Interspecific Hybridization in Lily (*Lilium*): Taxonomic and Commercial Aspects of Using Species Hybrids in Breeding

Ki-Byung Lim[1] • Rodrigo Barba-Gonzalez[2] • Shujun Zhou[3,4] • M. S. Ramanna[3] • Jaap M. van Tuyl[3*]

[1] School of Applied Biosciences, College of Agriculture and Life Science, Kyungpook National University, Daegu 702-701, Republic of Korea

[2] Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A.C. Biotecnología Vegetal, Av. Normalistas #800. Colinas de la Normal., Guadalajara, Jalisco, C.P. 44270, Mexico

[3] Plant Research International, Wageningen University and Research Centre, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

[4] College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, Zhejiang Province, China (310029)

Corresponding author: *** jaap.vantuyl@wur.nl**

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ABSTRACT

Lily comprises more than 80 species belonging to 7 sections. Within the sections cultivars bred from Sinomartagon, Archelirion, and Leucolirion are the most important in the commercial market. At this moment, the most promising breakthrough in lily breeding is the raising of new cultivar through interspecific hybridization with introgression of useful genetic traits from species or breeding materials belonging to the wild species which are not commonly used so far for commercial breeding. We have been crossing almost all different cross combinations and have succeeded in more than 28 cross combinations since 1980. The F_1 hybrids between the species have shown the intermediate phenotypic characteristics. Making interspecific or intergeneric hybrid is laborious but finding the clues, affecting to the most successful embryo formation, and growth is even more difficult and time consuming. Therefore research on the successful interspecific hybridization between distantly related species was carried out at several laboratories in the world. For the successful interspecific hybridization breeding, not only production of F_1 interspecific hybrids but also successful production of subsequent generations using interspecific hybrids to introgress valuable trait(s) is important. One of the promising crosses is of course between Orientals and several species such as *L. henryi*, Asiatics, and trumpet lilies. In this context, we demonstrate the possible methods and some valuable instances of the interspecific hybridization in lilies.

1. INTRODUCTION

Genus *Lilium* of the family Liliaceae comprises more than 80 species and these are divided into 7 sections (Comber 1949; Lighty 1968; de Jong 1974). The diversity of flower color, shape, fragrance and other phenotypic and physiological characteristics are found in the wild species which are dispersed in the Northern Hemisphere (10° to 60°), mainly in Asia, North America and Europe. Especially, China, Nepal, Korea and Japan are the gene centers of this genus around the world. In particular the Yunnan province is the famous habitat of the *Lilium* species in China.

All over the world the lily occupies a prominent place in horticulture as a cut flower, pot and garden plant. In 2005 more than 1.5 billion bulbs were produced around the world; in the first place in the Netherlands (in 2006 4161 ha; **Fig. 1**) together with Japan, the United States of America and more recently also in the Southern Hemisphere lands such as New Zealand and Chile. As a cut flower, lily is now ranked as the fourth most important crop in the Netherlands (statistical data from VBN 2006).

The breeding history of lily traces back to more than 200 years (Shimizu 1987). However, the real breakthroughs on lily breeding are only recent 50 years when assortments of Asiatic hybrids were bred (McRae 1998). To date, more than 9,465 lily cultivars have been registered (www.lilyregister.com). Among them, number of division I (Asiatic hybrids) is the most superior as compared to other divisions. Recently the number of cultivars involving interspecific hybrid has increased rapidly (**Fig. 1**). The reason is that interspecific hybrids that have more genetic variation and show distinct characteristics than the existing cultivars bred from the cultivars or species of each section alone.

