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Variation in Endogenous Gibberellins, Abscisic Acid, and Carbohydrate Content During the Growth Cycle of Colored *Zantedeschia* spp., a Tuberous Geophyte

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Abstract Phenologic changes and variation in the level of endogenous gibberellins (GAs), abscisic acid (ABA), carbohydrate content, and α-amylase activity were examined in colored *Zantedeschia* spp. cv. Cala Gold. These changes were examined in the primary bud tissues and in the attached tuber tissue during the growth cycle. Dormant tubers were dry-stored at 20°C for 3 months, planted in a phytotron, and grown under 22/16 ± 1°C. Plant development was monitored under continued irrigation until leaf senescence and tuber dormancy. GAs and ABA were extracted from the primary bud tissues, fractionated by HPLC, and analyzed using GC-SIM. Starch, glucose, soluble protein, and α-amylase activity were monitored in the tuber tissue attached to the primary bud. Endogenous changes in GAs and ABA in the primary bud were correlated with endogenous changes in carbohydrate content and α-amylase activity in the attached tuber tissue. These correlations were observed during the rest and the growth periods and were associated with developmental changes in the plant, that is, bud dormancy relaxation, bud growth, and inflorescence differentiation. ABA content decreased and a transient pulse of GA was measured in the primary bud concomitantly with the onset of shoot elongation in dry tubers during storage, before planting. The sharp increase of GAs in the bud preceded inflorescence differentiation as observed in dissected apices by about 15 days, as well as the increase in α-amylase activity in the attached tuber tissue. A steep decrease in starch level was measured in the tuber after planting, concomitantly with massive plant growth. These findings suggest a possible involvement of gibberellin in the initiation of α-amylase activity during dormancy relaxation in colored *Zantedeschia* and in the autonomous induction of flowering.

Keywords α-Amylase · Abscisic acid · Development · Flowering · Gibberellin · Tuber dormancy · *Zantedeschia*

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Introduction

In geophytes, cyclic patterns of growth, flowering, and rest periods involve a sequence of specific endogenous processes, which are expressed in the growth and differentiation of the shoot and in changes occurring in the storage organ (Le Nard and De Hertogh 1993; Flaishman and Kamenetsky 2006). These developmental transitions are frequently associated with changes in hormone, enzyme activity, and stored carbohydrate levels, which are probably involved in the control of flowering and dormancy (Hariprakash and Nambisan 1996; Okubo 2000; Robinson and others 2000; Kamenetsky and others 2003). In many geophytes, these developmental transitions follow seasonal changes in temperature and day-length (Le Nard and De Hertogh 1993; Flaishman and Kamenetsky 2006). Moreover, variation in the level of hormones like gibberellins (GAs) and abscisic acid (ABA) reflects plant responses to these changes in the environment (Levi and Dean 1998; Araki 2001; Yu and

others 2006) and are involved in these phenologic transitions (Ofir and Kigel 1998; Yamazaki and others 2002; Okubo 2000; Suttle 2004a; Ile and others 2006). However, in other geophytes, like colored *Zantedeschia*, developmental transitions are controlled by endogenous, autonomous processes that are not induced by specific environmental signals (Tjia 1989; Naor and Kigel 2002; Carrillo-Cornejo and others 2003; Halligan and others 2004). In these geophytes it is more difficult to identify physiologic markers that indicate the onset and cessation of developmental stages such as dormancy break and flowering because they cannot be manipulated by environmental signals under controlled conditions. Revealing the nature and time-course of these developmental changes would contribute to a better understanding of the role of different hormones and carbohydrate metabolism in controlling cyclic patterns of growth in autonomous geophytes. Furthermore, these changes might be used as physiological markers indicating the timing of developmental transitions, such as bud dormancy break, flowering and dormancy induction in this group of geophytes. Here we report on a quantitative study of the variation in the content of GAs and ABA in the shoot apex and in the level of starch, glucose, and α-amylase activity in the tuber tissue that occurs during the full growth cycle of colored *Zantedeschia* under controlled conditions. We focus particularly on the changes associated with dormancy and flowering.

