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A Simple Bioassay for Abscisic Acid Using Cucumber Hypocotyls

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Abstract. A simple bioassay based on the inhibition by abscisic acid (ABA) of cucumber (*Cucumis sativus* L., cv. National Pickling) hypocotyl elongation was developed. Sections of 3-day-old dark-grown cucumber hypocotyl taken from 0–5 mm immediately below the cotyledon were used for the assay. A dark incubation period of 20 h was followed by an exposure to light for 24 h. Under these conditions, the inhibition of hypocotyl elongation is proportional to the abscisic acid applied. The minimum detectable level of abscisic acid was 10^{-9} M, and the range of linear response to abscisic acid was between 10^{-7} and 10^{-3} M. This assay is 10 times more sensitive than the cucumber cotyledon greening bioassay for abscisic acid.

Inhibition of plant growth is often used as a basis for the qualitative bioassay of abscisic acid. Dörffling and Tietz (1983), in their recent review of ABA bioassay, indicated that some of the most frequently used bioassays are coleoptile straight growth (McWha et al. 1973, Tillberg 1975), *Lemna* growth (Van Staden and Bornman 1970, Tillberg 1975, Chen and Park 1976), lettuce hypocotyl growth inhibition (Goto 1978, Bakken and Boe 1982), inhibition of barley endosperm α-amylase induction (Chrispeels and Varner 1966, Sivori et al. 1971), and stomatal closure response (Tucker and Mansfield 1971, Dhawan and Paton 1980). A bioassay based on inhibition of cucumber cotyledon greening system has been reported by Fletcher et al. (1983).

Among the bioassay methods mentioned so far, either the assay procedures are too complicated and thus take too long per assay or the sensitivity ranges are too low, making them impractical for an ideal bioassay. We report a simple procedure for routine bioassay of ABA that has an acceptable sensitivity range, an acceptable incubation period, ease of analysis, range of linear response, and reliability within usable range.

Materials and Methods

ABA Bioassay

Cucumber (*Cucumis sativus* L., cv. National Pickling) seeds were obtained from Stokes Seeds Limited, St. Catharines, Ontario, Canada. The seeds were planted in vermiculite and germinated in the dark at 28°C for 3, 4, and 5 days. The hypocotyls from the seedlings of different ages were excised in dim green safe light. Three consecutive sections of hypocotyl at 5-mm intervals (0–5, 5–10, and 10–15 mm below the cotyledons) were excised. A group of seven hypocotyl sections with three replicates were placed in a 50-ml flask containing 1 ml of test solution for each trial. The test solution consisted of 2 mM sodium phosphate (pH 5.8), 40 mM KCl (Fletcher et al. 1983), and the ABA standard to be assayed. Bottles containing the test hypocotyls were placed on a shaker under the darkness at 28°C and shaken at 10 rpm for conditioning. After 20 h of incubation, the test samples were exposed to continuous rotation on a roller set at 1 rpm. The hypocotyl length was measured under a dissecting microscope with the aid of a micrometer after 24 h of exposure to light (average of 60 $\mu E \cdot m^{-2} \cdot s^{-1}$). A diagram of the procedures for bioassay of abscisic acid using cucumber hypocotyl is illustrated in Fig. 1.

From preliminary results, it was clear that the first 0- to 5-mm hypocotyl section immediately below the cotyledon of the 3-day-old seedling has a greater capacity for elongation than those from 4- or 5-day-old seedlings. Hence only the 0-to-5-mm hypocotyl sections of the 3-day-old seedlings were used for the assay material. A standard curve ranging from 10^{-9} M to 10^{-3} M of ABA and GA, respectively, as well as mixture of ABA and GA (0 M, 10^{-6} M and 10^{-4} M), was obtained based on the inhibition of hypocotyl elongation. A standard curve based on the inhibition of cucumber cotyledon greening system as reported by Fletcher et al. (1983) ranging from 10^{-9} M to 10^{-3} M of ABA was also obtained for comparison. Abscisic acid and gibberellic acid were purchased from the Sigma catalog—A-1012 (±)-2-*cis*, 4-*trans*-abscisic acid MW = 264.3, and G-3250 gibberellic acid MW = 364.4

Results and Discussion

The hypocotyl sections of 0–5 mm below the cotyledon from 3-day-old seedlings had the greatest capacity for elongation (Table 1; Fig. 2). ABA prevents the hypocotyl elongation, and the degree of inhibition is proportional to the concentration of ABA applied. A standard curve based on this result is shown (Table 2; Figs. 3, 4). From the standard assay procedure, the minimum detectable level of ABA with statistical significance is between 10^{-9} M and 10^{-7} M (Table 2), and the range of linear response to ABA is between 10^{-7} M and 10^{-3} M (Fig. 3).

