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Action Spectra for the Inhibition of Hypocotyl Growth by Continuous Irradiation in Light and Dark-Grown *Sinapis alba* L. Seedlings[1]

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ABSTRACT

Action spectra for the inhibition by continuous (24-hour) irradiation of hypocotyl growth in 54-hour-old *Sinapis alba* L. seedlings were measured using seedlings which had had four different pretreatments. These seedlings were either (*a*) dark-grown with a high total phytochrome level, (*b*) dark-grown with a low total phytochrome level, (*c*) light-grown with chlorophyll, or (*d*) light-grown with no chlorophyll [treated with 4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone (San 9789)].

The resulting action spectra show that the blue, red, and far-red (716 nm) wavebands are most inhibitory for dark-grown plants with high phytochrome content, whereas hypocotyl growth in dark-grown plants with a low phytochrome content are only slightly inhibited by blue and far-red radiation. In light-grown plants, the effectiveness of blue and far-red light almost disappears. The position of red light effectiveness in chlorophyll-containing plants is shifted to lower wavelengths compared with those containing no chlorophyll.

The so-called HIR[2] (14) responses of plant photomorphogenesis (*i.e.* those which require prolonged irradiation, show no reciprocity, and are not R/FR-reversible) have attracted considerable interest and controversy. This has been the case particularly with respect to such problems as the exact form of the action spectrum under various conditions, the identity of the photoreceptor(s), and the role of the HIR in the natural environment.

Most HIR action spectra have been measured in dark-grown seedlings and show action maxima in the FR and B wavebands with a shoulder in the R (*e.g.* refs. 18 and 24). The detailed action spectrum for inhibition of hypocotyl growth in *Lactuca* (8) shows only B and FR peaks with almost no action in the R region. The action spectrum for anthocyanin synthesis in *Sorghum* (4) shows only a B peak and, in appleskin, a peak appears in the R, but there is no activity in the FR (25). The ratio of the effectiveness of B/FR radiation also varies considerably from action spectrum to action spectrum, although this may be partially due to differences in irradiation time and mode (14, 15). A loss of the response to FR light with increasing age or after a light pretreatment has also been reported (2, 5, 27).

The identity of the photoreceptor(s) is not entirely clear. Candidates which have been considered are Chl/photosynthesis, phytochrome, and, for the B part of the spectrum, the "blue light receptor." Evidence suggests no direct role for photosynthesis (14), although it is possible that it may affect the final expression of responses in green plants. Hartmann (7–9) has shown that it is possible to explain the FR action peak on the basis of phytochrome alone. The receptor(s) responsible for the B action maximum has not been identified.

To clarify some of these points and also to determine the form of the action spectrum in green plants where screening by Chl will occur and total phytochrome will be lower than in dark-grown plants, action spectra for the inhibition of hypocotyl elongation in *Sinapis alba* L. seedlings have been measured. The measurements were carried out on (*a*) dark-grown plants with a high level of P_{tot}, (*b*) dark-grown plants with a low level of P_{tot}, (*c*) light-grown plants with a high Chl level, and (*d*) light-grown plants with a very low Chl level.

MATERIALS AND METHODS

Sinapis alba L. seeds (harvest 1975) were obtained from Asgrow Co., Freiburg-Ebnet, W. Germany, selected and sown on filter paper in plastic boxes as described by Mohr (19) except that a modified Hoagland solution (3) was used instead of distilled H_2O. For the herbicide-treated plants, 5 μM San 9789 was added to the Hoagland solution. This herbicide inhibits the synthesis of carotenoids, leaving the Chl open to photodestruction (1), thereby producing an essentially Chl-free, but white light grown, plant. Many phytochrome-modulated photomorphogenic responses, including hypocotyl growth, are not influenced significantly by San 9789 (10). The seeds were then transferred either to darkness at constant temperature (25 C) or to a growth chamber providing continuous white light (also at 25 C) for 54 h. The low P_{tot} dark-grown plants were given 5 min standard R light at 52 h after sowing, followed by 55 min darkness followed again by 5 min R light and 55 min darkness. This reduced the level of P_{tot} to 25% of that found in fully dark-grown seedlings (pretreatment D). The level in white light-grown, San 9789-treated plants (pretreatment H) was 2.5% of the level in dark-grown plants. All plants that had previously had a light treatment were then given a saturating irradiation with long wavelength FR light. At this point, all plants (including dark-grown plants) were transferred to the relevant monochromatic wavelength. After 24-h irradiation, the hypocotyl lengths were measured. The lengths of control plants which received no monochromatic irradiation were measured at 54 and 78

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[2] Abbreviations: HIR, high irradiance response; R, red; FR, far-red; B, blue; P_{tot}, total phytochrome; San 9789, 4-chloro-5(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone.

h after sowing.