The species within each section are easily crossable, their genomes are not much differentiated and the F_1 hybrids are fertile. Most of the diploid cultivars (2n=2x=24) in the three main groups of lilies, viz., Longiflorum, Asiatic and Oriental, are hybrids between closely related species

Abbreviations: AFLP, Amplified Fragment Length Polymorphisms; **CSM**, Cut Style Method; **OBF**, Oregon Bulb Farm

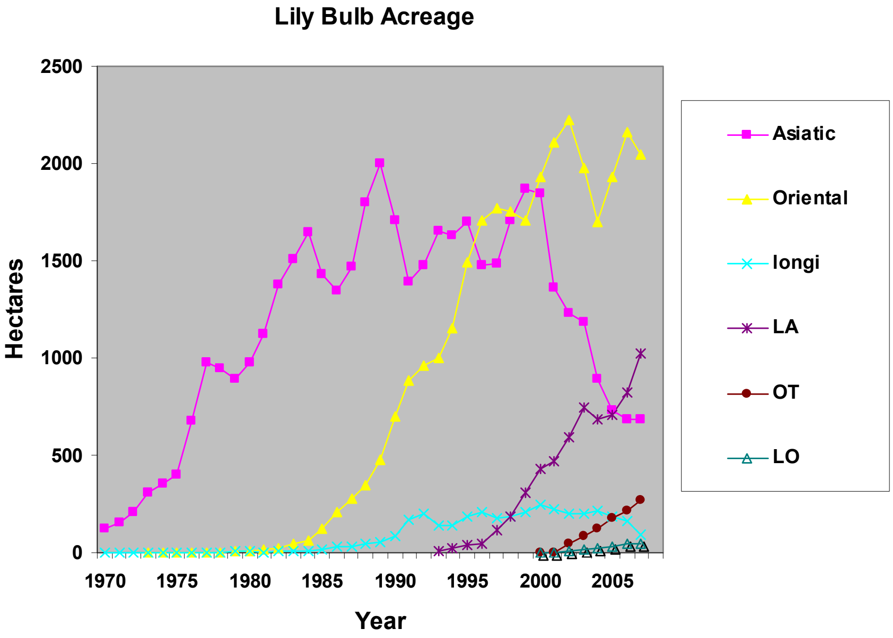

Fig. 1 Yearly changes of planting acreage of lily bulb production.

within the respective sections. These will not be referred to as 'hybrids' hence forth in this chapter. On the contrary, the species that belong to different sections are difficult to hybridize, their genomes are clearly differentiated and the F_1 hybrids are totally sterile (with very rare exceptions). These are indicated as hybrids. Because the species of the three main groups uniquely possess valuable horticultural characters and disease resistances, the main aim of lily breeding is to combine desirable traits from different sections into new cultivars. In addition, the species from other sections (i.e., *Lilium*, *Martagon* and *Pseudolirium*) might be potentially useful in the future. In this context, the crossing polygon (**Fig. 2**) clearly illustrates some of the successful crossings of the species as well as the taxonomic distances among the sections.

