INTERSPECIFIC HYBRIDIZATION OF FLOWER BULBS: A REVIEW

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Abstract

Interspecific and intergeneric crosses have been made to introduce new genetic variation into cultivated plants. For flower bulbs, interspecific hybridization is the most important source of genetic variation. Many of the cultivars have originated from complex species crosses which have given rise to a broad range of shapes and colours to plants and flowers (e.g., *Alstroemeria, Gladiolus, Hippeastrum, Lilium, Narcissus* and *Tulipa*).

Crossing barriers frequently occur when interspecific crosses are attempted. Sexual barriers preventing interspecific hybridization have been distinguished into pre- and post-fertilization barriers. The nature of the barrier determines the method to be used to overcome the specific barrier. A range of techniques such as cut-style pollination, the use of mentor pollen and grafting of the style have been applied successfully to overcome pre-fertilization barriers.

Lilium has been used as a model crop for the development of in vitro methods. Ovary, ovary slice, ovule and embryo culture are being exploited to overcome post-fertilization blocks which cause endosperm failure and embryo abortion. An integrated approach of *in vitro* pollination and fertilization followed by embryo rescue has been applied in many crosses.

Barriers occurring after a successful embryo rescue are hybrid breakdown (e.g., hybrid albinism in *Zantedeschia*, Yao *et al.*, 1995) and F1-sterility. Hybrid breakdown results in the loss of the hybrid before flowering and is a result of the unbalanced new genome combinations. F1-sterility of interspecific hybrids is very common and among others may be the consequence of reduced chromosome pairing during meiosis. In this case fertility can be restored by polyploidization, enabling pairing of homologous chromosomes in the allopolyploid hybrid. Breeding at polyploid levels is widely used in interspecific hybridization programmes of many bulb crops such as *Alstroemeria, Freesia, Gladiolus, Lilium*. Application of 2n-gametes (meiotic polyploidization) in

interspecific breeding programmes may be of great importance for the introgression of characters from diploids to tetraploids.

1. Introduction

The phenomena underlying crossing barriers are incompatibility and incongruity. Incompatibility operates in intraspecific crosses and is the result of the activity of S-alleles. Incongruity occurs in interspecific crosses as a result of a lack of genetic information in one partner to complete pre- and post-pollination processes in the other (Hogenboom, 1973). This review focuses on different methods used to overcome incongruity.

In breeding programmes for ornamental bulb crops, interspecific and intergeneric hybridization is the most important procedure for the introduction of genetic variation. Many of the cultivars have originated from complex species crosses which have given rise to a broad range of shapes and colours to plants and flowers (Ohri and Khoshoo, 1983ab; Van Eijk *et al.*, 1991; Van Creij *et al.* 1993). Many examples in this article are chosen from *Lilium*, because lily is used frequently as a model plant for style manipulations and *in vitro* culture methods (for more detailed information see Van Tuyl and De Jeu, 1996). Reviews of the entire field of reproductive barriers are written by Frankel and Galun (1977), Shivanna (1982), Williams *et al.* (1987) and Liedl and Anderson (1993).

2. Methods for Overcoming Interspecific Crossing Barriers

The sexual barriers hampering interspecific hybridization have been distinguished into pre-fertilization and post-fertilization barriers (Stebbins 1958). Many studies deal with methods for overcoming pre-fertilization barriers. Once fertilization has occurred, hybrid embryo growth may be restricted by post-fertilization barriers. Both embryo and endosperm have to develop an equilibrium for sharing nutrients in an undisturbed developmental process. In general the first division of the zygote is delayed to favour the first division cycles of the endosperm cells. When the equilibrium in the development of the zygote and endosperm is disturbed an abortion of the young embryo or disintegration of endosperm follows. This abortion can take place in various stages of development of the young seed. Depending on the stage of embryo abortion various *in vitro* techniques can be applied to rescue the abortive embryo.

2.1 Techniques for overcoming pre-fertilization barriers

2.1.1 Genetic variation in interspecific crossability

'The statement, that two species are not crossable, is controversial unless a broad genetic variation of the parental species has been used and the cross combinations have been carried out on a large scale under a wide range of environmental conditions'. This quotation of Hermsen (1984) implies that crossability is determined both by genetic and environmental factors. It is therefore necessary to test different accessions of both the parents for hybridization programs (Van Eijk *et al.* 1991). Unilateral incongruity is the phenomenon that a cross is successful in only one direction, whereas the reciprocal cross fails. In lily, crossing barriers can be overcome using cut-style pollination, but mostly in one cross direction only (Van Creij *et al.* 1993).

2.1.2 Use of mixed and mentor pollen

The use of mixed pollen i.e., mixture of compatible and incongruous pollen (Kunishige and Hirata, 1978) and mentor pollen, i.e., compatible pollen genetically inactivated by irradiation but still capable to pollen tube growth used together with incongruous pollen, is reported to overcome inhibition in the style in many plant species. For lily, mentor pollen was effective in overcoming self incompatibility but not in interspecific crosses (Van Tuyl *et al.* 1982).

