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Hormonal control of dormancy and apical dominance in tissue-cultured plants

G.-J. DE KLERK

Centre for Plant Tissue Culture Research, P.O. Box 85, 2160 AB Lisse, The Netherlands

SUMMARY

In many crops, it is important to control dormancy and/or apical dominance during micropropagation. The regulation of dormancy was studied in bulblets of lily, buds of apple and quince, and seeds of *Agrostemma githago*. The development of dormancy was enhanced by choosing the appropriate tissue-culture conditions, in particular temperature, and inhibited by application of fluridone, an inhibitor of abscisic-acid (ABA) synthesis. For the maintenance of dormancy, continuous synthesis of ABA was required. Dormancy was broken by treatment with gibberellins, or by cold storage under humid conditions. Apical dominance was studied in apple. As a pretreatment with fluridone promoted the outgrowth of axillary buds, ABA may also play a role in the development of apical dominance.

Key-words: Agrostemma githago, apical dominance, dormancy, *Lilium speciosum Malus*, micropropagation, tissue culture.

INTRODUCTION

In plants, shoot meristems are located in the apices and in the axils of leaves. Apical meristems often develop dormancy: even though the conditions are favourable, they do not show visible growth and only resume growth after a specific dormancy-breaking treatment (Villiers 1975). Dormant organs include buds, bulbs, tubers and seeds. It is believed that in the former three, dormancy is induced by environmental stimuli, in particular short day-length or cold temperatures (Powell 1987). In seeds, dormancy is not induced by an external stimulus but develops as part of seed maturation. It is desirable to control dormancy *in vitro* for the following reasons: (a) development of dormancy may result in reduction of growth; (b) in micropropagated bulblets and somatic embryos the development of a low level of dormancy *in vitro* is required to avoid precocious sprouting and germination respectively (Gerrits & De Klerk 1992); (c) the increased resistance of dormant tissues towards stress may be exploited (Sakai & Nishiyama 1978; Welander 1988). The occurrence of dormancy *in vitro* has only been examined in bulbous species (Hussey 1975; Aguettaz *et al.* 1990). In this article, I report on the occurrence of dormancy *in vitro* in woody species (apple and quince) and on the hormonal regulation of development, maintenance and breaking of dormancy in micropropagated lily bulblets. To examine the general applicability of the results, some experiments were done with seeds of *Agrostemma githago*.

Apical dominance concerns the inhibition of growth of axillary buds and lateral branches (Hillman 1984). Apical dominance is broken by damaging or removal of the

apical meristem. This shows that the control over the outgrowth of axillary meristems resides in the apical meristem. Axillary meristems may also develop dormancy which is superimposed over apical dominance (Hillman 1984). Micropropagation is usually based on axillary branching because propagation via adventitious buds may result in genetic aberrations (De Klerk 1990). However, in many plants axillary branching is difficult to achieve in spite of application of cytokinin or removal of the apex. Furthermore, apical dominance should be restored after planting in soil as bushy plants are undesirable (with some notable exceptions such as *Nephrolepis*). It is generally supposed that a high auxin: cytokinin ratio in the axillary bud results in blocking and a low ratio in activation of outgrowth (Wickson & Thimann 1958). The key roles of auxin and cytokinin have been corroborated by observations in transgenic plants overproducing auxin or cytokinin (Lincoln *et al.* 1990; Medford *et al.* 1989). However, recent data on hormone content and transport and on hormone-regulated genes demonstrate that this conventional view is unlikely (Pilate *et al.* 1989; Gocal *et al.* 1991; Tamas *et al.* 1992). Therefore, we examined the role of other hormones in shoot cultures of apple.

MATERIALS AND METHODS

Lilium speciosum

Regeneration of plantlets of *Lilium speciosum* 'Rubrum No. 10' on scale explants and determination of the dormancy status of the newly formed bulblets were done as described in detail previously (Aguettaz *et al.* 1990). In short, bulblets were regenerated *in vitro* on scale explants and harvested after 11 or 14 weeks. The dormancy status was evaluated by determining the percentage sprouting 10 weeks after planting in soil in samples of 30–50 bulblets.

In one experiment (Fig. 2), the bulblets were regenerated for 11 weeks at 20 or 15°C with or without fluridone (0·3 mg l^{-1}) or abscisic acid (ABA) (5 mg l^{-1}). In a second experiment (Fig. 5), the bulblets were regenerated under standard conditions for 14 weeks, harvested and then soaked for 24 h in a solution with the indicated concentration of plant growth regulator (50 bulblets in 50 ml solution) prior to planting.