Light Sources. White light in the phytochamber was provided by Xenon arc lamps (Osram XQO 10 kw), the light being filtered through heat-absorbing glass (3-mm KG 1, Schott and Gen. Mainz, W. Germany) and 6-mm thick Thermopane glass. The photon fluence rate was 370 μmol m^{-2} s^{-1} in the 400- to 800-nm wave band.

The source of standard R light was described by Mohr *et al.* (20) and produced 3.68 μmol m^{-2} s^{-1} (λ_{max} = 656nm).

Long wavelength FR light was obtained by filtering the light from a modified Zeiss Ikon Xenosol III 2.5-kw xenon arc source (23) through a Schott RG 9 color glass filter and a KG 1 heat-absorbing glass. Monochromatic light was obtained using Schott DAL filters. These had a half-bandwidth varying between 15 and 22 nm at 50% of maximum transmission. The light sources for the monochromatic experiments were as follows: for lower fluence rates at wavelengths above 500 nm, Leitz Prado 500-w Universal projectors as modified by Mohr and Schoser (21) were used; lower fluence rates at wavelengths less than 500 nm were obtained using Leitz-Wetzlar projectors with Osram XBO 450-w xenon arcs as modified by Mohr and Schoser (22); for higher irradiances, either a modified Zeiss Ikon Xenosol III or Xenosol V fitted with Osram XBO 2.5-kw or XBO 6.5-kw xenon arcs, respectively (23) was used. The spectral photon fluence rate distribution of the actinic light was measured with a Gamma Scientific (San Diego, Calif.) model 2900 digital photometer, 700-31 VTM scanning monochromator, 2020-10 C photomultiplier system (tube type, PM 6) and a flexible fiber optic tube fitted with miniature cosine-corrected receptor head (type 700-8 B). This instrument was calibrated against a Gamma Scientific standard source (traceable to standards possessed by Gamma Scientific). For cross-reference and daily monitoring of the actinic sources, a Tektronix J 16 photometer and J6512 radiant energy probe were used (Tektronix, Beaverton, Ore).

Production of the Action Spectra. For each treatment, 29 seedlings were measured and the longest two and shortest six (nongerminated counting as short) seedlings were rejected. From the remaining 21, the mean value was determined and, from the dark control values, percentage inhibition was estimated. Percentage inhibition was calculated as:

$$\% \text{ inhibition} = \frac{\Delta_D - \Delta_\lambda}{\Delta_D} \cdot 100$$

where Δ_D is hypocotyl growth (mm) in dark during the 24-h experimental period and Δ_λ is hypocotyl growth (mm) under the monochromatic light during the same period. Typical control growths were as shown in Table I.

Fluence rate-response curves were plotted as percentage inhibition against logarithmic photon fluence rate. The curves were fitted to the points by regression analysis in the part of the curve between 20 or 30 and 60% inhibition (26). Action spectra for 50% inhibition were constructed from these curves. The maxima and minima of the action spectra were found to be significant with respect to the sign test (26).

RESULTS AND DISCUSSION

Photon fluence rate-response curves are presented for plants which have received each of the four pretreatments in Figure 1 A to D. For clarity, data are shown only for wavelengths of action maxima and minima. Most of the curves appear, within the accuracy of our measurements, to be parallel. In some cases, however, this is not so. Previous fluence rate-response curves for HIR responses have often shown curves for different wavelengths to be nonparallel (14). Theory does not yet allow one prediction of whether or not one would expect the curves to be parallel.

The action spectrum for inhibition of hypocotyl growth in dark-grown plants (Fig. 2A) shows inhibition maxima in the B (λ_{max} = 448 nm), R (λ_{max} = 655 nm), and FR (λ_{max} = 716 nm) wavebands. Two brief exposures of dark-grown plants to R light (pretreatment X) resulted in a decreased response to both B and FR light (Fig. 2B). The R action peak remained unaffected.

