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Dry-matter partitioning between bulbs and leaves in plantlets of *Lilium speciosum* regenerated *in vitro*

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SUMMARY

Plantlets of *Lilium speciosum* regenerated *in vitro* consist of scales that may or may not bear a leaf. We studied the effects of several physical, nutritional and hormonal factors on dry-matter partitioning between bulbs and leaves. Both absolute bulb weight and relative bulb weight (bulb weight as a percentage of plant weight) were determined.

High temperature, culture in the dark and high osmolarity promoted relative bulb weight. Day-length had no effect. Amongst the nutritional factors sucrose promoted relative bulb weight. High concentration of MS-nutrients caused an increase in bulb fresh weight only. Bulb formation was under control of abscisic acid (ABA): ABA added to the medium completely blocked leaf formation, whereas fluridone, an inhibitor of ABA-synthesis, inhibited bulb formation. Paclobutrazol (PP333) blocked leaf formation completely. The PP333-effect was reversed by gibberellins A_{4+7}. Cytokinin (6-benzylaminopurine), auxin (indoleacetic acid), ethylene and methyl jasmonate had no effect.

Key-words: abscisic acid, bulb formation, dry-matter partitioning, gibberellins, *Lilium*, tissue culture.

INTRODUCTION

For multiplication of bulbous crops *in vitro*, five phases can be distinguished, namely initiation, multiplication, bulb formation, breaking of dormancy and transplantation to soil (Van Aartrijk & Van der Linde 1986). The initial explants are usually excised from bulb tissue or non-elongated flower stems. In the multiplication phase, often shoots are used because of their fast growth. In the final tissue-culture cycle, the shoots should form bulblets. A bulblet is a firm and compact structure and thus easy to handle. Furthermore, bulblets do require neither a rooting nor an acclimatization treatment after planting. Unlike bulblets, shoots are vulnerable and should be rooted and, after planting, weaned. In addition, shoots often form no or only incomplete bulblets after planting (Van der Linde & Schipper 1992).

Bulb formation is often difficult to achieve. Therefore, we examined bulb formation in *Lilium speciosum* as a model. Bulblets of lily consist of scales that may or may not bear a leaf. This system is particularly useful for studying the backgrounds of bulb formation as it offers the opportunity to examine partitioning of dry weight among bulbs and leaves by measuring the relative bulb weight (bulb weight as a percentage of plant weight). The absolute bulb weight has the disadvantage that it is also affected by the general growth rate of the plant.

Table 1. Fresh weights and relative weights of bulblets and number of bulblets regenerated per scale as affected by temperature (16 h light) and darkness (20°C)

Culture conditions	Bulb fresh weight (mg)	Relative bulb weight (%)	Number of bulbs per scale
Experiment 1			
15°C	25·8 ± 6·1	50·7 ± 5·0	3·9 ± 0·5
20°C	47·1 ± 10·2	63·6 ± 5·2	7·2 ± 0·8
25°C	63·2 ± 10·8	83·1 ± 4·4	9·3 ± 1·6
Experiment 2			
Light (16 h)	58·5 ± 10·0	65·2 ± 4·7	7·2 ± 0·8
Dark	83·8 ± 16·3	92·5 ± 4·9	3·5 ± 0·6

In this article we report on the effects of various physical, nutritional and hormonal factors on bulb formation.

MATERIALS AND METHODS

Tissue culture

Bulblets were regenerated on scale explants of *Lilium speciosum* Thunb. 'Rubrum no. 10' as described previously (Aguettaz *et al.* 1990). Explants were cultured on MS medium (Murashige & Skoog 1962) with addition of 3% (w/v) sucrose, 100 mg l^{-1} myo-inositol, 0·4 mg l^{-1} thiamine-HCl, 0·25 μM α-naphtaleneacetic acid (NAA), 0·6% (w/v) agar (BBL granulated) and a pH of 6·0 prior to autoclaving. Culture conditions were 20°C in light (20 μE $m^{-2}s^{-1}$ for 16 h day^{-1}). After 11 weeks of culture, plantlets were harvested. These standard conditions were changed as indicated.

Measurement of bulb formation

In each explant, the number of regenerated bulbs, plant fresh weight and bulb fresh weight were determined. Bulb fresh weight was also determined as a percentage of plant fresh weight (relative bulb weight). 'Bulb fresh weight' refers to the fresh weight of one bulblet without leaves. The values shown in the graphs are means obtained from 20–30 explants ± SE.

