Chapter 23

TULIP

Tulipa gesneriana and T. hybrids

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Abstract: Tulips are commonly associated with The Netherlands, even though they are native to Central Asia. This association began in 1594 and caused the famous 'tulipomania' in the 1600s. This vegetatively propagated crop is currently the most important bulbous geophyte in the world. Modern cultivars (predominantly *Tulipa gesneriana*) are grown for bulb production, cut flowers, flowering potted plants, and landscaping. The Netherlands and France are the primary tulip bulb producers. Continued breeding and improvement of *T. gesneriana* focus on disease resistance, improved floral longevity, and new flower shapes/colors. Interspecific hybridization is hampered by reproductive (pre- and post-pollination) and germination barriers (due to incongruity), and long generation times. Crossing barriers have been overcome with the use of techniques such as bud pollination, cut styles, grafted styles, placental pollination, and pollination of isolated ovules. Haploidization and molecular techniques are being used to create homozygous plants and conduct marker-assisted breeding, respectively.

Key words: Bulb, Geophyte, Interspecific hybridization, Liliaceae, Monocot, Mutation breeding

1. INTRODUCTION

1.1 Tulips – General Aspects

Generally, tulips are associated with The Netherlands. However, the primary gene centre of the genus *Tulipa* L. is located in the Pamir Alai and Tien Shan mountain ranges in Central Asia (Hoog, 1973). Diversification occurred from this

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region, resulting in a distribution from Morocco to Western Europe and eastward to western China. A secondary gene centre has been found in the Caucasus.

Tulips were introduced from Turkey into Europe. They flowered for the first time in The Netherlands in 1594. Around 1630, tulips were extremely popular. The highest price recorded in 1637 for one tulip bulb was fl 5200. - (€ 2360). This was about four times the yearly salary of a middle sized businessman (Dash, 1999). The introduced tulips have been grown and bred for a long time. This has resulted in a wide diversity of flowering, growth, vigour and flower shape. These tulips, whose original species have not been determined, are grouped together and are called *T. gesneriana* L. The current commercial assortment still consists mainly of cultivars from *T. gesneriana* (Fig. 23-1). The second group of cultivars, the Darwin hybrids, has been obtained from interspecific crosses between cultivars of *T. gesneriana* and genotypes of *T. fosteriana* Hoog ex W. Irving.

Tulips are grown either for (1) bulb production, (2) forcing as cut flower and potted plant and (3) landscaping. About 85% of the world bulb production is grown in The Netherlands (Le Nard and De Hertogh, 1993). Another important tulip production area is France. In 1995, the annual turnover for bulb production was about 600 million Dutch guilders and for cut flower production about 274 million Dutch guilders (Anonymous, 1996). More than 70% of the flower bulbs and cut flowers produced in The Netherlands are exported.

The tulip is the most important ornamental bulb crop in the world. The planted acreage in The Netherlands for the season 2001/2002 was about 10.700 hectares (Anonymous, 2002). The *T. gesneriana* and Darwin hybrids consist of more than 1100 cultivars (Van Scheepen, 1996). The 10 most popular cultivars, however, occupy more than 35% of the planted acreage. Only 7% (643 ha) of the total tulip area consist of species, of which *T. fosteriana*, *T. greigii* Regel and *T. kaufmanniana* Regel are the primary species.

1.2 Classification

The tulip is a monocotyledonous plant in the *Liliaceae* family. The number of species ranges from about 45 (Stork, 1984) to more than 100 (Hall, 1940, Botschantzeva, 1962). According to the taxonomic classification by Van Raamsdonk and De Vries (1992, 1995), the genus is divided into two subgenera: *Tulipa* and *Eriostemones* (Boissier). These subgenera are classified into eight sections (Table 23-1).

