Effect of Photoperiod on the Levels of Endogenous Gibberellins in Spinach as Measured by Combined Gas Chromatography-selected Ion Current Monitoring

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ABSTRACT

The changes in the levels of five endogenous gibberellins (GAs) in spinach in relation to photoperiodic treatment have been examined by combined gas chromatography-selected ion current monitoring. Long-day treatment caused a 5-fold decline in the level of GA15 while the levels of GA30 and GA20 increased dramatically during the same period. In absolute terms, the level of GA30 increased from 0.8 microgram per 100 grams dry weight in short days to 5.5 micrograms per 100 grams dry weight after 14 long days. The levels of GA17 and GA44 did not change significantly with long-day treatment. These results are consistent with the hypothesis that GA15 is converted to GA20 and that this conversion is under photoperiodic control. Since stem growth in spinach is correlated with an increase in the level of GA30, one major aspect of photoperiodic control of stem growth might be the availability of GA30 through regulation of the conversion of GA15 to GA30.

It has been demonstrated in a number of rosette plants, including Silene armeria (1), Agrostemma githago (9), and Spinacia oleracea (16), that photoperiodic control of stem growth is mediated by GAs. In spinach, LD3 treatment of plants previously maintained under SD does not increase the total GA level, but levels of individual GAs vary, indicating a profound change in GA metabolism (16, 17). It is assumed, therefore, that this change in GA metabolism is of physiological significance in the photoperiodic regulation of stem growth.

However, any study of photoperiodic control of GA metabolism requires preliminary information on the nature of GAs present in the plant and also on the quantitative changes in the levels of particular GAs with a change in photoperiod. Early work from this laboratory has shown by bioassay that changes occurred in levels of GA-like substances in spinach upon transfer from SD to LD (16, 17). Bioassays have, however, several limitations (5), most important of which is the difference in sensitivity to various GAs.

Recently, the identification of six C-13 hydroxylated GAs (Fig. 1) in spinach shoots have been reported (12). This set the stage for an analysis of the quantitative changes in the levels of those GAs by GLC-SICM.

MATERIALS AND METHODS

Plant Material and Photoperiodic Treatment. Seeds of spinach (Spinacia oleracea cv Savoy Hybrid 612, Harris Seed Co., Rochester, N. Y.) were sown in vermiculite. After 10 days, the seedlings were transferred to 340-ml plastic cups containing a gravel-vermiculite mixture (1:2) and were watered twice daily with half-strength Hoagland’s solution. The plants were maintained under SD conditions in growth chambers under the conditions described before (16) until ready for experimentation 6 weeks after sowing. SD treatments consisted of an 8-h period of light from fluorescent and incandescent lamps, followed by 16 h darkness. LD treatment consisted of the same 8-h illumination as in the SD treatment, followed by 16-h low intensity illumination from incandescent lamps (16). The LD treatments were staggered in such a way that all plants were harvested at the same time. In each treatment 10 plants were employed. At the end of an experiment, the stem length of each plant was determined. The shoots then were cut off.
at the soil level, frozen in liquid N₂, lyophilized, and stored at −15 C prior to extraction.

Extraction and Purification Procedures. The extraction and purification procedures were identical to those described previously (12). Methanolic extracts were reduced to a small aqueous residue and then purified by charcoal adsorption chromatography and silicic acid adsorption chromatography. The eluate resulting from silicic acid adsorption chromatography was fractionated by preparative reverse-phase HPLC (Bondapak C₁₈/PGISil B) as described before (8, 12). Fractions known to contain GA₄₄, GA₁₉, GA₁₇, and GA₂₀ (all monohydroxylated GAs) were combined and subjected to analytical reverse-phase HPLC (µBondapak C₁₈) as described before for monohydroxylated GAs (12). The fractions containing these four GAs then were combined. The fraction from preparative HPLC that contained GA₂₀ was also purified with analytical reverse-phase HPLC, using the gradient system described previously for dihydroxylated GAs (12).

The resulting final two fractions then were methylelated with ethereal diazomethane. The trimethylsilyl ethers of the methyl esters were prepared by addition of 20 μl pyridine-hexamethyl-disilazane-trimethylchlorosilazane (9:3:1, v/v) to methylelated samples dried in a capillary.

GLC-SICM. GLC-SICM was performed using a Hewlett-Packard 5985 mass spectrometer that was interfaced by a glass jet separator with a Hewlett-Packard 5840-A gas chromatograph. The four monohydroxylated GAs were chromatographed using a glass column (183 × 0.2 cm i.d.) packed with 2% SP-2401 on 100/120 Supelcoport. Samples (2 μl) were injected onto the column at 180 C. Following a 2-min isothermal hold, the temperature was programmed 10 C min⁻¹ until the column temperature was 205 C, whereupon the rate was slowed to 1 C min⁻¹. When the temperature reached 215 C, the rate was increased to 20 C min⁻¹ until the column reached the maximum temperature of 255 C. GA₂₀ was chromatographed on 2% SP-2100 with column conditions identical to those described before (12).

For each GA, three fragments with the following m/e values were monitored: GA₄₄, 432, 373, and 207; GA₁₉, 462, 434, and 374; GA₂₀, 418, 419, and 375; GA₁₇, 492, 460, and 208; GA₂₀, 506, 507, and 207. The dwell time of each fragment monitored was 200 ms. The relative level of each GA was calculated from the SICM response of the molecular ion of each compound, except for GA₁₉, in which case the base peak (m/e 434) was used. The ratios of the SICM response of the three fragments monitored for each GA were checked in every sample to ensure that interfering compounds did not affect the SICM response. Each sample was analyzed twice, and the average of the two readings was used in the subsequent calculations. No pair of readings ever differed by more than 5%. Other parameters of the mass spectrometer were the same as described before (12).