Apple and quince

Shoot cultures of apple (*Malus*) 'Jork' and quince (*Cydonia oblonga*) 'Leskovacz' were initiated in 1987 and cultured at 20°C as described previously (De Klerk *et al.* 1990). The shoots were subcultured every 6 weeks. In one experiment (Fig. 1), shoot tufts that had been cultured for 8 weeks (quince) or 12 weeks (apple), were cold-treated at 5°C under short day-length conditions (5 μE m^{-2} s^{-1} for 8 h day^{-1}). After 0, 1, 2, or 4 weeks of cold treatment, apices were excised and cultured under standard conditions at 20°C. After 6 weeks, the propagation factor was determined as the number of shoots originating from one transplanted apex. In another experiment (Fig. 6), shoots of apple were cultured for 4 weeks at 25°C with or without 0·03 mg l^{-1} fluridone. Then the apices were harvested. As fluridone inhibits ABA synthesis by blocking a step in carotenoid synthesis (Zeevaart & Creelman 1988), the effectiveness of fluridone is indicated by the inhibition of chlorophyll synthesis. Therefore, the apices were grouped according to their colour (green, green/white or white) and cultured on standard medium at 25°C. After 4 weeks, the propagation factor was determined.

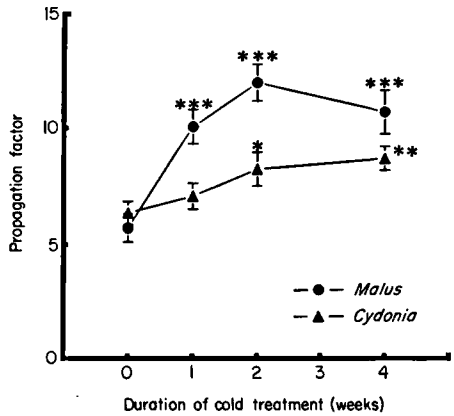

Fig. 1. Effect of a cold treatment on propagation factor in apple and quince. Cultures of apple and quince were transferred to 5°C and short-day condition. After various durations of cold treatment, apices were excised and transferred to fresh medium at 20°C. After 6 weeks of culture, the propagation factors were determined. Each value is the mean of *c.* 30 determinations ± SE. Values, significantly different from the control (0 weeks cold treatment) are indicated by $*P<0{\cdot}05$, $**P<0{\cdot}01$, and $***P<0{\cdot}001$.

Agrostemma githago

After harvest, seeds of *Agrostemma githago* (provenance Gatersleben, Germany) were stored for 9 months at room temperature to break dormancy (Borriss 1940). Samples of 50 seeds were imbibed in 9-cm Petri dishes on moistened filter paper in the dark. In one experiment (Fig. 3), fully after-ripened seeds were imbibed for 7 days at 30°C to induce secondary dormancy (Borriss 1940) in the presence of various concentrations of fluridone. Then the seeds were rinsed for 1 h in running tap water, dried and germinated on moistened filter paper at 20°C. The final germination percentages were determined after another 7 days. Each value is the mean of two samples of 50 seeds. In a second experiment (Fig. 4), seeds that had been after-ripened for only 3 months were imbibed at 20°C in the presence of increasing concentrations of fluridone. The final germination percentages were determined after 7 days. Each value is the mean of two samples of 50 seeds.

Plant growth regulators

±ABA, 6-benzylaminopurine (BAP), and indolebutyric acid (IBA) were from Sigma, gibberellin A_{4+7} (GA_{4+7}) from ICI and 1-methyl-3-phenyl-5-(3-[trifluoromethyl]phenyl)-4-(1H)-pyridone (fluridone) from Eli Lilly.

