LILY BREEDING RESEARCH IN THE NETHERLANDS.

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Abstract

At the Centre for Plant Breeding and Reproduction Research (CPRO-DLO) in Wageningen lily breeding research in the Netherlands is concentrated. To this end sources of germplasm (genetic material) are maintained in a lily species and cultivar collection. Research is done on long term storage of this collection by use of low temperature and minimal growth conditions in vitro.

Main themes of our breeding research are breeding for resistance (to *Fusarium oxysporum*, *Pythium* and virus diseases), breeding for quality traits (flower longevity lily; forcing ability; bulbgrowth of *Lilium longiflorum*), interspecific hybridization and genetic modification. A molecular marker system using RAPD's is developed in lily to link *Fusarium* resistance with molecular markers and to construct a genetic map of lily.

A system for in vitro pollination, fertilization and embryo rescue has been developed for lily. By combining pollination techniques to overcome prefertilization barriers with in vitro methods to overcome postfertilization barriers, wide interspecific crosses could be made more efficiently. This resulted into a range of new interspecific hybrids, which have led to completely new hybrid groups in the lily assortment. To overcome F1-sterility of interspecific hybrids, owing to lack of chromosome pairing during meiosis, colchicine and oryzalin are used for the induction of mitotic tetraploids. Interspecific crosses at tetraploid level between complex hybrids of *L. longiflorum, L. henryi, L. candidum*, Asiatic and Oriental hybrid lilies have been made. To develop efficient methods for breeding at tetraploid level, meiotic polyploidization is investigated.

Procedures of genetic modification of lily are developed in several directions. At the University of Leiden, for the introduction of virus resistances a transformation technique is in development by particle bombardment on bulb-scale explants. An approach followed by CPRO-DLO is pollen transformation: isolated pollen bombarded with particles coated with DNA containing the kanamycin resistance gene and the B-glucuronidase gene are used to obtain transgenic plants. Other biotechnological techniques are based on protoplast regeneration for either somatic hybridization or direct DNA transfer and microspore culture for the induction of haploidy.

1. Introduction

In the past 25 years the importance of the lily as a horticultural crop has increased enormously, especially in the Netherlands. In this country the lily bulb production area increased from about 100 ha in 1966 to about 3000 ha in 1993 resulting in about 800 million bulbs. In the list of the most important cut flowers in the Netherlands lily is placed on a fifth place, with a production of more than 300 million stems. The total economic value amounts to about 600 million Dutch guilders. Reasons for this increase are the progress in lily breeding, especially from the upright Asiatic and Oriental lilies, the improved possibilities for bulb production (including mechanization of the planting, digging and sorting) and the application of rapid clonal propagation by in vitro propagation.

In this review on lily breeding research in the Netherlands no data will presented about the current state and development of the lily assortment. The activities of the private breeding companies result in a stream of new lily cultivars which can be demonstrated by

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the number of new cultivars applied for breeder's right each year (the last decade that were more than 100 cultivars per year). The lily breeding research in the Netherlands is concentrated at the Centre for Plant Breeding and Reproduction Research (CPRO-DLO) in Wageningen. At CPRO-DLO (former IVT) lily breeding research was started 15 years ago. An overview and some results are presented in this paper and the most important publications are listed in the list of references. The last four years lily research increased enormously. This is especially due to the Urgency Programme for Bulb Diseases and Breeding Research, partially financed by the Dutch Board for Ornamental Crops. The current state of research in the following fields will be summarized: genetic resources, breeding for resistance, research on flower longevity, interspecific hybridization, polyploidization, genetic modification and other new techniques.

2. Genetic resources - collections

Well documented collections of genetic material form the base of each breeding programme. This means for lily, as for all commercially important ornamental, crops that material must be maintained vegetatively. The CPRO-DLO lily collections consists of more than 1000 species, cultivars and selections. This collection is very vulnerable, because material can easily be lost due to diseases. Moreover, high costs in labour are involved. Research in lily and tulip has been carried out by Bonnier et al. (1992) to develop methods to preserve vegetative material for several years, without yearly planting. Metabolic activity is minimized by in vivo storage at temperatures below zero and in vitro by applying specific combinations of temperature and medium components. Storage at -2° during three years seems possible without loss of vitality. A combination of a high sucrose concentration and a low salt concentration proved to minimize metabolic activity the best for in vitro storage of lily.

