FREEZING OF VEGETATIVE GERMPLASM OF LILY FOR 0 TO 4 YEARS

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Abstract

Germplasm collections of lily are usually maintained in the field leading to high labour costs and to risk of losses caused by diseases. Therefore research has been started aiming to develop storage methods for vegetative material of cultivars and species. In the present study, viability after 0 to 4 years of storage of bulbs in moist peat at -2°C was investigated. The cultivars used were 'Avignon', 'Connecticut King', 'Enchantment', 'Esther' and 'Mont Blanc' (Asiatic hybrids), 'Star Gazer' (Oriental hybrid) and *L. longiflorum* 'Gelria' and 'Snow Queen'. Viability was scored by the ability of scales to form bulblets. After 4 years of storage, more than 50 percent of the scales of 'Enchantment' and 'Esther' and only a few inner scales of 'Avignon', 'Connecticut King' and 'Mont Blanc' formed scale bulblets. Scales of 'Gelria', 'Snow Queen' and 'Star Gazer' showed enough regeneration for propagation after 2 years of storage, but hardly any regeneration of scales was found after 3 years of storage. These results suggest that Asiatic hybrids can be stored safely for at least 3 years in moist peat at -2°C. 'Star Gazer', 'Gelria' and 'Snow Queen' should not be stored longer than 2 years this way.

Additional index words

collection, frost, preservation, storage, viability

1. Introduction

All crop improvement is dependent on available genetic diversity. Therefore, breeding companies and governmental institutes preserve collections of lily genotypes. DLO - Centre for Plant Breeding and Reproduction Research (CPRO-DLO) maintains a lily collection of more than 1000 genotypes to support lily breeding and research. Genotypes of lily have to be preserved as clones, since lily genotypes are heterozygous Using seeds would break up unique genetic combinations which have evolved. Collections of clonal lines are usually maintained as field collections, leading to high investments of labour and space and risk of losses caused by diseases (Towill, 1988; Withers, 1991). An alternative method is conservation *in vitro* under controlled conditions. Although *in vitro* conservation has advantages, there are also some problems. At each transfer event, there is a risk of contamination with microbial organisms and attention is needed to maintain adequately controlled culture conditions over long periods of time. Furthermore, the establishment of an *in vitro* collection is labour-intensive and later on, each transfer operation will be laborious. In future also cryopreservation of lily meristems could be an alternative. A method has already been tested in which 8 percent of meristems of *L. speciosum* 'Rubrum' survived cryopreservation (Bouman and de Klerk, 1990).

In 1991 at CPRO-DLO, research has been started to study techniques for long term storage of vegetative material of lily, tulip and hyacinth (Bonnier et al., 1992). For lily, storage methods are being developed both for *in vitro* and *in vivo* material. In the past, a

lot of research has been carried out to develop a suitable method for lily bulbs for year round forcing. This resulted in a method in which bulbs of Asiatic hybrids, Oriental hybrids and *L. longiflorum* are stored in moist peat at -2°C for 1 year (Boontjes, 1983; Beattie and White, 1993). This storage method is probably also suitable for conservation of vegetative lily material for more than 1 year. For storage of vegetative collection material, regeneration of scale bulblets on scales is needed. In the present study, the relation between storage duration of bulbs at -2°C and the regeneration of scales of these bulbs is investigated.

2. Material and methods

2.1. Plant Material

During 4 years, bulbs of different cultivars with a circumference between 12 and 16 cm were obtained from commercial stocks and stored in moist peat at -2°C. Cultivars with different genetic backgrounds were used: Asiatic hybrids: 'Avignon', 'Connecticut King', 'Enchantment', 'Esther', 'Mont Blanc'; Oriental hybrids: 'Star Gazer'; *L. longiflorum:* 'Gelria', 'Snow Queen'.

2.2. Viability tests:

Regeneration of scale bulblets on scales was scored as follows: Bulbs were scaled and scales were placed for 10 minutes in a solution containing 1% Captan and 0.2% Sportak, to diminish growth of micro organisms during propagation. Then scales were dried for 1 hour and planted in soil or in vermiculite saturated with water. Scale bulblet formation was recorded after eight weeks of incubation at 25°C.

