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A Developmental Pattern of Flowering in Colored *Zantedeschia* spp.: Effects of Bud Position and Gibberellin

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Abstract

The restricted flowering of colored cultivars of *Zantedeschia* is a consequence of developmental constraints imposed by apical dominance of the primary bud on secondary buds in the tuber, and by the sympodial growth of individual shoots. GA_3 enhances flowering in *Zantedeschia* by increasing the number of flowering shoots per tuber and inflorescences per shoot. The effects of gibberellin on the pattern of flowering and on the developmental fate of differentiated inflorescences along the tuber axis and individual shoot axes were studied in GA_3 and Uniconazole-treated tubers. Inflorescence primordia and fully developed (emerged) floral stems produced during tuber storage and the plant growth period were recorded. Days to flowering, percent of flowering shoots and floral stem length decreased basipetally along the shoot and tuber axes. GA_3 prolonged the flowering period and increased both the number of flowering shoots per tuber and the differentiated inflorescences per shoot. Activated buds were GA_3 responsive regardless of meristem size or age. Uniconazole did not inhibit inflorescence differentiation but inhibited floral stem elongation. The results suggest that GA_3 has a dual action in the flowering process: induction of inflorescence differentiation and promotion of floral stem elongation. The flowering pattern could be a result of a gradient in the distribution of endogenous factors involved in inflorescence differentiation (possibly GAs) and in floral stem growth. This gradient along the tuber and shoot axes is probably controlled by apical dominance of the primary bud.

Key words: Bud position; Calla lily; Flowering; Gibberellin; tuber; Uniconazole; *Zantedeschia*

Introduction

The restricted flowering of colored cultivars of calla lily (*Zantedeschia* spp.) is a consequence of their developmental program (Funnell 1993). The plant growth cycle of colored *Zantedeschia* species is

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characterized by alternating growth and rest periods, unlike *Z. aethiopica* that, under suitable growing conditions, is potentially evergreen, and flowers continuously (Funnell 1993). Moreover, tubers of colored *Zantedeschia* spp. develop few shoots during the growth period, and their number is probably determined by the strength of apical dominance exerted by the primary shoot, as in *Zantedeschia aethiopica* (Ngamau 2001). In general, plants with moderate to strong apical dominance have limited or no lateral bud growth. Growth potential of lateral buds is correlated with their size, age and position along the stem axis, and is influenced by nutrient and hormonal gradients (Cline 1991). Thus, the degree of branching and shape of the shoot system are largely determined by the strength of apical dominance interacting with bud position.

Most of the studies on shoot organization in *Araceae* have examined the growth pattern of individual growing shoots (Ray 1987; Halligan and others 2004). However, little work has been done on positional growth patterns of buds along tubers and rhizomes of the Araceae (Ngamau 2001). Positional patterns of branching are observed also in *Zantedeschia* spp. tubers, considering the tuber as a condensed stem. Thus, depending on the cultivar, few to several secondary shoots may develop on the tuber, while the rest of the buds remain inhibited. Growing shoots of colored *Zantedeschia* are characterized by a sympodial growth habit in which apical inflorescence differentiation is followed by the activation of only 1 or 2 axillary buds below it (Ray 1987; Funnell 1993; Brooking and Cohen 2002; Halligan and 2004). These axillary buds develop into shoots usually consisting of a prophyll, up to 2 leaves and a terminal inflorescence. Not all differentiated inflorescences emerge, because of abortion that may occur in terminal and axillary buds of primary and secondary shoots (Brooking and Cohen 2002; Naor unpublished). Furthermore, the flowering potential generally decreases with decreasing tuber size (Corr and Widmer 1991). These developmental traits together with the short period of flowering result in a relatively low number of floral stems produced per tuber in colored calla lily.

Application of gibberellic acid is known to increase the number of floral stems produced per tuber in colored *Zantedeschia* (Corr and Widmer 1987, 1991; Brooking and Cohen 2002). It has been proposed that this increase is due to both a larger proportion of shoots reaching flowering and a greater number of inflorescences produced per shoot (Corr and Widmer 1987; Funnell 1993; Brooking and Cohen 2002). However, possible interactions between gibberellin and the flowering potential of buds, according to their position on the tuber and along the shoot axis, have not been studied so far. Furthermore, the effects of GA on specific processes determining the extent of flowering in *Zantedeschia* are not clear.

The objectives of the present study were: 1) to analyze the pattern of flowering in colored *Zantedeschia*, according to shoot order on the tuber axis (primary and secondary shoots) and bud position along the flowering shoots; 2) to examine the effects of GA_3 and Uniconazole (GA-biosynthesis inhibitor) on the patterns of growth and flowering; 3) to study the time-course of inflorescence differentiation and inflorescence fate in response to GA_3 application.

Materials and Methods

Plant Material

The cultivars Black Magic (BM, *Z. albomaculata* (Hook.) Baill., hybrid) and Calla Gold (CG, *Z. rehmanii* Engl. hybrid) were used in this study. Preliminary observations showed that the number of sprouting buds and floral stems per tuber, as well as variation in flowering due to tuber size and GA_3 treatments, were higher in CG than in BM. In BM the primary shoot exerts a stronger apical dominance with only a small number of shoots developing. Therefore, CG was used to study the effects of tuber size and GA_3 on ontogenetic gradients of flowering in the tuber and along primary and secondary shoots. The anatomical changes involved in the transition of the primary shoot apex to flowering in response to GA_3 were studied in BM.

In our study, the primary bud in the tuber is defined as the largest bud. It is the first bud to sprout, which later develops into the primary shoot. Secondary buds are the buds laterally positioned on the tuber axis, which may develop into secondary shoots. Axillary buds are the buds located along the growing shoot axis, which may flower or develop into the continuation buds for the next growth cycle.

Tubers were harvested during early winter in commercial farms in the Northeast of Israel (CG in Cohen Farm, December 2000 and 2001; BM in Sinai Farm, December 2002). The tubers were 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 temperature (20°C) in the dark during the experimental period (BM) or until planting (CG).

