# Genetic diversity of wild species and cultivated varieties of alstroemeria estimated through morphological descriptors and RAPD markers☆

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**Abstract**

In order to estimate the genetic diversity within the *Alstroemeriaceae* family, nine wild alstroemeria accessions, 10 commercial varieties and the monotype *Leontochir ovallei* were evaluated using two different methods, RAPD analysis and UPOVs morphological descriptors. DNA from leaves, roots and tepals were analyzed by RAPDs with eight primers that generated 236 RAPD bands. Dendrograms obtained allowed identification of five main clusters: *A. garaventae* alone, wild alstroemerias, commercial varieties, *A. exserens* and *A. spathulata* together, and *L. ovallei.* Twenty-five morphological descriptors related to stem, leaf and inflorescence characteristics were evaluated and a resulting dendrogram was analyzed containing two main clusters: one grouping all commercial varieties plus *A. magnifica* ssp. *magnifica* and the other one with the rest of the wild alstroemerias. With both methods, enough informative information data was obtained to place the wild alstroemerias and commercial varieties hierarchically in different clusters. In this respect, morphological analysis grouped all commercial varieties closer to each other. Also, morphological descriptors grouped all wild alstroemerias except *A. magnifica* ssp. *magnifica,* whereas RAPD markers grouped seven out of the nine wild alstroemerias, leaving in another cluster *A. garaventae, A. exserens* and *A. spathulata*. These results suggest that also in alstroemeria, RAPD markers are a useful tool for the protection of new releases from a breeding program.

*Keywords:* Alstroemeriaceae; Wild alstroemeria; Commercial varieties; Accession; Dendrogram and cluster

## 1. Introduction

The *Alstroemeriaceae* species have their origin in South America, with Chile and Brazil as the main diversity centers. This family includes three genera: *Alstroemeria* L., with about 60 described species; *Bomarea* Mirb., with about 100 species (Sanso and Hunziker, 1998) and the monotype genus *Leontochir* Phil. (Bayer, 1987; Aker and Healy, 1990).

All species are herbaceous, perennial and rhizomatous plants with big flowers, living in a wide range of habitats from rainy forest to desert areas and from the Andes Mountains to the coast (Muñoz and Moreira, 2003).

Alstroemeria varieties have been developed through interspecific hybridization (Burchi et al., 1997), selection of mutant sports and polyploidization (Broertjes and Verboom, 1974). Most of the new varieties have been obtained through interspecific crosses between Chilean genotypes (Han et al., 1999).

The taxonomic identification in the *Alstroemeria* genus is based on rhizome, stem, leaf, flower and fruit morphological characteristics. Furthermore, morphological characteristics have been employed to establish phylogeny of the *Alstroemeriaceae* family (Aagesen and Sanso, 2003). Nevertheless, such studies should be considered relatively subjective, because they depend on the morphological characteristics of a phenotype that can vary considerably in different environmental conditions (Bayer, 1987).

A complementary method is the use of molecular markers that allow genotype identification through DNA polymorphisms (Ferreira and Grattapaglia, 1998). Random amplified polymorphic DNA (RAPD) is a molecular marker based on the polymerase chain reaction (PCR) technique that has allowed genetic relationships among cultivated and wild *Alstroemeria* species (Dubouzet et al., 1998) and among alstroemeria

☆ *Leontochir ovallei* was not considered in this analysis because UPOV descriptors are specific for *Alstroemeria* genera.

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cultivars (Dubouzet et al., 1997) to be established. Also, this method has been used to detect natural and induced genetic variation in new alstroemeria varieties (Anastassopoulos and Keil, 1996). Burchi et al. (1997) considered RAPD analysis as a very effective tool for genotype identification and early detection of hybrids, useful for breeding programmes and breeder's rights protection purposes.

In order to discriminate among varieties and wild alstroemeria individual genotypes and estimate the genetic diversity within the *Alstroemeriaceae* family based on DNA polymorphisms and differences in morphological characteristics, RAPD analysis and UPOVs morphological descriptors were evaluated on nine wild alstroemeria accessions, 10 commercial varieties and the monotype *Leontochir ovallei*.

