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# Gibberellin-induced Inhibition and Promotion of Sprouting in Aerial Tubers of *Begonia evansiana* Andr. in Relation to Photoperiodic Treatment and Tuber Stage

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**Abstract.** Gibberellic-acid ($GA_3$) treatment, when applied within a period ranging from the start of short-day (SD) treatment until about 10 SD, $GA_3$ strongly inhibited formation of aerial tubers in response to SD and brought about sprouting of developing aerial tubers. In contrast, when applied after about 10 SD or more, $GA_3$ hastened the completion of the dormant state in the tubers and prolonged their dormancy. The dormancy-promoting effect of $GA_3$ on detached tubers increased with their degree of maturation. Application of growth retardants N-dimethylaminosuccinamic acid (B-9), 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine carboxylate methyl chloride (AMO-1618) and 2-chloroethyltrimethylammonium chloride (CCC) to the cuttings delayed the onset of dormancy in the aerial tubers. When the retardants were applied to detached aerial tubers, however, such a delay of dormancy was not observed, and $GA_3$ application did not inhibit sprouting in aerial tubers detached from CCC-treated cuttings.

**Key words:** *Begonia* – Dormancy (tubers) – Gibberellin – Growth retardants – Tubers.

## Introduction

The formation of aerial tubers in *Begonia evansiana* Andr. is induced by SD treatment (Esashi and Nagao, 1958; Esashi, 1961, 1966), and their sprouting is inhibited by $GA_3$ application (Nagao and Mitsui, 1959; Cho, 1970; Okagami, 1972; Okagami and Esashi, 1972; Okagami and Nagao, 1973). The present study was undertaken to examine how $GA_3$ affects the formation and the sprouting of aerial tubers of *B. evansiana* in relation to the number of SDs received and the stage of tuber growth.

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*Abbreviations*: GA, gibberellin; $GA_3$, gibberellic acid; SD, short day(s); LD, long day(s); SDP, short-day plant; LDP long-day plant; CCC, 2-chloroethyltrimethylammonium chloride; B-9, N-dimethylaminosuccinamic acid; AMO-1618, 2-isopropyl-4-dimethyl-amino-5-methylphenyl-1-piperidine carboxylate methyl chloride

## Materials and Methods

*Experiment with Cuttings*

One-node cuttings prepared according to the method of Esashi (1960) were used. The leaf of each cutting was cut to form a disc 10 cm in diameter. Before experimentation the cuttings were cultured for 7–10 days under continuous illumination (daylight extended with light from incandescent lamps, ca. 0.3 $W/m^2$). SDs (8.5 h light daily) were given by covering the cuttings with light-tight black cloth from 5:30 p.m. (17:30) to 9:00 a.m. (09:00) in a greenhouse shaded with reed blinds.

Each node of *B. evansiana* carries three axillary buds, a central and two lateral ones. During the preculture period, the central bud of each cutting developed into a shoot while the other two showed little or no development. Under SD, the central bud continues to grow (this is recorded in some experiments) but the other two do not. The terminal bud of the shoot developing from the central axillary bud, and the two lateral buds of each cutting were examined for tuberization and/or sprouting. The tubers which develop from these two kinds of buds will be referred to as the terminal and lateral tubers, respectively. The size of tubers was expressed in terms of the "tuber stage" (Esashi, 1960; the higher the value for tuber stage, the larger the tuber). Unless stated otherwise, data on tuber stage and sprouting percent of the buds of cuttings are the mean sums of the terminal and lateral tubers.

For treating with chemicals, the buds of the cuttings were wrapped with a small piece of absorbent cotton saturated with an aqueous solution of the desired chemical. For treatment of leaves, a sheet of absorbent cotton (5 × 5 cm) soaked with the test solution was placed on the leaf. The chemicals used were $GA_3$ (Kyowa Fermentation Industries, Tokyo), AMO-1618 (Rainbow Color & Chemical Co., Sepulveda, Cal., USA), B-9 (K & K Laboratories, Plainview, N.Y., USA), CCC (Tokyo Kasei Kogyo Co., Tokyo). Controls were treated with distilled water in the same way. After treatment, the treated leaves or buds were washed with

running tap water for a few minutes. In some experiments, CCC was applied to the culture solution of the cuttings (basal treatment) as described before (Nagao and Okagami, 1966). In all experiments, 8 cuttings were used for each treatment.

