Effect of Photoperiod and Growth Regulator on Growth and Flowering of *Stevia rebaudiana* Bertoni

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Stevia rebaudiana Bertoni is an endemic herb from Paraguay. Its leaves have a very sweet taste and are used by local people as a sweetener⁷⁾. A beverage made from the powdered leaves is used as an anticonceptional¹²⁾. The anticonceptional properties of *S. rebaudiana* are not well established. Although PLANAS and KUC¹²⁾ observed sterility in mice caused by a decoction of leaves, FARNSWORTH⁶⁾ was not able to confirm these results. According to BRIDEL and LAVIEILLE³⁾, RASENACK in 1908 isolated a sweet substance from leaves of *S. rebaudiana* known later as stevioside. BRIDEL and LAVIEILLE^{3,4)} obtained 60 to 65 g of stevioside per kilogram dry weight of leaves. Stevioside is more than three hundred times sweeter than sucrose. During recent years, stevioside has been considered to be a possible substitute for sugarcane or sugarbeet. It is the sweetest natural occurring substance known^{1,2,15)}. According to POMMARET and LAVIEILLE¹³⁾, it is not metabolized by the organism, although some toxicity is mentioned⁶⁾.

As a natural product stevioside has a promising future as a sweetening agent¹⁵⁾.

The aim of this work was the study of conditions for reproductive or vegetative growth of *Stevia rebaudiana*. Knowledge of these conditions makes it possible to manipulate the plants in order to produce seeds for propagation or leaves for stevioside production.

Materials and Methods

Stevia rebaudiana Bertoni from Paraguay was cultivated in the Instituto de Botanica of São Paulo.

Daylength: All treatments were subjected to

a daily 8 hours of natural light in the glasshouse. Photoperiods longer than 8h were obtained by complementary incandescent light (240 lux) in darkrooms. This illumination on the surface of the upper leaves was effective to supplement the photoperiod. Treatments with interrupted night (IN) were obtained by providing one hour of light in the middle of the dark period (8h of natural light+7.5h of darkness+1h of incandescent light of 240 lux+8.5h+7.5h of darkness).

Data on flowering were recorded when the blossoms could be seen macroscopically. **Growth regulators:** Plants with 4-5 or 10-12 pairs of leaves kept in continuous light (CL) were used in these experiments. 20 ml of a 2000 µg/ml solution of (2-chloroethyl) trimethyl ammonium chloride (CCC) were applied weekly as a soil drench, and gibberellic acid (GA₃), steviol (obtained from enzymatic hydrolysis of stevioside) and fusicoccin (kindly supplied by Dr. BALLIO, Università degli Studi di Roma) were also applied weekly as a drop on the apice of the plants.

Usually 8-11 plants per treatment were used throughout this work. Experiments were carried out all the year round.

Results

1. Effect of photoperiod on flowering

(1) **Critical daylength:** Preliminary experiments with plants kept in 8 h photoperiod (SD) and continuous light (CL) had shown that the plants kept in SD flowered while the ones in CL remained vegetative, which suggested that *S. rebaudiana* could be seen as a SD plant for flowering.

In order to make a more detailed study

of the effect of daylength upon the flowering, other experiments were performed. Seedlings with 4–5 pairs of leaves kept in CL since germination of the seeds (about 40 days from sowing) were transferred to photoperiods of 8, 10, 12, 14, 16 hours and 8 h IN for 65 cycles. Although flowering was more precocious in the 8 h photoperiod, higher flowering rates were obtained for the 12 h photoperiod. Plants kept under photoperiods of 14, 16 and 8 hIN remained vegetative (Fig. 1). A similar experiment was carried out with plants with 10–12 pairs of leaves growing in CL (about 70 days from sowing). These plants were subjected to photoperiods of 12, 13, 14, 15, 16 and 8 hIN for 50 cycles. Plants only flowered for the 12 and 13 h photoperiods. Earlier flowering and higher percentages of flowering plants occurred under the photoperiod of 12 h. All the remaining photoperiods failed to induce flowering (Fig. 2).

These results confirmed our previous observations upon the photoperiodic behavior of *S. rebaudiana*. Then, *S. rebaudiana* was a SD plant for flowering being the critical daylength between 13 and 14 h.