RESULTS

Control of development, maintenance and breaking of dormancy

Figure 1 shows that apple and quince shoots propagated faster after a cold treatment. This demonstrates that the cultures had developed a low level of dormancy. Dormancy also developed in lily bulblets regenerated *in vitro* on scale explants under standard conditions. These bulblets required a cold treatment (6 weeks at 2°C, De Klerk *et al.* 1990) prior to planting, but bulblets regenerated in the presence of fluridone were not dormant (Fig. 2). The effect of fluridone was due to inhibition of ABA-synthesis as shown by the development of dormancy after simultaneous addition of fluridone and ABA (Fig. 2). However,

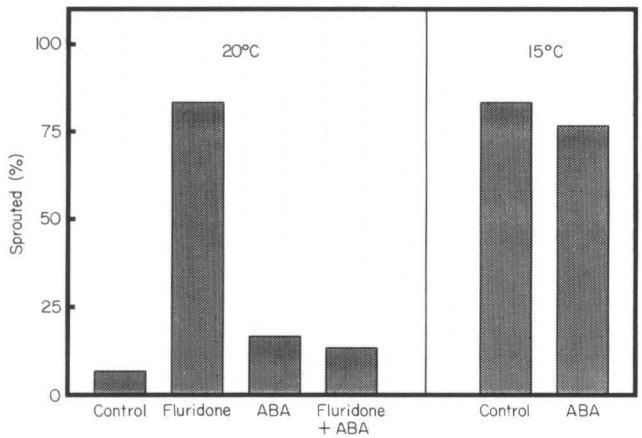

Fig. 2. Effect of fluridone, ABA and temperature on the dormancy status of *Lilium speciosum* bulblets. Bulblets were regenerated at 20°C at standard medium or with addition of fluridone (0·3 mg l^{-1}), ABA (5 mg l^{-1}) or fluridone + ABA (0·3 mg l^{-1} + 5 mg l^{-1}). Bulblets were also regenerated at 15°C on standard medium or with addition of 5 mg l^{-1} ABA. The dormancy status of noncold-treated bulblets was determined.

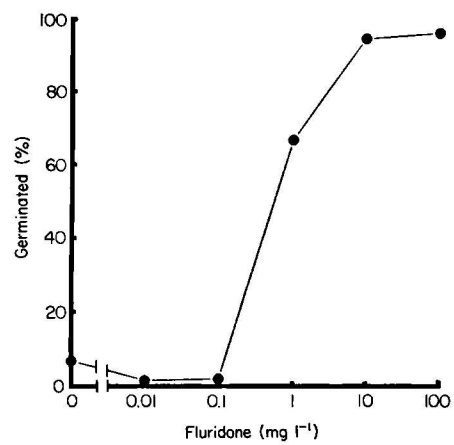

Fig. 3. Prevention of the development of secondary dormancy by fluridone in seeds of *Agrostemma githago* imbibed at 30°C. After-ripened seed of *Agrostemma githago* were imbibed for 7 days at 30°C with increasing concentrations of fluridone. After rinsing and drying, the seeds were allowed to germinate at 20°C. Seeds that had not been pre-imbibed at 30°C, germinated at 20°C to 90–100%.

in lily bulblets that did not develop dormancy because they were regenerated at 15 instead of 20°C, application of ABA did not restore dormancy (Fig. 2). In seeds, the effect of fluridone has been examined only in the development of primary dormancy in *Helianthus annuus* (Le Page-Degrivy & Garello 1991). In seeds of *Agrostemma githago* secondary dormancy develops during imbibition at 30°C (Borriss 1940). Secondary dormancy did not develop when fluridone was added during the imbibition at 30°C (Fig. 3). In *Agrostemma*, the development of primary dormancy was also inhibited by fluridone (data not shown).

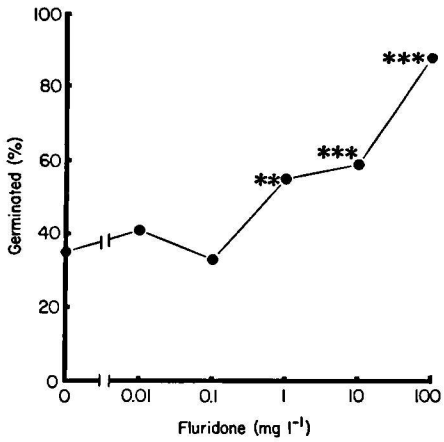

Fig. 4. Germination of three-months after-ripened seeds of *Agrostemma githago* imbibed in the presence of fluridone. Values, significantly different from the control (no fluridone added) are indicated by $^{**}P<0{\cdot}01$, and $^{***}P<0{\cdot}001$.

