi* E.MORREN

—Chromosome numbers of 80 cultivars and related wild species—

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Synopsis. Cytological study was carried out in cultivars of *P. sieboldi* E.Morr. and also in the wild species related closely to it. Tetraploid cultivar was reported for the first time in *P. sieboldi*, and triploid, diploid cultivars were added to the previous records. The size of stomata and the maximum number of nucleoli in a nucleus were investigated. The mechanism of the occurrence of triploid and tetraploid cultivars was discussed. On the basis of the karyotypes, hybridization between *P. sieboldi* and *P. jesoana* Miq. var. *jesoana* will be possible.

Introduction

Primula sieboldi E.Morr. belongs to the Cortusoides section in the genus *Primula*, and is distributed in Manchuria, Korea and Japan (SMITH 1929). In Japan, other thirteen wild species are also found (OHWI 1965), but only *P. sieboldi* was greatly appreciated of its beautiful flowers. Therefore, from the early "Edo period" (about 300 years ago), it was used as breeding material to develop new garden varieties. Under peaceful social conditions at that time, people in Edo collected enthusiastically it from marshy places along the River Arakawa, near Edo city, and carried out its breeding. The number of the cultivars was soon increased to five hundreds approximately. From the end of "Edo period", however, owing to a change of social fashion, growing of primroses declined gradually, and then its breeding was hardly taken in the consideration at that time (TORII 1970).

Most of the cultivars have already been extinguished, and, at present, about 250 cultivars are only known. Among these cultivars, has been done only by selection among the genetical differences arisen spontaneously, and a plenty of variable characters were combined so fully that production of a new cultivar was scarcely possible. Surpassing this limitation, the introduction of the quite new characters

from other species is desired at present. Then, keeping the view of this point, the cytological study is needed for breeding in many cultivars and wild species. In 1926, INUMA reported already eleven diploid cultivars ($2n=24$) and three triploid ones ($2n=36$), i.e. SHIROWASHI, SASA-NO-NAMI, and NURETSUBAME. In this paper, the chromosome numbers of 80 cultivars of *P. sieboldi* and some wild species related closely to it were reported.

Materials and Methods

Sources of materials are listed up in Table 1. Chromosome numbers were counted in the somatic cells of root tips. Root tips were pretreated in a 0.002M 8-hydroxyquinoline solution for three hours, fixed with an acetoalcohol (1:3) for several hours, and then stored in a 70% ethylalcohol at 5°C. After the hydrolyzation with 1/N hydrochloric acid for seven minutes at 60°C, root tips were stained by Feulgen-squash method. Chromosome length was measured directly on the photograph prints enlarged about 5000 times. The centromeric index was given as the ratio of the length of short arm to that of entire chromosome in percentage. Relative length value was also given in percentage of each chromosome length to the total length of one set of ($2n$) (LEVAN *et al.* 1964). Nucleolus was stained with a 1.5% light green solution for two or three minutes after the pretreatment

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Table 1. Chromosome numbers of 80 cultivars of *P. sieboldi* E. Morr. and wild species closely related to it

