Chromosome Association and Giemsa C-banding of Meiotic Chromosomes in Interspecific Hybrid of *Alstroemeria ligtu* L. hybrid and *A. pelegrina* L. var. *rosea*, its Amphidiploid, and Sesquidiploid between the Amphidiploid and the Parents

Takayuki Ishikawa¹⁾ and Hiroshi Ishizaka*

Horticultural Research Branch, Saitama Prefecture Agriculture and Forestry Research Center, 91 Rokumanbu, Kuki, Saitama 346-0037, Japan

¹⁾ Present address: Kazo Agriculture Improvement Center, 564-1 Fudoka, Kazo, Saitama 347-0054, Japan

Alstroemeria ligtu L. hybrid (LH, $2n=2x=16$), *A. pelegrina* L. var. *rosea* (PR, $2n=2x=16$) and their interspecific hybrids ($2n=2x=16$, $3x=24$, $4x=32$) were analyzed cytologically. In the parental species, the mean chromosome association per pollen mother cell (PMC) at metaphase I in meiosis was $0.04 \text{ I} + 7.98 \text{ II}$ in LH and $0.08 \text{ I} + 7.96 \text{ II}$ in PR. Meiosis proceeded normally in these 2 species and pollen fertility was 98.4% in the former and 94.9% in the latter. These 2 species produced many normal seeds by self-pollination. The hybrid ($2n=16$) showed partial chromosome association with a mean of $11.18 \text{ I} + 2.41 \text{ II}$. Abnormal chromosome behavior such as the formation of bridges and micronuclei was observed frequently at anaphase I and II of PMCs or at the early uninucleate stage of microspores, which resulted in low pollen fertility (0.6%). The hybrid did not produce any normal seeds when self-pollinated or backcrossed with either parent. In the amphidiploid ($2n=32$), the mean chromosome association per PMC was $0.82 \text{ I} + 15.59 \text{ II}$ at metaphase I and pollen fertility was 86.3%. Although the amphidiploid did not produce any normal seeds by self-pollination, it produced normal seeds by the reciprocal crosses with LH. The mean chromosome association in the sesquidiploid ($2n=24$) of LH $\times$ the amphidiploid was $8.24 \text{ I} + 7.85 \text{ II} + 0.02 \text{ III}$ and that of PR $\times$ the amphidiploid was $8.58 \text{ I} + 7.66 \text{ II} + 0.03 \text{ III}$. These sesquidiploids frequently showed chromosome bridges and micronuclei at anaphase I and II of PMCs or at the early uninucleate stage of microspores, and pollen fertility was 14.8% in the former and 13.0% in the latter. Although the two types of sesquidiploid did not produce any normal seeds by self-pollination, the former sesquidiploid produced normal seeds by the cross with LH. In LH, the Giemsa C-bands were observed in 7 out of 8 bivalents at metaphase I of PMCs and in 7-7 out of 8-8 chromosomes at anaphase I of PMCs, whereas no C-bands were observed in PR throughout these meiotic stages. These C-band patterns

were detected at metaphase I of PMCs in the hybrid, the amphidiploid, and the two types of sesquidiploids.

Key Words: *Alstroemeria ligtu* hybrid, *A. pelegrina* var. *rosea*, interspecific hybrid, amphidiploid, chromosome association, Giemsa C-banding.

Introduction

The genus *Alstroemeria* has about 93 species (Aker and Healy 1990). Many cultivars have been bred through crossing between several of these species (Goemans 1962, Hang and Tsuchiya 1988) or through irradiation treatments (Broertjes and Verboom 1974). Among the cultivated alstroemerias, the so-called *A. ligtu* hybrid (LH, $2n=2x=16$) is believed to have originated from a natural crossing between *A. ligtu* ssp. *ligtu* and *A. ligtu* ssp. *simsii* (Spreng.) Bayer (synonym *A. haemantha* Ruiz et Pav.) (Robinson 1963), and is now considered to be well established as cytologically homozygous lines because of the high frequency of chromosome pairing at metaphase I of pollen mother cells (PMCs) and high pollen fertility (Rustanius *et al.* 1991). This hybrid is widely utilized for cut flowers in Japan because of the favorable light color of its flowers. On the other hand, *A. pelegrina* var. *rosea* (PR, $2n=2x=16$) is dwarf and has large flowers; it is thus a valuable gene source for improving commercial alstroemerias as a cut flower or pot plant.

