The Effect of Gibberellin and Cytokinin on Floral Development in *Zantedeschia* spp. In Vivo and In Vitro

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Abstract

*Zantedeschia* spp. (*Araceae*) is a tuberous day-neutral plant in which flowering is not induced by environmental signals. Flower yield, however, is increased by treatments with gibberellin A$_3$ or gibberellin A$_4$+7 and benzyl adenine (BA). We examined the effect of GA$_3$ and BA on inflorescence differentiation in three experimental systems: sprouting tubers, intact growing plants and plantlets in tissue culture. A similar pattern of response was found in all the experimental systems. One application of GA$_3$ resulted in inflorescence differentiation in the apical bud of the main shoot on the tuber as well as in plantlets in vitro. Continuous application of BA weakened apical dominance imposed by the main shoot and thus enabled the sprouting of inhibited axillary buds. Application of BA accompanied by one application of GA$_3$ resulted in inflorescence differentiation in the apical and axillary buds in the main and secondary shoots of the tuber as well as in plantlet in vitro. Thus, BA affected flowering indirectly by promoting axillary bud growth that responded to the GA$_3$ treatment. These results suggest that in day-neutral *Zantedeschia* the meristem is responsive to GA$_3$ regardless of tuber size, plant age, or meristem position. A change in GA$_3$ level may play a central role in the transition from the vegetative to the reproductive stage, and both gibberellins and cytokinins are involved in floral initiation and development. Plantlets in vitro can serve, therefore, as a model system for studying inflorescence development in day-neutral plants.

INTRODUCTION

Flower yield in calla lily (*Zantedeschia* spp., *Araceae*) is relatively low due to developmental constrains related to branching and flowering control. A pre-determined developmental (i.e. branching) program determines the growth pattern of colored *Zantedeschia*. In several cultivars this pattern is characterized by strong apical dominance of the primary shoot. This results in massive growth of the primary shoot and in a gradually decreasing growth potential of secondary shoots on the tuber, with increasing distance from the primary shoot (Naor, unpublished). Thus, depending on the cultivar, few to several secondary shoots may develop on the tuber, while the rest of the buds remain inhibited. This limited production of secondary shoots constrains flower yield in colored calla lily. In other plant species, application of cytokinin is known to activate the growth of lateral buds inhibited by apical dominance (Cline, 1991). Thus, it is possible that cytokinin application can be used to increase the number of secondary shoots on the tuber and, therefore, increase flower yield. However, the effect of cytokinins on the sprouting of inhibited secondary shoots in *Zantedeschia* tubers, and on their subsequent flowering differentiation is not clear.

Colored *Zantedeschia* are tuberous day-neutral plants, in which flowering is not induced by environmental signals, suggesting that floral development occurs under suitable endogenous conditions. Lawson and Poethig (1996) suggested that gibberellin is involved in signaling the onset of the flowering process. Previous studies showed that in
day neutral plants, like sunflower (Almeida and Pareira, 1996) and *Zantedeschia* (Naor, unpublished data), endogenous gibberellins (GAs) increase prior to inflorescence differentiation, implying on the involvement of GAs in the flowering process. In *Zantedeschia*, inflorescence differentiation occurs in tissue culture plantlets following GA$_3$ application (Naor et al., 2004). Moreover, application of GA promotes flowering in other Araceae species (Funnell, 1993; Henny, 1999) and is used commercially to increase the flower yield of colored cultivars of calla lily (Funnell, 1993). However, due to the characteristically strong apical dominance, the increase in flower yield in colored *Zantedeschia* by GA is limited only to the elongating shoots. The increase in flower yield is relatively small compared to the number of buds and the potential of shoot production of the tuber. Furthermore, the influence of exogenous gibberellin on secondary bud activation and inflorescence differentiation in *Zantedeschia* tuber is not clear. The objectives of this study were to examine the separate and synergistic effects of GA$_3$ and the cytokinin benzyl adenine (BA) on bud sprouting and inflorescence differentiation in three experimental systems in *Zantedeschia*: tubers in storage, intact plants after natural flowering and plantlets in tissue culture.