ABA and GA are considered to be antagonistic in their roles during the processes of dormancy imposition in seeds and plants (Anderson and others 2001; Jacobsen and others 2002; Seo and others 2006). A high ABA level was found in dormant buds of several geophytes, and it declines at the end of the dormancy stage (Ofir and Kigel 1998; Fernie and Willimitzer 2001; Yamazaki and others 2002). However, a reduced ABA level is not always associated with growth resumption in dormant buds (Suttle 2004b). Application of GA often stimulates bud growth (Fernie and Willimitzer 2001), and its endogenous level increases concomitantly with bud elongation after dormancy release (Yamazaki and others 2002). In colored *Zantedeschia*, however, application of GA_3 to dormant tubers did not enhance bud sprout and flowering (Naor and others 2005), and application of ABA did not induce bud dormancy (Naor, unpublished). Nevertheless, GA is required for inflorescence differentiation in shoot apices of colored *Zantedeschia* grown in tissue culture (Naor and others 2005). This raises the question of whether changes in ABA and GA levels in the buds of colored *Zantedeschia* are associated with the regulation of dormancy and flowering. On the other hand, metabolic changes, particularly those related to storage compounds, have been frequently observed in association with dormancy break in geophytes (Kamenetsky and others 2003). In the *Araceae*, starch is the major storage compound in tuberous species (Mayo and others 1997). Indeed, variation in starch content occurs in tubers of colored *Zantedeschia* at harvest, planting, and flowering (Robinson and others 2000), but it was not studied in relation to imposition or break of bud dormancy. We hypothesized that the gibberellin level in the bud should be correlated with starch degradation in the tuber and onset of bud elongation, as it occurs in grass seeds in which gibberellin from the embryo signals the increase in α-amylase content in the endosperm aleuron cells (Jacobsen and others 1995). In geophytes, however, there are limited studies on the relationships between hormonal changes in the bud tissues and metabolic changes in the associated tuber tissues, which function as a metabolite source for the dormant bud. Few studies have been published in species with autonomous control of dormancy, as in colored *Zantedeschia.* Therefore, the objectives of this study were (1) to elucidate the variation in GAs and ABA levels in the tuber bud during a complete cycle in colored *Zantedeschia*, particularly during dormancy and flowering; (2) to study changes in carbohydrate levels and α-amylase activity in the tuber tissue during bud dormancy and growth; and (3) to unravel the relationships between the changes in hormonal and carbohydrate levels and bud dormancy and flowering.

Materials and Methods

Plant Material

To analyze hormonal, enzymatic, and carbohydrate changes in the tuber and bud tissues, we used tubers of Calla Gold (CG) from a commercial field. The tubers, size 12–14 (cm circumference), were harvested during early winter at a commercial farm in the northeastern Israel (Cohen Farm, December 2000). 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 in the dark at constant temperature (20°C) until planting in the phytotron. The tubers were planted in 1-L pots, 11 cm in diameter, filled with a mixture of volcanic tuff gravel and vermiculite #3 (1:1 v/v). Before planting, the tubers were randomly grouped into 11 samples of 15–20 tubers each. Plants were grown until the end of the growth cycle (that is, complete leaf senescence, 7 months after planting) under controlled conditions in a phytotron, in a glass-covered growth room under natural daylight, 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 grown under 22/16 ± 1°C (day/night) temperature, with the day temperature given between 08:00 and 16:00. Plants were kept under a screen shade transmitting 50% of

solar radiation, and irrigated twice a day with 50% Hoagland solution in the morning and tap water in late afternoon. To monitor bud development and changes in hormonal and carbohydrate levels, tuber samples were taken about every 15 days during storage and plant samples were collected about every 40 days during the growth period. Tuber weight and bud length, weight, and inflorescence development (after microscopic dissection) were recorded in the primary bud (Naor and others 2006).

Sampling of Bud and Tuber Tissue for Hormonal Analyses

Changes in endogenous levels of GAs and ABA were analyzed in the primary bud, whereas changes in α-amylase activity and glucose, starch, and soluble protein content were analyzed in the tuber tissues attached to the primary bud. At each sampling date during the storage and growth periods, the primary bud of six tubers or five plants, respectively, were carefully cut out, and dry outer scales or unfolded green young leaves and inner folded leaves larger than 4–5 cm were removed. The remaining tissue, including young primordial leaves and the vegetative or reproductive shoot apex, was weighed and immediately frozen in liquid N_2. Afterward, a cylindrical plug (15–20 mm long and 5 mm wide) of parenchymatous tuber tissue was cut just below the removed primary bud, weighed, and frozen in liquid N_2. The samples were freeze-dried and stored at −80°C until extracted and analyzed.

Gibberellin and ABA Extraction and Analysis from Apical Bud Tissue

Hormone extraction was based on the methods used by Koshioka and others (1994) and Grunzweig and others (1997), with minor modifications. Freeze-dried samples (average weight = 42 ± 4 mg) were extracted overnight in 80% methanol at 4°C. Standards of ABA ([$^2H_6$]ABA) and gibberellins ([17-$^2H_2$]GA_1, [17-$^2H_2$]GA_3, [17-$^2H_2$]GA_8, [17-$^2H_2$]GA_{19}, [17-$^2H_2$]GA_{20} and [17-$^2H_2$]GA_{29}) were added to the methanol filtrates at the beginning of the process. The standards were a gift from L. M. Mander Laboratory (Australian National University, Canberra, Australia). After methanol removal in vacuo, the aqueous phase was acidified to pH 2.6 with 1 M HCl. The aqueous residue was partitioned (3×) against hexane (0.5 vol). Combined aqueous fractions were partitioned (4×) with ethyl acetate (1 vol). The combined ethyl acetate phases were partitioned (3×) against 0.5 M phosphate buffer pH 8.3 (0.5 vol). Then 3 g of insoluble PVP (polyvinyl pyrrolidone) was added to the combined buffered phase and stirred at 4°C. The PVP was filtered out, the buffer phase was acidified to pH 2.6 by 5 M HCl, and partitioned (4×) against ethyl acetate. After drying with Na_2SO_4 and filtering, the ethyl acetate phase was evaporated and the residue dissolved in methanol and dried (2×). The residue was dissolved in methanol, loaded onto a Bond Elute DEA (diethylaminopropyl) column (Varian, Harbor City, CA, USA), and washed with methanol. The sample was eluted in methanol containing 0.5% acetic acid, filtered through a 0.45-mm Teflon filter, and reduced to dryness under N_2.

