

## Intergeneric hybridization among colchicaceous ornamentals, *Gloriosa* spp., *Littonia modesta* and *Sandersonia aurantiaca* via ovule culture

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**Abstract** Colchicaceous plants, *Gloriosa* spp., *Littonia modesta* and *Sandersonia aurantiaca*, are cultivated as ornamentals. However, unfortunately no large variations in horticultural traits are found within each genus. We examined intergeneric hybridization using 6 genotypes of *Gloriosa* spp., 1 genotype of *L. modesta* and 2 genotypes of *S. aurantiaca* to obtain wider variability and to develop novel cultivars in those groups. Following intergeneric cross-pollination, putative hybrid plantlets were obtained via ovule culture in various combinations. Early confirmation of the hybridity of ovule culture-derived plantlets was accomplished by flow cytometry and random amplified polymorphic DNA analyses. Several intergeneric hybrids have so far been produced flowers and subjected to morphological characterization. All the hybrids examined, i.e. *L. modesta* × *S. aurantiaca*, *L. modesta* × *S. aurantiaca* ‘Phoenix’, *L. modesta* × *G. superba* ‘Lutea’, *L. modesta* × *G. ‘Marron Gold’*, *S. aurantiaca* × *G. superba* ‘Lutea’, *S. aurantiaca* × *G. ‘Marron Gold’* and *S. aurantiaca* ‘Phoenix’ × *G. ‘Marron Gold’*, had novel morphological characteristics compared with their parents, some of which were horticulturally attractive. The results obtained in our series of studies indicate the validity of intergeneric hybridization in the improvement programs of colchicaceous ornamentals. We are now examining to develop a rapid and efficient micropropagation system and to restore fertility by artificial chromosome doubling of intergeneric hybrids that had been produced in our series of experiments.

**Key words:** *Gloriosa* spp., horticultural characterization, hybrid identification, *Littonia modesta*, *Sandersonia aurantiaca*.

Colchicaceae comprises 19 genera, which distribute worldwide except for South America (Vinnersten and Reeves 2003). Several genera of this family such as *Gloriosa*, *Sandersonia* and *Littonia* contain horticulturally important plants that are cultivated as pot or cut flowers. *Gloriosa* had been described to contain a number of species with different ploidy levels and morphologies, but most of them are currently regarded as forms of *G. superba* L. (Nordenstam 1998; Vinnersten and Reeves 2003). However, we followed the previous reports (Lemattre 1980; Nakamura et al. 2005) and adopted the former species and cultivar names for *Gloriosa* genotypes used in our series of studies. *Gloriosa* spp. are native to the tropics of Africa and Asia

(Nordenstam 1998). *Gloriosa* spp. have the following horticultural characteristics: tuberous perennials; plants show a climbing habit and have sessile leaves with a tendril at the tip; flowers are pendulous and yellow, red, purplish, or variously bicolored in color; tepals are undulate and reflexed turning upward and backward. *Gloriosa* spp. began to be cultivated commercially in Japan around 1980 (Azuma 1995a). Systems for year-round production of *Gloriosa* cut flowers have already been established in Japan. *S. aurantiaca* Hook. is a monotypic species native to Southern Africa (Nordenstam 1998). *S. aurantiaca* has the following horticultural characteristics: tuberous perennials; plants show a simple erect habit and have sessile leaves usually

Abbreviations: BA, 6-benzyladenine; FCM, flow cytometry; Gaf, *Gloriosa* ‘African Gold’; Gma, *Gloriosa* ‘Marron Gold’; Gro, *Gloriosa* rothschildiana; Grp, *Gloriosa* ‘Rothschildiana Pink’; Gsu, *Gloriosa* superba ‘Lutea’; Gve, *Gloriosa* ‘Verschild’; Lit, *Littonia modesta*; MS, Murashige and Skoog (1962); NAA,  $\alpha$ -naphthaleneacetic acid; PGR, plant growth regulator; PCR, polymerase chain reaction; RAPD, randomly amplified polymorphic DNA; Sau, *Sandersonia aurantiaca*; Sph, *Sandersonia aurantiaca* ‘Phoenix’.