RESULTS AND DISCUSSION

Using GLC-SICM, a standard calibration curve was constructed for GA₂₀ so that the absolute amount of GA₂₀ present in the plant material could be determined. This curve was linear over a range from 10 to 200 ng. The lower limit of sensitivity was 1 ng, which is about the same limit of sensitivity as the d-5 corn bioassay (unpublished observations). In contrast, at least 100 ng was necessary to get a good spectrum using repetitive scanning GLC-MS.

Because authentic samples of the other GAs present in spinach were either not available or were available in quantities too small to permit accurate weighing, standard calibration curves for these GAs could not be constructed. In these cases, only changes in the relative levels could be expressed. The largest SICM response for a given GA from the series of photoperiodic treatments was normalized to a unit dry weight basis and then arbitrarily assigned a value of 100. Normalized values from the other treatments then were expressed in proportion to the highest SICM value.

Figure 2 shows the changes in the relative levels of five GAs as well as stem height as a function of LD treatment. GA₂₀ occurred in quantities too small to be measured. The relative level of GA₁₉ declined 5-fold with LD treatment, whereas the levels of both GA₂₀ and GA₂₉ increased dramatically during the same period (Fig. 2). The levels of GA₁₇ and GA₂₉ remained fairly constant throughout photoperiodic treatment. In absolute terms, the level of GA₂₀ increased from 0.8 μg/100 g dry weight (30 ng/plant) to 5.5 μg/100 g dry weight (200 ng/plant), nearly a 7-fold increase. The relative changes in the levels of GA₁₉ and GA₂₀ with LD treatment as measured by GLC-SICM were also observed in a second experiment and confirm earlier work from this laboratory using the d-5 corn bioassay for quantitating the levels of GA-like substances (16, 17). However, GA₂₀, which reportedly has very little biological activity in the d-5 corn bioassay (15), would have been overlooked in the earlier work. Recent work has shown that the biological activity associated with spinach shoot extracts is due almost entirely to the combined effects of GA₁₉ and GA₂₀ (12). This, along with the fact that GA₁₉ and GA₂₀ give similar responses in the d-5 corn bioassay (2), indicates that, in absolute terms, GA₁₉ occurs in a similar, but inverse, range of quantities as GA₂₀.

In recent work from this laboratory (12), it was proposed that the following metabolic pathway based on structural considerations occurred in spinach: GA₂₀ → GA₂₉ → GA₁₉ → GA₁₇ → GA₂₀ → GA₂₀. The decline in the level of GA₁₉ with the concomitant rise in the level of GA₂₀ in spinach is indicative of a precursor-product relationship between the two GAs. Moreover, the co-occurrence of GA₁₉ and GA₂₀ in a number of disparate species, including Agrostemma githago (10), Pharbitis nil (8), Phaseolus coccineus (5), Pisum sativum (7), and Zea mays (13), is also circumstantial evidence supporting the notion that GA₁₉, a C₅₀₅-GA, is eventually converted to GA₂₀, a C₆₀₀-GA. However, as yet, no definitive biochemical evidence is available that proves such a conversion takes place (5, 6).

The initiation in the rise of the level of GA₂₀ lagged slightly behind the increase in the amount of GA₂₀ (Fig. 2) and was also

![Figure 2](https://www.plantphysiol.org/)

**Fig. 2.** Changes in the relative levels of five GAs in spinach as measured by GLC-SICM, and stem length as affected by different durations of LD treatment. The highest concentration (SICM response/unit dry weight) of each GA was arbitrarily assigned a value of 100 and the other concentrations were expressed in proportion to this value. The SICM response of the molecular ion was used in all calculations except for GA₁₉, in which case the base peak was used. Ten plants were used in each treatment.
observed in a second experiment (results not shown). This is consistent with the idea that GA_{20} is converted to GA_{30}. Indeed, this conversion has been demonstrated in a number of systems, including P. sativum (4), Bryophyllum daigremontianum (3), and Phaseolus vulgaris (14). Probably such a conversion is also a natural process in spinach. Inasmuch as 28-hydroxylated GAs, such as GA_{20}, are usually inactive in eliciting GA responses, conversions of the type proposed above are generally thought to be inactivation steps (6).

Stem growth is preceded by a steep rise in the level of GA_{30}. Because exogenous GA_{30} is able to cause stem growth in spinach plants maintained under SD (unpublished results), a major factor in the control of stem growth in spinach could be the availability of endogenous GA_{30}. The level of GA_{30} is controlled through a balance of production and metabolism (16, 17). If GA_{19} is a precursor of GA_{30}, the data presented in Figure 2 indicate that the steps GA_{19} → GA_{30} are under photoperiodic regulation. This suggests that one major aspect of photoperiodic control of stem growth in spinach is effected through regulation of this conversion. The fact that GA_{19} is at its highest level during SD could mean that it is itself inactive in promoting stem growth and must be converted to biologically active GA_{30}. Consequently, GA_{19} could serve in spinach as a “pool” gibberellin, a role postulated for GA_{19} in rice (11). Obviously, the exact metabolic relationship between the endogenous GAs must be ascertained before a clear picture of the mechanism of photoperiodic control of stem growth can be made.

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LITERATURE CITED


