

## Specificity of Gibberellin and Sucrose-promoted Flower Bud Growth in *Gladiolus*

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### ABSTRACT

A critical stage in flower bud growth in the spike of *Gladiolus* which is initiated by gibberellic acid (GA<sub>3</sub>) and sustained by sucrose has been identified. This corresponds to the stage at which separation of the outer bract occurs. In buds at different developmental stages isolated and held in water, it is the same bud stage that first shows increased growth. Buds not induced by light were shown to respond more significantly to GA<sub>3</sub> and sucrose than those induced by light. Since the separation of the outer bract results in light-induced amylase production and starch hydrolysis leading to petal growth, it is proposed that growth promotion by GA<sub>3</sub> is related to light-induced petal growth at this specific stage.

Key words: flower bud growth, *Gladiolus natalensis*, gibberellic acid, sucrose.

### INTRODUCTION

The control of growth and opening of a flower bud is not well understood and several serious gaps exist in our knowledge. Endogenous gibberellin levels seem to regulate flower growth (Jeffcoat, Scott and Harris, 1969; Sircar *et al.*, 1970; Murakami, 1973, 1975). Removal of stamens results in inhibition of corolla expansion. This can be overcome by the application of GA<sub>3</sub> (Plack 1957, 1958; Greyson and Tepfer, 1967). Bud opening is promoted in spikes of *Gladiolus* treated with GA<sub>3</sub> and sucrose (Rao and Mohan Ram, 1979). Recently, Rao and Mohan Ram (1980) noted a light-mediated step in the petal growth of *Gladiolus*. Separation of the outer bract, which encloses the immature flower bud and the spike axis, allows light to penetrate the bud. Light stimulates amylase synthesis in the petal epidermis, which hydrolyses the starch reserves in the ground parenchyma. It has been proposed that the reducing sugars thus released lower the osmotic potential leading to petal expansion and flower opening (Rao and Mohan Ram, 1980). Winkenbach (1970) had also reported that sugars are the principal osmotic regulator in the corolla in *Ipomoea*.

In the immature spikes of *Gladiolus* kept in sucrose solution, two distinct regions can be recognized – the lower bearing fully opened flowers and the upper containing buds with their corollas still enclosed within the bracts (Rao, 1979). In spikes held in sucrose plus GA<sub>3</sub>, the enclosed buds opened, suggesting the existence of a gibberellin-requiring phase in bud growth (Rao and Mohan Ram, 1979). To establish the relation between light and GA<sub>3</sub> in the growth and opening of flower buds, the effect of GA<sub>3</sub> and sucrose has been investigated further and it has been shown that the developmental stage of the flower bud promoted by GA<sub>3</sub> is that at which separation of the outer bract occurs.

### MATERIAL AND METHODS

Spikes of *Gladiolus natalensis* Hort. were used at the green-bud stage of development (harvested 1 day before the corolla of the lowest bud would have visibly emerged from

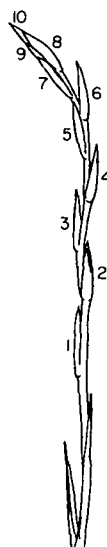


FIG. 1. *Gladiolus* spike at the green-bud stage showing the axis and the peduncle. Positions of buds corresponding to those in the isolated bud experiment are numbered.

the enveloping bracts, (Fig. 1). Experiments began 24 h after harvest during which time the spikes were kept upright in dark cardboard containers. Sets of 15 spikes were kept in individual glass tubes ( $2.5 \times 15.0$  cm) containing  $0.5$  M sucrose and  $10^{-5}$  M or  $10^{-4}$  M  $GA_3$ . Set A was placed directly in the treatment solutions at  $20 \pm 2$  °C and a 14 h photoperiod under cool-white fluorescent tubes (500 lx) for 15 days, and Set B was chilled at  $4 \pm 0.5$  °C for 1 week in the dark before placing in the test solutions at  $20 \pm 2$  °C for 15 days. Set B was included because earlier work showed that cold storage accentuated the differential response of the buds to sucrose and  $GA_3$  (Rao and Mohan Ram, 1979). The number of buds that opened each day was recorded.

To study the influence of  $GA_3$  and sucrose on the growth of isolated buds representing different stages of development, buds from positions 1 to 10 (Fig. 1) were excised so as to include a portion of the subjacent flowering axis. Fifteen excised buds for each treatment (Set C) were placed in injection vials containing 5 ml of water,  $0.25$  M sucrose,  $10^{-5}$  M  $GA_3$ , or sucrose plus  $GA_3$  solutions and kept in the same conditions as Set A. Another set of buds (Set D) was prepared from spikes which had been stored at  $4 \pm 0.5$  °C for one week and similarly treated. Set D contained 10 replicates.

