Chapter 19

LILY

Lilium hybrids

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Abstract: *Lilium* consists of ~80 species native to the Northern Hemisphere; commercial hybrids are used in a wide range of floricultural products, including flowering potted plants, cut flowers, and garden plants (herbaceous perennial). While lily breeding has been conducted for centuries, most breeding breakthroughs occurred during the past 50 years. These have included interspecific hybridization, intersectional hybridization, and various methods to overcome gametophytic self incompatibility and embryo abortion using pre-fertilization (cut style, grafted style), post-fertilization (ovary-slice culture, ovule culture, embryo culture) methods. The continued use of these and other modern techniques (polyploidization, molecular biology) will ensure creation of new phenotypes for the market.

Key words: cut style, embryo rescue, grafted styles, polyploidization, self incompatibility.

1. INTRODUCTION

1.1 Lilies – General Aspects

The lily has a history dating back at least 36 centuries. It can be traced to the Middle Minoan IIIA-B period (ca. 1750-1675 B.C.), when Cretan vases and frescoes illustrated its beauty, pure white colour and elegant fragrance (Evans, 1921, 1930; Woodcock and Stearn, 1950). The genus *Lilium* is in the family Liliaceae and comprises over 80 species (Comber, 1949; De Jong, 1974). The native *Lilium* species are spread over the Northern Hemisphere (10° to 60°) and centered mainly

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in Asia, North America, and Europe. Currently, the lily occupies a prominent place in horticulture as a cut flower, potted, and garden plant. In 2000 over 1500 million bulbs were produced worldwide. The Netherlands leads in bulb production with 4523 hectares in 2002. There is, however, production in Japan, the United States and more recently in the Southern Hemisphere, e.g. Australia, Chile and South Africa. As a cut flower, the lily is now the fourth most important crop in the Netherlands (Anonymous, 2002b).

1.2 Trends in Lily Hybridization for Commercial Market

Lily breeding goes back about 200 years (Shimizu, 1987). Significant breakthroughs are, however, only 50 years old, starting with the breeding of Asiatic hybrids (McRae, 1998). It has only been since the 1970's that the lily has become, after tulip, the most important flower bulb and cut flower. At present Dutch lily breeding companies are dominant. Over 100 new cultivars have been released annually for the market over the past two decades. Asiatic hybrids were the leading group until 1980's. Since then Oriental hybrids have become the most important group. This has been due to their outstanding flower shape and fragrance even though their forcing time is a few weeks longer than most Asiatic hybrids. This is especially true for old cultivars such as 'Casa Blanca' and 'Star Gazer'. Although 'Joy ('Le Reve')' has an early flowering habit, it has a rather short stem length, which is not desirable for cut flower production in high light and warm climates. For the last 10 years, many Oriental hybrid cultivars have been released with early flowering habits, large flowers and various flower colours.

Many researchers (Skirm, 1942; North and Wills, 1969; Ascher, 1973a; Asano and Myodo, 1977a,b; Asano, 1978, 1980; Van Tuyl et al., 1986, 1991, 2002) have successfully carried out interspecific hybridization studies and this opened the possibility of intersectional hybridization between, e.g. *L. longiflorum* x Asiatic hybrid. The cut style method (**CSM**) was developed to overcome pre-zygotic incongruity, which hinders pollen tube growth in the style between intersectional crosses. This technique in combination with embryo rescue methods such as embryo culture, ovule culture and ovary slice culture, has opened possibilities to develop numerous new hybrids. In the early 1990's a new class of lily cultivars were introduced to the market. They are called **LA** (**L**ongiflorum x **A**siatic) hybrids. In most cases **LA** hybrids are triploid and were derived from back crossing with the F_1 hybrid of *L. longiflorum* x Asiatic hybrid to an Asiatic hybrid (Genome composition of **LA** hybrids is indeed **ALA**). Although they have many desirable characteristics such as rapid bulb growth, healthy leaves, strong stems, large flowers and fragrance, they have negative characteristics such as flower malformation, weak petals and non pure flower colours. In the 1990's Dutch breeding companies focussed on Oriental hybrid breeding. Some outstanding cultivars released were 'Sorbonne' to replace

'Star Gazer' (both pink colour) and 'Siberia' to replace 'Casablanca' (both white). In 2002, the total bulb production area of lily in the Netherlands was 4368 ha (Anonymous 2002a). The **LA**-hybrids occupied 590 ha, which was a 90% increase when compared to 1999. This indicates that the production of these popular interspecific hybrids have increased over *L. longiflorum*, Asiatics, and Orientals in recent years.

2. GENETICS

2.1 Classification

The genus *Lilium* was classified into seven sections by Comber (1949) and later revised by Lighty (1968) and De Jong (1974). The seven sections are: Martagon, Pseudolirium, Lilium (Liriotypus), Archelirion, Sinomartagon, Leucolirion, and Daurolirion. This scientific classification is very different than the one used by Royal Horticultural Society (RHS), where the genera include a large number of species or many cultivars are often subdivided into groups, based on a particular characteristic or combination of characteristics. The RHS classification is reported in *The International Lily Register* (RHS 1982-2002). This classification is presented below:

(I) Early-flowering Asiatic Hybrids derived from *L. amabile*, *L. bulbiferum*, *L. cernuum*, *L. concolor*, *L. davidii*, *L. x hollandicum*, *L. lancifolium*, *L. leichtlinii*, *L. x maculatum* and *L. pumilum*
- (Ia) Upright flowers, borne singly or in an umbel
- (Ib) Outward-facing flowers
- (Ic) Pendant flowers

