

INTER-SPECIFIC HYBRIDS OF *LILIUM LANKONGENSE* FRANCHET PRODUCED BY EMBRYO-CULTURE

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SUMMARY

Lilium lankongense crossed with some related species of *Lilium* produced seeds without endosperm and with very small embryos which would not germinate in soil. Hybrid plants have been obtained from a number of these crosses by culturing excised embryos on sterile nutrient medium. It is suggested that the technique may prove useful in the production of hybrids from other lily species-combinations which are not normally viable.

INTRODUCTION

The *Sinomartagon* section of *Lilium* (COMBER, 1949) comprises some 22 species which have for long attracted the attention of plant breeders. Inter-specific hybrids between many of the members of this group can be obtained with relative ease and combinations of a number of the species have given rise to the important class of garden lilies known as the Asiatic hybrids. Although *L. lankongense* FRANCHET is also included in *Sinomartagon* and has certain attributes of potential value to the Asiatic hybrids, it has not, to our knowledge, previously been successfully used for breeding within the group.

Barriers to crossing due to differences in chromosome numbers were not anticipated since with one exception *Lilium* species and nearly all hybrids are diploid ($n = 12$) though trisomics occur occasionally.

At this Institute attempts to obtain hybrids between *L. lankongense* and related species have shown that, although hybrid zygotes may be formed following some crosses, it is unlikely that they can be induced to grow into plants without the aid of embryo culture techniques. However, by using such aids a number of species hybrids of *L. lankongense* have been raised, and the necessary techniques and the resulting hybrids are described in this paper.

MATERIALS AND METHODS

Flowers of *L. lankongense* were emasculated approximately 24 hours before anthesis and immediately cross-pollinated. Pollen was obtained from freshly dehisced anthers or from those which had been stored for several weeks in stoppered glass tubes con-

taining calcium chloride. Pollination was effected by dusting the anthers on the stigmas, and caps of aluminium foil were then placed over the pistils to prevent accidental contamination.

Capsules resulting from hand-pollination were allowed to grow on the plants until they reached the stage of maturity judged necessary for embryo removal. They were surface-sterilized by dipping in 97% alcohol and then opened under sterile conditions in the cabinet described below. Intact capsules were preferred to those which had begun to open on ripening, because seeds from the latter were frequently contaminated by pathogens.

Embryos were dissected from seeds with the aid of a stereomicroscope housed in a 'perspex' dissecting cabinet, 80 × 40 × 30 cm. Simple measures used to obtain sterile conditions gave an excellent control of micro-organisms and less than 1% of the cultures became contaminated.

Twenty-five ml screw-top wide-mouth glass vaccine bottles filled to about one quarter of their volume with medium, were prepared to take the embryos. A simple medium of 1% agar containing 2% sucrose and major mineral nutrients as described by EMSWELLER et al. (1962) was used. The bottles of medium were sterilized. Bottles containing the embryos were transferred to racks in the laboratory; no special temperature or light regimes were used.

RESULTS OF CROSSINGS

The first attempts to obtain hybrids between *L. lankongense* and several other species and hybrids were made here in 1966. Thirty different crosses were attempted and none produced germinable seed, but it was observed that capsules of *L. lankongense* sometimes began to swell after pollination with some other species, notably *L. davidii* Duchartre and its forms.

In 1967 emasculated flowers of *L. lankongense* were hand-pollinated in the greenhouse with pollen from 14 different species and hybrids. Again some capsules began to swell and eventually increased to 78–93% in length and 54–87% in width of capsules from fertile sib-crosses on the same plant. Some fully-grown but undehisced capsules from these crosses were opened and found to contain small flat seeds, of which approximately 10% had small embryos. All of the seeds, even those with embryos, lacked the endosperm which constitutes a large part of the bulk of normal seeds. The embryos were almost spherical and approximately 0.5 mm in diameter, whereas embryos from normal fertile crosses were cigar-shaped, generally grew to 4–5 mm. in length and were probably at least 10 times the volume. In 1968 some of the crosses were repeated and three new combinations made. Again these produced imperfect seed with small embryos, none of which germinated when sown in soil. As a result of the failure of these embryos to germinate in a normal manner it was decided in 1967 to investigate *in vitro* culture techniques in the hope of stimulating their growth and development.