The plants which had been grown under continuous white light (pretreatment W) (Fig. 2C) show negligible inhibition by B and FR light. Here, the major inhibitory waveband is in the R region (λ_{max} = 640 nm) with a subpeak in the yellow waveband (λ_{max} = 594 nm). Plants which have been grown under white light but contain little or no Chl (pretreatment H) have major (λ_{max} = 667 nm) and minor (λ_{max} = 609 nm) peaks, but these are at longer wavelengths than in the green plants (Fig. 2D).

Action Spectrum for Dark-grown Plants. The action spectrum for inhibition of hypocotyl growth in dark-grown plants shows action maxima in the B, R, and FR wavebands. In most previous action spectra, the effectiveness of R light is considerably lower than that of FR (*e.g.* refs. 4, 5, and 18). The detailed action spectrum of Hartmann (8) shows almost no activity in the R waveband for inhibition of hypocotyl growth in *Lactuca sativa.* Häcker *et al.* (6) demonstrated, however, that R light inhibits growth in the middle part of the hypocotyl but promotes growth in the upper (hook) region. These effects can compensate one another. The R region of Hartmann's spectrum must therefore be interpreted with care. The spectrum obtained here shows greatest similarity to that published by Jose and Vince-Prue (13) for inhibition of hypocotyl elongation in seedlings of *Raphanus sativus* L. Their spectrum shows strongest inhibition by R and FR light with a lesser peak in the B waveband. The small sub-peak around 600 nm cannot be explained. A similar peak was reported in several other action spectra (*e.g.* refs. 18, 24, and 25).

Action Spectra for Light-grown Plants. In dark-grown plants (pretreatment D), the level of phytochrome (P_{tot}) was high at the beginning of the irradiation period. In the other three treatments, the P_{tot} level had been lowered by a previous light treatment. The effects of these pretreatments is shown in the relevant action spectra. The action spectrum for low P_{tot} dark-grown plants (Fig. 2B, pretreatment X) shows the same qualitative features as that

Table I. *Lengths of Controls after Treatments Shown*

Age	Pretreatments			
	54 h Dark (D)	52 h Dark + 2 × (5 min R + 55 min dark) (X)	54 h White light (W)	54 h White light + San 9789 (H)
	mm[a]			
54-h pretreatment only	28.2 ± 0.7	27.9 ± 0.8	5.0 ± 0.1	5.1 ± 0.1
54-h pretreatment + 5 min RG9 + 24 h dark	56.9 ± 1.1	57.7 ± 1.4	16.3 ± 0.1	12.0 ± 0.3

[a] Values are ± SE.