## 2. Materials and methods

### 2.1. Plant material

Ten wild *Alstroemeriaceae* accessions showed in Table 1 and the commercial varieties from Royal Van Zanten: ''Belinda'', ''Olga'', ''Victoria''; from Van Staaveren: ''Diamond'', ''Yellow Libelle'', ''Irena'' and ''Sacha''; from Könst: ''Cuba'', ''Jamaica'' and ''Tobago'' were evaluated. Wild accessions were maintained in a greenhouse of the Agronomic Sciences Faculty, University of Chile and varieties were obtained from a commercial flower field.

### 2.2. RAPD analysis

#### 2.2.1. DNA extraction

Young leaves, tuberous roots and tepals from greenhouse-grown wild accessions and tepals from vase-life flowers of varieties accessions were collected for DNA extraction. 0.5 g of roots and 0.1 g of tepals and leaves were used for extraction and the samples were treated following Lodhi et al. (1994).

In order to estimate purity and concentration of DNA in the extracts, UV absorbance was measured using a Shimadzu UV-1601 spectophotometer. Optical density at 260 and 280 nm was obtained and relatioships $A_{260}/A_{280} > 1.71 \pm 0.1$ were considered useful (Lodhi et al., 1994).

Table 1
Accessions and origin of wild *Alstroemeriaceae* species used for RAPD and morphological analysis

| | Latitude | Altitude (m) | Origin |
|---|---|---|---|
| *A. garaventae* Bayer | 32°58′S to 33°12′S | 1.200 to 2.000 | Chile |
| *A. diluta* ssp. *chrysantha* Bayer | 28°08′S to 33°05′S | 0 to 800 | Chile |
| *A. magnifica* Herbert ssp. *magnifica* | 29°26′S to 30°18′S | 0 to 200 | Chile |
| *A. pelegrina* L. | 31°49′S to 33°09′S | 0 to 50 | Chile |
| *A. exserens* Meyen | 33°18′S to 36°04′S | 1.900 to 2.700 | Chile |
| *A. spathulata* Presl | 32°20′S to 33°05′S | 2.200 to 3.100 | Chile |
| *A. ligtu* L. ssp. *ligtu* | 35°14′S to 37°50′S | 0 to 1.300 | Chile |
| *A. ligtu* ssp. *incarnata* Bayer | 34°50′S to 35°50′S | 1.000 to 2.000 | Chile |
| *A. psittacina* | No information | No information | Brazil |
| *L. ovallei* Phil | No information | No information | Chile |

#### 2.2.2. RAPD protocol

PCR was carried out in a 10 uL mix containing 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 4.0 mM $MgCl_2$, 0.8 mM dNTP mix, 0.5 mM primer, 1.25 U AmpliTaq DNA polymerase (Invitrogen), 4.15 μL de-ionized water and 25 ng DNA extract. Primers used were OPA 04 (5′-AATCGGGCTG-3′), OPA 08 (5′-GTGACGTAGG-3′), OPB 05 (5′-TGCGCCCTTC-3′), OPB 07 (5′-GGTGACGCAG-3′), OPF 05 (5′-CCGAATTCCC-3′), OPF 08 (5′-GGGATATCGG-3′), OPF 14 (5′-TGCTGCAGGT-3′) and OPF 17 (5′-AACCCGGGAA-3′) (Operon Industries, USA).

Thermal cycling was performed using a PTC-100 (MJ Research Inc.), and the amplification was conducted following the thermal profile: three cycles of 60 s at 95 °C, 60 s at 37 °C and 80 s at 72 °C; 37 cycles of 35 s at 94 °C, 40 s at 40 °C and 80 s at 72 °C; one cycle of 7 min at 72 °C.

Amplification products were analyzed by electrophoresis on 1.5% agarose gels for 3 h at 100 V and then visualized by ethidium bromide staining (Sambrook et al., 1989). The resulting RAPD bands profiles were photographed under UV illumination and the pictures were analyzed with ''Kodak 1D Image Analysis Software. Version 3.5'' software, employing −2 sensibility and 75% band capture, to score the clearest and most consistent bands for presence (1) or absence (0).

