EXPLORATION OF *LIMONIUM* INTERSPECIFIC BREEDING POSSIBILITY

A thesis presented in partial fulfilment of
the requirement for the degree of Master
of science in plant science
at Massey University
Palmerston North
New Zealand

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1995

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Abstract

Interspecific crossability was investigated in the genus *Limonium* (Plumbaginaceae). Six *Limonium* species were chosen for this study, five of which are dimorphic and *L.perigrinum* which is monomorphic. Ovary, ovule and embryo development was investigated, as were *in vitro* pollen germination and pollen tube growth. Unilateral incompatibility was observed in 8 interspecific combinations. A high frequency of interspecific crossability was observed between *L.perezii* X *L.sinuatum* and *L.sinense* X *L.aureum*. Pollen tubes were frequently observed penetrating the ovules in these crosses. Pollen tube growth that terminated in the styles or was restricted to the stigmas was found in some *Limonium* interspecific crosses. Abnormalities of pollen tube growth in the interspecific crosses included heavy callose deposits at the tips of pollen tubes; pollen tube branching and pollen tube growing in the wrong direction.

Embryo, ovule and ovary development was studied with *L.perezii* plants following conspecific pollination. Three distinct groupings of florets can be recognised at the basis of their post-pollination growth and development. Twenty-six percent of conspecific pollinated florets showed no ovary and ovule growth. No embryo was found in this group. In eleven percent of florets, ovaries and ovules grew up to Day 12 after pollination and then shrivelled. No embryo was ever found in this group. Sixty-three percent florets produced embryos following conspecific pollination and developed normally.

The viability of *Limonium* pollen was assessed with Alexander's stain and fluorochromatic reaction (FCR) stain.

Optimal conditions for *in vitro L.perezii* pollen germination and tube growth were established. Poly-ethylene glycol and filter paper supports were of particular significance. *In vitro* pollen germination rate of about 40% was achieved. Plant growth regulators (IAA, GA_3 and ethylene), some minerals (manganese sulphate, copper sulphate) and prehydration treatment were used in experiments to improve pollen germination and tube growth. None of these factors, however, had positive effect on

either pollen germination or tube growth. It was found that while *L.perezii* pollen tube growth tolerates a wide range of temperature, there is an optimum between 20°C-25°C.

ACKNOWLEDGEMENTS

I am most grateful to my supervisor, Associate Professor D.W. Fountain for his excellent guidance, constructive criticism and patience while I was carrying out experiments and writing my thesis.

I wish also to offer my special thanks to Ed Morgan for his advice and extensive help with experimental work. I appreciate the suggestions he has made in the course of the writing of this thesis.

My sincere thanks are also extended to all the staff in the Plant Biology and Biotechnology Department for their help throughout the years of my study and to the staff in the Plant Growth Unit of Massey University for the work they performed in the glasshouse to maintain the necessary conditions for my work.

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GENERAL INTRODUCTION

The genus *Limonium* belongs to the family Plumbaginaceae. According to Baker(1953b), there are more than 150 *Limonium* species. *Limonium* species are believed to originate from several regions of the northern hemisphere. The overall distribution of the genus embraces all five continents (Baker 1953b). *Limonium* was formerly called *Statice*, but in 1947 Lawrence' proposal was approved at the International Botany Conference and the generic name was changed to *Limonium* (Tsurushima 1992a).

Most *Limonium* species are perennial herbs or shrubs, sometimes woody at the stem base, although there are also some annuals (Huxley *et al.* 1992). Apart from species which become upright with an elongate stem, most *Limonium* species produce a circular cluster of leaves and exhibit a rosette form. The inflorescences of *Limonium* are mostly corymbose panicles or spikes (Huxley *et al.* 1992). *Limonium* species can be divided into two types: seasonal and free flowering types on the basis of their flowering patterns. Seasonally flowering species mainly flower during summer, while the flowering of free flowering species is influenced by temperature. The free flowering species will keep producing flowers throughout the year if temperature and light are favourable (Harada 1992). Flowers of *Limonium* possess a tubular calyx and five petals united only at the base. Both sepals and corolla are small, most flowers are only 3-4mm in diameter. The flowers of *L.perigrinum* are considered the largest, being 15-16mm in diameter (Tsurushima 1992a). The sepals of *Limonium* flowers often have as bright a colour as the corolla has and often persist to fruiting after the petals have senesced, so, when flower colour is described, it often means the colour of the sepal. Many *Limonium* species are presently grown commercially around the world for use as cut flowers. The unique branching inflorescences of *Limonium* are perfect as fillers for bouquets, corsages, baskets and other flower arrangements.

Limonium crops have a fast growing market in many countries. In Japan in 1985 the area under cultivation nationwide was 188 hectares. This had increased to 280 hectares by 1988 and it is now estimated to be approaching 400 hectares (Tsurushima 1992a). It should be pointed out that the most widely grown *Limonium* species for cut flowers is *L.sinuatum*. It makes up about 80% of the Limonium under cultivation in the world.

The remaining 20% is made up of other species(Tsurushima 1992a). To cope with a rapid increase in market demand for new and novel flowers, generation of interspecific hybrids has become a more popular strategy for producing new forms and colours. The first marketed interspecific *Limonium* hybrid was "Misty Blue" which was released by Dainchi Engei company in 1984. This hybrid was from a cross between *L.latifolium* and *L.caspia* (Tsurushima 1992b). "Lemon Star" is a yellow interspecific hybrid of *L.aureum* and *L.sinense.* " Charm Blue" is a hybrid grown from seedlings obtained from a cross between *L.latifolium* and *L.gmelinii* (Harada 1992). The significance of interspecific breeding is that it provides a way to combine the respective merits possessed by different species into a hybrid. Some objectives in breeding programs may include increasing the range of flower colours, increasing flower size, increasing stem length long stems and abundant flower production. In addition, features including year round production, heat resistance of plants, cold resistance, disease resistance and good water uptake are also the targets for breeders (Tsurushima 1992a).

Interspecific hybridization often relies on techniques such: *in vitro* techniques as embryo rescue, ovule and ovary culture, isolation and fusion of protoplasts. "Blue Star" was obtained by culturing embryos produced by hybridization between *L.perezii* and *L.sinense* (Tsurushima 1992b). Recently a long stemmed form of *L.perigrinum* was produced by hybridization with *L.purpuratum* (Morgan *et al.* in press).

A successful interspecific hybridization program not only relies on biotechnological techniques but also on the knowledge of morphological features of the sexual reproductive organ, flowers; the understanding of physiological features of the reproduction process; and the understanding of pollen behaviour in interspecific crosses. The main purpose of this study is to explore the possibilities of producing hybrids between several *Limonium* species. Six *Limonium* species were chosen for this present study. They were

L. perigrinum (Bergius)

L. sinuatum (L.) Mill

L. perezii (Stapt)

L. aureum (L.) Hill

L. sinense (Girard) Kuntze

L. caspia (*L.bellidifolium*)

The objectives of this work were:

(1). To obtain knowledge about pollen behaviour on the stigmas and in the styles of related species.

(2). To determine the barriers to interspecific crosses

(3). To collect data for ovary, ovule and embryo growth in intraspecific crosses

(4). To assess techniques for testing *Limonium* pollen "viability"

(5). To investigate *Limonium* pollen germination *in vitro*

(6). To obtain knowledge relevant to taxonomic relationships and mechanisms of breeding incongruities