Chilean native species from the Andes Mountains: an attractive source of germplasm for ornamental plant breeding

D. Aros[a], C. Rivas, M. Toledo, C. Céspedes, C. Cortés, M.A. Santander, M. Vásquez, N. Álvarez, A. Donoso and M.L. Prat

Faculty of Agricultural Sciences, University of Chile, Santiago, Chile.

Abstract

Chile presents a rich biodiversity of vascular plants with almost 5,000 native species, about 50% of them being endemic. Many of these species are distributed throughout the Andes Mountains showing high adaptation to adverse environmental conditions. Particularly some geophytes and perennial species from the Andes Mountains close to the central part of Chile (between 33 and 34°S) show an attractive ornamental value. This research aimed to identify and characterize these plants as a possible source of germplasm for ornamental plant breeding purposes. Field trips during four flowering seasons (2013 to 2016), between December and February, were performed. Seeds and explants were collected and different propagation and breeding techniques were performed. Considering color and size of the flowers, and growth habits, among other characteristics, five genera were identified as attractive to use for ornamental plant breeding purposes: *Alstroemeria* and *Pasithea* (geophytes), and *Junellia*, *Malesherbia* and *Salpiglossis* (herbaceous perennials). In vitro seed germination was described for *Alstroemeria pallida* and *Alstroemeria exserens* (cold stratification), *Malesherbia linearifolia* (stratification with 100 mg L^{-1} GA_3), *Pasithea coerulea* (scarification with H_2SO_4 and cold stratification at 4°C) and *Salpiglossis* (stratification with 150 mg L^{-1} GA_3). In vitro and ex vitro propagation of *Junellia spathulata* was successfully performed by using cuttings. Hybridization and polyploidization were applied in *Alstroemeria* spp., while mutagenesis using gamma rays was applied to in vitro callus of *Alstroemeria* spp. Limitation on water supply during flowering and resistance to extreme cold conditions during winter, are also interesting characters to consider these species as promising germplasm to develop for the ornamental plant market.

Keywords: mutagenesis, hybridization, winter hardy, alstroemeria, *Pasithea*, *Junellia*, *Malesherbia*

INTRODUCTION

Chile presents a rich biodiversity of vascular plants with almost 5,000 native species, about 50% of them being endemic, mainly due to the geographical isolation of this country produced by the Pacific Ocean and the Andes Mountains. There are more than 40 genera of geophyte plants, including about 180 monocot species, which are concentrated in the central zone of Chile (Marticorena, 1990). The particular environmental conditions and presence of high endemism make it a hotspot of biodiversity (Mittermeier et al., 1999).

Species distributed throughout the Andes Mountains show high adaptation to adverse environmental conditions such as frozen environment (Sakai and Larcher, 2012; Arroyo et al., 1981), high solar radiation, and seasonality (Körner, 2007). Some geophytes and herbaceous perennial species from the Andes Mountains close to the central part of Chile (between 33 and 34°S) show an attractive ornamental value. However, many of these species

[a]E-mail: daros@uchile.cl

have not been collected and used in breeding programs of ornamental plants.

Pasithea coerulea, a monotypic species of the family *Xanthorrhoeaceae*, is a geophyte native to Chile and Peru, with high ornamental value for its inflorescence with deep blue flowers (Hoffmann, 1998).

Alstroemeria (*Alstroemeriaceae*) is a well-known genus in the ornamental market and nowadays is one of the most important cut flowers in many countries (Lim et al., 2012). *Alstroemeria pallida* is a rhizomatous species with pink flowers, growing between 1,500 and 2,800 m altitude in the Andes Mountains of Central Chile (Cavieres et al., 1998).

Many cultivars of alstroemeria have been developed by interspecific hybridization (Bridgen et al., 2009), induced mutagenesis (Aros et al., 2012) and polyploidization (Lu and Bridgen, 1997). However, species from the Andes Mountains, like *A. pallida* and *Alstroemeria umbellata*, are not sufficiently explored as parental species for interspecific crosses. Moreover, the character of scent has not been traditionally considered in breeding programs of this species.

