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Breeding perspectives of Liliums

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Abstract
Liliums are a broad group of perennial, leafy stemmed bulbous ornamentals which are classified into different types. Commercial varieties have common problems that reduce the marketability and thus becomes a major concern to the breeders which holds the following points as breeding objectives viz: Resistance to diseases, tolerance to physiological disorders like bud blasts, year round forcing ability, Flower longevity, ethylene resistance, pollen less cultivars, improved fragrance & flower colour, increased floral bud count on small sized bulbs**,** controlled plant height and tolerance to sub optimal growing conditions. In this paper an attempt has been made to review the details of conventional and non conventional breeding methods to improve the germplasm keeping the above breeding objectives in view.

Keywords: Conventional, non-conventional, breeding, germplasm

Introduction
The genus *Lilium* is in the family *Liliaceae* and comprises over 80 species (Comber,1949; De Jong, 1974) [4, 6]. The native *Lilium* species are spread over the Northern Hemisphere .Currently *Lilium* ranks second among bulbous crops in cut flowers in international market.

Botanical Classification
- Genus *Lilium*
- Family *Liliaceae*
- Sub family Lilioideae
- Tribe Lilieae
- Sub class Monocotyledonae
- Common Name Lily
- Chrom no. (2n=2x=24) Diploid
- Native Northern Hemisphere upto South Canada, Siberia, Florida and Nilgiri mountains of India

Description
Lilium are generally perennial, leafy-stemmed, bulbous herbs and can generally divided into either whorled or scattered leaf types. *L.martagon, L.canadense & L.hansonii* are examples of whorled-leaf types. While the majority of the Asiatics, from which modern green-house hybrids have been derived are scattered leaf types (Daniels, 1986) [5]. Basic structure of the lily plant consists of roots, stem, flowers and leaves. The storage organ can be rhizomes or bulbs. Depending on the point of origin, they also form bulblets (below ground) and bulbils (above ground).

Classification
The RHS classification as reported in *The International Lily Register* (RHS 1982-2002) 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. longiflorum*
IV. Hybrids of American species

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

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

Breeding Objectives

- Resistance to *Fusarium oxysporum*, *Pythium* & viral diseases.
- Tolerance to physiological disorders like bud blasts.
- Year round forcing ability.
- Flower longevity.
- Ethylene resistance.
- Pollen less cultivars.
- Improved fragrance & flower colour.
- Increased floral bud count on small sized bulbs
- Controlled plant height.
- Tolerance to sub optimal growing conditions.

Breeding Methods

A. Conventional

1. Introduction
   - Primary
   - Secondary
2. Selection
3. Hybridization: Interspecific
   - Intraspecific
   - Intergeneric
4. Mutation

B. Non-Conventional

1. Polyploidy breeding
2. Somatic embryogenesis
3. Genetic engineering
4. *In-vitro* techniques
5. Rescue method as Embryo, Ovary slice & Ovule culture

Interspecific Hybridization

Lim *et al*. (2000) [8] discussed the important horticultural characters are present in the different *Lilium* species. Commercial important characters include:

- Resistance to diseases such as bulb rot (*Fusarium*), *Botrytis* and several viruses (TBV, LSV and LVX)
- Phenotypic characteristics

