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A ROLE OF CAROTENOIDS IN PHOTOTROPISM OF *ARABIDOPSIS THALIANA* SEEDLINGS

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A carotenoid deficient mutant of *Arabidopsis thaliana* (Am 45-3) was used to investigate the role of carotenoids in phototropism and adaptation. The mutant seedlings appeared pale and contained about 2.5-3% of the amount of carotenoids present in the WT when grown in light. Phototropism of pale seedlings in response to unilateral BL pulse was similar to that of WT seedlings except that the amplitude of the response was lower. Pale seedlings retained their ability to undergo desensitization by BL irradiation as a part of adaptation. These seedlings also exhibited RL-induced enhancement of phototropism. Enhancement appeared to be associated with an increase of carotenoid content. These data are consistent with the conclusion that carotenoids are not the photoreceptor pigments for phototropism or desensitization, although the presence of carotenoids affects the amplitude of phototropism and mechanism for enhancement in *A. thaliana*.

Key words: *A. thaliana*, phototropism, carotenoids, blue light, red light

Ključne reči: *A. thaliana,* fototropizam, karotenoidi, plava svetlost, crvena svetlost

INTRODUCTION

Blue light induces many physiological responses including phototropism in plants (Kaufman, 1993). The identity of the photoreceptor pigment for phototropism has not yet been elucidated and it was recently suggested that mulitple photoreceptor pigments may mediate this process in *Phycomyces* and *Arabidopsis thaliana* (Galland and Lipson, 1987; Konjević et al., 1989). In addition, the existence of photoreceptor pigment was postulated that could control desensitization of *Arabidopsis* seedlings by the BL to a subsequent unilateral photostimulation (Poff et al., 1994).

Because of similarity of absorption spectra of carotenoids, and the action spectrum for phototropism, carotenoids were suggested to be the photoreceptor pigment mediating this process (Curry, 1969). However, absorption spectrum of β-carotene lacks the peak at about 370 nm present in the action spectrum for phototropism (Curry, 1969). Moreover, a mutant of *Phycomyces* that has less than $1x10^5$ of the WT carotenoid content still displayed normal sensitivity to phototropic stimulation (Presti et al., 1977). In addition, corn plants treated with a carotenoid synthesis inhibitor exhibit first and second positive phototropism similar to the response of untreated plants, the only difference being the amplitude of the response (Vierstra and Poff, 1981; Piening and Poff, 1988). These lines of evidence argued against the carotenoids as the chromophores of the photoreceptor for the BL in phototropism. A flavoprotein is now thought to be a photoreceptor pigment for phototropism as well as for other BL mediated processes (Song, 1984).

Adaptation in phototropism has been described in maize (Iino, 1988), *Phycomyces* (Galland, 1991) and *A. thaliana* (Janoudi and Poff, 1991). Irradiation of plants by light induces a change in their sensitivity and/or responsiveness to a subsequent irradiation (Galland, 1991; Janoudi and Poff, 1991). In maize both RL and BL are capable of inducing desensitization in phototropism (Iino, 1988). In *Arabidopsis* only BL can desensitize seedlings to a subsequent BL pulse (Janoudi and Poff, 1991). No attempt has yet been made to characterize the pigment responsible for desensitization in *Arabidopsis* seedlings.

A mutant strain of *A. thaliana* that has a pale phenotype was used to examine its carotenoid content and test for possible effect of decreased carotenoid levels on responses to BL. A mutant with a lower carotenoid content than the WT should be a good tool to test for carotenoid function in the BL absorption for phototropism or adaptation.

MATERIALS AND METHODS

Mutant population

A mixed population, Am 45-3, consisted of about 1/6 of seedlings that appeared pale and 5/6 that were normally pigmented. This fits the expected distribution if the pale seedling is a homozygous recessive which is incapable of maturing and setting seed (Chi-square test on the expected ratio of 5 normally pigmented : 1 pale seedling has

given probability value of 0.5). Two assumptions had to be made to allow comparison of observed ratio of two phenotypes in the Am 45-3 population with the predicted ratio 5:1. First, that the seeds obtained initially were progeny of a heterozygous plant and; second, that dominant homozygous and heterozygous plants have the same ability to set seeds. High probability value obtained in chi-square test is consistent with the hypothesis that Am 45-3 population probably consists of pale seedlings as homozygous recessive mutants, and normally pigmented seedlings representing a mixture of plants that are homozygous dominant and heterozygous for this genetic locus. Further in the text, the latter batch of seedlings is reffered to as normally pigmented, while carotenoid deficient mutants are reffered to as pale seedlings.