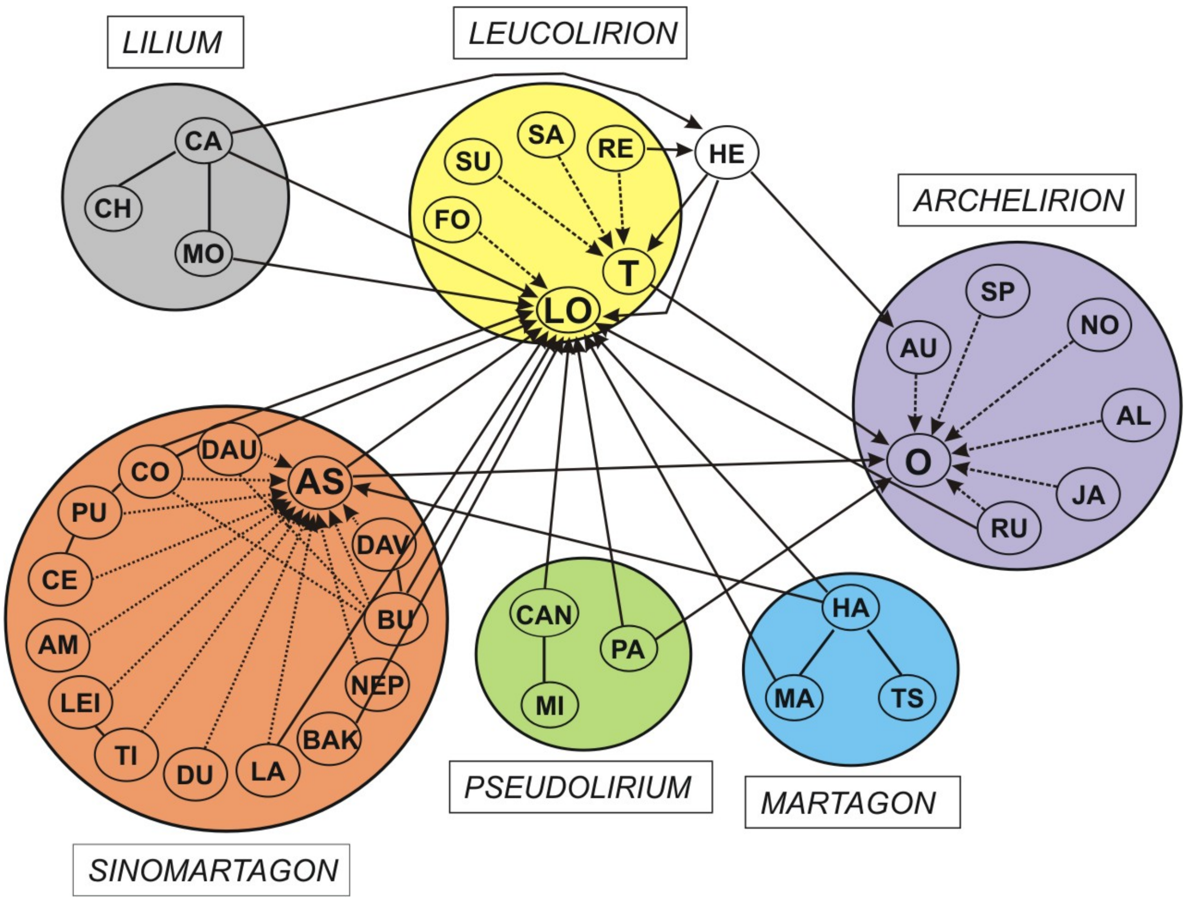

Fig. 2 A crossing polygon of the genus *Lilium* including all successful crosses of species between different sections developed at Plant Research International, The Netherlands. In this figure the connection between the Asiatic, Trumpet and Oriental hybrid groups (large ellipses) are shown by solid lines. In successful crosses between species (small circles) of different sections (large circles) the arrows point towards the female parent. Abbreviations: AL: *L. alexandrae*; AM: *L. amabile*; AS: Asiatic hybrids; AU: *L. auratum*; BAK: L. bakerianum; 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*; NEP: *L. nepalense*; NO: *L. nobilissimum*; O: Oriental hybrids; PA: *L. pardalinum*; PU: *L. pumilum*; RE: *L. regale*; RU: *L. rubellum*; SA: *L. sargentiae*; SP: *L. speciosum*; SU: *L. sulphureum*; T: Trumpet hybrid; TI: *L. tigrinum*; TS: *L. tsingtauense*.

2. TAXONOMIC ASPECTS OF INTERSPECIFIC HYBRIDIZATION IN LILY BREEDING

Both pre-fertilization and post-fertilization barriers hinder interspecific hybridization between different sections (Van Tuyl *et al*. 1991). Several techniques, such as the cut-style method (Asano and Myodo 1977a, 1997b), the grafted-style method and *in vitro* pollination techniques have been developed to overcome pre-fertilization barriers (Van Tuyl *et al*. 1991, 1992). However, even if fertilization is successful, post-fertilization barriers can hamper 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 1977ab; Asano 1978, 1980), ovary-slice culture and ovule culture have been developed to circumvent these barriers (Van Tuyl 1986; Van Tuyl *et al*. 1991). The first hybrid cultured from embryos was from a cross between *L. henryi* × *L. regale* which were rescued by Skirm (1942). Ascher (1973a, 1977b) succeeded in growing embryos of hybrid between *L*. 'Damson' × *L. longiflorum*.

