J Plant Growth Regul (1988) 7:197–201

Journal of
Plant
Growth
Regulation

Effects of Gibberellic Acid and Zeatin on the Growth Response of Cucumber Cotyledons

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Received January 27 1987; accepted February 19, 1988

Abstract. In contrast to cytokinin, gibberellic acid has no effect on the growth of the isolated cucumber cotyledon in darkness. Like cytokinins in light, gibberellic acid causes increases in fresh weight and area of the cotyledon at concentrations from 10^{-7} to 10^{-3} M. Radiant energies in the blue, red, and far-red regions of the spectrum all induce the growth responses to gibberellic acid. The effect of the far red is greater than that of the red, which is greater than that of the blue. Gibberellic acid is ineffective in the promotion of chlorophyll development, whereas cytokinins are very effective. Although zeatin and gibberellic acid both cause an increase in fresh weight and area of the cotyledons in light, they appear to have entirely separate actions in the growth responses.

Although there are many publications concerning the effects of cytokinins on isolated cotyledons, few exist on the effects of gibberellins. Kursanov et al. (1969) examined the effects of 6-benzylaminopurine (BAP) and gibberellic acid (GA) on cotyledons excised from imbibed seeds of pumpkin and found that for optimal concentrations of each, the GA caused an increase in fresh weight over the controls that was about half as great as that caused by BAP for one set of seeds but had no effect with another set and that BAP caused the same increase as in the first set. These effects were observed with cotyledons exposed to light. Banerji and Laloraya (1966), using very young seedlings of pumpkin grown in darkness, found that both kinetin and GA caused similar increases in the area of isolated cotyledons exposed to light.

The investigations reported here have examined the growth response to GA by isolated cucumber cotyledons from seedlings grown in darkness for 5 days.

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This response and the effect on chlorophyll development have been compared with the responses of the cotyledons to zeatin. Since the effects of red light on the growth of isolated cucumber cotyledons are very similar to the effects of cytokinins (Green and Muir 1979), the effects of light in the blue, red, and far-red regions of the spectrum on the growth of the cotyledons in the presence of GA or zeatin and their combination have been determined.

Materials and Methods

Cucumber (*Cucumis sativus* L. cv. straight eight) seeds were soaked for 2 h in aerated water and germinated in closed plastic boxes in darkness at 24°C for 5 days. The boxes were opened for brief periods each day. The cotyledons were excised under dim green light (500–560 nm, 1.8 μW cm^{-2}) and floated with the adaxial surface down on 12 ml of solution in covered Petri dishes (9 cm diam) for 3 days. The solutions contained 40 mM KCl and 10 mM $CaCl_2$, since these salts have been found to promote the response to cytokinins (Green and Muir 1978). Incubation of 12 cotyledons per sample occurred in darkness at 24°C or under fluorescent light (40 W Vitalite, 15.5 W m^{-2}) at 25 ± 1°C. Other light treatments utilized band-pass filters 25 cm in diameter (Ditric Optics). One filter transmitted 0.4 W m^{-2} at 660 nm with a band width of 5.6 nm, another filter transmitted 0.7 W m^{-2} at 730 nm with a band width of 12.9 nm, and a third filter transmitted 0.4 W m^{-2} at 430 nm with a band width of 7.4 nm. After incubation the cotyledons were blotted dry and individually weighed, and the area of each cotyledon was determined from tracings.

The chlorophyll content of the cotyledons was determined by a procedure that is used as a bioassay for cytokinins by many investigators (Fletcher et al. 1982). Ten cotyledons were floated on 4 ml of each test solution for 20 h in darkness at 24°C and then placed under the fluorescent light for 3 h. After the light treatment, the cotyledons were homogenized with a mortar and pestle at 4°C, extracted with 10 ml acetone, and centrifuged to remove the suspended material. The absorbance of the supernatant was measured at 665 nm.

Results and Discussion

No effect of increasing concentrations of GA on the fresh weight or the area was found for the cotyledons in darkness (Fig. 1). On the other hand, both the fresh weight and the area increased with increasing concentration of GA for the cotyledons in light. The fresh weight and area at 10^{-4} and 10^{-3} M GA were twice the values for cotyledons supplied only KCl and $CaCl_2$. This response to GA in light corresponds closely with the response of cucumber cotyledons in light observed earlier (Green and Muir 1979). Thus, the effects of GA on the growth of the cotyledons, although similar to those of cytokinins, differ in that they are totally dependent on light.