2n=24		2n=24	
AMAGASHITA	*(A. T.)	RASHOUMON	(T.)
ASAHI	(A. N.)	RURIDEN	(W.)
AWOBA-NO-FUE	(A.)	SAKURAGENJI	(N.)
AWO-UNABARA	(N. T.)	SAZANAMI	(N.)
AZUMA-NISHIKI	(N.)	SEIOUBO	(N.)
BENIJOHÔ	(N.)	SENSHOU	(N.)
BIJIN-KURABE	(N.)	SHIGA-NO-MIYAKO	(A.)
CHIDORI-ASOBI	(T.)	SHIKOUBAI	(N. T.)
CHIYOZURU	(W.)	SHISHI-FUNJIN	(N.)
CHÔ-NO-NEMURI	(N.)	SHI-UN-RYÛ	(T.)
DAIRIKI-MUSOU	(A.)	SHUCHÛ-NO-TAMA	(N.)
DEICHÛ-NO-TAMA	(N.)	SUMIZOME-GOROMO	(N.)
FUJIGOE	(N.)	TAGO-NO-URA	(A.)
FUJIMUSUME	(A.)	TAIMIN-NISHIKI	(N.)
GEKKEIKAN	(W.)	TAMAGAWAZOME	(T.)
GENJOURAKU	(T.)	TAMAKUJAKU	(T.)
GIN-PUKURIN	(T.)	TAMA-NO-MIYA	(A.)
GODAISHÛ	(T.)	TATSUTA-NO-YÛBE	(T.)
HANAGURUMA	(T.)	TÔYÔDEN	(W.)
HATAKE-NO-NISHIKI	(N.)	UCHÛ	(N. T.)
HATSU-SUGATA	(N.)	UKIMA-NO-HIKARI	(N.)
HATSU-ZAKURA	(N.)	YUUDAI-NO-YUME	(T.)
HI-NO-HAKAMA	(N.)	YUKI-NO-HADA	(N.)
HI-NO-KOROMO	(T.)	ZENDAIMIMON	(N.)
HINOMARU	(W.)		
HITOMARU	(T.)		
ISARIBI	(A.)	2n=36	
JÛNIHITO	(N. T.)	EKIRO-NO-SUZU	(N. W.)
JUSSHÛ-NO-SORA	(N.)	KAKUREMINO	(N.)
KAGEROU	(N.)	KASANE-SHIUN	(T.)
KAGETSUROU	(A.)	MEJIRODAI	(N. W.)
KAMIYO-NO-KAN-MURI	(T.)	RAN-ZAN	(N.)
KARIGINU	(W.)	SHISHIGASHIRA	(W.)
KINKEICHOU	(N.)		
KINSHÛKA	(W.)	2n=48	
KIYOMIGATA	(N.)	YAMATO-KAMIKAZE	(N.)
KOMADOME	(T.)		
KÔROHOU	(A.)	wild species	
KOTOBUKI	(T.)	Sect. CORTUSOIDES	
KUISAKEGAMI	(N. T.)	<i>P. sieboldi</i> E. Morr.	2n=24 24**, 36
KUMOIZURU	(T.)	<i>P. jesoana</i> Miq.	
KYUGANOKO	(N.)	var. <i>jesoana</i>	2n=24 24, 26
MATSU-NO-YUKI	(N. T.)	<i>P. kisoana</i> Miq.	
MIHO-NO-KOJI	(N. W.)	var. <i>kisoana</i>	2n=24 24
MIKUNI-NO-HOMARE	(N. T.)	<i>P. kisoana</i> Miq.	
MITAJIMAN	(N.)	var. <i>shikokiana</i> Makino	2n=24
MUSASHINO	(W.)	Sect. REINII	
NANKINKOZAKURA	(N. T.)	<i>P. tosaensis</i> Yatabe	2n=24
OMOI-NO-MAMA	(N.)	<i>P. reinii</i> Franch.	
		var. <i>reinii</i>	2n=24 24

* material sources

A.: from Mr. T. ARABARI (Gose, Nara Pref.)
N.: from Mr. C. NAKAMURA (Suita, Osaka Pref.)
T.: from Mr. T. TORII (Murayama, Tôkyô Pref.)
W.: from Mr. S. WATANABE (Sendai, Miyagi Pref.)

** past records cited from
BOLKHOVSKIKH (1969)

Fig. 1. Somatic chromosomes of *P. sieboldi* E. Morr. and *P. jesoana* Miq. var. *jesoana*

A : wild specimen of *P. sieboldi* $2n=24$

B : HATSU-SUGATA $2n=24$

D : MEJIRODAI $2n=36$

F : *P. jesoana* var. *jesoana* $2n=24$

B-E : cultivars of *P. sieboldi*

C : MITAJIMAN $2n=24$

E : YAMATO-KAMIKAZE $2n=48$

Δ shows ST-chromosomes. $\times 1750$

with a 5% sodium carbonate solution (NODA 1966).

Results

Chromosome numbers are presented in Table 1. Six cultivars, namely, EKIRO-NO-SUZU, KAKUREMINO, KASANE-SHIUN, MEJI-

RODAI, RANZAN, and SHISHIGASHIRA, were triploid ($2n=36$), one cultivar, YAMATO-KAMIKAZE, was tetraploid ($2n=48$), and all others were diploid ($2n=24$). Five wild *Primula* species related closely to *P. sieboldi* were also examined and they were all diploid ($2n=24$).