Effects of Tuber Size on Flowering (Experiment 1)

CG tubers ranging from 5 to 165 g (8-24 cm in circumference) were planted 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 the 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 screen shade, transmitting 50% solar radiation, and 16 h photoperiods were attained by extending the natural day-length with supplemental incandescent light (5 μmol m^{-2} s^{-1} at plant level). Plants were irrigated twice a day with 50% Hoagland solution in the morning and with tap water in late afternoon. To study the effects of GA_3 on the flowering of small tubers, tubers weighing 5-20 g were dipped in 0.6 mM GA_3 (Progib, CTS Ltd, Israel) for 30 min before planting. The number of floral stems produced by the primary and secondary shoots was recorded and floral stems were excised until the end of the growth period. A floral stem was defined as a peduncle bearing a spadix surrounded by an open-colored spathe, and was recorded just before anther dehiscence.

Effects of GA on Duration of the Flowering Period (Experiment 2)

Tubers of CG weighing 80–90 g were planted on 12 Jan 2000 under the same conditions as in Experiment 1. At the end of the first flowering cycle, half of the plants were leaf sprayed twice a week with a solution of 0.6 mM GA_3 and 0.01% Tween 20, and the rest of the plants were untreated and kept as a control. GA_3 leaf spray was continued for 14 months. Number of sprouting buds per tuber, number of shoots and leaves on the dominant shoots, and proportion of flowering plants and number of floral stems per plant were recorded. Tubers of control plants were harvested after leaf senescence, dry stored at 20°C, and bud sprouting was followed during storage.

Effects of GA_3 and Uniconazole on Flowering (Experiment 3)

CG tubers, weighing 40–50 g (16–18 cm in circumference) were planted on March 6, 2001 in 2 liter pots, 18 cm in diameter, filled with a mixture of volcanic tuff gravel and peat moss 2:1 (v/v). Plants were grown under shade (50% solar irradiation) and natural day-length and temperature during the spring and summer. Plants were drip irrigated (2 liter/h) twice a day with a N:P:K (150:90:240 ppm) solution. Tubers were dipped in 0.6 mM GA_3 (Progib, CTS Ltd, Israel) for 30 min, or in 4 mM Uniconazole (Agan, Israel) for 60 min, or in Uniconazole 4 mM for 60 min followed by GA_3 0.6 mM for 30 min (GA_3 + Uniconazole treatment). A 4 mM Uniconazole solution was used because similar inhibition of inflorescence differentiation was observed in preliminary experiments with 4 and 8 mM Uniconazole, but severe growth inhibition occurred at the higher concentration. For the GA_3, preliminary experiments using 0.06-3 mM GA_3 showed that under our experimental conditions 0.6 mM was the most effective concentration for flowering promotion. Growth regulator treatments were applied once, at the onset of the storage period or at planting time, or twice, before storage and before planting. The GA_3 + Uniconazole treatment was applied only once, before storage or before planting. In the control treatments, tubers were dipped in water for 30 min. Experiment design was in four randomized blocks, each plot with four plants.

Sprouting buds were dissected under a stereomicroscope to determine floral differentiation in the buds at the end of the storage period. After planting, the emerged floral stems were recorded at anthesis, were excised, and their length was measured. The petiole and blade dimension were measured in second leaf at anthesis to determine the effect of Uniconazloe and GA_3 on the plant growth. At the end of the experiment, non-emerged and primordial floral stems were recorded by dissecting each growing shoot of plants in the control and in Uniconazole treatments (10 plants per treatment).

Differentiated floral stems (primordial, emerged – that is, floral stems that reached anthesis or aborted) were recorded in the main shoot and in the secondary shoots of each tuber. In dissected tubers and plants, secondary shoot order (that is, starting from the main shoot) was determined according to the relative size of the sprouting buds and growing shoots, respectively. This generally reflected their phyllotactic position on the tuber. In each shoot, differentiated floral stems were recorded in basipetal order, starting from the apex.

Anatomical Changes in the Shoot Apex During Transition to Flowering (Experiment 4)

Tubers of BM, weighing 60–90 g (20–24 cm in circumference), were dipped in a GA_3 0.6 mM solution (Progib, CTS Ltd, Israel) for 30 min or in water

for 30 min (control), and stored at 20°C in darkness. Every 2 weeks the length of the primary sprouting bud was measured in 5 tubers, and the buds were fixed and stored in FAA aqueous solution (ethanol 50%, acetic acid 5% and formaldehyde 4%) for histological examination. Before embedding, 2–3 leaf scales were removed.

To study the stages of inflorescence differentiation in the bud apex, buds were examined under a light microscope. The samples were fixed in a solution of freshly prepared 2% paraformaldehyde in 0.1 M cacodylate buffer (pH = 7.0), dehydrated in a graded ethanol series and embedded in Paraplast. The sections were cut at 10 μm thickness with a rotary microtome (American Optical model 820, Spencer), stained in Safranin and Fast-Green, and mounted in Permount (Fisher Scientific, Cat. No. SP15–100) and were examined under a bright field and photographed under a Leitz Dialux 20 light microscope. The stages of inflorescence differentiation in the bud apex were recorded and correlated with the bud length.

Statistical Analysis

Factorial analysis of variance and regression analyses were carried out by SAS and Sigma plot software. Values presented in the tables are means ± se.

Results

Tuber Size and Flowering (Experiment 1)

The potential of flowering increased with tuber weight (Figure 1). Tubers smaller than 20 g had a very low flowering percentage (4%), while tubers weighing more than 40 g reached maximal flowering (80–100%). The proportion of flowering in small tubers (5–20 g) treated with GA_3 increased from 4% to 41%. The number of floral stems in this treatment increased from 1.0 to 1.7 per flowering plant. Floral stems developed from the apical and from the axillary bud of the first leaf below the apex. The number of floral stems increased in larger size tubers, although it did not exceed 2–3 floral stems even in tubers weighing more than 80 g. Secondary floral stems were produced only in tubers larger than 40 g (data not shown).