Attempts to raise plants by transferring the imperfectly developed entire seeds to nutrient medium, as described by SKIRM (1942) generally failed because the embryos would not readily commence to grow when left in the seed coat. The small embryos were therefore excised from the seed and transferred alone to the nutrient medium.

The embryos usually began to grow after 10 days and were transferred to pots of

soil compost about 2 months after excision. The young plants were kept in a warm, moist greenhouse atmosphere for several weeks after potting and then repotted and placed in a cool greenhouse.

THE HYBRIDS

Altogether 44 plants have been obtained; they represent the product of approximately 90 pollinations in 1967 and 1968 and the examination of nearly 10,000 seeds for the presence of embryos. The following combinations have been produced, all with *L. lankongense* as female parent:

- × *L. davidii* 'Maxwill' (Family Nos. 230, 253)
- × *L.* × *maxbile* (*L. maximowiczii* × *amabile*) (231)
- × *L.* 'Edith Cecilia' (*L. cernuum* hybrid) (232, 252)
- × *L.* 4/1 ('Redbird' × 'Fiesta hybrid') (233)
- × *L.* 26/1 (*L. tigrinum flaviflorum*) hybrid (234)
- × *L. duchartrei* (249)

It could not be concluded that these plants were true hybrids without some certain means of identification, especially since some lilies, notably *L. regale*, often produce apomictic seed when pollinated with a wide range of other species. With the above material it was impossible to distinguish between hybrid or any apomictic seedlings with any degree of confidence by comparison of morphological characters, at least until the plants had flowered. However, the distinction could be made quickly and confidently by the analysis of chromosome morphology at metaphase in orcein-stained root-tip squash preparations when the component parental genomes of hybrid karyotypes could be distinguished (Fig. 1). By this means some plants of families 230, 231, 232, 233 and 249 were confirmed as hybrids while families 234, 252 and 253 have not been examined; it is now highly probable that all are true hybrids.

It is interesting to note that whereas *L. lankongense* and *L. duchartrei* FRANCHET appear morphologically similar, and indeed the former has sometimes been classed as a variety of the latter, cross-pollination between these species in either direction did not produce normal viable seed. Very small embryos only developed and endosperm

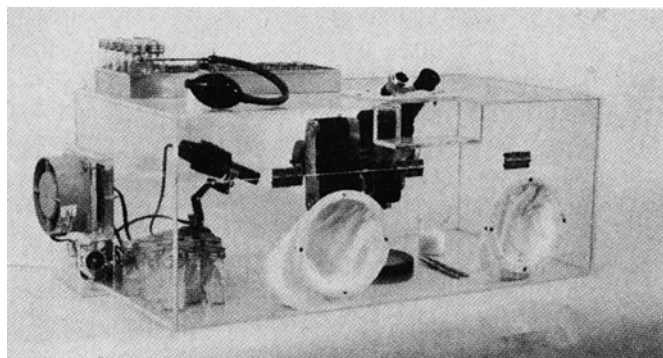


Fig. 1. Dissecting cabinet for transfer of *Lilium* embryos to in vitro culture.

EMBRYO CULTURE FOR LILIUM HYBRIDS

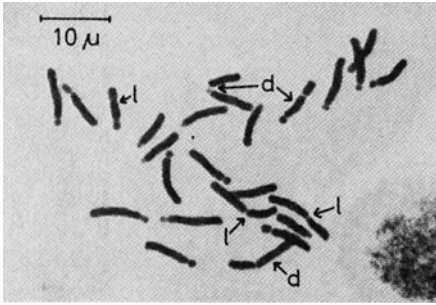


Fig. 2. Mitosis in hybrid of *L. lankongense* × *L. davidii* 'Maxwill' indicating specific chromosomes derived from each parent.

was absent when the capsules had reached maximum size. Crosses of *L. lankongense* with *L. wardii* and *L. taliense*, also included in Comber's *Sinomartagon* section of *Lilium*, failed even to give small embryos.