There is a cross-incompatibility among some species in the genus *Alstroemeria* (Buitendijk *et al.* 1995, De Jeu and Jacobsen 1995). The cross-incompatibility between *A. ligtu* hybrid and *A. pelegrina* var. *rosea* is associated with the abortion of the hybrid embryo. It has been overcome by ovule culture, and interspecific hybrid plants ($2n=16$) have been produced (Ishikawa *et al.* 1997). However, the hybrid plants could not be used as successive breeding material due to pollen and seed sterilities. Amphidiploids ($2n=32$) of LH $\times$ PR have been produced by chromosome doubling using colchicine, and two types of sesquidiploids ($2n=24$) were obtained from the crosses of LH ($2n=16$) and PR ($2n=16$) $\times$ the amphidiploids ($2n=32$) (Ishikawa *et al.*

Communicated by M. Shibata

Received June 5, 2001. Accepted August 21, 2001.

*Corresponding author (e-mail: shes@mail.raidway.ne.jp)

1999). These interspecific hybrids ($2n=16$, 24 and 32) showed various morphological features and different frequencies of pollen and seed fertilities (Ishikawa *et al.* 1999).

Pollen fertility of the interspecific hybrid is closely related to its meiosis, especially chromosome pairing at metaphase I and subsequent chromosome behavior, and the degree of chromosome pairing at metaphase I has been used for clarifying genetic relationships between the parents. Furthermore, the C-banding pattern has provided additional knowledge for the identification of individual chromosomes in many plants including the genus *Alstroemeria* (Buitendijk and Ramanna 1996). In this paper, we report the meiotic chromosome behavior, pollen and seed fertility, and banding pattern of meiotic chromosomes using the Giemsa C-banding technique in LH, PR, hybrid of LH $\times$ PR, its amphidiploid, sesquidiploid of LH $\times$ the amphidiploid, and that of PR $\times$ the amphidiploid.

Materials and Methods

Two parental species, *Alstroemeria ligtu* hybrid cv. 'Haruhigasa' (LH, $2n=2x=16$) and *A. pelegrina* var. *rosea* (PR, $2n=2x=16$), the LH $\times$ PR hybrid ($2n=16$), its amphidiploid ($2n=32$), sesquidiploid ($2n=24$) of LH $\times$ the amphidiploid and that ($2n=24$) of PR $\times$ the amphidiploid produced previously (Ishikawa *et al.* 1997, 1999) were grown in a greenhouse and used as plant materials.

Flower buds at appropriate stages were detached from the plants and fixed in ethanol/glacial acetic acid (3:1, v/v). Anthers were isolated from the flower bud and squashed on a slide glass. PMCs on the slide glass were then stained with acetocarmine for 5 min, warmed gently and squashed with a cover slip. Data on meiotic chromosome pairing behavior in PMCs at metaphase I, and the frequencies of meiotic chromosome irregularities such as bridges and micronuclei in PMCs at anaphase I and II and in microspores at the early uninucleate stage were recorded. They were observed in 5 anthers for each specimen.

Pollen grains were shed from the flowers just after anthesis on the slide glass, and stained with acetocarmine. About 1000 pollen grains were examined under a light microscope and the pollen grains containing nuclei stained with acetocarmine were considered to be fertile.

Seed fertility of LH, PR and their interspecific hybrids was investigated by self- and cross-pollination. Flowers were emasculated and covered with paper bags around 3 days before anthesis. When the stigma became swollen enough to hold pollen grains, the flowers were pollinated and covered again with paper bags to prevent further pollination by undesirable pollen.