The gibberellins and ABA were separated on a HPLC RP-18 column (10 mm i.d. × 250 mm; Merck, Germany) during a linear and slow increase of methanol concentration from 0 to 100%, containing 0.1% acetic acid. During 70 min of separation at a flow rate of 2 ml/min, 35 samples of 4 ml were collected every 2 min. Fractions 20–21 were pooled for ABA quantification, and fractions 15–20 and 23–28 were pooled for gibberellin identification and quantification. All the fractions were methylated by excess ethereal CH_2N_2 and evaporated under N_2. Pyridine-BSTFA containing trimethylchlorosilane (TMSi) 1% was added to the gibberellin fractions to form MeTMSi derivates. The derivatized samples were dissolved in CH_2Cl_2 and injected (1 µl) in spitless mode into a GC-MS SIM (Hewlett-Packard) with a DB-1 capillary column (0.25 mm i.d. × 30 m; J and W, Folson, CA, USA). Column head pressure of the He carrier gas was 70 kPa, column temperature was according to Gaskin and MacMillan (1991) with an initial temperature of 40°C, electron energy was 70 eV, and mass spectra were acquired every 1.3 s from 70 to 650 amu. Each sample contained 0.1 µl of parafilm solution dissolved in CH_2Cl_2 for the Kovats retention indices (KRI) determination. The endogenous level of the detected hormones was calculated by multiplying the peak area ratio (of the endogenous hormone and its standard) by the standard quantity, and divided by the tissue dry weight (DW; mg) of each sample.

Biochemical Analyses

To measure carbohydrates, soluble protein, and α-amylase levels in the tuber tissue, the dried samples were macerated and extracted (3×) in 0.5 ml Hepes buffer (pH 5) for 3 min and centrifuged (8000 rpm). The precipitate was used for starch analysis and the supernatant fractions were combined and used for glucose, soluble protein, and α-amylase analyses. The following methods of analysis were modified to test small quantities in ELISA polystyrene plates (Corning Inc.; 96 × 250-µl wells), with three replicate wells for each sample, standard, or control. Glucose was measured using the Glucose Assay Kit (Sigma G2020). This assay is based on enzymatic phosphorylation of the glucose and oxygenation of its product in the presence of NAD. The NADH level is proportional to the glucose level in the solution. For glucose determination, each well contained 20 µl sample crude extract and 200 µl test reagent or pH 5 buffer Hepes as a control. The absorbance of NADH was measured at 340 nm, after 15 min of incubation at

room temperature. For starch measurement, the precipitate of each sample was heated to 135°C for 60 min in the presence of 2.5 ml DD water for starch solubilization. After heating, the solution volume was completed to 10 ml, mixed, and 1 ml of the solution was centrifuged. To measure the starch level we used a Starch Assay Kit (Sigma S9144). The starch level is proportional to the NADH produced in the reaction. We incubated 25 µl from each 1-ml sample at 60°C for 15 min in the presence of 50 µl starch assay reagent containing α-amylase or in pH 5 buffer Hepes as a control. Then, 3 × 20 µl from each sample tube or control tube was incubated at room temperature in the presence of 200 µl glucose test reagent. Absorbance of NADH was read at 340 nm after 15 min at room temperature.

The presence of α-amylase in the crude extract was determined using the α-Amylase Reagent Kit (BioMerieux # 63114). The enzyme activity is proportional to the catalyzing product of 2-chloro-4-nitrophenyl maltotrioside. Each well contained 5 µl crude extract and 100 µl test reagent or 100 µl Hepes buffer (pH 5) as a control and incubated on a shaker at 30°C for 20 min. The reaction was stopped by adding 100 µl Tris buffer (pH 9) and absorbance was read at 405 nm. Soluble protein was determined by the method of Bradford (1976). In each well, 2 µl sample crude extract, 18 µl Hepes buffer (pH 5), and 200 µl Bradford reagent were incubated in the dark for 10 min at room temperature and absorbance at 595 nm was measured.

Statistical Analyses

Statistical analyses were carried out using Microsoft Excel and SigmaPlot software.

