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The effects of environmental conditions on sprouting of microprogated lily bulblets with various levels of dormancy

G. J. DE KLERK and A. PAFFEN

Centre for Plant Tissue Culture Research, PO Box 85, 2160 AB Lisse, The Netherlands

SUMMARY

We have examined the effects of environmental conditions on the sprouting of micropropagated bulblets of *Lilium speciosum* with various levels of dormancy. After planting at low temperature (15°C) or *in vitro*, a higher percentage of bulblets sprouted than after planting at high temperature (20 or 25°C) or in soil. Dormancy was relative, i.e. bulblets with deep dormancy were capable of sprouting at a limited range of conditions and bulblets with low or no dormancy at a wide range. The promotive effect of the *in vitro* cultivation was, to a large extent, caused by exposure of the bulblets to light which was absent after planting in soil because of coverage by a layer of soil.

Key-words: bulbs, dormancy, *Lilium speciosum*, tissue culture

INTRODUCTION

Plants of many species develop dormancy to overcome adverse climatic conditions. Dormant organs include seeds, buds, corms, tubers and bulbs. Dormancy is often 'relative' (Borriss 1940; Vegis 1964, 1973): dormant plants can grow in a narrow range of conditions and non-dormant plants in a wide range. In particular, after the release from dormancy growth may occur at a larger range of temperatures than before (Vegis 1964, 1973).

Bulblets of *Lilium speciosum* regenerated for 11 weeks under standard conditions (20°C and 3% sucrose), are dormant and require a cold treatment to achieve rapid sprouting after planting in soil at 17°C (Aguettaz *et al.* 1990; De Klerk *et al.* 1992). The temperature during regeneration influences the level of dormancy of the bulblets: at 15°C hardly any dormancy is induced, and at 25°C the bulblets develop deeper dormancy than at 20°C (Delvallée *et al.* 1990). To examine whether dormancy in lily bulblets is relative, we determined the sprouting performance of micropropagated lily bulblets with different levels of dormancy at various environmental conditions, viz., at various temperatures and in soil or *in vitro*. In addition, we examined the cause of the large difference between sprouting performance *in vitro* and in soil.

MATERIALS AND METHODS

Bulbs of *Lilium speciosum* Thunb. var. *rubrum* No. 10 were stored after harvest at 0·5°C until needed. Adventitious bulblets were regenerated *in vitro* on scale explants at 15, 20 or 25°C as described previously (Aguettaz *et al.* 1990).

Bulblets were excised from the original explant 11 weeks after the start of tissue culture and transferred to wooden containers with steamed potting soil, either directly or after an intervening cold treatment of 6 weeks at 2°C. The bulblets were covered with 2 cm of soil and cultured at 15, 20 or 25°C under standard light conditions (Philips TL 33, 30 μmol $m^{-2} s^{-1}$ for 16 h per day). The soil was moistened twice a week. Bulblets were recorded as sprouted when a leaf had emerged from the soil. Leaf emergence was recorded once a week. All values of sprouting refer to samples of 30 bulblets. The effects of the various conditions were determined at least twice.

Bulblets excised after 11 weeks of tissue culture were also transferred under sterile conditions to culture tubes with solidified medium, either directly or after an intervening cold treatment of 6 weeks at 2°C. The medium was composed of MS salts (Murashige & Skoog 1962) and 6 $g l^{-1}$ agar. The bulblets were allowed to sprout at 15, 20 or 25°C under standard light conditions (Philips TL 33, 30 μmol $m^{-2} s^{-1}$ for 16 h per day). Bulblets regenerated at 20°C were also planted at 15°C with addition of increasing concentrations of mannitol, or in the dark. Sprouting was scored once a week. Bulblets cultured *in vitro* were recorded as sprouted when the newly formed leaf had emerged from the bulblet for 2 mm. All values of sprouting refer to samples of 30 bulblets. The effects of the various conditions were determined at least twice.