Flower shapes
Sturdy stems
New colours and
Fragrance

1. Physiological characteristics

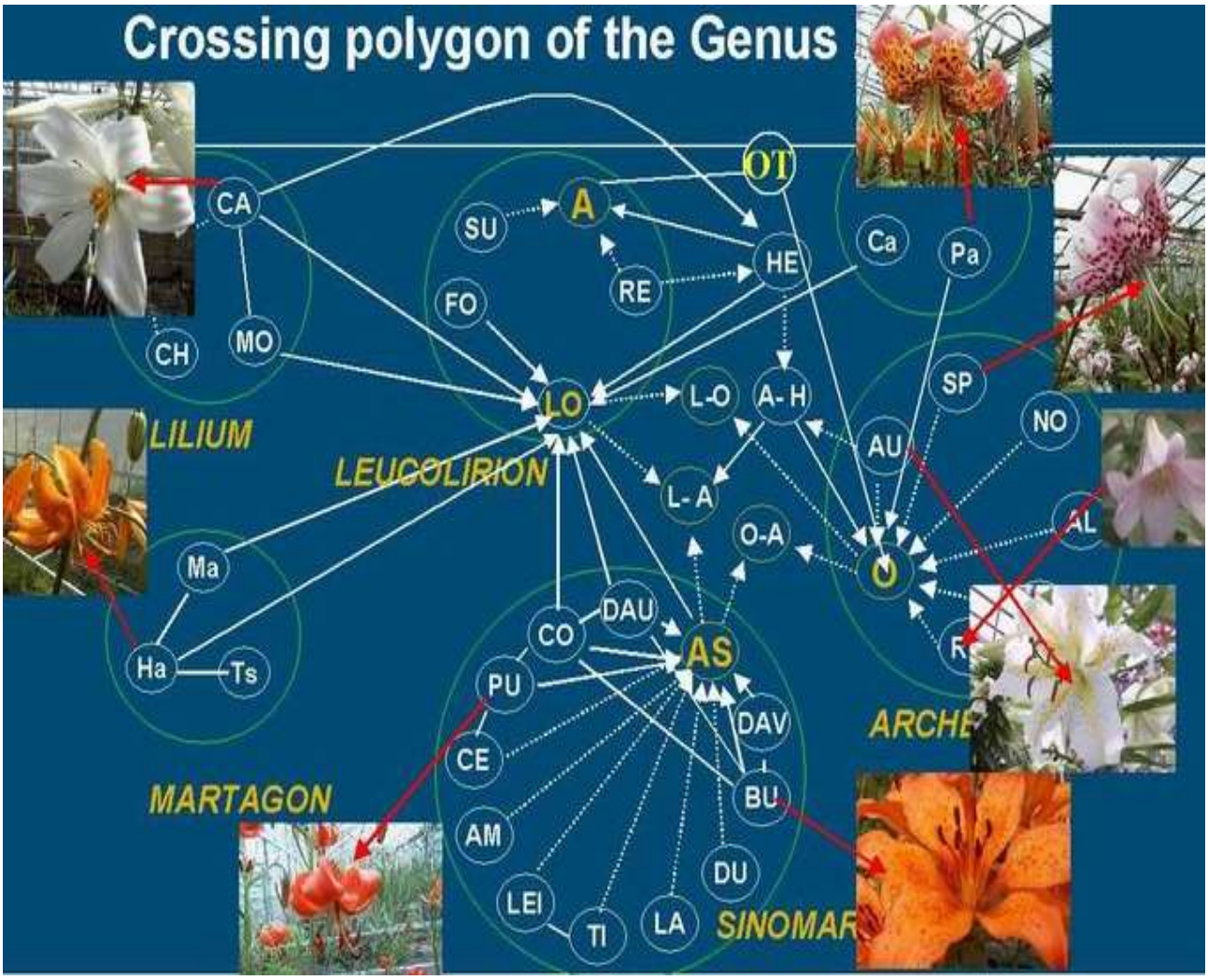
Tolerance to low-light intensity and heat
Leaf scorch
Year-round forcing ability
Long-term storage ability and
Rapid bulb growth

Table 1: To achieve the desirable characteristics in these interspecific hybrids, valuable characters of different species should be known. Some well-known examples of valuable characters among species are listed in table:

Species			Potential for breeding
	Desirable	Undesirable	
L.longiflorum	Low temp. tolerance, Flower shape, white	Susceptible to Fusarium, viruses	High
L. formosanum	Year round forcing, upright, growth,Vigour, fragrance	Weak stem, virus susceptible	Medium
Aurelion hybrid	Upright, yellow colour, fragrance	Susceptible to Fusarium, viruses, 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 vigour	Low
L. tigrinum	Vigour, resistance to virus, large Flower, bulbils formation, resistance to Fusarium	Hair, spots	Medium
L. callosum	Small, many flowers per stem, flower colour	Late flowering, weak growth Vigour	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

(Lim *et al*.,2002)

The conventional practice in lily breeding is in-section hybridization. Most of widely grown cultivars of Asiatic hybrid lilies originated from crosses between species of the Sinomartagon section. The next popular hybrid group is the Oriental hybrid lilies, obtained from crosses within the section Archelirion. In the section Leucolirion, *L. longiflorum* cultivars are an economically important group of lilies. The genus *Lilium* was classified into seven sections by De Jong (1974) [6].