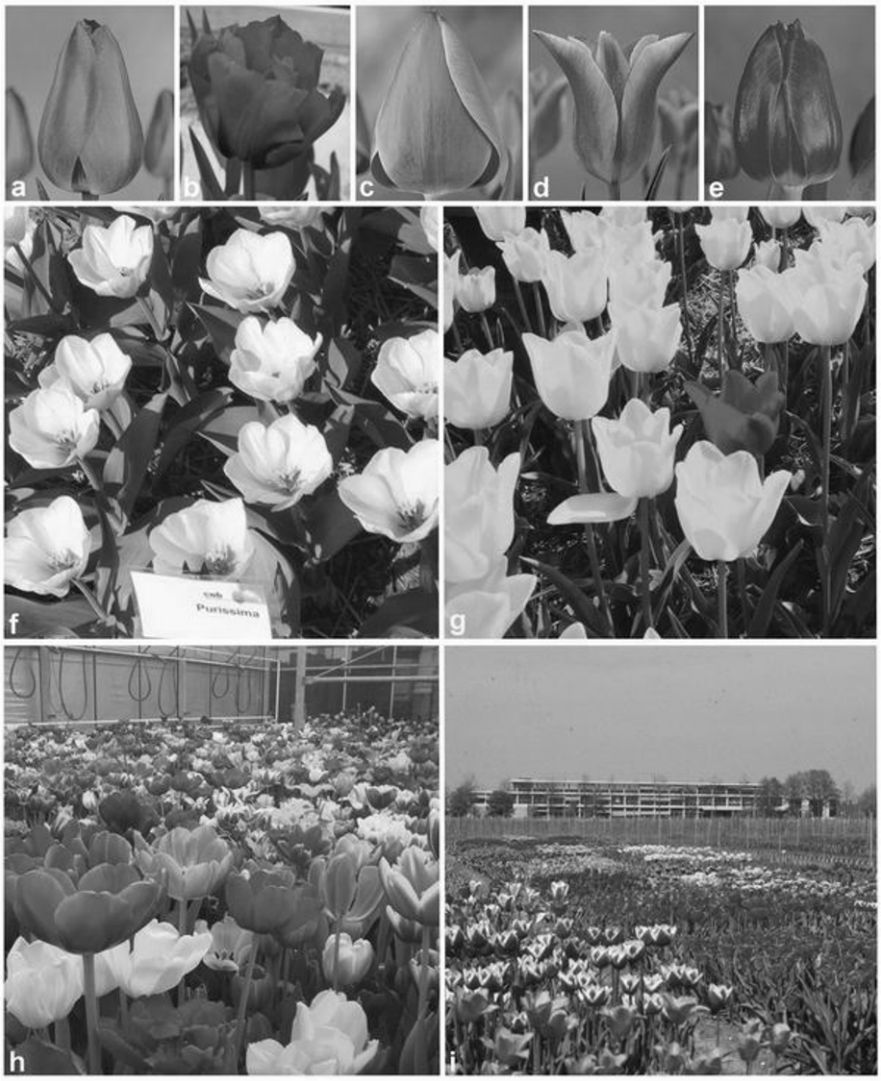

Figure 23-1. Some examples of the broad tulip assortment, all are *T. gesneriana* types except c and f. a. Barcelona b. Alliance c. Ad Rem (Darwin hybrid) d. China Pink e. Christmas Marvel f. Purissima (*fosteriana* hybrid); g. Mutation breeding: searching for sports a red sport of a new promising cultivar; h. Selecting a good new cultivar in a forcing experiment in the greenhouse; j. The tulip breeding fields of Plant Research International (Institute on background).

Table 23-1. The taxonomic classification of the species of the genus *Tulipa* in the sections (bold names) of the two subgenera, *Tulipa* and *Eriostemones*, according to Van Raamsdonk and De Vries (1992, 1995).

Subgenus *Tulipa*		
Tulipa	***Eichleres*** (Hall) Van Raamsdonk	***Tulipanum*** de Reboul
T. gesneriana L.	*T. ingens* Hoog	*T. agenensis* DC.
T. armena Boiss.	*T. lanata* Regel	*T. systola* Stapf
T. hungarica Borbas	*T. tubergeniana* Hoog	*T. kuschkensis* B. Fedtschenko
T. suaveolens Roth	*T. eichleri* Regel	*T. julia* C. Koch
T. didieri Jord.	*T. fosteriana* Hoog ex W. Irving	*T. aleppensis* Boiss. ex Regel
	T. greigii Regel	*T. praecox* Tenore
	T. albertii Regel	
	T. sosnovskyi Akhverdov et Mirzojeva	
	T. praestans Hoog	
	T. kaufmanniana Regel	
	T. tschimganica Bochantzeva	
	T. dubia Vvedensky	
	T. subpraestans Vvedensky	
Kolpakowskianae (Hall) Van Raamsdonk	***Clusianae*** Baker	
T. altaica Pall. Ex Sprengel	*T. clusiana* DC.	
T. lehmanniana Mercklin	*T. montana* Lindley	
T. tetraphylla Regel	*T. linifolia* Regel	
Subgenus *Eriostemones* (Boissier) Van Raamsdonk		
Australes sensu Hall	***Saxatiles*** sensu Hall	***Biflores*** sensu Hall
T. australis Link	*T. humilis* Herb.	*T. turkestanica* Regel
T. primulina Baker	*T. pulchella* Fenzl.	*T. polychroma* Stapf
T. biebersteiniana Schultes	*T. saxatilis* Sieb. ex Sprengel	*T. biflora* Pallas
T. sylvestris L.	*T. bakeri* A.D. Hall	*T. sogdiana* Bunge
T. whittalii (Dykes) A.D. Hall	*T. aucheriana* Baker	*T. neustrueva* Pob.
T. ophanidea Boiss. Ex Heldr.		*T. tarda* Stapf
T. hageri Heldr.		*T. dasystemon* Regel