Results

Shoot Development During Tuber Storage and Plant Growth

Elongation (>5 mm) of the primary bud in the tuber started approximately 20 days after storage onset (about 30 days from harvest in the field) and reached 33.0 ± 1.7 mm at planting time after 90 days in dry storage (Figure 1). After planting, the primary shoot arising from the apical bud produced 4.30 ± 0.24 leaves during 120 days of growth (ca. 210 days from onset of storage). The leaves senesced gradually during the following approximately100 days, as shown by the decrease in the number of green leaves (Figure 1B). Atrophy of leaf primordia (that is, necrosis starting at the tip of the primordium), an early indication of dormancy onset (Halligan and others 2004), was observed

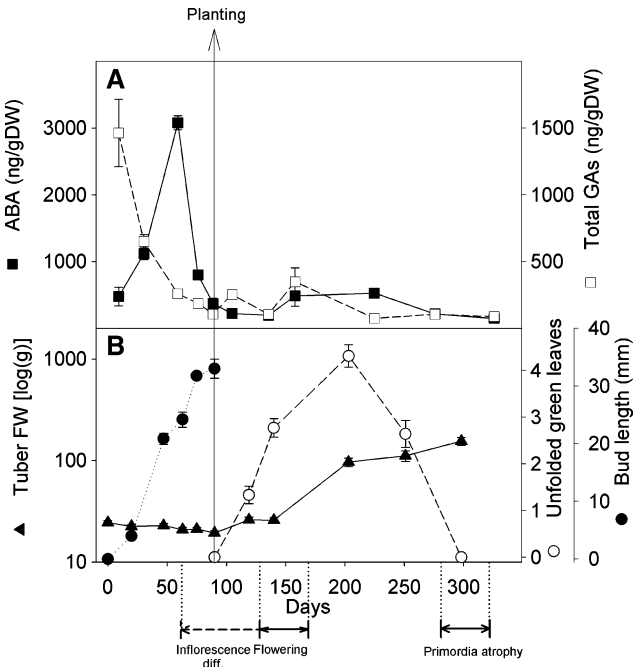

Fig. 1 Developmental changes in tuber weight, phenologic stages, and the level of endogenous hormone in the primary shoot during the growth cycle of colored *Zantedeschia* cv. CG. (**A**) ABA (solid square) and total GAs (open square) levels in the primary bud. (**B**) Changes in tuber FW (closed triangle), primary bud length (closed circle), and the number of green unfolded leaves (open circle)

in the apical bud about 150 days after planting (approximately 240 days from storage onset). The tubers gradually entered dormancy, even though they were continuously irrigated. Inflorescence differentiation in the primary bud was detected in about 30% of the stored tubers, starting approximately 60 days after onset of storage (Figure 1B), and 43% of the shoots reached flowering about 40 days after tuber planting. The flowering period lasted approximately 30 days.

During storage, initial tuber fresh weight (FW) decreased by 22% (Figure 1B) and tuber DW decreased by 13% (data not shown). The increase in the tuber FW commenced gradually after flowering onset, reaching a constant rate of 0.7 ± 0.07g day^{-1} ($p < 0.001$) until the completion of leaf senescence. Tuber FW at the end of the growth period (about 200 days from planting) was 155 ± 14 g, that is, 20 times higher than the initial weight (Figure 1B).

From this time-course analysis of the growth cycle, the following stages of development were defined, starting from onset of storage: (a) end of dormancy, about first 20 days; (b) bud sprouting and elongation, days 20–90; (c) intensive root and shoot growth after planting, days 90–140; (d) inflorescence emergence and flowering, days 140–170; (e) tuber growth, days 140–200; and (f) leaf senescence and dormancy imposition, days 200–300.

Gibberellins and ABA in the Bud Tissues

GAs in fractions 15–20 and 23–28 matched characteristics of specific known gibberellins by their KRI and ion relative abundances (Table 1). The GAs found in the bud tissues were from the 13-early hydroxylation group. In pretrials only GAs from this pathway were found in the bud tissues. Among GAs from the 13-early hydroxylation group, GA_3 and GA_1 are considered biologically active, GA_{19} and GA_{20} are precursors, and GA_{29} and GA_8 are nonactive metabolites of the active gibberellins (Sponsel 1995; Yamaguchi and Kamyia 2000). Levels of GAs in the primary bud varied during the growth cycle (Figure 1A). GA_3 was the most abundant gibberellin (Figure 2). During storage, GA_3, GA_1, and GA_{20} showed a rather sharp and brief increase (at about 50 days from storage onset). During the growth period, GAs levels in the primary bud were relatively low and constant. The peak levels of GA_3, GA_1, and GA_{20}, were ten times higher compared with the initial levels, and occurred approximately 15 days before inflorescence differentiation was observed in dissected apical buds of stored tubers (Figures 1 and 2). The level of GA_{19} fluctuated with no clear trends during the storage and growth periods (Figure 2). GA_{29} was inconsistently detected in very small quantities compared to the other GAs.

ABA content in the apical bud was highest in dry, dormant tubers at the onset of storage and strongly decreased within 50 days from 2927 ± 50 to 516 ± 29 ng/g DW ($p < 0.01$). The decrease in ABA was followed by an increase in the total GAs level from 238 ± 69 to 1538 ± 53 ng/g DW ($p < 0.01$; Figure 1A). These opposite changes were concomitant with the beginning of primary bud elongation during storage and were followed by inflorescence initiation (Figure 1B). After planting, GAs and ABA levels in the primary bud were relatively low until the end of the growth cycle. No increase in ABA was observed when the leaves senesced and dormancy was imposed (Figure 1A).