(II) Hybrids of Martagon type, one parent having been a form of *L. hansonii* or *L. martagon*

(III) Hybrids from *L. candidum*, *L. chalcedonicum* and other related European species (excluding *L. martagon and L. bulbiferum*)

(IV) Hybrids of American species

(V) Hybrids derived from *L. formosanum* and *L. longiflorum*

(VI) Hybrid Trumpet Lilies and Aurelian hybrids from Asiatic species, including *L. henryi* but excluding those from *L. auratum*, *L. japonicum*, *L. rubellum* and *L. speciosum*.
- (VIa) Plants with trumpet-shaped flowers
- (VIb) Plants with bowl-shaped flowers
- (VIc) Plants with flat flowers (or only the tips recurved)
- (VId) Plants with recurved flowers

(VII) Hybrids of Far Eastern species as *L. auratum*, *L. japonicum*, *L. rubellum* and *L. speciosum* (Oriental Hybrids)
(VIIa) Plants with trumpet-shaped flowers
(VIIb) Plants with bowl-shaped flowers
(VIIc) Plants with flat flowers
(VIId) Plants with recurved flowers
(VIII) All hybrids not in another division
(IX) All species and their varieties and forms

All *Lilium* species are diploid (2n=2x=24), except some triploid forms of *L. tigrinum* and *L. bulbiferum* existing in nature (Noda, 1966, 1978; Noda & Schmitzer, 1992). Early chromosome studies (cytogenetics) of lily were performed by Sato (1932) and Stewart (1947). The genome size of *Lilium* belongs to one of the largest in plant kingdom (Bennett & Smith, 1976, 1991). Differences in DNA-content can be measured efficiently using flow cytometry, which is used generally for discrimination between diploids and tetraploids (Van Tuyl et al., 1992). The variation in DNA content within the genus *Lilium* was studied by Van Tuyl & Boon (1997) and ranged from 69 to 96 pg/2C. It appeared that interspecific hybrids in general have intermediate DNA content of the parents. This method was used for the identification of the hybrid character of interspecific hybrids in a very early stage.

Lilium longiflorum has a strong gametophytic self incompatibility (SI) system and has been a model crop for studying the mechanism. Heat treatment and cutting the styles appeared to be methods to overcome this S-allele regulated system (Ascher, 1973a,b, 1977; Lindquist, 1991).

2.2 Interspecific Hybridization

Many important horticultural characters are present in the different *Lilium* species. Commercially important characters include:

1) Resistance to diseases such as bulb rot (*Fusarium*), *Botrytis* and several viruses (TBV, LSV and LVX) (Löffler et al., 1996);

2) Phenotypic characteristics such as flower shapes, sturdy stems, new colours, and fragrance;

3) Physiological characteristics such as tolerance to low-light intensity and heat, leaf scorch, year-round forcing ability, long-term storage ability, and rapid bulb growth.

Van Tuyl et al. (1986) have summarised the goals of interspecific hybridization in *Lilium.* They are as follows:

1) Introduction of desirable characters from various species into cultivars directly or indirectly (*i.e.*, bridge crosses);

2) Creation of new forms and types of lilies;

3) Overcoming F_1-sterility by mitotic and meiotic polyploidization;

4) Generation or expansion of the knowledge regarding on taxonomic relationships, inheritance mechanisms and introgression of specific genes.

Some well-known examples of valuable characters among species are listed in Table 19-1.

Table 19-1. Characteristics of *Lilium species* for commercial breeding.

Species	*Characteristics for breeding*		*Potential for breeding*
	Desirable	Undesirable	
L. longiflorum	Low temperature tolerance, flower shape. White	Susceptible to Fusarium, Virus	High
L. formosanum	Year round forcing, up right, growth vigour, fragrance	Weak stem, virus susceptible	Medium
Aurelion hybrid	Upright, yellow colour, fragrance, flower type	Susceptible to Fusarium, virus, weak stem	High
L. nepalense	Pea-green flower colour	Susceptible to virus, late flowering	Low
L. henryi	Vigour, virus and Fusarium resistance	Flower shape, weak stem	Medium
L. concolor	Upright flower, flower shape and size	Weak stem, leaf and growth vigor,	Low
L. tigrinum	Vigour, resistance to virus, large flower, bulbil formation, resistance to Fusarium	Hair, spots	Medium
L. callosum	Small, many flowers per stem, flower colour	Late flowering, weak growth vigor	Low
L. davidii	Resistance to Fusarium and virus	Short stem,	High
L. dauricum	Resistance to Fusarium	Short plant height	High
L. auratum	Large flower, fragrance, vigour, disease resistance, early flowering	Fusarium susceptible,	High
L. speciosum	Pink colour, fragrance	Spots, late flowering	Medium
L. nobilissimum	Pure white flower, fragrance, sturdy stem, upright	Late flowering	Medium
L. rubellum	Very early flowering, pink flower colour, fragrance	Short stem, susceptible to Fusarium	Medium
L. candidum	Low-temperature and low-light intensity tolerance, pure white, fragrance	Susceptible to Virus, weak growth vigor	Low
L. hansonii	Many flowers, long vase life	Flower fragrance, short stem, weak growth vigor, susceptible to virus	Low
L. martagon	Purple, small flower, long vase life, many flowers	Strong fragrance, susceptible to virus	Low
L. tsingtauense	Resistance to Botrytis	Short stem, weak growth, fragrance	Low