Fig. 2. Idiograms constructed on the basis of the measurements in Tables 2 and 3

A : *P. sieboldi* E. Morr.

B : *P. jesoana* Miq. var. *jesoana*

Chromosome complements of *P. sieboldi* and *P. jesoana* var. *jesoana*, both belong to the Cortusoides section, were studied (Fig. 2). All chromosomes of these two species had either median or submedian constrictions, and were divided into three groups in their size: A (3.3μ – 2.9μ), B (2.7μ – 2.2μ) and C (2.1μ – 1.8μ). In each species, one pair of the A group was ST-chromosome. Chromosome length showed gradual transition from 3.3μ to 1.8μ and the identification of each chromosome was rather difficult in both species. Provisional karyotype formulas derived from the measurement of their nuclei were as fol-

Fig. 3. The nucleoli in diploid (A), triploid (B) and tetraploid (C) cultivars of *P. sieboldi* E. Morr: RANZAN, EKIRO-NO-SUZU and YAMATO-KAMIKAZE, respectively. $\times 1750$

lows (Tables 2 and 3):

$$P. sieboldi \quad K(2n) = 2A_1 + 2^tA_2 + 2A_3 + 14B + 4C = 24$$

$$P. jesoana \quad K(2n) = 2A_1 + 2^tA_2 + 2A_3 + 14B + 4C = 24$$

Maximum number of nucleoli in the interphase nucleus was two in the diploid cultivars, SAZANAMI and AZUMA-NISHIKI, three in the triploid cultivars, EKIRO-NO-SUZU and four in the tetraploid cultivar YAMATO-KAMIKAZE (Fig. 3).

The average length and width of stomata were measured in ten diploid cultivars and two triploid cultivars of *P. sieboldi* (Fig. 4). No distinct gap could be recognized in the size of stomata between diploid and triploid cultivars. As a whole, however, size of stomata in triploid cultivars was larger than in diploid ones.

Discussion

Generally speaking, polyploidy plays the very important role in the various ornamental flowering plants. (HAYES *et al.*, 1955; STEBBINS, 1950). *P. sieboldi* is, as well known, a vegetatively propagated perennial plant. Because of this nature, the spontaneously induced polyploid individual has been maintained easily. In the previous breeding of cultivars of *P. sieboldi*, there was no trace of inter-specific hybridization.

Table 2. Mean length of arm and entire chromosome, relative length value and centromeric index of *P. sieboldi* E. Morren

Group	Short arm length (μ)	Long arm length (μ)	Entire chromosome length (μ)	Relative length value	Centromeric index
A {	1	1.39	1.84	3.23	5.43
	2	1.38	1.63	3.01	5.06
	3	1.26	1.67	2.93	4.94
B {	4	1.13	1.59	2.72	4.58
	5	1.11	1.43	2.54	4.29
	6	1.06	1.40	2.46	4.14
	7	1.08	1.28	2.37	3.99
	8	0.93	1.39	2.31	3.89
	9	1.02	1.18	2.19	3.69
	10	0.95	1.22	2.16	3.64
C {	11	0.87	1.08	1.95	3.28
	12	0.78	1.05	1.84	3.08
Average	1.08	1.40	2.48	4.17	43.5

*t: ST-chromosome

Table 3. Mean length of arm and entire chromosome, relative length value and centromeric index of *P. jesoana* Miq. var. *jesoana*

Group	Short arm length (μ)	Long arm length (μ)	Entire chromosome length (μ)	Relative length value	Centromeric index	
A	1	1.41	1.92	3.33	5.57	42.3
	^t 2	1.45	1.60	3.05	5.10	47.5
	3	1.32	1.61	2.93	4.90	45.1
B	4	1.18	1.36	2.54	4.25	46.5
	5	1.12	1.34	2.46	4.12	45.5
	6	1.05	1.32	2.37	3.97	44.3
	7	1.06	1.30	2.36	3.95	44.9
	8	1.09	1.25	2.34	3.92	46.6
	9	1.10	1.20	2.30	3.85	47.8
	10	1.04	1.19	2.23	3.73	46.6
C	11	0.95	1.16	2.11	3.53	45.0
	12	0.83	1.07	1.90	3.18	43.7
Average	1.13	1.36	2.49	4.17	45.5	

*t: ST-chromosome

Fig. 4. Mean length and width of stomata in μ of diploid (○) and triploid (●) cultivars of *P. sieboldi* E. Morr.

between it and other species. The present triploid and tetraploid cultivars had three and four ST-chromosomes in their complements of nuclei, respectively. Therefore, it was suggested that these triploid and tetraploid cultivars were auto-polyploid. The maximum number of nucleoli was parallel to the ploidy level in the cultivars of *P. sieboldi*. This result also ensured that triploid and tetraploid cultivars were auto-polyploid.

