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Gibberellic Acid-Induced Cell Elongation in Cotton Suspension Cultures

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Abstract. Gibberellic acid (GA_3) causes cell elongation in cotton suspension cultures derived from cotton ovule callus tissue of both auxin-dependent and -independent lines. Cell elongation was more pronounced in auxin-dependent cultures. Cells were cultured for a period of 14 days but differences in cell lengths could be detected after 6 days in culture. Cell elongation took place in cultures in which GA_3 was present throughout the culture period or only for the first 3 days. Auxins and cytokinin alone or in the presence of GA_3 did not promote cotton cell elongation above the value for the treatment with GA_3 alone.

One of the many physiological processes affected by gibberellins is stem elongation. Excised stem tissue has been used to analyze the mode of action of gibberellins on stem elongation (Jones and Moll 1983, Kaufman and Dayanandan 1983, Raskin and Kende 1984). However, stem sections are composed of a number of cell types which respond differently to exogenous gibberellins. The use of a simple biological system consisting of one cell type should allow the separation of the primary action of gibberellins from the secondary effects. Fry and Street (1980) attempted to define conditions which would promote gibberellin-induced cell expansion in suspension cultures. *Rosa* cells elongated and *Spinacia* cells expanded isodiametrically after gibberellic acid (GA_3) treatment; whereas cultures of *Daucus, Nicotiana, Vinca,* and *Catharanthus* were not sensitive to exogenous GA_3. Parsley cell suspensions treated with 1 μM GA_3 were 20 μm longer than untreated controls (Grossmann 1988).

Cells in suspension cultures initiated from cotton ovule callus tissue can be induced to elongate in the presence of GA_3 (Davidonis 1989, Trolinder et al. 1987). The purpose of this paper is to demonstrate the sensitivity of cotton cell cultures to GA_3 and other growth regulators which affect cell elongation.

Materials and Methods

Callus cultures were derived from *Gossypium hirsutum* L. (cv. Texas Marker 1) ovules removed 7 days preanthesis. Callus was initiated and subcultured on a modified Murashige and Skoog (MS) medium containing MS salts (Murashige and Skoog 1962), B5 vitamins (Gamborg et al. 1968), 0.55 mM myo-inositol, 167 mM glucose, 2.5 μM kinetin, 0.45 μM 2,4-dichlorophenoxyacetic acid (2,4-D), and 8.75 g/L agar (Davidonis 1989). After several subcultures, various callus phenotypes appeared. Cultures that were pale yellow, soft, and friable were subcultured every 2–3 weeks. Cultures were maintained in the dark at 32°C. Callus cells are not uniform in cell length and this variability in cell length was seen in suspension cultures derived from callus tissue.

Callus tissue (1.5–2.0 g) was harvested 2–3 weeks after transfer. Callus tissue was placed into 25 ml of modified Beasley and Ting salts with $CaCl_2$ reduced to 1.5 mM and vitamins (Beasley and Ting 1973), 1 mM myo-inositol, and 111 mM glucose (pH 5.9). The following plant growth regulators obtained from Sigma Chemical Company (St. Louis, MO, USA) were used: GA_3 (0.1–10 μM), 2,4-D (0.22 and 0.45 μM), naphthaleneacetic acid (NAA, 5.4 μM), 2(isopentyl)adenine (2ip, 1.2 and 2.5 μM), and 2(p-chlorophenoxy)-2 methylpropionic acid (PCIB, 5 and 10 μM). Cultures were incubated at 32°C in the dark on an orbital shaker (100 rpm) for 14 days. Each treatment consisted of three replicate flasks. One hundred cells were measured from each flask with an ocular micrometer. Cell lengths of single cells and cell aggregates of not more than four cells were measured. Due to the nonuniform distribution of cell lengths in the starting material, the results were reported as the percentage of cells in a certain length category. The average initial fresh weight was calculated, and tissue samples of that weight were dried (60°C) to give an initial dry weight. After 14 days the cells were harvested and dried.

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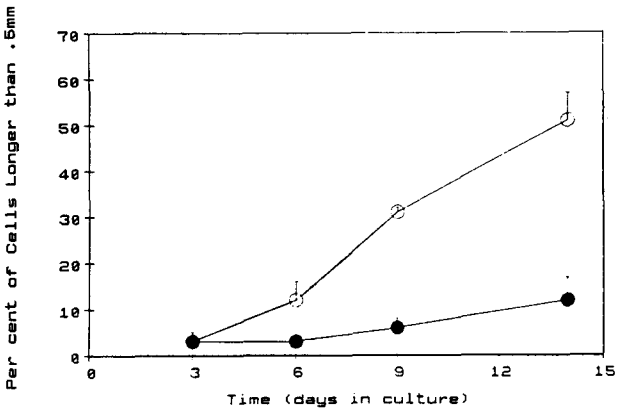

Fig. 1. Time course of cell elongation in auxin-dependent cotton suspension cultures treated with 5.7 μM GA_3 (○) or untreated (●). Results show means of three replicates ± SE.