2.1.3 Influence of environmental conditions

A positive effect of high temperature in overcoming incongruity has been detected and applied in breeding programmes of lily by pollinating at high temperatures (Van Tuyl *et al.* 1982; Okazaki and Murakami, 1992). Probably heat-sensitive inhibitors of pollen tube growth are inactivated. Comparable effects of floral aging on pollen tube growth are reported by Ascher and Peloquin (1966).

2.1.4 Style and ovary manipulations

Pollen tube growth inhibition in the style can be overcome using different pollination techniques in which style and ovary are manipulated (*Fritillaria*: Wietsma *et al.* 1994; *Lilium*: Myodo 1963; Van Tuyl *et al.* 1988, 1991; Janson *et al.* 1993). One of these manipulations involves removal of the stigma and a part or whole of the style and subsequently pollination of the cut end. This is referred as stump pollination, 'cut-style' or 'amputated-style' pollination. In a comparison of several pollination methods, it was shown that pre-fertilization barriers in lily can be circumvented by using the cut-style technique (Van Tuyl *et al.* 1991; Janson *et al.* 1993). Following this technique, many pollen tubes of e.g., lily and *Fritillaria* grow normally into the ovary. In this way pollen circumvents stylar and stigmatal barriers. However, a complication associated with this method in lily is the low seed set, probably caused by the premature arrival of pollen tubes in the ovary (Janson *et al.* 1993). A majority of the pollen tubes either did grow past the inner integument or did grow along, but not into the micropyle after cut-style pollination. Despite the low seed set, a large number of unique interspecific lily hybrids was obtained using this method (Asano and Myodo 1977ab; Asano 1980; Okazaki *et al.*, 1992, 1994; Van Creij *et al.*, 1993). Also Wietsma et al. (1994) were able to obtain interspecific hybrids using the cut-style technique in crosses between *Fritillaria imperialis* and *F. raddeana.*

The grafted style technique was applied successfully in order to improve the cut-style technique. (Van Tuyl *et al.*,1991). In this method, pollen grains are deposited on a compatible stigma. After one day, the style of the pollen donor is cut 1-2 mm above the ovary and grafted on the ovary of another incongruent plant. Style and stigma are joined *in vivo* using a piece of a straw filled with *L. longiflorum* stigmatic exudate or are stuck together with only the exudate. *In vitro* a piece of 'water agar' is placed on the style.

2.1.5 Chemical treatments

Application of growth regulators, such as auxins, cytokinins and gibberellins to the pedicel or the ovary at the time of or soon after pollination, may improve fruit and seed set after interspecific crosses of lily and tulip (Emsweller and Stuart 1948; Van Creij *et al.,* 1996 this Acta).

2.2 Techniques for overcoming post-fertilization barriers

A range of *in vitro* methods has been developed to overcome post-fertilization barriers in a number of plant species.

2.2.1 Ovary culture and ovary-slice culture

Ovary culture has been applied in many species: *Lilium, Nerine* and *Tulipa,* (Van Tuyl *et al.* 1990; Van Creij *et al.* 1996b). Ovary-slice culture was applied by Kanoh *et al.* (1988), and Van Tuyl *et al.* (1991) for the production of interspecific *Lilium* hybrids. Ovaries were harvested 7-40 days after cut-style pollination and, after surface sterilising, sliced into 2 mm thick disks. Seed germination occurred 30-150 days after pollination. By this method plantlets were obtained from very small embryos.

2.2.2 Ovule culture

In those crops in which the fruit is aborted before embryo culture can be applied, ovule culture is an easy and fast method. This technique is applied in *Alstroemeria* (Bridgen *et al.* 1989, Buitendijk *et al.* 1995; De Jeu and Jacobsen, 1996), lily and *Nerine* (Van Tuyl *et al.* 1990) and tulip (Van Tuyl *et al.* 1993, Van Creij *et al.* 1996b).

2.2.3 Embryo culture

Embryo culture can be applied successfully in crosses in which pollinated flowers can stay on the plant for a notable time. In most cases embryos can be rescued when the globular stage is reached. This method has been applied in a large number of crops. Some examples in flower bulbs are: *Allium* (Nomura and Oosawa 1990), *Alstroemeria* (Buitendijk *et al.* 1992), *Freesia* (Reiser and Ziessler 1989), *Hippeastrum* (Bell, 1972), *Lilium* (Van Tuyl *et al.* 1991), *Tulipa* (Custers *et al.* 1995) and *Zantedeschia* (Yao, *et al.* 1995).