Effects of GA_3 on Duration of Growth and Flowering Period (Experiment 2)

In colored *Zantedeschia* the plant growth cycle consists of a growth period followed by a rest period. In the control plants one growth cycle occurred during the experimental period, with 50% of the tubers reaching flowering. In these tubers, one wave of flowering was recorded, starting at about 40 days after planting and continuing for another 2.5 months (Figure 2A). All the leaves senesced 7 months after planting, tubers became dormant (no sprouting buds) and were harvested (Figures 2B, C). Renewal of bud sprouting occurred during dry storage and all tubers sprouted approximately 50 days after complete leaf senescence (Fig. 2B).

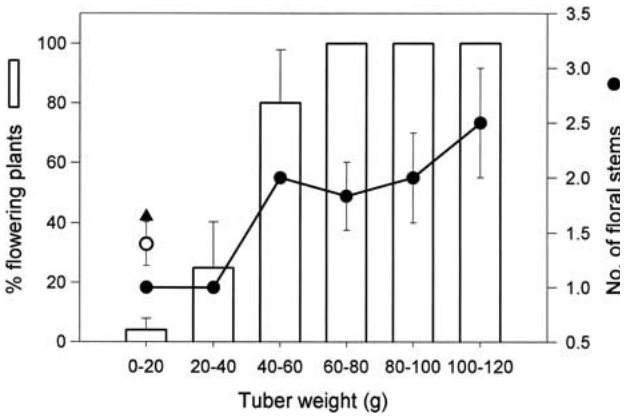

Figure 1. The effect of tuber size and GA_3 treatment on the number of floral stems and the percent of flowering plants in cv. CG. (□–% flowering plants; ●- number of floral stems per flowering plant; ▲- % of flowering plants in GA_3-treated tubers weighing 0-20 g; O- number of floral stems per flowering plants in GA_3-treated tubers weighing 0-20 g.

Continuous application of GA_3 for approximately 14 months resulted in two growth cycles separated by a short rest period, even though the plants were continuously irrigated (Figure 2C). GA_3 prolonged the first growth cycle as compared to the control, delaying leaf senescence of the primary shoot (9–10 vs. 7 months from planting, respectively) and of secondary shoots (12 months after planting) (Figure 2B, C). Secondary shoots continued to flower concomitantly with primary shoot senescence (Figure 2a). During the first growth cycle, two waves of flowering occurred in GA-treated tubers compared to one in the control (Figure 2A, C). The floral stems developed even in shoots with senescing leaves. At the end of the first growth cycle, 86% of the tubers reached flowering, compared to 50% in the controls. The second growth cycle in GA_3-treated tubers started after about 2 months of limited bud sprouting (only 40% of tubers had sprouting buds) (Figure 2B), and spanned 3.5 months. During this period, new shoots were produced and reached flowering in approximately 80%

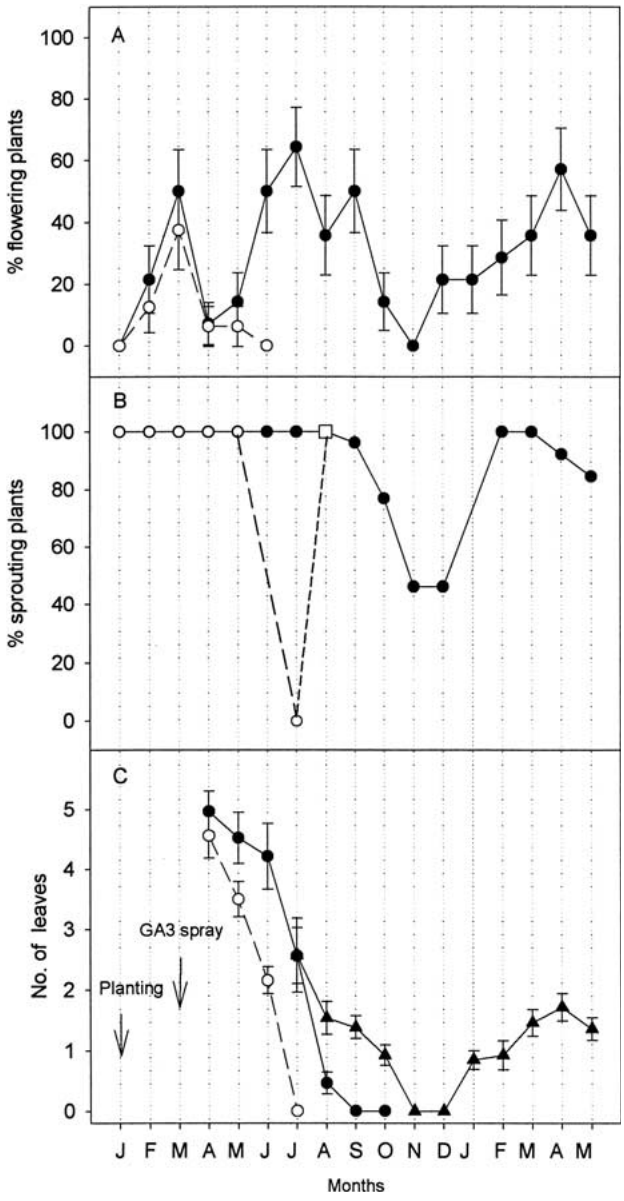

Figure 2. The effect of continuous application of GA_3 on the vegetative and floral development. Plants of cv. CG were given GA_3 as a foliage spray twice a week for 14 months. A. The percent of flowering plants. ○- Control; ●- GA_3 treated plants. B. % of sprouting plants. ○- Control; ●- GA_3 treated plants. Dashed line: tubers were harvested and dry stored during dormancy and the renewal of bud sprouting occurred during storage. C. Number of leaves in the primary shoot. ○-Control; ●-leaves in the primary shoot of GA_3 treated plants; ▲-leaves in the 2nd primary shoot of GA_3 treated plants. Arrows mark the planting date and the onset of foliage spray.

of the plants. These results indicate that continuous GA_3 application delayed bud dormancy, promoted floral stem elongation and prolonged the flowering period.