The acetocarmine-stained slides with PMCs at metaphase I and anaphase I were subjected to C-banding treatment. Immediately after removal of coverslips by CO₂-freezing, the slides were placed in 99% ethanol, left for 24 h, and air-dried at room temperature for a few minutes. The staining procedure reported by Giraldez *et al.* (1979) was

used with the following modifications. The slides were incubated in 0.2N HCl at 60°C for 2.5 min, in 5% Ba(OH)₂ solution at 25°C for 7 min, then in $2 \times$ SSC solution at 60°C for 30 min. In this procedure, the slides were rinsed with tap water after each step of the treatment. Treated slides were stained with 2% Giemsa solution (Fisher) diluted with 1/15 M phosphate buffer (Na₂HPO₄/KH₂PO₄, pH 7.0) for 30 min at room temperature, then briefly rinsed and air dried. The concentration of Giemsa solution and the duration of the staining were appropriately adjusted within the extents of 2–4% and 30–40 min, respectively, according to the quality of the slide and Giemsa solution. Five and more well-banded plates were observed for each sample.

Results

Meiosis

Meiosis of the parental species was normal. The mean chromosome association per PMC at metaphase I was 0.04 I + 7.98 II in LH and 0.08 I + 7.96 II in PR (Table 1, Fig. 1A, 1C). These plants formed normal tetrads, followed by the formation of normal microspores at the early uninucleate stage. Frequencies of normal PMCs, without either bridges or micronuclei, at anaphase I and II were both 98.3% in LH, and 98.4 and 99.1%, respectively, in PR (Table 2). Frequencies of microspores without micronuclei at the early uninucleate stage were 98.7% in LH and 99.3% in PR (Table 3). The fertility of pollen grains at the mature stage was 98.4% in LH and 94.9% in PR (Table 3). These species produced many normal seeds by self-pollination (Table 4).

Meiosis of the LH $\times$ PR hybrid was irregular. Many univalents and a few bivalents were observed at metaphase I of PMCs, but neither trivalents nor multivalents was detected. The mean number of chromosome associations per PMC was 11.18 I + 2.41 II (Table 1, Fig. 1E). Frequencies of abnormal PMCs with bridges and/or micronuclei were 88.4 and 87.5% at anaphase I and II, respectively (Table 2). Although almost all of the microspores at the early uninucleate stage were viable, they had nuclei of different sizes. Furthermore, 32.4% of the microspores contained micronuclei (Table 3). The fertility of pollen grains at the mature stage was 0.6% (Table 3). No seeds were obtained from any of the cross-combinations using the hybrid (Table 4).

By contrast, the amphidiploid showed normal meiosis. Sixteen bivalents were predominantly detected at metaphase I of PMCs, and no multivalents were detected. The mean number of chromosome associations per PMC was 0.82 I + 15.59 II (Table 1, Fig. 1G). Frequencies of PMCs without bridges and/or micronuclei were 80.1 and 81.9% at anaphase I and II, respectively (Table 2). Almost all of the microspores at the early uninucleate stage were viable and the frequency of microspores without micronuclei was 92.8%, producing fertile pollen grains at a high frequency (86.3%) (Table 3). Normal seeds were not obtained from the amphidiploid by self-pollination and the reciprocal crosses of the amphidiploid $\times$ PR, whereas 2 and 110 normal seeds

Table 1. Observation of chromosome association at metaphase I of PMCs in *Alstroemeria ligtu* hybrid, *A. pelegrina* var. *rosea*, their interspecific hybrid, amphidiploid and sesquidiploids between the amphidiploid and the parents

Plant	No. of PMCs observed	Chromosome association at metaphase I		
		I ¹⁾	II ²⁾	III ³⁾
<i>A. ligtu</i> hybrid 2n=2x=16	98	0.04	7.98	0
<i>A. pelegrina</i> var. <i>rosea</i> 2n=2x=16	105	0.08	7.96	0
Hybrid 2n=2x=16	149	11.18	2.41	0
Amphidiploid 2n=4x=32	100	0.82	15.59	0
Sesquidiploid-1 ⁴⁾ 2n=3x=24	188	8.24	7.85	0.02
Sesquidiploid-2 ⁵⁾ 2n=3x=24	148	8.58	7.66	0.03

¹⁾ I=univalent ²⁾ II=bivalent ³⁾ I=trivalent⁴⁾ *A. ligtu* hybrid × the amphidiploid⁵⁾ *A. pelegrina* var. *rosea* × the amphidiploid**Table 2.** Observation of PMCs at anaphase I and II in *Alstroemeria ligtu* hybrid, *A. pelegrina* var. *rosea*, their interspecific hybrid, amphidiploid and sesquidiploids between the amphidiploid and the parents