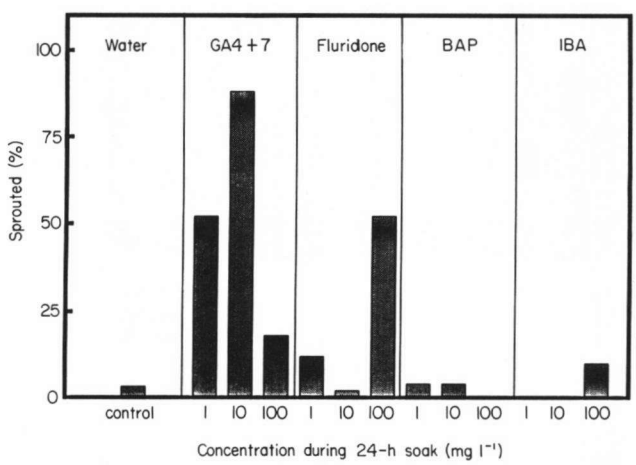

Fig. 5. Effect of a 24-h soak in solution of fluridone, GA_{4+7}, IBA or BAP on the sprouting percentage of dormant bulblets of *Lilium speciosum*.

A greater percentage of lily bulblets that were transplanted to medium with fluridone after the induction of dormancy sprouted than did bulblets transferred to standard medium (Gerrits *et al.* 1992). In *Agrostemma*, fully dormant seeds did not germinate when fluridone was added. In partially after-ripened seeds, though, the germination percentage increased significantly when fluridone was added during imbibition (Fig. 4). This shows that continuous ABA synthesis was necessary to maintain dormancy. In mature lily bulblets, dormancy was broken by a 24 h soak in GA_{4+7}, whereas fluridone had only a small effect at very high concentration (Fig. 5). BAP and IBA had no effect at all.

Control of apical dominance

After addition of fluridone to shoot cultures of apple, the plants were often very bushy. The propagation factor of fluridone-treated plants was significantly higher (Fig. 6a). This

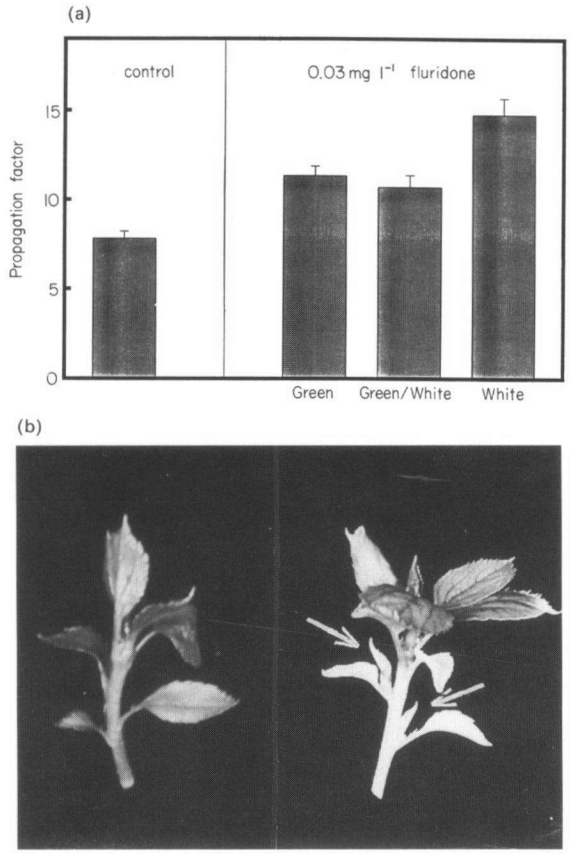

Fig. 6. Effect of fluridone on the outgrowth of axillary buds in apple. Fluridone was added at various concentrations and the rate of proliferation was determined after 6 weeks (a). In panel (b) a long shoot grown in the presence of fluridone is shown. Note that the axillary buds have grown (indicated by arrow).

might have been caused by reduction of dormancy. However, in elongated shoots, grown in the presence of fluridone, axillary buds had started to grow indicating that the axillary buds had been affected specifically (Fig. 6b). Furthermore, high concentrations of fluridone (0·05 mg l^{-1}) often resulted in extremely bushy tufts with non-elongated internodes, similar to tufts at high concentrations of cytokinin. Such tufts were not observed after a cold treatment.

DISCUSSION

It is assumed that proliferation of perennial crops *in vitro* often slows down or stops because of the development of dormancy (Margara 1982). It should be noted that dormancy is usually, at least partly, broken at subculturing by the excision and by the high level of cytokinin in the fresh propagation medium (cf. Lavender & Silim 1987). A cold treatment enhanced propagation in well established cultures of apple and quince (Fig. 1). This shows that these cultures had developed a low level of dormancy. Figure 1 also indicates that dormancy may often remain unnoticed as growth was only slowed down. It

is interesting to note that dormancy developed without an apparent environmental stimulus.

