Ion Leakage as a Criterion for Viability of Lily Bulb Scales after Storage at -2°C for 0.5, 1.5, and 2.5 Years

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Additional index words. *Lilium*, germplasm, conductivity, collection

Abstract. The viability of 'Avignon' and 'Esther' (Asiatic hybrids), 'Star Gazer' (Oriental hybrid), and 'Snow Queen' lily bulb scales (*L. longiflorum* Thunb.) was determined after storage at -2°C for 0.5, 1.5, and 2.5 years. Ion leakage, the percentage of scales that formed bulblets, and the number and weight of these bulblets were determined on scales from the inner, middle, and outer part of bulbs. During storage, the outer scales of all cultivars and the inner and middle scales of 'Snow Queen' showed increased ion leakage accompanied by a decreased ability to form scale bulblets during storage. Concurrently, the percentage of scales forming bulblets declined, and more and smaller scale bulblets were formed per regenerative scale. Thus, ion leakage is a useful criterion to measure viability of lily scales.

The preservation of a broad genetic variation in germplasm collections is important for breeding crops and combatting genetic erosion (Grout, 1991; Wehner, 1988). Collections of clonal lines are usually maintained in the field (Towill, 1988), where there are high labor and space costs and risk of disease losses. Hence, storage of clonal material for several years would be very useful. A project was started at the Centre for Plant Breeding and Reproduction Research in 1991 with the goal of developing storage methods for lilies.

Techniques for measuring the viability are necessary to detect decline of vigor under diverse circumstances. In addition to growth characteristics, ion leakage, which is used as a standard test for determining seed viability, might be a usable criterion (Schmidt and Tracy, 1988; Tracy and Juvik, 1988). Ion leakage measured by conductivity and K leakage can also be used to measure damage of ozone exposure in bean (*Phaseolus vulgaris* L.) (McKersie et al., 1981). Forney and Peterson (1990) reported that enhanced leakage of K^{+} ions was an effective indicator of chilling injury in grapefruit (*Citrus paradisi* Macf.) callus. However, McCollum and McDonald (1991) found that electrolyte leakage from chilled fruit measured by conductivity did not increase significantly until chilling injury had become severe. Electrolyte leakage has also been shown to be a good indicator for temperature stress in leaf disks of red pepper (*Capsicum annuum* L.) (Nanaiah and Anderson, 1992). Bonnier et al. (1992) showed that ion

leakage measured by conductivity or K leakage can be used to determine severe injury in lily scales artificially damaged by frost, heat, or desiccation.

To retard flowering, Asiatic and Oriental lily bulbs are usually stored at -2°C (Beattie and White, 1993). *Lilium longiflorum* can also tolerate this temperature (Miller, 1993). The objective of our study was to test whether ion leakage of bulb scales is a satisfactory criterion for testing viability of lily bulbs stored at -2°C . Therefore, effects of storage duration were tested on viability of lily scales measured by ion leakage and formation of scale bulblets.

Materials and Methods

Plant material. 'Avignon', 'Esther', 'Snow Queen', and 'Star Gazer' bulbs were obtained from commercial stocks. Of each cultivar, 300 bulbs with a circumference of 14 to 16 cm, harvested in Oct. or Nov. 1989, 1990, and 1991, were packed in moist peat and acclimatized for 6 weeks at 0°C and then stored at -2°C for 2.5, 1.5, and 0.5 years, respectively.

In May 1992, 12 bulbs were sampled per cultivar and year of harvest. One scale from the inner 20% of the scales (inner scale), one scale not belonging to the 40% inner or outer scales (middle scale), and one scale from the outer 20% of the scales (outer scale) were selected per bulb. The fresh weight, ion leakage, and the formation of scale bulblets were measured for each scale.

Ion leakage. Scales were washed with water and stored during the night in a polyethylene bag at 18 to 20°C to give fresh wounds time to heal. The next day each scale was washed in distilled water and then put in 100 ml distilled water at 20°C for 1.5 h. Subsequently, the conductivity of the samples was measured using a digital conductivity meter (Philips PW9526 with electrode PW9514/60). The conductivity of each scale was corrected by subtracting the conductivity of the distilled water

($1.85\ \mu\text{S}\cdot\text{cm}^{-1}$) and then dividing by the fresh weight of the scale.