Growth conditions

Seedlings used in experiments for measurement of phototropism and gravitropism were grown as described previously (K h u r a n a et al., 1989) with one difference. Seedlings with the pale phenotype were grown for 4 h longer (43 h) than the normally pigmented seedlings (39 h) in order to bring them to approximately same size. Difference in pigmentation between two phenotypes of etiolated seedlings was obvious and easy to score. Pale plants that were light grown for 4 months also had a distinctive phenotype. Although these plants in culture looked morphologically similar to WT plants, except being smaller in size, their stems always shriveled quickly after development of flowers which made it impossible to do any crossing experiment. These seedlings were grown on Murashige-Skoog 1X medium (Gibco BRL) supplemented with 3% (w/v) sucrose. Because the pale seedlings died under the light conditions in which the WT-Col seedlings were grown, the following procedure was used for their culture. Forty hours after they germinated in darkness, pale seedlings were selected from the mixed population according to the colour of their cotyledons and placed into plastic containers with nutrient medium. These containers were then covered with a green plexiglas (Rohm Gmbh Plexigas gs, DIN 4102-B2) that had low transmittance throughout the visible part of the spectrum.

Light sources

White light that was used for growth of seedlings and to potentiate germination (60 μmol $\cdot$ $m^{-2}.s^{-1}$) was obtained from GE (Cleveland, OH) DeLux Cool-white fluorescent tubes. The BL source consisted of a projector equipped with a Sylvania (GTE Products, Danvers, MA) 300 W ELH tungsten halogen lamp and 450 nm interference filter with a half-band width of 10 nm (PTR Optics Waltham, MA). Red light (0.6 μmol $\cdot$ $m^{-2}s^{-1}$) used for pre-irradiation of seedlings was obtained from one gold fluorescent tube (GTE, Sylvania) wrapped with red cellophane (Highland Supply Corp., Highland, IL). This source provides radiation from 560 to 720 nm with maximum output at 620 nm. The duration of actinic BL was controlled with a Uniblitz shutter (Vincent Associates, Rochester, NY). Fluence rates were measured with a Li-Cor (Lincoln, NE) LI-190 SA in combination with a LI 1000 Datalogger.

Measurement of curvature

Phototropic and gravitropic curvature of hypocotyls were measured as described by K h u r a n a et al., (1989), except that a curvature was allowed to develop for 80 min in phototropism experiments.

High Performance Liquid Chromatography Analysis

HPLC analysis was performed as previously described (R o c k , 1991), with the spectrophotometer set at 436 nm for detection of carotenoids. Identification of carotenoids was done according to retention times of peaks on chromatograms. The area under the relevant peaks of absorbance on the chromatograms of the tested carotenoids was used as an indicator of their amounts. Quantification of individual carotenoids was done from the standard curves generated in the laboratory.

RESULTS

HPLC analysis

The content of 10 carotenoids was compared in light grown WT-Col and pale Am 45-3 seedlings. Chromatography revealed that the carotenoid content in the WT-Col plants was approximately 35-40 times higher than that in the pale mutant plants (Fig. 1. a and b). The amount of β-carotene was approximately 2.5 times higher in the WT-Col plants than in the pale plants when expressed as percentage of total carotenoids while the amounts of lutein and antheraxanthin were lower when expressed on the same basis (Fig. 1. a and b).

Relative quantities of individual carotenoids as a percentage of total amount of 9 tested carotenoids in the RL-irradiated seedlings were not different from those in the etiolated seedligns of WT-Col (Tab. 1). However, RL-irradiated WT-Col seedlings had approximately 40% higher quantities of carotenoids than etiolated seedlings (Tab. 2).

Tab. 1. – The quantity of individual carotenoid as percent of total amount of nine tested carotenoids in etiolated (-RL) and red light- irradiated seedlings (+RL) of WT-Col. Values in the table are means ± 1 SE; n=2.

	- RL (%)	+ RL
β-carotene	4.1 ± 1.9	4.7 ± 0.5
lutein	42.7 ± 0.4	42.1 ± 1.5
zeaxanthin	0.6 ± 0.0	1.2 ± 0.6
antheraxanthin	3.2 ± 0.5	3.2 ± 0.1
lutein epoxide	7.7 ± 0.9	8.0 ± 0.2
all-trans violaxanthin	29.4 ± 0.5	28.5 ± 0.1
9 cis-violaxanthin	3.6 ± 0.5	3.5 ± 0.5
13 cis-violaxanthin	3.6 ± 0.8	3.6 ± 0.8
9 cis-neoxanthin	5.3 ± 0.1	5.1 ± 0.0

