Dormancy Release of Micropropagated Bulblets of *Lilium speciosum* after Long Culture in Soil

Geert-Jan de Klerk, Isabelle Delvallée, and Annie Paffen

Central Research Laboratory for Tissue Culture of Horticultural Crops,
P. O. Box 85, 2160 AB Lisse, The Netherlands

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Abstract. Micropropagated *Lilium speciosum* Thunb. bulblets were cold-treated at 2C for various periods before being planted in soil. About 20% of noncold-treated bulblets and 100% of bulblets cold-treated for 6 weeks sprouted within 10 weeks after planting. Intermediate lengths of cold treatment resulted in intermediate percentages of emergence. The remaining 80% of the noncold-treated bulblets sprouted synchronously from 25 to 33 weeks after planting. In these bulblets, the number of scales increased before sprouting. A sample of bulblets that had received a short cold treatment (2 or 3 weeks) also showed biphasic emergence, with the second period between 25 and 33 weeks. When the cold treatment was interrupted by 3 weeks at 17C, the percentage of emergence during the first period was reduced and the timing of the second period advanced.

In The Netherlands, 16 million lily bulblets were produced by micropropagation in 1989 (Pierik, 1990). Other bulbous crops are likely to be micropropagated in large numbers in the near future. Since sprouted bulblets are difficult to handle and require acclimatization, micropropagated bulblets should be dormant at harvest. Knowledge of the factors that determine dormancy in micropropagated bulblets is limited. Therefore, we initiated a research program on the development of dormancy during culture in vitro using *L. speciosum* as a model. We have reported previously that temperature, sucrose concentration, gibberellic acid concentration, and duration of culture have a significant effect on the development of dormancy (Aguettaz et al., 1990; Delvallée et al., 1990). Our article describes dormancy release in micropropagated bulblets. Experiments were conducted to investigate 1) the kinetics of dormancy release during chilling, 2) the effect of an intermittent warm treatment during chilling, and 3) the fate of dormant, nonsprouted bulblets after protracted culture in soil.

Plant material. Field-grown bulbs (circumference 18 to 20 cm) of *L. speciosum* 'Rubrum' no. 10 were harvested in Nov. 1988 and stored at 0.5C in moistened peat until use. The dormancy status of micropropagated bulblets was not affected by the duration of storage of the starting material (I.

Delvallée, unpublished observations).

Tissue culture conditions. Scales were surface-sterilized for 30 min in 1% (w/v) NaOCl. Explants of 7 × 7 mm were placed with the abaxial side on 15 ml of medium in 22-mm-diameter culture tubes. The medium was composed of MS macro- and microelements (Murashige and Skoog, 1962), and (mg·liter⁻¹) 30,000 sucrose, 0.4 thiamine, 100 myo-inositol, 7000 agar (Difco-Bacto), and 0.05 1-naphthaleneacetic acid. The explants were cultured at 20C (unless indicated otherwise) at a light intensity of 30 μmol·m⁻²·s⁻¹ and a 16-h photoperiod from cool-white fluorescent lamps (Philips TL 33).

Cold treatments. Bulblets were harvested from the scales after 15 weeks (unless indicated otherwise) and the leaves and roots removed. Samples of 30 bulblets were treated for 20 min with 0.2% (w/v) Benlate solution, transferred to moistened filter paper in 9-cm-diameter petri dishes, and stored at 2C. In two experiments, the durations of chilling were 0, 1, 2, 3, 4, 5, or 6 weeks, in two other experiments 0, 2, 4, or 6 weeks, and in a fifth experiment 0, 1, 3, or 6 weeks.

Fig. 1. Emergence of *Lilium speciosum* bulblets after increasing duration of cold treatment at 2C. Emergence was recorded 10 weeks after transplanting to soil. Each value represents the mean of several determinations with confidence limits at 95% level, indicated by vertical bars.

Data from the five experiments were combined.

For the chilling treatments of 2 and 3 weeks, an additional experiment was carried out in which chilling was interrupted after 1 week and 2 weeks, respectively, by 3 weeks at 17C.

Culture in soil. After the cold treatments, the samples were planted in steamed potting soil in wooden containers of 23 × 23 × 10 cm and cultured at 17C at a light intensity of 20 μmol·m⁻²·s⁻¹ (cool-white fluorescent lamps) for 16 h·day⁻¹. The soil was moistened twice a week. Leaf emergence was recorded once per week. All values of emergence refer to samples of 30 bulblets. Each experiment was repeated at least once.