2. GROWING TULIPS

Normally tulips are vegetatively propagated. It is only for breeding purposes that tulips are crossed and seeds are harvested. After sowing, seeds require a period of low temperature to induce germination and to initiate a bulb primordium (Niimi, 1978). The embryo produces one cotyledonary leaf, a primary root, and a hollow diverticulum called a 'dropper'. The bulb primordium is positioned at the tip of the dropper. As the dropper grows further into the soil a bulblet is produced at the end of the dropper (Taillandier and Riviere, 1981). This small tulip bulb requires 4 to 5 years of growth before it reaches the critical minimal size for flowering. The minimal size depends on the genotype, but typically *T. gesneriana* bulbs must reach a circumference between 6 and 8 cm. Tulips grown for bulb production, commercial forcing and landscaping are vegetatively propagated. Daughter bulbs develop from the buds which are located in the axil of the bulb scales are used for vegetative propagation. The average propagation rate of most tulip cultivars is between two and three bulbs per year (Le Nard and De Hertogh, 1993).

The tulip bulb has an annual replacement cycle, which can be divided into three phases (Le Nard and De Hertogh, 1993):

(1) Mother bulbs are planted in autumn, when soil temperature decreases. Subsequently, the roots of the mother-bulb grow rapidly until November-December. Concurrently the fully differentiated shoot elongates slowly and the daughter-bulbs exhibit a slight growth. The scales of the mother bulbs begin to senescence slowly.

(2) During early spring, when temperatures increase and after an extend period of low temperature, plant growth becomes very active. Rapid shoot and floral bud elongation occurs prior to flowering. Flowering tulips form two or more leaves after flowering. The growth rate of the daughter bulbs increases to the maximum. Concurrently the mother bulb scales shrivel and progressively disappear.

(3) At the end of the spring, the aerial organs [stem, leaves and flower(s)] of the mother bulbs senescence and growth of the daughter bulbs ceases. During this period the daughter bulbs undergo initiation and differentiation of floral and vegetative buds and root primordia. All these organs are present in the daughter bulbs by the end of summer.

A very important factor affecting the growth and development and flowering of tulip is temperature. For flower initiation (phase 3), a relatively high temperature (17-23 °C or higher) is needed. Thereafter (phase 1), a period of low temperature is required (2-9 °C). Physiological changes occur at low temperatures that are required for optimal floral stalk elongation and flower development at the subsequent higher temperatures (14-20 °C) (phase 2). For commercial flower and potted plant production, flowering is controlled by simulating the temperature conditions required in nature (this called forcing). The cold period can be given partially by storing the bulbs in temperature controlled and highly ventilated rooms, prior to planting the bulbs. This called "precooling". The optimal length of the total cold

treatment varies with the genotype. Le Nard and De Hertogh (1993) have published a review of the physiology of tulips.

3. SEXUAL REPRODUCTION

Normally each tulip flower has one pistil and six anthers. There are variations in semi- and full-double cultivars. The pistil consists of a stigma, a short style and an ovary. The stigma and short style comprise about 20% of the total pistil length. The ovary has three carpels, each containing two rows of ovules. One ovary contains between 150 ovules *(T. turkestanica*) to 210-270 ovules (most species) to 300-450 ovules (*T. gesneriana*).

A central bundle of pollen tubes develops in the ovarian cavity after compatible pollination. Pollen tubes bend sideways and grow towards and into the ovules. At 15˚C, they reach the first ovules at 1-3 days after pollination. This temperature is optimal for most tulip crosses (Kho and Baër, 1971). The lowest ovules are reached about 7-11 days after pollination. The number of ovules penetrated by a pollen tube increases from 3 to 9 days after pollination. On average 68%-83% of the ovules is ultimately penetrated by a pollen tube (Van Creij et al., 1997a). In general, fertilization takes place after penetration of the ovule by a pollen tube. However, Van Creij et al. (1997b) observed no fertilization in several percent of the ovules with pollen tube penetration. Pecenicyn (1972) has described the fertilization process of several *Tulipa* species.

In tulips, the development from zygote to mature embryo follows a different pathway than found in most monocot angiosperms. The zygote of most species divides transversely resulting in an apical cell, which gives rise directly to the embryo, and a basal cell, which divides to form the suspensor. In tulips, the basal cell develops into a proembryonal cell complex and the apical cell gives rise to a part of this cell complex, the suspensor, and the embryo (Ernst, 1901, Haccius and Hausner, 1972, Wafai and Koul, 1982).