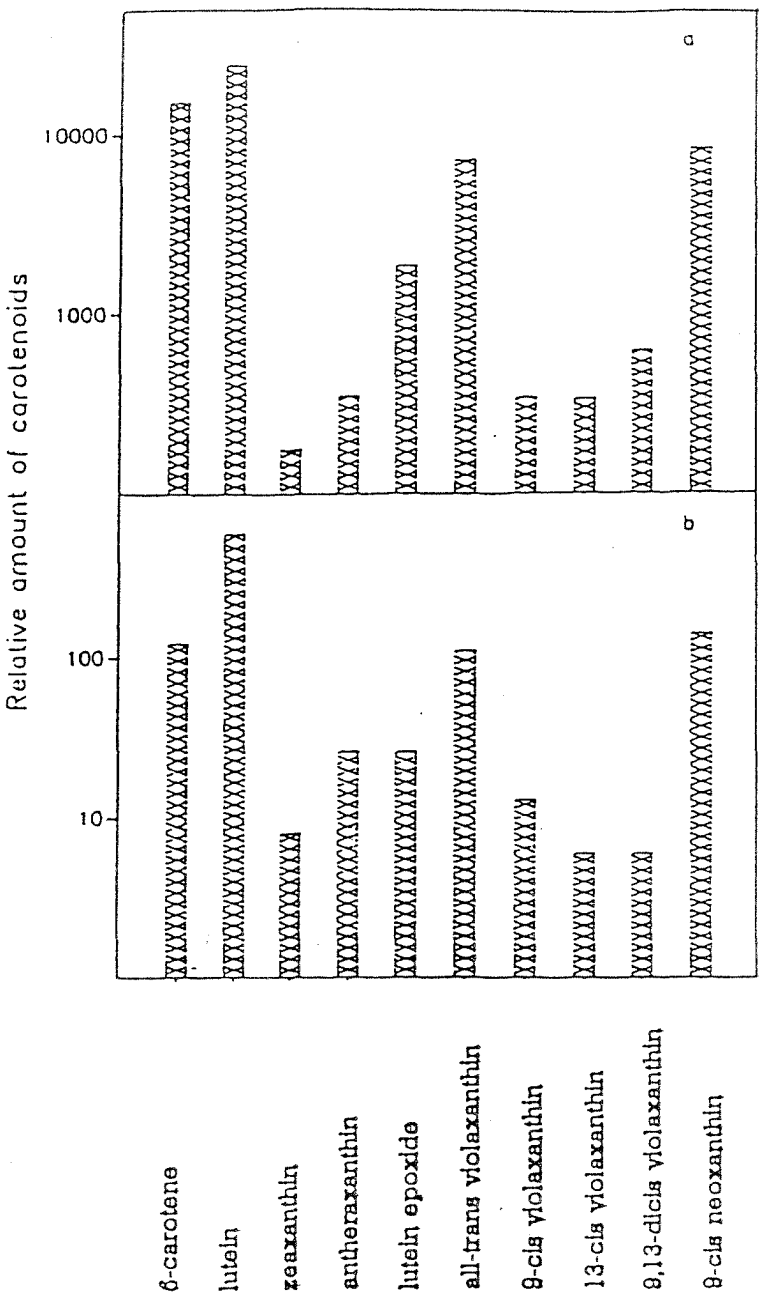

Fig. 1. – The relative amounts of 10 tested carotenoids in light grown (WT-Col)-(a) and pale-(b) seedlings

Tab. 2. – The quantity of individual carotenoids in etiolated (-RL) and red light-irradiated (+RL) seedlings of WT-Col. Values in the table are means ± 1 SE; n=2.

	- RL (mg · gFW^{-1})	+ RL
β-carotene	0.076 ± 0.011	0.097 ± 0.046
lutein	1.367 ± 0.008	2.014 ± 0.062
antheraxanthin	0.144 ± 0.008	0.205 ± 0.026
all-trans violaxanthin	1.707 ± 0.045	2.590 ± 0.047
9-cis violaxanthin	0.072 ± 0.013	0.106 ± 0.012
9-cis neoxanthin	0.287 ± 0.018	0.412 ± 0.002

Fluence-response relationships

The fluence-response relationships for induction of first positive phototropism for the etiolated seedlings from Am 45-3 population with the normally pigmented phenotype and the WT-Col seedlings were similar (Fig. 2. a and b). Fluence requirements for initiation of phototropism as well as for maximum response and descending arm of the curve corresponded very well in these two batches of seedlings (Fig. 2. and b). Based on these data it was decided to further compare responses of pale seedlings to BL and responses of the normally pigmented seedlings to BL assuming that the normally pigmented seedlings have wild type ability to perceive BL. The fluence-response relationship for first positive phototropism to a BL pulse was also measured for pale seedlings (Fig. 2. c). Response of pale seedlings was lower than that of both WT-Col and normally pigmented mutant seedlings (Fig. 2. a, b and c).

