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

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Abstract. The viability of 'Avignon' and 'Esther' (Asiatic hybrids), 'Star Gazer' (Oriental hybrid), and 'Snow Queen' bulb scales (L. longiflorum Thunb.) was determined after storage at –2C 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 combating 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 useful 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.) (Nanaih 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 bulb scales are usually stored at –2C (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 –2C. 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 0C and then stored at –2C 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 20C 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 20C 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 μS cm⁻¹) and then dividing by the fresh weight of the scale.

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 formed per scale is considered.
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 \( \mu \text{S/cm} \cdot \text{g}^{-1} \) (conductivity class 0–1 in Table 2). 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 high conductivity values formed more than one bulblet per gram scale, supporting 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 1.23 [ln(123) = 4.81] and 2.72 [ln(272) = 5.61] \( \mu \text{S/cm} \cdot \text{g}^{-1} \) 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.

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

<table>
<thead>
<tr>
<th>Conductivity class</th>
<th>Scales (no.)</th>
<th>Regenerative scales (%)</th>
<th>Bulblets/gram scale (no.)</th>
<th>Mean wt/ scale bulblet (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>279</td>
<td>99</td>
<td>1.9 ± 0.1*</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>1–3</td>
<td>86</td>
<td>91</td>
<td>2.4 ± 0.3</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>3–9</td>
<td>40</td>
<td>68</td>
<td>3.1 ± 0.5</td>
<td>0.14 ± 0.06</td>
</tr>
<tr>
<td>&gt;9</td>
<td>22</td>
<td>27</td>
<td>5.4 ± 1.2</td>
<td>0.10 ± 0.15</td>
</tr>
</tbody>
</table>

*95% confidence intervals.