The first division of the zygote results in the formation of the basal cell, which is taller and contains more plasma than the apical cell. Haccius and Hausner (1972) observed in *T. tarda* that cell multiplication proceeded from the base to the apex. The developmental sequence of cell division is precisely ordered in *T. tarda*, while it is more variable in *T. altaica* (former name *T. kolpakowskiana* (Van Raamsdonk and De Vries, 1995). Irregularities in division order are frequently found in *T. gesneriana*. These first divisions result in the formation of an irregularly segmented cell complex, the so-called proembryonal cell mass (Van Creij et al. 1997b). Van Creij et al. (1997b) observed ovules containing a proembryonal cell mass starting three weeks following pollination at 15°C. The suspensor is formed at the chalazal side of the proembryonal cell mass starting about 3 to 6 weeks after pollination. The globular embryo develops on top of the suspensor from mostly six weeks after

pollination. In this stage, the suspensor and embryo are surrounded by endosperm. The suspensor degenerates at the advanced globular embryo stage. The globular embryo will elongate in length and develop into a spindle-shaped embryo. Most spindle-shaped embryos are found beginning nine weeks after pollination. Subsequently, the embryo sac is almost completely filled with endosperm at the spindle-shaped embryo stage. The endosperm around the embryo is digested and, ultimately, the embryo is positioned in a cavity filled with fluid. Mature seeds can be harvested about 12 weeks after pollination (Van Creij et al., 1997b).

Aberrations in embryo development were found in compatible crosses within *T. gesneriana* and within *T. fosteriana* (Sayama et al., 1982, Van Creij et al., 1997b). Sayama et al. (1982) observed seeds with endosperm but without embryo in both compatible crosses. Van Creij et al. (1997b) found the percentage of ovules with developing embryos in compatible *T. gesneriana* crosses varying between 16% and 50%. Aberrations in embryo and/or endosperm development were found in about 5% of the ovules with embryos. Most of these ovules exhibited abnormal endosperm (4%) and the majority also had a deformed embryo (3%). The development from zygote to spindle-shaped embryo was retarded in several ovules. This resulted in the appearance of ovules containing a proembryonal cell mass or a globular embryo at the stage seed pods are harvested.

4. BREEDING

4.1 Improvement of Tulips

The production of flowers and bulbs of an optimal quality can be seriously affected by many pathogens. Therefore, disease resistances are important breeding objectives. Introduction of genes for resistance should improve the commercial assortment of tulips. In addition the use of pesticides required should be significantly decreased. Beside the introduction of disease resistance, a shorter cold requirement and forcing period, an improved flower longevity, and new flower shapes and flower colours are important goals for tulip breeding.

Most tulip breeders focus on breeding within *T. gesneriana.* Parents are chosen on basis of forcing qualities, flower colour, flower form and disease resistances. Each year several thousands flowers are hand pollinated resulting in several ten thousands seedlings. After growing the seeds till adult plants, most breeders select around 0.1% - 1% of the best plants (plant habit, colour etc.) during forcing as cutflower in the greenhouses. After vegetative propagation further selection takes place for other characters as disease resistance, bulb production etc.

A very important method for tulip improvement is the exploitation of the genetic variation of other tulip species through interspecific hybridization (see 4.4).

Crossing barriers, however, have prevented the formation of hybrids in many interspecific tulip crosses. *T. gesneriana* has been crossed successfully with only 12 out of the approximately 55 tulip species by using conventional breeding methods (Van Eijk et al., 1991, Van Raamsdonk et al., 1995). Crossing barriers impeding sexual reproduction in interspecific tulip crosses are due to incongruity between the crossed species (Hogenboom, 1973).

The rapid introduction of new cultivars enriched with desirable traits for the tulip production is also hampered by several other factors. The main hindrance is the long period required for the development of a new cultivar. After crossing, 5 to 6 years are needed to obtain a flowering bulb. Subsequently, it takes another 10 to 20 years to screen the tulips for desirable characters and to propagate the bulbs for commercial release. The production of large numbers of bulbs needed for the introduction of a new cultivar could be enhanced if the multiplication rate could be increased. Despite many efforts to develop a rapid multiplication system *in vitro* (Baker et al., 1990, Hulscher and Krijgsheld, 1995, Chanteloube et al., 1995, Kuijpers and Langens-Gerrits, 1997), the production of tulip bulbs still occurs primarily by propagation in fields.

