Opportunities to Introduce New Traits With Wide Crosses©

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INTRODUCTION

Interspecific hybridisation is the most important source for introducing new characteristics to many ornamental crops. For example, there are over 1500 begonia species, many of which have been hybridised to produce the wide range of taxa we see today. Similarly the current range of summer-flowering *Zantedeschia* cultivars, with their diverse colours and forms, are based on hybridisation between five species (Funnell, 1993).

Opportunities for interspecific hybridisation may be limited by many barriers but there are a number of strategies available to assist in overcoming these barriers. These breeding barriers include:

- Pollination barriers.
  - Difference in length of style between two species.
  - Inhibition of pollen germination on the stigma.
  - Inhibition of pollen tube elongation down the style.
- Embryo abortion.
- Sterile F1 hybrids or lack of introgression due to chromosomal "incompatibilities."
- Albino hybrid plants.
  - Plastome-genome incompatibility.
- Hybrid necrosis.

Observation of the fate of flowers following pollination with self or foreign pollen, and developing an understanding of the pollination and post-pollination floral biology of the plants being worked with, are important early steps in a wide crossing programme (e.g., Burge and Morgan, 1993).

TECHNIQUES TO OVERCOME BREEDING BARRIERS

Techniques have been developed to overcome many of the barriers to sexual hybridisation (Burge et al., 1995; Morgan 2004; Morgan et al., 1995, 1998, 2001b, 2006; van Tuyl, 1997; van Tuyl et al., 1991). This includes a range of techniques to overcome or bypass pollination barriers.

Pollination Barriers. There many different barriers to interspecific pollination, but those that are more difficult to overcome are related to pollen-stigma interactions at pollination. Stigmatal and stylar barriers have been successfully circumvented by the cut-style technique, in which the stigma and part of the style is removed, and subsequently pollen is applied on the end of the cut style (van Tuyl, 1997). An improvement on this technique has been the grafted-style technique. With this technique, pollen is applied to the stigma of the same species, and after about 1 day the style of this pollen donor is cut (usually close to the ovary) and grafted onto the ovary of a related species to allow growth of the pollen tubes to the ovule and fertilisation of the related species.

There may also be physical barriers, e.g., the style of one species may be considerably longer than that of related species, e.g., *Gloriosa* styles are much longer than those of *Sandersonia*, and we were not successful at producing hybrids when *Gloriosa* was the female parent even though pollen germination occurred (Morgan et al., 2006).

Embryo Abortion. In the event that pollen tubes reach the ovary, fertilisation occurs, giving rise to the hybrid embryo. However, the embryos of many interspecific hybrids abort prior to seed maturation because of a lack of endosperm development. This may be circumvented by ovary, ovule, or embryo culture. Ovary and ovule culture are used when the embryos abort very early in development or for species with very small embryos, whereas embryo rescue is generally used when the abortion occurs later. The ovaries, ovules, or embryos are placed on a medium which supplies the essential ingredients for continued growth and development of the embryos (Morgan et al., 1995, 1998, 2001b, 2003, 2006; van Tuyl et al., 1991; van Tuyl, 1997).

Sterile Hybrid Plants. Many interspecific hybrids are sterile, which restricts subsequent breeding and development of superior hybrid varieties. Fertility of interspecific hybrids can often be restored by doubling chromosome numbers (Morgan et al., 2001a). However, the resulting plants are tetraploid and may be difficult to include in the breeding programme later.

Incompatibilities. Genetic incompatibility can occur with interspecific hybrids. This includes the formation of albino plants due to plastome-genome incompatibility (Yao and Cohen, 2000). Albino hybrids cannot be grown out of culture and so these hybrids cannot be used in breeding programmes. Similarly, hybrid necrosis — if severe — can prevent plants from flowering and so restrict the use of these hybrids in an ongoing breeding programme (Bomblies and Weigel, 2007).

NEW HYBRIDS AND CULTIVARS

We have successfully developed a range of interspecific hybrids using techniques to overcome pre- and post-fertilisation barriers. This includes *Limonium perigrinum* × *L. purpuratum* hybrids. Stem length of the hybrid plants varied from 40 to 70 cm (Morgan et al., 1995) and one of the longer stemmed plants was developed as the cut flower cultivar Chorus Magenta (Seelye et al., 2000). An interspecific hybrid between *L. perezii* and *L. sinuatum* was the start of a breeding programme to produce novel statice cultivars (Morgan et al., 1998, 2001a). Fertility of the F1 hybrid was restored by doubling the chromosome number and this has been followed by four backcross generations in our ongoing *Limonium* breeding programme. Embryo rescue techniques have also been used to produce novel *Sandersonia* intergeneric hybrids (Morgan et al., 2001b, 2002, 2006) and *Gentiana* hybrids (Morgan, 2004; Eason et al., 2007). A number of gentian varieties have been released with novel flower colours (Eason et al., 2007).

Confirmation of Hybrids. Following the production of a putative hybrid, breeders usually like to confirm that the plant is a true hybrid, because pollen contamination may occur if insufficient care is taken with emasculation. Identifying and discarding plants that are not hybrids as early as possible avoids the costs of growing-on plants that are of no value. A range of molecular markers are now being used to confirm interspecific hybrids and to monitor introgression (van Tuyl et al., 2002). We commonly use flow cytometry to confirm that plants are hybrids (Morgan 2004;

Morgan et al., 1995, 1998, 2001b) or that we have produced polyploid plants (Morgan et al., 2003, 2004). Flow cytometry measures the nuclear DNA content of plant cells. The cells of each species have a characteristic amount of DNA and the nuclear DNA content of an interspecific hybrid is usually intermediate between the two parents. Therefore, by measuring the DNA content of each parent and the putative hybrid it is possible to confirm that a plant is a hybrid provided that the two parents have different nuclear DNA contents. Where the parent species have similar nuclear DNA contents, a range of molecular techniques is available to look for the presence of genetic material from both parents. Random amplification of polymorphic DNA (RAPD) is a technique commonly used by plant breeders to ensure that putative hybrid plants contain genetic material from both parents.

CONCLUSIONS

Sexual hybridisation has been the main method for introducing novel traits into ornamental crops. This is likely to continue as our understanding of breeding barriers improves and as we develop improved techniques to overcome these barriers. Molecular tools are now an important part of breeding by sexual hybridisation, through their use for early confirmation of interspecific hybrids.

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