Plant	Stage	No. of PMCs observed	No. of normal PMCs (%)	No. of abnormal PMCs		
				PMCs with micronuclei (%)	PMCs with bridges (%)	PMCs with micronuclei and bridges (%)
<i>A. ligtu</i> hybrid	Anaphase I	751	738 (98.3)	7 (0.9)	6 (0.8)	0 (0)
2n=2x=16	Anaphase II	649	638 (98.3)	11 (1.7)	0 (0)	0 (0)
<i>A. pelegrina</i> var. <i>rosea</i>	Anaphase I	828	815 (98.4)	8 (1.0)	5 (0.6)	0 (0)
2n=2x=16	Anaphase II	961	952 (99.1)	9 (0.9)	0 (0)	0 (0)
Hybrid	Anaphase I	599	70 (11.7)	446 (74.5)	58 (9.7)	25 (4.2)
2n=2x=16	Anaphase II	432	54 (12.5)	308 (71.3)	51 (11.8)	19 (4.4)
Amphidiploid	Anaphase I	512	410 (80.1)	76 (14.8)	20 (3.9)	6 (1.2)
2n=4x=32	Anaphase II	625	512 (81.9)	113 (18.1)	0 (0)	0 (0)
Sesquidiploid-1 ¹⁾	Anaphase I	455	108 (23.7)	283 (62.2)	32 (7.0)	32 (7.0)
2n=3x=24	Anaphase II	624	137 (22.0)	443 (71.0)	17 (2.7)	27 (4.3)
Sesquidiploid-2 ²⁾	Anaphase I	646	124 (19.2)	455 (70.4)	39 (6.0)	28 (4.3)
2n=3x=24	Anaphase II	583	91 (15.6)	418 (71.7)	29 (5.0)	45 (7.7)

¹⁾ *A. ligtu* hybrid × the amphidiploid²⁾ *A. pelegrina* var. *rosea* × the amphidiploid

were obtained from the reciprocal crosses of the amphidiploid × LH, respectively (Table 4).

In the sesquidiploid of LH × the amphidiploid and that of PR × the amphidiploid, PMCs with 8 bivalents and 8 univalents were observed most frequently at metaphase I, although those with trivalents were hardly observed. The mean number of chromosome associations per PMC was 8.24 I + 7.85 II + 0.02 III in the former sesquidiploid and 8.58 I + 7.66 II + 0.03 III in the latter sesquidiploid (Table 1, Fig. 2A, 2C). The frequency of abnormal PMCs with bridges and/or micronuclei at anaphase I and II was 76.2 and 78.0%, respectively, in the sesquidiploid of LH × the amphidiploid, and 80.7 and 84.4%, respectively, in the sesquidiploid of PR × the amphidiploid (Table 2). Almost all of the

microspores at the early uninucleate stage were viable, but they contained micronuclei at 39.7% in the former and at 21.3% in the latter (Table 3). The frequency of fertile pollen grains at the mature stage was 14.8% in the sesquidiploid of LH × the amphidiploid and 13.0% in the sesquidiploid of PR × the amphidiploid (Table 3). Seeds were not obtained from the former sesquidiploid by self-pollination, the cross of the former sesquidiploid × LH and the reciprocal crosses of the former sesquidiploid × PR, whereas 2 normal seeds were obtained from the cross of LH × the former sesquidiploid (Table 4). Seeds were not obtained from any of the cross-combinations using the latter sesquidiploid.

Table 3. Observation of microspore at early uninucleate stage and pollen grain at mature stage in *Alstroemeria ligtu* hybrid, *A. pelegrina* var. *rosea*, their interspecific hybrid, amphidiploid and sesquidiploids between the amphidiploid and the parents