Changes in Carbohydrates, α-Amylase, and Soluble Proteins in Tuber Tissue

Changes in the levels of carbohydrates, soluble proteins, and α-amylase activity were monitored in the tuber tissue adjacent to the primary bud during the storage and growth periods (Figure 3). During the storage period and the onset on bud elongation, minor or no changes were measured in tuber DW, glucose, starch, or soluble protein concentrations in the tuber tissue, whereas major changes were observed in these parameters after planting. During storage, starch and soluble protein concentrations did not change significantly, and glucose concentration increased by 30% (from 11.5 ± 0.4 to 16.4 ± 0.8 mg/g DW, $p < 0.004$; Figure 3A, B). The activity of α-amylase in the sampled plug of tuber tissue was constant during the first 75 days of storage and increased by approximately 40% (0.048 ± 0.002 to 0.068 ± 0.003 U/mg protein, $p < 0.004$) at day 90, just before planting. This increase occurred following the sharp increase in total GAs in the elongating bud about 15 days earlier (Figure 3C).

One month after planting, the DW of the tuber tissue below the primary bud (that is, cylindric tuber plug) decreased by 50% (from 309 ± 51 to 123 ± 8 mg, $p < 0.02$) simultaneously with the massive growth of the plant shoot (Figure 3A). The soluble protein concentration decreased by 30% compared with the preplanting average level. This

Table 1 Kovats Retention Indices (KRI) and Relative Intensities of Characteristic Ions for MeTMSi Derivatives of Gibberellins in the Buds of Colored *Zantedeschia* cv. Calla Gold and Published Data (Gaskin and MacMillan 1991)

Gibberellin	HPLC fraction	KRI	Ions *m/z* (% relative abundance)					
GA_1	15–20	2673[a]	506 (100)	491 (7)	448 (14)	377 (12)	313 (10)	235 (19)
		2669[b]	506 (100)	491 (9)	448 (18)	377 (12)	313 (9)	235 (6)
GA_3	15–20	2695	504 (100)	489 (6)	475 (3)	460 (7)	431 (12)	387 (10)
		2692	504 (100)	489 (7)	475 (12)	460 (9)	431 (9)	387 (11)
GA_8	15–20	2823	594 (100)	535 (7)	448 (14)	375 (3)	311 (7)	268 (9)
		2818	594 (100)	535 (6)	448 (14)	375 (5)	311 (4)	268 (5)
GA_{29}	15–20	2684	506 (100)	491 (7)	477 (9)	389 (17)	375 (22)	235 (44)
		2684	506 (100)	491 (11)	477 (3)	389 (6)	375 (15)	235 (10)
GA_{19}	23–28	2604	462 (11)	434 (100)	402 (29)	374 (75)	345 (32)	
		2596	462 (7)	434 (100)	402 (37)	374 (64)	345 (24)	
GA_{20}	23–28	2481	418 (100)	403 (25)	375 (71)	359 (17)	301 (25)	235 (6)
		2482	418 (100)	403 (16)	375 (46)	359 (12)	301 (12)	235 (8)

