

# Growth Retardant-Induced Changes in Phototropic Reaction of *Vigna radiata* Seedlings<sup>1</sup>

R. Konjević\*, D. Grubišić, and Mirjana Nešković

Institute of Botany, Faculty of Science and Institute for Biological Research "S. Stanković", University of Belgrade, 29. Novembra 142, 11060 Belgrade, Yugoslavia

## ABSTRACT

The effect of growth retardants on phototropism has been studied in mung bean (*Vigna radiata*) seedlings. Ancymidol, tetcyclacis, and paclobutrazol inhibited phototropism while AMO 1618 and CCC were ineffective. The fluence-response relationships for phototropism of etiolated seedlings were similar to those previously described for monocots and other dicots. Ancymidol caused a shift in the maximum phototropic response to higher fluence of light. It is suggested that ancymidol may affect phototropism through an effect on the photoreceptor system.

The present paper was initiated by the observation that ancymidol<sup>2</sup> inhibited phototropism of mung bean seedlings and that this inhibition could not completely be reversed by gibberellic acid. Based on this observation, the idea was developed that growth retardants, in addition to their inhibition of gibberellin biosynthesis, might have a separate effect on one of the first steps in the phototropism transduction chain. Two of the growth retardants (AMO 1618 and CCC) inhibit gibberellin biosynthesis by preventing the formation of *ent*-kaurene via kaurene synthetase (6, 12). The other three growth retardants (ancymidol, tetcyclacis, and paclobutrazol) are known to inhibit gibberellin biosynthesis by inhibition of mixed function oxidases, which convert *ent*-kaurene to *ent*-kaurenoic acid (3–5, 18). A component of this enzyme system is cytochrome P-450. In recent literature, there is some evidence that cytochrome P-450 may be involved in blue light perception (23).

The results presented here are consistent with the hypothesis that ancymidol, tetcyclacis, and paclobutrazol act on phototropism via an effect on cytochrome P-450 and not via an effect on gibberellin biosynthesis.

## MATERIALS AND METHODS

Mung bean (*Vigna radiata*) seeds were germinated for 48 h in Petri dishes on two layers of moistened filter paper.

<sup>1</sup> This work was supported by Republic Fund for Scientific Research (RZNS).

<sup>2</sup> Abbreviations: ancymidol,  $\alpha$ -cyclopropyl- $\alpha$ -(*p*-methoxyphenyl)-5-pyrimidine methyl alcohol; AMO 1618, 2'-isopropyl-4'-(trimethylammonium chloride)-5'-methylphenyl piperidine-1-carboxylate; CCC,  $\beta$ -chloroethyltrimethylammonium chloride; tetcyclacis, 5-(4-chlorophenyl)-3,4,5,9,10-pentaazatetracyclo-5,4,1,0<sup>2,6</sup>,0<sup>8,11</sup>-dodeca-3,9-diene; paclobutrazol, (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-ol.

Seedlings were transferred into plastic boxes with seed holders containing distilled water for an additional 3 d. From the time of sowing the seedlings were exposed either to various light sources (white, blue, or red), or kept in darkness until the first internode started elongating (5 d after sowing). Phototropic induction was performed using carefully selected, straight seedlings. The lower hypocotyl parts of the seedlings were wrapped with cotton wool and inserted into test tubes containing either water or test solutions. The seedlings were oriented such that the light direction was perpendicular to the expanding primary leaves. Throughout all experimental period, temperature was kept at  $25 \pm 2^\circ\text{C}$ .

Two types of experiment were performed. In one, the effects of growth retardants on the so-called second positive phototropic response were studied. The seedlings were provided test substances 12 h prior to the onset of unilateral blue light stimulation. The shoot curvature was measured from shadowgraphs taken after 5 h of continuous stimulation. In a separate experiment designed to study the possible effect of ancymidol on the fluence response relationship for phototropism in etiolated plants, control seedlings were kept in water while test plantlets were supplied with ancymidol 12 h prior to phototropic stimulation. The seedlings were irradiated at a fixed fluence rate of  $0.23 \mu\text{mol m}^{-2} \text{s}^{-1}$  with various exposure times and returned to darkness to develop curvatures. The curvatures were measured from shadowgraphs taken 150 min after irradiation.

The effect of growth retardants on growth rate and geotropism of white-light-grown seedlings was tested. For growth-rate determinations, 4.5-d-old seedlings were placed in test tubes containing either water or test solutions. After an additional 12 h of incubation, the growth increment was determined. Geotropism was tested by placing the plantlets in the horizontal position in darkness for 2 h, and curvature measured from shadowgraphs taken at the end of this period.

All manipulations with seedlings, preceding the phototropic induction, were performed either under the light sources used for the pretreatments or under a green safe light in the case of etiolated seedlings.