Irradiation of seedlings with the RL for 1 hour resulted in higher amplitude of response to a subsequent BL pulse (Fig. 3). This RL-induced enhancement of phototropism was used in all following experiments as the higher curvature was easier to score. The fluence-response curve describing first and second positive phototropism to the BL of the RL pre-irradiated seedlings was measured by varying the duration of pulses at constant fluence rate (Fig. 3). For the normally pigmented mutant seedlings threshold for the first positive response was about 0.01 μmol·m^{-2} and for second positive the threshold was about 100 μmol·m^{-2} (Fig. 3.a). The region of the maximum for the first positive response shows two distinctive peaks followed by the descending arm (Fig. 3. a). The pale seedlings exibited similar response to the unilateral BL pulses after the RL pre-iradiation (Fig. 3.b). The fluence requirements for initiation of the first and second positive responses as well as the fine structure for first positive phototropism corresponded well to those of the normally pigmented seedlings (Fig. 3.a). The major difference between the responses of these two batches of seedlings was the amplitude, with pale seedlings exhibiting a lower response than that of the normally pigmented seedlings (Fig. 3).

The effect of a desensitizing irradiation pulse was examined for both phenotypes from Am 45-3 population (Fig. 4. a and b). Seedlings were first irradiated for 1 hour with RL, and then were given a BL pulse from above followed within 2.5 min by a unilateral BL pulse of 0.3 μmol · m^{-2} to induce phototropism. By varying the desensitizing BL from above a fluence-response relationship for desensitization was measured. The fluence-response curves for desensitization in both phenotypes are similar (Fig. 4). As the fluence of BL administered from above increases, the response to the

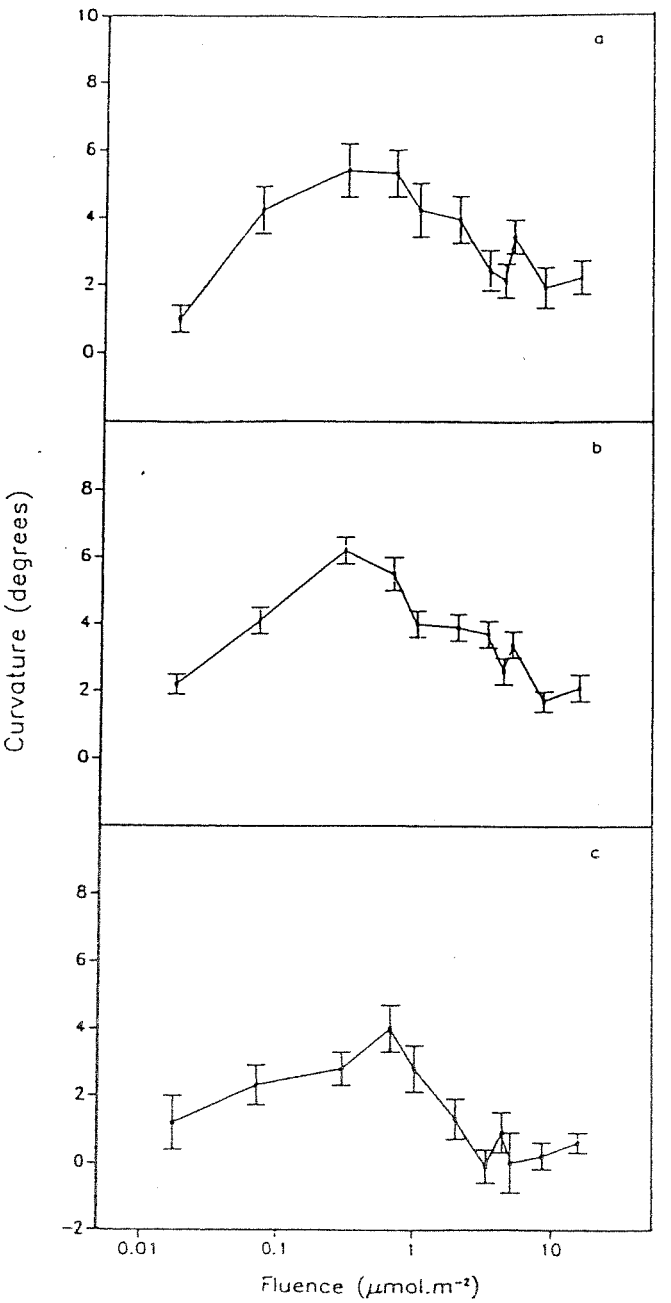

Fig. 2. - Fluence-response relationship for induction of first positive phototropism of etiolated: WT-Col-(a), normally pigmented-(b) and pale-(c) seedlings