4.2 Disease Resistance

Tulips can be affected by several diseases e.g. bulb-rot, fire, and viral diseases. Host resistance is the best approach to prevent such diseases. The most important pathogens are *Fusarium oxysporum* (bulb-rot), *Botrytis tulipae* and Tulip Breaking Virus (TBV). Also other fungi (*Pythium* spp., *Rhizoctonia tuliparum*/*solani*), viruses (Tobacco Necrosis Virus (TNV) and Tobacco Rattle Virus (TRV)), mites and nematodes (*Trichodoridae*, *Pratylenchus penetrans* and *Ditylenchus dipsaci*) can cause economic losses. The use of resistant cultivars reduces the use of chemical control, increases bulb production, and requires less labor for sorting and selecting harvested bulbs. Resistant cultivars are also important for bulb exports. Bulbs infected with *Fusarium* cause extra efforts to clean the stock and give rise to complaints from consumers.

Breeding research for *Fusarium* resistance was carried out by Van Eijk and co-workers (Van Eijk et al., 1983, Romanov et al., 1991). They produced reliable screening tests not only for clones but also for juvenile seedlings at the pre-selection stage. In these tests, bulbs are planted in *Fusarium*-infested soil and grown under standardized conditions for the entire season. After harvest, bulbs are examined for *Fusarium* infection. Almost absolute resistance was found within the *T. gesneriana* assortment. Since seedling selection produces some susceptible plants (escapes), selected plants have to be re-tested at the clonal level. The inheritance of *Fusarium* resistance was investigated and it was found that the use of one resistant parent can result in resistant descendants.

Research on tulip breaking virus (TBV) (Romanov et al., 1991, Straathof et al., 1997, Eikelboom et al., 1992) resulted in reliable screening tests at the clonal and seedling level. Viruliferous aphids inoculate leaves of flowering plants. Flower breaking was observed one year after inoculation. Absolute TBV resistance was found in several *T. fosteriana* cultivars, e.g. 'Cantata' and 'Princeps'. In crosses between *T. gesneriana* x *T. fosteriana* highly resistant genotypes were found. These crosses, also called Darwin hybrids, are mostly triploid and suffer from F1-sterility. Artificial doubling of the chromosome could solve this problem in the future (Van Tuyl, 1996, Van Tuyl and De Jeu, 1997).

An assay for screening tulips for resistance to *Botrytis tulipae* (Straathof et al., 2002) has been developed. Absolute resistance was found in *T. tarda.* However, this species can not be crossed with the assortment of *T. gesneriana*. In some cultivars of *T. gesneriana* and *T. kaufmanniana* partial resistance was found. At Plant Research International (PRI) breeding for *Botrytis* resistance is in progress.

For the future, resistance to the three main diseases of tulip must be combined in order to have multi-resistant tulips. PRI has been conducting research to accomplish that task in co-operation with a group of tulip growers.

4.3 Flower Longevity

Flower longevity, the life of a cut flower or potted plant is one of the most important characteristics for the consumer. Research has shown that large genetic variation is available in the cultivar assortment (Van Eijk and Eikelboom, 1976, Van der Meulen and Van Oeveren, 1993, Van der Meulen et al., 1997). They developed screening tests in which the vase life of a genotype could be estimated. A good correlation was found between the longevity of the flower still attached to the bulb and the vase life of the flower (Van Eijk and Eikelboom, 1976). The variation in flower longevity among cultivars varied from 8 to 16 days, when evaluated at 14°C. Studying segregating populations derived from these cultivars, the flower longevity varied from 6 to 22 days (Van der Meulen et al., 1997). This means that selection for longer flower longevity, based on additive effects of several genes, is a promising method.

4.4 Methods for Overcoming Crossing Barriers After Interspecific Hybridization

A wide range of techniques has been developed to bypass crossing barriers in many crops. Manipulation of the fertilization process is rather difficult. Most techniques focus, therefore, on bypassing crossing barriers prior to fertilization or post-fertilization.

Pre-fertilization barriers have been bypassed in several different interspecific crosses after bud-pollination (Sink et al., 1978), the use of the cut-style method or the grafted-style method (Van Tuyl et al., 1991, Wietsma et al., 1994), placental

pollination (Zenkteler, 1990, Sink et al., 1978) and pollination of isolated ovules (Stewart, 1981). In tulip, the cut-style method and placental pollination have been studied (Van Creij et al., 2000a). Following the cut-style method, the style is cut above the ovary and subsequent pollinated at the cut surface of the remaining portion of the style. The percentage of ovules with pollen tube penetration did not increase in crosses between *T. gesneriana* and five other *Tulipa* species after the application of the cut-style method. For placental pollination, ovaries were cut longitudinally into six sectors and placed *in vitro*. Each sector contained a placenta with a row of ovules and the ovary wall. Pollen was applied on the placenta. Pollen tube penetration percentages were not increased after placental pollination compared to stigmatic pollination. However, after placental pollination, most of the ovules with pollen tube penetration showed subsequent embryo germination.