Origin of triploid cultivars without crosses between diploid and tetraploid varieties has been discussed by many workers in various cultivated plants, such as: *Morus* (SEKI 1959

cited from MATSUOKA 1971), *Hemerocallis* (MATSUOKA 1971), *Lilium* (NODA 1966), *Petasites* (IMAZU and FUJISHITA 1962), *Tea* (SIMURA 1935) etc. They suggested that these triploid cultivars were derived from the sexual fusion of haploid gametes with unreduced diploid gametes. Also in *P. sieboldi*, the induction of diploid gametes may occur by chance in the diploid cultivars, because the giant pollen grain which seemed to be fertile was observed in this study (Fig. 5). The triploid cultivars of *P. sieboldi* might be originated in the sexual fusion between normal gametes and diploid gametes which occa-

sionally induced under some unfavorable environmental conditions. These unreduced gametes occasionally induced by chance might also have been the cause of the origin of the tetraploid cultivar of *P. sieboldi*, however, it could not be denied that it was produced by the somatic-doubling in some diploid cultivar.

In many polyploid plants, increase in the size of cells, especially stomata, has been reported and was distinctly parallel to their ploidy level. As to cultivars of *P. sieboldi*, there was no distinct gap in size of stomata between diploid and tetraploid cultivars. In diploids, it ranged from 38μ to 47μ in length

Fig. 5. A giant pollen grain appeared in the diploid KINKEICHŌ. $\times 1500$

and from 34μ to 39μ in width and the largest one was equal to the mean size of triploids. As a whole, however, the mean size of stomata was larger in triploids than in diploids.

Since karyotype formulas of *P. sieboldi* and *P. jesoana* var. *jesoana* showed good similarity to each other in size and form of chromosomes and the number of ST-chromosomes, they were suggested to be related closely to each other and able to be crossed. If so, the two or three umbels-formed inflorescence, orbicular leaf, and deep crimson flower color of *P. jesoana* could be introduced into cultivars of *P. sieboldi*. To clarify such a speculation, further study will be necessary.

Summary

Among 80 cultivars of *P. sieboldi*, EKIRO-NO-SUZU, KAKUREMINO, KASANE-SHI-UN, MEJIRODAI, RANZAN, and SHISHI-GASHIRA, were triploid ($2n=36$), and YAMATO-KAMIKAZE was tetraploid ($2n=48$). The other cultivars were diploid. Tetraploidy in *P. sieboldi* was reported here for the first time. As a whole, size of stomata in triploid cultivars was larger than in diploid ones. The maximum number of nucleoli, and the number of ST-chromosomes were both parallel to their ploidy level. These polyploid cultivars were reasonably suggested to be autopolyploid. Unreduced gametes may have played an important role on the occurrence of them.

Five wild Japanese *Primula* species related closely to *P. sieboldi* have somatic chromosome number of 24, respectively. Since *P. jesoana* var. *jesoana* shows good similarity to *P. sieboldi* in the karyotypes, they may be crossed each other. Production of a quite new cultivar is expected from the inter-specific hybridization.