2.2.4 Integrated techniques for overcoming pre- and post-fertilization barriers

In many interspecific and intergeneric crosses, integrated techniques to manipulate both pre- and post-fertilization barriers have been applied. *In vitro* pollination and fertilization is one such technique (Zenkteler, 1990). Unlike the other techniques, which retain the zone of inhibition (stigma and style) and manipulate pollen germination and pollen tube growth to overcome pre-fertilization barriers, *in vitro* pollination brings pollen grains in direct contact with the ovules, and is, therefore, considered more effective.

In *Lilium* various combinations of *in vitro* pollination (cut-style and grafted-style method) and embryo rescue (ovary, ovule and embryo culture, placental pollination), were applied in order to control the whole fertilization process (Janson 1993; Van Tuyl *et al.* 1991). This resulted into a range of new interspecific hybrids (Van Creij *et al.* 1993; Van Tuyl *et al.* 1996 this Acta). Similar results were obtained from interspecific crosses in *Tulipa* and intergeneric crosses between *Nerine* and *Amaryllis* (Van Tuyl *et al.* 1990, 1991; Van Creij *et al.* 1996b). Until now *in vitro* fertilization for bulbous crops has not been achieved using isolated sperms and eggs.

2.3 Techniques for overcoming F1-sterility

Barriers occurring after a successful embryo rescue are hybrid breakdown and F1-sterility. F1-sterility of interspecific hybrids is very common and may be the result of reduced chromosome pairing during meiosis. Breeding at polyploid levels is widely used in interspecific hybridization programmes of many bulbous crops such as *Alstroemeria, Freesia, Gladiolus* and *Lilium.*

2.3.1 Chromosome doubling

Interspecific F1-hybrids may display sterility owing to lack of chromosome pairing during meiosis. This sterility hampers further breeding. Somatic (mitotic) chromosome doubling may induce homologous pairing of chromosomes and therefore restores fertility (Hermsen 1984). Colchicine was used successfully to produce fertile allotetraploids in many crops such as *Lilium* (Asano 1982; Van Tuyl 1989) and *Iris* (Eikelboom and Van Eijk, 1990). As an alternative for colchicine, oryzalin, a herbicide with anti-mitotic activity, was recently used successfully (Van Tuyl *et al.* 1992). Crossability may be improved in the process of plant breeding by equalizing the functional ploidy level of the parents. Allopolyploids may function as fertile bridges for gene introgression into the cultivar assortment (Hermsen, 1984).

2.3.2 Application of 2n-gametes

Application of meiotic polyploidization in interspecific breeding programmes may be of great importance for the introgression of characters from diploids to tetraploids (Hermsen, 1984; Veilleux, 1985). In many species, polyploidization is the result of functional 2n-gametes in one or both the parents. Such gametes may result from mechanisms of meiotic restitution. Normally, the frequency of 2n-gametes is low. Wide interspecific lily hybrids are usually completely male and female sterile. In rare cases, however, some fertile pollen is detected. In a group of more than 50 hybrids from the cross *L. longiflorum* x *L. candidum*, raised through embryo culture, only one hybrid showed pollen fertility of 25%. Meiosis in this hybrid was highly irregular and all pollen contained 2n-gametes (Asano, 1984; Van Tuyl, 1989). Comparable cases were found by backcrossing oriental hybrids with 'Shikayama' x *L. henryi* and *L. auratum* x *L. henryi* (Asano, 1982) resulting in triploid progenies. Backcrossing these triploids with *L.*

auratum x *L. henryi* resulted in aneuploids with chromosome numbers intermediate between 36 and 48 (Van Tuyl, 1989). In contrast to the wide interspecific crosses, the hybrids between the Asiatic hybrid 'Enchantment' and the related *Lilium pumilum* produced fertile pollen. Meiotic studies of several of these hybrids showed that not only haploid pollen was formed but also relatively high percentages of 2n-pollen (Van Tuyl *et al.* 1989).

5. Growth in the Field

The breeding of flower bulbs started centuries ago. Reliable data are only available from the last 50-100 years. Moreover, most of the breeding efforts have been carried out by private firms or hobbyists. Therefore it is not know or not certain which interspecific crosses are the basis of the cultivars which are used nowadays. It is however clear that interspecific crosses are the basis of most cultivated flower bulbs e.g., tulip, lily, *Gladiolus* (Ohri and Khoshoo, 1983ab), *Narcissus* (Coleman, 1964; Brandham, 1986), freesia (Goemans, 1979), *Hippeastrum* (Traub, 1958) and *Alstroemeria* (De Jeu and Jacobsen, 1996). Except for tulip and lily during the evolutionary process of cultivating the flower bulbs interspecific hybridization went hand in hand with polyploidization. The vegetative propagation of flower bulbs is favourable for the development of a polyploid assortment, because fertility is less important for reproduction. Recently some of these processes are more or fully controlled and carried out in more goal-directed breeding programmes (*Tulipa*: Van Creij *et al.* 1996ab; *Fritillaria*: Wietsma *et al.* 1994; *Iris*: Eikelboom and Van Eijk 1990; *Lilium*: Van Tuyl *et al.* 1997; *Alstroemeria*: Bridgen *et al.* 1989; Buitendijk *et al.* 1995; De Jeu *et al.* 1992; *Nerine*, *Amaryllis*: Coertze and Louw, 1990; Van Tuyl *et al.* 1992; *Ornithogalum, Lachenalia*: Ferreira and Hancke, 1986; *Zantedeschia*: Yao *et al.* 1995). To trace the ancestors of cultivars nnew techniques are available nowadays. Ørgaard (1995) analysed the hybrid origin of two cultivars by molecular techniques including genomic southerns and in situ hybridization. In lily, section-specific random amplified polymorphic DNA (RAPD) markers are detected, which can identify the parental sections of inter-section hybrids (Yamagishi, 1995).