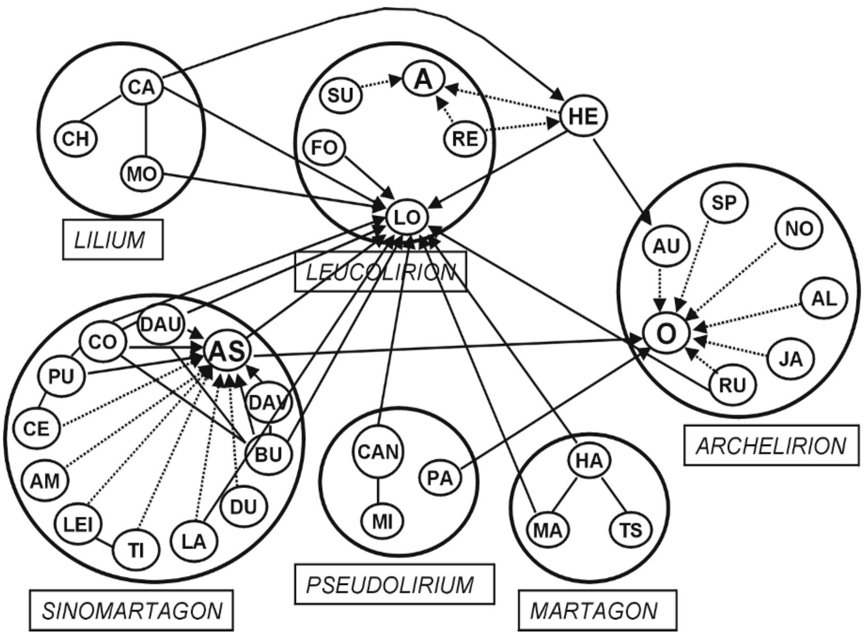

Figure 19-1. A crossing polygon of the genus Lilium including all successful crosses of species between different sections of the genus Lilium developed at Plant Research International, The Netherlands. In this figure, the connection between the Asiatic, Aurelian, and Oriental hybrid groups (large ellipses) are shown by dotted lines. In successful crosses between species (small circles) of different sections (large circles) the arrows point towards the female parent. Abbreviations: A: Aurelian hybrids; AL*:* *L. alexandrae*; AM: *L. amabile*; AS: Asiatic hybrids; AU: *L. auratum*; BU: *L. bulbiferum*; CA: *L. candidum*; CAN: *L. canadense;* CE: *L. cernuum*; CH: *L. chalcedonicum*; CO: *L. concolor*; DAU: *L. dauricum*; DAV: *L. davidii*; DU: *L. duchartrei*; FO: *L. formosanum*; HA: *L. hansonii*; HE: *L. henryi*; JA: *L. japonicum*; LA: *L. lankongense*; LEI: *L. leichtlinii*; LO: *L. longiflorum*; MA: *L. martagon*; MI: L. michiganense; MO: *L. monadelphum*; NO: *L. nobilissimum*; O: Oriental hybrids; PA: *L. pardalinum*; PU: *L. pumilum*; RE*:* *L. regale*; RU*:* *L. rubellum*; SP*:* *L. speciosum*; SU: *L. sulphurenum*; TI: *L. tigrinum*; TS: *L. tsingtauense*.

Both pre-fertilization and post-fertilization barriers restrict interspecific hybridization between the different sections (Van Tuyl et al., 1991). Several techniques, such as the cut-style method (Asano and Myodo 1977a,b), the grafted-style method, and *in vitro* pollination techniques have been developed to overcome pre-fertilization barriers (Van Tuyl et al., 1991). However, even if fertilization is successful, post-fertilization barriers can inhibit the growth of hybrid embryos (Van Tuyl et al., 1991). *In vitro* pollination and rescue methods such as embryo culture. (Skirm, 1942; North and Wills, 1969; Ascher, 1973a; Asano and Myodo, 1977a,b; Asano, 1978, 1980), ovary-slice culture, and ovule culture have been developed to circumvent these barriers (Van Tuyl et al., 1991). Plants were obtained from cultured embryos of *L. henryi* × *L. regale* by Skirm (1942). Ascher (1973a,b) succeeded in obtaining plants of *L.* 'Damson' × *L. longiflorum*. Asano and Myodo (1977b) reported the culture of immature hybrid embryos between *L. longiflorum* × *L.* 'Sugehime' and *L.* 'Shikayama' × *L. henryi*. Asano (1980) produced many interspecific hybrids between *L. longiflorum* × *L. dauricum*, *L. longiflorum* × *L. amabile*, *L. longiflorum* × *L. pumilum*, *L. longiflorum* × *L. candidum*, *L. auratum* × *L. henryi*, *L.* 'Sasatame' × *L. henryi*, *L.* 'Royal Gold' × *L. speciosum* and *L. regale* × *L. leichtlinii maximowiczii*.