Formation of scale bulblets. Scale bulblet formation was recorded after 8 weeks of incubation in vermiculite saturated with water at 25°C . This method induces bulblet formation for Asiatic hybrids and *L. longiflorum* (Van Tuyl, 1983) and Oriental hybrids (Hendriks, 1988). The number and weight of the formed bulblets were determined per scale.

Statistical methods. Effects of storage duration on ion leakage and the formation of scale bulblets were analyzed by standard analysis of variance and LSD. To get a normal distribution, conductivity values were log-transformed. The mean conductivity values expressed in Table 1 are back-transformed mean logarithm values. The number, weight, and size of the scale bulblets formed per scale were analyzed, excluding scales that did not form bulblets. First, the model (cultivar $\times$ scale $\times$ -2°C storage duration) was analyzed, but for all characteristics, a significant interaction was found between effects of storage time and of scale position. Therefore, statistical analyses were carried out separately for the inner, middle, and outer scales. In the absence of an interaction between effects of cultivar and storage time, significances are displayed for main effects of storage time per scale type. Otherwise, significance is also presented per cultivar (Table 1). The percentage of scales forming bulblets was not statistically analyzed per single scale because of extreme data (0% and 100%). The percentages were calculated per treatment.

To analyze the relation between conductivity and the formation of scale bulblets, the scales were classified into four categories of conductivity. Regression analysis was carried out with the number of bulblets formed per gram per regenerative scale, fresh weight of the scale bulblets formed per gram per regenerative scale, and the mean fresh weight per scale bulblet using the model (category $\times$ cultivar $\times$ scale). Since all interactions were either nonsignificant or small compared to main effects, means and a 95% confidence interval were calculated over all scales per category.

Results

Effects of storage. Ion leakage, expressed as conductivity, increased significantly with storage duration for outer scales of all cultivars (Table 1). For 'Snow Queen', ion leakage of inner and middle scales increased also significantly with storage duration (Table 1). For the other cultivars, there was no significant effect of storage duration on ion leakage of inner and middle scales.

The regeneration percentage for inner and middle scales was more than 80% in all treatments (Table 1). For outer scales, this percentage decreased with storage duration depending on the cultivar. For outer scales of 'Esther', it was still 83% after 2.5 years of storage, but for 'Snow Queen' it was curtailed to 18%.

The effect of storage time on the number of scale bulblets varied with cultivar (Table 1).

Received for publication 19 Jan. 1994. Accepted for publication 15 June 1994. This work was financially supported by Dutch bulb growers and breeders and the Dutch government via the Urgency Program for Research on Diseases and Breeding of Flower Bulbs. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Table 1. Mean values of conductivity and the formation of scale bulblets for 12 inner, middle, and outer scales after storage of bulbs of four lily cultivars at -2C.

Characteristic	Storage duration (years)	Inner scales					Middle scales					Outer scales				
		Cultivar					Cultivar					Cultivar				
		AV ²	ES	SQ	SG	Mean	AV	ES	SQ	SG	Mean	AV	ES	SQ	SG	Mean
Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$)	0.5	0.76 b ^y	0.63 a	1.00 a	0.65 a	0.75 a	0.71 b	0.45 ab	0.52 a	0.57 a	0.55 a	0.42 a	0.48 a	1.17 a	0.37 a	0.54 a
	1.5	0.42 a	0.59 a	1.80 b	0.63 a	0.73 a	0.34 a	0.26 a	0.90 b	0.69 a	0.49 a	0.65 a	0.70 ab	2.57 b	1.55 b	1.16 b
	2.5	0.65 ab	1.03 a	5.12 c	0.67 a	1.23 b	0.61 b	0.61 b	4.13 c	0.65 a	1.00 b	1.94 b	1.36 b	23.59 c	2.43 b	3.50 c
Regeneration (%)	0.5	100	100	100	92	98	100	100	100	100	100	100	100	100	92	98
	1.5	100	92	92	92	94	100	100	92	83	94	100	100	83	92	94
	2.5	100	100	83	92	94	100	100	83	92	94	50	83	18	50	51
Scale bulblets/gram scale (no.)	0.5	3.3 b	2.9 a	2.8 a	0.9 a	2.5 a	3.6 b	2.0 b	1.6 a	0.6 a	2.0 b	2.5	1.8	2.6	0.9	2.0 a
	1.5	2.1 a	2.7 a	2.5 a	1.2 a	2.1 a	2.2 a	1.1 a	1.5 a	1.2 a	1.5 a	2.9	2.7	2.6	1.8	2.5 b
	2.5	2.1 a	2.2 a	4.7 b	1.3 a	2.5 a	1.8 a	1.9 b	3.2 b	0.8 a	1.9 b	3.0	1.5	4.0	1.3	2.0 ab
Mean wt/scale bulblet (g)	0.5	0.06	0.13	0.08	0.09	0.09 a	0.09 a	0.24 a	0.14 a	0.23 a	0.17 a	0.20 a	0.21 a	0.24 a	0.47 b	0.28 a
	1.5	0.16	0.12	0.09	0.18	0.14 b	0.29 b	0.48 b	0.17 a	0.40 b	0.33 b	0.28 a	0.26 ab	0.24 a	0.26 a	0.26 a
	2.5	0.19	0.18	0.08	0.16	0.16 b	0.40 b	0.30 a	0.12 a	0.40 b	0.31 b	0.18 a	0.36 b	0.18 a	0.32 a	0.30 a