Plant growth regulators

± Abscisic acid (ABA, Sigma); gibberellins A_3 and A_{4+7} (GA_3, Sigma and GA_{4+7}, ICI); 6-benzylaminopurine (BAP, Sigma); α-naphtaleneacetic acid (NAA, Sigma); indole-acetic acid (IAA, Sigma); L-α-(2-aminoethoxyvinyl)-glycine (AVG, Sigma); ethephon; 1-methyl-3-phenyl-5-(3-[trifluoromethyl]phenyl)-4-(1H)-pyridone (fluridone, Eli Lilly); (2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1H-1,2,3-triazol-1-yl) penta-3-ol (paclobutrazol = PP333, ICI); methyl jasmonate (Firmenich s.a.).

RESULTS

Physical factors

At high temperatures, leaf formation was significantly reduced ($P < 0·05$) as shown by the increased relative bulb weight (Table 1). Bulb fresh weight and number of bulblets per

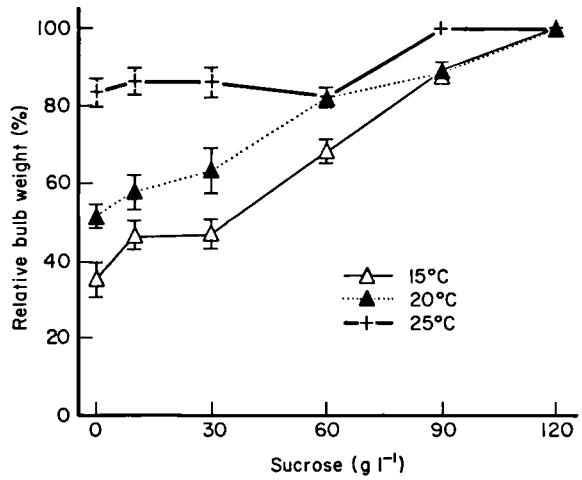

Fig. 1. Relative bulb weight (bulb weight as a percentage of plant weight) of *L. speciosum* bulblets cultured *in vitro* at 15, 20 or 25°C with addition of increasing concentrations of sucrose.

explant also increased with temperature. In Table 1, the effect of culture in the dark is also shown. In the dark, bulb fresh weight was *c.* 50% higher than in the light. The number of bulblets in the dark was much lower than in light. In the dark only few bulblets formed leaves. Day-length (8 h or 16 h) did not affect dry-matter partitioning significantly (data not shown).

Nutritional factors

The concentration of sucrose in the medium had a strong effect on dry-matter partitioning (Fig. 1). Leaf formation was completely blocked at 9% sucrose (25°C) or 12% sucrose (15°C and 20°C). Sucrose also enhanced bulb fresh weight. At 15°C, 20°C or 25°C the highest fresh weight was obtained at 6% sucrose ($25{\cdot}7 \pm 3{\cdot}8$ mg), 9% sucrose ($75{\cdot}9 \pm 11{\cdot}4$ mg) or 12% sucrose ($68{\cdot}2 \pm 12{\cdot}8$ mg) respectively. The concentration of MS-nutrients (0, 0·5, 1·0, 1·5, 2·0-times the normal concentration) had no effect on dry-matter partitioning but stimulated both the number of plantlets regenerated per scale and bulb fresh weights (data not shown).

To evaluate a possible effect of the osmotic value of the medium, we added mannitol together with 1% sucrose. Mannitol enhanced the relative bulb weight and had, on a weight basis, the same effect as sucrose (Fig. 2). It should be noted that the highest concentration of mannitol reduced bulb fresh weight by 65%.

Hormonal factors

ABA blocked leaf formation almost completely at high concentration (Fig. 3). Fluridone, an inhibitor of ABA-synthesis (Zeevaart & Creelman 1988), blocked bulb formation and promoted leaf formation (Figs 4 and 5). The effect of fluridone on dry-matter partitioning was reversed by simultaneous addition of ABA (Fig. 4). ABA reduced bulb fresh weight by *c.* 20% Fluridone reduced bulb fresh weight by *c.* 35% but total plant fresh weight (bulb + leaves) increased by 30%.