3. Breeding for resistance

As model for breeding for resistance to soil-borne fungi in bulbous crops *Fusarium oxysporum* in lily has been chosen. This soil-borne fungus causes the serious basal rot in lily. For this system a reliable screening method at clonal level has been developed to detect *Fusarium* resistance (Straathof & Löffler, 1994a; Löffler et al. 1993b). Using those methods, resistance was found both in cultivated varieties and in wild species of lily (Straathof et al. 1993, Straathof & Van Tuyl, 1993, 1994). Besides, research was done to develop seedling tests for optimizing selection for resistance (Straathof & Löffler, 1994b). This selection can even be improved when molecular markers, which are linked with the resistance genes on the genome, can be used. In this research, carried out by Sandbrink and co-workers mainly the technique of Random Amplified Polymorphic DNA (RAPDs) is used. (see also Straathof et al. in this Acta). Using the molecular marker techniques *Fusarium* resistance of the Asiatic cultivar 'Connecticut King' is being marked at the lily genome. Three out of 213 RAPD markers were significantly ($p < 0.005$) linked to *Fusarium* resistance explaining approximately 24 % of the total phenotypic variance of the resistance. Herewith the first start has been made for an efficient screening method at seedling level.

The durability of the applied resistance is of high practical importance. Therefore the capacity of the fungus to adapt to *Fusarium* resistance in lily is investigated in greenhouse tests. No races of the fungus were found (Löffler & Rumine, 1991). Furthermore, the fungus was not able to adapt easily to the resistant host plant (Löffler et al., in preparation). After 5 infection and re-isolation cycles on partial resistant lilies, the aggressiveness of the fungus was not increased. So it was concluded that there are no reasons to doubt on the durability of the resistance found in lily.

Possibly *Fusarium* can affect bulbs by the production of toxins. The toxin fusaric acid is produced in vitro and can be demonstrated in the culture filtrate. HPLC analysis suggests that this toxin is also produced in plants. Since this toxin equalled the effects of culture

filtrate in the bioassay, it is concluded that fusaric acid is responsible for the toxic effect in the culture filtrate (Löffler et al. 1993a; Löffler & Mouris, 1990, 1992). In vitro selection for resistance against *Fusarium oxysporum* is investigated at CPRO-DLO for Gladiolus in more detail (Remotti & Löffler, in preparation). In lily the possibilities are also promising and especially for the Oriental hybrids, where no resistance is available, this might be a promising approach.

4. Breeding for quality traits

4.1 Flower longevity

The longevity of cut flowers is a complex quality character. Internationally, hardly any breeding research activities take place. At CPRO-DLO research is going on to elucidate this complex character. To prevent the influence of environmental conditions as much as possible preharvest and postharvest conditions are standardized and experiments are performed in climate rooms (Van der Meulen et al. 1992). The influence of temperature during cultivation of the flower on individual flower longevity was minimal. The influence of temperature on longevity of cutted flower was measured at three temperatures (14, 17 and 20°C). 35 Genotypes were used to determine the temperature most distinctive for selection on flower longevity. At a temperature of 17°C, the variation in longevity of the 35 genotypes was most distinctive. Hereby two criteria have been used to define end of flower life: loss of turgor and flower deformation. Both criteria were highly correlated but the latter was easier to assess. For lily forcing, the most optimal constant temperature in practice, 17°C, was chosen as standard. An increase in light intensity (15, 20, 25, 30 W/m^2 HPI-T (400 W), during 16 hours) only slightly improved flower longevity in some of the tested genotypes. Therefore for light intensity 24 W/m^2 was chosen as standard. Origin and duration of storage of the bulbs hardly influenced the longevity of individual flowers, although it did effect the percentage flowering buds and number of buds per stem. The genotypes tested did not show any difference in longevity of the individual flowers when different stages of maturity of the stems were compared. The individual flower therefore seems to be a better criterion than the complete stem for the comparison of flower longevity of different lily genotypes (see also Van der Meulen et. al in this Acta). Using these criteria, the so called potential flower longevity of a range of lily cultivars was determined. It appeared that the differences found between cultivars through the year and between the years were similar (Van der Meulen & Van Oeveren, 1993ab). Research has also been done to the effect of other components like the ethylene sensitivity, stress (for example after dry storage) and the presence of sugars. The influence of ethylene treatment on the mean longevity of the individual flower was of no importance in tulip, while in lily significant lily differences were found between cultivars. Research is started to determine the longevity of individual lily seedlings, in order to study the inheritance of the various components of flower longevity.