Later, Van Tuyl and coworkers (1991 and 2002) succeeded in making numerous new intersectional hybrids between many sections of the genus *Lilium* by the use of various pollination and embryo rescue methods. Examples include *L. longiflorum* (Leucolirion section) × *L. monadelphum* (Lilium section), *L. longiflorum* × *L. lankongense* (Sinomartagon section), *L. longiflorum* × *L. martagon* (Martagon section), *L. longiflorum* × *L. candidum* (Lilium section), *L. henryi* (Leucolirion section) × *L. candidum*, *L. longiflorum* × *L. rubellum* (Archelirion section), *L. longiflorum* × Oriental hybrid, Oriental × Asiatic hybrid, *L. longiflorum* × *L. canadense* (Pseudolirium section) and Oriental hybrid × *L. pardalinum* (Pseudolirium section). The crossing polygon (Figure 19-1) shows the crossing compatibility within and between the sections achieved by our research group so far (Van Tuyl et al., 2002).

2.3 Overcoming Pre-Fertilization Barriers

2.3.1 Cut Style Method (CSM)

CSM is the most commonly used technique to overcome pre-fertilization barriers that exist mainly on the stigma and in the style. The inhibiting chemicals are eliminated by cutting off most of style on the day of flowering and subsequently pollinated by placing pollen paste consisting of mixed pollen and stigmatic exudate on top of the cut surface. In this case, the number of germinating pollen can be reduced; however, the numbers of germinating pollen, which penetrate into ovary, is increased. Recent studies have been carried out by comparing of normal and CSM pollination for the production of F_1 and BC_1 interspecific hybrids. The results indicate that CSM is superior for the generating F_1 hybrids and normal pollination method is superior for generating BC populations. This finding can be explained by the crossing barriers between species. Once the genome composition is heterozygous, such as in case of F_1 interspecific hybrids, backcrossing by normal pollination method showed normal pollen germination and pollen tube growth through the style.

2.3.2 Grafted Style Method (GSM)

The GSM was developed because the CSM normally produced only a few embryos per pod. When using the CSM, pollen tubes remain short and most of them do not penetrate the micropyle. GSM should be used in combination with *in vitro* pollination (Van Tuyl et al., 1991). This method is carried out as follows: desirable pollen from the donor plant is pollinated onto compatible stigma in vitro for 1 to 2 days and then the style is cut above 1-2 mm above the ovary. Subsequently, the cut style is joined to the short cut style with ovary of recipient. This graft should be kept for two days, until the pollen bundle enters the ovary of the recipient completely. After five days, the ovary is cultured following the ovary-slice method. This technique is a highly delegated method and labour intensive and only for combinations, which have been unsuccessful with other techniques.

2.4 OverComing Post-Fertilization Barriers

2.4.1 Ovary-Slice Culture

Ovary-slice culture was applied by Kanoh et al. (1988) and Van Tuyl et al. (1991) for the production of interspecific *Lilium* hybrids. Ovaries are harvested 7 to 10 days after pollination, sliced into 2 mm thick disks, and placed on a medium containing 10% sucrose. Within 30 days, ovules or embryos can be separated from the ovary-slice and cultured individually until germination.

2.4.2 Ovule Culture

The ovule culture method must be applied during the course of embryo growth and before the embryo is degenerated. The time for ovule culture is dependent on the cross combination and ranges from 30 to 45 days after pollination. Because the embryo rescue method is labour consuming, ovule culture method can be used when large numbers of hybridized ovules have to be carried out within a short period. A drawback, however, is that the germination efficiency of ovule culture method is lower than embryo culture (Figure 19-2a, b).

2.4.3 Embryo Culture

North and Wills (1969) have reported the successful culture of embryos from seeds without an endosperm and originating from interspecific crosses involving *L. lankongense*. Embryo culture can be applied successfully in crosses in which the degeneration of embryo progresses slowly (Figure 19-2c). This normally occurs with crosses between relatively closely-related species. In most cases, embryos can be rescued when the globular stage is reached. This technique is very reliable and embryos grow fast without any abnormal development (Figure 19-2d). The best time for embryo rescue method is about 40 to 60 days after pollination.

2.5 General Methodology to Overcome F_1 Sterility

A primary impasse to achieving introgression by backcrossing is sterility of interspecific hybrids. This can be due to several reasons, e.g. as chromosome aberrations, genetic incongruity (genic sterility), or other unknown factors (Asano 1982). Meiotic division of the wide interspecific hybrids is often disturbed due to factors such as unbalanced chromosome assortment, chromosome bridges, chromosome lagging during anaphase I and II, time discrepancy between chromosome movement, and cytokinesis (Asano, 1982). Any pollen generated through these disturbances is lethal. Although the chromosomes of two distantly related genomes have high levels of chromosome association, the pollen will be predominantly sterile or unbalanced due to the random distribution of homoeologous chromosomes during meiotic division (Asano, 1982; Hermsen, 1984; Ramanna, personal communication). Unreduced gamete formation is an exception in this type of material and circumvents these balance disturbances. This phenomenon was demonstrated by 83.6% of restituted pollen in the hybrid of *L. auratum* (Archelirion section) × *L. henryi*, and 52 % in *L. longiflorum* (Leucolirion section) × *L. leichtlinii* (Sinomartagon section) (Asano, 1982), *L.* 'Connecticut Yankee' (Sinomartagon section) × *L. longiflorum* (Leucolirion section) and *L. aurelianense* (Leucolirion section) × *L. longiflorum* (Ascher 1973a,b and 1977). When in natural conditions fertile pollen formation by abnormal meiosis is rare in intersectional hybrids. However, researchers are developing artificial methods using temperature fluctuations and chemical treatments at the optimum stage of flower bud development.