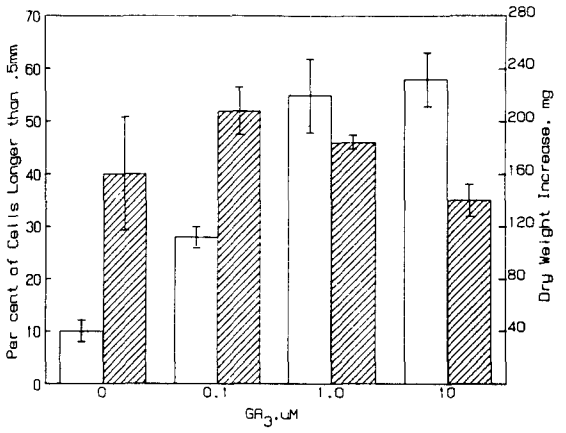

Fig. 2. Effects 0.1, 1.0, and 10 μM GA_3 on cell length and dry weight of auxin-dependent cotton suspension cultures. Cell length, open bars; dry weight, hatched bars. Results show means of three replicates ± SE. An ANOVA or ranks of actual data values showed that at $p = 0.05$, 0.1 μM GA_3 was not significantly different from the no GA_3 treatment (cell length) and that dry weight increase means were not significantly different.

The results of several experiments comparing GA_3 treatment with other growth regulator treatments were combined. This procedure was valid based on the outcome of chi-square tests for GA_3 treatments. This procedure was also used to check the agreement of replicates for a given treatment. Treatments resulting in a p-value of $<.05$ were considered to contain an outlier replicate. In each case the outlier replicate was omitted. P-values from the chi-square test comparing each treatment to GA_3 are listed in the Results and Discussion.

Results and Discussion

Callus tissues derived from preanthesis cotton ovules were subcultured on agar-solidified medium over a period of 1 year. Periodically, callus cell lines were checked for auxin-independence. Auxin-independent cell lines grew well on a medium containing only kinetin. Suspension cultures were derived from auxin-dependent and auxin-independent callus cultures. After 3 days in suspension culture, GA_3-treated auxin-dependent cells were not distinguished from untreated cells (Fig. 1). A difference in cell length distribution was seen after 6 days in culture. Untreated cells showed some elongation but the percentage of GA_3-treated cells longer than 0.5 mm was five times greater after 14 days in culture. Untreated cells showed no changes in cell widths during the culture period. After 6 days in culture, GA_3-treated cells were slightly wider than untreated cells. Suspension cultures (auxin-dependent cells) were initiated in the presence of GA_3 (5.7 μM), after 3 days the medium was replaced with fresh media containing no GA_3 or GA_3 (5.7 μM), and the cells were cultured an additional 11 days. The percentage of cells longer than 0.5 mm was the same for cultures treated for 3 days with GA_3 or 14 days with GA_3. Callus cells from auxin-dependent and auxin-independent cell lines were placed into liquid media containing 0.1–10 μM GA_3. The percentage of cells longer than 0.5 mm increased with an increase in GA_3 concentration (Figs. 2 and 3). An ANOVA on the ranks of actual data values was used to determine if the cell length percentage and dry weight increase means were significantly different. An increase in GA_3 concentration coincided with a decrease in dry weight in auxin-independent cultures but not in auxin-dependent cultures. Cotton cells remained GA-sensitive for over 1 year.

The results of similar sets of experiments with a GA_3 control treatment showed some culture variations, but it is clear from the results that GA_3 promoted cell elongation (Table 1). Auxin and cytokinin concentrations used alone or in combination with GA_3 were concentrations normally used in plant tissue culture. Low auxin concentrations did not promote cell elongation above the GA_3 level. Treatment with the antiauxin PCIB (5 and 10 μM) alone or in combination with GA_3 did not enhance elongation above the GA_3 level. Isopentenyl adenine at the lower concentration (1.2 μM) did not inhibit the GA_3 response, but 2.5 μM 2ip did counteract GA_3-induced elongation as did 0.45 μM 2,4-D and 5.4 μM NAA. A pattern emerged and showed that the maximum response to GA_3 was realized when GA_3 was used alone or in combination with 0.22 μM 2,4-D, 1.2 μM 2ip, and PCIB.