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with an apical tendril; flowers are pendulous, bell-shaped and generally orange–yellow in color; tepals are fused. *S. aurantiaca* has been introduced from New Zealand in the 1980s, and then has been cultivated commercially in Japan (Azuma 1995b). Year-round commercial production systems of *S. aurantiaca* cut flowers have also already been established. Cut flowers of *Gloriosa* spp. and *S. aurantiaca* have high marketability, and they are traded as important items in the commercial market. *Littonia* consists of about 8 species that are distributed in Africa, namely from South Africa to Senegal and Arabia (Nordenstam 1998). Among these species, *L. modesta* Hook. is sometimes cultivated as an ornamental (Morgan et al. 2003). *L. modesta* has the following horticultural characteristics: tuberous perennials; plants show a climbing habit with sessile leaves and apical tendrils; flowers are pendulous, campanulate, not opening widely and orange–yellow in color. *L. modesta* is still only rarely cultivated in Japan. For *Gloriosa* spp. and *S. aurantiaca*, some cultivars have so far been produced by intra- and interspecific hybridization or sport selection (Lemattre 1980; Morgan et al. 2002; Morgan et al. 2004). However, there are still few variations in horticultural traits, such as plant form, flower color and shape, within each genus.

For ornamentals, cross-breeding is one of the most important means for introducing desirable traits and producing novel cultivars. Although cross-breeding is generally achieved within the species, wide hybridization such as interspecific or intergeneric hybridization may be more effective for widening the variability in horticultural traits. Wide hybridization is often prevented by pre- and post-fertilization barriers, but the latter barrier, in which hybrid embryos obtained after fertilization degenerate during their development, can sometimes be overcome by embryo rescue techniques such as embryo or ovule culture. In some ornamental plants, such as *Lilium* spp. (Van Tuyl et al. 1991), *Primula* spp. (Kato et al. 2008), *Cyclamen* spp. (Ishizaka 2008), interspecific hybrid cultivars have so far been produced via embryo rescue. In colchicaceous ornamentals, intergeneric hybridization has recently been performed for widening the variability in horticultural traits: *Santonia* ‘Golden Lights’, an intergeneric hybrid cultivar of *S. aurantiaca* × *L. modesta*, was developed via ovule culture (Eason et al. 2001; Morgan et al. 2001, 2003; Clark et al. 2005; Burge et al. 2008); intergeneric hybrids between *S. aurantiaca* and *Gloriosa* spp. were also produced via ovule culture (Nakamura et al. 2005; Burge et al. 2008). However, hybrids have been produced only in limited intergeneric combinations. In the present mini review, we summarize our recent studies on intergeneric hybridization in colchicaceous ornamentals using 6 genotypes of *Gloriosa* spp., 1 genotype of *L. modesta* and 2 genotypes of *S. aurantiaca*.

## Ovule culture

Reciprocal intergeneric cross-pollination was performed using 6 genotypes of *Gloriosa* spp. (Gsu, Gaf, Gma, Gro, Grp and Gve), 1 genotype of *L. modesta* (Lit) and 2 genotypes of *S. aurantiaca* (Sau and Sph), which are listed in Table 1 (Kuwayama et al. 2005). Although *S. aurantiaca* has leaves usually with a tendril at the tip, we used *S. aurantiaca* genotypes without leaf tip tendrils. Tubers of all genotypes were planted in pots and cultivated in the greenhouse without heating.

A total of 411 flowers of seed parent were used for intergeneric cross-pollination. Flowers of the seed parent were emasculated 2 days before anthesis, and cross-pollination was carried out on the day at anthesis using fresh pollen. Ovaries were harvested 2 weeks after pollination. When Sau, Sph and Lit were used as a seed parent, many enlarged ovules were obtained. On the other hand, only a few or no enlarged ovules were obtained when *Gloriosa* genotypes except for Gsu were used as a seed parent. Totally 3,860 enlarged ovules were obtained in 32 intergeneric cross-combinations, in which ovule culture was carried out. For ovule culture, enlarged ovules with placental tissues were prepared from the ovaries and cultured on half-strength MS medium containing  $0.01 \text{ mg L}^{-1}$  NAA and  $0.01 \text{ mg L}^{-1}$  BA at  $25^\circ\text{C}$  in the dark (Nakamura et al. 2005).