## Light Sources

For white light Sylvania Grolux tubes were used at a fluence rate of  $24.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; red light at  $11.6 \mu\text{mol m}^{-2} \text{s}^{-1}$  ( $\lambda_{\text{max}} = 660 \text{ nm}$ ) was obtained from Philips fluorescent tubes TL 20/15 used in combination with a 3-mm thick Rohm & Haas (Darmstadt, FRG) plastic filter No. 501; blue light at 2.25

$\mu\text{mol m}^{-2} \text{s}^{-1}$  for blue light pretreatment or  $0.225 \mu\text{mol m}^{-2} \text{s}^{-1}$  for unilateral blue light stimulation ( $\lambda_{\text{max}} = 450 \text{ nm}$ ) was obtained by using Philips fluorescent tubes TL 20/18 in combination with 3-mm thick Rohm & Haas plastic filter No. 627.

### Chemicals

Ancymidol was obtained as a gift from Eli Lilly & Co; tetcyclacis was provided by BASF AG (Ludwigshafen, FRG); paclobutrazol was a gift from ICI. AMO 1618 was purchased from Fluka and CCC from BDH.

### Statistics

In each experiment lots of 30 to 40 seedlings were used for each point; means are based on 3 to 4 independent experiments.

## RESULTS

To confirm that growth retardants have a specific effect on phototropism it was necessary to rule out their possible effects on straight growth rate and differential growth under gravitropic stimulation. The results show that the growth rate of white-light-grown seedlings was not affected within 12 h by incubation in growth retardants (Table I). Similarly, the capacity for differential growth, as judged by curvature in gravitropism was also unaffected (Table I).

In contrast with these results, phototropism was inhibited by ancymidol, tetcyclacis, and paclobutrazol in all cases. However, this inhibition in red-light-grown seedlings was not very pronounced (Table II). On the other hand, AMO 1618 and CCC had no effect on phototropism with any pretreatment (Table II).

Since the effect of ancymidol was the most pronounced (Table II) its effect on fluence-response relationships for etiolated seedlings was measured. A fluence response curve, measured for control plants (Fig. 1), is similar to other fluence-response relationships already described in the literature (1, 20), although the low fluence response, typically referred to as first positive curvature, may be subdivided into two peaks.

**Table I. Effect of Retardants on the Growth Rate and Gravitropism of *Vigna radiata* Seedlings**

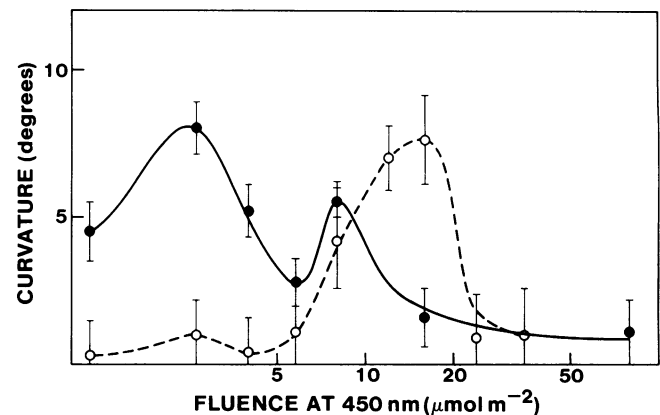
White-light-grown seedlings were treated with growth retardants for 12 h; the length of seedlings was recorded at the beginning and at the end of this period and the growth expressed as growth increment; for gravitropism, curvature was measured after 2 h of stimulation; retardants were applied, through the roots, as  $10 \mu\text{M}$  solutions, except for CCC which was applied as  $100 \mu\text{M}$ .

Treatment	Growth Increment in mm $\pm$ SE	Gravitropism Degree of $\pm$ SE Curvature
H <sub>2</sub> O	7.1 $\pm$ 0.5	53.5 $\pm$ 5.3
CCC	8.6 $\pm$ 2.3	56.2 $\pm$ 5.4
AMO 1618	8.0 $\pm$ 1.4	52.3 $\pm$ 5.1
Ancymidol	6.4 $\pm$ 0.9	56.9 $\pm$ 5.0
Tetcyclacis	7.6 $\pm$ 0.8	56.7 $\pm$ 5.6
Paclobutrazol	6.9 $\pm$ 1.1	58.4 $\pm$ 5.8

**Table II. Effect of Growth Retardants on Phototropic Reaction of *Vigna radiata* Seedlings**

Growth retardants were applied through the roots, 12 h prior to the onset of phototropic stimulus, as  $10 \mu\text{M}$  solutions, except for CCC which was applied as a  $100 \mu\text{M}$  solution; curvature was measured 5 h after the onset of unilateral blue light.