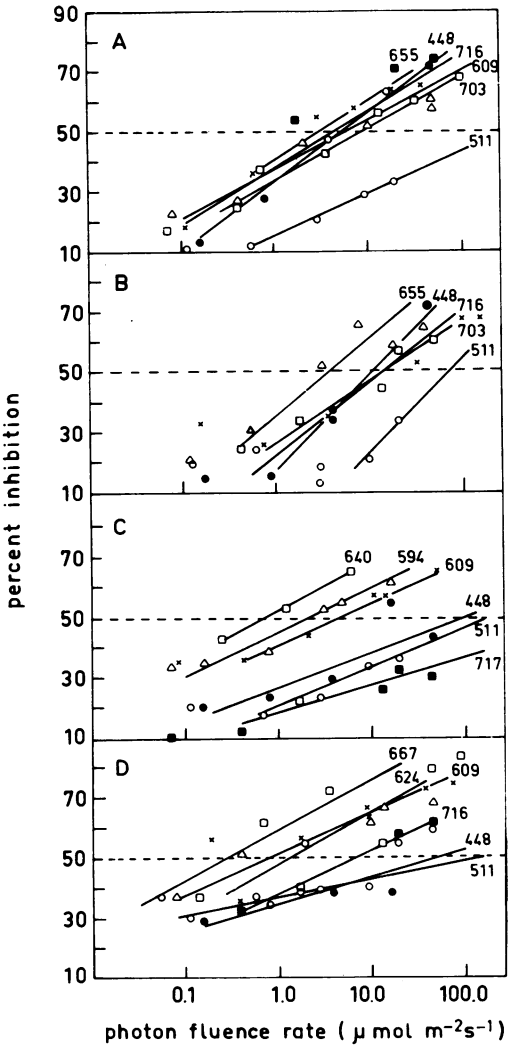

FIG. 1. Fluence rate-response curves for light inhibition of hypocotyl elongation. A: For treatment D (54 h dark + 24 h N_λ); (●), 448 nm; (○), 511 nm; (△), 609 nm; (×), 655 nm; (□), 703 nm; (■), 716 nm. B: For treatment X (52 h dark + 5 min R + 55 min dark + 5 min R + 55 min dark + 24 h N_λ); (●), 448 nm; (○), 511 nm; (△), 655 nm; (×), 703 nm; (□), 716 nm. C: For treatment W (54 h white light + 24 h N_λ); (●), 448 nm; (○), 511 nm; (△), 594 nm; (×), 609 nm; (□), 640 nm; (■), 716 nm. D: For treatment H (54 h white light + 24 h N_λ in presence of San 9789); (●), 448 nm; (○), 511 nm; (△), 609 nm; (×), 624 nm; (□), 667 nm; (■), 716 nm.

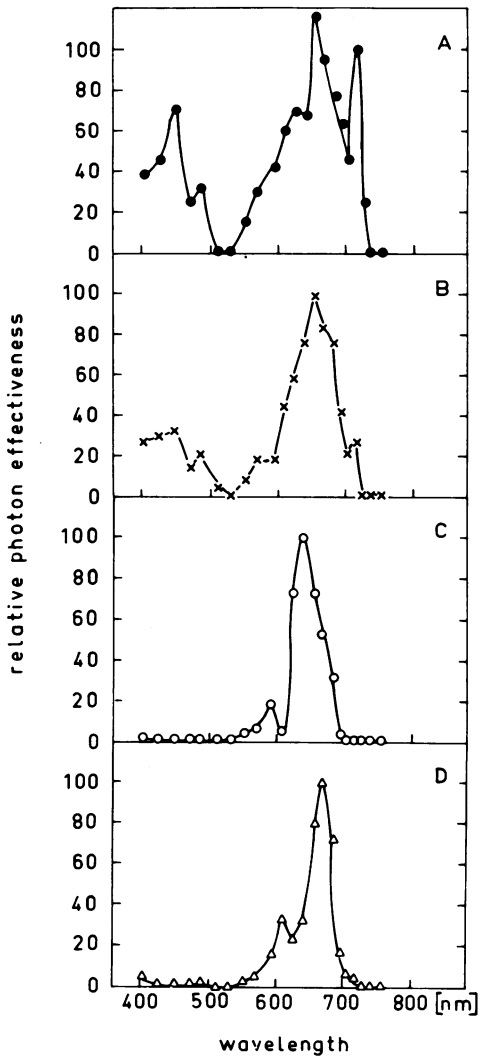

FIG. 2. Action spectra for light inhibition of hypocotyl elongation plotted from fluence rate-response curves of the type shown in Figure 1. A: For dark-grown (pretreatment D) seedlings; relative photon effectiveness normalized to 716 nm = 100%. B: For dark-grown (pretreatment X) seedlings; relative photon effectiveness normalized to 655 nm = 100%. C: For white-light-grown, green (pretreatment W) seedlings; relative photon effectiveness normalized to 640 nm = 100%. D: For white-light-grown, San 9789 (pretreatment H) seedlings; relative photon effectiveness normalized to 667 nm = 100%.

for high P_{tot} dark-grown plants, but both the B and FR peaks are considerably smaller. In the plants which had been treated previously with white light (Fig. 2C, pretreatment W), the effectiveness of both B and FR light disappears almost entirely. The resulting action spectra show similarities to that for anthocyanin production in "light-grown" apple skin (25).