Two months after initiation of ovule culture, rhizome-like structures were developed from cultured ovules in 30 out of 32 cross-combinations. Ovules with rhizome-like structures were transferred to half-strength MS medium containing  $0.25 \text{ mg L}^{-1}$  NAA and  $2.5 \text{ mg L}^{-1}$  BA and cultured for shoot induction at  $25^\circ\text{C}$  under a 16-h photoperiod with fluorescent lighting ( $50 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ). After 1–6 months of transfer, shoots were regenerated from rhizome-like structures. Regenerated shoots were transferred for rooting to half-strength MS medium without PGRs and cultured under the same conditions. Most shoots produced roots within 2 weeks, and plantlets thus obtained produced small tubers at the shoot base. Finally ovule culture-derived plantlets were obtained in 19 intergeneric cross-combinations. When Gsu, Lit and Sau were used as a seed parent, relatively large numbers of plantlets were obtained. Irrespective of the seed parent, no plantlets were obtained when Lit was used as a pollen parent, although Morgan et al. (2001) succeeded in the production of intergeneric hybrids of Sau × Lit. Several ovule culture-derived plantlets unfortunately died during culture on PGR-free medium.

## Early confirmation of the hybridity of ovule culture-derived plantlets

Colchicaceous ornamentals usually require 2–3 years from sowing to flowering stages (Azuma 1995a,

Table 1. Ploidy level, chromosome number and some horticultural characteristics of colchicaceous ornamentals and intergeneric hybrids.

