

Review Paper on Lily Breeding**Rija Rehman¹**¹ PMAS Arid Agriculture university Rawalpindi***DOI: <https://doi.org/10.63163/jpehss.v3i3.1138>*****Abstract:**

Lilies (Liliaceae family) are economically very important bulbous ornamental crop. Cultivars of this crop are used as cut flowers and garden plants, and are now indispensable for horticultural use. Genus *Lilium* of the family Liliaceae consists of more than 80 species and these species are divided into 7 sections (Comber 1949; Lighty 1968; de Jong 1974). The diversity of flower shape, colour, fragrance and other physiological and phenotypic characteristics are found in its wild species which are dispersed in the Northern Hemisphere (10 to 60 °), mainly in North America and Europe, Asia. Especially, China, Japan, Korea and Nepal are the gene centres of this genus around the whole world. Yunnan province is the famous habitat of the *Lilium* species in China in particular. This crop has been subjected to extensive interspecific hybridization followed by selection. Additionally, spontaneous polyploidization has played a role in its evolution. In lilies, there is a tendency to replace diploids with polyploid cultivars. The introduction of molecular cytogenetic techniques such as genomic in situ hybridization (GISH) permitted the detailed studies of genome composition in lily. In addition, this review presents the information about the future development regarding breeding in lilies.

Introduction:

Around the world, lily is considered as a prominent plant 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 Chile and New Zealand. 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 is observed in 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 superior as compared to other divisions. There are seven sections of genus *Lilium* in which 80 species are present. The sections of *Lilium*, according to Comber's classification (1949) are Martagon, Pseudolirium, Sinomartagon, Leucolirion, Liriotypus, Archelirion and Daurolirion. In 1974 De Jon revised this classification and the sections according to his classifications are as follows: Martagon, Pseudolirium, *Lilium*, Archelirion, Sinomartagon, Leucolirion and Oxypetala. Some phylogenetic studies in the genus *Lilium* were performed by Nishikawa (1990, 2007) by using sequences of the internal transcribed spacer region of nuclear ribosomal DNA. The species position was improved in Sinomartagon.



Phylogeny of liliun species:

Phylogeny of *Lilium* species Lilies (the species of genus *Lilium*) are distributed throughout the temperate and cold regions of the Northern Hemisphere. Approximately 100 known species are known. Based on morphological characters lilies are classified by several authorities (Baker 1871, Endlicher 1836, Wilson 1925). Genus *Lilium* was classified by Comber (1949), based on 15 morphological characters, and it is considered to be the most authoritative. He classified the genus into seven sections by each, *L. martagon*; section *Pseudolirium*, by *L. philadelphicum*; section *Liriotypus*, lectotype; section *Martagon*, by *L. auratum*; section *Sinomartagon*, by *L. candidum*; section *Archelirion*, by *L. davidii*; section *Leucolirion*, by *L. longiorum*; and section *Daurolirion*, by *L. dauricum*. Sections *Pseudolirium*, *Sinomartagon*, and *Leucolirion* were divided into four (2a–2d), three (5a–5c), and two (6a and 6b) subsections, respectively. However, there is some dispute regarding Comber's classification (Haw 1986), and molecular phylogenetic estimations have provided more precise relationships among *Lilium* species. Nishikawa et al. (1999) reported first comprehensive molecular phylogeny, who inferred phylogenetic relationships among 55 species of *Lilium* and two relatives from nucleotide sequence variations in the internal transcribed spacer (ITS) regions of 18S–25S nuclear ribosomal DNA.

Cross Breeding in lilies:

To create a new lily with characteristics of each parent plant hybridizers cross-breed lilies (*Lilium* spp.). Dutch lily breeders began a revolution, cross-breeding among the four kinds of lilies – longiflorum, Asiatic, Oriental and trumpet. With up to 70 new lily varieties produced every year, according to the University of Illinois Extension website, lilies are available in a large variety of shapes, colours and other characteristics, in addition with improved disease resistance.