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Literature Cited

- BOLKHOVSKIKH, Z. 1969. Chromosome numbers of flowering plants. Acad. Sci., USSR.
- HAYES, H. K., F. R. IMMER and D. C. SMITH 1955. Methods of plant breeding. McGraw-hill Book Comp., Inc., New York. pp. 551
- IINUMA, M. 1926. Triploidy of chromosome number in garden varieties of *Primula Sieboldii*, E. Morr. Sci. Rep. Tohoku Imp. Univ. Ser. 4, 2: 189~194
- IMAZU, T. and N. FUJISHITA 1962. Morphological, ecological and cytological studies on cultivated and wild butterburs, *Petasites japonicus* IV. On the chromosome numbers (in Japanese with English summary). Jour. Jap. Soc. Hort. Sci. 31: 293~302
- LEVAN, A., K. FREDGE and A. A. SANDBERG 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52: 201~220
- MATSUOKA, M. 1971. Spontaneous occurrence of triploid *Hemerocallis* in Japan (in Japanese with English summary). Jap. J. Breed. 21: 275~284
- NODA, S. 1966. Cytogenetics on the origin of triploid *Lilium tigrinum* (in Japanese with English summary). Bull. Osaka Gakuin Univ. 9: 85~140
- OHWI, J. 1965. Flora of Japan. Shibun-dô, Tôkyô.
- SIMURA, T. 1935. Citologia Studio pri la Teplantoj (in Japanese with Latin summary). Proc. Crop Soc. Japan 7: 121~133
- SMITH, W. W. and G. FORREST 1929. The sections of the genus *Primula*. Jour. R. H. S. London 54: 4~50
- STEBBINS, G. L., Jr. 1950. Variation and evolution in plants. Oxford Univ. Press, London. pp. 643
- TORII, T. 1970. Siebold's primrose (in Japanese). Encyclopedia of horticulture Vol. 5. Seibundô-shinkôsha, Tôkyô. p. 2216~2225

ニホンサクラソウの細胞遺伝学的研究

—園芸品種および近縁野生種の染色体数—

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ニホンサクラソウの育種に役立てるため、ニホンサクラソウの園芸品種ならびに、その近縁野生種の染色体数を調べた。園芸品種のなかには、二倍体以外に三倍体、四倍体のものが含まれていた。四倍体の報告は今回が初めてである。結果は次の通りである。

二倍性品種 ($2n=24$)

天ヶ下	鳥の錦	寿	獅子奮迅
朝日	初姿	喰裂紙	紫雲竜
青葉の笛	初桜	雲井鶴	手中の玉
青海原	緋の袴	京鹿子	墨染衣
東錦	緋の衣	松の雪	田子の浦
紅女王	日の丸	三保の古事	大明錦
美人競	人丸	御国の誉	玉川染
千鳥遊	漁火	三田自慢	玉孔雀
千代鶴	十二単重	武蔵野	玉の宮
蝶の眠	十州の空	南京小桜	竜田の夕
大力無双	陽炎	思いの儘	東陽殿
泥中の玉	花月楼	羅生門	宇宙
富士越	神代の冠	瑠璃殿	浮間の光
藤娘	狩衣	桜源氏	瑤台の夢
月桂冠	錦鶏鳥	漣	雪の肌
還城楽	金秀花	西王母	前代未聞
銀覆輪	清見渦	戦勝	
五大州	駒止	志賀の都	
花車	香炉峰	紫光梅	

三倍性品種 ($2n=36$)

駅路の鈴	隠れ簀	重紫雲	目白台
嵐山	獅子頭		

四倍性品種 ($2n=48$)

大和神風

園芸品種間では、倍数性レベルと仁の最大数とに比例関係が認められ、また全般的に見て三倍性品種は、二倍性品種に比して大きな気孔を有している。二倍性、三倍性および四倍性品種は、それぞれ二本、三本、四本の仁染色体を有しており、同質倍数性が暗示される。倍数性品種の起原には、非還元性配偶子が大きく関与していると思われる。

近縁野生種の染色体数は次の通りであつた。

Sect. *Caortusoides*

<i>P. sieboldi</i> E. Morr.	$2n=24$
<i>P. jesoana</i> Miq. var. <i>jesoana</i>	$2n=24$
<i>P. kisoana</i> Miq. var. <i>kisoana</i>	$2n=24$
<i>P. kisoana</i> Miq. var. <i>shikokiana</i>	$2n=24$

Sect. *Reinii*

<i>P. tosaensis</i> Yatabe	$2n=24$
<i>P. reinii</i> Franch. et Savat.	
var. <i>reinii</i>	$2n=24$

P. sieboldi と *P. jesoana* var. *jesoana* との核型は、ほぼ一致しており、類縁性が高いと思われ、両者の間に交雑の可能性が推定される。従つて、*P. jesoana* の特徴である、段咲き、円形の葉、濃紅色の花弁などの形質をニホンサクラソウに導入して、全く新しい園芸品種を作出する可能性がある。