| Parent and hybrid <sup>a</sup> | Ploidy level and chromosome number (2n) | Plant form | Leaf tip tendrils <sup>b</sup> | Flower form  | Flower color <sup>c</sup> |                         |
|--------------------------------|---|------------|--------------------------------|--|---------------------------|-------------------------|
|                                |   |            |                                |  | JHS Color Chart No.       | ISCC-NBS color name     |
| Parent                         |   |            |                                |  |                           |                         |
| Gsu                            | 2x=22                                   | Climbing   | +                              | Pendulous; undulate and reflexed tepals              | 2505                      | Bright yellow           |
| Gaf                            | 2x <sup>d</sup>                         | Climbing   | +                              | Pendulous; undulate and reflexed tepals              | 2505                      | Bright yellow           |
| Gma                            | 4x=44                                   | Climbing   | +                              | Pendulous; slightly undulate and reflexed tepals     | 1016                      | Grayish brown           |
| Gro                            | 6x=66                                   | Climbing   | +                              | Pendulous; undulate and reflexed tepals              | 2906                      | Vivid greenish yellow   |
| Grp                            | 6x <sup>d</sup>                         | Climbing   | +                              | Pendulous; undulate and reflexed tepals              | 0106                      | Bright red              |
| Gve                            | 7x <sup>d</sup>                         | Climbing   | +                              | Pendulous; undulate and reflexed tepals              | 2505                      | Bright yellow           |
| Lit                            | 2x=22                                   | Climbing   | +                              | Pendulous; undulate and reflexed tepals              | 0107                      | Vivid red               |
| Sau                            | 2x=24                                   | Erect      | -                              | Pendulous; undulate and reflexed tepals              | 2703                      | Light greenish yellow   |
| Sph                            | 2x=24                                   | Erect      | -                              | Pendulous; undulate and reflexed tepals              | 0407                      | Vivid red               |
| Hybrid                         |   |            |                                |  | 2505                      | Bright yellow           |
| Lit×Sau                        | 2x=23                                   | Climbing   | +                              | Pendulous; campanulate; not opening widely           | 1605                      | Vivid orange            |
| Lit×Sph                        | 2x=23                                   | Climbing   | +                              | Pendulous; campanulate; fused tepals                 | 2205                      | Vivid reddish yellow    |
| Lit×Gsu                        | 2x=22                                   | Climbing   | +                              | Pendulous; bell-shaped; fused tepals                 | 1605                      | Vivid orange            |
| Lit×Gma-1                      | 3x <sup>d</sup>                         | Climbing   | +                              | Pendulous; bell-shaped; fused tepals                 | 2205                      | Vivid reddish yellow    |
| Lit×Gma-2                      | 3x <sup>d</sup>                         | Climbing   | +                              | Pendulous; bell-shaped; fused tepals                 | 2511                      | Light yellow            |
| Sau×Gsu                        | 2x=23                                   | Climbing   | +                              | Pendulous; campanulate; fused tepals at the base     | 1605                      | Vivid orange            |
| Sau×Gma                        | 3x <sup>d</sup>                         | Climbing   | +                              | Pendulous; campanulate; fused tepals at the base     | 1905                      | Bright yellowish orange |
| Sph×Gma                        | 3x <sup>d</sup>                         | Climbing   | +                              | Pendulous; undulate and horizontally expanded tepals | 2505                      | Bright yellow           |
|                                |   |            | +                              | Pendulous; undulate and slightly reflexed tepals     | 0419                      | Grayish red             |
|                                |   |            | +                              | Pendulous; undulate and slightly reflexed tepals     | 2704                      | Bright greenish yellow  |
|                                |   |            | +                              | Pendulous; undulate and slightly reflexed tepals     | 2703                      | Light greenish yellow   |
|                                |   |            | +                              | Pendulous; undulate and slightly reflexed tepals     | 8911                      | Strong reddish purple   |
|                                |   |            | +                              | Pendulous; undulate tepals fused at the base         | 2511                      | Light yellow            |
|                                |   |            | +                              | Pendulous; slightly undulate and reflexed tepals     | 1016                      | Grayish brown           |
|                                |   |            | +                              | Pendulous; slightly undulate and reflexed tepals     | 2705                      | Bright greenish yellow  |
|                                |   |            | +                              | Pendulous; slightly undulate and reflexed tepals     | 1015                      | Light reddish brown     |
|                                |   |            | +                              | Pendulous; slightly undulate and reflexed tepals     | 2704                      | Bright greenish yellow  |

<sup>a</sup> Gsu, *Gloriosa superba* 'Lutea'; Gaf, *Gloriosa* 'African Gold'; Gma, *Gloriosa* 'Marron Gold'; Gro, *G. rothschildiana*; Grp, *G. 'Rothschildiana* Pink'; Gve, *Gloriosa* 'Versehild'; Lit, *Littonia modesta*; Sau, *Sandersonia aurantiaca*; Sph, *S. aurantiaca* 'Phoenix'.

<sup>b</sup> +, with leaf tip tendrils; -, without leaf tip tendrils.

<sup>c</sup> Flower color was checked visually with an aid of the JHS Color Chart (Japan Color Research Institute 1984). For Lit and Sau, flower colors were graded from vivid orange (flower base; upper line) to vivid reddish yellow (flower tip; lower line). For Gma, Lit×Gma-1, Sau×Gma, and Sph×Gma, flower colors were clearly different between the center (upper line) and rim (lower line) of the tepal. For Lit×Gma-2, flower color was light greenish yellow (upper line) with strong reddish purple spots (lower line).

<sup>d</sup> Ploidy level was estimated by FCM analysis.