Figure 4 illustrates the appearance of excised hypocotyl after incubation in ABA standard solution (10^{-7} M to 10^{-3} M) for 20 h and illumination for 24 h. Repeated assays showed that the procedure is relatively simple compared to other assay methods of a similar sensitivity range. The total assay time is less

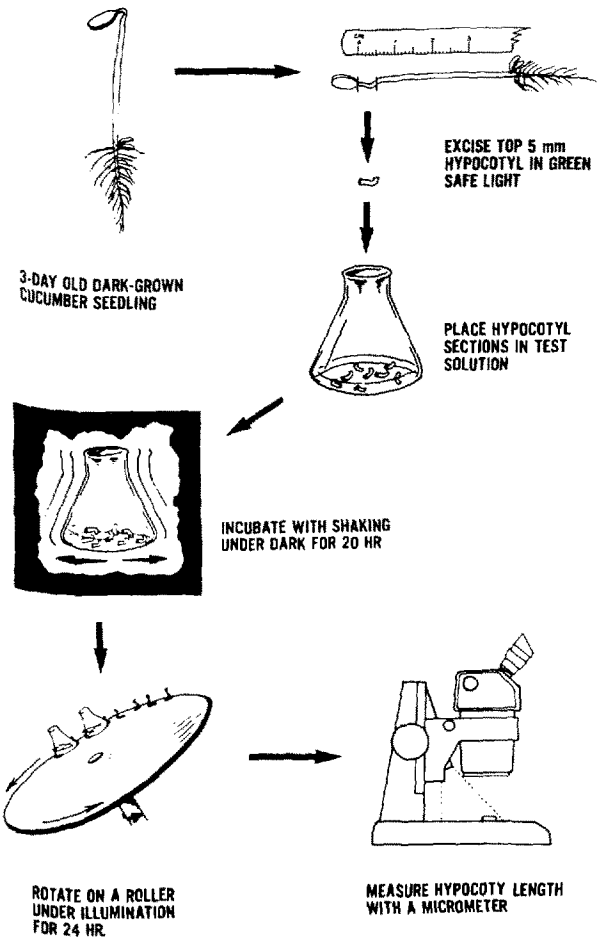

Fig. 1. A diagram of the procedures for bioassay of abscisic acid using cucumber hypocotyl.

Table 1. Net elongation (mm) of hypocotyl sections of 3-, 4-, and 5-day-old cucumber hypocotyl taken from different positions below the cotyledons.

Distance of hypocotyl section below the cotyledons (mm)	Age of seedling (days)*		
	3	4	5
0–5	5.27[a]	2.95[b]	2.08[c]
5–10	1.38[d]	1.17[d]	0.89[de]
10–15	0.31[eg]	0.40[eg]	0.19[fg]

Means with same letters are no significantly different at the 5% level of Duncan's multiple range test.

* Cucumber hypocotyl excised from three seedling age groups were incubated in the dark in buffer solution for 20 h and then illuminated for 24 h.

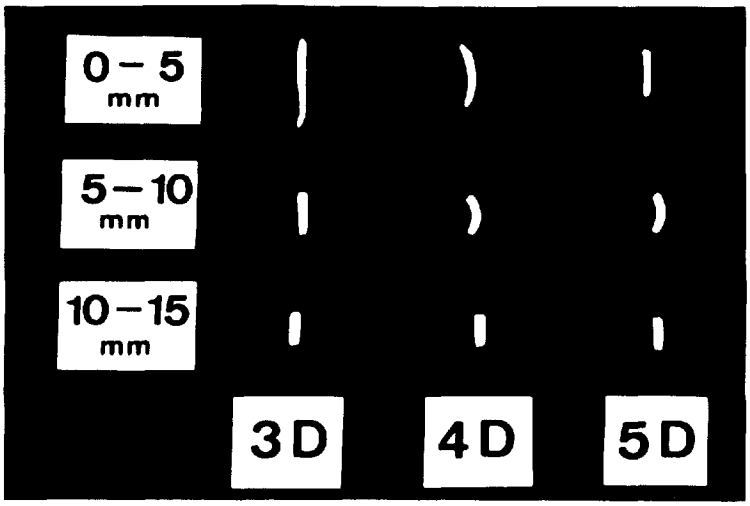

Fig. 2. The appearance of hypocotyl sections excised from three seedling age groups at different distances below the cotyledon. Sections were incubated in the dark in buffer solution for 20 h and then illuminated for 24 h. The hypocotyl section 0–5 mm below the cotyledon of the 3-day-old seedling shows the greatest capacity for elongation.

Table 2. Relationship of ABA concentration to cucumber hypocotyl elongation and chlorophyll levels in cucumber cotyledons.