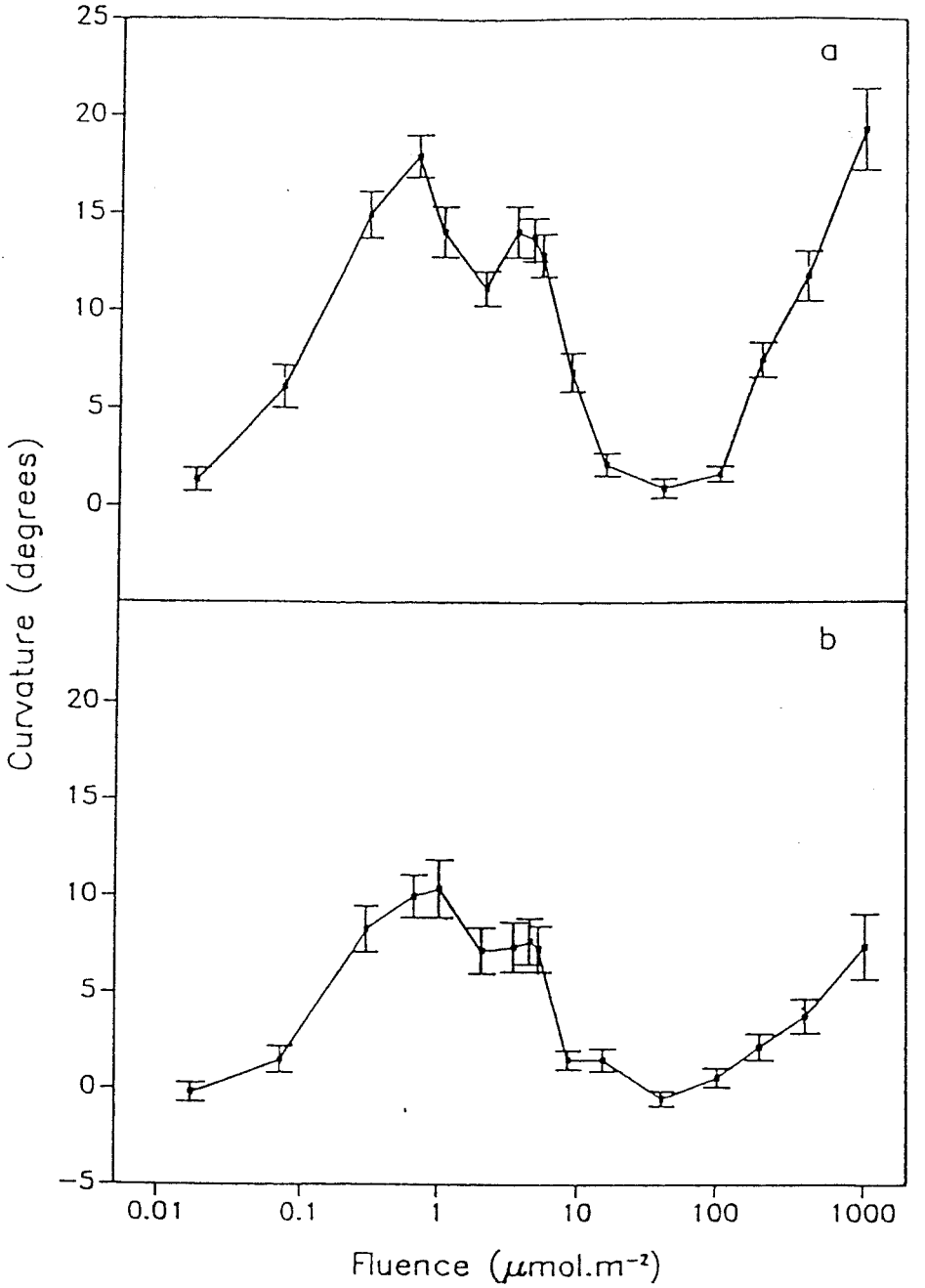

Fig. 3. – Fluence-response relationship for induction of phototropism of RL pre-irradiated: normally pigmented-(a) and pale-(b) seedlings; n>60; vertical bars represent ± 1 SE