1995b), and therefore, early identification of hybrids is necessary for efficient cross-breeding. We examined early confirmation of the hybridity of ovule culture-derived plantlets by FCM and RAPD analyses (Amano et al. 2007a). FCM analysis of leaf tissues was performed using a flow cytometer PA (Partec GmbH, Münster, Germany) according to Saito et al. (2003). Since neither cytochimeras nor polysomaty were found in all the 9 colchicaceous genotypes, the position of the single peak corresponding to nuclei in the G0/G1 phase of the cell cycle may be usable as an index for hybrid identification. The G0/G1 peaks of most colchicaceous genotypes appeared at apparently different positions. However, the G0/G1 peaks of 2 diploid *Gloriosa* genotypes (Gsu and Gaf) and Lit appeared at almost the same positions. Therefore, for ovule culture-derived plantlets from intergeneric crosses between genotypes with the G0/G1 peaks of almost the same positions, such as Lit×Gsu and Lit×Gaf, the hybridity could not be verified by FCM analysis. For most ovule culture-derived plantlets from crosses between genotypes with the G0/G1 peaks of different positions, the G0/G1 peak appeared at an intermediate position between the corresponding parents, indicating that these plantlets were hybrids. However, the G0/G1 peak of some plantlets appeared at the same position as the seed parent, indicating that they were not hybrids and may be derived from self-pollination of the seed parent. When Gve was used as one parent, the G0/G1 peak of ovule culture-derived plantlets appeared at near intermediate positions between the parents. Since Gve was estimated to be heptaploid, these plantlets may be hybrids with 3–4 genomes of Gve. When Sau was used as one parent, the G0/G1 peak of some ovule culture-derived plantlets appeared at an unexpected position, which was lower or higher than an intermediate position between the parents. Thus, the hybridity of these plantlets could not be verified by FCM analysis. In consequence, a total of 42 independent intergeneric hybrid plantlets were identified only by FCM analysis.

For verifying the hybridity of ovule culture-derived plantlets, which could not be identified as hybrids by FCM analysis, as well as for confirming the results of FCM analysis, RAPD analysis was carried out according to Obata et al. (2000). Intergeneric polymorphisms in the RAPD profile were obtained using several primers. The hybridity of all the 42 independent plantlets, which were identified as hybrids by FCM analysis, was also confirmed by RAPD analysis. In addition, RAPD analysis facilitated verification of the hybridity of some ovule culture-derived plantlets, which could not be identified as hybrids by FCM analysis. Totally, 50 independent intergeneric hybrid plantlets have so far been identified by FCM and/or RAPD analyses. These results indicate that FCM in combination with RAPD analyses offer simple and rapid means for the early

detection of intergeneric hybrids in colchicaceous ornamentals. The effectiveness of these analyses for hybrid identification has also been reported for some ornamentals such as *Primula* spp. (Kato et al. 2008) and *Dianthus* spp. (Nimura et al. 2003).

## Horticultural characterization of some intergeneric hybrid plants

Intergeneric hybrid plantlets with a small tuber were acclimatized, transplanted to pots, and cultivated in the greenhouse without heating. After 2 years of cultivation, tubers of intergeneric hybrids were divided and transplanted to new pots. Some hybrid plants, including those of Lit×Sau, Lit×Sph, Lit×Gsu, Lit×Gma, Sau×Gsu, Sau×Gma and Sph×Gma, produced flowers 2–5 years after cultivation in the greenhouse. We carried out horticultural characterization of these hybrid plants at the flowering stage (Table 1, Figure 1) (Amano et al. 2007b, 2008; Nakazawa et al. 2008).

### *Lit* × *Sau*

Three independent hybrid plants were examined. There were no apparent morphological differences among the 3 hybrids. All the hybrids produced flowers in mid-June, whereas Lit and Sau did in mid-June and early June, respectively. The hybrids generally resemble *Santonia* ‘Golden Lights’, a hybrid cultivar of Sau×Lit, in plant form and leaf and flower morphologies (Eason et al. 2001; Morgan et al. 2001, 2003; Clark et al. 2005; Burge et al. 2008). The hybrids showed a climbing habit and had sessile leaves with leaf tip tendrils. Hybrid flowers were pendulous and had a nearly intermediate morphology between the parents, but they were distinguishable from parental flowers. Tepals of hybrid flowers were fused for basal half of their length, whereas those of Lit and Sau were entirely separate and fused, respectively. The tip of tepals of hybrid flowers slightly curved outwardly. Flower color of the hybrids was vivid orange (JHS 1605) like those of the flower base of both parents. The hybrids had yellow, non-dehiscent anthers, which turned brown in senescent flowers.