Many cases of interspecific hybrids are recorded by Skirm (1942), Ascher (1973a, 1973b), Asano (1982a, 1982b, 1984), and Van Tuyl *et al*. (1991, 2000) with successful new combinations between sections of the genus *Lilium* by the use of various pollination and embryo rescue methods. Examples of intersectional hybrids include *L. longiflorum* (Leucolirion) × *L. monadelphum* (Lilium), *L. longiflorum* × *L. lankongense* (Sinomartagon), *L. longiflorum* × *L. martagon* (Martagon), *L. longiflorum* × *L. candidum* (Lilium), *L. henryi* (Leucolirion) × *L. candidum*, *L. longiflorum* × *L. rubellum* (Archelirion), *L. longiflorum* × Oriental hybrid, Oriental × Asiatic hybrid, *L. longiflorum* × *L. canadense* (Pseudolirium), Oriental hybrid × *L. pardalinum* (Pseudolirium), Asiatics × *L. hansonii* and *L. longiflorum* × *L. hansonii* among others. The crossing polygon given in **Fig. 2** shows the crossing compatibility within and between the sections achieved by our research group so far.

Based on the distances of relatedness by classical and molecular classification, the ability of interspecific hybridization could be measured. Numerous interspecific or intersectional crosses are carried out all over the world every year. Wide interspecific (intersectional) crosses are done mostly by cut style method (CSM) to circumvent the pre-fertilization barrier. Many parts of interspecific crosses are relying on the case by case, which means that some cross combinations are always successful but, not with different cultivar as one of the parent, although the type of crosses is the same. Probably there are two ways for the introgression by interspecific hybridization. Firstly, crossing between distantly related species belonging to different sections and move back to one parental genome by backcrossing. Secondly, crossing between closely related species within each section, and then further crossing to more distantly related species of a different section. In this case, we need more detail information on the relatedness of each species within the sections. There are several reports on phylogeny of *Lilium* species (Hayashi and Kawano 2000; Nishikawa *et al*. 2001, 2002). Nishikawa *et al*. (2002) reported the genetic distance within the section Archelirion where they found *L. rubellum* is closer to *L. auratum* var. *auratum* than to *L. auratum* var. *platyphyllum* which belongs to the same subgroup with *L. japonicum*, *L. nobilissimum*, *L. speciosum*, and *L. alexandrae*. Nishikawa *et al*. (2001) analyzed 64 *Lilium* taxa by molecular phylogenetic analysis. They concluded that section Leucolirion including *L. henryi* is closer to the section Archelirion. Both *L. nanum* and *L. oxypetalum* are inserted between sections Archelirion and Pseudolirium. Martagon species are connecting a group of major Sinomartagon section and the other group containing *L. duchartrei*, *L. lankongense*, *L. amoenum*, *L. wardii* and *L. taliense*. It seems that overall relationships among *Lilium* species coincide with the results of interspecific crosses made by us.

Production of F_1 hybrid of interspecific crosses seems to be not a major problem. The problem is keeping the fertility of both male and female parents in the subsequent crossings. Because small scale crosses are not enough to produce commercially competitive cultivars, breeders need more crosses and progenies from F_1 interspecific hybrids, either backcrossing to the pollen parent or embryo sac parent. The detailed methods to overcome F_1 sterility are described by Barba-Gonzalez *et al*., in the other chapter of this series. **Fig. 3** shows the genetic flow of certain group of *Lilium* through back crossing (in this case, between *L. longiflorum* and Asiatic group, and *L. longiflorum* and Orientals). The genome composition of the interspecific F_1 hybrids is 50:50 between two parental species. However, the genome composition of subsequent hybrids will be close to one of the recurrent parent. When the *2n* gametes are used for back-

