**LIMONIUM BREEDING: NEW OPTIONS FOR A WELL KNOWN GENUS**

E.R. Morgan, G.K. Burge and J.F. Seelye  
New Zealand Institute for Crop & Food Research Ltd  
Private Bag 11 600, Palmerston North  
New Zealand  
morgane@crop.cri.nz

**Keywords:** Limonium perigrinum, L. purpuratum, L sinuatum, L. perezii, oryzalin, polyploidy, interspecific hybrid

**Abstract**

Limonium is a genus that is well known in international cut flower markets. There has been considerable investment in breeding new varieties over many years and the objective has been to try to incorporate traits from less well known species into new cultivars. Limonium perigrinum was hybridized with L. purpuratum and a plant with stems in excess of 60 cm selected from this cross for further development. This plant was later released under PVR as ‘Chorus Magenta’. Subsequent breeding has had two aims - to increase the flower color range, but maintain the form of ‘Chorus Magenta’, and to generate a range of Limonium hybrids for the potted plant market. There are four selections in trials as potted plants in New Zealand and Australia at present. Limonium perezii was hybridized with L. sinuatum using embryo rescue techniques. The hybrid plants were sterile. Use of spindle toxins resulted in a doubling of the nuclear DNA contents of the hybrids and a large proportion of the pollen staining with Alexander’s stain. Reciprocal crosses between the DNA tetraploids and their diploid parent species are producing embryos.

1. Introduction

Limonium is distributed in tropical and temperate zones throughout Europe, the Middle East, China, Japan, North and South America, and Australia. A small number of species are used in commerce, but opportunities exist to incorporate features from lesser-known species into new hybrids.

Embryo rescue or ovule culture techniques have been used to produce new cultivars of flower crops, e.g. Lilium (Van Tuyl et al., 1990, 1991) and Gypsophila (Kishi et al., 1994). A number of interspecific Limonium hybrids have been reported (Harada 1992) and there are two reports on embryo culture in Limonium (Morgan et al. 1995, 1998).

Limonium perigrinum and L. purpuratum were hybridized and the cultivar ‘Chorus Magenta’ was selected from the hybrids (Morgan et al., 1995, Seelye et al., 2000). The aim of these crosses had been to produce a long flower stemmed plant with an inflorescence similar in form to that of L. perigrinum. ‘Chorus Magenta’ has been granted PVR in New Zealand, Israel and the European Union with further applications for PVR pending.

Opportunities exist to produce Limonium as a flowering potted plant. Limonium has the advantage of maintaining flower color for a very long time because the colored calyces remain open after the flowers have senesced. Thus, even though only one or a few inflorescences may be produced, the plants retain their color for a very long time. We are investigating breeding potted plants of Limonium using L. perigrinum.

Limonium perezii and L. sinuatum were hybridized to produce a range of hybrid plants that were sterile and were very similar in form to L. sinuatum (Morgan et al., 1998). Several selections are currently in commercial trials but further progress in
breeding from these hybrids was dependent upon restoring fertility in these hybrids.

This paper reviews progress in breeding these different lines of Limonium and
describes recent results from the two breeding programs.

2. Materials and methods

2.1. Limonium perigrinum x L. purpuratum

Hybrids between Limonium perigrinum and L. purpuratum have been produced
and the characteristics of one of these plants, ‘Chorus Magenta’, described in detail by
Morgan et al. (1995) and Seelye et al. (2000). Plants of ‘Chorus Magenta’ and one of its
F1 siblings were crossed with L. perigrinum. Pollen sterility in ‘Chorus Magenta’ and its
siblings meant that L. perigrinum was used as the male parent. Approximately 140 plants
were produced then grown on for two years in pots and in the soil for evaluation.

2.2. Limonium perezi x L. sinuatum

Pollination of L. perezi with L. sinuatum gave rise to a number of sterile hybrid
plants through in ovulo embryo culture (Morgan et al., 1998).

Shoots from in vitro grown hybrid plantlets were proliferated on a modified MS
medium supplemented with 0.05 mg l⁻¹ IBA, 0.1 mg l⁻¹ GA3 and 0.3 mg l⁻¹ BAP as
described by Morgan et al., (1998). Shoots were transferred to a similar medium
supplemented with 15.5 mg l⁻¹ oryzalin for 14 days before transfer to proliferation
medium lacking oryzalin for further growth. After six weeks of growth shoots were
transferred to a root induction medium consisting of modified MS medium containing 1
mg l⁻¹ IBA (Morgan et al., 1998). Plantlets were deflasked after six weeks on this
medium. Plants were grown in pots in a greenhouse for approximately 6 months before
being tested for polyploidy by flow cytometry using an established protocol (Morgan et
al., 1998).