Ion abundances were obtained from GC-SIM spectra

[a] Experimental data

[b] Published data

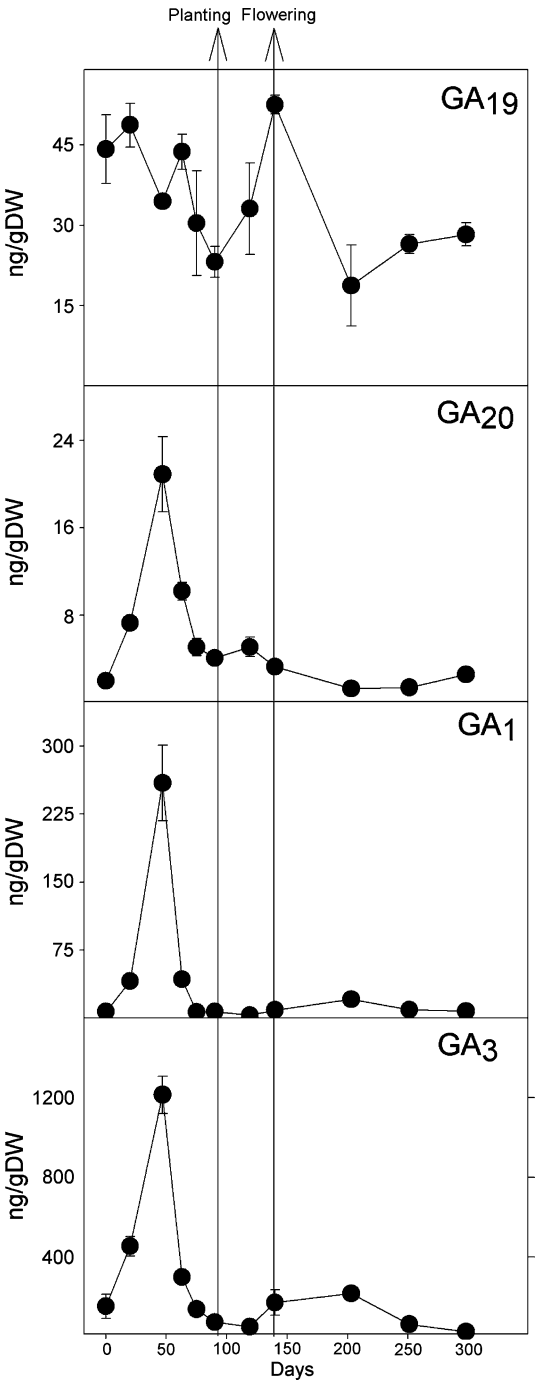

Fig. 2 Developmental changes in the level of endogenous gibberellins in the primary bud of colored *Zantedeschia* cv. CG during the growth cycle

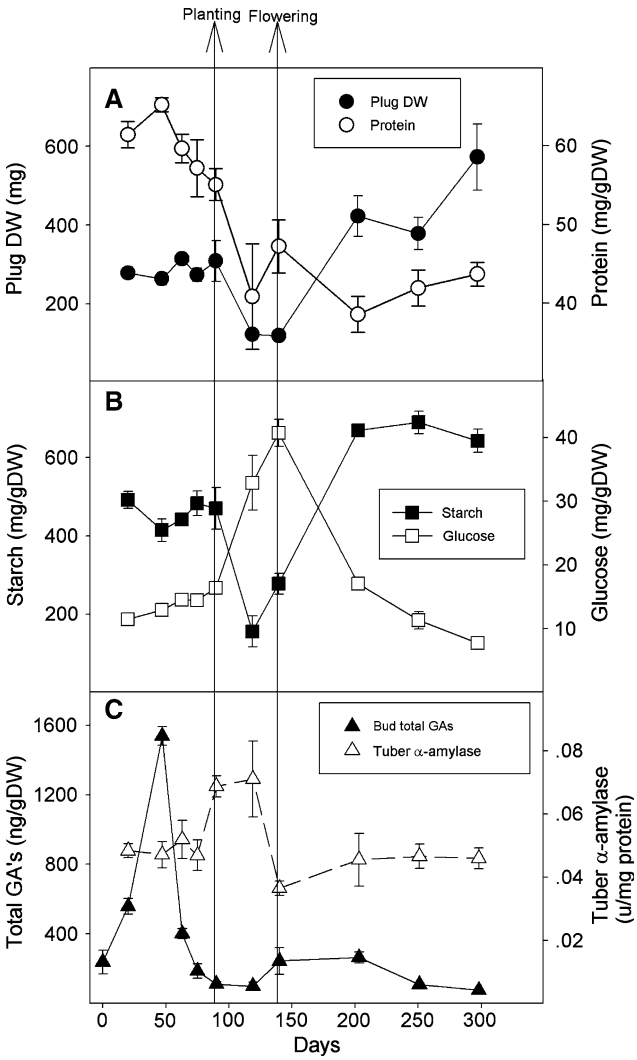

Fig. 3 Developmental changes during the plant growth cycle of colored *Zantedeschia* cv. CG. (**A**) Changes in the weight of the tuber tissue (tissue plug) below the primary bud (solid circle) and the level of soluble protein (open circle). (**B**) Level of starch (solid square) and glucose (open square). (**C**) Changes in α-amylase activity (open triangle) in the plug tissue, and in total GAs in the primary bud (solid triangle)

decrease was accompanied by a sharp reduction of 66% in starch level and by a reciprocal sharp 136% increase in glucose level. These changes in starch and glucose were preceded by an increase in α-amylase activity, which began in the dry-stored tubers before planting and continued afterward for a short period (Figure 3C). Stages after flowering were characterized by a gradual and continued increase in starch concentration (Figure 3B). Starch accumulation was accompanied by a concomitant decrease in glucose to the levels found during storage. In contrast, soluble protein concentration and α-amylase activity remained relatively low and constant after flowering, until the onset of leaf senescence and bud dormancy. At the end of the growth cycle, the sampled tuber plug DW was approximately five times heavier compared with its weight at flowering, and two times its weight at the beginning of storage (119 ± 12 and 279 ± 15 mg, respectively). Similarly, the starch concentration at the end of the growth cycle (643 ± 30) was approximately 50% higher compared with its initial level (492 ± 22 mg/g DW), and four times higher compared with the level at flowering (156 ± 40 mg/g DW). In contrast, the soluble protein

concentration at the end of the growth cycle was 29% lower compared with the initial level during storage (Figure 3A).

Discussion

In this study with colored *Zantedeschia*, we showed that endogenous changes in GAs and ABA in the apical bud correlate with endogenous changes in carbohydrate content and α-amylase activity in the tuber tissue. These correlations were observed during the rest and the growth periods and were associated with developmental changes in the plant, that is, bud dormancy, shoot and tuber growth, and inflorescence differentiation.

