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Changes in the gibberellin content of *Bryophyllum daigremontianum* in connection with floral induction

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Summary

Changes in the content of gibberellin(GA)-like substances of the long-short-day plant *Bryophyllum daigremontianum* during photoinduction were investigated by extraction and bioassay on d5-corn. After transfer from LD to SD the GA content increased to a maximum at 14 SD, which is the minimal duration of inductive treatment necessary for flowering. At the time flower primordia started to differentiate, the GA level reached a second peak. Between the two maxima the GA content dropped to a low level at 20 SD. Under LD conditions the GA content showed little variation.
Plants shifted to SD at a night temperature of 23°C did not flower, and the GA content diminished to that of plants grown in SD.
Variations in the GA level were due to changes in fraction II, which is probably identical with GA_5. No significant changes in the level of fraction I were observed.

Introduction

Flower formation in the long-short-day plant *Bryophyllum daigremontianum* can be induced by the shift from long-day (LD) to short-day (SD) conditions, or by application of gibberellin A_3 (GA_3) to plants in SD. Thus, GA_3 can replace the LD, but not the SD step of photoinduction, suggesting that GA is the limiting factor for floral induction in SD. Direct evidence to support this idea has been obtained in extraction experiments which indicate that the level of endogenous GA-like substances is much higher in plants grown in LD than in those grown in SD (Skene and Lang, 1964; Zeevaart, 1969b).
Following the transfer from LD to SD, internodes of *Bryophyllum* elongate considerably more than under permanent LD, and it was suggested therefore that photoinduction may be accompanied by an increase in the level of GA-like substances (Zeevaart and Lang, 1962).
The present work was performed to determine possible changes in the content of GA-like substances in connection with floral induction and the appearance of flower primordia.

Material and methods

Plants of *Bryophyllum daigremontianum* (R. Hamet et Perr.) Berg. were of the same strain as used in earlier experiments (Zeevaart and Lang, 1962).

All plants were initially grown in a greenhouse (Zeevaart, 1969b). Plants to be used for extraction experiments were first moved to a growth room with 16 hours light from fluorescent and incandescent lamps (intensity 3000 ft-c) at 25° C, and 8 hours darkness at 18° C for at least 10 days, before they were transferred to flower-inducing conditions.

Photoperiodic regimes and temperatures in growth chambers during the experimental treatments were the same as described previously (Zeevaart, 1969a).

At the beginning of an experiment the lower leaves were removed and only the uppermost 6 leaf pairs, including the youngest pair longer than 2 cm, were retained. Plants were grown under inductive conditions for various periods of time until harvested. A few plants were returned to LD conditions at each harvest to observe the flowering response. Two samples of plant material were harvested in each treatment: 1) the basal 3 leaf pairs, called 'old leaves'; 2) the upper leaves, including the stem and shoot apex, called 'shoot tips'. The material was frozen in liquid nitrogen, lyophilized and the dry weight determined.

Extraction procedures and preparation of the acidic fractions containing GA-like substances have been described in detail (Zeevaart, 1969b). Preparative thin-layer chromatography (TLC) was carried out on silica gel H 20 × 20 cm plates which were 400 μm thick and were developed in solvent system 1 for 15 cm. Ten equal zones were scraped off, eluted and routinely assayed on d5-corn (Zeevaart, 1969b). Active fractions were occasionally also tested on d1-corn, on dwarf pea and in the α-amylase assay (Jones and Varner, 1967). The data are expressed as μg GA_3-equivalents per 100 g dry weight, or per 10 plants. The solvent systems used for TLC were the following:

Solvent system 1 (Sembdner et al., 1962): Chloroform - ethyl acetate - acetic acid (60 : 40 : 5).

Solvent system 2 (Ikekawa et al., 1963): Carbon tetrachloride - acetic acid - water (8 : 3 : 5) were shaken in a separatory funnel and separated into 2 phases. The TLC plates were equilibrated with the upper phase overnight, and then developed with a mixture of the lower phase (5 parts) and ethyl acetate (1 part). Following preparative TLC in solvent system 1, fraction II (R_F 0.4–0.6), was re-chromatographed on TLC plates coated with a 250 μm layer of silica gel H. Fractions were applied as a narrow band 15 cm long. Gibberellin standards were spotted in the middle of the band. To improve the resolution, the plates were pre-developed with acetone - acetic acid (100 : 1) until the zone of application was in a narrow line about 0.5 cm from the origin (Verbiscar et al., 1967). After drying, the plates were equilibrated and developed to 15 cm in solvent 2. Zones of 1 cm were scraped off and prepared for bioassay, leaving a central zone across the plate with the reference GA's intact. This part of the chromatogram was sprayed with 5% (v/v) H_2SO_4 in ethanol, heated at 120° C for 10 minutes and the fluorescent GA spots viewed in UV light.