Regeneration is defined enough for propagation if the percentage of bulbs with regeneration is at least 37 percent and the percentage of scales with regeneration is at least 5 percent. If 10 bulbs or 100 scales per genotype are stored, the risk of losing a genotype is than smaller than 1 percent.

Several experiments were carried out in which viability of bulbs stored for zero to more than 4 years was determined by the percentage of bulbs with at least one regenerative scale and the percentage of regenerative scales. This study contains the joined results of these experiments. The numbers of bulbs and scales used are summarized in Table 1.

3. Results

3.1. Asiatic hybrids

After a storage duration of 4 years, regeneration of bulbs was 100 percent for 'Enchantment' and 88 percent for 'Esther' (Figure 1A). For scales these values were 66 percent and 55 percent respectively (Figure 1 B). Regeneration of 'Mont Blanc' was 50 percent for bulbs and 33 percent for scales (Figure 1A,B). This seems enough for propagation, but these results are only based on 2 bulbs and 24 scales (Table 1). Regeneration of 'Avignon' and 'Connecticut King' was limited to 1 scale of 86 scales and 3 scales of 109 scales respectively (Figure 1B). After a storage duration of 3 years, regeneration of 'Avignon' was curtailed, but still enough for propagation i.e. 47 percent of the bulbs had regenerative scales and 18 percent of the scales regenerated scale bulblets (Figure 1A,B). Regeneration of 'Connecticut King' and 'Mont Blanc' was better than of 'Avignon' both after 2 years and 4 years of storage, but no data are available for a storage duration of 3 years (Figure 1A,B). After a storage duration of 2 years, regeneration of all cultivars was enough for propagation.

3.2. 'Star Gazer' and *L. longiflorum* cultivars

Regeneration of 'Star Gazer' had declined, but was still enough for propagation after 2 years of storage, whereas there was hardly any regeneration after a storage duration of more than 3 years (Figure 1C,D). Regeneration of 'Gelria' and 'Snow Queen' was better than 'Star Gazer' after 2 years of storage, but after 3 years of storage no regeneration was found.

4. Discussion

After 4 years of storage regeneration of 'Esther' and 'Enchantment' was still sufficient for propagation, whereas only a few scales of 'Avignon', 'Connecticut King' and 'Mont Blanc' formed scale bulblets. Regeneration of 'Avignon' was still enough for propagation after a storage duration of 3 years. Since regeneration of 'Connecticut King' and 'Mont Blanc' was better than of 'Avignon' both after 2 years and 4 years of storage, it seems likely that regeneration of 'Connecticut King' and 'Mont Blanc' would have been enough for propagation after a storage duration of 3 years. This means that a field collection of Asiatic hybrids can probably be maintained by planting bulbs only one season per 3 years. Bulbs of the Oriental hybrid 'Star Gazer' and *L. longiflorum* 'Gelria' and 'Snow Queen' should not be stored longer than 2 years at -2°C.

The storage method used in the present study is only tested on 8 cultivars. To store a complete collection for several years at -2°C, all cultivars of the collection should first be tested on their ability to survive 2 and 3 years storage. During the test period the collection needs still be maintained by yearly planting of bulbs. After the test period, the maintenance of a field collection of lily will be much cheaper than in the past.

Towill (1988) mentioned the importance of the concept of maintaining both an active and base collection for all crops as is stated by the International Board for Plant Genetic Resources. An active collection contains plant accessions available for distribution, evaluation and documentation, and a base collection is used as a duplicate for long term storage of diversity. The maintenance of a base collection *in vitro* would be preferable, as it would reduce the risk of losses caused by or diseases. Therefore, storage methods for in vitro material of lily are also being investigated.

Cryopreservation could be an alternative in future (Bouman and de Klerk, 1990).

Acknowledgements

Part of the work was supported by the Dutch Urgency Programme for Research on Diseases and Breeding of Flower Bulbs.

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Table 1. The number of observations of bulbs and scales used.