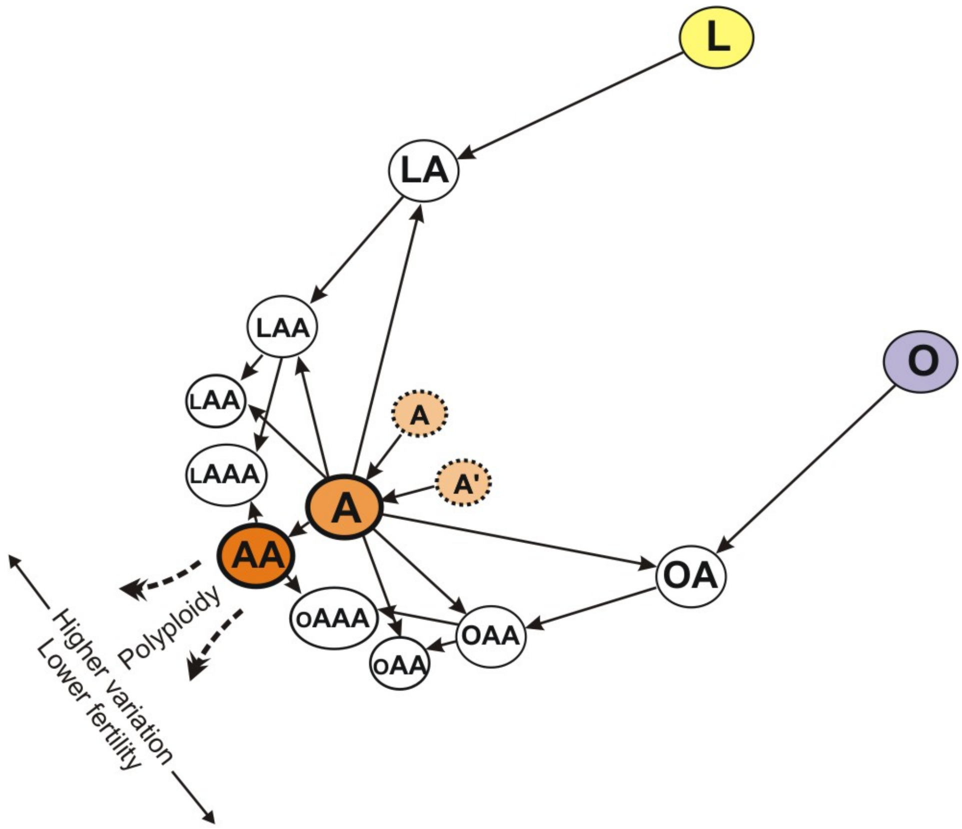

Fig. 3 Schematic genetic flows by interspecific hybridization of *Lilium* species between *L*. Orientals and *L*. Asiatics, and *L. longiflorum* and *L*. Asiatics. The first step of interspecific hybridization in this figure is between *L. longiflorum* and Asiatics (**LA**) and Orientals and Asiatics (**OA**), respectively. **LA** and **OA** hybrids then backcrossed to Asiatics to produce **LAA** and **OAA** hybrids. Asiatic hybrids (designated as A in solid circle) were derived from crosses of unknown Asiatic species (A or A' in dotted circles) and later on became tetraploid (designated as AA) by chromosome doubling.

crossing, the ploidy level of the subsequent progeny would be higher but the fertility is decreased, so that further crossing will be more difficult. We have to consider three concepts in interspecific hybridization; 1) maintain stable ploidy level that is as euploid as possible, 2) ploidy level should be as low as possible so that, on further crossing, both tri- or tetra-ploid level can be achieved and 3) keeping the fertility of male and female parent as high as possible. It may be pointed out in this context that when the cultivars are highly sterile, the future lily breeding using certain intersectional hybrids will be more difficult to imitate or to make similar ones.