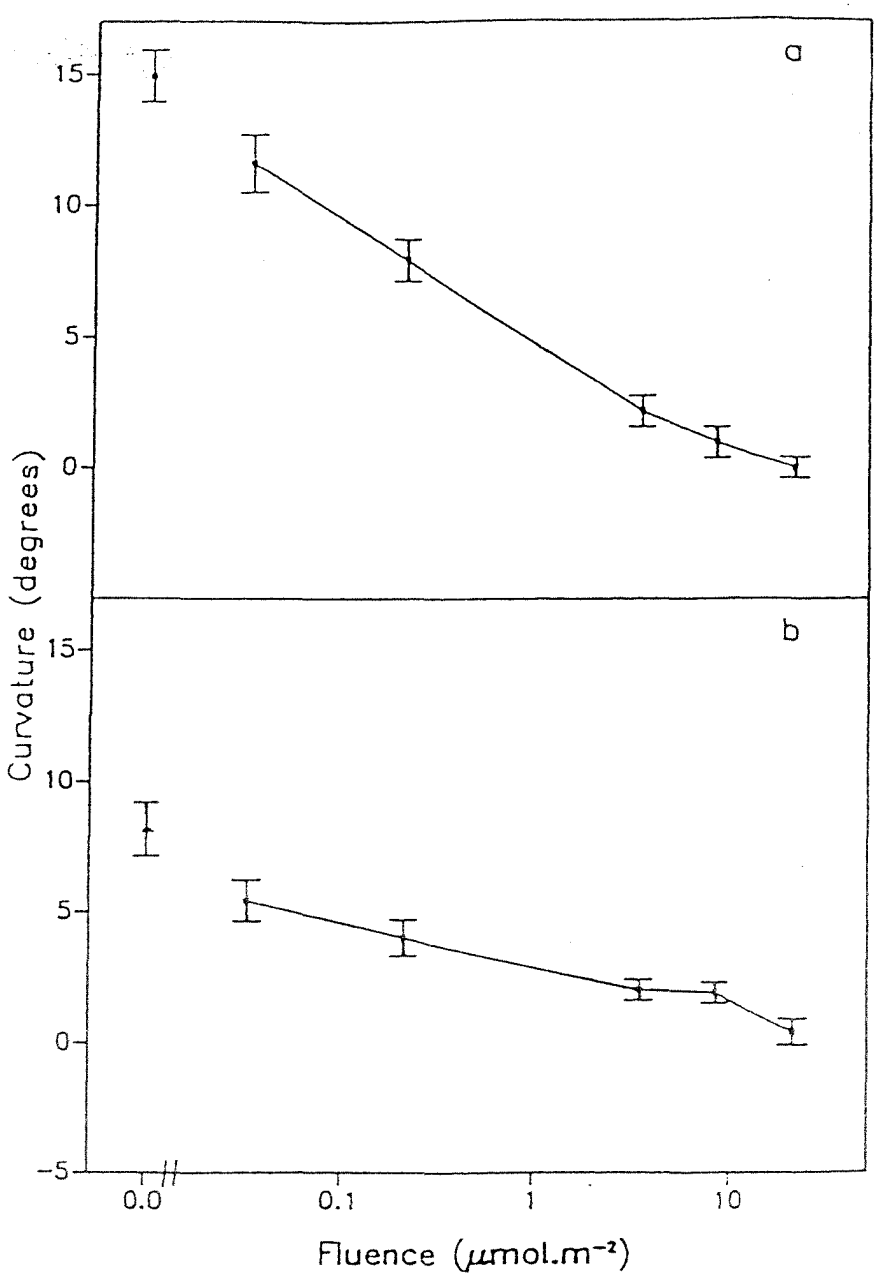

Fig. 4. – Fluence-response relationship for induction of phototropism following the BL pulse from above: normally pigmented-(a) and pale-(b) seedlings; n>60; vertical bars represent ± 1 SE

subsequent unilateral pulse decreases. Even the lowest fluence of BL applied from above (0.033 μmol · m^{-2}), in both batches of seedlings induced desensitization. The highest fluence of BL applied from above (21 μmol · m^{-2}) completely desensitized seedlings to the subsequent unilateral BL pulse (Fig. 4). Pale seedlings in this type of experiment again exhibited a lower amplitude of response than the normally pigmented seedlings (Fig. 4).

Gravitropism measurements

The ability of hypocotyls of pale seedlings to exhibit curvature was tested by exposing them to the constant 90 degrees gravity stimulation for 2 hours. Their response was not different from the response exerted by normally pigmented seedling (Table 3) to the same stimulus, suggesting that the mechanism of differential growth in pale seedlings was not impaired.

Tab. 3. – Gravitropic response to constant 90 degrees stimulation for 2 hours of two mutant phenotypes both RL pre-irradiated. Values in the table are means ± 1 SE; n>60

	degrees of curvature
normally pigmented phenotype	12.8 ± 0.8
pale phenotype	12.6 ± 1.2

DISCUSSION

The results of this study indicate that carotenoids do not play a major role in BL-induced phototropism and desensitization in *A. thaliana*. Although the level of 10 carotenoids in pale mutant seedlings was 35-40 times lower than in WT-Col plants they retained a WT sensitivity to unilateral BL and their phototropic response was only about two times lower in amplitude (Figs. 1. and 3).