Methods for bypassing post-fertilization barriers focus on the survival of hybrid embryos and on restoring the fertility of F_1-hybrids. Embryo culture, ovule culture, ovary-slice culture and ovary culture have been developed to enable hybrid embryos to survive *in vitro* (for reviews see Williams et al., 1987, Sharma et al., 1996). The application of embryo rescue techniques in tulip breeding has been reported by Van Tuyl et al. (1990), Custers et al. (1992, 1995) and Van Creij et al. (1999, 2000b). More embryos could be rescued from an earlier developmental stage (4 wk. post-pollination) with ovule culture, as compared to embryo culture. Also, more embryos could be rescued at each culture date with ovule culture in comparison with embryo culture (Custers et al., 1995). The efficiency of direct ovule culture and ovary-slice culture followed by ovule culture has studied by Van Creij et al. (1999). For ovary-slice culture, ovaries were cut transversely in eight sections and placed on medium. The percentage of germinating embryos increased, in most cases, significantly with a more advanced developmental stage of the embryos at the start of the culture. The results of ovary-slice culture, started at various dates after pollination, were comparable to or better than the results of direct ovule culture. By using ovary-slice culture and/or ovule culture, unique hybrids have been obtained from the crosses *T. gesneriana* x *T. agenensis* and *T. gesneriana* x *T. praestans* (Van Creij et al., 1999*)*.

Post-fertilization barriers may cause sterility of F_1-hybrids. Sterility of F_1-hybrids can be caused by the lack of chromosome pairing during meiosis. In many crops, chromosome doubling has restored fertility. Recently, tetraploid tulip cultivars have been produced after treating tulip stems from bulbs *in vitro* with oryzalin or colchicine (Eikelboom et al., 2001, Van Tuyl et al., 2002).

Various techniques have in most cases to be applied for the production of viable hybrid plants of a specific cross. When prefertilization barriers hinder interspecific hybridization, they must be bypassed. However, once prefertilization barriers are bypassed, embryo rescue techniques must often be used to save the hybrid embryos from a premature death. Finally, sterility of the F_1-hybrids must often be overcome.

In vitro pollination offers the prospects to perform an integrated system of pollination, fertilization and embryo-rescue techniques under optimal environmental

conditions. Van Creij (1997) describes a procedure for *in vitro* pollination of tulip, using compatible intraspecific *Tulipa gesneriana* crosses as model system. Application of *in vitro* pollination offers good prospects for tulip breeding. Pollination methods, such as intra-ovarian pollination, which might bypass prefertilization barriers can be developed. Interspecific crosses showing post-fertilization barriers can be made *in vitro*, or ovary culture can be started at early culture dates. The number of embryos that germinated was doubled after *in vitro* pollination compared to the application of ovary-slice culture followed by ovule culture started 3 weeks after pollination. The bulblets obtained *in vitro* can be used for polyploidization treatments *in vitro*.

4.5 Interspecific Hybridization

Many crosses between *T. gesneriana* and other tulip species have been carried out to enrich the commercial assortment with desirable traits from these species. However, incongruity barriers impede sexual reproduction in many interspecific tulip crosses either in whole or in part. These barriers can prevent or diminish the formation of viable seeds prior to fertilization (pre-fertilization barriers), during fertilization, or post-fertilization. Liedl and Anderson (1993) published a review concerning reproductive barriers.

Crosses between cultivars from *T. gesneriana* and species from all eight sections of the genus *Tulipa* have been carried out by Van Eijk et al. (1991) and Van Raamsdonk et al. (1995). Pre-fertilization barriers and post-fertilization barriers have been studied in interspecific tulip crosses, with *T. gesneriana* as one of the parents (Van Creij et al., 1997a, 1997b). Beside cultivars of *T. gesneriana*, the commercial assortment consists of the Darwin hybrids (crosses between *T. gesneriana* and *T. fosteriana*), which are mostly triploid. The use of triploid cultivars for further breeding is impossible due to F_1 –sterility. In contrast, *T. gesneriana* was found to be compatible with other species of the same section (*Tulipa*). Analysis of pollen tube growth in the pistil and pollen tube penetration in the ovules in reciprocal crosses between *T. gesneriana* and *T. didieri* exhibited pollen tube growth percentages comparable to intraspecific *T. gesneriana* crosses (Van Creij et al. 1997a). Van Raamsdonk et al. (1995), however, found that fewer hybrid F_1 bulbs were produced in crosses with *T. didieri* as pistillate parent when compared to crosses with *T. didieri* as pollen donor. Apparently, post-fertilization barriers diminish the number of viable F_1 bulbs obtained from the cross *T. didieri* x *T. gesneriana*.