**MATERIAL AND METHODS**

Two cultivars of colored *Zantedeschia* differing in strength of apical dominance were used: Calla Gold (CG, *Z. rehmanii* Engl. hybrid) a red cultivar with a relatively weak apical dominance (Exp. 1, 2 & 3), and Black Magic (BM, *Z. albomaculata* (Hook.) Baill hybrid), a yellow cultivar with strong apical dominance (Exp. 1).

**Morphological Definitions**

Floral stem – a spadix surrounded by a colored spathe at the top of a leafless peduncle.

Primary bud – the apical and largest bud of the tuber that becomes the primary shoot. The primary shoot is the first to grow; it is the most developed shoot and often the only shoot that elongates on the tuber.

Secondary buds – the axillary buds along the tuber axis, that develop according to the phyllotactic order along the tuber axis. Most of these buds remain inhibited during the growth period.

**Plant Material**

Dormant tubers of CG and BM, weighing 20 to 30 g, were harvested in a commercial farm in Israel during early winter (CG in Cohen Farm, December 2000 and 2001; BM in Sinai Farm, December 2002). The tubers were cleaned and dipped in Sterner 0.15% (oxolinic acid) for 15 min and in Captan 1% for 30 min before storage, and again before planting, to prevent Erwinia and fungal contamination, respectively. Treated tubers were stored for 3 months at constant 20°C in the dark during the experimental period or until planting. For the in vitro studies, plantlets of *Zantedeschia* cv. CG grown in vitro were obtained from Northern R&D Tissue Culture Laboratory, Israel.

**The Effect of BA and GA$_3$ on Inflorescence Development in Sprouting Tubers during Storage (Exp. 1)**

To study the effect of BA and GA$_3$ on inflorescence development in secondary shoots during storage, CG and BM tubers were dipped in a solution of GA$_3$ 0.6 mM (200 ppm) and Tween-20 0.15% for 30 min before storage or dipped in Tween-20 0.15%. Afterwards and during ca. 3 months, half of the tubers from each treatment were dipped in BA 0.9 mM (200 ppm; Bongro, 4% BA distributed by Agan Ltd.) and Tween-20 0.15% every two weeks, while the other half were dipped in Tween-20 0.15% for 30 min. At the end of the storage period, the tubers were planted for 5-6 weeks to intensify the treatment effect on the sprouting buds and then harvested. The harvested tubers were dissected under a stereomicroscope and the number of secondary sprouting buds and the number of floral stems produced by the primary and secondary shoots was recorded.
The Effect of BA and GA₃ on Inflorescence Development in Intact Plant (Exp. 2)

CG tubers were planted in early summer in 1 liter pots, 11 cm in diameter, filled with a mixture of volcanic tuff gravel and vermiculite #3 1:1 (v/v). Plants were grown under controlled conditions in a phytotron, in a glass covered growth room under natural daylight, at 22/16 ± 1°C (day/night) temperature. Day temperature was given between 08:00 and 16:00. Plants were kept under shade (50% solar radiation) and 16 h photoperiods, attained by extending the natural day-length with supplemental incandescent light (5 μmol m⁻² s⁻¹ at plant level). Plants were irrigated twice daily, with 50% Hoagland solution in the morning and with tap water in late afternoon.

To study the effects of BA and GA₃ on the flowering of secondary shoots, plants were leaf sprayed either with a solution of BA 0.9 mM (200 ppm) and Tween-20 0.15%, or water and Tween-20 0.15%, twice a week starting 40 days after tuber planting. At the end of flowering (ca. 90 days after planting), half of the BA treated plants (6 plants) and half of the water treated plants (6 plants) were leaf sprayed twice with GA₃ 0.6 mM (200 ppm) and Tween 0.15% and the other half with water and Tween-20. BA and water sprays continued for further 30 days after GA₃ treatment, until the end of the experiment. At the end of the experiment, the tubers were dissected under a stereomicroscope and the number of secondary buds and of inflorescences produced by the primary and secondary shoots were recorded.