In one experiment, 25 to 30 noncold-treated bulblets were planted in soil and dug up after 12 weeks. The bulblets were dissected and the number of scales determined. Sprouted bulblets were omitted.

Effect of duration of cold treatment. Both conventionally produced lily bulbs (Kamerbeek et al., 1970) and micropropagated bulblets (Higgins and Stimart, 1990; Niimi et al., 1988; Stimart and Ascher, 1981) require chilling to achieve rapid sprouting after planting. The percentage of sprouted bulblets 10 weeks after planting increased with the length of cold treatment (Fig. 1). All bulblets cold-treated for 6 weeks emerged within 10 weeks.

Sprouting of dormant bulblets after protracted culture in soil. About 20% of the noncold-treated bulblets sprouted during the first 10 weeks after planting. After this, few bulblets sprouted until 24 weeks. Then, within a period of a few weeks (up to 33 weeks), all previously dormant bulblets sprouted (Fig. 2). Samples that had received a cold treatment insufficient to break dormancy in all bulblets (2 or 3 weeks at 2C) also showed biphasic emergence. The timing of the second period was not advanced by the cold treatment. In bulblets cold-treated for 4 weeks, however, additional emergence after the first period commenced 12 weeks after planting and extended over a long period.

Increase in the number of scales in dormant bulblets. In vitro lily bulblets continue to form new primordia and scales after the

Fig. 2. Emergence of *Lilium speciosum* bulblets after transplanting into soil. The bulblets were cold-treated at 2C for 0, 2, 3, 4, 5, or 6 weeks. Emergence was recorded weekly.

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Fig. 3. Emergence of *Lilium speciosum* bulblets after transplanting into soil. The bulblets were treated for 2 weeks at 2C with or without an intermittent period of 3 weeks at 17C. (A) 2 = 2 weeks at 2C; 1-3-1 = 1 week at 2C-3 weeks at 17C-1 week at 2C or for 3 weeks at 2C with or without an intermittent period of 3 weeks at 17C. (B) 3 = 3 weeks at 2C; 2-3-1 = 2 weeks at 2C-3 weeks at 17C-1 week at 2C.

Table 1. Number of scales of dormant *Lilium speciosum* bulblets at the time of transplanting into soil and 12 weeks after transplanting. The bulblets were harvested 11 weeks after the start of tissue culture.

	No. scales/bulblet ^z	
	20C	25C
Time of transplanting	4.4 ± 0.2	4.1 ± 0.2
12 weeks after transplanting	5.0 ± 0.1*	5.3 ± 0.3**

^zValues represent the mean of 25 to 30 bulblets ±SE.

*,**Increase during culture in soil is significant at $P = 0.05$ or 0.01 , respectively according to Student's t test.

induction of dormancy (Delvallée et al., 1990). Consequently, induction of dormancy does not stop growth. We concluded that dormancy in lily corresponds with the inability of the primordium to form a leaf (Delvallée et al., 1990). After planting dormant bulbs in soil, the number of scales increased (Table 1). The increase was similar for bulblets regenerated at 20 and 25C, even though dormancy is deeper in the latter (Aguettaz et al., 1990). The actual number of newly formed scales may be higher than indicated, since the outermost scales may have degraded. Leaves that emerged 25 to 33 weeks after planting did not originate from the primordium present at the time of planting, but from a primordium formed in the soil after planting.

Effect of a cold treatment interrupted by

a warm period. When a 2C treatment of 2 weeks was interrupted by 3 weeks at 17C, the timing of the second period was advanced relative to no interruption (Fig. 3A). Similar results were obtained in an experiment in which a cold treatment of 3 weeks was interrupted by 3 weeks at 17C (Fig. 3B). These data show that the timing of the second period depends only on the number of weeks at high temperature and not on the weeks at 2C.

Apart from a marked effect on the timing of the second period, an intermittent warm period also reduced the effect of the chilling treatment. Similar results have been reported for buds in woody crops (Saure, 1985). We have previously observed that a short period at 20C induces dormancy in bulblets that were not dormant (Delvallée et al., 1990). Possibly the intermittent period at 17C induced dormancy and thereby reduced the emergence during the first period.

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