*Experiments with Detached Aerial Tubers*

Tubers were collected from plants grown under artificial or natural SD conditions. About 30–50 tubers were used for each treatment. They were soaked in a $GA_3$ solution or distilled water (control) in a beaker for 30 min at room temperature, were then placed on a net and throughly washed with running tap water for 1 min, rinsed with distilled water for a few seconds, placed on water-saturated absorbent cotton in 6- or 9-cm Petri dishes, and incubated at 28° C in light (1.1 W/m$^2$) from "daylight" fluorescent lamps (40-D-SDL, Toshiba, Tokyo) or in the dark. For CCC treatment the tubers were incubated on absorbent cotton soaked with aqueous solution of CCC throughout the period of experiment, the cotton being renewed at intervals of about 20 days.

## Results

### *Effect of $GA_3$ Given at the Start of the SD Treatment on Tuberization and Shoot Elongation*

When leaves of cuttings kept under SDs were treated with different concentrations of $GA_3$ during the dark periods of the first 3 SDs (Fig. 1), tuberization was inhibited and shoot elongation was promoted, the effects increasing with the $GA_3$ concentration. The lateral buds were more sensitive to $GA_3$ inhibition of tuberization than was the terminal one. Tuberization was inhibited also when $GA_3$ was applied to buds (Table 1); the bud treatment was, however, much more effective than the leaf treatment. The promotion of shoot growth by $GA_3$ application concerned both the elongation of the shoot and the number of nodes, both under LD and SD conditions (Table 2).

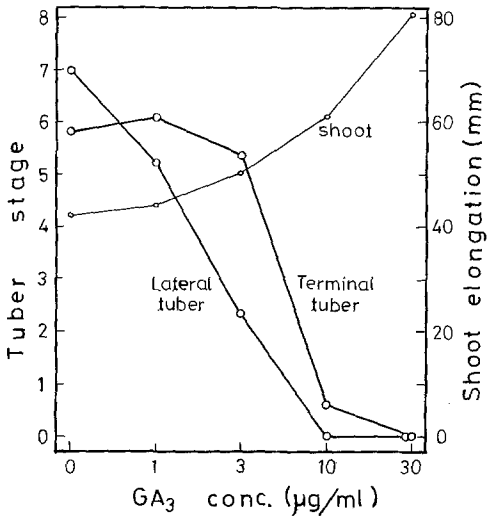


**Fig. 1.** Effect of $GA_3$ given at the start of the SD treatment on tuberization and shoot elongation under SD. $GA_3$ was applied to leaves during the dark periods of the first 3 SD. Observations after 18 SD.

**Table 1.** Effect on tuberization under SD of $GA_3$ given at the beginning of the SD treatment

$GA_3$ applied to buds or leaves during the dark period of the first 1 or 3 SD. Tuber stage determined after 20 SDs

| Concentration of $GA_3$ (μg/ml) | $GA_3$ treatment (in days) | Tuber stage | |
|---|---|---|---|
| | | Bud treatment | Leaf treatment |
| 0 | 0 | 6.7 | 6.7 |
| 1 | 1 | 1.6 | – |
| 1 | 3 | 0.2 | 1.7 |
| 3 | 1 | 0.5 | 2.5 |
| 3 | 3 | – | 1.5 |
| 10 | 1 | – | 0.3 |

**Table 2.** Effect of $GA_3$ on the increase in number of nodes in the shoot of cuttings kept under LD and SD

$GA_3$ solution was applied to buds during the first 3 SD or LD. The increase in number of nodes was observed after 28 LD or SD. Each value represents the mean of 8 cuttings with the 90% confidence limits

| Concentration of $GA_3$ (μg/ml) | Increase in node number | |
|---|---|---|
| | LD | SD |
| 0 (control) | 2.0±0.0 | 0.8±0.2 |
| 0.3 | 2.3±0.3 | 1.4±0.3 |
| 1 | 2.3±0.2 | 1.5±0.3 |

### *Effect on Tuberization of $GA_3$ Given Various Days after the Start of the SD Treatment*

Buds of cuttings growing under SD were treated with 0.5, 1 or 100 μg/ml $GA_3$ during one dark period only but on different days after the start of the SD treatment. $GA_3$ at 100 μg/ml completely inhibited tuberization when given before 9 SD or less (Fig. 2A). In lower concentrations (0.5 or 1 μg/ml), $GA_3$ was less effective in inhibiting tuberization and caused a maximum inhibition of tuber enlargement when applied after the second SD (Fig. 2B and C).