Hybrids were produced in several crosses between *T. gesneriana* and representatives of the section *Eichleres* (Van Eijk et al., 1991, Van Raamsdonk et al., 1995). Crosses between *T. gesneriana* (pistillate parent) and *T. kaufmanniana* and *T. fosteriana* exhibited high pollen tube penetration percentages (Van Creij et al., 1997a). Small numbers of seeds were obtained from these crosses (Van

Raamsdonk et al., 1995). Seed production has never been reported for the cross *T. gesneriana* x *T. praestans*, despite relatively high percentages of ovules with pollen tube penetration (Van Creij et al., 1997b). Apparently, post-fertilization barriers hinder or prevent the formation of viable seeds in these crosses. Custers et al. (1995) studied the ovule content of swollen ovules of the cross *T. gesneriana* x *T. kaufmanniana*, six weeks after pollination. About half of the swollen ovules contained embryos that ceased to grow. Others showed remnants of embryo tissue or were empty. Embryos continued to grow in only 10-25% of the swollen ovules. However, the sizes of these embryos were highly variable and most of them remained smaller than control embryos. Most of these ovules did not produce germinating seeds. This confirms the presence of post-fertilization barriers. Either no or a low number of ovules were penetrated by a pollen tube after pollination of pistils of *T. fosteriana*, *T. praestans* or *T. kaufmanniana* with pollen from *T. gesneriana* (Van Creij et al., 1997a). This indicates the occurrence of prefertilization barriers.

Crosses between *T. gesneriana* and species from the section *Tulipanum* did not produce verified hybrids, except for *T. systola* Stapf (=*T. stapfii* Turrill; Van Raamsdonk and De Vries, 1995). Relatively high percentages of pollen tube penetration were found in the cross *T. gesneriana* x *T. agenensis*. Embryogenesis has been studied in this cross and compared with embryogenesis in a compatible *T. gesneriana* cross. Fewer embryos were formed from the cross *T. gesneriana* x *T. agenensis* when compared to the compatible cross. Embryogenesis was also retarded. The first globular embryos and spindle shaped embryos were found at later dates and the relatively lower number of spindle shaped embryos in mature seeds had a shorter average length than in the compatible cross. This retarded development, in combination with the higher percentages of ovules with aberrations in development from 4.5 weeks after pollination, mainly in endosperm formation, reveal that post-fertilization barriers occur at this level. Pre-fertilization barriers prevented pollen tube penetration in the ovules in the reciprocal cross. This resulted in pollen tube penetration percentages lower than 1% (Van Creij et al., 1997b).

Crosses between *T. gesneriana* and species from the sections *Kolpakowskianae* and *Clusianae* and from the subgenus *Eriostemones* have never been successful (Van Eijk et al., 1991, Van Raamsdonk et al., 1995). Pre-fertilization barriers prevented normal pollen tube growth in crosses between *T. gesneriana* as the pistillate parent and *T. altaica*, *T. clusiana, T. sylvestris*, *T. pulchella* and *T. turkestanica*. Pollen tubes had reached no more than 7% of the ovules in some flowers of these crosses. However, pollen tube penetration percentages up to 31% (*T. pulchella*) or 87% (*T. clusiana*) were found in a low number of flowers of the reciprocal crosses (Van Creij et al., 1997a).

Crosses between several other tulip species were also carried out to investigate the possibility of using these species hybrids in bridge crosses. Species of the same section can often be crossed (Van Raamsdonk et al., 1995). The crosses exploited

between species of the section *Eichleres* and the section *Tulipanum* did not succeed. F_1 bulblets were produced from crosses between species of the section *Eichleres* and *Clusianae*. They were, however, never verified on hybrid origin. Crosses made between *T. systola* (section *Tulipanum*) and some species of the section *Eichleres* were also not successful (Van Raamsdonk et al., 1995). Nevertheless, *T. systola* might be used in bridge crosses between species of the section *Tulipanum* and *T. gesneriana*, The species of the section *Eichleres,* which can be crossed with *T. gesneriana,* can only be used in bridge crosses with species of the same section.

Several factors proved to influence the rate of pollen tube growth and pollen tube penetration and the number of seeds obtained in tulip crosses. Reciprocal differences were found in the numbers of ovules with pollen tube penetration. Van Raamsdonk et al. (1995) found reciprocal differences in seed set on the plant. The maternal genotype affected the percentages with pollen tube penetration (Van Creij et al., 1997a). They also found the percentages of ovules with pollen tube penetration differed largely between the different flowers of the specific crosses and between both years. Cultivar-effects, effects of accessions, and year-effects were found to influence seed set in interspecific tulip crosses (Van Eijk et al., 1991, Custers et al., 1995).