(2) *Induction period*: The aim of these experiments was to verify the number of SD necessary to induce flowering. Plants with 8 pairs of leaves kept in CL were subjected to 1, 2, 4 and 6 SD. After this SD treatment the plants returned to CL. As we can see in Fig. 3, two SD were able to induce flowering although precocity and higher percentages of flowering plants were obtained with more cycles of SD. One SD was not completely sufficient to induce flowering in *S. rebaudiana*.

(3) *Earliness of flowering*: It was shown earlier that plants with 4 to 12 pairs of leaves can be induced to flower in SD conditions. In these experiments, seedlings with 3, 4, 5 and 6 pairs of leaves growing in CL were subjected to 9 inductive cycles of SD and then returned to CL. Although in the previous experiment 6 cycles of SD were effective in bringing about 100% of flowering, in this experiment 9 cycles were used for safety. Results were recorded after 50 days from the beginning of the SD

Fig. 1. Effect of different photoperiods on flowering of plants of *S. rebaudiana* at the stage of 4–5 pairs of leaves. In the abscissa, days of treatment means the number of days from the beginning of treatments with different photoperiods. (○) 8h; (●) 10h; (▲) 12h; (■) 14,16 and 8hIN.

Fig. 2. Effect of different photoperiods on flowering of plants of *S. rebaudiana* at the stage of 10–12 pairs of leaves. In the abscissa, days of treatment means the number of days from the beginning of treatments with different photoperiods. (●) 12h; (▲) 13h; (■) 14, 15, 16 and hIN.

treatment. Plants with 3 pairs of leaves could not be induced to flower by SD. With 4 pairs of leaves the plants became sensitive to SD and at the 6th pair of leaves onward the plants reached the maximum sensitivity to flowering (Table 1).

(4) *Effect of growth regulators on flowering*: Seedlings with 5 pairs of leaves growing in CL were transferred to SD conditions where they received 4 applications of growth regulators at five day intervals. 100 μ g of

Table 1. Effect of the number of leaf pairs on the induction of flowering in *Stevia rebaudiana*.

Pairs of leaves	% of flowering plants
3	0
4	20
5	70
6	100

GA₃ and steviol were applied as a droplet on the apice and 40 mg of CCC as a soil drench at each application. Percentages of flowering plants were higher in GA₃-treated plants than in the control at least for 35 days from the beginning of the treatments. CCC and steviol delayed flowering, steviol being the agent which caused the more drastic effect (Fig. 4).

2. Effect of growth regulators on growth of plants kept in CL

(1) *Effect of GA₃*: Seedlings with 4 pairs of leaves received 5 applications of 100 µg of GA₃ applied as a droplet on the apice at 3 day intervals. Results are shown in Fig. 5. Data are statistically significant at the 5% level for GA₃ from the seventh day onward. Similar results were obtained for older plants with 10–12 pairs of leaves (8.80 cm for control and 27.40 cm for GA₃-treated plants).

(2) *Effect of CCC and GA₃*: Seedlings with 4 pairs of leaves received 5 applications of 40 mg of CCC as a soil drench and 5 applications of 25 µg of GA₃ as a droplet on the apice once a week. Results can be seen in Fig. 6. CCC alone strongly inhibited growth of *S. rebaudiana* and this inhibitory effect was counteracted by applications of GA₃. Data for CCC are statistically significant in relation to the control from the seventh day onward but differences between CCC and CCC+GA₃ are significant from the fourteenth day of treatment. Besides GA₃ and CCC, steviol and fusicoccin were also tested on the growth of *S. rebaudiana*. Steviol inhibited growth and fusicoccin had no effect. Nevertheless fusicoccin reversed the inhibitory effect caused by CCC (Table 2).

Fig. 3. Effect of different number of SD cycles on induction of flowering in *S. rebaudiana*. In the abscissa, days of treatment means the number of long days after the SD cycles.

(○) 0 and 1 SD; (■) 2SD; (●) 4SD; (▲) 6SD.

Fig. 4. Effect of some growth regulators on flowering of *S. rebaudiana*. In the abscissa days of treatment means the number of days from the beginning of growth regulators application.

(●) GA₃; (▲) CCC; (■) steviol; (○) control.

Discussion

Stevia rebaudiana Bertoni is a SD plant for flowering. This is confirmed by treatments of SD and SD with interrupted night⁸⁾. Plants with 4 to 12 pairs of leaves can be induced to flower in photoperiods shorter than 13 hours but remain vegetative in photoperiods longer than 14 hours. Then, *S. rebaudiana* was a short day plant with a critical day-length between 13 and 14 hours for flowering. Two SD cycles were partially effective to induce flowering. Increasing

Fig. 5. Effect of GA_3 on stem elongation of *S. rebaudiana*. In the abscissa, days of treatment means the number of days from the beginning of gibberellic acid application.