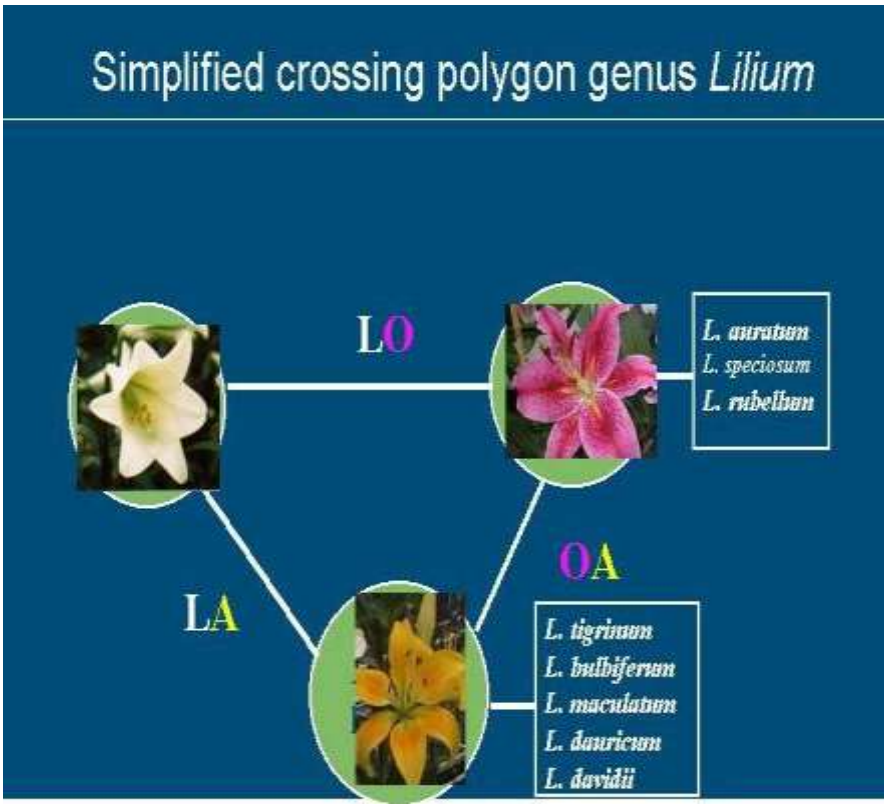


(Tuyl *et al*., 2002)

Plate 1: A crossing polygon of 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 arrow points towards the female parent.

Abbreviations

A: Aurelian hybridsA; A-H: *L. auratum* x *L. henryi*; 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:*H henryi;* JA: *L. japonicum;* LA: *L. lankongense*; LEI: *L. leichtlinii*; LO: *L. longiflorum*; MA: *L. martagon;* MI: *L. michiganense*; MO: *L. monadelphum;NO:* L. nobilissiium; O: Oriental hybrids; OT= Oriental-trumpet hybrids; PA: *L. pardalinum*;PU: *L. pumilum;* RE*: L. regale*; RU: *L. rubellum*;SP: *L. speciosum*; TS *L. tsing-tauense*; TI: *L. tigrinum.*



(Tuyl *et al*., 2002) (www.liliumbreeding. nl/ polygon)

Plate 2: The simplified crossing polygon of Genus *Lilium* shows the crossing combination between the important sections of genus *Lilium* including only *L. longifolium*, Oriental hybrids and Asiatic hybrids which are mainly used for the production of commercial hybrids.

Most of the present day commercial cultivars are the result of these intersectional crosses. Some of the important commercial interspecific hybrids are listed in the table below:

Table 2: Commercial hybrids of *Lilium*

Asiatic	Allround, Amarone, Barbon, Beatrix, Black Out, Blue Eyes, Caliente, Cannes, Canova, Cardinal, King, Compass, Detroit, Mona, Morden Butterfly, Novana
LA hybrids (Longiflorum x Asiatic)	Aerobic, Ballroom, Batistero, Best Seller, Birgi, Canilo, Dream Catcher, Glossy Wings, Golden Tycoon, Indian Diamond Money Maker, Modern Sryle
LO hybrids (Longiflorum x Oriental)	Deliana, Divine, Eagle, Elegant Lily, Pink Brilliant, Prince Promise, Sea Treasure, Triumphator
Martagon hybrids	Attiwaw, Autumn Color, Black Prince, Blush, Cadense, Dalhansoni, Gaybird, Glacier, Hantsing, Pink Attraction, Pink Taurade, Red Patti, Sunset Glow
Asiapets (Aurelian x Asiatic)	Fiery Belles, Ivory Belles, Silky Belles
OA Hybrids (Oriental x Asiatic)	Elegent Crown, Fancy Crown, Fuego Crown, First Crown, Sunny Crown
Longipets (Trumpet x Longiflorum)	Easter Morn
Orienpets (Oriental x Trumpet)	Altari, American Bandstand, American Dream, Black Beauty, Concad'OR, Donato, Elite, Garden Pleasure, Orange Jewel, Indian Summer, Starburst Sensation