Breeding Procedure

Identifying the Reproductive Organs:

Before cross-breeding lilies you should identify the reproductive parts of lilies necessarily. Arising from the lily flower's centre are stalked organs. They are the stamens, which is the male part, and the pistils, which are the female parts. At the top of the pistil, stigma is present, which is the pollen attractor; it is attached by a hollow, slender style to the lily ovary, where seeds develop. There are several stamens surrounding the pistil. At the top of hair-like structures, called filaments, are pollen-filled anthers.

Choosing Parent Plants:

By selecting two lily varieties, cultivars or species cross-breeding lilies begin with. Then one plant is selected to be the seed-bearing plant or pod parent, and the other is selected to be the pollen donor, or pollen parent. Although the seed-bearing plant has a short period of receptivity to pollen, dry pollen can be stored for two to three months at least. The process to store pollen begins with placing a desiccant, such as calcium chloride, in the bottom of a container and holding it in place with cotton. Place anthers severed from a lily plant on top of the cotton, and put another layer of cotton on top of the anthers. Packed in that manner, pollen can be preserved in a refrigerator or shipped to another location.

Pollinating the Pod Parent:

Pollinating the pod parent has two goals. First is to remove pollen from the pod parent, which may lead to self-pollination. Second is to remove pollen from all other plants surrounding pollen parent, except the selected pollen parent. When a pod parent shows sign of flower bud opening, gently pull its petals apart, and snip out the anthers with the help of small scissors that were dipped in isopropyl alcohol for sterilization. Cover the flower bud with cotton bag to protect its injured parts from contamination. After the flower opening, the stigma exudes a sticky substance, showing its receptivity to fertilization with pollen. Using sterilized small scissors and tweezers, exclude a ripe anther from a pollen plant. A ripe anther has pollen granules spilling out of its sac. Rub the anther over the sticky stigma of pod parent until pollen granules adhere to the stigma. Cover the flower with cotton bag until the flower petals begin to drop and the pod parent's ovary swells.



Collecting Seeds and Growing Little Lilies:

Once the ovary swells into a mature seedpod, and its colour turns brown, exclude the pod from the plant before the pod splits open. Until splits open, the seedpod needs to be in a dry place, naturally when its seeds are ripe. Either sow the seeds in a garden that has well-drained, fertile soil with a pH level of 6.5 to 7.0 or sprout the seeds in a germinating medium under fluorescent lights. The temperature must remain 65 to 70 degrees Fahrenheit in the germinating environment.

For future reference keeping records of the names of the parent plants is important.

Breeding methods in Lilies:

Interspecific crosses:

The number of cultivars involving interspecific hybrid recently has increased rapidly as interspecific hybrids show distinct characteristics and have more genetic variation than the species of each section alone or existing cultivars bred from the cultivars. The species of same section are easily crossable, their genomes are not much differentiated and they have fertile F1 hybrids. Most of the diploid cultivars ($2n=2x=24$) in the three major groups of lilies, viz., Longiflorum, Oriental and Asiatic are hybrids between closely related species within the respective sections. On the contrary, the species that belong to different sections are difficult to hybridize, their F1 hybrids are totally sterile (with very rare exceptions) and genomes are clearly differentiated. These are symbolized as hybrids. Because the species of the three major groups uniquely have valuable horticultural characters and disease resistances, the main purpose of lily breeding is to combine desirable traits from different sections into new cultivars. In the future, the species from other sections (i.e., *Lilium*, *Martagon* and *Pseudolirium*) might be potentially useful. In this context, the crossing polygon clearly illustrates some of the successful crossings of the species as well as the taxonomic distances among the sections.