4.6 Mutation Breeding

Tulip is a vegetatively propagated crop and during cultivation hundreds of natural mutants (sports) were selected (Van Scheepen, 1996). Mutations can exhibit difference in flower (edge) colour or flower shape (parrots, fringed and double). Differences exist in the mutation sensitivity of cultivars. Many mutants are known from specific cultivars, e.g. ‘Bartigon’, ‘William Copland’, ‘Murillo’ and ‘Apeldoorn’.

In order to produce mutations artificially, the possibilities of Röntgen (X-rays) for mutation breeding in tulips have been investigated (Broertjes and Alkema, 1970, Van Harten and Broertjes, 1989). Mother bulbs, as well as daughter bulbs, can be used for mutation induction. Also the dosage required varies from 350 to 550 rad. The radiation can be applied early in the planting season (August) or late (November). Besides mutations in flower colour and flower shape, mutations also occur in the colour of the leaf edge, in plant height and in bulb production. In the 1970’s PRI released several radiation mutants of ‘Preludium’ and ‘Lustige Witwe’. Private breeders are still using mutation breeding techniques (Straathof and Eikelboom, 1997).

5. PLOIDIZATION TECHNIQUES

5.1 Polyploidization

The number of chromosomes of many cultivars and *Tulipa* species have been investigated (Zeilinga and Schouten, 1968a). The assortment consists mainly of diploids (two sets of 12 chromosomes; $2n = 2x = 24$), some triploids (mainly Darwin hybrids) and a rare tetraploid ($2n = 4x = 48$). The production of tetraploids using laughing gas was described by Zeilinga and Schouten (1968b). By placing plants, which were pollinated a week previously, for one day in a cylinder with laughing gas (N_2O) and 5 to 6 atmospheric pressure, tetraploid seedlings were obtained. To improve the fertility of these tetraploids, they were crossed mutually. Highly fertile tetraploid cultivars of *T. gesneriana* and tetraploids of *T. fosteriana* and *T. kaufmanniana* were released to Dutch tulip companies in 1989 and 1991 (Straathof and Eikelboom, 1997).

Recently, using *in vitro* polyploidization techniques, similar as in *Lilium*, tetraploid tulip cultivars have been produced (Eikelboom et al., 2001, Van Tuyl et al., 2002). For future tulip breeding, this technique will undoubtedly have an important role in overcoming interspecific crossing barriers.

5.2 Haploidization Using Microspore Culture

The production of *in vitro* haploid plants using micropore culture is a technique used in many plant species in order to produce homozygous plants. Research showed that tulip was more promising to develop via this technique these plants than lily (Van den Bulk et al., 1992, Van den Bulk and Van Tuyl, 1997). Embryo-like structures from young pollen (microspores) were obtained in tissue culture. However, due to the difficulties with the *in vitro* propagation of tulips, this research was completed without obtaining homozygous doubled haploids.

6. MOLECULAR BREEDING METHODS

6.1 Transformation

Wilmink and co-worker (Wilmink et al., 1992, 1995), have investigated genetic modification of tulips. Using a Particle Delivery System, transient expression of the reporter gene for beta-glucuronidase was demonstrated. It was shown that the CAMV 35S as well as the TR2' promoter were active in flower stem explants. Regeneration and bulb formation of tulip *in vitro* is, however, extremely difficult and it takes many years for a flowering tulip will be obtained. Seven years after selecting

Gus-positive and PPT-resistant explants only one complete transgenic tulip plant flowered. It proved, however, that stable integration was established.

6.2 Marker-Assisted Breeding

Tulip and the long juvenile period is highly suitable for using molecular marker techniques and this makes pre-selection possible. Marker-assisted breeding has been investigated using AFLP-markers in lily and tulip (Van Heusden at al., 2002). AFLP-markers were found over the entire tulip genome (12 linkage groups). Several AFLP-markers were linked with TBV-resistance (Van Heusden, pers. comm.). In the future, the application of PCR-derived markers for various characters will speed up the breeding process of tulips.

7. FUTURE PROSPECTS

Tulip is a crop with a long juvenile period. It takes at least 25 years to develop a new cultivar after carrying out a cross. When disease resistance tests are performed, this will take even longer. To reduce the use of pesticides, restricted by many governments, disease resistance will be required in the future. Currently, *in vitro* propagation is not an economical method to speed up this process. Therefore, for the future, the application of molecular assisted breeding techniques can be an important tool for reaching the goal of developing a tulip assortment, which can be grown without large disease problems.

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