(▲) GA_3 ; (●) control.

Fig. 6. Effect of CCC and CCC+ GA_3 on stem elongation of *S. rebaudiana*. In the abscissa, days of treatment means the number of days from the beginning of growth regulators application.

(▲) CCC+ GA_3 ; (■) CCC; (●) control.

number of SD cycles increased precocity and the percentage of flowering plants. This is also known for other SD plants such as *Pharbitis nil*¹⁰⁾. This fact could be explained by an increase in the total number of hours of darkness which could accelerate reactions leading the plants to flower⁹⁾ or else to overcome the inhibitory effect of long days¹⁵⁾.

Precocity in flowering was attained when the plants were in the stage of 4 pairs of leaves and reached its maximum at the 6th pair onward. Also in *Lolium temulentum* older plants are more sensitive to the photoperiodic stimulus than younger ones⁶⁾. Growth of plants with 4 or 12 pairs of leaves was stimulated by relatively high concentrations of GA_3 . This promotive

Table 2. Effect of some growth regulators on stem elongation of *Stevia rebaudiana*.

Treatments	Stem elongation % of control
Steviol	68
Fusicoccin	95
CCC	70
Fusicoccin+CCC	117

effect was about the same for young or old plants. CCC reduced growth probably by inhibition of synthesis of endogenous gibberellins. As was expected, GA_3 counteracted the inhibitory effect caused by CCC. Steviol, known to have some gibberellin-like activity¹⁴⁾, inhibited growth of *S. rebaudiana*. Another GA-opposite effect of steviol was observed on flowering of *S. rebaudiana*. While GA_3 enhanced flowering steviol drastically delayed it.

Fusicoccin is known to have some GA-like properties¹¹⁾. In *Stevia* fusicoccin was ineffective at least in the two concentrations used (1.8 and 18.0 μ g) but like GA_3 counteracted the inhibitory effect of CCC.

None of the growth regulators tested affected the number or rate of leaf initiation in *S. rebaudiana*.

In conclusion, it is advisable to grow *S. rebaudiana* with a photoperiod of 14 or more hours or SD with interruption of the night for vegetative growth. When seeds are needed for propagation, plants of *Stevia* with more than 4 pairs of leaves shall be submitted to at least two photoperiodic cycles of less than 14 h in order to induce flowering.

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Summary

To obtain some information for the vegetative and reproduction growth of *Stevia rebaudiana* Bertoni, the effects of photoperiod and some growth regulators were examined. *Stevia rebaudiana* flowers when the daylight

is shorter than 14 hours (Figs. 1 and 2), which suggested that *S. rebaudiana* could be seen as a short day plant for flowering. According to the detailed studies, it was ascertained that a minimum of 2 inductive shortday cycles are necessary for flowering induction (Fig. 3). They can be induced to flower from the 4 pairs of leaves stage onward (Table 1).

(2-chloroethyl-trimethyl ammonium chloride (CCC) and enzymatic hydrolysis of stevioside (steviol) inhibit vegetative and reproductive growth while gibberellic acid (GA_3) enhances the growth (Figs. 4, 5 and 6). Fusicoccin partially counteracted the inhibitory effect of CCC (Table 2).

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〔和 文 摘 要〕

ステビアの生長と開花に及ぼす日長と生長調節物質の効果

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ステビオサイド生産に対する基礎的知見を得るために、パラグアイ産の系統を用いて日長と数種の生長調節物質が栄養生長と生殖生長に及ぼす影響を調査した。

ステビアは 14 時間より短い日長では開花するがそれ以上の日長では開花しない事から (第 1・2 図), 短日植物の一種であることが判った。なお詳細な分析結果からみて, 最少必要短日処理は 2 回である事 (第 3 図) および 4 対葉の時期から日長に感応し始める事 (第 1 表) が明らかとなった。

生長調節物質の影響についてみると, CCC とステブイオールは栄養生長及び開花を抑制するがジベレリンはいづれも促進する事 (第 4・5・6 図), 及びフシコシンは CCC の抑制作用を部分的に阻止する働きがある事 (第 2 表) が確認された。