The fluence requirements for the initiation of first and second positive phototropic response as well as for induction of two peaks and descending arm in the range of first positive phototropism were similar in pale seedlings and normally pigmented seedlings (Fig. 3. a and b). If any of the tested carotenoids were responsible for perception of unilateral BL that results in curvature, then some (or all) of the features of the fluence-response curve would be expected to move towards higher fluences according to the decrease in the amount of pigment(s).

Although carotenoids probably are not the photoreceptor pigments for phototropism in *Arabidopsis* they do affect the amplitude of the phototropic response (Figs. 1, 3 and 4). In order to detect the direction of light stimulation and respond phototropically, the seedling must detect the difference in absorption of light on its lighted and shaded side. If carotenoids were a screening agent across the seedling then their absence or decreased presence would result in lack of, or decrease in the light gradient. Results describing lower response to unilateral BL of the pale mutant seedlings when compared to the response of the normally pigmented seedlings (Fig. 3) are consistent with such a role of carotenoids.

The data describing the diference in levels of carotenoids in etiolated and RL-irradiated seedlings of WT-Col seedlings also support the theory that carotenoids may play a role as screening agents. One of the possible ways of enhancing the

phototropism could be by increasing the levels of carotenoids in the tissue, resulting in the steeper light gradient across the seedling. Higher levels of carotenoids are detected in the RL pre-irradiated WT-Col seedlings than in the etiolated WT-Col seedlings (Table 2). These results can be correlated with larger phototropic response of RL pre-irradiated normally pigmented mutant seedlings when compared to the etiolated WT-Col seedlings (Figs. 2. a and 3.a). Although these two strains differ genetically this difference has not affected their first positive phototropic response (Fig. 2. a and b). Moreover, seedlings of the Estland ecotype showed the same difference in magnitude of phototropism with and without RL pre-irradiation (J a n o u d i and P o f f, 1991). Enhancement of phototropism by RL may be due to the increased carotenoid content of the tissue (Figs. 2. a and 3. a).

However, there is a discrepancy between ratios of carotenoid levels in two different phenotypes and amplitudes of their responses to unilateral BL pulses. The explanation for this disagreement in the case of decrease of carotenoid content may be that carotenoids are not the only compounds that absorb the light from the blue part of the spectrum. When levels of carotenoids are very low, backscatter of light or the other molecular species such as pterins might prevent the disappearance of light gradient across the seedling which would result in complete absence of the response. Another explanation could be that the ratio of carotenoids in light grown pale vs. normally pigmented plants is not a good indicator of the ratio in etiolated tissue. Therefore the light gradient across the seedling established due to a presence of some molecular species that absorbs incident light although required, will not necessarily determine the magnitude of phototropism.

Pale mutant seedlings also retained the ability to undergo desensitization. Adaptation in phototropism of *Arabidopsis* seedlings includes: desensitization, refractory period, recovery and enhancement (J a n o u d i and P o f f, 1991). The complex shape of fluence-response curve for phototropism has been proposed to be due to adaptation (P o f f et al., 1994). Fluence requirements for desensitization overlap with fluences that induce enhancement. Thus plant is responding to the BL as a stimulus that induces and enhances phototropism and simultaneously causes desensitization. Therefore, response of a plant to a phototropic stimulation is a function of all components in both induction and adaptation of phototropism. If carotenoids were responsible for the perception of light that induces desensitization, pale seedlings would exhibit threshold for desensitization changed corresponding to the changed amount of carotenoids. Since similar fluence requirements for desensitization were exhibited by the pale and normally pigmented seedlings (Fig. 4) suggesting that carotenoids probably are not responsible for mediation of this process.

The quantities of individual carotenoids as a percentage of total amount of carotenoids have not changed due to 1 hour of RL (Table 1). These results indicate that the possible action of carotenoids in enhancement of phototropism is not mediated by single molecular species which would require the increased amount of that pigment.

Some carotenoids are known to be synthesized in plant organs in the absence of light (B r i t t o n, 1988). However, light is known to induce a large increase in carotenogenesis (B r i t t o n, 1988). For example, Oelmuller and Mohr have reported an increase in β-carotene in milo seedlings grown for 72 hours under RL as opposed to the plants grown in darkness (O e l m u l l e r and M o h r, 1985). We have measured the difference in carotenoid content between pale seedlings and the WT-Col seedlings in light grown tissue and related this difference to the magnitude of phototropism of etiolated seedling. The assumption is that the inductive effect of white light on

carotenogenesis is equal in both tested phenotypes. This is supported by the fact that the pale seedlings retain their sensitivity to BL (Fig. 3) and responsiveness to RL (Figs. 2. c and 3. b).