RESULTS

Sprouting in soil and in vitro *at various temperatures*

Non-cold-treated bulblets regenerated for 11 weeks at 15, 20 or 25°C were planted in soil and allowed to sprout at 15, 20 or 25°C (Fig. 1a–c). The percentage of sprouted bulblets decreased with increasing temperature during regeneration *in vitro* and with increasing temperature after planting. Bulblets regenerated at 25°C sprouted at all three temperatures only to a small percentage (less than 10%). After planting at 25°C, bulblets regenerated at all three temperatures sprouted also to a low percentage (less than 15%).

When the non-cold-treated bulblets were planted *in vitro* on solidified medium, they always sprouted better than in soil (Fig. 1d–f). Bulblets planted at low temperature sprouted to a higher percentage than bulblets planted at high temperature. After planting *in vitro* at 15°C, bulblets regenerated at all three temperatures sprouted within 10 weeks after planting to 100 or almost 100%. However, the bulblets regenerated at low temperature had a higher rate of sprouting than those regenerated at high temperature (Fig. 1d). After planting *in vitro* at 20°C, the bulblets regenerated at 15°C sprouted to 100% within 2 weeks (Fig. 1e). Bulblets regenerated at 20 or 25°C and planted *in vitro* at 20°C, showed a biphasic sprouting, the first wave of sprouting occurring shortly after planting and the second after 15 weeks (Fig. 1e). After planting *in vitro* at 25°C, bulblets regenerated at 15°C had a sprouting percentage of 70, whereas bulblets regenerated at 25°C showed a better sprouting performance than those regenerated at 20°C (Fig. 1f).

A cold treatment of 6 weeks at 2°C prior to planting usually resulted in a better sprouting performance. Figure 2 shows, as examples, the effect of the cold treatment on sprouting of bulblets regenerated at 15°C or 25°C and planted at 25°C to 15°C, respectively. The cold treatment resulted in almost 100% sprouting in bulblets regenerated at 15°C and planted *in vitro* at 25°C, but in only 40% sprouting in bulblets planted in soil at 25°C (Fig. 2a). For bulblets regenerated at 25°C and planted at 15°C, the cold