The interactions of radiant energy at different wavelengths with GA or GA and mixed isomers of zeatin on the fresh weight and area of cucumber coty-

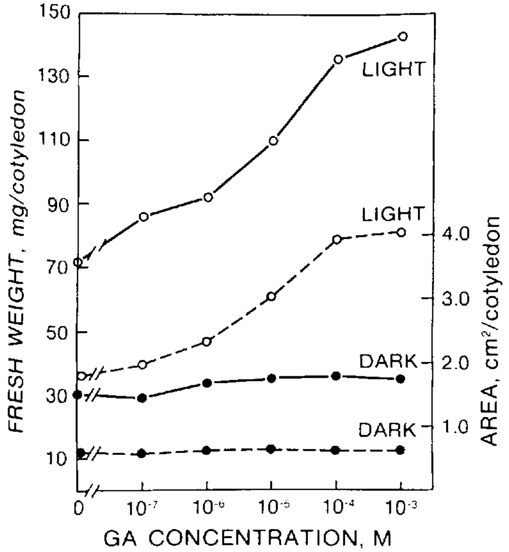

Fig. 1. Effects of increasing concentrations of GA on the fresh weight (solid lines) and area (dashed lines) of cucumber cotyledons in light and in darkness.

ledons are given in Table 1 for a representative experiment. The high energy level of the fluorescent light resulted in large effects in all situations. The low energy levels of the radiation at 730 nm were sufficient to induce the promotive effects of GA on the fresh weight and area of the cotyledons, but the radiation at 430 nm was less effective in inducing the responses to GA. When the mixed isomers of zeatin were added to the solutions containing GA, they caused an increase in fresh weight of the cotyledons that was nearly the same for all light treatments (~50 mg). The isomers also caused similar increases in the area of the cotyledons for all light treatments except under the high energy flux of the fluorescent light.

The effects of the *trans* isomer of zeatin were examined in similar manner, and the data of a representative experiment are given in Table 2. The responses of the cotyledons to the presence of the *trans* isomer were very similar to the

Table 1. Effects of light on the fresh weight and area of cucumber cotyledons induced by gibberellic acid (10^{-4} M) and with the mixed isomers of zeatin (10 mg/L). The control treatment consisted of cotyledons in 40 mM KCl and 10 mM $CaCl_2$. Values are the averages for 12 cotyledons ± SE.

Treatment	Dark	Vitalite	430 nm	730 nm
Fresh weight: mg/cotyledon				
Control	34.1 ± 1.0	92.3 ± 4.7	39.3 ± 1.3	51.3 ± 3.1
GA	43.6 ± 1.6	137.4 ± 5.7	56.3 ± 2.7	84.5 ± 3.9
GA + zeatin	115.1 ± 5.6	187.6 ± 10.8	107.7 ± 10.6	138.0 ± 4.0
Area: cm²/cotyledon				
Control	0.65 ± 0.01	2.11 ± 0.09	0.75 ± 0.03	1.16 ± 0.09
GA	0.76 ± 0.01	3.78 ± 0.13	1.13 ± 0.08	2.06 ± 0.06
GA + zeatin	1.97 ± 0.09	3.82 ± 0.19	2.00 ± 0.10	2.76 ± 0.07

Table 2. Effects of light on the fresh weight and area of cucumber cotyledons induced by gibberellic acid (10^{-4} M), the *trans* isomer of zeatin, and their combination. Otherwise as for Table 1.