In contrast, $GA_3$ (1 or 100 μg/ml) had little or no inhibitory effect on tuber enlargement when given after 11 SD or more. Moreover, $GA_3$ at all concentrations used accelerated both browning and abscission of tubers. These results indicate that $GA_3$ application hastens the dormancy development in the aerial tubers of *B. evansiana*.

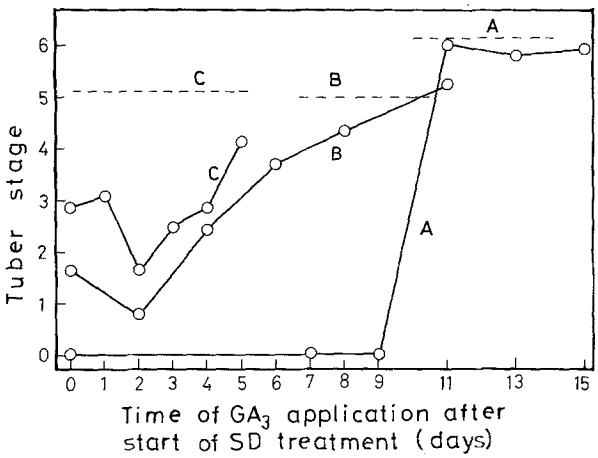


**Fig. 2.** Effect on tuberization under SD of $GA_3$ given various days after the start of the SD treatment. Buds were treated with 100 (curve A), 1 (curve B) or 0.5 µg/ml $GA_3$ (curve C) during the dark period of the SD specified. Observation was made after 19 (A) or 13 SD (B, C). Broken lines-tuber stage of the respective control

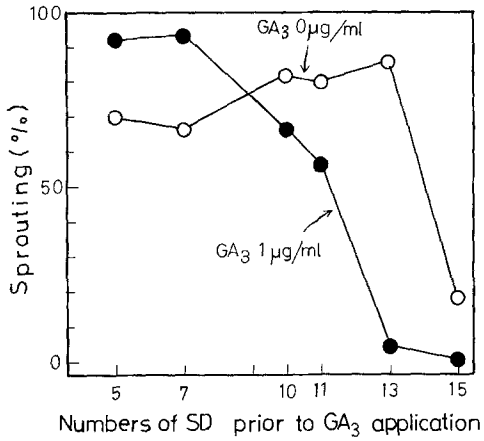


**Fig. 3.** Effect of $GA_3$ on sprouting of tuberizing buds under LD. Cuttings kept under SD for different numbers of days were transferred to LD, and their tuberizing buds were treated with $GA_3$ (1 µg/ml) during the first 1-h period. Sprouting was examined after 37 LD

## *Effects of $GA_3$ on the LD-induced Sprouting of Tuberizing Buds*

When cuttings of *B. evansiana* are transferred to LD conditions after having been exposed to SD below certain minimum number, the tuberizing buds begin to sprout (Esashi, 1962). The effect of $GA_3$ on such sprouting was examined in the following experiment. Cuttings were exposed to 5–15 SDs and were then transferred to LD (continuous light); their tuberizing buds were treated with 1 µg/ml $GA_3$ during the first 1-h period of the continuous illumination (Fig. 3). In cuttings given 5 or 7 SD, $GA_3$ had a stimulative

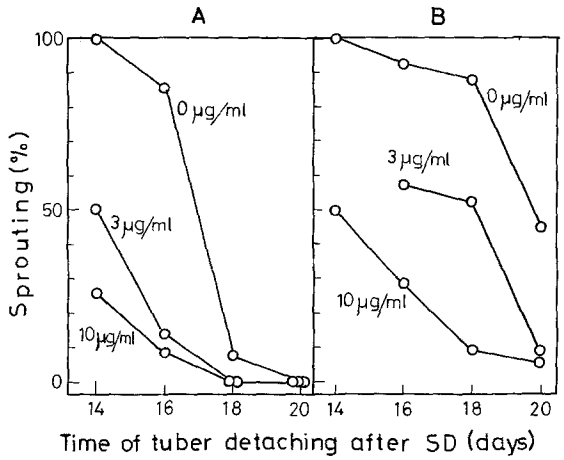


**Fig. 4A and B.** Effect of $GA_3$ on the sprouting of detached aerial tubers at different developmental stages. Tubers were detached from the cuttings after receiving the specified number of SD (abscissa). **A** Tubers incubated at 28° C in the light for 40 days. **B** Tubers which failed to sprout under the above conditions were exposed to low temperature (2° C) for 60 days and then incubated at 28° C in the light for 45 days. Sprouting percentages include those given in **A**

effect on sprouting; in those subjected to 10 SDs or more, on the contrary, $GA_3$ inhibited sprouting, progressively with the increasing number of SDs given.