Malesherbia linearifolia or 'blue star of the Cordillera' belongs to the family *Passifloraceae* and is an endemic species with blue and purple flowers reaching up to 1 m tall (Hoffmann, 1998).

Salpiglossis sinuata belongs to the family *Solanaceae* and is described as a herbaceous perennial with a stem up to 80 cm, displaying dark blue or purple flowers (Navas, 2001).

Junellia spathulata belongs to the family *Verbenaceae*, native to Chile and Argentina and grows as a small shrub up 70 cm tall, showing blue and scented flowers (Peralta et al., 2008).

Considering the importance of conservation and sustainable use of native plants, this research aimed to identify, propagate and characterize native species from the Andes Mountains in Chile as possible source of germplasm for ornamental plant breeding purposes.

MATERIALS AND METHODS

Collection of plant material

Field trips to the Andes Mountains located in the Central Region of Chile (between 33-34°S) were performed during the last four seasons (2013 to 2016), between December and February. Seeds and explants were collected and different propagation and breeding techniques were performed.

Seed propagation

Considering the seed dormancy described for the species studied, different pre-germinative techniques, both stratification and scarification were applied for seed propagation of *Alstroemeria* spp., *Malesherbia linearifolia*, *Pasithea coerulea* and *Salpiglossis sinuata*. Germination under dark and light conditions was evaluated in *Salpiglossis sinuata.* Stratification considered different concentrations of gibberellic acid (0.0, 0.5, 1.0 and 1.5 mg L^{-1} GA_3) and cold stratification, while scarification considered application of sulfuric acid (H_2SO_4) and soaking with water (Table 1). Results were expressed as % germination after 8 weeks of evaluation.

Vegetative propagation

In vitro and ex vitro propagation of *Junellia spathulata* was performed by using cuttings and different concentrations of auxins (indolbutyric acid (IBA)) were evaluated. Moreover, different concentrations of cytokinin (benzyl aminopurine (BAP), 0.0, 0.5, 1.0 and 2.0 mg L^{-1}) were assessed for in vitro propagation of *Alstroemeria pallida* using rhizomes. The weight of the explants and % sprouting was evaluated after 8 weeks.

Table 1. Treatments of stratification (GA_3 and cold), scarification (H_2SO_4 and water) and growth conditions (dark and light) applied to seeds of four native species from the Andes Mountains.

Species	Stratification		Scarification		Growth conditions
	GA_3	Cold	H_2SO_4	Water	Dark/light
Alstroemeria spp.		x		x	x
Malesherbia linearifolia	x	x	x	x	
Pasithea coerulea		x	x	x	
Salpiglossis sinuata	x				x

Breeding techniques

Interspecific hybridization followed by in vitro rescue of embryos was performed using different genotypes of *Alstroemeria* spp., focusing the crosses between scented and non-scented individuals. Crosses have been performed during the last three seasons (2014 to 2016) and numbers of embryo germination, acclimation and flowering were recorded. Different concentrations of oryzalin (0, 5, 10 and 20 mg L^{-1}) were applied for 3 and 6 days on in vitro calli of *Alstroemeria* spp. to produce polyploidization. Moreover, gamma rays were applied on in vitro calli of alstroemeria hybrids to calculate the appropriate dosage for breeding purposes.

RESULTS AND DISCUSSION

Seed propagation

Malesherbia linearifolia was difficult to propagate through seeds and the maximum percentage of germination was only 11.48% by applying stratification with 100 mg L^{-1} GA_3. Scarification using sulfuric acid did not show positive results (1.11%).

Pasithea coerulea responded positively to scarification treatments performed with sulfuric acid and soaking with water, reaching a percentage of germination up to 93.3%. Stratification for 4 weeks at 4°C also showed an important effect reaching 69.3% germination, significantly different from the treatment without stratification (12.0%).