The Effect of BA and GA₃ on Inflorescence Development in Plantlets In Vitro (Exp. 3)

Plantlets of Zantedeschia cv. CG that were subcultured for the experiments, were 2-3 cm high, had 1-2 leaves, a vegetative apical meristem and roots. Plantlets (5-10) were grown in 0.5 l culture boxes (10 cm diameter x 6 cm height) in MS medium (Sigma M5524), supplemented with vitamins and sucrose (3%) and solidified with 0.65% agar (w/v) (Cohen, 1981). The experiment was performed under controlled conditions at constant temperature (22 ± 1°C) and 16h light / 8h dark photoperiod. Light intensity was 60-90 μmol m⁻² s⁻¹ at plant level, provided by cool white fluorescent lamps (Philips 40W/33WC). To study the effects of BA and GA₃ on inflorescence development in the primary and secondary shoots in vitro, plantlets were dipped for 30 min in 0.6 mM (200 ppm) GA₃ (Sigma G-7645) solution or in water and planted in media containing BA 1.3 μM (Sigma B-3408, added before autoclaving), or in BA-free medium. Control plantlets were planted in BA-free medium without a pre-planting treatment. The developmental stages of the shoot apex were recorded by dissecting the buds under a stereomicroscope. Plantlets were dissected 30-50 days after GA treatment as no further changes in inflorescence development were observed at later dates of dissection.

Table 1 presents a summary of the hormonal treatments in the three experimental systems.

Statistical Methods

Standard error analyses were carried out by SAS and Sigma plot software.

RESULTS AND DISCUSSION

The Effect of BA on Bud Sprouting

The control of apical dominance is associated with different levels of growth regulators, either suppressing (i.e. auxins) or promoting (i.e. cytokinins) lateral bud growth (Cline, 1991, 1994). In Zantedeschia, application of BA enhanced bud sprouting (Funnell et al., 1992) as in other Araceae (Henny, 1986; Henny and Fooshee, 1985). In the present study, continuous application of BA promoted bud sprouting in CG tubers during storage, as well as in intact plants (Fig. 1 and 4). This increase was primarily the result of enhanced secondary bud sprouting that was 15-25 times greater as compared to the control or GA₃ treatments. Similar results were found in BM tubers (data not shown).
The enhancement of secondary bud sprouting and elongation by BA in tubers during storage and in intact plants indicates that these buds were non-dormant, but inhibited by apical dominance. Furthermore, scooping of the primary bud in tubers resulted in secondary bud sprouting, similarly to BA application (Naor, unpublished). It is possible that exogenous supply of BA or removal of the apex changed the ratio between growth suppressors (auxin?) and growth promoters (cytokinins) resulting in secondary bud sprouting.

In plantlets in vitro, a high concentration of BA (13.3 µM) in the medium resulted in massive proliferation of axillary and possibly adventitious buds. This resulted in clusters containing 20-50 buds, as compared to 0-10 buds in a medium with low BA concentration (1.33 µM; Fig. 4). In this tissue culture system, BA promoted bud differentiation and probably also released them from the inhibition exerted by existing buds.

**The Effects of BA and GA3 on Inflorescence Differentiation**

The effects of GA3 and BA on inflorescence differentiation were similar in all the experimental systems studied. In the control treatments, the proportion of plants with inflorescences and the number of inflorescences in the primary and secondary shoots were very low in all the experimental systems (Fig. 2 and 3). In contrast, following one application of GA3, inflorescences differentiated in the primary buds of 75-100% of the tubers, in 60% of intact plants and in 90% of in vitro plantlets (Fig. 2). The proportion of plants with inflorescences in secondary shoots after GA3 treatment was relatively high in CG plants and tubers, compared to control plants. In plantlets in vitro, inflorescence development occurred only in GA3 treated plantlets and always in the primary shoot (Fig. 2d). The development of the inflorescence primordium in these plantlets was similar to the pattern observed in intact plants during normal development. However, inflorescence development in vitro ceased after reaching 2-5 mm length, and remained enclosed within the last leaf primordium (Naor et al., 2004).