2.6 Importance of Introgression Breeding

Introgression is one of the main goals in interspecific hybridization in order to introduce a restricted number of traits from the donor species to the recipient. For example, in the Oriental hybrid group (Archelirion section) no orange flower colour is present. In addition they are susceptible to *Fusarium*, but resistant to *Botrytis*. The Asiatic hybrids (Sinomartagon section), however, are available in a range of colour, but are generally susceptible to *Botrytis* and resistant to *Fusarium*. Thus, to combine these traits is clear that interspecific hybridization must be used, especially when compared to alternative methods such as genetic transformation or mutation breeding.

2.7 Polyploidization

2.7.1 Mitotic Polyploidization

As indicated most interspecific hybrids between distantly related *Lilium* species are highly sterile. Therefore, even after successful interspecific hybridization between diploid species (2n=2x=24), the sterility of interspecific hybrids imposes a significant restriction to introgression breeding. Polyploidization can solve this problem, which can be distinguished into mitotic and meiotic polyploidization. The former is obtained through artificial chromosome doubling by treatment of vegetative tissue with spindle inhibitors such as colchicine (Blakeslee and Avery 1937; Emsweller and Brierley (1941) or oryzalin (Van Tuyl et al., 1992). In many cases, however, there are serious problems for the homoeologous recombination between parental species in allotetraploid of F_1 interspecific hybrid (Lim et al., 2001b). Due to preferential pairing between homologous chromosomes at metaphase I of the meiosis, homoeologous chromosomes have absolute or almost no pairing and therefore homoeologous recombination is reduced dramatically. In most cases allotriploid progenies are formed between diploid and allotetraploid, and finally triploid plants might not be fertile. This is not relevant for further crossing. Tetraploidization can contribute some advantages to make triploid progeny. They are more vigorous, healthier, and have larger organs than diploids. But in most cases triploid cultivars are not fertile, which commercial breeders prefer to avoid further crossings by competitors. From a theoretical point of view, combining three genomes (Longiflorum, Asiatic, Oriental) have an increased opportunity to bring diverse characters into one plant.

2.7.2 Meiotic Polyploidization

An alternative and preferred method to use interspecific hybrids involves the use of 2n-gametes that occur occasionally in interspecific hybrids of lilies (Van Tuyl et al., 1989). The plants derived from crosses with 2n-gametes are in their parental characteristics genetically more homozygous (SDR effect), or more heterozygous with some degree of heterozygosity depending on recombinations (FDR effect) (Hermsen, 1984). An important feature of 2n-gametes is that, depending on the mode of origin, a certain level of intergenomic recombination can occur during the meiosis I division. Fertile n- and 2n-gametes have been produced in interspecific lily hybrids (*L*. 'Enchantment' × *L. pumilum*) within the Sinomartagon section (Van Tuyl et al., 1989). In fact, fertile n-gametes with homoeologous recombination are ideal for introgression breeding without increasing the ploidy level of the succeeding generation. However, in most cases, unreduced (2n) gametes are predominantly produced.

BC_1 plants derived from 2n-gamete producing F_1 intersectional hybrids showed a range of fertility and are used for BC_2 progeny. By GISH analysis, BC_2 progeny possess relatively large amounts of donor species chromosome segments together with a number of whole chromosomes. This can be used for the introgression breeding of desirable genes into cultivar (Lim et al., 2001a).

2.8 Chromosome Analysis by Genomic *in situ* Hybridization (GISH) Technique

Genomic *in situ* hybridization (GISH) can distinguish the parental genomes of interspecific hybrids. This technique, which utilizes total genomic DNA from one of the parental species as a probe and from the other counterpart species as a block, provides a new technique for effective parental genome analysis in both sexual and somatic hybrids. This technique also detects translocations involving chromosomes from different genomes is useful to monitor chromosome behavior during meiosis. Therefore, the level of introgression in back-crossed progenies between different species can be measured by GISH analysis. Due to its large chromosome size, lily has advantages in the analysis of the number of parental chromosome composition and homoeologous recombination breakpoints. This is beneficial to determine whether or not recombination has taken place between parental chromosomes. Lim et al. (2000, 2001a,b) have utilized this procedure in their studies.

3. BREEDING

3.1 Important Traits for Commercial Breeding

3.1.1 Flower Colour

In lily breeding flower colour is one of the most important characters in combination with flower shape and size. In order to produce a desirable colour in the next generations or understanding of the inheritance of flower colour is needed. The genetics of flower colour has been determined differently in the hybrid groups. In the Asiatic hybrids, orange flowers are the dominant wild type, while yellow is determined by a single-recessive gene. Another gene determines the absence of carotenoid flower colours resulting in white or pink flowers. In Orientals, dark pink is dominant over white. In this group, the carotenoid colours have a minor role (Banba, 1975).