Salpiglossis sinuata reached a high germination rate when seeds were exposed to light (56.7%) in comparison with germination under dark conditions (36.3%). Moreover, stratification with 150 mg L^{-1} GA_3 was the best treatment in both dark and light conditions, reaching 50 and 85% germination, respectively (Figure 1).

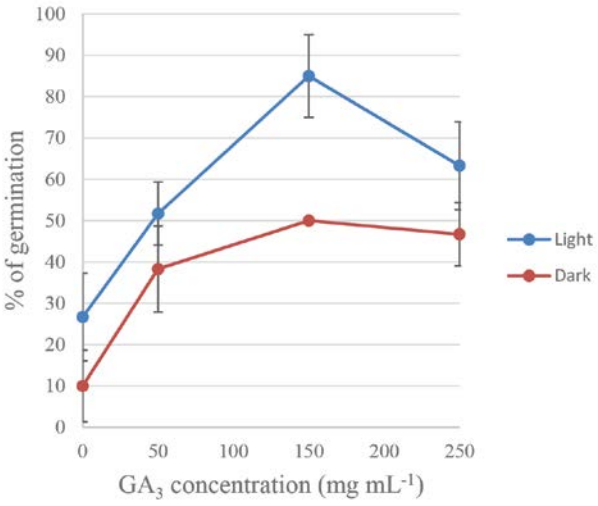

Figure 1. Percentage of germination observed in *Salpiglossis sinuata* seeds after the application of different concentrations of GA_3.

Alstroemeria pallida and *A. umbellata* seeds showed up to 26.7 and 61.7% germination, respectively, when using stratification (4 weeks at 13°C) and scarification (soaking with water).

Vegetative propagation

Application of IBA did not show an effect on the sprouting of *Junellia spathulata* cuttings cultured on substrate, which reached a sprouting of 44.7% and an average root length of 2.23 cm.

In vitro culture of *Alstroemeria pallida* rhizomes showed the highest weight of the explants after 8 weeks with the application of BAP at 2.0 mg L^{-1}, which was significantly different from the other treatments applied (Table 2).

Table 2. Increase in weight observed in *Alstroemeria pallida* rhizomes cultured in vitro with MS medium supplemented with different concentrations of BAP.

BAP (mg L^{-1})	Weight of explant (g)	
	0 week	8 weeks
0	1.06a	2.40b
0.5	1.11a	2.92b
1.0	1.05a	3.14b
2.0	1.26a	4.59a

Breeding techniques

A total of 262 crosses have been performed between different alstroemeria genotypes, resulting in 3,669 embryos rescued, 46 acclimated plants and 22 flowering plants during the last four seasons (2013-2016) (Table 3).

Table 3. Summary of data recorded for the cross-pollinations performed using different alstroemeria genotypes.

Season	Crosses	Rescued embryos	Germinated embryos	In vitro plants	Plants at greenhouse	Flowering plants
2013	28	420	60	72	22	12
2014	59	880	268	90	18	10
2015	102	1420	771	102	6	0
2016	73	949	262	72	0	0
Total	262	3669	1361	336	46	22

In vitro calli of alstroemeria hybrids cultured with 10 mg L^{-1} oryzalin for 3 days showed the most promising results and were able to regenerate through somatic embryogenesis. Confirmation of polyploidization has been performed using flow cytometry, confirming polyploidy of two alstroemeria lines.

CONCLUSIONS

Considering the germination rate, all the species showed better results when stratification methods were applied. Therefore, there is an endogenous dormancy developed by these species, probably due to the natural environment where they live. The breeding techniques described in this study have been successfully applied to different wild species of the Andes Mountains. Ornamental value of the new lines obtained must be evaluated to develop new cultivars for the ornamental market.

Apart from the ornamental value of the species characterized in this study, limitation on water supply during flowering and resistance to extreme cold conditions during winter, are also interesting characters to consider these species as promising germplasm to develop for the ornamental plant market.

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