## *Effect of $GA_3$ on the Sprouting of Isolated Aerial Tubers at Various Stages of Maturation*

Tubers were detached from cuttings given 14, 16, 18 or 20 SDs and incubated in the light at 28° C after being treated with 0 (control), 3 µg/ml and 10 µg/ml $GA_3$ for 30 min (Fig. 4A). The sprouting of the control tubers decreased with the progress of their maturation, as reported previously (Esashi, 1962). $GA_3$ strongly inhibited the sprouting, and this effect was enhanced with the maturation of the tubers treated. The tubers which failed to sprout in the above experiment were subjected to a low-temperature treatment (2° C) in the dark for 60 days and then incubated at 28° C in the light for a further 45 days (Fig. 4B). The sprouting tendency of these chilled tubers was similar to that obtained with the unchilled tubers, although their sprouting level was generally higher.

## *Effects of Growth Retardants on the Sprouting of Aerial Tubers of Cuttings*

CCC induces sprouting of aerial tubers of *B. evansiana* (Nagao and Okagami, 1966). In the present

**Table 3.** Effect of growth retardants on the sprouting of tuberizing buds of cuttings

The buds of the cuttings were treated with various concentrations of B-9 or AMO-1618 during the first 10 of 17 SD, the cuttings were then maintained under continuous light for 6 days

| Concentration of growth retardant (mM) | Sprouting (%) | |
|---|---|---|
| | B-9 | AMO-1618 |
| 0 | 25.0 | 25.0 |
| 0.1 | 25.0 | 47.6 |
| 1 | 83.3 | 59.6 |
| 10 | 95.6 | 82.3 |

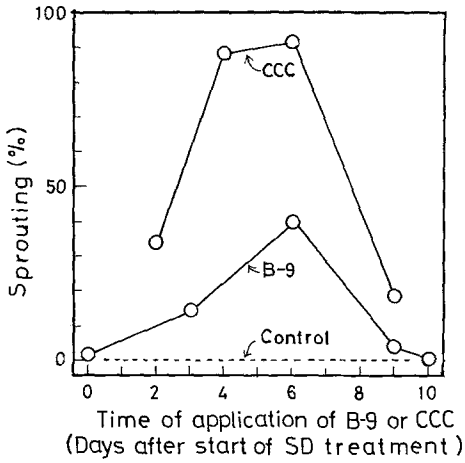


**Fig. 5.** Effect on sprouting of B-9 or CCC given various days after the start of the SD treatment. The buds of the cuttings were treated with 3 mM B-9 or CCC for 3 days. The cuttings were exposed to 21 SDs and subsequently grown under continuous light for 7 days

**Table 4.** Effect of $GA_3$ on the sprouting of aerial tubers detached from CCC-treated cuttings

CCC (1 mM) was applied to the basal part of cuttings during the last 10 of 15 SDs. Tubers detached from these cuttings were treated with $GA_3$ and then incubated in the light at 28° C for 50 days

| Concentration of $GA_3$ (μg/ml) | Sprouting (%) | |
|---|---|---|
| | Tubers of control cuttings | Tubers of CCC-treated cuttings |
| 0 | 68.9 | 95.0 |
| 0.3 | 66.5 | 95.0 |
| 1 | 28.5 | 90.0 |

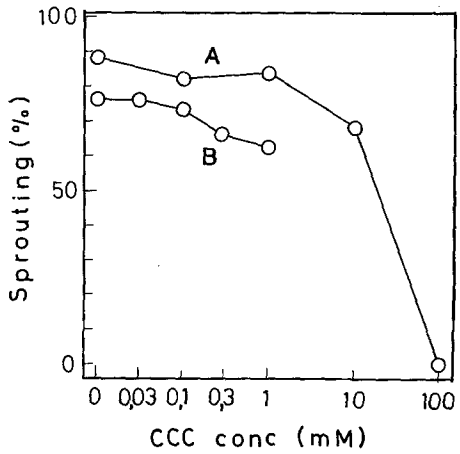


**Fig. 6.** Effect of CCC on the sprouting of detached aerial tubers. Detached tubers at the photo-sprouting stage were incubated with various concentrations of CCC in the light at 28° C for 90 (curve A) or 60 days (curve B)

experiments, B-9 and AMO-1618 at 0.1, 1 or 10 mM were applied to the buds of cuttings during the first 10 of 17 SD; these cuttings were then kept in continuos light for 6 more days (Table 3). The sprouting percentages increased with increasing concentration of the growth retardants.