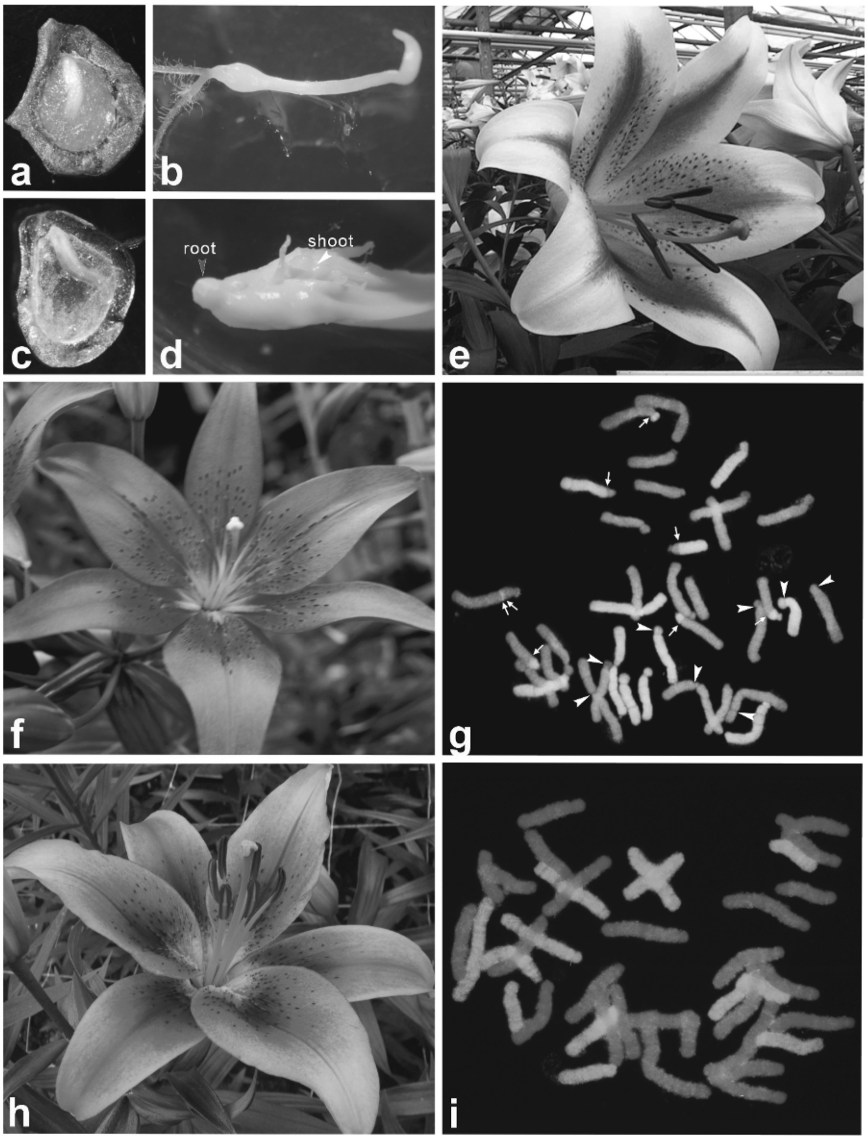

Figure 19-2. a. Normal embryo at 50 to 60 days after pollination, b. a hybrid plant with roots and bulb, c. abnormal embryo growth from an interspecific hybrid, d. a germinated seedling showing ambiguous shoot and root formation, e. super lily (OLO) with a 27cm diameter flowers, f and g. ALA triploid derived from meiotic polyploidization possessing many homoeologous recombinations (arrows). Arrow-heads indicate nucleolar organizer regions (NORs), h. An interspecific triploid hybrid between Asiatic and Oriental (AOA) derived from mitotic polyploidization of an OA hybrid. i. Chromosome painting showing chromosome composition -Asiatic (red) and Orientals (yellow).

3.1.2 Disease Resistance

Fusarium oxysporum var *lilii*, *Botrytis* and viruses are the most severe pathogens for lilies. Research has been performed at Plant Research International for the evaluation of disease resistance for *Fusarium* and *Botrytis* and useful screening techniques has been developed (Straathof and Van Tuyl, 1992, 1994). A high degree of *Fusarium* resistance was found in some Asiatic cultivars and in some species e.g. *L. dauricum.* In contrast, all Oriental hybrids were relatively susceptible. On the other, hand Oriental hybrids were highly resistant to *Botrytis*, while many Asiatic hybrids and some species e.g., *L. pumilum*, *L cernuum* were very susceptible (Van Tuyl and Lim, unpublished results). Resistance to Lily Mottle Virus (Tulip Breaking Virus) was found in some Asiatic cultivars and it segregates as a single gene (Van Heusden et al., 2002).

3.1.3 Flower form, Shape, and Forcing Ability

Due to a wide range of flower shapes, size, colour and morphological characteristics in combination with strong growth habits and relative high levels of resistance to diseases, the sections Leucolirion, Sinomartagon, and Archelirion sections are the most important groups economically. Interspecific hybrids within the sections, especially Asiatic hybrids, have been bred since the early 1800's (Shimizu, 1987). The distinctive characters of three important hybrids groups for cut flowers are:

1) The Longiflorum hybrids (**L**-genome) group in the Leucolirion section have trumpet-shaped, pure white flowers, a distinctive fragrance, year-round forcing capabilities, and mostly outward-facing flowers;

2) The Asiatic hybrids (**A**-genome) group in the Sinomartagon section possess a range of flower colours (orange, yellow, white, pink, red, purple and salmon), mostly upright-facing flowers, and are early (*L. pumilum*, *L. cernuum*) to late (*L. callosum*) flowering types;

3) The Oriental hybrids (**O**-genome) group in the Archelirion section have large, pink or white flowers, a strong fragrance, sturdy stems, wide dark-green leaf shapes, and early (*L. rubellum*) to late flowering (*L. nobilissimum*) habits.

The commercial Asiatic hybrids originated from interspecific crosses between species of the Sinomartagon section, Oriental hybrids were derived from crosses in the Archelirion section, and *Longiflorum* hybrids obtained from *L. longiflorum* Thunb. or crosses of *L. longiflorum* and *L. formosanum* Wallace.