Intersectional Hybridization:

Due to the development of embryo and pollination polyploidization and rescue methods (Asano, 1978, 1980; Myodo and Asano, 1977a,b; Barba-Gonzalez, 2005; Barba-Gonzalez et al., 2004,

2005, 2006; Lim, 2000; Van Tuyl and Lim, 2004, 2006; Lim et al., 2001, 2004, 2007; Boon and

Van Tuyl, 1997; Van Tuyl et al., 1989, 1991, 1992, 2002, 2003), a range of intersectional hybrids were introduced. The first group of intersectional hybrids, the Longiflorum × Asiatic hybrids (LA hybrids), was developed after two decades of the Oriental hybrids, by interspecific hybridization between hybrids of different sections, the Asiatic and the Longiflorum group. The ploidy level of these lilies is triploid, since they are backcrosses from a chromosome doubled F1 LA hybrid with an Asiatic hybrid. This group became more important than the Asiatic hybrid group and have in large part essentially replaced them in the industry during last ten years. Similarly, other intersectional crosses, mostly triploid hybrids (which possess a superior plant habit and growth vigour) have been bred, such as the OT (Oriental × Trumpet), LO (Longiflorum × Oriental), and OA (Oriental × Asiatic) hybrids. Like the LA hybrids have

replaced the Asiatic hybrids and the LO- hybrids the Longiflorums, it can be expected that in the future the Orientals will (partly) be replaced by the OTs. Besides, the development of the main seven hybrid groups (O, L, A, T, LO, LA and OT) a number of other species have been bred successfully. Some species that are bred successfully but not included in the commercial assortment yet are *L. candidum* (section *Lilium*) crosses with *L. henryi* and with *L. longiflorum*. Many successful crosses were made with Longiflorum as mother and *L. auratum*, *L. dauricum*, *L. henryi*, *L. kelloggii*, *L. lankongense*, *L. lophophorum*, *L. monadelphum*, *L. bakerianum*, *L. canadense*, *L. concolor*, *L. pardalinum*, *L. pumilum*, *L. martagon*, *L. hansonii*, *L. nepalense* and *L. sempervivoideum* as father. With Oriental as mother, crosses were made with *L. nepalense*, *L. dauricum* and *L. pardalinum*. Some of these new combinations can be expected in the assortment for the future.

Future developments:

As breeding plays an important role in the development of the assortment. In the last 15 years due to advanced breeding techniques, the assortment has totally changed dramatically. Whereas Asiatic hybrids have been replaced by LA-hybrids (in 2010 for 75%), a similar process is visible with the OT-hybrids which will probably replace the Orientals (in 2010 for 15%) and with the LO-hybrids which will likely replace the Longiflorums (in 2010 for 50%). All these new hybrids are triploid inter-sectional hybrids. Finally for the future, besides the development of the main seven hybrid groups (O, A, L, T, LA, LO and OT), a number of other species have been successfully used. Species which were crossed successfully with a high potential to be used in new hybrid groups are: *L. pardalinum* and *L. canadense* (section *Pseudolirium*), *L. martagon* and *L. hansonii* (section *Martagon*), *L. candidum* and *L. monadelphum* (section *Lilium*), *L. nepalense* and *L. bakerianum* and *L. henryi*. Techniques which are being used to increase the introgression of characters from wild species to the assortment are molecular cytogenetics (Lim, 2000; Barba-Gonzalez, 2005; Zhou, 2007; Khan, 2009) and molecular assisted breeding (Shahin et al., 2009). Recently, comprehensive genetic maps of 2 lily populations including 6 QTLs for Fusarium-resistance were published (Shahin et al., 2010). While genetic transformation is a technique investigated in lily for more than 25 years (Cohen, 2011), it is used in genetic studies but until now transformation has played no role in the development of new cultivars. In the near future, knowledge of DNA-sequences linked to horticultural traits will play a key role in bridging the genomes of lily. This will enable combinations of favourable complex traits from the wide genetic variation available to breeders.