Major Breeding Problems

1) Self- Incompatibility

It refers to failure of pollen from a flower to fertilize the same flower or the other flower of same plant. It is either due to pre -fertilization or post -fertilization barriers.

a). Pre-fertilization barriers like :

Pollen-Stigma Interaction (in this pollens fail to germinate on stigma) Pollen-Style Interaction (if pollens germinate then tube fails enter style or grows slowly to effect fertilization)

b). Post- fertilization barriers

(Includes degeneration of embryo at an early stage of development)

2) F_1-Hybrid sterility

Most of the hybrids of distantly related species are sterile and sterility results due to failure of chromosome pairing during meiosis which leads to formation of spores with unbalanced chromosome constitution thus cause sterility.

3) Long juvenile period

4) Low propagation rate

Self- Incompatibility

Both pre-fertilization and post-fertilization barriers restricts interspecific hybridization

To overcome pre-fertilization Barriers:

1. Use of mixed and mentor pollen

Mixed pollen (compatible pollen + incongruous pollen) Mentor pollen (irradiated pollen**)**

2. Influence of environmental conditions

Use of high temperature treatment during crossing can overcome the barrier

3. Style and ovary manipulation

Using different pollination techniques like stump pollination, Grafted-style pollination & intrastylar pollination

4. Chemical treatment

Certain chemicals or lipids like trilinolein, protein, polysaccharide, phenolic compounds and growth regulators like auxins, cytokinins *etc.* may improve the fruit and seed sets.

1. Use of mixed and mentor pollen:

Table 3 Number of *L. longiflorum* flower pollinated, number of pods with seed, total number of seed of each treatment at 18 ºC

Treatment No & Description	No. of flower Pollinated	Pods with Seeds	Total Seeds
1-C	10	10	2278
2-S	10	0	0
3-Cr	10	0	0
4-Cr+C	10	10	2011
5-Cr+S	10	1	11
6-Cr/8C	10	10	1995
7-Cr/8S	10	1	9
8-Cr/24C	10	10	2115
9-Cr/24S	10	2	25
10-Cr/72C	10	10	1190
11-Cr/72S	10	0	0

(Van Tuyl *et al.,* 1984) [13]

C = compatible pollen 'White American' S = self-incompatible pollen 'White Europe', Cr = irradiated (1000 krad pollen 'White American', Cr+____= mentor pollen applied immediately before a 2nd pollen, and Cr/_____ = pioneer pollen applied 8, 24, or 72 hrs before a 2nd pollen Van Tuyl *et al.* (1984) [13] investigated the features of using irradiated mentor-pollen and irradiated pioneer pollen to overcome incompatibility and incongruity in *Lilium.* No seed production resulted from single applications of self-incompatible pollen at 18^0 C. In contrast, the irradiated mentor or the irradiated pioneer-pollen techniques produced seed at the same temperature. These results are consistent with the concept of irradiated mentor or pioneer pollen inducing favourable conditions for incompatible pollen tube growth.

Table 4: The effect of the temperature in the phytotron glasshouses on fruit and seed set of Lilium cultivars after cross and self –pollination

Treatment	Crossed[1] Number of				Selfed			
	flowers	fruits	seeds	Seeds/fruit	flowers	fruits	seeds	Seeds/fruit
L. x 'Enchantment'								
10 ^{0}C	10	0	-	-	10	0	-	-
14 ^{0}C	10	9	3	0.3	10	0	-	-
17 ^{0}C	10	8	13	1.6	10	0	-	-
20 ^{0}C	10	10	190	19	10	9	9	1
23 ^{0}C	10	10	172	17	10	8	73	8
26 ^{0}C	10	10	4123	41	10	9	225	25
L. longiflorum x 'White Europe'								
10 ^{0}C	4	0	-	-	5	0	-	-
14 ^{0}C	3	2	0	-	5	0	-	-
17 ^{0}C	5	3	292	77	4	0	-	-
20 ^{0}C	8	7	255	36	7	2	0	-
23 ^{0}C	10	9	601	60	8	2	16	8
26 ^{0}C	8	8	808	101	7	2	95	48

'Enchantment' was crossed with 'Connecticut King' and 'White Europe' with 'Ace' (Tuyl *et al.,* 1982) [14]

Tuyl *et al.,* (1982) [14] reported the effect of the temperature in the phytotron glasshouses on fruit and seed set of Lilium cultivars after cross and self–pollination. The pollinations were made in the greenhouse from August 21 to September 2, 1981. The mean daily temperature of the greenhouse during this period varied from 17 to 24^0C. It was recorded that self incompatibility is largely overcome when the prevailing temperature is high enough.