3.1 Lilium

The history of lily breeding is relatively young. It is an example how advanced techniques, in a relatively short period, can exploit the variation of species within one genus. The Asiatic and Oriental hybrid groups are, the last 50 years, developed from interspecific crosses within the sections Sinomartagon and Archelirion, respectively, of the genus *Lilium*. The development of these hybrid groups resulted in the increase of the lily acreage of 100 ha in 1970 till more than 3500 ha in The Netherlands in 1995. The last 15 years, wide interspecific crosses of genotypes of different sections have been made by the application of a range of pollination and embryo-rescue techniques. Especially *L. longiflorum* played an important role in these interspecific crosses (Van Tuyl *et al.* 1997). Commercial cultivars from crosses between *L. longiflorum* and Asiatic hybrids (LA-hybrids) are already obtained. At the same time breeding research on polyploidy in lilies was started (Van Tuyl, 1989). For overcoming of F1-sterility and the introgression of traits in the assortment breeding at polyploid level is essential. For the near future also LO-hybrids (crosses of *L. longiflorum* x Oriental hybrids) and OA-hybrids (crosses of Oriental x Asiatic hybrids) will completely innovate the lily assortment.

3.2 Tulipa

The history of tulip breeding goes back to the 12th or 13th century and it is unknown whether or which interspecific crosses were made for what is called now *T. gesneriana*. The assortment of *Tulipa* consists mainly of cultivars from the 'species' *T. gesneriana* and of (sterile and triploid) Darwin hybrids, obtained after interspecific hybridization between *T. gesneriana* and *T. fosteriana*. Within the subgenus *Tulipa* (Van Raamsdonk and De Vries, 1992, 1995) the possibilities for interspecific hybridization are studied by Van Eijk *et al.* (1991) and by Van Raamsdonk *et al.* (1995). Recently pre- and post-fertilization barriers have been identified by Van Creij *et al.* (1996a). *In vitro* embryo-rescue methods are developed and new unique hybrids e.g., *T. gesneriana* x *T. praestans* and *T. gesneriana* x *T. agenensis* are created (Van Creij *et al.* 1996b; Custers *et al.* 1995). For future tulip breeding these techniques combined with polyploidization methods will undoubtedly play an important role.

3.3 Other bulb crops

During the last one and a half century, interspecific hybridization and polyploidization played a major role in the development of the modern large-flowered (tetraploid) *Gladiolus* assortment (Le Nard and Cohat, 1977; De Hertogh and Le Nard, 1993; Ohri and Khoshoo, 1983b). In *Gladiolus*, *Narcissus* (Brandham, 1986) *Alstroemeria* (Ramanna, 1992) and *Freesia* (Goemans, 1979; Sparnaaij, 1979) polyploidization occurred spontaneously during interspecific hybridization, probably due to the occurrence of 2n-gametes which appeared to be the main factor in the evolution of the cultivated assortment.

4. Concluding Remarks

For bulbous crops, interspecific hybridization is an effective method for the introduction of desired characters into breeding material and achieving crop improvement (Van Tuyl and De Jeu, 1996). The possibilities for interspecific crosses are nevertheless limited due to various crossing barriers. The application of various pollination methods combined with embryo rescue (integrated techniques) is a very powerful breeding approach. A more controlled environmental condition during the processes of pollination, fertilization and embryo development would result in repeatability of experiements almost independent of the season. Conditions for each process can be optimised, and crossing barriers can be studied more systematically. *In vitro* methods enable the development of an integrated procedure for overcoming pre- and post-fertilization barriers.

Breeding at polyploid levels is widely used in interspecific hybridization programmes of many bulb crops such as *Alstroemeria, Freesia, Gladiolus, Iris, Narcissus* and *Lilium*. Application of 2n-gametes (meiotic polyploidization) in interspecific breeding programmes may be of great importance for the introgression of characters from diploids to tetraploids.

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