Plant	Microspore at early uninucleate stage				Pollen grain at mature stage	
	No.of microspores observed	No.of normal microspores (%)	No.of abnormal microspores		No.of pollen grains observed	Frequency of fertile pollen grains (%)
			Microspores with 1 micronucleus (%)	Microspores with 2 micronuclei (%)		
<i>A. ligtu</i> hybrid 2n=2x=16	942	930 (98.7)	12 (1.3)	0 (0)	1005	98.4
<i>A. pelegrina</i> var. <i>rosea</i> 2n=2x=16	828	822 (99.3)	6 (0.7)	0 (0)	1008	94.9
Hybrid 2n=2x=16	1256	849 (67.6)	357 (28.4)	50 (4.0)	1016	0.6
Amphidiploid 2n=4x=32	1075	998 (92.8)	69 (6.4)	8 (0.7)	1013	86.3
Sesquidiploid-1 ¹⁾ 2n=3x=24	1192	719 (60.3)	419 (35.2)	54 (4.5)	997	14.8
Sesquidiploid-2 ²⁾ 2n=3x=24	1257	990 (78.8)	251 (20.0)	16 (1.3)	1012	13.0

¹⁾ *A. ligtu* hybrid × the amphidiploid²⁾ *A. pelegrina* var. *rosea* × the amphidiploid**Table 4.** Seed fertility of *Alstroemeria ligtu* hybrid, *A. pelegrina* var. *rosea*, their interspecific hybrid, amphidiploid and sesquidiploids between the amphidiploid and the parents

Cross-combination	No. of flowers pollinated	No. of capsules obtained	No. of seeds obtained	
			Total	Normal
LH self	5	5	111	98
PR self	5	5	61	52
Hybrid self	163	0	—	—
Hybrid × LH	49	0	—	—
LH × Hybrid	50	0	—	—
Hybrid × PR	95	0	—	—
PR × Hybrid	61	0	—	—
Amphidiploid self	65	29	66	0
Amphidiploid × LH	29	26	65	2
LH × Amphidiploid	17	17	137	110
Amphidiploid × PR	16	0	—	—
PR × Amphidiploid	14	0	—	—
Sesquidiploid-1 ¹⁾ self	45	0	—	—
Sesquidiploid-1 ¹⁾ × LH	41	0	—	—
LH×Sesquidiploid-1 ¹⁾	17	2	8	2
Sesquidiploid-1 ¹⁾ × PR	63	0	—	—
PR × Sesquidiploid-1 ¹⁾	30	0	—	—
Sesquidiploid-2 ²⁾ self	44	0	—	—
Sesquidiploid-2 ²⁾ × LH	38	0	—	—
LH×Sesquidiploid-2 ²⁾	15	0	—	—
Sesquidiploid-2 ²⁾ × PR	40	0	—	—
PR×Sesquidiploid-2 ²⁾	91	0	—	—

¹⁾ *A. ligtu* hybrid × the amphidiploid²⁾ *A. pelegrina* var. *rosea* × the amphidiploid

C-banding

A cytological comparison of the parental species revealed differences between LH (2n=16) and PR (2n=16) in C-banding pattern at metaphase I and anaphase I of PMCs. Prominent C-bands were detected on 7 out of 8 bivalents at metaphase I of PMC in LH but not on 1 bivalent

(Fig. 1A, 1B). Thereafter, these 8 bivalents divided equally into 8-8 chromosomes at anaphase I and prominent C-bands were detected on 7-7 out of 8-8 chromosomes in LH (Fig. 3A, 3B). In contrast, prominent C-bands were not detected on 8 bivalents at metaphase I and on 8-8 chromosomes at anaphase I of PMCs in PR (Fig. 1C, 1D, 3C, 3D).

Fig. 1. Meiotic chromosomes at metaphase I of PMCs of *A. ligu* hybrid (A and B, 8 II), *A. pelegrina* var. *rosea* (C and D, 8 II), their hybrids (E and F, 12 I + 2 II), and the amphidiploids (G and H, 16 II). A, C, E and G are acetocarmine-stained plates (arrows indicate bivalents), and B, D, F and H are the same plates subsequently stained by C-banding (arrows indicate prominent bands that appeared after C-banding treatment). Bars=20 µm.

Fig. 3. Meiotic chromosomes at anaphase I of PMCs of *A. ligu* hybrid (A and B, 2n=16), *A. pelegrina* var. *rosea* (C and D, 2n=16), and the amphidiploids (E and F, 2n=32). A, C and E are acetocarmine-stained plates, and B, D and F are the same plates subsequently stained by C-banding. Arrows indicate prominent bands that appeared after C-banding treatment. Bars=20 µm.