#### 2.2.3. Data analysis

A binary matrix was obtained to estimate the relationship between each pair of accessions and genetic distances (GD) values were calculated with ''PC-Ntsys. Versión 2.02'' software using the formula derived from the Dice similarity index. Finally, dendrograms were built through the unweighted pair group method using arithmetic averages (UPGMA) (Rohlf, 1993).

### 2.3. Morphological descriptors

#### 2.3.1. Evaluation

The International Union for the Protection of New Varieties of Plants (UPOV, 2003) is an organization that protects breeder's rights publishing a guide with morphological descriptors to conduct a new variety's registration test. In this study 22 UPOV morphological descriptors, and another three descriptors considered as informative were selected and evaluated on three to five plants per accession. Information obtained was compared to published data (Bayer, 1987; Muñoz and Moreira, 2003).

UPOV descriptors considered characteristics of length, thickness and density of foliage in the stem; length, width and shape of the leaf; number and length of branches in the umbel; length of pedicel; color, size and tepal spread of the flower; shape, depth of emargination and stripes of the outer and inner tepals; color and presence of spots in the filament. The other three descriptors were: size of the mucro of the outer tepals, overlap of inner tepals with respect to the

outer high tepal and number of stripes of the inner medium tepal.

### 2.3.2. Data analysis

The data were analyzed with ''SPSS 12.0 for Windows'' statistical software to obtain all phenotypic distances among accessions through squared euclidean distance coefficient and the information was recorded in a similarity matrix. A dendrogram was built by Hierarchical cluster analysis and furthest neighbor method.

## 3. Results and discussion

### 3.1. RAPD analysis

In total 236 polymorphic RAPD bands were generated with the eight primers used: OPA 04, OPA 08, OPB 05, OPB 07, OPF 05, OPF 08, OPF 14 and OPF 17 with 55, 31, 26, 15, 28, 18, 33 and 30 polymorphic RAPD bands, respectively. A disadvantage of the method is showed considering an average of 29.5 bands per primer is a very high number for RAPD analysis, where we cannot ensure that the anonymous bands obtained has certainly the same size, even more when distant genotypes are used, like in this research.

According to the genetic distances (GD) obtained and the relative position of each accession in the dendrogram, five clusters were identified: *A. garaventae* alone, wild alstroemerias, commercial varieties, *A. exserens* and *A. spathulata* together; *L. ovallei* (Fig. 1).

*A. garaventae* showed a GD of 0.58 from the closest group (wild and varieties) and 0.64 from *A. exserens* and *A. spathulata.* This species inhabits a restricted area of the Central Coast Mountains, between 32°58′S and 33°12′S (Muñoz and Moreira, 2003) (Table 1).

*A. diluta* ssp. *chrysantha, A. psittacina*, *A. ligtu* ssp. *ligtu, A. ligtu* ssp. *incarnata, A. magnifica* ssp. *magnifica* and *A. pelegrina*, were clustered in the wild alstroemeria group with a GD of 0.45 from the commercial varieties, except ''Jamaica'' and ''Sacha''.

The commercial varieties group (''Diamond'', ''Olga'', ''Belinda'', ''Cuba'', ''Yellow Libelle'', ''Victoria'', ''Tobago'' and ''Irena'') were consistently assigned to one cluster with a GD of 0.35 and 0.17 between them. This result supports the idea of a similar genetic base for all this group. ''Jamaica'' and ''Sacha'' however, are clustered in a subgroup insert in the wild group with a GD of 0.30 among them. The lowest GD observed among the commercial varieties was between Diamond and Irena (GD: 0.17), both these accessions belong to the same breeding program (Royal Van Zanten).

*L. ovallei* showed the largest GD value compared to the rest of the accessions (GD: 0.75). Han et al. (1999) in a study with AFLP markers, included *L. ovallei* and *B. salsilla* in an outgroup, showing large genetic distances (GD: 0.82) within the *Alstroemeriaceae* family. Furthermore, Muñoz and Moreira (2003) suggest that *L. ovallei* has been reproductively isolated in the coast of Atacama region, evolving to its present form. GD observed between wild alstroemerias and *L. ovallei*, reflects the wide genetic diversity within the *Alstroemeriaceae* family (GD: 0.04–0.69) and within the *Alstroemeria* genus (GD: 0.04–0.58).