Effects of GA_3 and Uniconazole on Inflorescence Differentiation (Experiment 3)

Inflorescence Differentiation During Storage. Inflorescence differentiation was observed during storage in sprouting buds (25–35 mm) of both GA_3 and Uniconazole-treated tubers (Table 1). No significant differences were observed between control and Uniconazole-treated tubers in the proportion of tubers with differentiated inflorescences (ca. 50%), nor in the number of inflorescences produced in the sprouting buds before planting. In contrast, inflorescences developed in all GA_3-treated tubers and the number of inflorescences in the sprouting buds increased 4–5 fold. This increase was due to a two-fold increase in the number of inflorescence differentiated in the main and first secondary buds, as well as by promoting inflorescence development in higher order secondary buds that were vegetative in the control treatment. In tubers treated with GA_3 + Uniconazole, the proportion of tubers with inflorescences and the number of inflorescences per sprouting bud were similar to GA_3-treated tubers.

Floral Stem Production After Planting. Floral stems were produced in 80–100% of the tubers in the control and GA_3 treatments in contrast to 38–75% in Uniconazole treated tubers (Table 2A). Factorial analysis of variance showed that GA_3, Uniconazole and GA_3 + Uniconazole treatments significantly ($P < 0.05$) affected the number of floral stems produced per flowering tuber, while the time of application (before storage vs. before planting) and the number of applications had no effect (Table 2B). No significant interaction was observed between the growth regulators and the number of applications ($P = 0.24$).

Uniconazole reduced the number of floral stems by approximately 40% compared to the control ($P < 0.05$), while GA_3 and GA_3 + Uniconazole treatments increased it by approximately 2.5 fold and 1.8-fold, respectively ($P < 0.05$). The smaller increase in floral stems due to simultaneous application of GA_3 + Uniconazole compared to GA_3 was significant ($P < 0.05$). In the control treatment, the number of floral stems produced during plant development was larger than the number of inflorescences differentiated in the tuber at the end of the storage period (2.4 vs. 1.3). In contrast, in the GA_3-treated tubers the number of inflorescences at the end of the storage period did not increase during the growth period (5.9; Tables 1 and 2).

The possibility that the lower number of floral stems produced by Uniconazole-treated tubers was due either to growth inhibition or to inhibition of

Table 1. Percent of Tubers with Differentiated Inflorescences and the Number of Inflorescences per Bud in the Primary (1) and Secondary Buds (2 to 8) of Sprouting Tubers, before Planting

	Treatments			
Bud position on tuber	Control	GA_3	Uniconazole	GA_3 + Uniconazole
	Tubers with inflorescences (%)			
Primary bud 1	54	100	50	100
Bud 2	8	92	7	64
Bud 3	0	62	7	42
Bud 4	0	31	0	14
Buds 5–8	0	31	0	7
	Inflorescences per bud			
Primary bud 1	1.1 ± 0.1	2.1 ± 0.2	1.0 ± 0	2.3 ± 0.2
Bud 2	1.0 ± 0.0	1.8 ± 0.2	1.0 ± 0	2.1 ± 0.3
Bud 3	–	1.3 ± 0.2	1.0 ± 0	1.0 ± 0.0
Bud 4	–	1.5 ± 0.3	–	1.0 ± 0.0
Buds 5–8	–	2.8 ± 0.9	–	1.0 ± 0.0
Inflorescences tubers[1]	1.3 ± 0.2	5.9 ± 1.1	1.1 ± 0.1	5.0 ± 0.9

N = 13–14 plants per treatment. At the onset of the storage period, tubers were dipped for 30 min in 0.6 mM GA_3 solution, or 60 min in 4 mM Uniconazole solution, or 60 min in 4 mM Uniconazole followed by 30 min in 0.6 mM GA_3.
[1]The number of inflorescences per tuber were calculated only from tubers with differentiated inflorescences.

Table 2. The effect of 0.6 mM GA_3 and 4 mM Uniconazole on A. the Percent of Flowering Plants and on B. the Number of Floral Stems per Flowering Plant

	Treatments				
Time of application	Control	GA_3	Uniconazole	GA_3+Uniconazole	Main effect of application
	A. % flowering plants				
Before storage	81 ± 10	100	75 ± 11	100	88 ± 4
Before planting	100	94 ± 6	63 ± 12	88 ± 4	89 ± 4
Before storage and before planting	–	100	38 ± 12	–	69 ± 8
Main effect of treatment	91 ± 5	98 ± 2	58 ± 7	97 ± 3	–
	B. Number of floral stems				
Before storage	2.0 A[1]	5.6 A	1.4 AB	4.7 A	3.7 a
Before planting	2.8 A	5.8 A	1.6 A	4.0 A	3.6 a
Before storage and before planting	–	6.2 A	1.1 B	–	3.4 a
Main effect of treatment	2.4c[2]	5.9 a	1.4 d	4.3 b	

Statistical main effects were calculated since the interaction treatment X application was not significant (P = 0.24).
[1]Different capital letters indicate significant differences (α = 0.05) between main effects of applications within the hormonal treatment.
[2]Different small letters indicate significant differences (α = 0.05) between main effects of treatments or between main effects of applications.

inflorescence differentiation was examined. Shoot dissection at the end of the experiment showed that inflorescences differentiated in 70–90% of the tubers treated with one or two applications of Uniconazole. However, Uniconazole inhibited the development of 25–44% of the primordial inflorescences, and subsequently they aborted (Table 3). Uniconazole applied to the tubers before storage and/or planting also reduced the size of the leaves that emerged later on (petiole length by ca. 55 ± 3%, blade length by 19 ± 2%, blade width by 13 ± 2%, and blade area by 28 ± 4%), thus indicating that gibberellin biosynthesis was inhibited by Uniconzole that penetrated into the bud tissues.