Fig. 2. Meiotic chromosomes at metaphase I of PMCs of the sesquidiploids of *A. ligu* hybrid x the amphidiploids (A and B, 8 I + 8 II) and the sesquidiploids of *A. pelegrina* var. *rosea* x the amphidiploids (C and D, 8 I + 8 II). A and C are acetocarmine-stained plates, and B and D are the same plates subsequently stained by C-banding. Arrows indicate prominent bands that appeared after C-banding treatment. Bars=20 µm.

The hybrid (2n=16) formed many univalents and a few bivalents at metaphase I of PMCs (Table 1). PMCs shown in the plates (Fig. 1E, 1F) formed 12 univalents and 2 bivalents, and prominent C-bands were detected on 5 univalents and 2 bivalents. The 7 signals of C-bands corresponded to 7 signals of C-bands which appeared on 8 bivalents at metaphase I of PMCs in LH. These findings suggest that 5 univalents with C-bands derived from LH and 2 bivalents with C-bands were formed by pairing between chromosomes of PR and LH with C-bands.

In the amphidiploid (2n=32), prominent C-bands were detected only on 7 bivalents among 16 regular bivalents at metaphase I of PMC (Fig. 1G, 1H). Thereafter, these 16 bivalents separated into 16-16 chromosomes at anaphase I and prominent C-bands were detected on 7-7 out of 16-16 chromosomes at anaphase I (Fig. 3E, 3F). These findings suggest that 7 bivalents at metaphase I and 7-7 chromosomes at anaphase I derived from LH.

The sesquidiploids (LH x the amphidiploid and PR x the amphidiploid) predominantly formed 8 univalents and 8

bivalents at metaphase I of PMCs (Fig. 2A, 2C). Prominent C-bands were detected on 7 out of 8 bivalents but not on 8 univalents at metaphase I of PMCs in the sesquidiploid of LH $\times$ the amphidiploid (Fig. 2B). Conversely, prominent C-bands were detected on 7 out of 8 univalents but not on 8 bivalents at metaphase I of PMCs in the sesquidiploid of PR $\times$ the amphidiploid (Fig. 2D). These findings suggest that 7 bivalents with C-bands in the former sesquidiploid and 7 univalents with C-bands in the latter sesquidiploid derived from LH.

Discussion

LH ($2n=16$) and PR ($2n=16$) formed 8 bivalents predominantly at metaphase I of PMCs and showed normal meiosis, as reported by Rustanius *et al.* (1991) and Stephens *et al.* (1993). The LH $\times$ PR hybrid ($2n=16$) showed abnormal chromosome behaviors at meiosis; low frequency of chromosome pairing at metaphase I and formation of bridges and/or micronuclei at anaphase I and II, which probably led to the formation of microspores with incomplete genomes at the early uninucleate stage. Sterile pollen grains observed at the mature stage might be due to the abortion of the nucleus in these microspores before pollen mitosis. These findings suggest that LH is genetically different from PR, although both chromosomes retain some degree of homology.

By contrast, the amphidiploid ($2n=32$) of LH $\times$ PR showed a high frequency of chromosome pairing at metaphase I of PMCs, which seemed to result from the induction of homologous chromosomes of LH and PR by amphidiploidization. Successful chromosome pairing probably led to normal cell division without bridges and/or micronuclei at anaphase I and II and resulted in the production of fertile pollen grains. Lu and Bridgen (1997) reported that chromosome pairing at metaphase I in the amphidiploid ($2n=32$) of *A. aurea* Graham ($2n=16$) $\times$ *A. caryophyllaea* Jacq. ($2n=16$) was nearly normal, but the amphidiploid showed low pollen fertility (12%) due to irregular chromosome behavior such as the formation of bridges and lagging chromosomes at anaphase I or tetrad stage and did not set any seeds by self-pollination or backcross with the parent *A. aurea*. They suggested that sterility genes might be present in the amphidiploid of *A. aurea* $\times$ *A. caryophyllaea*. In the present study, the amphidiploid of LH $\times$ PR showed normal chromosome behavior at meiosis and high pollen fertility (86.3%) at the mature stage of the pollen grain. Empty seeds were derived from self-pollinated ovaries of the amphidiploid, probably due to the degeneration of endosperm after fertilization, while progenies of the amphidiploid were obtained by the embryo rescue technique (Ishikawa *et al.* 1999). Normal seeds were obtained by the reciprocal crosses of the amphidiploid $\times$ LH (Table 4). Therefore, we considered that the amphidiploid did not have the cytological instability at meiosis and sterility genes reported by Lu and Bridgen (1997), and that male and female gametes of the

amphidiploid were normal.