Results

Results of an experiment in which harvests were made after increasing durations of inductive SD conditions, are given in Fig. 1. Flower formation took place in plants exposed to 14 or more SD, but flower primordia did not become macroscopically visible until after 35 days. Considerable variation in the level of GA-like material occurred after the shift LD → SD, both in the shoot tips and in the old leaves. An

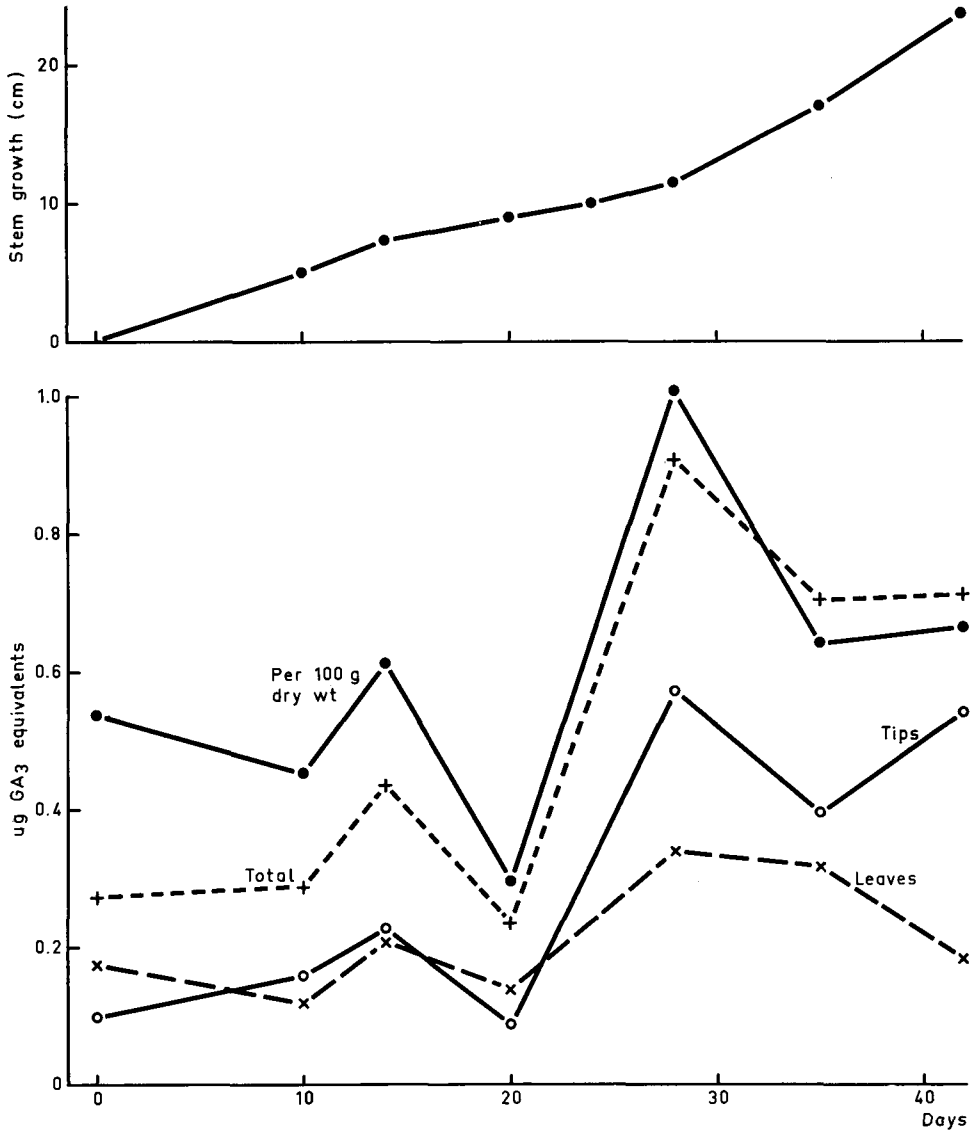

Fig. 1 Growth (top) and content of gibberellin-like substances (bottom) in Bryophyllum daigremontianum *following transfer from long-day to short-day conditions at a night temperature of 15° C. Data calculated as μg GA_3-equivalents per 10 plants. Total GA content also plotted as μg GA_3-equivalents per 100 g dry weight. Six plants harvested per treatment.*