References:

- Asano, Y. 1978. Studies on crosses between distantly related species of lilies. III. New hybrids obtained through embryo culture. *J. Japan. Soc. Hort. Sci.* 47:401-414.
- Asano, Y. 1980. Studies on crosses between distantly related species of lilies. V. Characteristics of newly obtained hybrids through embryo culture. *J. Japan. Soc. Hort. Sci.* 49:241-250.
- Asano, Y. and Myodo, H. 1977a. Studies on crosses between distantly related species of lilies. I. For the intrastylar pollination technique. *J. Japan. Soc. Hort. Sci.* 46:59-65.
- Asano, Y. and Myodo, H. 1977b. Studies on crosses between distantly related species of lilies. II. The culture of immature hybrid embryos. *J. Japan. Soc. Hort. Sci.* 46:267-273.
- Barba-Gonzalez, R. 2005. The use of 2n gametes for introgression breeding in Oriental × Asiatic lilies. Ph.D.-thesis, Wageningen University, 111p.
- Barba-Gonzalez, R., Lokker, B.H., Lim, K.B., Ramanna, M.S. and Van Tuyl, J.M. 2004. Use of 2n gametes for the production of sexual polyploids from sterile Oriental × Asiatic hybrids of lilies (*Lilium*). *Theor. Appl. Genet.* 109:1125-1132.
- Barba-Gonzalez, R., Lim, K.-B., Ramanna, M.S., Visser, R.G.F. and Van Tuyl, J.M. 2005. Occurrence of 2n gametes in the F1 hybrids of Oriental × Asiatic lilies (*Lilium*): Relevance to intergenomic recombination and backcrossing. *Euphytica* 143:67-73.

- Barba-Gonzalez, R., Van Silfhout, A.A., Ramanna, M.S., Visser, R.G.F. and Van Tuyl, J.M. 2006. Progenies of allotriploids of Oriental × Asiatic lilies (*Lilium*) examined by GISH analysis. *Euphytica* 151:243-250.
- BKD. 1970-2010. Beplante oppervlakten Bloembollen:lelie. Cohen, A. 2011. Biotechnology in lilies - dreams vs. reality. *Acta Hort.* 900:149-160.
- Comber, H.F. 1947. A new classification of the *Lilium*. *Lily Yearbook*, Royal Hort. Soc. London 15:86-105. De Jong, P.C. 1974. Some notes on the evolution of lilies. *Yb. North Amer. Lily Soc.* 27:23-28.
- De Graaff, J. 1970 Looking backwards. *Yb. North Amer. Lily Soc.* 23:7-20.
- Emsweller, S.L. and Brierley, P. 1941. Colchicine - induced tetraploidy in *Lilium*. *J. Hered.* 31:223-230.
- Khan, M.N. 2009. A molecular cytogenetic study of intergenomic recombination and introgression of chromosomal segments in lilies (*Lilium*). Ph.D.-thesis, Wageningen University, 121p.
- Leslie, A.C. 1982. The international lily register. 3rd edition, including 17 additions (1984-1998) The Royal Horticultural Society, London www.rhs.org.uk/plants/registration.
- Lim, Ki-Byung. 2000. Introgression breeding through interspecific polyploidisation in 226 lily: a molecular cytogenetic study. Ph.D. thesis, Wageningen University, 120p.
- Lim, K.B., Chung, J.D., Van Kronenburg, B.C.E., Ramanna, M.S., De Jong, J.H. and Van Tuyl, J.M. 2000. Introgression of *Lilium rubellum* Baker chromosomes into *L. longiflorum* Thunb.: a genome painting study of the F1 hybrid, BC1 and BC2 progenies. *Chromosome Res.* 8:119-125.
- Lim, K.B., Ramanna, M.S., De Jong, J.H., Jacobsen, E. and Van Tuyl, J.M. 2001. Indeterminate meiotic restitution (IMR): a novel type of meiotic nuclear restitution mechanism detected in interspecific lily hybrids by GISH. *Theor. Appl. Genet.* 103:219-230.
- Lim, K.B., Barba-Gonzalez, R., Ramanna, M.S., Shen, T.M. and Van Tuyl, J.M. 2004. Occurrence of SDR 2N- gametes in *Lilium* hybrids. *Breeding Science* 54:13-18.
- Lim, Ki-Byung and Van Tuyl, J.M. 2006. Lily, *Lilium* hybrids. p.517-537. In: N.O. Anderson (ed.), *Flower breeding and genetics: issues, challenges and opportunities for the 21st century*, Chapter 19. Kluwer Academic Publishers, Dordrecht.
- Lim, Ki-Byung and Van Tuyl, J.M. 2004. Elegant lady a pink longiflorum lily cultivar suitable for cut flower forcing. *Korean J. Breed.* 36:123-124.
- Lim, Ki-Byung, Barba-Gonzalez, R., Zhou, S., Ramanna, M.S. and Van Tuyl, J.M. 2008. Interspecific hybridization in lily (*Lilium*): taxonomic and commercial aspects of using species hybrids in breeding. p.146-151. In: J.A. Teixeira de Silva (ed.), *Floriculture, ornamental and plant biotechnology: advances and topical issues* (1st Ed., Vol. 5), Global Science Books, Isleworth, UK.
- McRae, E.A. 1998. *Lilies: a guide for growers and collectors*, Timber press, Portland, Oregon, 392p.
- Nishikawa, T. 2007. Molecular phylogeny of the genus *Lilium* and its methodical application to other taxon. Thesis United Graduate School of Agricultural Science Tokyo University of Agriculture and Technology, Japan, 95p.
- Nishikawa, T., Okazaki, K., Uchino, T., Arakawa, K. and Nagamine, T. 1999. A molecular phylogeny of *Lilium* in the internal transcribed spacer region of nuclear ribosomal DNA. *J. Mol. Evol.* 49:238-249.
- Rockwell, F.F., Grayson, E.C. and De Graaff, 1961. *The complete book of lilies*, New York, 352p.