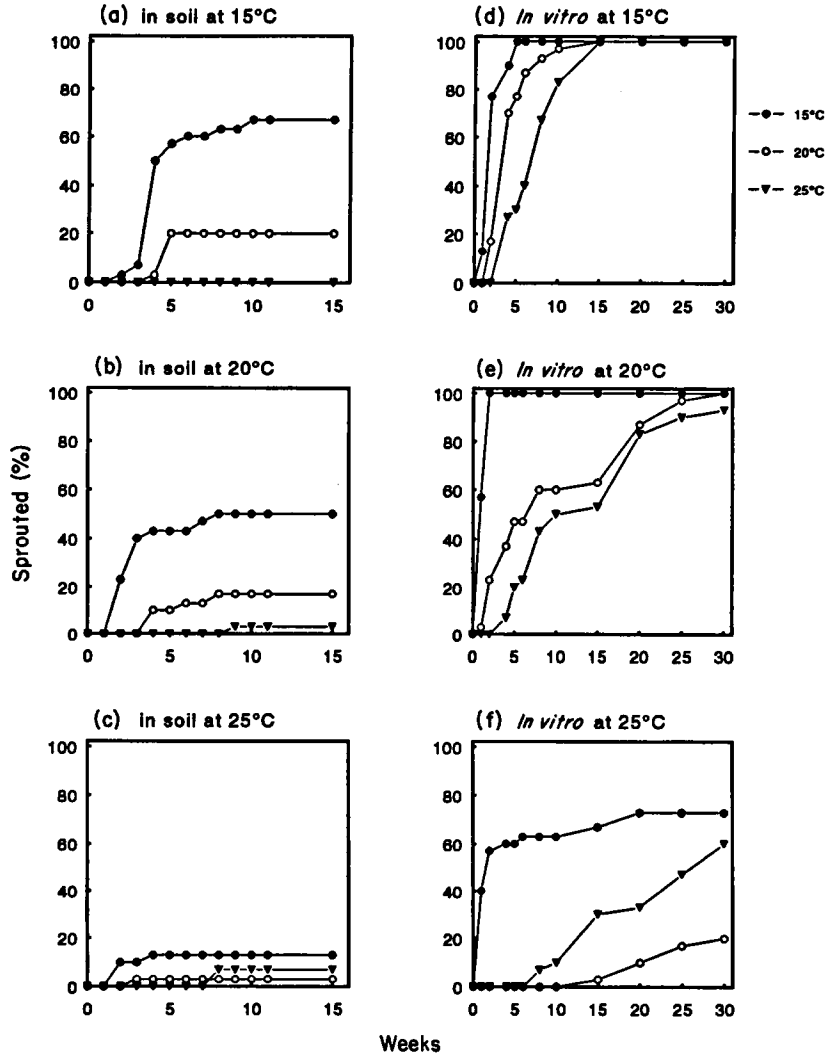

Fig. 1. Sprouting of bulblets of *Lilium speciosum* in soil (a–c) or *in vitro* (d–e) at 15°C (a and d), 20°C (b and e) or 25°C (c and f). The bulblets had been regenerated *in vitro* from scale explants at 15 (●), 20 (○) or 25°C (▼) and had not been cold-treated.

treatment was necessary to achieve sprouting in soil and increased the rate of sprouting *in vitro* (Fig. 2b).

Effects of the presence of osmoticum or darkness

The improved sprouting *in vitro* may be caused by greater water availability *in vitro*. To reduce the water potential *in vitro*, mannitol was added to the medium. Figure 3a shows that mannitol did not affect the sprouting percentage but only the rate of sprouting. When the exposure to mannitol was preceded by a cold treatment of 6 weeks at 2°C, sprouting occurred faster than in non-cold-treated bulblets (Fig. 3b). At high mannitol concentrations, many of the cold-treated bulblets died, as shown by their yellow-brown

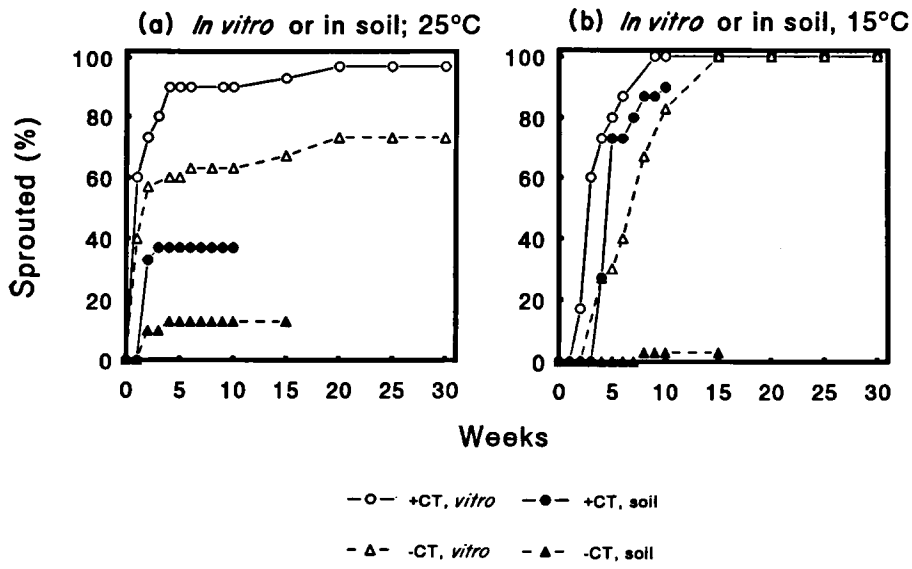

Fig. 2. Sprouting of bulblets of *Lilium speciosum* in soil (●, ▲) or *in vitro* (○, △) at 25°C (a) or 15°C (b). The bulblets had been regenerated from scale explants at 15°C (a) or 25°C (b) and planted without (△, ▲) or with (○, ●) an intervening cold treatment (CT) of 6 weeks at 2°C.