²AV = 'Avignon'; ES = 'Esther'; SQ = 'Snow Queen'; SG = 'Star Gazer'.

^yMean separation between different storage durations by protected LSD test, $P = 0.05$.

With 'Snow Queen', the number of scale bulblets increased on scales stored for 2.5 years for all scale types, although not significantly for the outer scales. Significant differences in the number of scale bulblets of the other cultivars appeared to be effects of differences between samples grown in different seasons rather than effects of storage time. Differences in the number of scale bulblets formed per gram between scale types were small.

The weight of the scale bulblets formed on inner scales increased significantly with storage time (Table 1). For 'Avignon' and 'Star Gazer', the same effect was found for middle scales. For outer scales, significant effects of storage duration on the weight of the scale bulblets were found only for 'Esther' and 'Star Gazer'. These significant differences are probably caused by sample differences. For all cultivars, scale bulblets formed were heavier on middle and outer scales than on inner scales (Table 1).

Relation between conductivity and the formation of scale bulblets. For most of the scales, the conductivity value was lower than $1 \mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$ (conductivity class 0-1 in Table 2). With the exception of two scales, all the scales in this class regenerated scale bulblets. With increasing conductivity values, the number of regenerative scales declined relatively, and the regenerative scales appeared to form more, but smaller, scale bulblets (Table 2).

Discussion

Effects of storage. Ion leakage of outer scales increased with storage duration and was accompanied by a decrease in the ability of scales to form new bulblets. With inner and middle scales, the increase in ion leakage and the loss of the regeneration percentage were limited to 'Snow Queen'. For all cultivars, effects of storage duration were most significant with outer scales. The faster decline of viability of outer scales compared to inner and middle scales was also found by Matsuo and Arisumi (1978) after storage of *L. longiflorum* bulbs at 25C. There may be several causes for

this effect. First, outer scales are formed before the middle and inner scales and are therefore older. Second, outer scales are more exposed to environmental conditions and micro-organisms.

For inner scales of all cultivars and for middle scales of 'Avignon' and 'Star Gazer', the weight of the bulblets formed increased with storage duration (Table 1). This increase in the weight of the bulblets was not accompanied by a decrease in the number of bulblets formed, except for 'Avignon'. The increase in the production of scale bulblets with storage duration has not been reported before.

Relation between conductivity and the formation of scale bulblets. Since the percentage of regenerative scales declined with conductivity (Table 2), conductivity values can be used to predict regeneration of scales and to measure the effects of treatments on the viability of scales. However, ion leakage is not an absolute criterion to predict regeneration of scales. Some scales with high conductivity values still produced scale bulblets and two scales with conductivity $<1 \mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$ did not (Table 2).

The observation that scales with high conductivity values formed more and smaller scale bulblets supports the results of Matsuo and Arisumi (1978). They found the same tendency for scales of *L. longiflorum* after treatment with hot water. It might be a general feature of lily scales with lowered viability.