As many as 12 species of the Sinomartagon section are involved in the currently available cultivars of Asiatic hybrids: *L. amabile* Palibin (Korean lily; orange or yellow coloured), *L. bulbiferum* Linn. (Orange, upright-facing), *L. cernuum* (early flowering, white or pink colour), *L. concolor* Salisb. (small flower, early and upright-facing), *L. dauricum* Ker-Gawler (early flowering, upright-facing, hairy, and

Fusarium resistant), *L. davidii* Elwes (orange with black spots), *L. lancifolium* Thunb. (strong stem, vigorous, hairy and bulbil forming), *L. lankongense* Franchet (pink with fire spots), *L. leichtlinii* Hook. (citron-yellow or orange with hairy buds), *L. maculatum* Thunb. (upright-facing) and *L. pumilum* (orange or yellow flower colours and dwarf) (Woodcock and Stearn, 1950).

At least five species of the Archelirion section - *L. alexandrae* Wallace*, L. auratum* Lindley*, L. nobilissimum*, *L. rubellum* and *L. speciosum* Thunb. - have been intercrossed (Beattie and White, 1993). These hybrids are referred to as Oriental hybrids. *L. japonicum* Thunb. was also used as parent for the Oriental hybrids (McRae 1998).

3.2 Commercial Breeding

Commercial breeding of lilies nowadays focus on the one hand on the development of new (interspecific) hybrids like LA, LO, OT, OA etc. for which embryo rescue techniques are needed and can be called "In vitro breeding" (see Section 2.2). On the other hand improvement of the Oriental, Asiatic and longiflorum-hybrid groups are made using classical techniques. This means normal hand pollinations are performed, seeds are sown and in the second (in case of longiflorum) or third (in case of Asiatic) or in the fourth year (in case of Orientals) after crossing seedlings can be selected. The size of these programmes vary from a few thousand to sometimes a half million seedlings per year. Selection for forcing quality is done under greenhouse forcing conditions. In the further processes of selection and propagation (using scaling or in vitro propagation), growth characteristics and disease resistances will be evaluated. Before deciding large-scale propagation, selections will be tested year-round and at different locations (preferably in different countries). The last 5-10 years of the around 100 lily cultivars which are commercialised with breeder's right (see 3.4) about half originates from interspecific crosses (in vitro breeding) and about half from classical breeding. It is obvious that the technique of *in vitro* breeding is mostly done on a smaller scale, but also that in advanced generations with improved fertility normal sowing procedures is or will be applied. The most important breeders on this moment are Vletter & Den Haan, Marklily, Worldflower, Royal van Zanten, Bischoff Tulleken, De Jong Lilies and Sande (Peterse, 2000). In the VS, Australia, New-Sealand, Germany and some other countries small companies and hobby breeders focus on lilies for the garden market. Depending on the climate these garden lilies originate from a wide range of species and species hybrids. The production is relative small. These cultivars are only registered without application for breeders'rights. A last effort of lily breeding is the seed propagated *L x formolongi* group. In Japan and Korea this type of lilies are grown on a relative small scale. For this purpose upfacing longiflorum hybrids (Raizan, Augusta) are developed by Daichii (Japan).

3.3 Molecular Breeding

Molecular markers linked to resistance traits, especially polygenic complex traits which are difficult to determine, can reduce the breeding and selection processes indirectly, by the so-called, marker assisted selection (MAS). Molecular markers have been used in lily for tracing parentages (Kazuhisa et al., 1998), identifying diversity (Wen and Hsiao, 2001) and for finding linked RAPD-markers to *Fusarium oxysporum* resistance in Asiatic hybrids (Straathof et al., 1996; Jansen, 1996). Straathof et al. (1996) studied the inheritance of *Fusarium* resistance resulting in at least 3 QTL's, which was a difficult trait to repeat during different years. Van Heusden et al. (2002) could repeat these results using AFLP-markers. TBV resistance was clearly a monogenic trait and could reliably to be mapped on linkage group 9. The closest linked marker, however, was still at a distance of about 10 cM. Despite the difficulty screening for *Fusarium* resistance, four significant QTLs were detected. For additional studies of the four QTLs and their individual contributions to the resistance, it will be necessary to convert the linked AFLP-markers to more robust PCR markers (Van Heusden et al., 2002).

Genetic transformation could be a powerful technique for the insertion of genes that do not exist in lily. Both *Agrobacterium* and microprojectile mediated transformation in lily have been accomplished by several researchers (Bino et al., 1992; Van der Leede-Plegt et al., 1992; Watad et al., 1998). The GUS gene was successfully transmitted into next generation. For future applications, a marker-free system is required. Research at PRI is currently focused on developing marker-free technology.

3.4 Lily Registration

Since 1960, at least 7000 cultivars have been cited in the RHS Lily register. There are two organisations that deal with lily cultivar registration either in the RHS lily register or in UPOV (Union for the Protection of New Varieties of Plants). Every year, over 100 new cultivars are registered in UPOV to obtain the breeder's right for commercialisation. In contrast about 200 cultivars are registered in RHS lily register in order to registrar the cultivar name, where the same cultivar name is not allowed. Therefore, RHS lily registration is based on non-profit activity, while breeder's right is primarily for the commercial basis. RHS lily registration does not require a fee, while UPOV registration requires qualification of the commercial value as a new cultivar for one year under standard forcing condition with control cultivars as a comparison. The breeder must provide enough bulbs (50 bulbs, 12-14cm in circumference) for the trial and the judging committee records distinctive characters for the new cultivar, e.g. disease resistance, physiological characteristics, mutation, uniformity and other phenotypic characters. If the first round of evaluation is not satisfactory, the committee can retest the selection. Additional information can be found at http://www.upov.int/eng/index.htm. Once registered in UPOV, the breeder's right is valid for 25 years, unless cancelled by the applicant.