Using different pollination techniques:

Stump pollination:

- Removal of stigma and a part or whole of style
- Pollination of cut end

Cut style pollination method (CSM)

To eliminate inhibiting chemicals

Method

1. Cut the style with razor blade 0-2 mm above the ovary
2. Apply stigmatic fluid followed by pollen application.

Drawback

- Produces few embryos per pod

Grafted style method (GSM)

To have more number of embryos per pod.

Method

- Pollens are deposited on compatible stigma
- Cut the style with germinating pollen 1-2 mm above ovary after 1 day
- Attach the cut end to an ovary of the mother

Table 5: Effect of *in vitro* pollination methods on capsule formation in intergeneric cross between *Hemerocallis* and *Lilium*

Parent		% Capsule Formation	
Hemercallis	***Lilium***	**Stigmatic**	**Cut Style**
H.dumortieri	Oriented hybrid lily 'Casablanca'	0	16.7
H.thunbergii	Oriented hybrid lily 'Casablanca'	0	50
H.minor	Oriented hybrid lily 'Casablanca'	5	33.3

(Ahn *et al.*, 2003) [1]

Ahn *et al.*, 2003 [1] studied effect of *in vitro* pollination methods on capsule formation in intergeneric cross between *Hemerocallis* and *Lilium. H. dumortieri* and *H. minor* were selected for the female parents and *Lilium* **'**Casablanca' (Oriental hybrid lily**)** were chosen for the male parent. The process of cut style pollination had showed higher capsule formation than that of the stigmatic pollination.

Table 6: Pollen tube growth in *L. longiflorum*, 5 days after pollination at 3 constant phytotron temperature, with just opened (0 days old) and 5 days old flower, with 3 pollination techniques and 3 pollen parents.

Pollen parent		Self			Enchantment			Mont Blanc		
Pollination tech.	*****	**18^0**	**22^0**	**26^0**	**18^0**	**22^0**	**26^0**	**18^0**	**22^0**	**26^0**
Normal	0	2	2	2	1	2	2	1+	2	2
	5	2	2+	2+	1+	2	2	1+	2	2
Mentor	0	2+	2+	2	2+	2+	3+	2+	3+	3+
	5	2+	3+	2+	3+	2+	3+	3+	3+	3+
Cut style	0	2+	3	3+	2+	3+	3+	2+	3+	3+
	5	3+	3+	3+	3+	3+	3+	3+	3+	3+

*Age of the flower 0, 5 days (Tuyl *et al.*, 1982) [14]

Where,

1 = germination into stigma
1+ =some pollen tubes in style
2 = many pollen tubes in style
2+ = some pollen tubes in ovary
3 = many pollen tube in ovary
3+ = some pollen tubes in ovule

Tuyl *et al.*, (1982) [14] reported growth of pollen tube in *L. longiflorum*, 5 days after pollination at 3 constant phytotron temperatures, with just opened (0 days old) and 5 days old flower, with 3 pollination techniques and 3 pollen parents. Cut style method was always necessary to obtain seed setting followed by embryo culture. Also positive influence of high light intensities and temperature was seen in all the three cases. Ahn *et al.*, (2003) [1] reported the effect of pollination methods on capsule formation in intergeneric crosses between *Hemerocallis* and *Lilium*. The % capsule formation was maximum in case of cut style pollination method followed by ½ style method in each cross. No or very little capsule formation was in case of stigmatic pollination method. Therefore cut style pollination method was recommended for future research.