4.2 Bulb growth of *Lilium longiflorum*

The acreage of *Lilium longiflorum* in the Netherlands has increased during the last seven years more than tenfold, covering in 1992 more than 200 hectares. The Netherlands now produce their own *L. longiflorum* bulbs and are no longer dependent on bulbs imported from Japan. Fifteen years ago, problems with this lily species indicated the need to give the highest priority to breeding research on this species. One of the problems in growing *L. longiflorum* under the relatively cool conditions in the Netherlands is the lack of vigour and the premature sprouting of the daughter bulbs. Therefore a new scale propagation method was developed for *L. longiflorum* adapted to the Dutch climate conditions. A high initial temperature for a long period followed by a relatively short cold period produced the highest bulb yield. This could be explained by the fact that all bulbs sprout with no 'sleepers' and most bulblets produce stems (Van Tuyl, 1985). The scale propagation

method, which now has been used for more than ten years as a standard in the research, comprises 12 weeks at 26° C, four weeks at 17°C, four weeks at 5°C, after which planting out in the field takes place around mid-April. In this way, the bulbs grow in the warmest months of the year, from June through September. After investigating a range of *L. longiflorum* origins in a comparative trial for sensitivity to summer sprouting and growth vigour, the best five cultivars have been chosen for inter-crossing. A selection procedure was performed for summer sprouting, bulb growth and forcing qualities resulting in the selection of suitable material. The aims of this research project were attained in 1985 and the project was concluded with the release of suitable material (e.g. the cvs 'Gelria' and 'Longivetta') to the Dutch growers (Van Tuyl, 1985, 1990a, 1992).

5. Interspecific hybridization

Within the genus *Lilium* the about 85 species can be classified into seven sections. In the Netherlands the group of the Asiatic hybrid cultivars, which is derived from species of the section Sinomartagon and the Oriental hybrids, derived from species of the Archelirion section, are economically the most important groups for cut flower production. The lily assortment, however, might be considerably improved by exploiting economically important traits from other *Lilium* species. The species used in our interspecific hybridization programme (Van Tuyl et al. 1986, 1990b) have been chosen on the basis of their respective characters: *L. candidum* (section *Lilium*) with pure whiteness, fragrance and low light and temperature tolerance; *L. longiflorum* (section Leucolirion) with forcing ability and growth vigour; *L. henryi* (section Archelirion) with resistance to virus diseases and bulb rot (caused by *Fusarium oxysporum*) and *L. pumilum* with earliness and a bright red colour (section Sinomartagon). Fundamental research on fertilization processes in lily was done in close collaboration with the department of Plant Cytology and Morphology of the Agricultural University of Wageningen (Janson et al. 1992, 1993).
The following topics illustrate the CPRO research on interspecific hybridization:
The use of in vitro pollination techniques (5.1), the development of the LA-hybrids (5.2), results of crosses with 5 *Lilium* species (5.3).

5.1. In vitro pollination techniques

The use of a complete and integrated in vitro system for pollination, fertilization and embryo rescue in lily was examined. By combining pollination techniques (cut-style and grafted style) to overcome prefertilization barriers with in vitro methods to overcome postfertilization barriers, both under fully controlled conditions, interspecific lily crosses could be made very efficiently. In vitro, cut-style pollination and in vitro grafted style technique were developed and applied on various interspecific crosses using *L. longiflorum*, and both Asiatic and Oriental hybrids as parents. In addition, methods for ovary culture, ovary-slice culture and ovule culture were generated. Ovule swelling score in ovary culture was used to evaluate media effects on ovule development (Van Tuyl et al, 1988, 1990ac, 1991ab).