Effect of Lowering P_{tot}. It has been reported frequently in the literature that the effectiveness of FR light decreases or disappears after exposure to light (2, 11, 27) and with age (2, 5). Both of these factors decrease P_{tot} levels. The level of phytochrome (P_{tot}) in dark-grown *Sinapis* decreases after 48 h (29). Also, R light leads to phytochrome destruction, thereby reducing the P_{tot} level. It is possible that the decrease in P_{tot} is responsible for the disappearance of the FR peak. Jose (11), however, could find no effect of aging on the FR peak, although it did disappear after pretreatment with R light. The results of Evans *et al.* (5) showed that the FR peak disappeared with age but that aging had no effect on the B peak.

Significance of B and FR Peaks. A comparison of the four spectra shows that although the FR and B light peaks disappear more or less synchronously following light pretreatment, the R peak remains stable. This could be interpreted as implying the existence of two separate pigments, one responsible for B/FR effects and one for R. However, although the disappearance of the FR peak with age or after a light pretreatment is well documented, such effects have not been reported for this B peak. [Indeed, in *Lactuca* (27) this appears not to be the case.] In *Sinapis*, the B peak behaves in the same way as the FR peak. The synchronous disappearance of the B and FR action maxima supports the hypothesis that they are due to the same photoreceptor. Wavelength-dependent Chl production will result in differential screening effects during the treatment period. However, it is unlikely that a difference in greening was enough to account for the considerable drop in B light effectiveness noticeable between dark-grown and R light pretreated seedlings.

Hartmann (7–9) has provided convincing evidence that the FR peak can be explained on the basis of phytochrome in *Lactuca*. This suggests that the B peak in *Sinapis* (at this age and for this response) may also be due to phytochrome. Wildermann *et al.* (28) have previously provided evidence that the B light receptor in *Sinapis* is phytochrome. In other plants, such as *Cucumis*, the evidence for a separate blue receptor is greater (17), although confounding effects caused by a phototropic response cannot be excluded. The results presented here do not require the postulation of a separate blue receptor. If one is present, it plays a very small role.

Significance of R Peak. If the FR peaks are due to phytochrome, the question arises as to which photoreceptor is responsible for the response in the R waveband. Chl is an unlikely candidate; indeed, the similarity in the responses of the herbicide-treated plants and the green plants provides strong evidence that Chl and photosynthesis are generally not involved in the continuous light responses. The inhibition response was lower in green plants than in herbicide-treated plants and its action maximum was shifted about 20 to 30 nm (*i.e.* away from the Chl absorption maximum) to lower wavelengths. Those effects are presumably due to screening of the photoreceptor by Chl. Such effects have already been reported for induction responses (see ref. 12 for examples). It seems most likely that phytochrome is indeed the photoreceptor for the R peak, although it probably acts through a different mechanism than that suggested by Hartmann (7–9) to explain the HIR responses. A likely possibility would be multiple induction effects.

The white light-grown plants thus showed a fluence rate-dependent response to continuous white light that was not due to photosynthesis. This supports the suggestion of McLaren and Smith (16) that, in the control of photoresponses in the natural environment, both light quantity and light quality may be important.