At this moment, most of the interspecific (intersectional) cultivars are derived from crosses by using *2n* gametes from the F_1 hybrids. As was pointed out earlier, such crosses give rise to triploids that are mostly sterile and difficult to use as parents. On the other hand, if the F_1 hybrids that produce fertile *n* gametes are detected, they can be valuable for the introgression breeding because the ploidy of the subsequent progenies can be maintained at the diploid level (Zhou 2007). Fertile *n* gametes have several benefits. They can be directly backcrossed to one of the parents without increasing the ploidy level; the fertility of subsequent progenies is not affected; selection of the desirable genotypes can be achieved at the diploid level and, finally, inheritance of characters can be monitored at the diploid level. Fortunately, we have found fertile F_1 interspecific hybrids that produce *n* gametes. Some genotypes of **LA** hybrids have been found to form 12 bivalents in a considerable number of pollen mother cells and also produce low frequencies of haploid gametes together with *2n* gametes. From these genotypes it was possible to produce BC_1 progenies that possess diploid (2n=2x=24) as well as triploid (2n=3x=36) chromosome numbers (Zhou 2007). The occurrence of normal chromosome pairing and formation of haploid gametes in F_1 hybrids crossed between taxonomically distant species is a relatively rare phenomenon but the instance of **LA** hybrids is by no means unique. For example, both interspecific and intergeneric hybrids have been reported to produce haploid gametes in the past: the interspecific hybrids of *Allium cepa* x *A. fistulosum* (Emsweller and Jones 1945; Khrustaleva and Kik 2000), intergeneric hybrids, *Lycopersicon esculentum* x *Solanum lycopersiciodes* (Chetelat *et al.* 1997) and *Festuca* x *Lolium* (Jauhar, 1993). In case of using *2n* gametes rather than *n* gametes, the ploidy level will be increased unintentionally by the breeders. Higher ploidy level (triploid) in the subsequent progenies is more problematic to make crosses further. The progenies in that case might have odd number of ploidy level (3x, 5x) or aneuploidy, and dramatic decrease of fertility. The polyploid *Lilium* such as penta- and hexaploid showed normally delayed growth of stem and malformation of organs and tissues. Therefore, the ideal interspecific crosses are using viable *n* gamete containing homoeologous recombinations between parental genomes.