increase at 14 SD was followed by a sharp decline at 20 SD. It is of interest to note that the minimum in GA content at 20 SD was associated with a lower growth rate (Fig. 1). The peak in GA content at 14 SD and the low level around 20 SD

have been observed in other experiments as well, although the magnitude of the changes varied considerably from one experiment to another. In one case the level after 14 SD had increased 96% over that of the initial harvest when calculated on a dry weight basis.

A second maximum in the GA content was reached after 28 SD, but in another experiment it was found after 35 SD. A higher GA level was always due to the higher activity of fraction II (R_F 0.4–0.6). The level of fraction I (R_F 0.1–0.3) was always low in comparison to that of fraction II, and no clear-cut changes in the level of this fraction were noticed. Since the shift LD → SD did not only involve a change in daylength, but also a shift to lower temperature, an additional experiment was performed to compare plants grown in LD and in SD under identical temperature conditions. Furthermore, a SD treatment with a night temperature of 23°C was included. It is known (Zeevaart and Lang, 1962) that plants transferred from LD to this SD environment are not induced to flower formation.

The plants were harvested after 14 and 36 days. Results of GA determinations are given in Table 1. The GA content of plants grown in LD at 15°C remained quite constant when expressed on a dry weight basis. On the other hand, plants shifted to SD at 15°C had an increased GA content after 14 SD as compared to plants in LD. After 36 SD the level had increased much further. This shows that the changes in GA content observed after the shift LD → SD are not merely temperature effects, but are related to photoinduction.

The plants shifted from LD → SD with a 23°C night temperature remained vegetative and exhibited a SD growth habit after 36 days. The GA content of the shoot tips was at that time the same as that of plants grown in continuous SD (Table 1).

Table 1 Growth, flowering response and levels of gibberellin-like substances in Bryophyllum daigremontianum *as affected by different photoperiods and night temperatures. Seven plants harvested per treatment.*

Treatment [1]		*Shoot growth (cm)*	*Flowering response at harvest* [2]	*GA_3-equivalents per 100 g dry wt (µg)*	*GA content of 14 LD (%)*
LD → 14 LD 15°C	Tips	7.2	V	0.27	100
	Leaves			0.20	100
LD → 36 LD 15°C	Tips	21.0	V	0.30	111
	Leaves			0.23	115
LD → 14 SD 15°C	Tips	6.1	V	0.35	129
	Leaves			0.25	125
LD → 36 SD 15°C	Tips	17.6	F	1.36	504
	Leaves			0.37	185
LD → 14 SD 23°C	Tips	7.4	V	0.11	40
	Leaves			0.17	85
LD → 36 SD 23°C	Tips	13.7	V	0.02	7
	Leaves			0.04	20
36 SD 15°C	Tips	6.4	V	0.02	7
	Leaves			0.02	10

[1] Temperatures indicated are for the 16-hour long night for SD treatments, or in the case of LD for 8 hours of supplementary light + 8 hours darkness.

[2] V = vegetative; F = flower buds.