Table 7: Effect of pollination methods on capsule formation in intergeneric crosses between *Hemerocallis* and *Lilium*

Parent		Pollination Method	No. of Flowers Pollinated	No. of capsules obtained	% capsule formation
Hemerocallis	***Lilium***				
♀	♂				
H.dumortieri	Oriental Hybrid Lily 'Casablanca'	Stigmatic	68	2	2.9
		1/2 style	60	5	8.3
		Cut Style	73	38	52.1
H.dumortieri	Asiatic hybrid lily 'London'	Stigmatic	65	0	0
		1/2 style	65	5	7.7
		Cut Style	65	30	46.1
H.minor	Oriental Hybrid Lily 'Casablanca'	Stigmatic	75	2	2.7
		1/2 style	75	7	9.3
		Cut Style	77	46	59.7
H.minor	Asiatic hybrid lily 'London'	Stigmatic	68	0	0
		1/2 style	65	4	6.2
		Cut Style	65	26	40

(Ahn *et al.*, 2003) [1]

Table 8: Fruits and seeds produced after self pollination of *Lilium longiflorum* cultivar 'Georgia' flowers and treatment with naphthalene acetamide (NAM) or potassium gibberellate (GK) or both.

Treatment	Pollination	Fruits	Total Seeds	Seeds per Fruit	Seeds per Pollination
Seeds with visible Embryos					
None	143	5	53	10.6	0.4
GK	142	10	251	25.1	1.8
NAM	172	91	2033	22.3	11.8
GK+NAM	173	82	1550	18.9	9

Seeds without visible Embryos					
None	143	5	187	37.4	1.3
GK	142	10	536	53.6	3.8
NAM	172	111	4786	43.1	27.8
GK+NAM	173	103	3651	35.4	21.1

Emsweller *et al*.., 1959 [7]

Emsweller *et al.* (1959) [7] studied the number of fruits and seeds produced after self pollination of *Lilium longiflorum* cultivar 'Georgia' flowers and treatment with naphthalene acetamide (NAM) or potassium gibberellate (GK) or both. More capsules and seeds were produced from the naphthalene acetamide treatment than from the others. Selfing alone produced low yields. Potassium gibberellate at the concentrations and times used improved seed set only slightly.

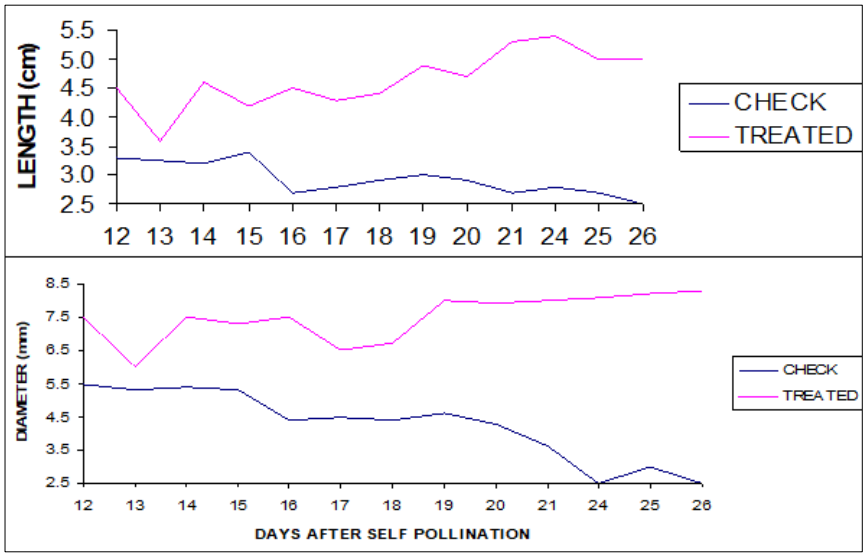


Fig 3: Growth curves of NAM treated and untreated capsules of *L. longiflorum* 'Georgia' from 12 to 26 days after self pollination.

The growth curve shows differences in length and diameter of capsules from pollinations treated with naphthalene acetamide and the controls were evident for 8 to 10 days after pollination. From then on, the capsules from the treated pollinations continued to increase in size and the controls began a steady decrease.

Overcoming post-fertilization Barriers

1. Ovary and ovary slice culture:
   - Harvest ovaries 7-40 days after cut- style pollination
   - Slice them into 2-3 mm thick disks after surface sterilization
   - Culture in 10% sucrose for 30 days.