Ontogenesis of Flowering in the Primary and Secondary Shoots. The timing of floral stem development on the primary and secondary shoots varied according to their phyllotactic position on the tuber and along the axis of each shoot. The period between floral stem production by consecutive shoots on the tuber axis was approximately 5 days, while

Table 3. Effects of 4 mM Uniconazole on Inflorescence Differentiation in Primary and Secondary Shoots on the Tuber

	Uniconazole treatments			
Parameter	Control	Before storage	Before planting	Before storage and planting
Sprouting buds/plant	3.1 ± 0.8	1.5 ± 0.4	3.3 ± 0.5	1.8 ± 0.4
Flowering plants (%)	80 ± 13	80 ± 13	90 ± 10	70 ± 15
Inflorescences in main shoot[1]	1.9 ± 0.4	1.1 ± 0.1	1.1 ± 0.2	1.0 ± 0.0
Plants with inflorescences in secondary shoots 2–3[1](%)	38	38	55	14
Inflorescences in shoots 2–3 [1]	1.7 ± 0.3	1.3 ± 0.3	1.2 ± 0.2	1 ± 0.0
Aborted floral stems[1] (%)	12	25	44	43
Aborted floral stems/differentiated buds (%)	10	23	22	38

Number of inflorescences is the sum of emerged floral stems and differentiated inflorescences determined by dissection at end of the experiment (aborted inflorescences included). N = 10 plants/treatment.
[1]In plants with reproductive shoots.

along the primary and secondary shoots, floral stems emerged approximately every 11 days (Figure 3). The percent of floral stems per shoot decreased basipetally along the tuber axis, from the primary to lower order secondary shoots, and also along individual shoot axes (Figure 4). A similar positional gradient occurred also for floral stem length across the tuber axis, while within each shoot the floral stem length remained constant (Figure 5).

This developmental pattern was observed in tubers from all treatments, but it was more pronounced in the GA_3 than in Uniconazole-treated tubers or in control tubers that produced fewer flowering shoots.

Anatomy of Floral Bud Development (Experiment 4)

The effect of GA_3 on inflorescence development during storage was studied microscopically in the primary bud of BM. Inflorescence development during storage was correlated with bud elongation in the non-dormant tubers. In the control plants, the apical meristem remained flat during 65 days of storage (Figure 6A,B,D,F,H) while the bud elongated to 11 ± 3 mm. In GA_3-treated tubers, in contrast, the primary bud elongated to 18 ± 3 mm during this period, and apical as well as axillary inflorescences were clearly detected (Figure 6A,C,E,G,I). In the GA_3-treated tubers, a change in the apex morphology was noticed 15 days after GA_3 application (Figure 6C) and was clearly evident at 33 days (Figure 6E). At this stage, the meristem elongated into a dome-shaped apex, the elongating peduncle lifted the apex towards the tuber surface, the spathe surrounding the primordial inflorescence was already formed and production of new leaf primordia ceased. After 45 days, a second inflorescence was already formed from the axillary bud of the uppermost primordial leaf that is, below the apical meristem (Figure 6G). After 65 days, floret meristems could be seen along the primordial inflorescence axis (Figure 6I).

Discussion

This study on the ontogeny of floral bud development in *Zantedeschia* highlights effects of bud position along the shoot and of the position of individual shoots on the tuber axis. Developmental correlations between shoots in relation to flowering were studied, taking into consideration the whole tuber and its shoots as one growing unit. Most studies with aroid species referred only to the primary shoot as the growing unit and examined developmental patterns along its axis (Ray 1987a b; Scribailo and Tomlinson 1992; Lemon and Posluszny 2000; Brooking and Cohen 2002; Dufour and Guerin 2003; Halligan and others 2004). In *Zantedeschia* the analysis of flowering patterns is constrained by the low flowering potential that characterizes normal development and by the tuber size. In our study this limitation was overcome by the use of larger tubers and by the application of GA_3, which promotes flowering in calla lily (Corr and Widmer 1987, 1991; Funnell 1993). Application of GA_3 allowed a better expression of the flowering potential of the plants, as shown by the increase in the number of shoots bearing floral stems per tuber and in the number of floral stems

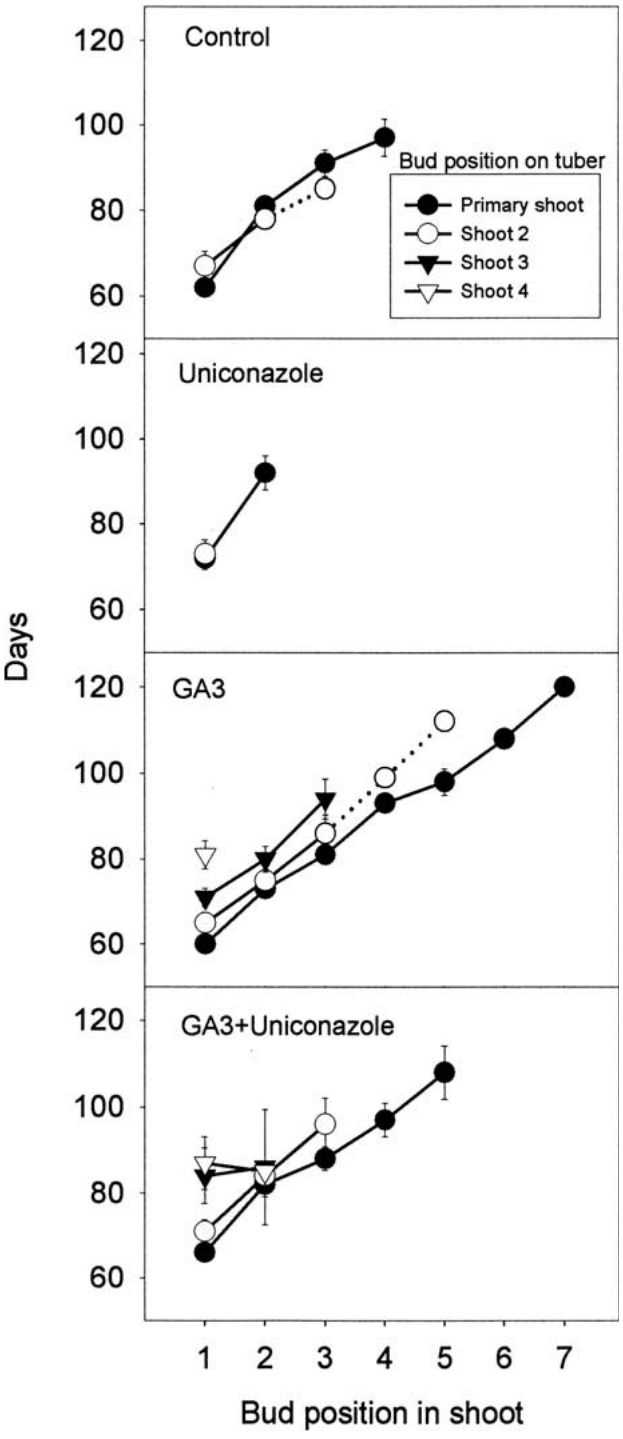

Figure 3. Days to floral stem anthesis according to the bud position along the tuber and the shoot axes, ●-primary shoot; ○-shoot 2; ▼-shoot 3; ▽-shoot 4. Dashed line indicates results of one plant.