The isolated buds were weighed each day for 15 days and as the buds were at different stages of development, fresh weight was expressed as a percentage of the highest increment over the initial weight. Analysis of variance of the data was carried out and the least significant differences between means and confidence intervals were calculated at  $P \leq 0.05$ .

## RESULTS

### *Number of buds opening*

*Set A.* A rhythmic pattern of bud opening was noted (Fig. 2). The initial two peaks of opening in the control and in the  $GA_3$ -treated spikes occurred on days 3 and 6. Spikes held in sucrose alone, or sucrose plus  $GA_3$  exhibited the second peak on day 5. On days 10, 13 and 14 conspicuous bud opening was recorded only in the spikes placed in sucrose plus  $GA_3$  ( $10^{-4}$  M).

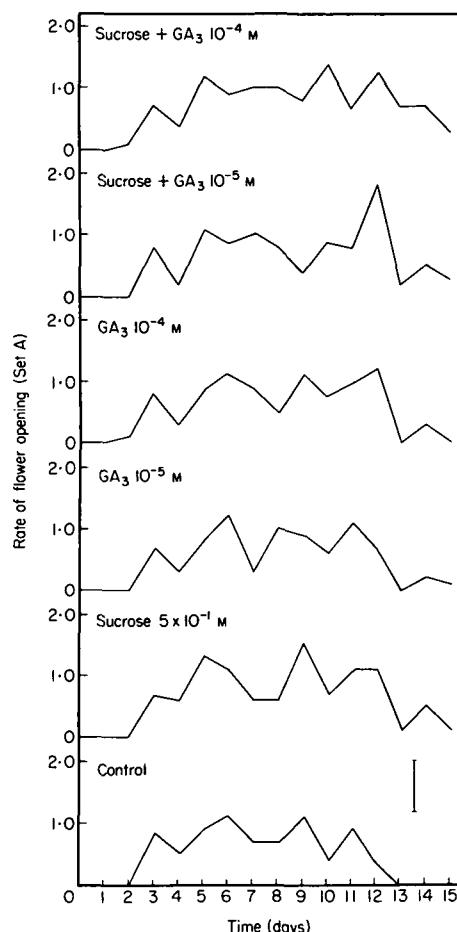


FIG. 2. Mean numbers of flowers opening on each day in spikes treated with sucrose and  $GA_3$  individually and together (Set A). Vertical bar indicates least significant difference between means ( $P \leq 0.05$ ).

*Set B.* Bud opening in Set B spikes commenced on day 1 (Fig. 3). Three major peaks, followed by a decline, were recorded for the control, sucrose and  $GA_3$ -treated spikes. In the spikes held in sucrose plus  $GA_3$ , opening continued until day 8. On this day opening ceased in the control, and dropped in the spikes treated with sucrose or  $GA_3$  individually. Importantly, however, a very sharp rise in opening was noted on day 9 in spikes kept in sucrose plus  $GA_3$  (both combinations), and this was followed by a decline.

From the data in Fig. 3 the total number and positions of buds on the spikes affected by the various treatments were calculated. As buds always open sequentially, the total number of flowers opened indicates their position. In the control,  $10^{-5}$  and  $10^{-4}$  M  $GA_3$ , 5.5, 6.2 and 6.4 flowers had opened respectively on day 8. The corresponding figures on day 9 were 5.7, 6.5 and 6.8. By day 15 the values attained were 5.7, 6.8 and 7.2 flowers respectively. In the spikes held in sucrose, buds up to position 5.9 had opened on day 8 and this increased to 6.3 on the following day. The comparable positions on day 8 for the spikes held in sucrose plus  $GA_3$  ( $10^{-5}$  M) and sucrose plus  $GA_3$  ( $10^{-4}$  M) were 6.1 and 6.9 respectively. These increased to 8.1 and 8.9 on day 9. By day 15 the figures for sucrose,