During the suspension culture period, the length of parsley cells did not increase during the stationary phase of growth (Grossmann 1988). Thus, after

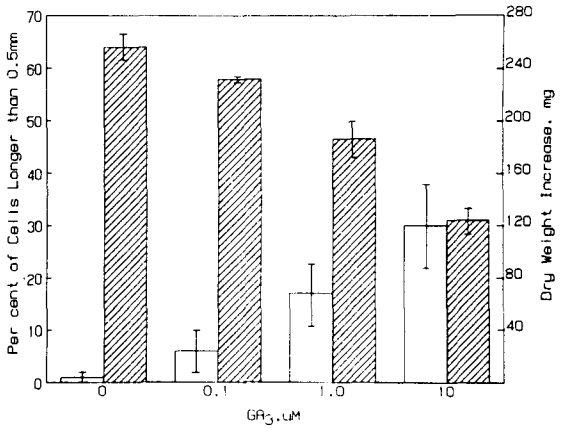

Fig. 3. Effects of GA_3 on cell length and dry weight of auxin-independent cotton suspension cultures. Cell length, open bars; dry weight, hatched bars. Results show means of three replicates ± SE. An ANOVA on ranks of actual data values showed that at $p = 0.05$, all GA_3 treatments were significantly different from the no GA_3 treatment (cell length and dry weight increase).

Table 1. Elongation of auxin-dependent cotton cells after the simultaneous addition of GA_3 and other growth regulators.

Treatment	Numbers of cells (frequency)		
	Cell length (mm)		
	<0.5	0.5–1.0	>1.0
5.7 μM GA_3	33	53	14
0.22 μM 2,4-D	73	24	3[a]
0.45 μM 2,4-D	73	24	3[a]
5.4 μM NAA	90	9	1[a]
1.2 μM 2ip	93	7	0[a]
2.5 μM 2ip	67	29	4[a]
5 μM PCIB	80	20	0[a]
10 μM PCIB	87	13	0[a]
5.7 μM GA_3 + 0.22 μM 2,4-D	35	53	12
5.7 μM GA_3 + 0.45 μM 2,4-D	49	46	5[a]
5.7 μM GA_3 + 5.4 μM NAA	44	46	10[a]
5.7 μM GA_3 + 1.2 μM 2ip	35	55	10
5.7 μM GA_3 + 2.5 μM 2ip	43	47	10[a]
5.7 μM GA_3 + 5 μM PCIB	32	54	13
5.7 μM GA_3 + 10 μM PCIB	35	55	10
No regulators	79	21	0[a]

[a] Frequency distributions significantly different from 5.7 μM GA_3 at $p = 0.05$. See Materials and Methods for discussion of the data. Cells were grown in the presence or absence of growth regulators and measured after 14 days.

4 days in culture parsley cells treated with GA_3 were 36-μm long and after 12 days 60-μm long. Parsley cell suspensions were sensitive to GA_3 in the range of 1–100 μM and an increase in the concentration above 1 μM did not enhance cell elongation but led to a decrease in cell number. A similar situation may exist in cotton cell suspensions, since a difference in length was detected after 6 days in culture and a decrease in the dry weight of auxin-independent cotton cells treated with GA_3 was noted.

The manner in which cotton cell suspensions responded to GA_3 (0.1–10 μM) compares favorably with excised stem segments. Lettuce hypocotyls elongated in the presence of 0.1–1 μM GA_3, and oat internodes were sensitive in the concentration range of 10 μM–1 mM (Jones and Moll 1983, Kaufman and Dayanandan 1983). A sustained growth response was elicited in lettuce hypocotyls and oat internodes subjected to a short pulse of GA_3 (Montague et al. 1973, Silk et al. 1977). In the current study cotton suspension cultures treated with GA_3 for 3 days responded in the same manner as cells treated continuously with GA_3. Auxin and NAA, in combination with GA_3, did not enhance elongation above the GA_3 control and was inhibitory at concentrations above 1 μM in lettuce hypocotyl sections (Jones 1980). Also PCIB (0.001–100 μM) did not affect GA_3-induced growth in lettuce hypocotyl sections. In the cotton cell suspension system, neither auxins nor PCIB enhanced elongation. The cotton cell suspension cultures described in this paper should serve as an excellent system for cell elongation. Other cotton cell suspensions derived from cotton hypocotyl cells did not elongate to the same degree as those derived from cotton ovules (Davidonis 1989).

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