### *Lit* × *Sph*

Two out of four independent hybrid plants have so far produced flowers and subjected to horticultural characterization. There were no apparent morphological differences between the 2 hybrids. Both hybrids produced flowers in late June, whereas Lit and Sph did in mid-June. The Lit×Sph hybrids generally resembled the Lit×Sau hybrids described above. However, leaves and flowers of the Lit×Sph hybrids were slightly larger than the Lit×Sau hybrids. In addition, flower color of the Lit×Sph hybrids (brilliant orange yellow; JHS 1905)



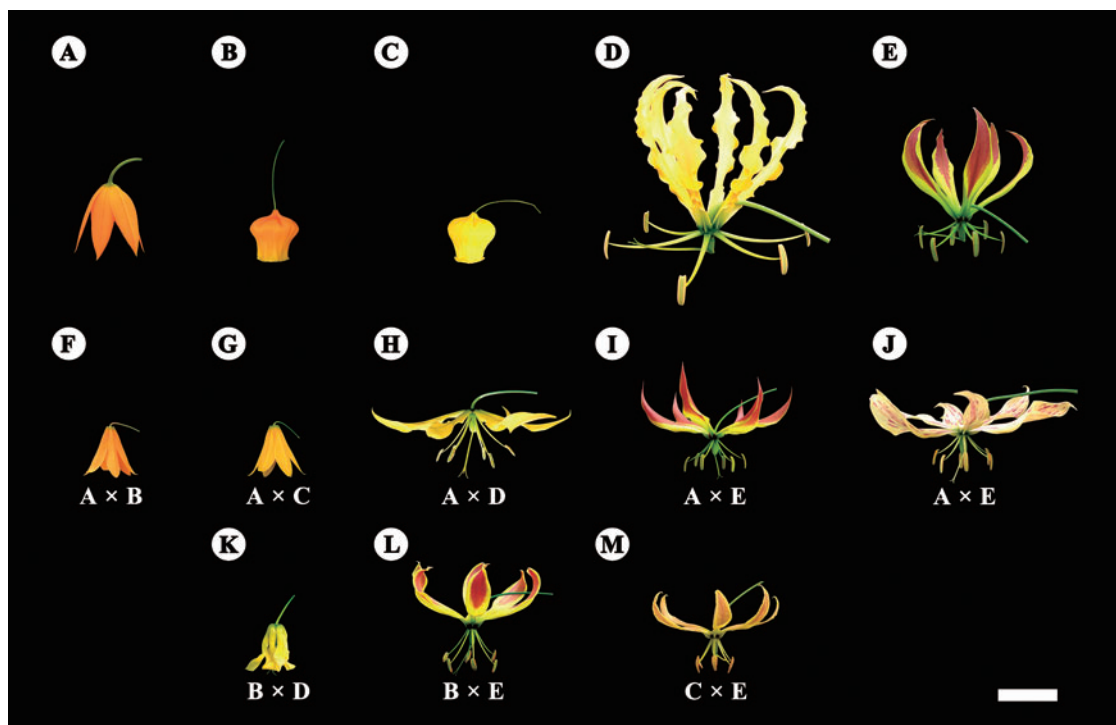


Figure 1. Flowers of intergeneric hybrids and their parents in colchicaceous ornamentals. (A) *Littonia modesta* (Lit). (B) *Sandersonia aurantiaca* (Sau). (C) *S. aurantiaca* 'Phoenix' (Sph). (D) *Gloriosa superba* 'Lutea' (Gsu). (E) *Gloriosa* 'Marron Gold' (Gma). (F) Lit×Sau. (G) Lit×Sph. (H) Lit×Gsu. (I) Lit×Gma-1. (J) Lit×Gma-2. (K) Sau×Gsu. (L) Sau×Gma. (M) Sph×Gma. Bar=3 cm.

was clearly different from that of the Lit×Sau hybrids. The hybrids had yellow, non-dehiscent anthers, which turned brown in senescent flowers.