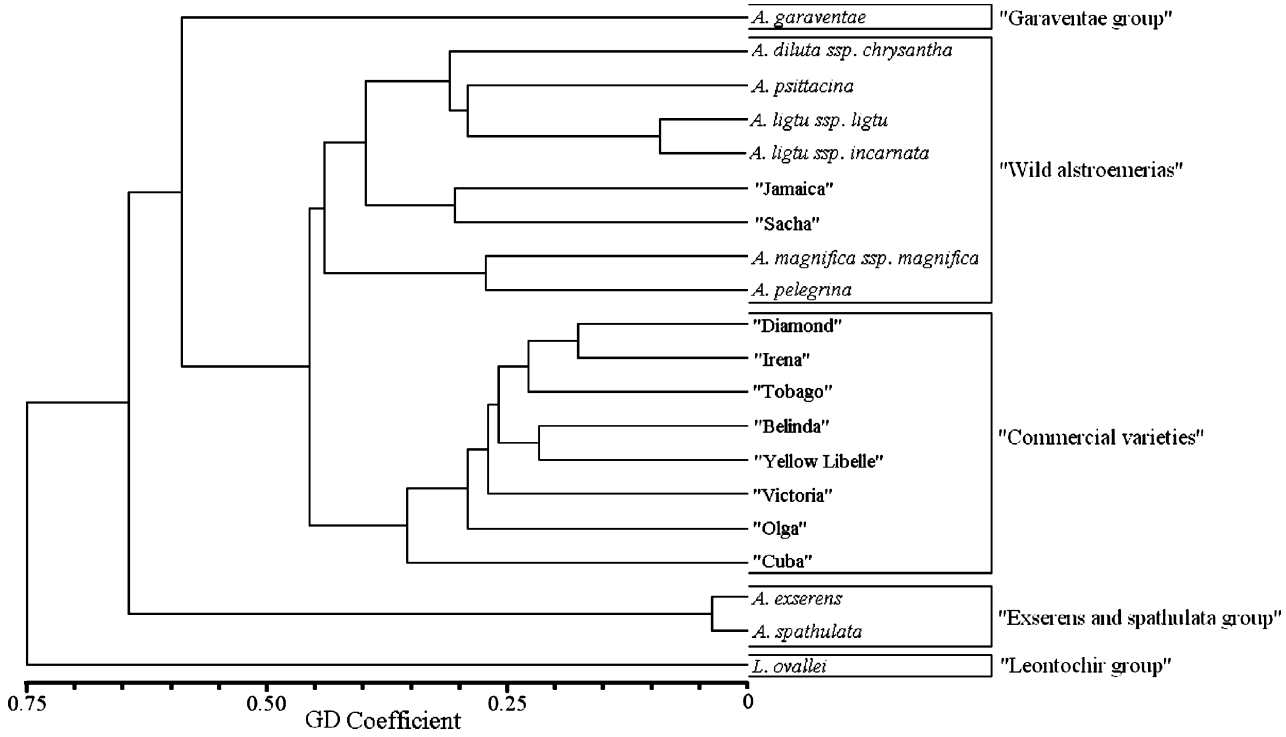


Fig. 1. Dendrogram of nine wild alstroemerias, 10 commercial varieties and *L. ovallei* resulting from a UPGMA cluster analysis based on Dice's genetic distances obtained from 236 RAPD bands.

*A. exserens* and *A. spathulata* showed the lowest GD (0.04) and were assigned as sister species in an isolated group from the rest of accessions. *A. exserens* is found between 33°18′S and 36°04′S and between 1.900 and 2.700 m. *A. spathulata* is found between 32°20′S and 33°05′S and between 2.200 and 3.100 m (Muñoz and Moreira, 2003). However, in this study both species were collected from populations growing together in ''Valle Nevado'' location, explaining perhaps their genetic similarity.

A close GD between *A. ligtu* ssp. *ligtu* and *A. ligtu* ssp. *incarnata* was observed (GD: 0.10) clustering these accessions in a subgroup within the wild alstroemeria group. Han et al. (1999) in their study identified the ''ligtu group'', including *A. ligtu* ssp. *simsii*, *A. ligtu* ssp. *incarnata*, *A. ligtu* ssp. *ligtu* and *A. haemantha,* showing a GD values between 0.38 and 0.50. Furthermore, the close relationship between *A. ligtu* ssp. *ligtu* and *A. ligtu* ssp. *incarnata* could be explained due to their taxonomical proximity.