Next, an experiment was conducted to determine the application time when growth retardants were most effective in causing the tuberizing buds of cuttings to sprout. B-9 or CCC at a concentration of 3 mM was applied to the buds of cuttings during 3 days, at various times after the start of SDs. Figure 5 shows that these retardants were most effective when applied to cuttings which had been subjected to 4–6 SD.

### *Effect of $GA_3$ on the Sprouting of Aerial Tubers Detached from CCC-Treated Cuttings*

The basal part of cuttings was treated with 1 mM CCC during the last 10 of 15 SD. Then the tubers were detached, treated with $GA_3$, and examined for sprouting (Table 4). $GA_3$ had no inhibitory effect on the sprouting of tubers detached from CCC-treated cuttings.

### *Effect of CCC on the sprouting of Detached Aerial Tubers*

Tubers detached at the "photo-sprouting stage" (Esashi, 1962) were incubated in various concentrations of CCC. These tubers, in contrast to those attached to the CCC treated cuttings (Nagao and Okagami, 1966 Fig. 5), showed no promotion of sprouting by CCC (Fig. 6). All tubers treated with 100 mM CCC died.

## Discussion

When *B. evansiana* cuttings were treated with $GA_3$ in buds or leaves before they received about 10 SDs,

$GA_3$ inhibited both the initiation and enlargement of aerial tubers and caused sprouting of tuberous buds and increased shoot elongation and node formation (Table 1, 2; Figs. 1–3). Thus, $GA_3$ application in SD induced the same morphogenetic events as those occurring under LD conditions. However, $GA_3$ applied after about 10 SDs produced little effect on the tuber enlargement, inhibited sprouting, and hastened the development of dormancy (Fig. 2–4). The change in the response of tuberizing buds to $GA_3$ corresponds with the time at which the tubers on the cuttings progress from the "dark-sprouting" stage to the "photo-sprouting" stage (Esashi, 1962). $GA_3$ application has the same action as LD in flowering of many plants, generally promoting it in LDP and inhibiting or delaying it in SDP (e.g., Lang, 1957, 1965; Wittwer and Bukovac 1957; Harder and Bünsow, 1958). The inhibition of tuberization in *B. evansiana*, a SD-dependent process, by $GA_3$ application during its earlier stages is in general agreement with these observations. However, in some SDP, e.g. *Xanthium* (Greulach and Haesloop, 1958) and Biloxi soybean (Bharti and Garg, 1970), it has been found that $GA_3$ given under non-inductive LD conditions after an insufficient SD treatment can induce flower formation, i.e. partly substitute for SD requirement. The $GA_3$-induced dormancy in the tubers of *B. evansiana* when the hormone is applied under LD following an SD treatment (Fig. 3) can also be considered as partial substitution for an SD requirement. However, in *Begonia* as in *Xanthium* and in soybean, the transition from vegetative growth to "reproductive" development cannot be brought about by $GA_3$ treatment under continuous LD (data not shown). It appears that whether or not $GA_3$ application promotes tuberization in *B. evansiana* is dependent upon the state of the buds at the time of the $GA_3$ treatment.

$GA_3$-induced dormancy of the aerial tubers of *B. evansiana* is in contrast to the breaking of dormancy by GA found in many other plants. However, this species is not the only plant in which $GA_3$ exerts an inhibiting action on sprouting; the resting buds of some woody plants (e.g., Weaver, 1959; Brian et al., 1959), and the bulbils, subterranean dormant organs and seeds of several species of *Dioscorea* (Okagami and Nagao, 1971; Okagami and Tanno, 1977; Okagami and Kawai, 1977) also respond to $GA_3$ in a way similar to the tubers of *Begonia*.

From the inhibitory action of CCC on dormancy induction in *Begonia* tubers (Nagao and Okagami, 1966) it has been assumed that endogenous GA is acting as dormancy inducer. This assumption is supported by an present results with B-9 and AMO-1618 (Table 3, Fig. 5). The result in Figure 5 indicate that endogenous GA synthesis, as related to dormancy induction, occurs mainly between 4–10 days from the start of the SD treatment. When CCC was applied to detached tubers (Fig. 6), however, sprouting was not promoted, indicating that the endogenous GA which participates in dormancy induction cannot be synthesized in the tubers themselves when these are detached from mother plant.

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