#### *Lit*×*Gsu*

Three independent hybrid plants were examined. There were no apparent morphological differences among the 3 hybrids. All the hybrids produced flowers in late August, whereas Lit and Gsu did in mid-June and early July, respectively. The hybrids showed a climbing habit, and had a few vegetative shoots below the first flower and sessile leaves with leaf tip tendrils. Hybrid flowers were pendulous and had nearly horizontally expanded tepals, which were apparently distinguishable from parental flowers. Tepals of hybrid flowers were undulate and entirely separate like those of Gsu, but the tip of hybrid tepals was only slightly reflexed. Flower color of the hybrids was bright yellow (JHS 2505) like those of Gsu. Although all the hybrid plants had partly dehiscent anthers, their pollen fertility was only 2.4% as assessed with acetocarmine staining.

#### *Lit*×*Gma*

Two independent hybrid plants (Lit×Gma-1 and Lit×Gma-2), which showed apparently different morphologies, were examined. Hybrids produced flowers in late July (Lit×Gma-1) or late June (Lit×Gma-2), whereas Lit and Gma did in mid-June and early July,

respectively. The Lit×Gma-1 hybrid showed a climbing habit, and had a few vegetative shoots below the first flower and sessile leaves with leaf tip tendrils. Hybrid flowers were pendulous and had tepals moderately reflexed turning upward and backward, which were apparently distinguishable from parental flowers. Flower color of the Lit×Gma-1 hybrid was grayish red (JHS 0419) for the tepal center and brilliant greenish yellow (JHS 2704) for the tepal rim. Although the Lit×Gma-1 hybrid had dehiscent anthers, its pollen fertility was only 0.9% as assessed with acetocarmine staining. The Lit×Gma-2 hybrid also showed a climbing habit, and had a few vegetative shoots below the first flower and sessile leaves with leaf tip tendrils. However, plant height and leaf size of the Lit×Gma-2 hybrid were larger than those of the Lit×Gma-1 hybrid. The hybrid flowers were pendulous and had undulate and slightly reflexed tepals, which were clearly distinguishable from flowers of the parents and the Lit×Gma-1 hybrid. Flower color of the Lit×Gma-2 hybrid was light greenish yellow (JHS 2703) with strong reddish purple spots (JHS 8911). The Lit×Gma-2 hybrid had dehiscent anthers, and its pollen fertility was 7.0%.

#### *Sau*×*Gsu*

One out of six independent hybrid plants have so far produced flowers and subjected to horticultural characterization. This hybrid produced flowers in mid-

August, whereas Sau and Gsu did in early June and early July, respectively. The hybrid showed a climbing habit, and had a vegetative shoot below the first flower and sessile leaves with leaf tip tendrils. Hybrid flowers were pendulous and had an intermediate morphology between the parents, but they were clearly distinguishable from parental flowers. Tepals of hybrid flowers were undulate and fused at the base. Some tepals did not expand completely. Flower color of the hybrid was light yellow (JHS 2511). Although the hybrid had partly dehiscent anthers, its pollen grains were shrunken and not stained with acetocarmine.

### **Sau × Gma**

One out of thirteen independent hybrid plants have so far produced flowers and subjected to morphological characterization. This hybrid produced flowers in early July, whereas Sau and Gma did in early June and early July, respectively. The hybrid showed a climbing habit, and had a vegetative shoot below the first flower and sessile leaves with leaf tip tendrils. Hybrid flowers were pendulous and had slightly undulate and reflexed tepals like those of Gma, but they were apparently distinguishable from parental flowers. Flower color of the hybrid was grayish reddish brown (JHS 1016) for the tepal center and brilliant greenish yellow (JHS 2705) for the tepal rim. The hybrid had dehiscent anthers, and its pollen fertility was 8.3% as assessed with acetocarmine staining.