In seeds that require dry storage, dormancy does not develop when the level of endogenous ABA is reduced by mutation (Karssen *et al.* 1983) or by treatment with the ABA-synthesis inhibitor fluridone (Le Page-Degrivy & Garello 1991). The role of ABA in dormancy development had not yet been demonstrated in buds, bulbs or seeds that require cold treatment to break dormancy (Powell 1987). Figure 2 shows unambiguously that in lily bulblets, the presence of ABA was required for the development of dormancy: when the level of ABA was reduced by addition of fluridone, the bulblets did not develop dormancy, whereas the effect of fluridone was overcome by simultaneous addition of ABA. The control over the establishment of dormancy is, however, more complex: in bulblets regenerated at 15°C addition of high levels of ABA did not enhance the development of dormancy. This was unlikely due to low transport or rapid catabolism of ABA, as at 15°C added ABA has a marked effect on plantlet morphology (Gerrits & De Klerk 1992). Various reports indicate roles of antagonistic hormones, in particular GAs (Juntilla 1981; Cairns & De Villiers 1989; Aguettaz *et al.* 1990) and hormone sensitivity (Walker-Simmons 1987).

Only few reports concern the control over the maintenance of dormancy. In wheat seeds (Morris *et al.* 1991) and lily bulblets (Gerrits *et al.* 1992) ABA is possibly involved. In seeds of *Agrostemma* ABA also controlled the maintenance of dormancy. In the breaking of dormancy, GA has a main role, but seeds and buds may also be released from dormancy by addition of cytokinins and ethylene (Bewley & Black 1982; Powell 1987). In GA-deficient mutant seeds of tomato and *Arabidopsis*, addition of GA is required for germination (Karssen & Lacka 1986). In lily bulblets, a short treatment with GA broke dormancy, whereas IBA and BAP had no effect (Fig. 5).

The term 'dormancy' is used for a wide range of phenomena differing with regard to the type of organ, the nature of dormancy (arrest of growth in seeds or change in the direction of development in buds and bulbs; Young *et al.* 1974; Delvallée *et al.* 1990), the inducing stimulus and the conditions for release (cold storage under humid conditions or dry storage at room temperature). Thus, the question arises whether there is a general hormonal mechanism underlying dormancy. It is striking that ABA plays a key role in the development of dormancy in both seeds (Karssen *et al.* 1983) and bulbs (Fig. 2). ABA may be also involved in the maintenance of dormancy in seeds (Fig. 4, Morris *et al.* 1991) and bulbs (Gerrits *et al.* 1992). A dormancy-breaking treatment can be substituted by a GA treatment in seeds (Bewley & Black 1982), buds (Powell 1987) and bulbs (Fig. 5). These data indicate close parallels between the various types of dormancy. A possible explanation of this is that they have evolved from a single type of stress response. In this respect it is interesting to note that plants accumulate ABA in response to drought stress (Zeevaart & Creelman 1988).

Recently, evidence has been obtained that the conventional concept of apical dominance (the ratio of auxin:cytokinin in the axillary bud controls outgrowth) is incorrect (Pilate *et al.* 1989; Gocal *et al.* 1991; Tamas *et al.* 1992). Knowledge about the actual control of apical dominance may result in a better control of the outgrowth of axillary buds both during the culture *in vitro* and after planting of micropropagated plantlets in soil. It has been suggested that polarity in the stem controls the outgrowth (Morris & Johnson 1990; Tamas *et al.* 1992). We found that in apple, fluridone released the inhibition in axillary buds (Fig. 6). Furthermore, silver thiosulphate (an inhibitor of ethylene action) also releases axillary buds from inhibition in rose and apple (Van Telgen *et al.* 1993). These

data indicate that there are much more possibilities to influence apical dominance than addition of cytokinin or auxin and removal of the apex.

Plants may have evolved similar or different mechanisms to control growth of apical and axillary meristems. It should be noted that apical dominance is imposed by another part of the plant, whereas dormancy is imposed by an environmental stimulus or forms part of a developmental programme in the organ itself. This indicates different mechanisms. Nevertheless, the promotion of outgrowth of axillary buds by fluridone suggests that ABA also plays a part in apical dominance.

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