ABA conc. (M)	Mean hypocotyl elongation (mm)	μg Chl./cotyledon pair
0	6.77[a]	59.9[a]
10^{-9}	5.78[b]	53.8[a]
10^{-8}	5.58[b]	57.1[a]
10^{-7}	5.52[b]	59.3[a]
10^{-6}	4.94[c]	57.3[a]
10^{-5}	3.50[d]	40.3[b]
10^{-4}	1.90[e]	21.8[c]
10^{-3}	0.62[f]	13.1[c]

Means within column with same letters are not significantly different at the 5% level of Duncan's multiple range test.

than 5 days, the shortest complete assay time required available for ABA bioassay. The assay procedure does not require sophisticated equipment, and no complicated technique is involved. Thus it is suitable for routine and rapid ABA bioassay.

In many assays gibberellic acid (GA) from the plant extract interferes with ABA bioassay (Goto 1978, Dörffling and Tietz, 1983). In this study, in which a serial concentration of exogenous GA standard was assayed against the hypocotyl elongation, the hypocotyl elongation did not proportionally increase to the increment of GA concentration. A Duncan's multiple-range test analysis showed that GA treatment at 10^{-9} M, 10^{-8} M, 10^{-7} M, and 10^{-6} M did not

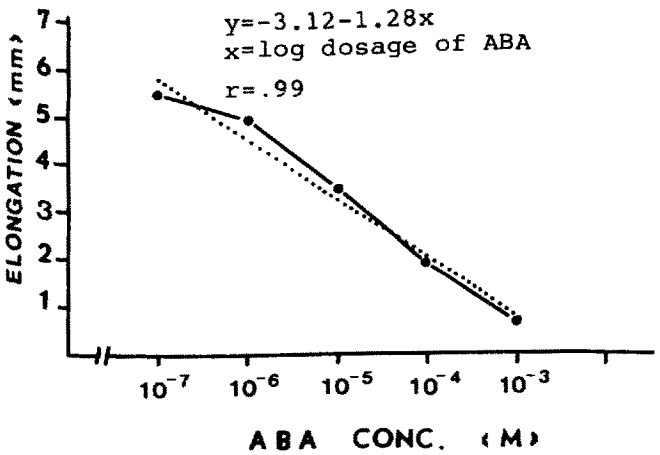

Fig. 3. Net elongation (mm) in cucumber hypocotyl treated with various concentrations of ABA (10^{-7} M to 10^{-3} M) for 20 h in the dark and illuminated for 24 h. Hypocotyl sections excised 0–5 mm immediately below the cotyledon of the 3-day-old seedlings were used for the assay material.

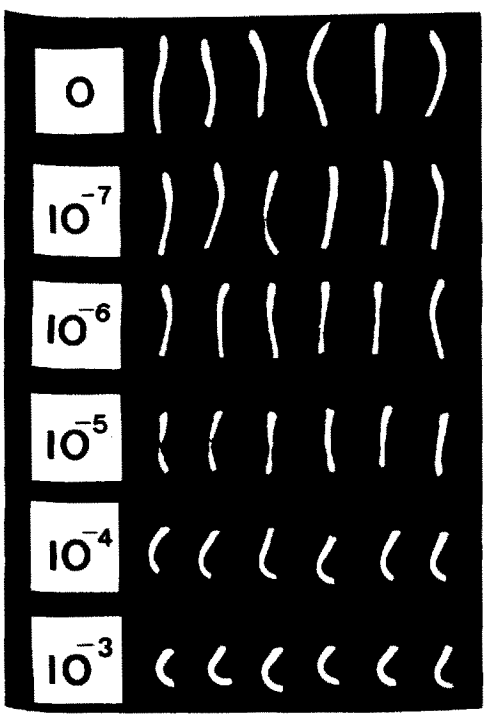

Fig. 4. Growth of cucumber hypocotyl excised from 3-day-old dark-grown seedlings. They were incubated in the dark in ABA solutions (10^{-7} M to 10^{-3} M) for 20 h and then illuminated for 24 h.

Table 3. Relationship of GA concentration to cucumber hypocotyl elongation.

GA conc. (M)	Mean hypocotyl elongation (mm)
0	6.15[a]
10^{-9}	6.39[ab]
10^{-8}	6.12[a]
10^{-7}	6.38[ab]
10^{-6}	6.65[ab]
10^{-5}	6.93[b]
10^{-4}	7.55[c]
10^{-3}	6.05[a]*

Means within column with same letters are not significantly different at the 5% level of Duncan's multiple range test.
* Hypocotyl section in 10^{-3} M GA solution showed symptoms of rotting at the end of the experimentation period.

differ significantly from the untreated check (Table 3). Although there is a little enhancement of hypocotyl elongation by 10^{-4} M GA, hypocotyl section in 10^{-3} M GA solution showed symptoms of rotting at the end of the experimentation period. In a parallel experiment, serial concentrations of ABA combined with GA (0 M, 10^{-6} M, and 10^{-4} M, respectively) were used to study the interaction between two plant hormones on cucumber hypocotyl elongation. GA concentrations below 10^{-6} M did not significantly relieve the inhibition of cucumber hypocotyl elongation caused by ABA at 10^{-5} M or less (Table 4). GA concentrations above 10^{-4} M in the mixed solution enhanced the hypocotyl elongations to some extent, which were similar to those when GA was assayed alone (Table 3). Thus the interference of endogenous GA from the hypocotyl is not a major concern.