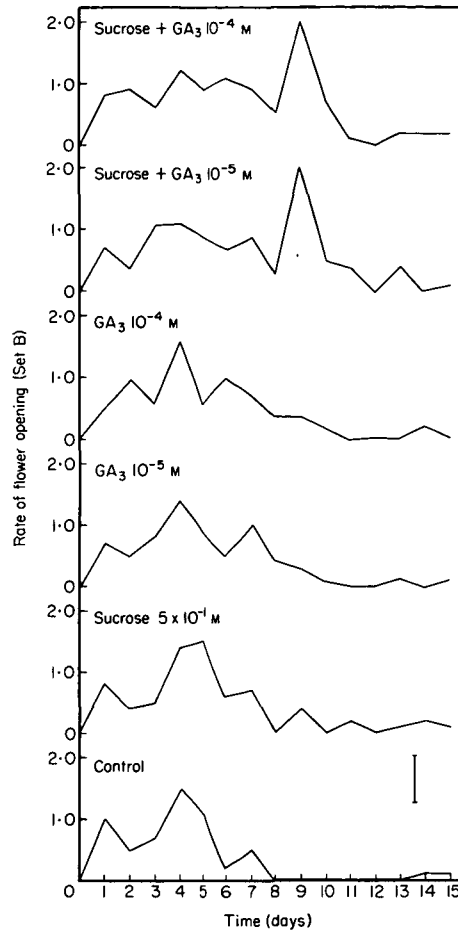


FIG. 3. Mean numbers of flowers opening on each day in spikes treated with sucrose and  $GA_3$  individually and together, after chilling at  $4^\circ C$  for one week (Set B). Vertical bar indicates least significant difference between means ( $P \leq 0.05$ ).

sucrose plus  $GA_3$  ( $10^{-5}$  M) and sucrose plus  $GA_3$  ( $10^{-4}$  M) treated spikes were 6.9, 9.5 and 10.3 respectively. Thus when sucrose or  $GA_3$  were used independently buds beyond position 7 did not open. However, when sucrose and  $GA_3$  were used together, younger buds were stimulated to bloom. Even in the buds which failed to open in response to  $GA_3$  or sucrose, there was a marked difference in the growth of the corolla. Whereas  $GA_3$  caused elongation of the petals beyond the bracts, in sucrose treatment the buds failed to grow.

#### Growth of excised flower buds

*Set C.* In water a decreasing growth response occurred in buds 1 to 7 (Fig. 4). Remarkably, however buds 8 to 10 showed a progressive increment in fresh weight. In general, sucrose was more effective in promoting the growth of the older buds; with  $GA_3$  the growth of the younger buds was more influenced. In sucrose plus  $GA_3$  an enhancement in fresh weight occurred in all the buds, with a marked stimulation in buds 8–10 in which the highest fresh weight in all the treatments was recorded.

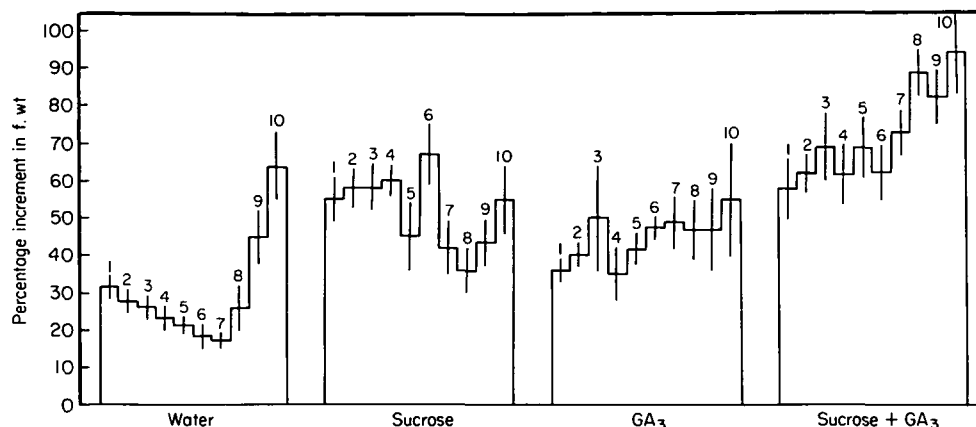


FIG. 4. Percentage increase in fresh weight of excised buds (Set C) at different stages of development (buds 1-10) in response to water, sucrose,  $GA_3$  and sucrose plus  $GA_3$ . Vertical bars indicate confidence interval of the means ( $P \leq 0.05$ ).