2. **Ovule culture**
   - ovules are cultured in a similar way
   - Easy and fast method
   - Done 30-45 days after pollination

3. **Embryo culture**
   - Better than above methods as germination efficiency is mor

Table 9: Total number of *in-vitro* plantlets and the number of plants which produced flowering plants obtained from several interspecific cross combinations, using ovary-slice culture and other embryo rescue techniques

Successful Combinations	Number	
	Plantlets	Flowering
L. longiflorum x Asiatic Hybrid	>200	>150
L. longiflorum x L candidum	>100	28
L. longiflorum x L. concolor	21	8
L. longiflorum x L. henryi	16	15
L. longiflorum x L. dauricum	>100	56
L. longiflorum x L. rubellum	5	4
L. longiflorum x L. bulbiferum	3	2
L. longiflorum x L. martagon	1	1
L. longiflorum x L. canadense	1	1
L. henryi x L. candidum	3	2
L. longiflorum x Oriental hybrid	25	15
Oriental hybrid *x L. pardalinum*	15	1
Oriental hybrid *x L. hansonii*	10	1
Oriental x Asiatic Hybrid	>500	-

(Tuyl *et al*., 1999)

Tuyl *et al*., (1999) obtained flowering plants from several Interspecific combinations .The number of obtained plantlets and the number of these plants which have flowered are listed in the table. The plants, especially the OA-hybrids, have not flowered yet, so the number of flowering plants will increase.

Table 10: The size of ovules and the number of ovules that regenerated into plantlets and flowered from the interspecific cross between *L. longiflorum* and *L. callosum*

Ovary slice culture week after pollination	Ovule no[z]	Size (mm)	No. of plantlets flowered in 1991z
1	0.2	1.1 x 1.8	0.2
2	0.4	1.8 x 2.3	0.2
3	3.3	1.8 x 2.6	0.7
4	2.7	2.7 x 3.5	2.3
5	3.2	3.3 x 4.6	3.8

[Z] Number of plants per flower pollinated
(Roh *et al.*, 1996) [9]

Roh *et al.*, (1996) [9] studied the size of ovules and the number of ovules that regenerated into plantlets and flowered from the interspecific cross between *L. longiflorum* and *L. callosum* .Interspecific LC hybrids were obtained from ovaries cultured within a week after pollination. However, greatest success was obtained with ovaries cultured 4 week after pollination.

Table 11: Embryo rescue culture by *in vitro* ovary culture in distant lily hybrids

Combination	Capsules	Embryo seed	Regeneration			
			callusing	browning	plantlet	survival (%)
#10 x Asterix	7	19	10	6	3	16
#10 x Purple Sensation	5	11	3	3	5	45
#10 x Sancerre	1	3	-	-	3	100
Lkm x Asterix	11	57	15	26	16	28
Nellie white x Sancerre	1	3	-	-	3	100

#10 = *L. longiflorum* (local strains) **(**Chin *et al*., 1997) [3]

Chin *et al*. (1997) [3] studied distant hybridization between *Lilium longifolium* and Asiatic or Oriental lilies accompalished by *in vitro* pollination and ovary culture. *Lilium longifolium* was preferred as the maternal parent and five local strains of *Lilium longifolium* –Ld, Lkm, Lchin, cv 'Nellie White' and a Dutch clone designated #10,three Asiatic hybrids- 'Astrix'(Ast),' Purple Sensation' and "Sancerre'(San) were used. The frequency of regeneration from five combinations is listed in the table. The survival rate varied from 16 to 100%, depending on the combination.

Overcoming F1 sterility
Mitotic polyploidization

- Obtained through artificial chromosome doubling
- (Treatment of mitotic cells of vegetative tissue with spindle inhibitors such as colchicine (Blakeslee and Avery; 1937) [2] or Oryzalin (Van Tuyl *et al.*,1992) [10]

Meiotic Polyploidization:

- Application of 2n gametes for the production of sexual progeny either through crossing or selfing

Advantages of Polyploidy in lily breeding

- large flowers
- stronger stems
- restoration of F1 sterility at tetraploid level

Table 12: *In-vitro* chromosome doubling of sterile interspecific hybrids of lily using colchicine and oryzalin

Level of Polyploidy	Treatments (%)				
	Col 0.1	Ory 0.01	Ory 0.005	Control	Total
Tetra	3	-	4	-	7
Di_tetra	3	6	9	-	18
Di_octa	-	-	1	-	1
Tetra_octa	3	-	-	-	3
Octa	2	-	2	-	2
Total poly	9	6	16	-	31
Total	13	41	38	15	107

(Tuyl *et al*., 1992)

Tuyl *et al.*(1992) studied *In-vitro* chromosome doubling of sterile interspecific hybrids of lily using colchicine and oryzalin. Thirty one lily plants of 92 regenerated oryzalin and colchicines treated plants appeared to be polyploid. A high percentage is chimeric probably because of juvenility of the material. More number of polyploids were obtained by the use of oryzalin at lower concentration then by the use of colchicines at higher concentration.