*Alstroemeria psittacina* is a Brazilian species and Han et al. (1999) classified it together with other Brazilian species in a separate cluster from the Chilean species group, and Buitendijk and Ramana (1996) suggested that the Chilean and Brazilian species form distinct lineages. However, Dubouzet et al. (1998) placed the Brazilian species *A. inodora* and *A. brasiliensis* in the same group as the Chilean species *A. ligtu*. In this study, both *A. ligtu* ssp. *ligtu* and *A. ligtu* ssp. *incarnata* were the closest accessions to *A. psittacina,* thus this species could not be clustered in a separate group from Chilean species.

### 3.2. Morphological descriptors

The most variable descriptor was ''Flower: main color'' with eight different classes, identifying ''light pink'' as the most frequent color. ''Stem: length'' also showed wide variability with seven different classes.

Low polymorphism was observed between the commercial varieties, furthermore there were some monomorphic descriptors for all this group like ''Inflorescence: length of branches in umbel'' and ''Outer tepal: shape of blade''.

The wild alstroemeria group showed a high level of polymorphism but the descriptor ''Stamens: small spots on filament'' was monomorphic for all this group.

According to the morphological data obtained and the relative position of each accession in the dendrogram, two main clusters were identified: one grouping all commercial varieties and *A. magnifica* ssp. *magnifica* and the other eight remaining wild alstroemerias (Fig. 2).

The close relationship between *A. magnifica* ssp. *magnifica* and the commercial varieties group is supported mainly by the similarity of quantitative characters, like stem thickness and length; leaf length and width; inflorescence size, flower and tepal stripe. A clear distinction related to exuberant vigour of commercial varieties and *A. magnifica* ssp. *magnifica*, compared to wild alstroemerias.

Within the commercial varieties group, two subgroups were separated by *A. magnifica* ssp. *magnifica*, one included ''Belinda'', ''Yellow Libelle'', ''Diamond'' and ''Victoria'', with phenotypic distances (PD) values between 0.06 and 0.27.