Tuber Size and Storage Compounds

Starch content in tubers of colored *Zantedeschia* ranged between 45 and 65% of tuber DW, indicating its role as a major storage compound, as in other tubers (Jenner 1982). Soluble protein was about 5% of the tuber DW, within the range found in *Araceae* (that is, 1–5%) as well as in other tuberous species like yam, tapioca, sweet potato, and potato (Miege 1982). Large differences in the DW of the tuber tissue and in starch and soluble protein concentrations were observed between field-grown tubers at the onset of storage and phytotron-grown tubers at the end of the growth cycle. The DW of the tuber tissue plug sampled at the end of the growth cycle was about 2 times higher than at the onset of storage, and the starch concentration in the plug was around 1.4 higher. Thus, total starch content in the tuber at the end of the growth cycle was about 8 times higher than at the onset of storage. In contrast, the soluble protein concentration in the plug was lower at the end of the growth cycle (about 4.4 vs. 6.1%), but its total content in the tuber was 1.25 times higher than at the onset of storage. This indicates that unlike yam tubers (Treche and Agbor-Egbe 1996), the protein concentration in *Zantedeschia* tubers was "diluted" by the increase in the content of other storage compounds, like starch. These trends can be explained by assuming that growth conditions in the field, where the experimental tubers were produced, constrained plant development and accumulation of photosynthates in the tuber compared to conditions in the phytotron. In the phytotron, the improved growth conditions (optimal temperature of 22/16°C D/N) increased the accumulation of starch and other storage compounds, resulting in higher DW of the tuber tissue. In contrast to starch, the increase in soluble protein concentration was much smaller, suggesting that either N was a limiting factor in the soil substrate or a given level of protein is allocated by the plant per unit of tuber tissue weight, with little variation in this amount in response to growth conditions. Similar trends have been reported for wheat grains (Zhao-Hui and others 2006) in which starch levels are very responsive to growth conditions, whereas the amount of protein allocated to each grain is more stable. On the other hand, stressed growth due to late planting reduces protein content in potato tubers (Ezekiel and Bhargava 1992).

Endogenous Changes in Soluble Proteins, Carbohydrates, and α-Amylase

Onset of bud elongation is a visual indication of the end of bud dormancy and is usually preceded by endogenous changes occurring in both the bud and tuber tissues (Suttle 2004a, b). In colored *Zantedeschia*, bud elongation starts in the dry tuber before planting, indicating that stored assimilates and water in the tuber tissues were used for the growth process. However, no significant decrease in tuber DW and starch level and just a slight decrease in soluble protein level were observed during 90 days of storage before planting, despite the onset of bud elongation. A similar trend was found for starch in yam during storage (Treche and Agbor-Egbe 1996), in contrast to other geophytes in which high starch degradation occurs throughout storage, concomitantly with bud sprouting (Hariprakash and Nambisan 1996; Neilsen and others 1997). The apparent lack of starch and soluble protein degradation during storage in *Zantedeschia* can be explained by considering the large differences in relative bud and tuber sizes. By the end of the storage period, the DW of the sprouting primary bud was approximately 315 mg, representing 5% of the total tuber DW. Thus, the expected proportional small changes in starch and protein levels in the tuber tissue are difficult to detect because they are in the range of the sampling and measurement errors.

After planting, a steep decrease in starch and soluble protein content occurred concomitantly with massive shoot and root growth, showing that tuber water content was a limiting factor for growth. The starch and soluble protein in the tuber decreased until flowering, indicating that the growing shoot and roots and the developing inflorescence are strong sinks, and the tuber continued to function as a main source of metabolites, in addition to photosynthesis by the new foliage (Robinson 2000). After flowering, DW and starch content of the tuber tissue increased gradually, indicating that the tuber became a new sink for photosynthates transported from the shoot (Funnell and others 2002). The glucose concentration in the tuber was negatively correlated with starch concentration and α-amylase activity. This suggests that glucose levels were regulated by α-amylase degradation of starch and probably also by other starch-degrading enzymes. Because these changes in carbohydrate metabolism occurred after the onset of bud elongation, they are likely not involved in the transduction chain signaling for dormancy break in colored *Zantedeschia*. Therefore, they cannot be used as an reliable signal for dormancy break.