Cultivar	Storage duration (years)						Storage duration (years)				
	0.5	1.5	2	2.5	3	4	0.5	1.5	2	3	4
	Bulbs #						*Scales #*				
Avignon	39	38	27	29	15	7	135	277	276	197	86
Connecticut King	0	0	5	9	0	5	0	0	146	0	109
Enchantment	8	9	5	9	5	5	0	0	253	196	180
Esther	18	25	17	9	5	8	106	62	132	134	157
Mont Blanc	8	0	5	9	0	2	0	0	129	0	24
Star Gazer	42	25	24	9	15	5	169	154	147	90	0
Gelria	8	9	4	0	5	5	0	0	109	0	0
Snow Queen	21	17	12	0	5	5	0	0	129	0	0

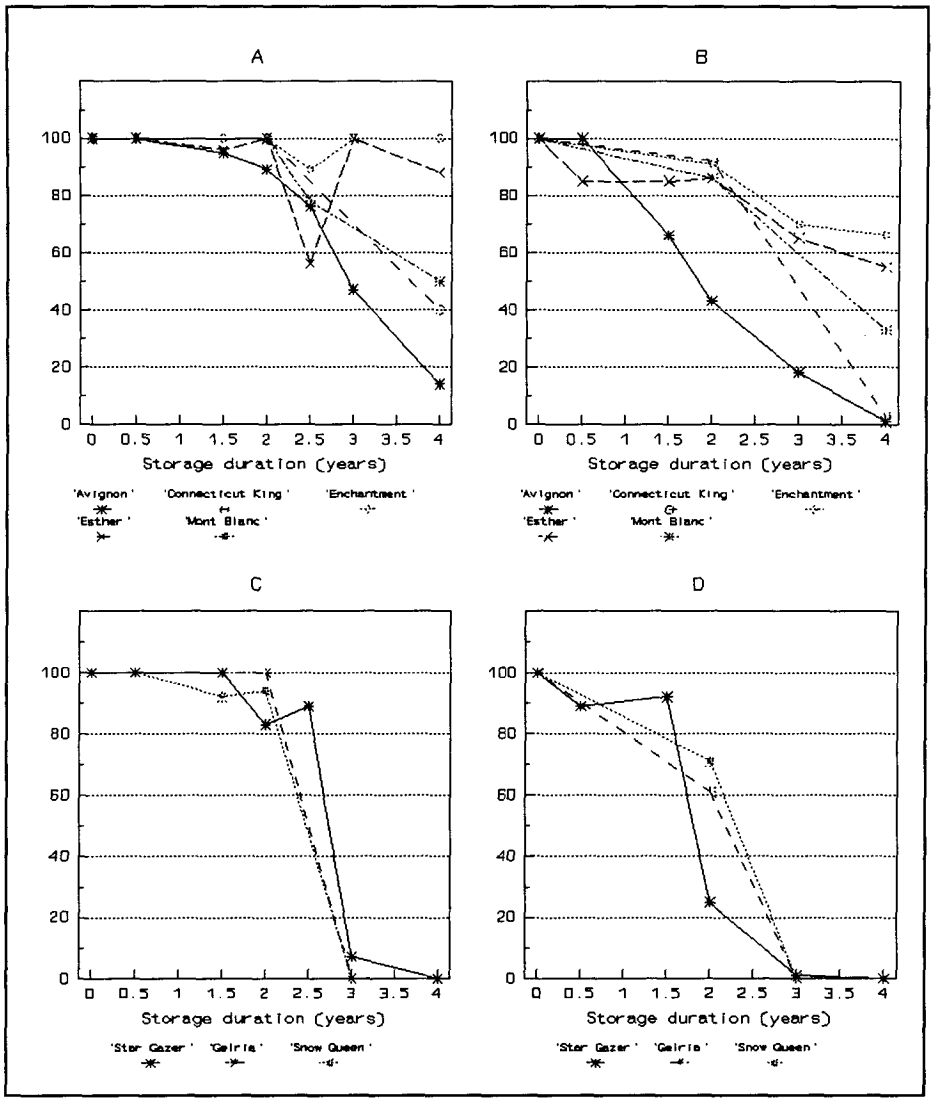

Figure 1. The relation between storage duration at -2°C and viability of lily scales. A: percentage of bulbs with at least one regenerative scale for 5 Asiatic hybrids.

B: percentage of regenerative scales for 5 Asiatic hybrids. C: percentage of bulbs with at least one regenerative scale for one Oriental hybrid and the *L. longiflorum* cultivars. D: percentage of regenerative scales for one Oriental hybrid and the *L. longiflorum* cultivars.