### **Sph × Gma**

One hybrid plant was examined. The hybrid produced flowers in late June, whereas Sph and Gma did in mid-June and early July, respectively. The Sph × Gma hybrid generally resembled the Sau × Gma hybrid described above. However, leaves of the Sph × Gma hybrid were larger than the Sau × Gma hybrid, whereas flowers of the Sph × Gma hybrid were smaller than the Sau × Gma hybrid. Flower color of the Sph × Gma hybrid was light reddish brown (JHS 1015) for the tepal center and brilliant greenish yellow (JHS 2704) for the tepal rim, which was slightly different from that of the Sau × Gma hybrid. Although the hybrid had dehiscent anthers, its pollen fertility was only 1.0% as assessed with acetocarmine staining.

## **Conclusion and prospects**

In our series of studies, a number of hybrids in various intergeneric combinations among colchicaceous ornamentals, *Gloriosa* spp., *L. modesta* and *S. aurantiaca*, were successfully obtained via ovule culture. Early confirmation of the hybridity of ovule culture-derived plantlets was accomplished by FCM in combination with RAPD analyses. Thus ovule culture

and hybrid identification techniques established in our series of studies may allow efficient cross-breeding in colchicaceous ornamentals.

An informal classification of Colchicaceae incorporating classifications by Nordenstam (1998) and Dahlgren et al. (1985) showed that *Gloriosa*, *Littonia* and *Sandersonia* were included in the tribe Iphigenieae (Vinnersten and Reeves 2003). Phylogenetic analysis of Colchicaceae by sequencing of 3 regions of plastid DNA revealed that *Gloriosa* and *Littonia* form a clade, whereas *Sandersonia* form a different clade with *Ornithoglossum* (Vinnersten and Reeves 2003). However, production of a number of intergeneric hybrids in our series of studies as well as in the previous studies (Morgan et al. 2001; Nakamura et al. 2005) strongly suggests that all the 3 genera, *Gloriosa*, *Littonia* and *Sandersonia*, are closely related to each other.

All the intergeneric hybrids examined in our series of studies were clearly distinguishable from the corresponding parents and had novel morphological characteristics, some of which were horticulturally attractive. The results obtained in our series of studies and the previous reports on production and characterization of *Santonia* 'Golden Lights', an intergeneric hybrid cultivar of Sau × Lit (Eason et al. 2001; Morgan et al. 2001, 2003; Clark et al. 2005; Burge et al. 2008) indicate that intergeneric hybridization may be an useful means for producing novel cultivars in colchicaceous ornamentals. Since some morphological characteristics were affected by growing environment, planting season and tuber size in *Santonia* 'Golden Lights' (Morgan et al. 2003; Clark et al. 2005), further characterization of our intergeneric hybrids should be performed using tubers with different sizes under various culture conditions.

Intergeneric hybrids obtained in our series of studies may be useable not only directly as a unique cut flower or a potted plant, but also as materials for further breeding of colchicaceous ornamentals. However, all the hybrids showed only poor or no pollen fertility as assessed with acetocarmine staining. In addition, no plants could be obtained after self-pollination and backcross-pollination of some intergeneric hybrids (unpublished). Therefore, it is necessary to restore fertility of intergeneric hybrids for further breeding. Since fertility restoration in interspecific hybrids is sometimes accomplished by artificial chromosome doubling of the hybrids (Van Tuyl and Lim 2003), we are now trying to induce chromosome doubling by chemical treatments of the intergeneric hybrids.

Since only very low multiplication rates could be obtained by traditional vegetative propagation via tubers in the intergeneric hybrids obtained in our series of studies, establishment of a rapid and efficient clonal propagation system is necessary for efficient breeding

and rapid development of cultivars. There have been some reports on *in vitro* propagation of *Gloriosa* and *Sandersonia* (Finnie and Staden 1989; Custers and Bergervoet 1994; Okumura *et al.* 2000; Jadhav and Hegde 2001; Sivakumar *et al.* 2003; Ghosh *et al.* 2007). We are now examining to develop a rapid and efficient propagation system in the produced intergeneric hybrids according to these reports.

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