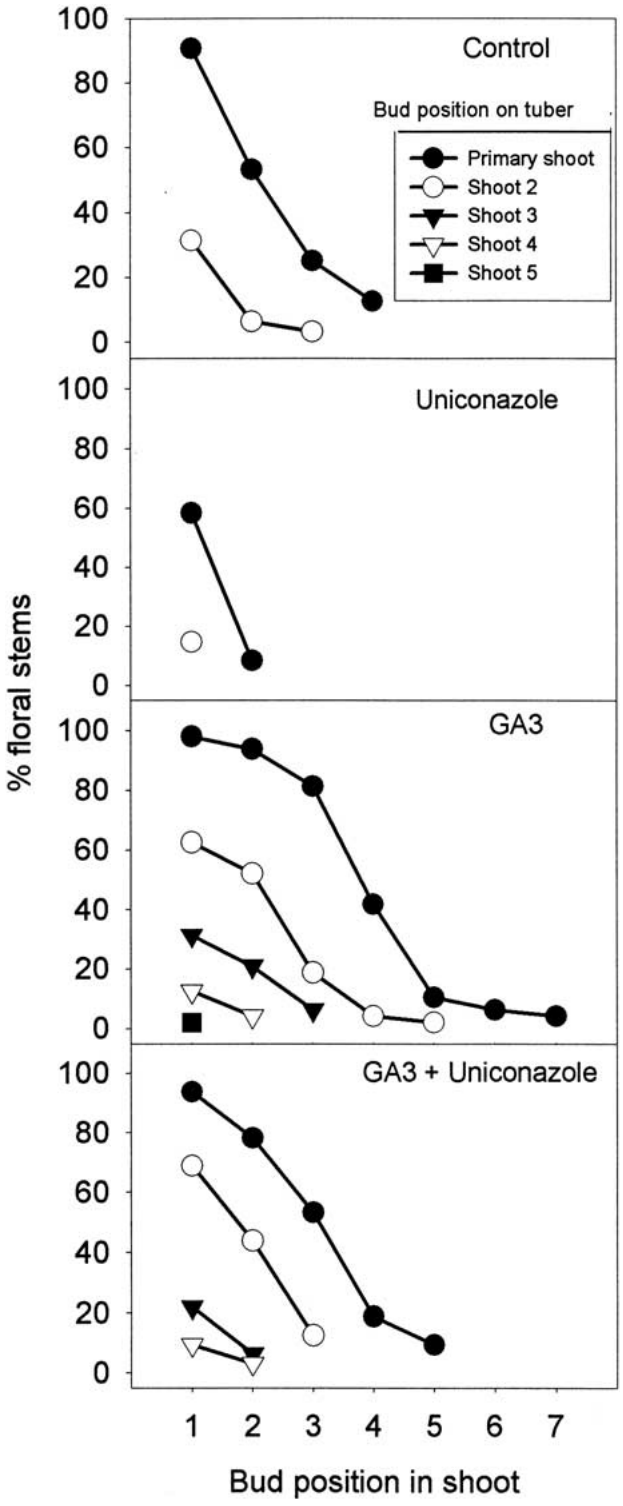

Figure 4. Percent of plants with flowering shoots according to the bud position across the tuber and along the shoot axis. ●- primary shoot; ○ -shoot 2; ▼ - shoot 3; ▽-shoot 4; ■- shoot 5.

produced per shoot (Table 1) (Corr and Widmer 1987). However, this increase was constrained by apical dominance.