In the BA treatment, the proportion of tubers with a differentiated inflorescence in the primary bud increased during storage from 0 to 50% in BM (Fig. 2b) and from 10 to 40% in CG (Fig. 2a) as compared to the control treatment. Similarly, in intact CG plants it increased from 20% to 60% (Fig. 2c). The number of inflorescences in sprouting tubers and growing intact plants was low, and they differentiated in only 10 and 25% of the activated buds, respectively (Fig. 3). The enhancement of flowering in tubers by BA was probably due to a synergistic effect between BA and endogenous gibberellins in the buds (Funnell et al., 1992). This hypothesis is supported by the number of inflorescences in secondary shoots in sprouting tubers or intact plants, following the GA3 + BA treatment compared to the number of activated shoots (Fig. 3). On the other hand, addition of BA to the media did not promote inflorescence differentiation in plantlets in vitro. This lack of response can be attributed to a low level of endogenous gibberellins in the plantlets compared to sprouting buds in the tuber (Naor, unpublished).

**CONCLUSIONS**

BA affects flowering indirectly by weakening apical dominance thus promoting secondary bud sprouting. The response of the activated buds to the GA3 treatment resulted in an increase in the number of differentiated inflorescences per tuber. However, further studies are required to implement this finding in order to increase the floral yield per tuber, while maintaining the high quality of floral stems. The meristem in the activated bud is responsive to GA3 regardless of tuber size, plant age or bud position in sprouting tubers or intact plants. In addition, a change in endogenous gibberellin level may play a central role in the transition from vegetative to reproductive stage. The similar pattern of inflorescence differentiation in different experimental systems suggests that plantlets of *Zantedeschia* in vitro can serve as a model system to study the control of flowering in day neutral plants.
Literature Cited

Tables

Table 1. A summary of the hormonal treatments given in the three experimental systems.

<table>
<thead>
<tr>
<th>Experiment system</th>
<th>Control</th>
<th>GA$_3$ (1-2 applications)</th>
<th>BA (continuous)</th>
<th>BA + GA$_3$ treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1: Sprouting tubers in storage</td>
<td>Water dip</td>
<td>One pre-storage dip</td>
<td>Two week interval dips</td>
<td>BA + GA$_3$ treatments</td>
</tr>
<tr>
<td>Exp. 2: Intact plants</td>
<td>Leaf water spray</td>
<td>Two foliage sprays 90 days after planting</td>
<td>Foliage spray twice a week</td>
<td>BA + GA$_3$ treatments</td>
</tr>
<tr>
<td>Exp. 3: Plantlets in vitro</td>
<td>Planting on BA free medium</td>
<td>One dip before planting on BA free medium</td>
<td>Water dip and planting on BA medium</td>
<td>One GA$_3$ dip before planting on BA medium</td>
</tr>
</tbody>
</table>
Fig. 1. The number of sprouting buds in CG tubers during storage (Exp. 1) and the number of elongating shoots in CG intact plants during the growth period (Exp. 2). Bars indicate SE.
Fig. 2. Percent of tubers with differentiated inflorescences in the primary bud or shoot and the percent of differentiated inflorescences in the secondary buds or shoots in: (A) CG sprouting tubers in storage; (B) BM sprouting tubers in storage; (C) CG intact plants during the growth period; (D) CG plantlets in tissue culture. Bars indicate SE.
Fig. 3. The number of inflorescences in the primary bud or shoot and in the secondary buds or shoots in: (A) CG sprouting tubers in storage; (B) BM sprouting tubers in storage; (C) CG intact plants during the growth period; (D) CG plantlets in tissue culture. Bars indicate SE.
Fig. 4. The effect of BA on secondary bud sprouting. BA was applied for ca. 90 days as a foliage spray at two-week intervals to intact plants, as a dip to tubers in storage or as medium component in vitro. (A) Control intact plants; (B) BA treated intact plants; (C) Control tuber in storage; (D) BA treated tube in storage; (E) low BA to plantlets; (F) high BA to plantlets. pb - Primary bud; sb - Secondary bud.