5.2. The development of LA-hybrids

Within the genus *Lilium, L. longiflorum* is one of the most interesting species carrying many characters required in horticulture. Parallel to the breeding programme with *L. longiflorum* aimed at improving bulb production under Dutch climate conditions (see 4.2), interspecific crosses were made at the former IVT with other white lilies viz. *L. candidum* and the white Asiatic hybrids 'Mont Blanc' and 'Whilito' (Van Tuyl et al.1990b). In the summer of 1980, 9 cut-style pollinations produced 3 set pods using *L. longiflorum* 'White

Europe' and 'Mont Blanc'. 42 Days after pollination, 11 embryos were rescued by in vitro culture. In 1982, 8 hybrids of this cross came into flower. General characteristics of these hybrids are: white or creamy white flower colour with fewer spots than 'Mont Blanc', 'floppy' flower size, little or no direct ornamental value, sterile pollen, and often dark green *L. longiflorum* foliage. The only hybrid with some ornamental value, which we called 'Loblanca', has large Asiatic type flowers. In 1984 this hybrid was found to be female fertile and backcrossed to 'Mont Blanc' and *L. longiflorum*. The cross with 'Mont Blanc' produced 25 embryos. 'Lomonta' is one of these large flowered triploid, upfacing hybrids which came into flower in 1986. From bulb growth experiments it was demonstrated that these hybrids have a growth vigour comparable with that of *L. longiflorum*. Various clones from these crosses were released to the Dutch private breeding firms (Van Tuyl, 1990a). Using colchicine a tetraploid 'Loblanca' was developed which showed complete restoration of the pollen fertility. Crosses with tetraploid F1-hybrids appeared to be more successful than similar ones at the diploid level. The latter crosses mainly produce sterile triploids. Meanwhile several Dutch breeding companies have already developed commercial cultivars from this material in combination with their own material. This group of hybrids is called LA-hybrids because of their *L. longiflorum* and Asiatic origin and are characterized by large flower sizes, pastel flower colours and a striking growth vigour. Comparable crosses can be made between *L. longiflorum* and Oriental hybrids, called LO-hybrids.

5.3. Recent results of wide interspecific crosses

Using ovary- and ovule culture in combination with certain pollination techniques wide interspecific crosses were made between several lily species (*L. longiflorum, L. dauricum, L. henryi, L. rubellum, L. candidum* and *L. concolor*) and cultivars (e.g. the Asiatic hybrid 'Whilito')(Van Tuyl et al. 1991ab; Van Creij et al. 1993). A major part of the hybrids (more than 100) obtained after culturing more than 50.000 ovules flowered in 1991. Except two all in vitro cultured ovules, appeared to be hybrids. This can be determined visually rather easily. Remarkable was the large variation in flower shape, spotting and colour observed in the cross of *L. longiflorum* with *L. dauricum*. The white coloured *L. longiflorum* and the yellow coloured and heavily spotted *L. dauricum* produced a progeny of plants with a complete different spotting and flower colour. The flower colour ranged from almost white, via cream, light yellow, pink, bicoloured yellow-pink to dark purple and violet. The latter colours are virtually unknown within the Asiatic hybrid group (see also Löffler et al. in this Acta about the variation for *Fusarium* resistance in this progeny). The hybrids originating from the cross of *L. longiflorum* and *L. concolor* all showed a light red flower colour and a flower size intermediate between both parents. Interesting combinations are those in which *L. henryi* is used as a parent because of the enormous vigour and the intermediate or high levels of resistance of this species (for *Fusarium*, virus and *Botrytis*). Several hybrids from the cross *L. longiflorum* with *L. henryi* came into flower. Although these hybrids didn't show a perfect flower form, the vigour was remarkable and they were even partly fertile.

In general, wide interspecific hybrids in lily present sterility because of cytogenetical difficulties during meiosis. This sterility hampers further breeding with these hybrids. Because these problems were expected all hybrids were tested for pollen fertility. Research showed that when some fertility was found this was caused by the occurrence of 2N-gametes. This means that the pollen grains formed have the double number of chromosomes. Further breeding with this material is only possible at tetraploid level. The sterile hybrids of *L. henryi x L. candidum* and *L. longiflorum x L. henryi* demonstrated fully restored fertility after in vitro chromosome doubling (see 6. mitotic polyploidization).

In Figure 1 a crossing polygon is presented of the genus *Lilium* with results of our wide interspecific crosses which were only successful using special pollination or embryo rescue methods.

In cooperation with 11 Dutch lily breeding companies, this year research has been

started to overcome crossing barriers between the Asiatic and the Oriental hybrids using LA's, LO's *L. henryi x L. candidum* and *L. longiflorum x L. henryi* as bridges (see Fig. 1).