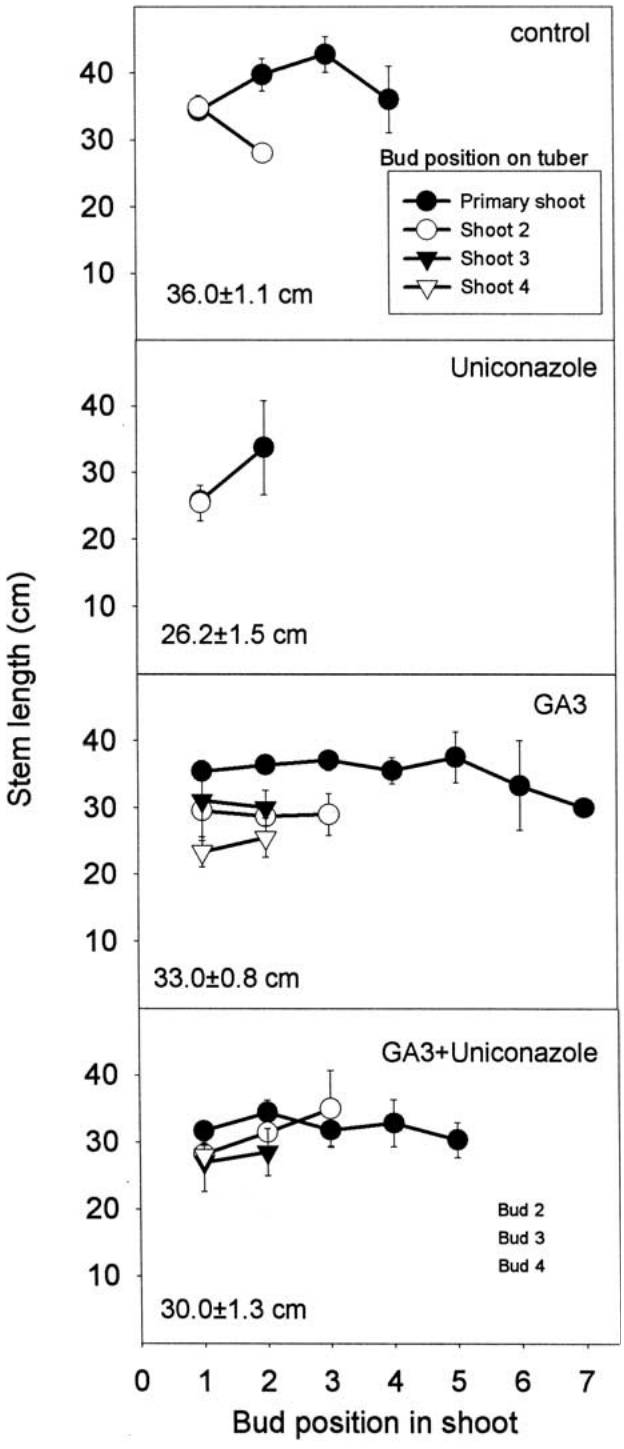

Figure 5. Floral stem length according to the bud position across the tuber and along the shoot axis, ●-primary shoot; ○- shoot 2; ▼-shoot 3; ▽-shoot 4. Average stem length per treatment ± s.e. is indicated in the bottom left corner of each treatment.

Shoot dominance

In colored cultivars of *Zantedeschia*, the strong apical dominance of the primary shoot controls the growth pattern of secondary shoots on the tuber and bud activation along the axis of each shoot. The phenomenon of apical dominance and the inhibition of secondary bud development was previously described and reviewed by Cline (1991). In CG tubers, this dominance resulted in massive growth of the primary shoot and was associated with a gradual decrease of the growth potential of secondary shoots on the tuber, with increasing phyllotactic distance from the primary shoot. In individual shoots, apical flowering activates sympodial growth from 1 or 2 axillary buds (Brooking and Cohen 2002), as was found in other *Araceae* (Ray 1987a, b; Scribailo and Tomlinson 1992; Lemon and Posluszny 2000; Dufour and Guerin 2003). In GA_3 treated plants, however, flowering occurred in 2 to 6 axillary buds, including side shoots on the activated sympodia. This suggests that more buds are active following apical inflorescence differentiation in the individual shoots.

The largest number of flowering buds developed on the primary shoot, and their number decreased gradually according to shoot position on the tuber. In each tuber, the apical bud of the primary shoot produced the first floral stem. This was followed by apical flowering in secondary shoots and occurred progressively later with increasing phyllotactic distance from the primary shoot. A similar positional pattern was found for the proportion of floral stems produced by shoots along the tuber and by buds along each flowering shoot.

This programmed flowering pattern in colored *Zantedeschia* cannot be explained by differences in meristem size or age, because inflorescence differentiation *in vitro* occurs even from very small apical meristems (Naor and others 2004), as well as in very young small tubers provided with GA (Brooking and Cohen 2002). Furthermore, continuous application of GA_3 prolonged the flowering period by approximately 6 months, showing that meristem age did not limit flowering. On the other hand, if gibberellin is involved in signaling the onset of the flowering process (Lawson and Poethig 1995; King and Evans 2003), the flowering pattern in *Zantedeschia* could be a result of a gradient in the distribution of endogenous factors involved in inflorescence differentiation (possibly GAs) that is imposed by apical dominance along the tuber and shoot axes.

Floral stem length decreased along the tuber axis, from the primary to the secondary shoots, but was