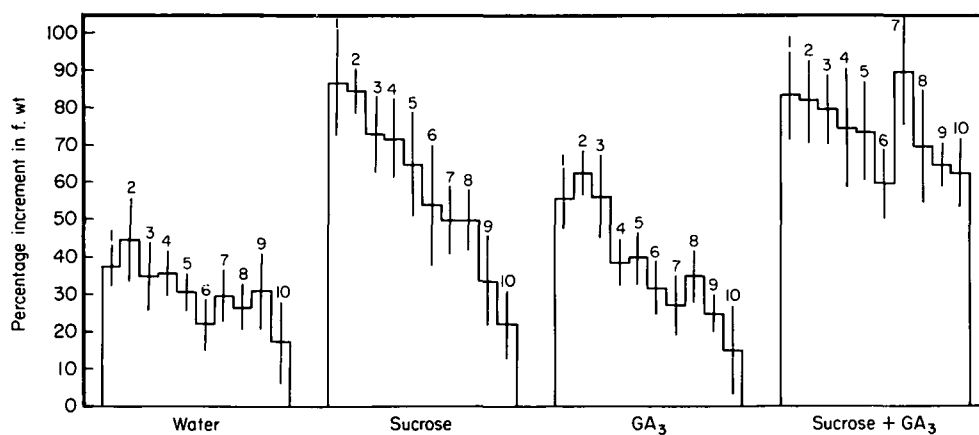


FIG. 5. Percentage increase in fresh weight of excised buds after chilling at 4 °C for 1 week (Set D) at different stages of development (buds 1-10) in response to water, sucrose,  $GA_3$  and sucrose plus  $GA_3$ . Vertical bars indicate confidence interval of the means ( $P \leq 0.05$ ).

*Set D.* In general, the control buds of Set D showed a decreasing growth response from positions 1 to 10 in contrast to the differential response noted in the controls of Set C (Fig. 5). With sucrose there was an increase in the fresh weights of all buds between positions 1 and 8 over those of the controls. The growth of buds 1-3 was slightly increased over the controls by  $GA_3$  treatment. However, the fresh weight increment in the remaining buds held in sucrose or  $GA_3$  was similar to that of the controls. The use of sucrose plus  $GA_3$  resulted in a remarkable restoration of the growth potential of the younger buds (Fig. 5). It was also observed that in Sets C and D buds up to position number 9 had opened in sucrose plus  $GA_3$ , in contrast with the control in which buds up to position 3 had opened.

## DISCUSSION

The present work has shown that GA<sub>3</sub> in combination with sucrose promotes flower bud opening in immature cut spikes. Buds situated higher up on the spike are more responsive to GA<sub>3</sub> and sucrose than those lower down. As isolated buds also show a similar response to sucrose plus GA<sub>3</sub> with a marked promotion in the growth of the younger buds, it may be inferred that the stage of development of a bud is an important factor regulating its growth response. A combined effect of GA<sub>3</sub> and sucrose has been shown in the elongation of hypocotyl segments (Purves and Hillman, 1958; Kazama and Katsumi, 1973) and in the linear growth of staminal filaments (Murakami, 1973).

In a harvested green-bud spike the outer bracts of buds 1–7 are found to be separated (Fig. 1) and that of buds 8–10, not separated (Rao and Mohan Ram, 1981). The peak of opening in response to sucrose plus GA<sub>3</sub> on day 9 in Set B, and its absence in the other treatments, demonstrates clearly the presence of a critical stage of bud development at which these substances are limiting. In this treatment the earliest stage of bud, which is stimulated to open, corresponds to the oldest unseparated bud (position 8). A significant feature here is the differential growth response of isolated buds in water (Set C). Again, it is the isolated bud from position 8 that is the first to show a conspicuous increase in growth. Earlier evidence showed that separation of the outer bract permits light-induced amylase production and starch hydrolysis (Rao and Mahan Ram, 1980), and it is quite probable that the GA<sub>3</sub> action in the stage-specific growth promotion is related to the former. The observation that the buds not induced by light (buds 8–10) respond more to sucrose plus GA<sub>3</sub> than those induced by light, and the resurgence in growth of the buds in Set D in response to sucrose plus GA<sub>3</sub>, provide further evidence for the above proposition. It is possible that GA<sub>3</sub> is able to substitute for light in the non-induced buds, but this remains to be tested.

Changes in endogenous gibberellins (Murakami, 1973, 1975) and their promotory action on the growth of petals (Plack 1958; Greyson and Tepfer, 1967) have been recorded. Nevertheless, a specific stage-dependent action of gibberellin and its correlation with light has not been demonstrated. In the present work, it is important to examine the role played by GA<sub>3</sub> and sucrose and decide which is the initiating factor and which is the limiting factor. The evidence that GA<sub>3</sub> is the initiating factor comes from the observation that corolla growth occurs in the younger buds in GA<sub>3</sub>-treated spikes but it falls short of the extent necessary for opening since sucrose is limiting. Green-bud spikes have been shown to lack adequate carbohydrate reserves for flower opening (Rao and Mohan Ram, 1981). By contrast, when spikes were held in sucrose only the unseparated buds showed no growth, presumably because endogenous gibberellins were inadequate. When the two were used together the growth initiated by GA<sub>3</sub> was sustained by sucrose leading to complete flower opening.

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