3.5 History of the Pink Longiflorum 'Elegant Lady'

One of the successful examples of interspecific hybridization is 'Elegant Lady' (triploid, **LLR),** it was derived from crossing *L. longiflorum* and a **LLRR** F_1 interspecific hybrid. *L. longiflorum* possesses long, white, tubular shaped flowers with a pleasing fragrance, and *L. rubellum* has a very early flowering habit (ca. 35 days) and a pink flower with a pleasing fragrance. The **LR** hybrid has pink flower and early forcing habit. Since the **LR** F_1 hybrid is sterile, it was necessary to make a tetraploid by mitotic polyploidization to recover fertile pollen. The amphidiploid (**LLRR**) was fertile and was crossed with *L. longiflorum* to make the hybrid 'Elegant lady' which possess a tubular shaped pink *longiflorum* flower. Genomic *in situ* hybridization (GISH) confirmed that the **LLR** triploid was composed of two sets of *L. longiflorum* chromosomes and one set of *L. rubellum* chromosomes without any homoeologous recombination between parental chromosomes (Lim et al, 2000). The characters were intermediate between *L. longiflorum* and *L. rubellum* (Table 19-2). This plant exhibits elegant tubular-pink flower with very early flowering and a pleasing fragrance. This hybrid was named 'Elegant lady' and was commercially released in 2000.

Table 19-2. Characteristics of *L. longiflorum* and its F_1 and BC_1 hybrids.

Genotype	Plant height (cm)	Flower length (cm)	Forcing time (days)
'Gelria'	94.0	15.9	95.0
'Snow Queen'	116.3	18.2	96.7
LR (F_1)	47.9	11.2	51.7
LLR 'Elegant lady'	79.4	15.9	75.2

3.6 History of ALA Hybrids Derived from Meiotic Polyploidization

LA interspecific hybrids have been commercially available for a decade and currently about 30 cultivars are marketed. Most of them are triploids derived from backcrossing by mitotic or meiotic polyploidization. We analyzed one (**A**)**LA** hybrid 'Fangio' (2n=3x=36, triploid, **A** genome=24, **L** genome=12) and confirmed by GISH that there are many recombinant chromosomes between the **L** and **A** genomes (Figure 19-2f, g). Based on GISH analysis data, it was concluded that this hybrid was derived from spontaneous meiotic polyploidization (Lim & Van Tuyl, unpublished results).

3.7 Breeding Trends

Since 1960 about 7.000 lily cultivars has been registered since 1960 (Leslie, 1982; Mynett, 1996). Active lily breeding work started in Japan between the 1920's and 1940's, in Australia and New Zealand during the 1950's and 1960's and in the United States in the 1960's to 1970's. In the past 25 years lily breeding has been predominantly carried out in the Netherlands. Due to the release of many tetraploid clones from Plant Research International (formerly IVT, CPRO-DLO) to Dutch commercial breeders, the number of polyploid cultivars has steadily increased during the last decade (Van Tuyl et al., 1991; Schmitzer, 1991). With Asiatic hybrids many of the diploid cultivars have been replaced by tri- and tetraploid hybrids, while the **LA**-hybrids are mostly triploids. In contrast, all commercial *L. longiflorum* and Oriental hybrids are still diploid.

Interspecific polyploid cultivars have been produced by the recent employment of new hybridization techniques (Figure 19-2e,f,g,h,i). Examples include, respectively, **LA**-, **LO**-, **OA**- and **OT**-hybrids derived from *L. longiflorum* (**L**) and Asiatic hybrids (**A**), *L. longiflorum* (**L**) and Oriental hybrids (**O**), Oriental hybrids (**O**) and Asiatic hybrids (**A**), and Oriental hybrids (**O**) and Trumpet hybrids (**T**; Leucolirion section).

A major goal of lily breeding is to combine the three distinctive groups, so-called, Longiflorum-, Asiatic- and Oriental-hybrids. For example, **LA**-hybrids have become popular in the market over the past 10 years because of their flower shape and size, upright-facing flowers, sturdy-long stems, early flowering habit and a pleasing fragrance which was not available in Asiatic hybrids. By expanding interspecific hybridization between **LO**-hybrids and **OT**-hybrids, new types of interspecific hybrids will appear in the market together with **OA**-hybrids. **OLA**-hybrids derived from merging the three hybrid groups are also being developed as commercial cultivars.

3.8 Future Prospects

Looking to the future it is clear that after the increase of the Asiatic hybrids in the 1970's and 1980's, which was followed by the exotic and large flowered Oriental lilies, the time for more complex hybrids is here. The LA-hybrids, which have increased from 10 ha in 1995 to more than 590 ha in 2002, demonstrates this trend. The OT and OA hybrids are emerging. It will not take long before one group of hybrid lilies will be developed in which the different species and hybrid groups cannot be distinguished anymore. At the same time, breeding for resistance to virus, *Botrytis, Fusarium* will utilize improved systems like molecular assisted breeding and GISH-techniques.

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