Advantages of Oryzalin over Colchicine

Tuyl *et al*. (1992) observed the following advantages of using oryzalin over colchicine

- Restoration of F-1 sterility successfully in *Lilium henryi x L. candidum* hybrid using oryzalin.
- Oryzalin inhibited plant cell division much more effective than colchicine.
- Its lower concentration (i.e. 0.001-0.005) is as effective as higher concentration of colchicine (i.e. 0.1 per cent).
- Growth abnormalities caused by colchicine are not seen in this case.
- Oryzalin in less toxic than colchicine.

Advantages of Meiotic polyploidization over Mitotic polyploidization

Tuyl *et al*. (1990) discussed the following advantages of meiotic polyploidization over mitotic polyploidization

- No occurrence of chimeras.
- No decreased growth and fertility caused by the toxic effect of colchicine.
- Genotypic and environmental dependency (Van Tuyl and Stekelenbugr, 1989) and
- Outbreeding effect in contrast to mitotic polyploidization where inbreeding takes place.

Table 13: Commercial Mutants of Lilium

Name of the new cultivar	Place of date of release (or approval) and name of principal worker (s) and institute	Conditions and date of mutagenic treatment [Parent cultivar]	Main improved attributes of cultivar
Mies Bouwman	The Netherlands, 1977 A.J. Bischoff- tulleken, wieringerwerf*	X-rays, 1968 [Tabasco]	Orange flower colour, Excellent forcing qualities
TX 68-1	The Neatherlands, 1977 A.J. BBischoff- tulleken, wieringerwerf*	X-rays, 1968 [Tabasco]	Orange flower colour, Excellent forcing qualities

* In co-operation with the Association Euratom- ITAL, Wageningen.

Sources: IAEA (1972, pp. 536-544) and *Mutation Breeding Newsletter*, 1 (1972)-9 (1977) and Association Euratom-ITAL, Wageningen.

Achievements

1. **Wide range of production of interspecific LA, LO and** OA hybrids with improved characters.

- Incorporation of fragrance of *L. longiflorum, L. speciosum* into Asiatic and Trumpet groups.
  - *e.g.* Batistero (LA)
  - La Reine (LA)
  - Boogie Woogie (OT)
- Vigour and disease resistance of *L. henryi* into other species.
  - *e.g.* Easter Morn
- Cultivars with improved winter hardiness based on crosses with *L. tigrinum, L. cernum* and *L. pumilum*.
- Incorporation of colour to Oriental hybrids
  e.g. *O x A hybrids: Fancy Crown (Deep red)*
  Fuego Crown (red)
  First Crown (red)

O x T hybrids: *Black Beauty (deep crimpson)*
Boogie Woogie (Bright Yellow)
Orania (Tangerine Orange)
Red Hot (red)
L x O hybrid: *Divine (deep purple)*
Bergamo (yellow\orange)

- **Development of golden yellow cultivars**

e.g. *Pisa (Vletter and Den Haan)*

Asiatic hybrids with low light requirement for flowering are developed by crossing with *L. candidum*.

e.g. *America, Colosseo, Brunello, Novana, Nive*

2. **Development of screening techniques for resistance to *Fusarium* and *Cylindrocarpon***

3. **Development of early forcing cultivars**

e.g. Prima (*L. hollandicum x L. tabasco*) (Mynett 1986)

4. **Development of pollen-less cultivars or low-pollen cultivars**

e.g. Yellito,
China (Mak Leek Inc.)

5. Production of Polyploids

- Tetraploids *e.g.* Angel's Touch, Bronze Medel, My Dear, Peachy Keen, Nut Cracker

- Triploids *e.g.* Easter Morn, Elegant Lady, Fangio

6. Production of early flowering Orientals using *L. rubellum* as a parent.

- *e.g.* Egypt, Dame Blanche, Roseto, Tiber, Woodriff's Memory

Future Prospects

- Development of new types of Interspecific hybrids by expanding hybridization between LO, LA, OT, and OA hybrids *e.g.*, OLA hybrids
- Development of Ethylene-insensitive cultivars
- Improvement of fragrance like
- Reduction in Orientals
- Incorporation in Asiatic
- Introduction of Colour in Orientals
- Improved vase-life
- Development of Polyploids
- Development of Pollen less or with little pollen cultivars

Conclusion

From breeding perspective, most important breeding method can be considered as Inter-specific hybridization:

- *in-vitro* pollination techniques (cut- style and grafted style pollination)
- *in-vitro* culture techniques (ovary- slice culture and ovule culture)

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