Fletcher et al (1982) showed that addition of 40 mM KCl in the cytokinin assay solution enhanced chlorophyll formation of cucumber cotyledon. Longer exposure (24 h) to light and in the presence of 40 mM KCl, the inhibition of growth and chlorophyll production by ABA is enhanced (Fletcher et al, 1983). It is believed that ABA interferes with potassium uptake (Green and Muir 1978, Fletcher et al. 1983). We adapted Fletcher's assay solution by using 2 mM sodium phosphate buffer, pH 5.8, to stabilize the pH, and 40 mM of KCl to increase the sensitivity of ABA. We found that the assay solution gave consistent results. To avoid anaerobiosis during incubation and greening (Dei, 1978), we incubated the hypocotyl sections in test solution on a shaker, shaking at 10 rpm, and put on a roller during greening. The hypocotyl section is quite straight at the end of the greening period, especially those under low ABA dosage, which makes the elongation measurement much easier. An important technical trick for consistent results is to cut the hypocotyl at uniform lengths for each treatment.

For a direct comparison, a standard curve based on Fletcher's (1983) chlorophyll greening system of cucumber cotyledon was obtained. As shown in Table 2, the range of linear response to ABA is between 10^{-6} M and 10^{-3} M and agrees quite well with Fletcher's results. Using hypocotyl of 3-day-old seed-

Table 4. Interaction between ABA and GA concentrations to cucumber hypocotyl elongation.

ABA (M)	GA concentration (M)		
	0	10^{-6}	10^{-4}
0	6.49[a]	7.46[a]	9.78
10^{-7}	6.53[b]	7.19[b]	10.16
10^{-6}	5.42[c]	6.08[c]	8.11
10^{-5}	3.35[d]	4.03[d]	5.60
10^{-4}	1.78[e]	2.22[f]	3.81[g]
10^{-3}	1.27[h]	1.93[i]	2.22[i]

Means within row with same letters are not significantly different at the 5% level of Duncan's multiple range test.

lings in this assay, it is now possible with good reproducibility to detect quantitatively as little as 10^{-7} M of ABA. Hence the cucumber hypocotyl elongation assay provides a sensitive, rapid, and simple bioassay for abscisic acid.

The great capacity for elongation of the hypocotyl section and the low interference by GA suggest that this simple bioassay system could be a convenient one to use to study how ABA works.

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References

Bakken TJ, Boe AA (1982) Two bioassay techniques for determining abscisic acid concentration J Am Soc Hort Sci 107(1):109–112

Chen SSC, Park WM (1976) Dual effects of abscisic acid on the growth of a duckweed. Taiwania 21:50–51

Chrispeels MJ, Varner JE (1966) Inhibition of gibberellic acid–induced formation of α-amylase by abscisin II. Nature 212:1066–1067

Dei M (1978) Inter-organ control of greening in etiolated cucumber cotyledons. Physiol Plant 43:94–98

Dhawan AK, Paton KM (1980) A simple bioassay for abscisic acid and other antitranspirants. Ann Bot 45:493–495

Dörffling K, Tietz D (1983) Methods for the detection and estimation of abscisic acid and related compounds. In: Addicott FT (ed) Abscisic acid, pp 23–78

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

Fletcher RA, Venkatarayappa T, Kallidumbil V (1983) Comparative effects of abscisic acid and methyl jasmonate in greening cucumber cotyledon and its application to a bioassay for abscisic acid. Plant Cell Physiol 24:1057–1064

Goto N (1978) Gibberellins and inhibitors in leaves of tall and dwarf beans. Physiol Plant 42:359–364

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

McWha JA, Phililpson JJ, Hillman JR, Wilkins MB (1973) Molecular requirements for abscisic acid activity in two bioassay systems. Planta 109:327–336

Sivori EM, Sonvico V, Fernandez NO (1971) Determination of abscisic acid following Paleg's method. Plant Cell Physiol 12:993–996

Tillberg E (1975) An abscisic acid–like substance in dry and soaked *Phaseolus vulgaris* seeds determined by the Lemna growth bioassay. Physiol Plant 34:192–195

Tucker DJ, Mansfield TA (1971) A simple bioassay for detecting "antitranspirant" activity of naturally occurring compounds such as abscisic acid. Planta 98:157–163

Van Staden J, Bornman CH (1970) Spirodela growth test: A possible bioassay for abscisic acid. JS Afr Bot 36:9–12