In summary, we report that seedlings with the pale phenotype from the population of mutant Am 45-3 have 35-40 times lower level of 10 tested carotenoids and exhibit two times lower phototropism than the normally pigmented seedlings from the same population. Pale seedlings retained their abiltiy to: undergo desensitization by BL, exhibit RL-mediated enhancement of phototropism and respond gravitropically in the WT fashion. Red-light-mediated enhancement of phototropism may be in part due to the increase of carotenoid content in the tissue. On the basis of these data it is concluded that carotenoids are not photoreceptor pigments for phototropism or desensitization in *A. thaliana* although they appear to affect the amplitude of phototropic response.

REFERENCES

Britton, G. (1988): Biosynthesis of carotenoids. *In* Plant Pigments (T.W. Goodwin, ed). pp. 133-182. – Academic Press, London.

Curry, G.M. (1969): Phototropism. *In* The physiology of plant growth and development (M. B. Willkins, ed.) pp. 245-273. – McGraw-Hill, New York.

Galland, P. & Lipson, E. D. (1987): Blue-light reception in *Phyconyces* phototropism: Evidence for two photosystems operating in low- and high-intensity ranges. – Proceedings of National Academy of Sciences USA *84*: 104-108.

Galland, P. (1991): Photosensory adaptation in aneuaral orgnaisms. – Photochemistry and Photobiology *54*: 1119-1134.

Iino, M. (1988): Desensitization by red and blue light in phototropism in maize celeoptiles. – Planta *176*: 183-188.

Janoudi, A. & Poff, K. L. (1991): Characterization of adaptation in phototropism of *Arabidopsis thaliana*. – Plant Physiology *95*: 517-521.

Kaufman, L. S. (1993): Transduction of blue-light signals. – Plant Physiology *102*: 333-337.

Khurana, J. P., Best, T. R. & Poff, K. L. (1989): Influence of hook position on phototropic and gravitropic curvature by etiolated hypocotyls of *Arabidopsis thaliana*. – Plant Physiology *90*: 376-379.

Konjević, R., Steinitz, B. & Poff, K. L. (1989): Dependence of the phototropic response of *Arabidopsis thaliana* on fluence rate and wavelength. – Proceedings of National Academy of Sciences USA *86*: 9876-9880.

Oelmuller, R. & Mohr, H. (1985): Carotenoid composition in milo (*Sorghum vulgare*) shoots as affected by phytochrome and chlorophyll. – Planta *164*: 390-395.

Piening, C. J. & Poff, K. L. (1988): Mechanism of detecting light direction in first positive phototropism in *Zea mays* L. – Plant, Cell and Environment *11*: 143-146.

Poff, K. L., Janoudi, A., Rosen, E. S., Orbović, V., Konjević, R., Fortin, M. C. & Scott, T. K. (1994): The physiology of tropisms. *In Arabidopsis* (E. Meyerowitz and C. R. Somerville, eds.). – Cold Spring Harbor Laboratory Press, Cold Spring Harbor-New York.

Presti, D., Hsu, W. J. & Delbruck, M. (1977): Phototropism in *Phycomyces* mutants lacking b-carotene. – Photochemistry and Photobiology *26*: 403-405.

Rock, C.D. (1991): Ph. D. Thesis. Michigan State University, East Lansing.

Song, P. S. (1984): Photophysical aspects of blue light receptors: The old question (Flavins versus Carotenoids) re-examined. *In* Blue light effects in biological systems. (H. Senger, ed.). – Springer, Berlin.

Vierstra, R. D. & Poff, K. L. (1981): Role of carotenoids in the phototropic response of corn seedlings. – Plant Physiology *68*: 798-801.

Rezime

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ULOGA KAROTENOIDA U FOTOTROPIZMU KLIJANACA *ARABIDOPSIS THALIANA*

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Mutant *Arabidopsis thaliana* (Am 45-3) koji je deficijentan u karotenoidima je korišćen za ispitivanje uloge karotenoida u fototropizmu i adaptaciji. Klijanci mutanta su bledi i sadrže oko 2.5-3% količine karotenoida koji su prisutni u divljem tipu koji je gajen na belom svetlu. Fototropski odgovor bledih klijanaca na plavu svetlost je bio sličan odgovoru divljeg tipa sem što je amplituda odgovora bila manja. Bledi klijanci zadržali su svoju sposobnost desenzitizacije plavom svetlošću kao elemenat adaptacije. Ovi klijanci su takođe pokazali povećanje fototropskog odgovora indukovano crvenim svetlom. Povećanje fototropizma je izgleda povezano sa povećanjem količine karotenoida. Naši rezultati su u saglasnosti sa zaključkom da karotenoidi nisu fotoreceptorni pigmenti za fototropizam ili desenzitizaciju iako prisustvo karotenoida utiče na amplitudu fototropizma i mehanizam njegovog povećanja kod *A. thaliana*.