3. COMMERCIAL ASPECTS OF INTERSPECIFIC HYBRIDIZATION IN LILY BREEDING

After World War II, Jan de Graaff, the owner of Oregon Bulb Farm (OBF), a devoted lily breeder, attempted to make new lily hybrids. Until that time lilies sold on the market were strains which were propagated by seed or clones from species. OBF released “Enchantment” in 1947, the first Mid-Century hybrid. Other Asiatic hybrids by OBF were ‘Tabasco’, ‘Showboat’, ‘Destiny’ and ‘Sterling Star’. Only since the 1970’s lily became an important cut flower in the commercial market. ‘Sans Souci’ was one of the Oriental pot lily bred by Leslie Woodriff in the mid fifties. He also bred the famous cultivars such as ‘Black Beauty’, ‘Stargazer’ and ‘Le Reve’. In the early 1980s the Dutch lily breeding companies became dominant. Since 1975 in lily more than 2,500 applications for Breeders Right were registered for 95% by the Dutch breeding industry. The most important lily breeding companies of the last 15 years are Vletter & Den Haan, Mak Breeding (took over Bischoff Tulleken in 2005), World Breeding, Mark Lily, Royal van Zanten and De Jong Lilies. Piet Schenk, almost 30 years’ lily breeder for Bischoff Tulleken, played a prominent role in the growth of lily breeding and production in the Netherlands. Over 100 new cultivars each year have been released on the market during the last two decades. The new cultivars derived from interespecific hybridization are increasing year by year. The first interspecific hybrid in the market were the **LA** hybrids (*Lilium longiflorum* x Asiatic hybrid): The genome composition of **LA** hybrids is indeed **ALA** or **LAA**, all of them are triploid, since they are derived from back crossing F_1 hybrid of **LA** hybrid to an diploid Asiatic hybrid. Although several desirable characteristics such as fast bulb growth, healthy leaves, strong stem, large flower and fragrance, they also possessed undesirable characters such as flower malformation, weak petals and unclear flower color. Focusing on Oriental hybrid breeding was the most important aim of the Dutch breeding companies in the 1990’s. One of outstanding cultivars among many was ‘Sorbonne’ after ‘Star Gazer’ (both pink color) and ‘Siberia’ following ‘Casablanca’ (both white). In the year 2006, the total bulbs production acreage of lily in the Netherlands was 4,161 ha. Among them **LA** hybrids, **LO** hybrids, **OA** hybrids, and **OT** hybrids occupy ca. 780 ha, 39 ha, 1.2 ha, and 187 ha, respectively. Altogether more than 24% of total lily bulb production occupies interspecific hybrids and over 14% increased as compared 2005. It means that lily grows rapidly to triploid interspecific assortments (**Fig. 1**). New types of interspecific hybrid are introduced in recent years, especially **LO** and **OT** hybrids. Oriental hybrids are enriched with good characteristics such as flower size, flower color and attractive fragrance. Examples are ‘Conca D’or’ and ‘Robina’, both are **OT** hybrids between Oriental and Trumpet lily. Most of those interspecific hybrids cultivars including **LA**, **LO** and **OT** are derived from back crosses to Asiatics or Orientals. They are triploid BC_1 progenies with a genome composition **ALA**, **LLO** and **OOT**, respectively. *L. rubellum* has several good traits such as early flowering, fragrance, pink color (Lim *et al.* 2000). One of the successful interspecific crossings was ‘Elegant lady’ of which genome composition is **LLR** derived from backcrossing between tetraploid *L. longiflorum* and tetraploid **LR** hybrid. This hybrid cultivar shows early flowering, fascinating pink, and *longiflorum* flower shape.

4. FUTURE PROSPECTS

It has been well recognized that some of the important horticultural characters and disease resistances are present in species belonging to different sections. Thus, for example, Longiflorum hybrids (Leucolirion) have long trumpet shaped flowers with characteristic fragrance; the Asiatic hybrids (Sinomartagon) which are known for early flowering also possess a wide range of flower colours and most importantly, resistance against *Fusarium oxysporium* as well as some viruses. The Oriental hybrids (Archelirion) are known for their resistance against *Botrytis elliptica* as well as variation for flower colours (**Table 1**). Some of these characters from the three sections can be more efficiently combined into new cultivars when they can be localized on the respective chromosome or linkage groups. Such knowledge can be helpful for monitoring their presence, or absence, in the segregating progenies and thus enhance the efficiency of selection. For the construction of molecular linkage maps, amplified fragment length polymorphisms (AFLP) have already been successfully used in lily (unpublished). These maps are particularly attractive in lily because of the large chromosomes, together with their amenability for GISH and fluorescent in situ hybridization (FISH), so that

Table 1 Characteristics and some useful traits for the commercialization of *Lilium* species.

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

physical maps can be constructed. In view of the availability of these techniques, even in spite of the difficulties for introgression of characters from taxonomically distant species, success might be achieved in lily because more rational breeding methods can be applied. Lily breeding through interspecific hybridization in recent year has greatly increased. Interspecific hybridization techniques can facilitate introgression of the useful traits within short time. The speed of new cultivar creation is now faster. Lily breeding companies developed **LA**, **LO** and **OT**, and also the acreage of those cultivars have increased rapidly. Interspecific hybridization will give abundant chances to make new cultivars between species of different sections that were not used so far. For example, the material from *L. nepalense*, *L. bakerianum* and L. martagon section would be the one for the creation of new cultivar type. There are of course limitations on the creation of new type of interspecific hybrid that might be due to its sterility and limited chances of homoeologous recombinations. However, we have accumulated numerous crossing data from every interspecific cross and use it for the development of new breakthroughs in the field of introgression breeding.

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