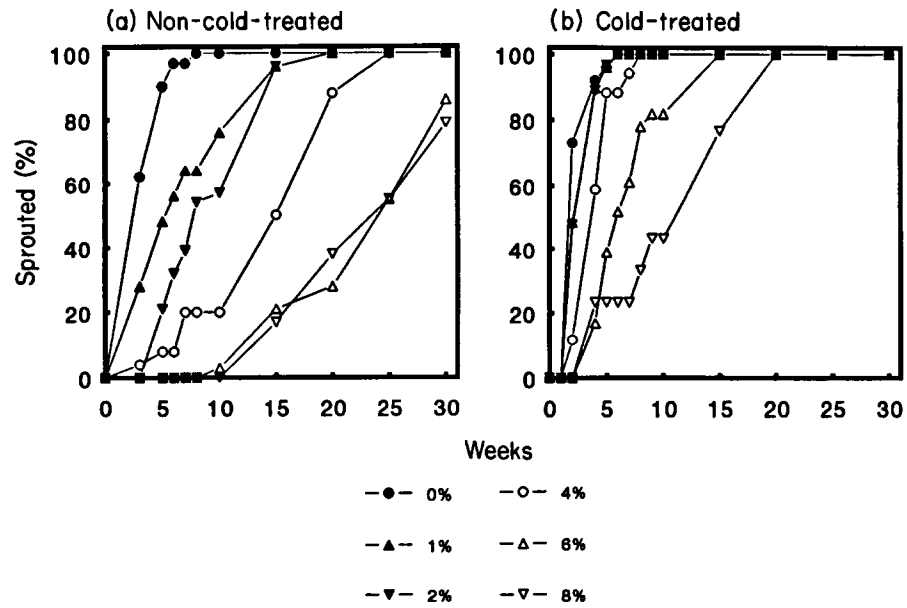

Fig. 3. Sprouting of viable bulblets of *Lilium speciosum in vitro* at 15°C with addition of increasing concentrations of mannitol. The bulblets had been regenerated from scale explants at 20°C and had been planted without (a) or with (b) an intervening cold treatment of 6 weeks at 2°C. The bulblets that did not survive (see Table 1 for the percentages dead bulblets), have been omitted from the calculation.

colour at the end of the experiment, 30 weeks after planting (Table 1). At 8% mannitol, only 30% of the cold-treated bulblets survived and sprouted, whereas the remaining 70%

Table 1. Percentage of dead bulblets after 30 weeks of culture *in vitro* with addition of increasing concentrations of mannitol. Prior to the culture on medium with mannitol, the bulblets had been either cold-treated or not

Mannitol conc. (%)	Dead bulblets (%)	
	Non-cold-treated	cold-treated
0	0	0
1	0	7
2	0	13
4	0	13
6	0	23
8	7	70

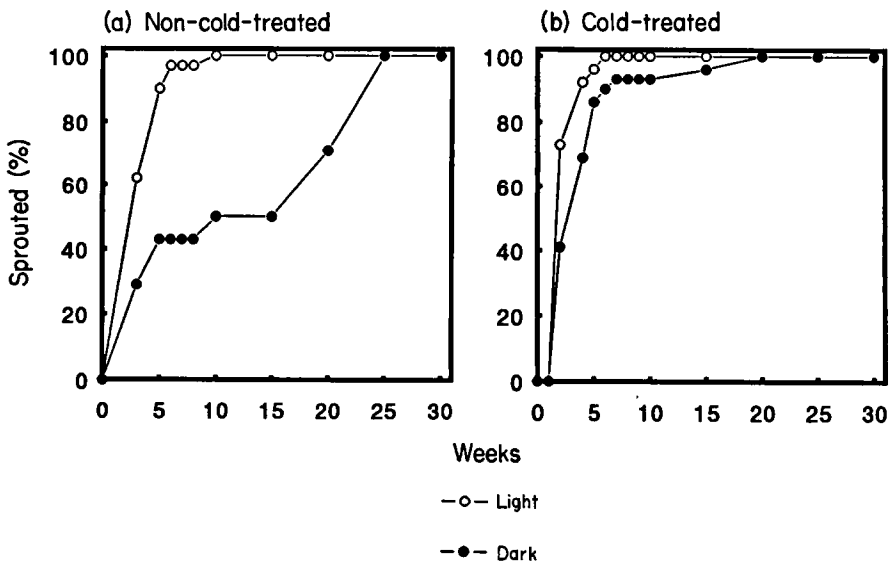

Fig. 4. Sprouting of bulblets of *Lilium speciosum in vitro* at 15°C in the dark (●) or in the light (○). The bulblets had been regenerated from scale explants at 20°C and planted without (a) or with (b) an intervening cold treatment of 6 weeks at 2°C.

of the bulblets had died. It should be noted that only a few of the non-cold-treated bulblets (7%) died at 8% mannitol.