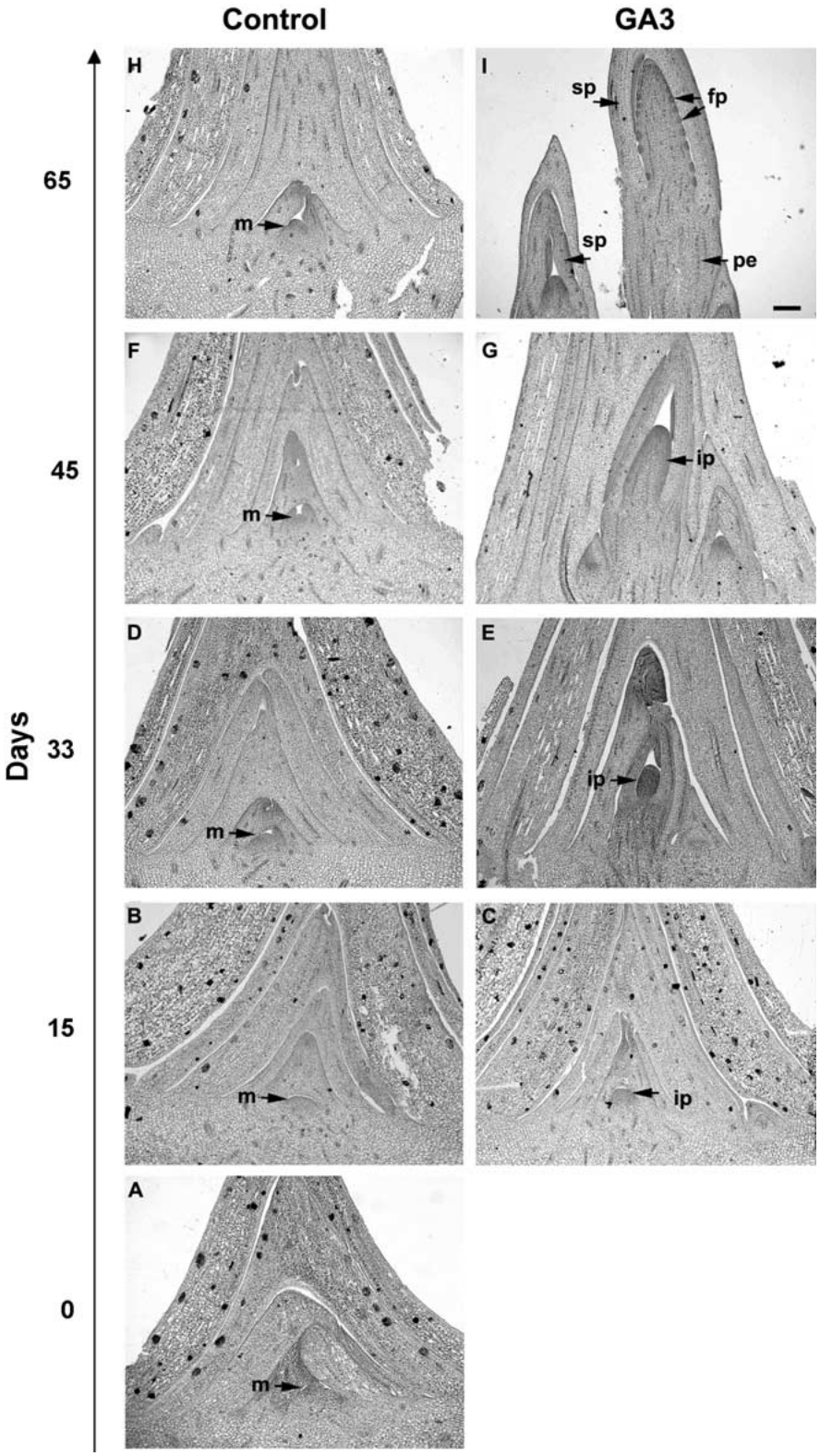

Figure 6. Changes in the apical bud anatomy following a GA_3 application to BM tubers in storage. Control: (**A**) <0.5 mm, (**B**) <0.5 mm, (**D**) 2.6 mm, (**F**) 5.6 mm, (**H**) 10.6 mm; GA_3-treated tubers: (**C**) <0.5 mm, (**E**) 7.4 mm, (**G**) 14.0 mm, (**I**) 18.0 mm. The numbers indicate the bud length on the day of measurement. Days after GA_3 application: (**A**) day 0; (**B,C**) day 15; (**D,E**) day 33; (**F,G**) day 45; (**H,I**) day 65. m-meristem. ip - inflorescence primordium; pe - peduncle; sp - spathe; fp – floret primordium. Bar = 200 μm.

more or less constant along each shoot axis. It is suggested that this positional change in the length of the floral stem is also imposed by the dominance of the primary shoot and is probably related to its sink strength or to a differential distribution of gibberellins and other growth regulators.

Effects of GA on Inflorescence Differentiation and Floral Stem Elongation

In BM tubers, in which inflorescence differentiation does not occur during storage, histological examination of the primary bud showed a change from vegetative to reproductive stage within 15 days after GA_3 treatment to the tubers. Also, small tubers (less than 20 g) did not flower unless provided with GA_3. This supports the assumption that GAs play a central role in inflorescence differentiation in *Zantedeschia* (Brooking and Cohen 2002; Naor and others 2004). Because GAs also enhance stem elongation, it has been proposed that their effect on floral stem development can be separated into two distinct stages: inflorescence differentiation during the early stages of shoot growth, and elongation of the floral stem at later stages (*Zantedeschia* cv. Black Magic - Brooking and Cohen 2002; *Lolium temulentum* - King and Evans 2003). This hypothesis is also supported by our findings with CG tubers. Inflorescence differentiation was not suppressed when Uniconazole was applied at the onset of the storage period, but stem elongation was inhibited. Consequently, the proportion of tubers with differentiated inflorescences was similar to control tubers, while the proportion of the emerged floral stems was reduced. Uniconazole lowered the endogenous gibberellins in tubers (4 nM) and in plantlets *in vitro* (0.03 mM) by approximately 40% (Naor unpublished), thus suggesting that inflorescence differentiation resulted from gibberellins already present in the tuber at the onset of storage, before Uniconazole application, while stem elongation depended on gibberellin synthesis at later stages and was therefore inhibited by Uniconazole.

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REFERENCES

Brooking IR, Cohen D. 2002. Gibberellin-induced flowering in small tubers of *Zantedeschia* ''Black Magic'' Sci Hort 95:63–67.

Cline MG. 1991. Apical dominance Bot Rev 57:318–358.

Corr BE, Widmer RE. 1987. Gibberellic acid increases flower number in *Zantedeschia elliottiana* and *Z. rehmannii.* HortScience 22:605–607.

Corr BE, Widmer RE. 1991. Paclobutrazole, gibberellic acid and rhizome size affect growth and flowering of *Zantedeschia* HortScience 26:133–135.

Dufour L, Guerin V. 2003. Growth, development and flower production of *Anthurium andreanum* Lind. in tropical conditions Sci Hort 98:23–35.

Funnell, KA (1993) "Zantedeschia" In: Hertogh, A, Le Nard, M (eds.), *Physiology of flowering bulbs*, Elsevier, Amsterdam, The Netherlands, pp 683-704.

Halligan EA, Brooking IR, Funnell KA, Catley JL. 2004. Vegetative and floral development of *Zantedeschia* ''Black Magic'' Sci Hort 99:55–65.

King RW, Evans LT. 2003. Gibberellin and flowering of grasses and cereals: prizing open the lid of the ''florigen' black box Ann Rev Plant Biol 54:307–328.

Lawson EJR, Poethig RS. 1995. Shoot development in plants: time for a change Trends Genet 11:263–268.

Lemon GD, Posluszny U. 2000. Shoot development and evolution in *Pistia stratiotes* (*Araceae*). Int J Plant Sc. 161:721–732.

Ngamau K. 2001. Promoting side shoot development in *Zantedeschia aethiopica* 'Green Goddess' Gartenbauwiessenschaft 66:85–92.

Naor V, Kigel J, Ziv M. 2004. Hormonal control of inflorescence development in plantlets of calla lily (*Zantedeschia* spp) grown *in vitro* J Plant Growth Reg 42:7–14.

Ray TS. 1987a. Leaf type in *Araceae.* Am J Bot 74:1359–1372.

Ray TS. 1987b. Diversity of shoot organization in the *Araceae.* Am J Bot 74:1373–1387.

Scribailo RW, Tomlinson PB. 1992. Shoot and floral development in *Calla palustris* (*Araceae-Calloideae*). Int J Plant Sci 153:1–13.