Conductivity values are often presented as a percentage of a maximum conductivity based on leakage after freezing or heating (Forney

and Peterson, 1990). In our study, conductivity values are not corrected for maximum conductivity values for two reasons. First, maximum conductivity values of the scales could not be established, as the scales were used for propagation after measuring conductivity. Second, ion leakage of healthy scales was very low and probably limited to the outer cell layers of the scales. In that case, correction for maximum conductivity values of these scales would not be valid. Conductivity values that varied between 123 [$\ln(123) = 4.81$] and 272 [$\ln(272) = 5.61$] $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$ per scale were found after autoclaving and grinding other lily scales (results not shown). Since conductivity values were distributed normally after a transformation to their logarithms, the distinction between their logarithms is more relevant for the variation in maximum conductivity than the distinction between the nontransformed values.

In conclusion, ion leakage measured by conductivity appeared to be a useful criterion to measure viability of lily scales. It can be used to investigate further the development of suitable storage methods for lilies.

Literature Cited

- Beattie, D.J. and J.W. White. 1993. *Lilium*—Hybrids and species, p. 423-454. In: A. De Hertogh and M. Le Nard (eds.). The physiology of flower bulbs. Elsevier, Amsterdam.
- Bonnier, F.J.M., J. Keller, and J.M. van Tuyl. 1992. Conductivity and potassium leakage as indicators for viability of vegetative material of lily, onion and tulip. *Acta Hort.* 325:643-648.

Table 2. Relation between ion leakage and formation of scale bulblets of lily scales, combined for four cultivars stored at -2C for 0.5, 1.5, or 2.5 years, classified in four categories of conductivity ($\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$).

Conductivity class	Scales (no.)	Regenerative scales (%)	Bulblets/gram scale (no.)	Mean wt/scale bulblet (g)
0-1	279	99	1.9 ± 0.1^z	0.30 ± 0.02
1-3	86	91	2.4 ± 0.3	0.29 ± 0.03
3-9	40	68	3.1 ± 0.5	0.14 ± 0.06
>9	22	27	5.4 ± 1.2	0.10 ± 0.15

^z95% confidence intervals.

- Forney, C.F. and S.J. Peterson. 1990. Chilling-induced potassium leakage of cultured citrus cells. *Physiol. Plant.* 78:193-196.
- Grout, W.V. 1991. Conservation in vitro. *Acta Hort.* 289:171-178.
- Hendriks, C.H.M. 1988. The ideal temperature treatment for lily scales varies markedly (in Dutch). *Bloembollencultuur* 99:30-31.
- Matsuo, E. and K. Arisumi. 1978. Studies on growth and development of bulbs in the easter lily (*Lilium longiflorum* Thunb.). VII Scale position dependence of the effect of the hot water treatments on the growth behaviour of the scale bulb-lets. *Mem. Fac. Agr. Kagoshima Univ.* 14:69-76.
- McCollum, T.G. and R.E. McDonald. 1991. Electrolyte leakage, respiration, and ethylene production as indices of chilling injury in grapefruit. *HortScience* 26:1191-1192.
- McKersie, B.D., P. Hucl, and W.D. Beversdorf. 1981. Solute leakage from susceptible and tolerant cultivars of *Phaseolus vulgaris* following ozone exposure. *Can. J. Bot.* 60:73-78.
- Miller, W.B. 1993. *Lilium longiflorum*, p. 391-422. In: A. De Hertogh and M. Le Nard (eds.). *The physiology of flower bulbs*. Elsevier, Amsterdam.
- Nanaiah, G.K. and J.A. Anderson. 1992. Electrolyte leakage and evolution of ethylene and ethane from pepper leaf discs following temperature stress and fatty acid infiltration. *J. Amer. Soc. Hort. Sci.* 117:846-851.
- Schmidt, D.H. and W.F. Tracy. 1988. Endosperm type, inbred background, and leakage of seed electrolytes during imbibition in sweet corn. *J. Amer. Soc. Hort. Sci.* 113:269-272.
- Towill, L.E. 1988. Genetic considerations for germplasm preservation of clonal materials. *HortScience* 23:91-95.
- Tracy, W.F. and J.A. Juvik. 1988. Electrolyte leakage and seed quality in a shrunken-2 maize selected for improved field emergence. *HortScience* 23:391-392.
- Van Tuyl, J.M. 1983. Effect of temperature treatments on the scale propagation of *Lilium longiflorum* 'White Europe' and *Lilium* x 'Enchantment'. *HortScience* 18:754-756.
- Wehner, T.C. 1988. Genetic considerations in germplasm collection and maintenance: A summary. *HortScience* 23:95-97.