Most of the plants raised in 1967 were from crosses between *L. lankongense* and *L. davidii* 'Maxwill'. Twelve of the 18 plants from this cross flowered in the glasshouse 13 months after embryo excision (Fig. 3). All apparently had the stoloniferous habit of *L. lankognense* but the leaf margins were hairy as in the pollen parent. Individuals varied in colour, size and form of the flowers; the colour was always pale, ranging from white to mauve or honey-colour. All had a scent similar to, but less pronounced than, that of *L. lankongense*.

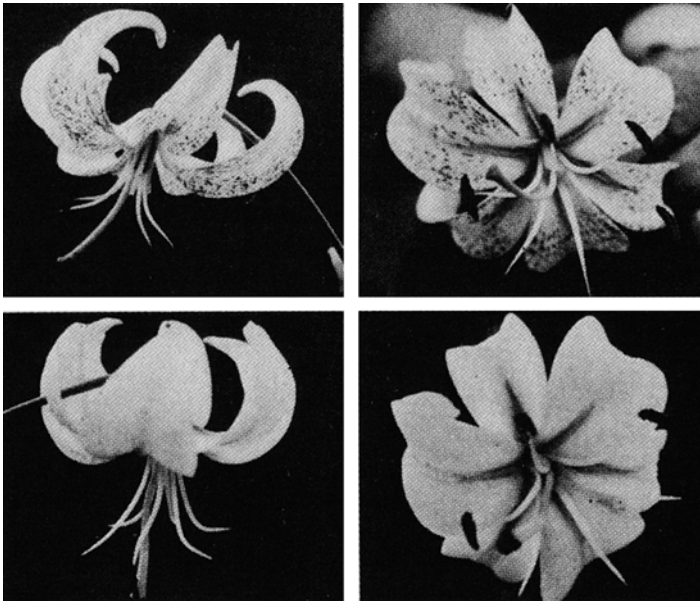


Fig. 3. Flowers of the hybrid *L. lankongense* × *L. davidii* 'Maxwill' (Family 230).

These hybrids were sib-crossed but produced very little seed. This may have been due in part to gametophytic pollen-style incompatibility but even when seed was formed it contained no endosperm and only small embryos. Attempts are being made to raise plants from these embryos.

DISCUSSION

EMSWELLER et al. (1962) have successfully used embryo culture to obtain hybrids between *L. auratum* and *L. speciosum*. The cross produces seeds with fully developed embryo and endosperm but these very rarely germinate when sown in the normal way because the endosperm produces a toxin which damages the embryo and prevents it from growing. Excised mature embryos, and seeds which have been leached in water to remove the toxin, will grow on sterile agar. Thus their technique seems to fulfil a somewhat different role in this species-combination to our technique, because the *L. lankongense* hybrids produced only very small embryos associated with endosperm abortion at an early stage of development. Placing these abnormally small embryos on a sterile medium provided the nutrient supply which in normal seeds is made available by the endosperm, and thus enabled them to develop into plants.

BROCK (1954) noted chromosome breakage in the endosperm of *L. regale* and an Asiatic hybrid lily Phyllis Cox which resulted in the failure of the endosperm and ultimately led to the death of the embryo. He suggests that this sequence of events is probably a common cause of failures in inter-specific hybridization. The results described in this paper support his views and indicate that embryo culture may have considerable importance for the lily breeder who wishes to make use of a wider range of germ plasm.

It is already evident that the *L. lankongense* hybrids may have some garden value in their own right, but it is not yet known whether they can be used to transfer the useful characters of that species to a wide range of Asiatic hybrids. *L. lankongense* has scented flowers with a basic white coloration; two features which are lacking in the main hybrid group. It also appears to possess considerable field resistance to *Botrytis elliptica* which causes serious damage to many lilies unless a regular spraying routine is carried out.

It is intended to exploit this technique further and six other new hybrid combinations have already been produced.

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