The sesquidiploid ($2n=24$) of LH $\times$ the amphidiploid and that of PR $\times$ the amphidiploid predominantly formed 8 bivalents and 8 univalents. The former plant should have 2 genomes of LH and 1 genome of PR and the latter plant should have 1 genome of LH and 2 genomes of PR. Thus, it is reasonable to assume that the bivalents of the former or latter sesquidiploid derived from pairing between homologous chromosomes in LH or PR, and that the univalents of the former or latter sesquidiploid derived from chromosomes of LH or PR lacking the pairing partner. These assumptions are supported by the results of C-banding. Micronuclei or chromosome bridges were detected at anaphase I and II of PMCs in the two types of sesquidiploids. Microspores with incomplete genomes probably led to irregular chromosome behavior at pollen mitosis, resulting in the induction of sterile pollen grains. In the seed fertility of the two types of sesquidiploids, normal seeds were obtained from the cross of LH $\times$ the sesquidiploid (LH $\times$ the amphidiploid), suggesting that pollen grains produced in this sesquidiploid possess a competence as gamete. However, seeds were not obtained from other cross-combinations as shown in Table 4. Thus, the reproductive ability of male and female gametes in the two types of sesquidiploids should be confirmed by histological observation of embryonic development after self-pollination or the reciprocal crosses between the sesquidiploids and the parents. In the triploid consisting of 3 genomes with strong homology, especially in the autotriploid, trivalents are supposed to appear frequently at metaphase I of PMC. In the present study, the frequency of trivalents was very low at metaphase I of PMCs in the two types of sesquidiploids, suggesting that the homology between the genome of LH and PR is low and LH is genetically different from PR.

Buitendijk *et al.* (1995, 1998) and Buitendijk and Ramanna (1996) reported that polymorphic C-band patterns were present on root tip chromosomes in *Alstroemeria*. They showed the presence of prominent C-bands with a slight gradation of intensity on 8 pairs of root tip chromosomes in *A. ligtu* ssp. *ligtu* ($2n=16$) and they were present on 7 or 8 pairs in *A. ligtu* ssp. *simisii* ($2n=16$). In the present study, C-bands appeared in 7 out of 8 bivalents at metaphase I of PMCs in LH and 7 out of 8 pairs of chromosomes at anaphase I. This finding is in agreement with the results reported by Buitendijk *et al.* (1998) and Buitendijk and Ramanna (1996) because the *A. ligtu* hybrid (LH) is believed to have originated from a natural crossing between *A. ligtu* ssp. *ligtu* and *A. ligtu* ssp. *simisii* (Robinson 1963). Buitendijk and Ramanna (1996) reported that terminal C-bands appeared in 5 pairs of acrocentric chromosomes of root tip cells among 8 pairs of chromosomes in *A. pelegriana*. In the present study, no C-bands were present on 8 bivalents at metaphase I or 8 pairs of chromosomes at anaphase I in PR. This may be due to the disappearance of C-bands by chromosome condensation at metaphase I and anaphase I. The Giemsa C-banding technique, which has facilitated the identification of

individual chromosomes in many plant species and the identification of parent chromosomes in interspecific hybrids, has been applied to root tip chromosomes (Singh 1993). As above mentioned, it was possible to identify 7 meiotic chromosomes deriving from LH using Giemsa C-banding in the interspecific hybrid of LH $\times$ PR, but it was impossible to identify meiotic chromosomes of PR. Kamstra *et al.* (1997, 1999) identified meiotic or mitotic chromosomes of interspecific hybrids of *Alstroemeria* by fluorescence *in situ* hybridization using probes of ribosomal DNA or species-specific DNA. Thus, use of fluorescence *in situ* hybridization and the development of the original probes should play an important role for identifying meiotic chromosomes in interspecific hybrids of the genus *Alstroemeria* including the LH $\times$ PR hybrid.