To study the effect of GAs, we added PP333, an inhibitor of GA-synthesis, to the medium. PP333 strongly inhibited leaf formation (Fig. 6). Surprisingly, its effect was completed

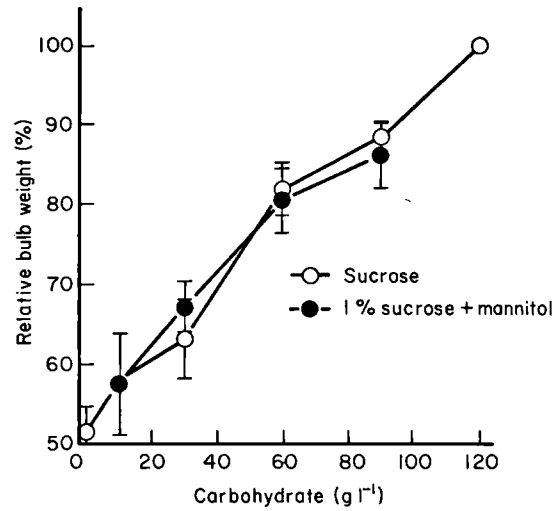

Fig. 2. Relative bulb weight (bulb weight as a percentage of plant weight) of *L. speciosum* bulblets cultured on increasing concentrations of sucrose or on 10 g l^{-1} sucrose with increasing concentrations of mannitol. X-axis refers to total carbohydrate concentrations (g l^{-1}).

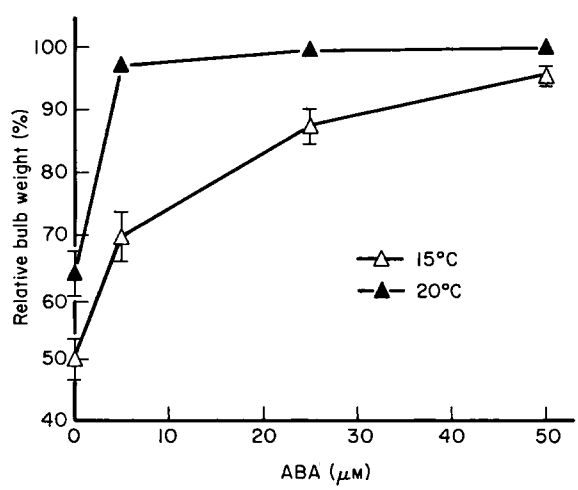

Fig. 3. Relative bulb weight (bulb weight as a percentage of plant weight) of *L. speciosum* bulblets cultured *in vitro* with addition of increasing concentrations of ABA.

within a narrow range: at 0·3 mg l^{-1} dry-matter partitioning and bulblet morphology were little affected. At 0·5 mg l^{-1}, however, leaf formation was almost completely blocked and bulblets had very short and thick scales. To examine whether PP333 had an effect by inhibition of GA-synthesis or by side-effects, we added PP333 and GA_{4+7} simultaneously. The effect of PP333 was significantly reversed ($P < 0{\cdot}01$) by 0·1 mg l^{-1} GA_{4+7} (Fig. 7). PP333 reduced bulb fresh weight especially in the range 0–0·5 mg l^{-1} but higher concentrations up to 1·0 mg l^{-1} had no additional effect (Table 2). PP333 and GA_{4+7} added

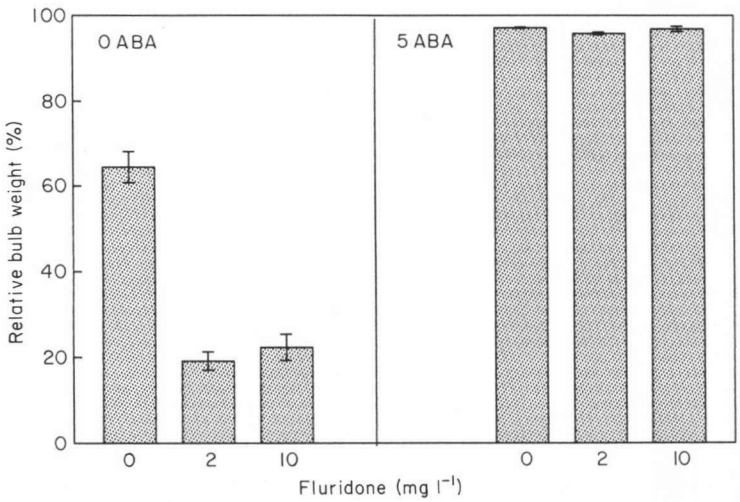

Fig. 4. Relative bulb weight (bulb weight as a percentage of plant weight) of *L. speciosum* bulblets cultured on medium with fluridone or on medium with fluridone and 5 μM ABA.