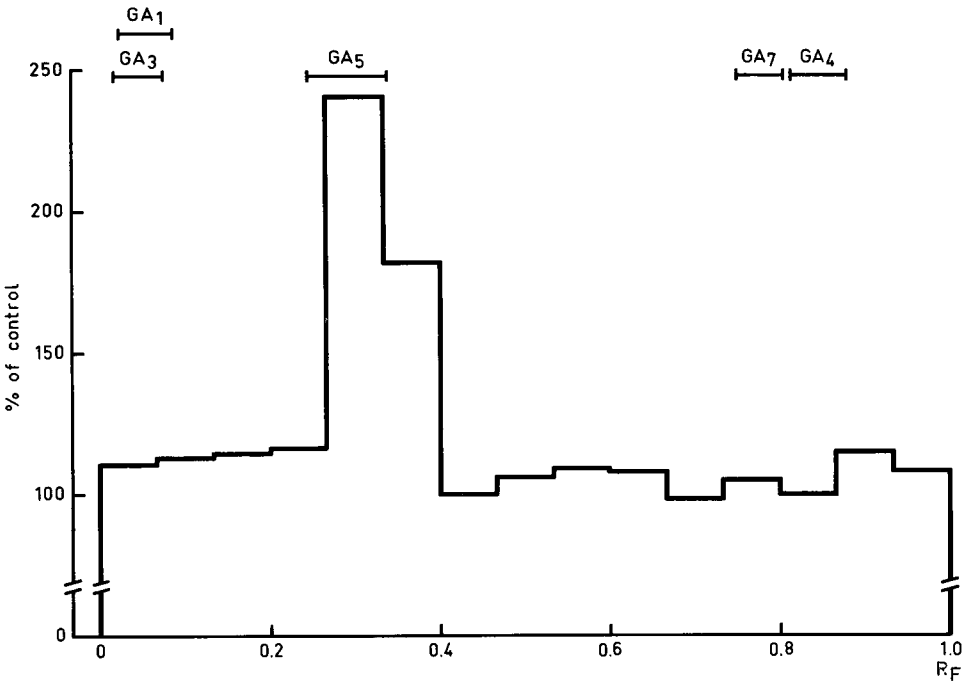

Fig. 2 D5-corn bioassay of Bryophyllum *fraction II following re-chromatography on a 250 μm layer of silica gel H in solvent system 2. The horizontal bars indicate the positions of co-chromatographed authentic gibberellins.*

After re-chromatography of fraction II in solvent 2, only one zone with biological activity was obtained (Fig. 2); it had the R_F (0.30) of authentic GA_5. Biological properties of fraction II also indicated that it is similar to GA_5: active in d5-corn, but little or no activity in dl-corn, dwarf pea, or in the α-amylase release assay.
Fraction I, on the other hand, is quite active in the release of α-amylase by barley half seeds. Insufficient amounts of fraction I were available for more extensive studies.

Discussion

The data presented above show that after the shift LD → SD, the GA content reaches a maximum after 14 SD, which coincides with the minimal number of SD necessary for photoinduction. A second peak was observed after 28 SD, or later, when floral primordia started to differentiate.
Plants transferred to SD at a night temperature of 23°C did not flower and the GA content dropped sharply, particularly in the shoot tips. This suggests that the low level of GA is due to decreased synthesis rather than increased metabolism. In the latter case, the GA content of old leaves would have been low, too.
The amount of GA-like substances extractable from plants grown continuously in LD remained practically constant when expressed on a dry weight basis, and the plants continued growing at the same rate for long periods of time. Applied GA_3

had little effect on shoot growth in LD. E.g., application of 100 μg GA_3 per plant increased shoot growth by 40% over a 50-day period, as compared to a growth increment of 560% in plants treated with 40 μg GA_3 in SD. Thus, the growth rates of plants grown in LD and in SD conditions were correlated with the amounts of extractable GA-like substances, and also with the effects of applied GA_3. However, according to the data in Table 1 more stem growth took place in LD than after the shift LD $\rightarrow$ SD 15°C, despite the fact that the latter group of plants had a higher GA content.

Major changes occurred in the level of fraction II. Although the evidence is not conclusive, results of TLC and bioassays strongly suggest that fraction II is identical with GA_5, or the related *Pharbitis* gibberellin, GA_{20} (Murofushi et al., 1968). Since different levels of fraction II were associated with different growth rates, one might expect this GA-like substance to be highly active. However, GA_5 applied to *Bryophyllum* was at least 10 times less active than GA_3 in causing stem growth and flower formation (Zeevaart, unpublished data). Also, in plants treated with GA_3 in SD, fraction II could be detected only in trace amounts while the level in photoinduced plants was high (Zeevaart, 1969b). Therefore, fraction II is presumably not essential for flowering. One possible interpretation is that fraction II itself has little or no biological activity, but must first be converted to fraction I, which has GA_3-like properties. This leaves unexplained, however, the observation that there are no significant changes in the level of fraction I during photoinduction.

In conclusion, changes in the level of fraction II in *Bryophyllum* are correlated with growth and developmental responses, but the precise physiological significance of these findings is not clear at present.

Acknowledgments

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