Endogenous Changes in ABA and GA in Dormant and Sprouting Primary Buds

Endogenous changes in GAs and ABA that correlate with developmental changes have been found in geophytes (Yamazaki and others 2002; Suttle 2004a, b; Kim and Kim 2005; Xia-YiPing and others 2005). In these geophytes, dormancy, flowering, and tuberization were variously associated with endogenous changes in gibberellin, ABA, cytokinin, auxin, and jasmonic acid. Correlation between bud sprouting and high GA levels was found in several geophytes (Yamazaki and others 2002; Suttle 2004b), whereas ABA was correlated with inhibition of bud growth, tuberization, or dormancy induction and maintenance (Yamazaki and others 2002; Suttle 2004b). The resemblance between colored *Zantedeschia* and other geophytes in the patterns of change in ABA and GAs levels in relation to developmental stages suggests similar functions. The association between high levels of ABA in the primary tuber bud and bud dormancy shortly after tuber harvesting in the field supports the hypothesis that high ABA content inhibits bud sprouting in colored *Zantedeschia*. This assumption is supported by previous findings in *Zantedeschia*, showing a negative correlation between ABA level and length of the growth period, that is, onset of dormancy (Naor, unpublished). However, in the present study, no increase in ABA level was observed toward the end of the growth cycle, during leaf senescence and dormancy onset under mild temperature (22/16°C D/N) and continuous irrigation in the phytotron. This can be explained by the different growth conditions in the phytotron compared to the field conditions under which the dormant tubers used for the experiment were produced. In the phytotron, plants were grown continuously under moderate temperature and irrigated until complete leaf senescence, whereas under field conditions they were exposed to rising temperatures and terminal drought. It can be argued that under field conditions higher ABA content is reached in the tuber buds as a response to dehydration. Consequently, these tubers showed deeper dormancy compared with tubers with weaker dormancy produced in the phytotron under constant irrigation (Naor and Kigel 2002). However, it is not clear whether the large pulse in endogenous gibberellin observed during storage at the time of dormancy break also occurs in less dormant tubers produced under milder conditions. Thus, further investigation of colored *Zantedeschia* is needed to unravel the hormonal changes caused by conditions at the end of the growth cycle that are involved in the imposition and extent of bud dormancy.

The endogenous gibberellins found in the bud are of the early-13 hydroxylation biosynthetic pathway. In this group of GAs, biological active GA_1 and GA_3 are formed from a common precursor, GA_{20} (Sponsel 1995). Therefore, it is not surprising that GA_{20}, GA_3, and GA_1 showed similar patterns of change in the bud along the growth cycle. However, during sampling only 30% of the buds had differentiating inflorescences and it was not possible to distinguish between vegetative and future reproductive buds. GA_1 has a lower florigenic action compared with GA_3 (Mander and others 1995), is involved mostly in cell (Or and others 2000) and stem elongation (Suttle 2004a), and enhances α-amylase production in aleurone cells of germinating cereal seeds (Choi and others 1996). The increase in GA_1 level prior to the increase in α-amylase activity in the dry tuber suggests a similar role as enzyme enhancer. Its endogenous level increases in correlation with bud growth in other geophytes (Suttle 2004a, b), after dormancy is released (Yamazaki and others 2002; Suttle 2004a) and ABA level declines (Suttle 2004a, b).

The endogenous changes in ABA and gibberellins in the sprouting buds of colored *Zantedeschia* occurred in the dry tuber during storage under constant conditions, independent of an environmental cue. The rapid increase in content of GA_1 and GA_3 in the elongating bud was measured prior to morphologic, enzymatic, and biochemical changes in the bud and tuber tissue. It was followed by an increase in α-amylase activity in the tuber tissue, starch degradation, and inflorescence differentiation. In colored *Zantedeschia*, onset of bud dormancy, bud sprouting, and inflorescence differentiation are autoregulated and do not require environmental signals (Tjia 1989; Naor and Kigel 2002; Carrillo-Cornejo and others 2003; Halligan and others 2004). Application of GA_3 to tubers did not enhance bud sprouting, but application to growing plants inhibited bud dormancy initiation (Naor and others 2005). It is therefore suggested that the increase in GA level during onset of bud elongation serves as a signal mediator in an internal control system in the bud apex. This signal regulates several physiologic processes involved in early bud growth and reproductive differentiation.

Gibberellins and Flowering

An important finding in this research was that a sharp and transient increase in gibberellin level occurred in elongating buds of tubers during storage. This increase occurred shortly before (about 15 days) primordial inflorescences were observed in dissected apices. This finding suggests a possible involvement of gibberellin in autonomous flowering induction in colored *Zantedeschia*. Gibberellin is known as an alternate pathway in flowering control in *Arabidopsis thaliana* (Yu and others 2006), whereas in day-neutral plants gibberellin was proposed as the internal factor controlling flowering (Lawson and Poethig 1995). The involvement of gibberellin in flowering of colored *Zantedeschia* is supported by several facts: (1) gibberellin

is required in vitro for flowering of plantlets (Naor and others 2003); (2) application of GA_3 to growing plants enhances inflorescence differentiation in nondormant buds, regardless of their size, age, and bud position on the shoot (Corr and Widmer 1987; Funnell 1993; Brooking and Cohen 2002; Naor and Kigel 2002; Naor and others 2005); (3) gibberellin application enhances flowering of small-size tubers that otherwise do not flower (Brooking and Cohen 2002; Naor and others 2003, 2005); and (4) exogenous GA_3 enhances elongation of the inflorescence peduncle (Naor and others 2005). The minimal tuber size required for flowering in *Zantedeschia* suggests that a threshold level of gibberellin is required in the apex and that this threshold is reached above a certain tuber size. Further research is required to determine if gibberellins act as a multipurpose signal enhancing bud sprouting and carbohydrate metabolism as well as flowering induction, and whether different specific gibberellins are involved in these processes.

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