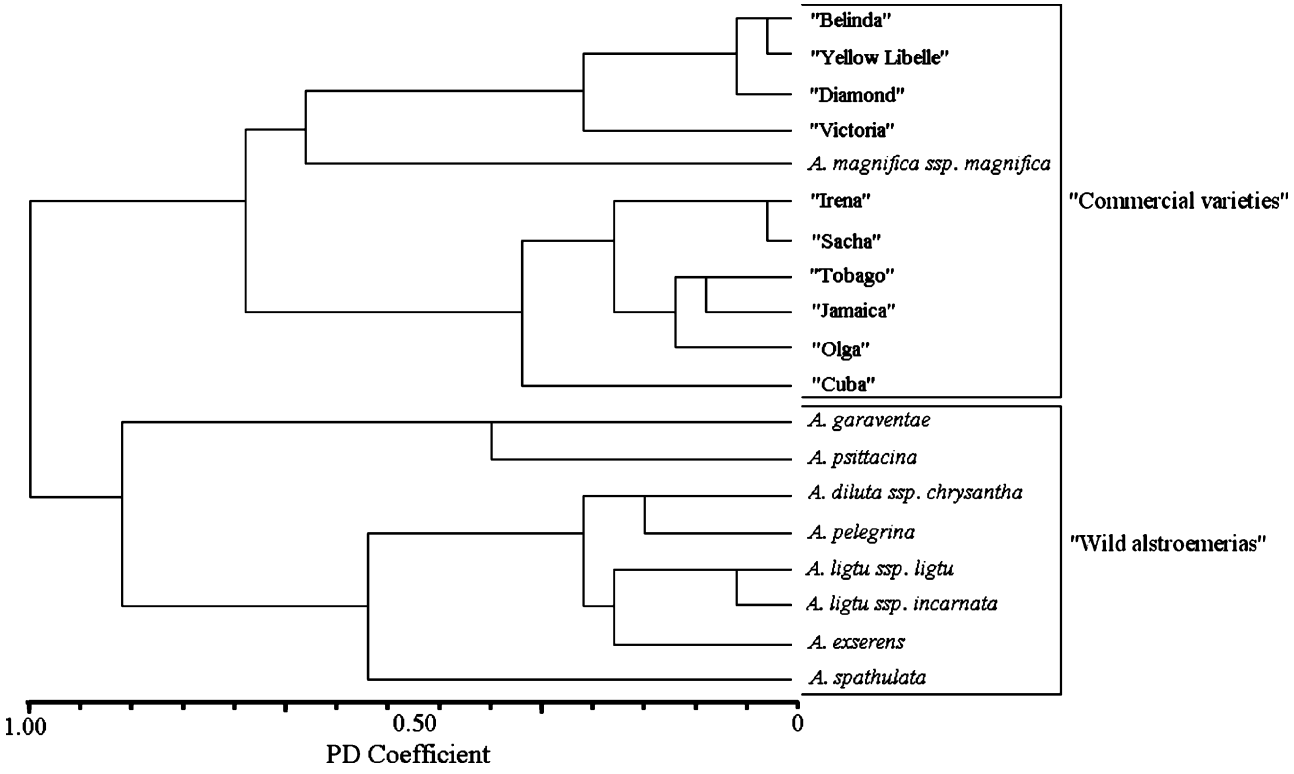


Fig. 2. Dendrogram of nine wild alstroemerias and 10 commercial varieties, based on a Hierarchical cluster analysis of 25 informative morphological descriptors.

The other subgroup included ''Irena'', ''Sacha'', ''Tobago'', ''Jamaica'', ''Olga'' and ''Cuba'' with PD values between 0.04 and 0.34.

The lowest PD was obtained between ''Belinda'' and ''Yellow Libelle'' (PD: 0.06) with 20 coincident descriptors. This finding agrees with the RAPD analysis that also grouped these varieties closely. ''Irena'' and ''Sacha'' were also much closer (PD: 0.04) with the morphological descriptors than with the RAPD analysis.

Among the wild alstroemerias group, *A. ligtu* ssp. *ligtu* and *A. ligtu* ssp. *incarnata* were the nearest accessions (PD: 0.06) sharing the same results for 17 descriptors and differing mainly in descriptors related to quantitative characters as flower and filament color. These results show that, RAPD and morphological analysis methods clustered correctly *A. ligtu* ssp. *ligtu* and *A. ligtu* ssp. *incarnata* as sister accessions, members of the same genus and species.

*A. garaventae* and *A. psittacina* were clustered in a subgroup because they were located further away from the rest of the wild alstroemerias (PD: 0.63), even when between them there was no closer PD (PD: 0.39). Thus neither method was able to place *A. psittacina* in a different group from the Chilean wild alstroemerias. However whereas RAPD analysis grouped *A. exserens* and *A. spathulata* much closer, morphological descriptors grouped them only with a PD value of 0.35 (Figs. 1 and 2). From the RAPD data *A. garaventae* looks very different from almost all the other alstroemerias, it branches off the dendrogram only just after *A. exserens* and *A. spathulata*, so it is placed quite differently in the RAPD compared to the morphological analysis.

## 4. Conclusions

Considering that RAPD markers allowed discrimination between all commercial varieties and wild species, fingerprints obtained are useful for the protection of new releases from a breeding program, particularly in vegetative propagated crops such as alstroemeria. Furthermore, these fingerprints could be used as an adequate tool for the identification of putative hybrids derived from interspecific crosses.

In general clusters observed in both dendrograms based on RAPD markers and morphological descriptors, fit expected results, with the exception of the Brazilian species which were located closer to the Chilean group than predicted from other studies (Han et al., 1999; Buitendijk and Ramana, 1996).

Using molecular markers, commercial varieties were clustered closer together than wild species, suggesting they share a relatively narrow and common genetic background. Furthermore this suggests that wild germplasm is still a rich source for breeding programs.

Commercial varieties were grouped through UPOV descriptors in a cluster with very similar morphological characteristics, belonging to a unique typology, suggesting commercial opportunities for breeding new alstroemeria types with different attributes.

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