Acknowledgement

We thank Dr. Masahiro Mii, Plant Cell Technology, Faculty of Horticulture, Chiba Univ. for his valuable suggestions.

Literature Cited

- Aker, S. and W. Healy (1990) The phylogeography of the genus *Alstroemeria*. *Herbertia* 46: 76-87.
- Broertjes, C. and H. Verboom (1974) Mutation breeding of *Alstroemeria*. *Euphytica* 23: 39-44.
- Buitendijk, J.H., N. Pinsonneaux, A.C. van Donk, M.S. Ramanna and A.A.M. van Lammeren (1995) Embryo rescue by half-ovule culture for the production of interspecific hybrids in *Alstroemeria*. *Sci. Hortic.* 64: 65-75.
- Buitendijk, J.H. and M.S. Ramanna (1996) Giemsa C-banded karyotypes of eight species of *Alstroemeria* L. and some of their hybrids. *Ann. Bot.* 78: 449-457.
- Buitendijk, J.H., A. Peters, R.J. Quene and M.S. Ramanna (1998) Genome size variation and C-band polymorphism in *Alstroemeria aurea*, *A. ligtu* and *A. magnifica* (*Alstroemeriaceae*). *Pl. Syst. Evol.* 212: 87-106.
- De Jeu, M.J. and E. Jacobsen (1995) Early postfertilization ovule culture in *Alstroemeria* L. and barriers to interspecific hybridization. *Euphytica* 86: 15-23.
- Giraldez, R., M.C. Cermeño and J. Orellana (1979) Comparison of C-banding pattern in the chromosomes of inbred lines and open pollinated varieties of rye. *Z. Pflanzenzucht* 83: 40-48.
- Goemans, J.A.M. (1962) Breeding of *alstroemerias*. *J. Roy. Hort. Soc.* 87: 282-284.
- Hang, A. and T. Tsuchiya (1988) Chromosome studies in the genus *Alstroemeria*. II. Chromosome constitutions of eleven additional cultivars. *Plant Breed.* 100: 273-279.
- Ishikawa, T., T. Takayama, H. Ishizaka, K. Ishikawa and M. Mii (1997) Production of interspecific hybrids between *Alstroemeria ligtu* L. hybrid and *A. pelegrina* L. var. *rosea* by ovule culture. *Breed. Sci.* 47: 15-20.
- Ishikawa, T., T. Takayama and H. Ishizaka (1999) Amphidiploids between *Alstroemeria ligtu* L. hybrid and *A. pelegrina* L. var. *rosea* induced through colchicine treatment and their reproductive characteristics. *Sci. Hortic.* 80: 235-246.
- Kamstra, S.A., A.G.J. Kuipers, M.J. De Jeu, M.S. Ramanna and E. Jacobsen (1997) Physical localization of repetitive DNA sequences in *Alstroemeria*: karyotyping of two species with species-specific and ribosomal DNA. *Genome* 40: 652-658.
- Kamstra, S.A., M.S. Ramanna, M.J. De Jeu, A.G.J. Kuipers and E. Jacobsen (1999) Homoeologous chromosome pairing in the distant hybrid *Alstroemeria aurea* $\times$ *A. inodora* and the genome composition of its backcross derivatives determined by fluorescence *in situ* hybridization with species-specific probes. *Heredity* 82: 69-78.
- Lu, C.S. and M.P. Bridgen (1997) Chromosome doubling and fertility study of *Alstroemeria aurea* $\times$ *A. caryophyllaea*. *Euphytica* 94: 75-81.
- Robinson, G.W. (1963) *Alstroemeria*. *J. Roy. Hort. Soc.* 88: 490-494.
- Rustanius, P., A. Hang, H.G. Hughes and T. Tsuchiya (1991) Chromosome analysis of *Alstroemeria ligtu* hybrids. *HortSci.* 26: 902-904.
- Singh, R.J. (1993) Chapter 2. The handling of plant chromosomes. In "Plant cytogenetics" Singh, R. J. (ed.), CRC Press, Florida. p. 7-23.
- Stephens, J.L., T. Tsuchiya and H.G. Hughes (1993) Chromosome studies in *Alstroemeria pelegrina* L.. *Int. J. Plant Sci.* 154: 565-571.