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LITERATURE CITED

1. BARTELS PG, A HYDE 1970 Chloroplast development in 4-chloro-5-(dimethylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H) pyridazinone (Sandoz 6708)-treated wheat seedlings. Plant Physiol 45: 807–810
2. BLACK M, JE SHUTTLEWORTH 1974 The role of the cotyledons in the photocontrol of hypocotyl extension in *Cucumis sativus* L. Planta 117: 57–66
3. CUMMING BG 1967 Early-flowering plants. *In* FH Wilt, NK Wessels, eds, Methods in Developmental Biology. Thomas Y Crowell Company, New York, pp 277–299
4. DOWNS RJ, HW SIEGELMAN 1963 Photocontrol of anthocyanin synthesis in Milo seedlings. Plant Physiol 38: 25–30
5. EVANS LT, SB HENDRICKS, HA BORTHWICK 1965 The role of light in suppressing hypocotyl elongation in Lettuce and *Petunia*. Planta 64: 201–218
6. HÄCKER M, KM HARTMANN, H MOHR 1964 Zellteilung und Zellwachstum im Hypokotyl von *Lactuca sativa* L. unter dem Einfluß des Lichtes. Planta 63: 253–268
7. HARTMANN KM 1966 A general hypothesis to interpret "high energy phenomena" of photomorphogenesis on the basis of phytochrome. Photochem Photobiol 5: 349–366
8. HARTMANN KM 1967 Ein Wirkungsspektrum der Photomorphogenese unter Hochenergiebedingungen und seine Interpretation auf der Basis des Phytochroms (Hypokotylwachstumshemmung bei *Lactuca sativa* L.). Z Naturforsch 22b: 1172–1175
9. HARTMANN KM 1967 Photoreceptor problems in photomorphogenic responses under high energy conditions (UV-blue-far red). Abstracts European Photobiology Symposium. Hvar, Yugoslavia, pp 29–32
10. JABBEN M, GF DEITZER 1979 Effects of the herbicide SAN 9789 on photomorphogenic responses. Plant Physiol 63: 481–485
11. JOSE AM 1977 Photoreception and photoresponses in the radish hypocotyl. Planta 136: 125–129
12. JOSE AM, E SCHÄFER 1978 Distorted phytochrome action spectra in green plants. Planta 138: 25–28
13. JOSE AM, D VINCE-PRUE 1977 Action spectra for the inhibition of growth in radish hypocotyls. Planta 136: 131–134
14. MANCINELLI AL, I RABINO 1978 The "high irradiance responses" of plant photomorphogenesis. Bot Rev 44: 129–180
15. MANCINELLI AL, L WALSH 1979 Photocontrol of anthocyanin synthesis. VII. Factors affecting the spectral sensitivity of anthocyanin synthesis in young seedlings. Plant Physiol 63: 841–846
16. MCLAREN JS, H SMITH 1978 Phytochrome control of the growth and development of *Rumex obtusifolius* under simulated canopy light environments. Plant Cell Environ 1: 61–67
17. MEIJER G 1968 Rapid growth inhibition of gherkin hypocotyls in blue light. Acta Bot Néerl 17(1): 9–14
18. MOHR H 1957 Der Einfluß monochromatischer Strahlung auf das Längenwachstum des Hypocotyls und auf die Anthocyanbildung bei Keimlingen von *Sinapis alba* L. (= *Brassica alba* Boiss.). Planta 49: 389–405
19. MOHR H 1966 Untersuchungen zur phytochrominduzierten Photomorphogenese des Senfkeimlings (*Sinapis alba* L.). Z Pflanzenphysiol 54: 63–83
20. MOHR H, U MEYER, K HARTMANN 1964 Die Beeinflussung der Farnsporen-Keimung *Osmunda cinnamomea* (L) und *O. claytoniana* (L.) über das Phytochromsystem und die Photosynthese. Planta 60: 483–496
21. MOHR H, G SCHOSER 1959 Ein Interferenzfilter Monochromatoranlage für photobiologische Zwecke. Planta 53: 1–17
22. MOHR H, G SCHOSER 1960 Eine mit Xenonbögen ausgerüstete Interferenzfilter Monochromatoranlage für kurzwellige sichtbare und langwellige ultraviolette Strahlung. Planta 55: 143–152
23. RASCHKE K 1967 Eine Anlage zur monochromatischen Bestrahlung biologischer Objekte ausgerüstet mit Interferenzfiltern und einer elektronisch geregelten. 2.5 KW Xenonlampe. Planta 75: 55–72
24. SIEGELMAN HW, SB HENDRICKS 1957 Photocontrol of anthocyanin formation in turnip and red cabbage seedlings. Plant Physiol 32: 393–398
25. SIEGELMAN HW, SB HENDRICKS 1958 Photocontrol of anthocyanin synthesis in apple skin. Plant Physiol 33: 185–190
26. SNEDECOR GW, WG COCHRAN 1967 Statistical Methods, Ed 6 Chap 5 and 6. Iowa State University Press, Ames
27. TURNER MR, D VINCE 1969 Photosensory mechanisms in the lettuce seedling hypocotyl. Planta 84: 368–382
28. WILDERMANN A, H DRUMM, E SCHÄFER, H MOHR 1978 Control by light of hypocotyl growth in de-etiolated mustard seedlings. I. Phytochrome as the only photoreceptor pigment. Planta 141: 211–216
29. WILDERMANN A, H DRUMM, E SCHÄFER, H MOHR 1978 Control by light of hypocotyl growth in de-etiolated mustard seedlings. II. Sensitivity for newly formed phytochrome after a light to dark transition. Planta 141: 217–223