Plants identified as DNA tetraploids were grown on to flowering. Pollen staining
was assessed using Alexander stain (Alexander 1969). Pollen germination after transfer to
the stigma of a compatible L. perezi plant was assessed using the protocol of Kho and
Baer (1968).

Reciprocal crosses were carried out between L. perezi and the DNA tetraploid
hybrids with flowers being dissected to assess the success of pollination approximately
two weeks after the crosses were made. Embryos, within their ovules, were transferred to
in vitro culture using established protocols (Morgan et al., 1998) to ensure that plants
were produced from these crosses.

3. Results

3.1. Limonium perigrinum x L. purpuratum

Plants derived from the cross between ‘Chorus Magenta’ and its sibling, and L.
perigrinum were grown in greenhouses for 2 years to assess their potential as either a cut
flower crop or as potted plants. None of these plants had the stem lengths of ‘Chorus
Magenta’ (60 cm) with some being as short as 15 cm. There was considerable variation in
the form of the inflorescence of these plants though most tended to be more like the
compact form of L. perigrinum than the elongated inflorescence of ‘Chorus Magenta’.
There was a small number of plants with a L. perigrinum like inflorescence that when
grown from cuttings produced flowering plants with a total height from potting medium
to top of inflorescence of approximately 30 cm, with foliage to a height of 18-20 cm.
There was some variation in flower colour and one line with a particularly strong red/pink
colour has been retained for further breeding.
3.2. *Limonium perezii* x *L. sinuatum*

The diploid hybrids had a mean nuclear DNA content of 7.59 pg DNA (Morgan et al., 1998) and the DNA tetraploid hybrids had a nuclear DNA content of 16.3 pg DNA. A greater percentage of pollen from the DNA tetraploid hybrids stained with Alexander stain (approximately 82% staining red) than from the diploid form of these hybrids (less than 1% staining red). Pollen germination was also observed on the stigmata of compatible *L. perezii* plants brushed with anthers from the DNA tetraploid hybrid plants.

Embryos were produced from crosses between the DNA tetraploid hybrids and both *L. perezii* or *L. sinuatum*. Reciprocal crosses also gave rise to embryos.

4. Discussion

4.1. *Limonium perigrinum* x *L. purpuratum*

The development of *Limonium* as a potted plant provides another commercial opportunity for this genus. None of the plants produced had the stem lengths of ‘Chorus Magenta’ (60 cm) with some as short as 15 cm. The form of the inflorescence of these plants tended to be more like *L. perigrinum* than ‘Chorus Magenta’. There was some variation in flower colour and one promising form has been retained for further breeding.

Plants grown from cuttings can flower within 4 months producing small attractive plants that may be suitable as potted plants. One or two inflorescences usually develop soon after the flowers on the first inflorescence open. The time taken for the inflorescence to appear and the number of inflorescences that develop can be modified by the form of the cutting as well as environmental factors such as temperature. The potential of these hybrids for the potted plant market is being evaluated by companies in New Zealand, Australia and Israel.

4.2. *Limonium perezii* x *L. sinuatum*

The aim of this cross was to produce plants with the form of *L. perezii*, but the colour range of *L. sinuatum*. However, the phenotype obtained was difficult to distinguish from *L. sinuatum*. Additionally pollen staining was less than 1% with Alexander stain and all attempts to back cross the hybrids to the parents failed. The oryzalin treatments applied to the *in vitro* grown plants resulted in a number of plants with 2C nuclei contents approximately double that of the untreated plant material. The increase in nuclear DNA content coupled with the restoration of fertility suggests an increase in ploidy to the tetraploid state for these plants though this has not been confirmed with chromosome counts. The DNA tetraploid plants were difficult to distinguish from the diploid versions on the basis of morphological features such as internode lengths, leaf thickness, etc. and could only be identified using flow cytometry.

5. Conclusions

The development of *Limonium* varieties bred from *L. perigrinum* as potted plants offers potential new markets. However, progress from this point will depend on market response to the varieties currently on offer.

There is a number of diploid hybrids between *L. perezii* and *L. sinuatum* with three different colours, pink, blue and white, included in this group of plants. The restoration of fertility, the ability to produce embryos using the DNA tetraploids and the techniques for embryo rescue that are already developed mean that we are now well placed to move forward with breeding new forms of *L. perezii*. 
References