6. Mitotic and meiotic polyploidization

The reasons for using polyploidy in lily breeding are the larger flowers and the stronger stems of polyploid plants (especially important for forcing under low light conditions during the winter period (Van Tuyl et al., 1985). In interspecific hybridization, the F1-sterility at the diploid level is restored at tetraploid level. Tetraploids can be obtained by colchicine or oryzalin treatment of mitotic cells (mitotic polyploidization). An important advantage of the developed in-vitro techniques is that arising sterility can be restored by in-vitro chromosome doubling. This can be done in the same year in which ovules or embryos are cultured. As a consequence a considerable speed up of the breeding process can be realized. In the experiments for this purpose colchicine and oryzalin was used.

Oryzalin (3,5-dinitro-N^4,N^4-dipropylsulfanilamide) is developed as a herbicide and is found to be a metaphase inhibitor, like colchicine. Oryzalin however is characterized by a lower toxicity than colchicine, can be applied in lower concentrations and must be considered as an alternative for colchicine (Verhoeven, et al.1990). In an experiment with several sterile interspecific hybrids, colchicine (0.1%) and oryzalin (0.01% and 0.005%) was employed. The regenerated bulblets were tested for their level of ploidy using flow cytometry Of 92 regenerated lily plants, 31 appeared to be polyploid. A high percentage is chimeric probably because of juvenility of the material. Oryzalin appeared to be a less inhibiting regeneration than oryzalin, and also the number of polyploids was higher. Disadvantageous effects like growth abnormalities caused by mutation induction possibly do not occur using oryzalin (Van Tuyl et al. 1992, 1993). Meanwhile several tetraploid hybrids e.g. of *L. henryi x L. candidum* and of *L. longiflorum x L. henryi* came into flower and showed a fully restored fertility. In Figure 1 the hybrids of which tetraploids are produced are indicated with a T. At tetraploid level these groups are intercrossed in our current breeding programme.

Many species produce 2N-gametes (meiotic polyploidization) because of irregularities in meiotic division. Normally, the frequency of 2N-gametes is low. The frequency can be increased by environmental conditions like high temperatures and by selection of the most suitable genotypes. In our lily breeding research programme, individual plants producing 2N-gametes were traced by crossing a series of diploid Asiatic hybrids with tetraploid parents. Several genotypes are selected from self pollinated populations which produce 30-50% 2N-gametes. The mechanism of restitution is being investigated.

Wide interspecific lily hybrids are usually completely male and female sterile. In rare cases, however, some fertile pollen can be detected. In a group of more than 50 embryo cultured hybrids of the cross *L. longiflorum* x *L. candidum* only one hybrid showed a pollen fertility of 25%. Meiotic studies revealed in this case general irregularities during meiotic division and all pollen produced in this hybrid contained 2N-gametes. Comparable cases were found in the interspecific hybrids 'Shikayama' x *L. henryi* and *L. auratum* x L. henryi ; used as pollen parents, these produced triploid progenies. Backcrossing these triploids with *L. auratum* x *L. henryi* gave a number of aneuploids with a chromosome number between 36 and 48. In contrast to the wide interspecific hybrids, seedlings from the interspecific cross of the Asiatic hybrid 'Enchantment' and the related *L. pumilum*, produced fertile pollen. Meiotic studies of several of these hybrids showed, that not only haploid pollen was formed but also relatively high percentages 2N-gametes. Application of meiotic polyploidization in lily breeding can be of great importance for further developments in lily culture (Van Tuyl, 1990, 1993).

In order to detect possible 2N-pollen producing genotypes, measurements with a flow cytometer were executed. Differences between diploid and tetraploid genotypes can be detected easily using leaf samples (Fig. 2A) which were chopped and coloured with

DAPI. In samples of lily pollen two peaks were produced (Fig. 2C). These peaks may represent the 1C and 2C DNA level (Bino et al. 1990). Apparently in the bicellulate lily pollen the two nuclei have a different DNA content. We suggest that the vegetative nucleus is haploid and is responsible for the 1C peak, while the generative nucleus is in the S-phase and gives the 2C peak. Analyzing the pollen of 2N-gametes producing genotypes, we found an additional peak at the 4C level (Fig. 2C). Pollen of the interspecific hybrid (*L. auratum* x *L. henryi*) only presented 2C and 4C-peaks, indicating that this interspecific hybrid only produces 2N-gametes. Results of a meiotic analysis, a number of crossing experiments and pollen measurements agreed with the outcome of the flow cytometric determinations. The flow cytometry method enables the large scale screening of collections of genotypes for their potential of 2N-pollen production (Van Tuyl et al. 1989).