Soil inhibits the transmission of light. Since light is known to stimulate sprouting of buds (e.g. Pierik 1976), the low sprouting percentage in soil may have been caused by the darkness imposed on the bulblets that are covered by 2 cm of soil. Culture of excised bulblets *in vitro* in the dark indeed resulted in a strong reduction of the sprouting performance of non-cold-treated bulblets 10 weeks after planting (Fig. 4a). However, these bulblets showed a biphasic sprouting and reached 100% sprouting in the period between 15 and 25 weeks after planting. In cold-treated bulblets, the rate

of sprouting was only somewhat reduced in the dark relative to the non-cold-treated ones (Fig. 4b).

DISCUSSION

In bulblets of *L. speciosum* regenerated *in vitro*, the dormancy status depends upon the temperature at which the bulblets have been regenerated: at high temperature (25°C) a high level of dormancy develops and at low temperature (15°C) a low level (Fig. 1; Delvallée *et al.* 1990). The present article shows that dormant lily bulblets can sprout to a high percentage under certain conditions. For example, bulblets regenerated at 25°C sprouted to less than 5% when planted in soil at 15°C (Fig. 1a) and required a cold treatment to achieve 100% sprouting (Fig. 2b). However, the non-cold-treated bulblets were capable of rapid sprouting to 100% when planted *in vitro* at 15°C (Fig. 1d). Thus, in lily bulblets, dormancy is not absolute but relative (cf. Borriss 1940; Vegis 1964).

Favourable conditions for sprouting include low temperature and the *in vitro* environment. Sprouting of dormant buds and germination of dormant seeds in a narrow range of conditions has been reported previously (Vegis 1964, 1973). Similar to our observations for dormant and non-dormant bulblets of *Lilium speciosum*, it has been reported for various species that dormant buds and seeds can grow at low temperature and non-dormant ones both at low and high temperature.

Two major differences between culture in soil and *in vitro* are availability of water and presence of light. In soil, water availability is much lower than *in vitro* because of the rapid diffusion of water in the agar medium. The low water availability is a particularly important factor in lily bulblets since they are planted without roots. Reducing the water availability *in vitro* by addition of mannitol did not affect the sprouting percentage but only the rate of sprouting (Fig. 3). This shows that the water availability is of little or no importance with respect to the large difference in sprouting percentage between soil and the *in vitro* environment. With respect to light, it should be noted that bulblets cultured in soil are in almost complete darkness because light is unable to penetrate through soil (Bliss & Smith 1985). Planting of bulblets *in vitro* in the dark resulted in a reduction of the sprouting percentage during the first 15 weeks of culture (Fig. 4). Therefore, the low sprouting percentage in soil can be traced back to a large extent, to the absence of light in soil.

We have previously reported on the occurrence of two waves of emergence after planting of dormant bulblets in soil, the first occurring shortly after planting and the second from 25 to 35 weeks (De Klerk *et al.* 1992). The nature of the biphasic emergence is not clear. In the present paper, we also observed on several occasions biphasic emergence in bulblets planted *in vitro* (Figs 1e and 4a). However, the second wave occurred much earlier, viz. between 15 and 25 weeks. Possibly, the ample water availability allowed the second wave to occur earlier.

An interesting finding in this study is the death of cold-treated bulblets at high mannitol concentrations in contrast to non-cold-treated ones (Table 1). This shows that the occurrence of dormancy enables plants to withstand environmental stress and that after the breaking of dormancy the plants are again vulnerable. When severe manipulations are imposed on bulblets—for example, to remove pathogens by a warm water treatment (Hol & Van der Linde 1992) or to cryopreserve meristems (Bouman & De Klerk 1990)—this preferably should be done with dormant bulblets.

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