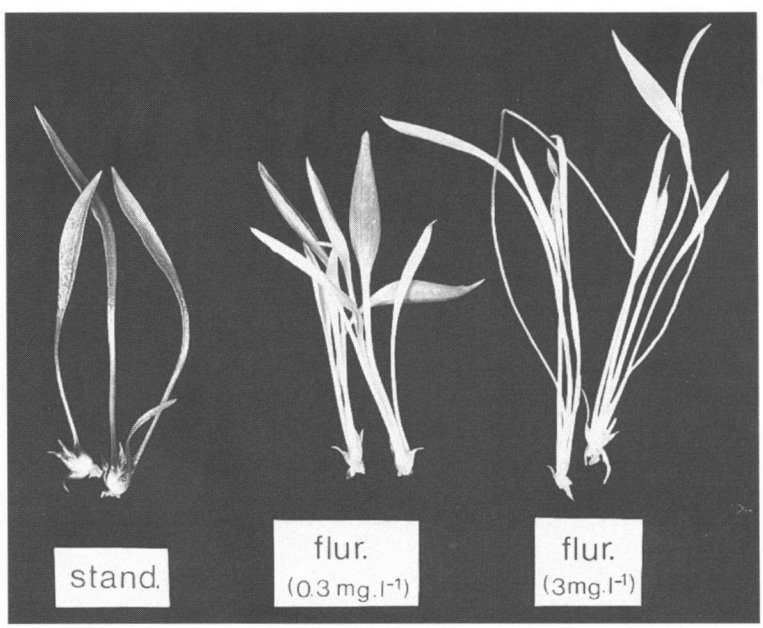

Fig. 5. Plantlets of *L. speciosum* regenerated *in vitro* with addition of 0·3 and 3·0 mg l^{-1} fluridone.

together also reduced bulb fresh weight (Table 2). Addition of GA_3 or GA_{4+7} alone had no consistent effect on relative bulb weight and bulb fresh weight at low concentrations; at high concentrations of GA_{4+7} (1·0 mg l^{-1}) bulb fresh weight decreased.

We also studied the effect of auxin (IAA, 0–25 μM), cytokinin (BAP, 0–2·5 μM), methyl jasmonate (0–5 μM) and ethylene (AVG, 0–3 μM; ethephon, 0–1·2 mg l^{-1}). These growth regulators had no distinct effect on bulb formation (data not shown).

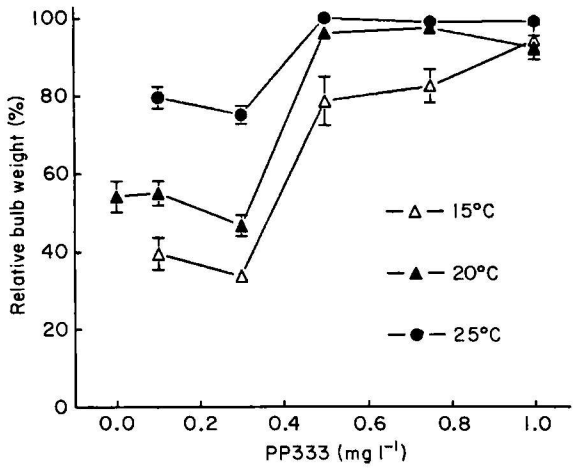

Fig. 6. Relative bulb weight (bulb weight as a percentage of plant weight) of *L. speciosum* bulblets cultured at 15, 20 or 25°C with addition of increasing concentrations of PP333.

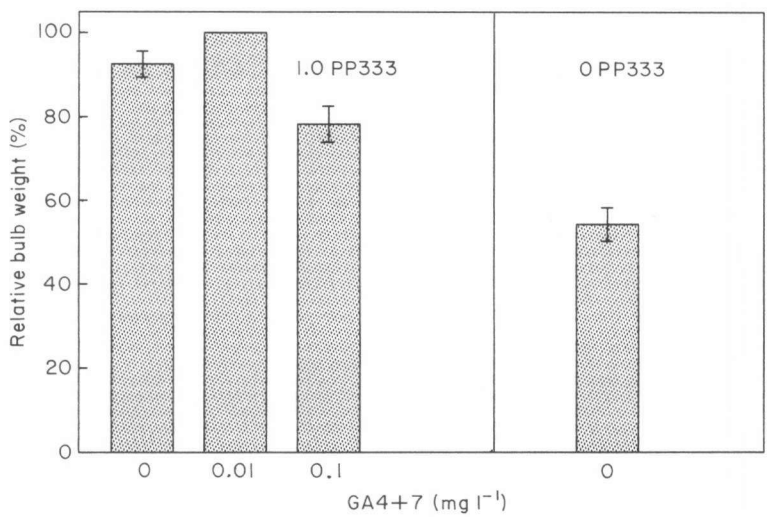

Fig. 7. Relative bulb weight (bulb weight as a percentage of plant weight) of *L. speciosum* bulblets cultured on medium with 1·0 mg l^{-1} PP333 and increasing concentrations of GA_{4+7}.