7. Genetic modification and other new techniques

Procedures of genetic modification of lily are developed in several directions. At the University of Leiden for the introduction of virus resistances the coat protein method has been used by Langeveld. Contructs are made containing the DNA encoding for the coat proteins of LSV, TBV and LVX. A transformation technique is in development using particle bombardment on bulb-scale explants. Another approach, followed at CPRO-DLO, is pollen transformation: isolated pollen bombarded with particles coated with DNA containing the kanamycin resistance gene and the B-glucuronidase gene have been used to obtain transgenic plants (Bino et al. 1993). 400.000 *Lilium longiflorum* seeds were obtained after pollination with bombarded pollen and after screening for kanamycin resistance of the first 65.000 seedlings three kanamycin resistant plants were obtained. These plants showed also the activity of the Gus-gene and using PCR the presence of this gene could be confirmed at molecular level. Currently the behaviour of these introduced genes after generative reproduction is studied. The progeny will be examined molecularly and tested for kanamycin resistance to investigate the segregation of this character.

Other new techniques at CPRO-DLO are protoplast isolation and regeneration for either somatic hybridization or direct DNA transfer (see Famelaer et al. in this Acta) and microspore culture for the induction of haploidy (Van den Bulk et al. 1992). Using microspore culture of the Asiatic cultivar 'Whilito' it is possible to change their normal developmental pattern. This change can result in the formation of multinucleate microspores, which possibly is an indication for sporophytic development leading to embryo formation (Van den Bulk et al, 1993).

8. Concluding remarks

The Netherlands are country number one in the world for lily culture. Research on culture and breeding in combination with a well organized bulb industry has attributed to this success. Present research is directed to reduce the use of chemical control in bulb culture (within the Urgency Program for Diseases and Breeding of Bulbs).

At CPRO-DLO extensive research is carried out towards breeding for resistance, quality traits (e.g. flower longevity), interspecific hybridization and new breeding techniques. Interspecific hybridization research proves to be very promising and resulted in a new hybrid group of lily, the LA-hybrids and other unique hybrids. In current research, in close cooperation with breeding companies it is attempted to combine Asiatic and Oriental hybrids in one group. Methods for genetic modification and other new techniques e.g. transformation and protoplast fusion are under investigation and promise new possibilities for application in lily breeding for the future.

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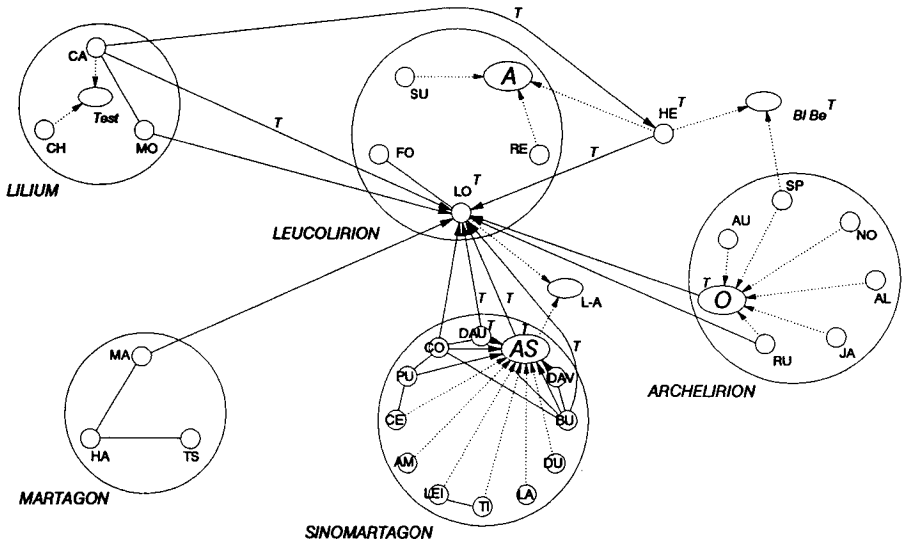