	Degrees of Curvature $\pm$ SE			
	Pretreatment			
	Darkness	Blue light	White light	Red light
H <sub>2</sub> O	51.7 $\pm$ 3.1	48.2 $\pm$ 2.7	46.6 $\pm$ 1.8	50.7 $\pm$ 3.2
CCC	52.7 $\pm$ 2.7	52.3 $\pm$ 3.0	48.9 $\pm$ 2.0	
AMO1618	57.4 $\pm$ 2.2	51.3 $\pm$ 2.1	44.7 $\pm$ 2.1	
Ancymidol	32.1 $\pm$ 2.9	26.5 $\pm$ 2.5	15.8 $\pm$ 2.9	39.0 $\pm$ 3.4
Tetcyclacis	37.2 $\pm$ 2.4	27.5 $\pm$ 2.7	28.4 $\pm$ 2.9	34.5 $\pm$ 3.7
Paclobutrazol	40.3 $\pm$ 2.3	38.1 $\pm$ 2.3	20.0 $\pm$ 3.5	40.1 $\pm$ 3.5



**Figure 1. Fluence response curve for phototropism of etiolated mung bean seedlings; (●), control plantlets; (○), ancymidol treated seedlings; ancymidol ( $10 \mu\text{M}$ ) was applied 12 h prior to the onset of blue light; curvature was measured 150 min after phototropic stimulation; each vertical bar represents  $\pm$ SE.**

Ancymidol showed a dramatic effect on the fluence response relationship (Fig. 1) shifting the response to higher fluence.

## DISCUSSION

Based on the results presented above, the effects of the growth retardants, ancymidol, tetcyclacis, and paclobutrazol, on phototropism of *Vigna radiata* seedlings are not due to the consequence of their effects on growth *per se*, and thus not due to their effects on gibberellin biosynthesis.

Several lines of evidence support this conclusion. First, the time of incubation with these substances was relatively short. Growth retardants were applied only 12 h before phototropic stimulation, and after the seedlings have already synthesized endogenous gibberellins. Therefore, the reduced curvature could not be a consequence of impaired gibberellin biosynthesis, since the plantlets have an existing pool of these hormones. This conclusion is further corroborated by the finding that neither growth rate nor geotropism were affected by the inhibitors. In addition, phototropism was not affected by all retardants. AMO 1618 and CCC had no effect whatso-

ever. This outcome is consistent with the different sites of action of these inhibitors, as already noted.

The photo-reduction of a *b*-type Cyt was initially reported by Poff and Butler (16, 17) in *Phycomyces* and *Dictyostelium*. Subsequently, this photoreducible cytochrome was found to be associated mostly with membrane fractions of plant tissue (2). In addition, light-induced phenylacetic acid binding sites, which may be associated with a blue light receptor, are mostly located at plasma membranes of corn coleoptiles (21). Additional evidence for participation of a flavin-Cyt complex in blue light photoreception for phototropism comes from studies of Leong and Briggs (13, 14) and Leong *et al.* (15) on acifluorfen action in corn membranes. It has since been suggested that a photoreducible Cyt P-450 is associated with plasma membranes (22). The action spectrum for the plasma membrane bound LIAC is similar to the action spectra for phototropism and also similar to the absorption spectra for flavins (23). As has already been mentioned, ancymidol, tetcyclacis, and paclobutrazol inhibit a 'mixed function oxidases'-catalyzed reaction involving Cyt P-450. Therefore, it seems that our data are consistent with the concept of a flavin-Cyt P-450 complex as a receptor mediating blue light action in phototropism. Additional support for this hypothesis is the evidence presented by Coolbaugh *et al.* (4), who showed a direct effect of ancymidol on Cyt P-450 in the microsomal fraction of immature seeds of *Marah macrocarpus*.

It is well known that a light pretreatment causes a shift in the fluence-response relationship (1, 7, 11, 19) in the range of first positive curvature. The effect of ancymidol on the fluence-response curve is similar to that of the light pretreatment, a result that indicates that it might affect the photoreceptor system. However, one cannot, at present, exclude the possibility that ancymidol may also affect later stages in transduction steps, such as the responsiveness of the tissue to unilateral irradiation.

#### ACKNOWLEDGMENTS

The authors wish to thank Eli Lilly & Co., BASF AG and ICI for their gifts of chemicals. We are very obliged to Dr. Kenneth L. Poff for his critical and helpful discussion of the manuscript.