Treatment	Dark	Vitalite	730 nm	660 nm
Fresh weight: mg/cotyledon				
GA	42.6 ± 1.8	131.8 ± 3.4	86.8 ± 3.9	83.7 ± 6.1
1 mg/L zeatin	54.1 ± 2.7	135.3 ± 6.1	96.6 ± 5.1	83.3 ± 4.6
10 mg/L zeatin	79.1 ± 5.4	152.8 ± 6.0	115.0 ± 6.3	106.9 ± 6.5
GA + 10 mg/L zeatin	102.4 ± 10.5	188.6 ± 7.3	142.1 ± 3.2	120.8 ± 3.3
Area: cm^2/cotyledon				
GA	0.72 ± 0.02	3.99 ± 0.11	2.22 ± 0.08	2.05 ± 0.12
1 mg/L zeatin	1.18 ± 0.06	2.99 ± 0.08	2.26 ± 0.11	1.92 ± 0.09
10 mg/L zeatin	1.66 ± 0.07	3.40 ± 0.06	2.56 ± 0.08	2.19 ± 0.11
Ga + 10 mg/L zeatin	2.03 ± 0.14	4.20 ± 0.09	3.00 ± 0.06	2.59 ± 0.07

effects of the mixed isomers on both fresh weight and area. In this experiment the effect of radiation at 660 nm was found to be less that at 730 nm.

The data for the effects of GA and *trans* zeatin on the development of chlorophyll in the cucumber cotyledon are given in Table 3. The promotive effect of 40 nm KCl and 10 mM $CaCl_2$ on chlorophyll development resulting from the presence of zeatin corresponds with the results obtained for kinetin (Green and Muir 1978) and those obtained for 6-benzylaminopurine (Fletcher et al. 1982). In contrast, the effect of GA on chlorophyll development either alone or in combination with *trans* zeatin is not significant at the 1% level. These results are also of interest in relation to the finding by Back and Richmond (1969) that kinetin and GA both inhibited chlorophyll degradation in leaf discs of *Tropaeolum* and *Taraxacum* in darkness. The inhibition of chlorophyll degradation by GA is, then, an effect separate from its action in chlorophyll development.

The dependence on light by GA for its effects in the growth of cucumber cotyledons clearly distinguishes its action from that of the cytokinins. The cytokinin action may be primarily through an increase in the internal membranes of the chloroplast, as suggested by Farineau and Rousseaux (1975), and the action of GA may be through the stimulation of enzyme activity, as found by Martin and Northcote (1982). The isolated cotyledon of the cucumber (and

Table 3. Chlorophyll formation in etiolated cucumber cotyledons induced by gibberellic acid (10^{-4} M) and *trans* zeatin (10 mg/L) in water solutions and in solutions containing 40 mM KCl and 10 mM $CaCl_2$. Each value is the mean of four determinations ± SE. Dark value was 0.005 ± 0.002.

	Chlorophyll absorbance	
Treatment	Without KCl and $CaCl_2$	With KCl and $CaCl_2$
	0.157 ± 0.02	0.203 ± 0.02
GA	0.205 ± 0.02	0.281 ± 0.02
Zeatin	0.352 ± 0.02	0.728 ± 0.03
GA + zeatin	—	0.718 ± 0.03

perhaps other cucurbits) is an excellent tissue in which to distinguish the molecular action of cytokinins from that of gibberellins.

References

Back A, Richmond A (1969) An interaction between the effects of kinetin and gibberellin in retarding leaf senescence. Physiol Plant 22:1207–1216

Banerji D, Laloraya MM (1966) Comparative effect of indole-3-acetic acid, kinetin and gibberellic acid on the growth of isolated Cucurbita pepo cotyledons. Curr Sci (India) 35:601–602

Farineau N, Rousseaux R (1975) Influence de la 6-benzylaminopurine sur la differentiation plastidiale dans les cotyledons de concombre. Physiol Plant 33:194–202

Fletcher RA, Kallidumbil V, Steele P (1982) An improved bioassay for cytokinins using cucumber cotyledons. Plant Physiol 69:675–677

Green JM, Muir RM (1978) The effect of potassium on cotyledon expansion induced by cytokinins. Physiol Plant 43:213–218

Green JM, Muir RM (1979) An analysis of the role of potassium in the growth effects of cytokinin, light and abscisic acid on cotyledon expansion. Physiol Plant 46:19–24

Kursanov AL, Kulaeva ON, Mikulovich TP (1969) Combined effect of 6-benzylaminopurine, gibberellic acid and 3-indoleacetic acid on the expansion of isolated pumpkin cotyledons. Am J Bot 56:767–772

Martin C, Northcote DM (1982) The action of exogenous gibberellic acid on protein and mRNA in germinating castor bean seeds. Planta 154:168–173