Figure 1

Figure 1: Crossing polygon of the genus *Lilium* Large circles denote sections. Circles: species; small ellipses: species hybrids; large ellipses: hybrid groups. Dotted lines: parentage, connection between hybrids and their parents; solid lines: successful cross combinations, arrows indicate direction of pollen flow. In this figure the connection between the Asiatic, Aurelian and Oriental hybrid groups (large ellipses) are shown by dotted lines. Some hybrids (small ellipses) were made not belonging to one of the major hybrid groups. In successful crosses between species (small circles) of different sections (large circles) the arrow points towards the female parent.

Abbreviations: A: Aurelian hybrids; AL: *L. alexandrae*; AM: *L. amabile*; AS: Asiatic hybrids; AU: *L. auratum*; BU: *L. bulbiferum*; Bl Be: 'Black Beauty'; CA: *L. candidum*; 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*; MO: *L. monadelphum*; NO: *L. nobilissimum*; O: Oriental hybrids; PU: *L. pumilum*; RE: *L. regale*; RU: *L. rubellum*; SP: *L. speciosum*; SU: *L. sulphureum*; TI: *L. tigrinum*; TS: *L. tsingtauense*; Test: *L. x testaceum*; T means that from species or F1-hybrids besides a diploid also a tetraploid form is developed.

(figure designed by L.W.D. van Raamsdonk, CPRO-DLO, Wageningen).

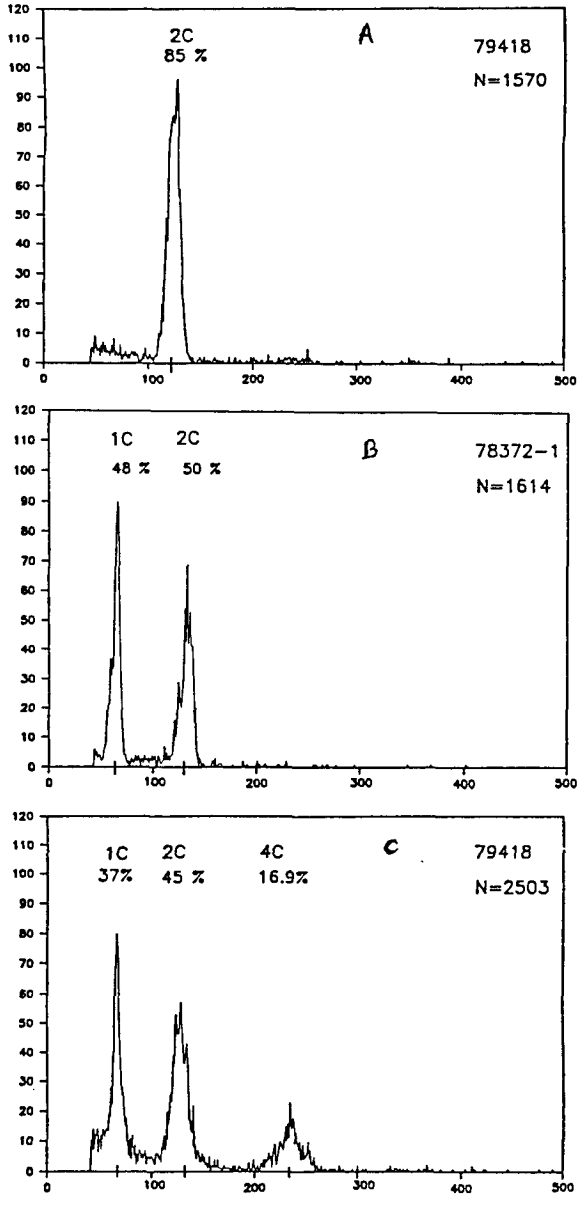

Figure 2

Flow cytometric of measurements of nuclear DNA contents of the leaf tissue of 'Enchantment' x *L. pumilum* (A), of vegetative and generative cells of pollen of *L. longiflorum* 'Gelria' (B) and of pollen of an 'Enchantment' x *L. pumilum* hybrid which produce 2N-pollen (C). X-axis: relative DNA amount per nucleus, Y-axis: number of nuclei. N= total number of counts registered in channels 40 to 500; percentages in all figures related to N.