- Shahin, A., Arens, P., Van Heusden, S. and Van Tuyl, J.M. 2009. Conversion of molecular markers linked to Fusarium and virus resistance in Asiatic lily hybrids. *Acta Hort.* 836:131-136.
- Shahin, A., Arens, P., Van Heusden, A.W., Van der Linden, G., Van Kaauwen, M., Khan, N., Schouten, H., Van de Weg, E., Visser, R. and Van Tuyl, J.M. 2010. Genetic mapping in *Lilium*: mapping of major genes and QTL for several ornamental traits and disease resistances. *Plant Breeding*, in press.
- Van Tuyl, J.M., De Vries, J.N., Bino, R.J. and Kwakkenbos, A.A.M. 1989. Identification of 2n pollen producing interspecific hybrids of *Lilium* using flow cytometry. *Cytologia* 54:737-745.
- Van Tuyl, J.M., Van Diën, M.P., Van Creijl, M.G.M., Van Kleinwee, T.C.M., Franken, J. and Bino, R.J. 1991. Application of in vitro pollination, ovary culture, ovule culture and embryo rescue for overcoming incongruity barriers in interspecific *Lilium* crosses. *Plant Science* 74:115-126.
- Van Tuyl, J.M., Meijer, H. and Van Diën, M.P. 1992. The use of oryzalin as an alternative for colchicine in in-vitro chromosome doubling of *Lilium* and *Nerine*. *Acta Hort.* 325:625-630.
- Van Tuyl, J.M. and Boon, E. 1997. Variation in DNA-content in the genus *Lilium*. *Acta Hort.* 430:829-835. Van Tuyl, J.M., Lim, K.B. and Ramanna, M.S. 2002. Interspecific hybridization and introgression. p.85-103.
- In: A. Vainstein (ed.), *Breeding for ornamentals: classical and molecular approaches*, Kluwer Academic Publishers, Dordrecht/Boston.
- Van Tuyl, J.M., Chung, M.Y., Chung, J.D. and Lim, K.B. 2002. Introgression studies using GISH in interspecific *Lilium* hybrids of *L. longiflorum* × Asiatic, *L. longiflorum* × *L.*