DISCUSSION

Bulb formation can be evaluated in different ways. From a practical point of view, dry weight and the content of storage compounds (sugars, starch) are important. To study the mechanisms that are involved in the partitioning of dry matter among leaves and scales we determined the relative bulb weight. In this way we could discriminate between the influence of a treatment on dry-matter partitioning and possible effects on total-plant growth.

Leaf formation decreased with increasing temperature. Similar results have been obtained with *Lilium longiflorum* (Stimart & Ascher 1981) and crocus corms (Plessner *et al.* 1990).

Table 2. Bulb fresh weights of bulblets regenerated on scale explants as affected by PP333 and PP333 + GA_{4+7}

PP333 (mg l^{-1})	GA_{4+7} (mg l^{-1})	Bulb fresh weight (mg)
0	0	57·9 ± 5·6
0·1	0	49·5 ± 4·4
0·3	0	36·9 ± 3·4
0·5	0	22·2 ± 3·0
0·75	0	30·2 ± 2·3
1·0	0	21·9 ± 2·7
1·0	0·01	33·2 ± 3·3
1·0	0·1	34·7 ± 5·5

Previously, Delvallée *et al.* (1990) reported that in bulblets of *L. speciosum* cultured *in vitro* the development of dormancy depends on temperature. At 15°C no dormancy occurs. At 20 or 25°C dormancy develops after 7 or 5 weeks of culture respectively. After induction of dormancy the primordia lose the ability to form a leaf and always develop into a scale. This might explain the decrease in leaf formation at high temperatures: at 15°C leaf formation proceeds during the entire culture period as no dormancy developed, at 25°C dormancy was induced earlier than at 20°C so fewer leaves were formed.

The stimulation of storage organ formation by sucrose has been reported for bulbs (Takayama & Misawa 1979; Chow *et al.* 1992), tubers (Asahira & Yazawa 1979; Vreugdenhil & Helder 1992) and seeds (Xu *et al.* 1990). It is obvious that an increased supply of carbohydrates may lead to the formation of an organ to store this supply. The mechanism by which the plant translates the signal of a high sucrose concentration is not known. It might be that in lily bulblets sucrose affects dry-matter partitioning by its osmotic effect. Mannitol had, on a weight basis, the same effect as sucrose (Fig. 2). A high concentration of osmoticum might increase the level of ABA in the tissue (cf. Zeevaart & Creelman 1988). ABA added to the medium shifted allocation of dry matter completely to the bulblets (Fig. 3).

ABA and GA play an important role in bulb formation of *L. speciosum*, whereas other hormones had little or no effect. The role of ABA is shown by addition of ABA or fluridone (Figs 3 and 4). This finding contrasts with results obtained in other storage organs. ABA has no effect on tuber formation from single-nodes of potato (Ewing 1987; Vreugdenhil & Struik 1989). Ginzburg & Ziv (1973) found no effect of ABA on tuberization in stolon tips of gladiolus. In tulip, addition of ABA to cold-treated sprouts even decreased the number of sprouts forming a bulb (Nishiuchi 1983). Xu *et al.* (1990) showed that ABA does not play a role in accumulation of storage proteins in seeds of alfalfa. With regard to tulip, it should be noted that tulip scales are swollen leaves whereas lily scales are swollen petioles. To evaluate the role of ABA further, experiments are in progress to determine endogenous levels in bulblets regenerated at 15, 20 and 25°C.

Addition of GAs had no consistent effect on bulb formation. In some experiments, we observed an increase in leaf formation, but in other experiments no effect was found (unpublished data). Inhibition of the synthesis of GAs by PP333 (Fig. 6), always completely inhibited leaf formation. It is remarkable that PP333 had its effect in a very narrow range (0·3 mg l^{-1}–0·5 mg l^{-1}). This effect was caused by inhibition of GA-synthesis and not by side effects since simultaneous addition of GA_{4+7} restored leaf formation. In

gladiolus, PP333 promotes corm formation and shifts assimilate allocation towards the growing corm (Steinitz *et al.* 1991). This PP333-effect is also reversed by simultaneous addition of gibberellin (Steinitz & Lilien-Kipnis 1989). In single-node explants of potato, GA decreases tuberization (Ewing 1987). Together these observations indicate an inhibiting effect of GA on accumulation of storage material.

Bulblets should have a high sink activity during the tissue culture period to obtain large, viable bulblets. After transfer to soil, the bulblets should shift from sink to source and mobilize their reserves to sustain the growing sprout. Then the bulblet should become sink again and accumulate carbohydrates produced by photosynthesis. Currently we examine the hormonal control of these transitions.

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