#### LITERATURE CITED

1. Blaauw OH, Blaauw-Jansen G (1970) Third positive (C-type) phototropism in the Avena coleoptile. *Acta Bot Neerl* 19: 764-775
2. Brain RD, Freeberg JA, Weiss C, Briggs WR (1977) Blue light induced absorbance changes in membrane fractions from corn and Neurospora. *Plant Physiol* 55: 948-952
3. Coolbaugh RC, Hamilton R (1976) Inhibition of *ent*-kaurene oxidation and growth by  $\alpha$ -cyclopropyl- $\alpha$ -(*p*-methoxyphenyl)-5-pyrimidine methyl alcohol. *Plant Physiol* 57: 245-248
4. Coolbaugh RC, Hirano SS, West CA (1978) Studies on the specificity and site of action of  $\alpha$ -cyclopropyl- $\alpha$ -[*p*-methoxyphenyl]-5-pyrimidine methyl alcohol (ancymidol), a plant growth regulator. *Plant Physiol* 62: 571-576
5. Dalziel L, Lawrence DK (1984) Biochemical and biological effects of kaurene oxidase inhibitors, such as paclobutrazol. *In* R Menhenett, DK Lawrence, eds, *Biochemical Aspects of Synthetic and Naturally Occurring Plant Growth Regulators*. British Plant Growth Regulator Group, Monograph 11, Wantage, pp 43-57
6. Dennis DT, Upper CD, West CA (1965) An enzymic site of inhibition of gibberellin biosynthesis by AMO 1618 and other plant growth retardants. *Plant Physiol* 40: 948-952
7. Ellis RJ (1987) Comparison of fluence-response relationships of phototropism in light- and dark-grown buckwheat. *Plant Physiol* 85: 689-692
8. Fall RR, West CA (1971) Purification and properties of kaurene synthetase from *Fusarium moniliforme*. *J Biol Chem* 246: 6913-6928
9. Frost RG, West CA (1977) Properties of kaurene synthetase from *Marah macrocarpus*. *Plant Physiol* 59: 22-29
10. Hedden P, Graebe JE (1985) Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of *Cucurbita maxima* and *Malus pumila* embryos. *J Plant Growth Regul* 4: 111-122
11. Hofmann E, Schafer E (1987) Red light-induced shift of the fluence-response curve for first positive curvature of maize coleoptiles. *Plant Cell Physiol* 28: 37-45
12. Kende H, Ninnemann H, Lang A (1963) Inhibition of gibberellic acid biosynthesis in *Fusarium moniliforme* by AMO 1618 and CCC. *Naturwissenschaften* 50: 599-600
13. Leong TY, Briggs WR (1981) Partial purification and characterization of a blue light-sensitive cytochrome-flavin complex from corn membranes. *Plant Physiol* 67: 1042-1046
14. Leong TY, Briggs WR (1982) Evidence from studies with acifluorfen for participation of a flavin-cytochrome complex in blue light photoreception for phototropism of oat coleoptiles. *Plant Physiol* 70: 875-881
15. Leong TY, Vierstra RD, Briggs WR (1981) Blue light-sensitive cytochrome-flavin complex from corn coleoptiles. Further characterization. *Photochem Photobiol* 34: 696-703
16. Poff K, Butler WL (1974) Absorbance changes induced by blue light in *Phycomyces blakesleanus* and *Dictyostelium discoideum*. *Nature* 248: 799-801
17. Poff K, Butler WL (1975) Spectral characterization of the photoreducible *b*-type cytochrome of *Dictyostelium discoideum*. *Plant Physiol* 55: 427-429
18. Rademacher W, Jung J, Graebe JE, Schwenen L (1984) On the mode of action of tetcyclacis and triazole growth retardants. *In* R Menhenett, DK Lawrence, eds, *Biochemical Aspects of Synthetic and Naturally Occurring Plant Growth Regulators*. British Plant Growth Regulator Group, Monograph 11, Wantage, pp 1-11
19. Schafer E, Iino M, Briggs WR (1984) Red light-induced shift of the fluence-response curve for first positive phototropic curvature of maize coleoptiles. *In* H Senger, ed, *Blue Light Effects in Biological Systems*. Springer-Verlag, Berlin, pp 476-479
20. Steinitz B, Poff KL (1986) A single positive phototropic response induced with pulsed light in hypocotyls of *Arabidopsis thaliana* seedlings. *Planta* 168: 305-315
21. Vierstra RD, Poff KL (1984) Localization of light-induced phenylacetic acid-binding sites in corn coleoptile membranes: Possible association of the blue light photoreceptor pigment with the plasma membrane. *In* H Senger, ed, *Blue Light Effects in Biological Systems*. Springer-Verlag, Berlin, pp 185-195
22. Widell S, Caubergs RJ, Larsson C (1983) Spectral characterization of light reducible cytochrome in a plasma membrane-enriched fraction and in other membrane from cauliflower inflorescences. *Photochem Photobiol* 38: 95-98
23. Widell S, Larsson C (1984) Blue light effects and the role of membranes. *In* H Senger, ed, *Blue Light Effects in Biological Systems*. Springer-Verlag, Berlin, pp 177-184