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THE INFLUENCE OF LIGHT ON THE CONTENT OF GROWTH SUBSTANCES IN PEA SHOOTS. I. EFFECT OF RED LIGHT ON THE EXTRACTABLE INDOLE AUXINS

It is well known that light has a marked effect on the elongation of plant stems, which is on the other hand regulated by growth hormones. However, the attempts to correlate the light effects with the metabolism of hormones have so far produced conflicting and contradictory results (Hillman and Galston, 1961). This may partly be due to the fact that the techniques used in different laboratories were rather dissimilar. First, light treatments applied by different authors varied very much in respect to the quality and intensity of light, as well as in respect to the duration of illumination. Second, a variety of methods for obtaining, purifying and estimating the content of growth substances makes a comparison between different results very difficult; in earlier literature growth substances were often referred to as »auxins« and it is not clear which substances are in fact involved.

It is not our intention to give here a complete review of the previous work. The present paper will not deal with the light treatments bringing about the photoperiodic responses or active in producing photosynthesis. Only the relationship between morphogenetic effects of light and the changes in endogenous growth substances will be considered.

Several authors have found that light decreases the content of growth substances in plants. Van Overbeek (1936) exposed *Avena* coleoptiles to the light of wavelengts longer than 575 nm for 3 hours; irradiated coleoptile tips yielded less auxin by diffusion than the non-irradiated ones. Blaauw-Jansen (1959) applied monochromatic red light (660 nm) and found in coleoptile extracts a decrease in the amount of one active substance, presumably IAA. Similar results were obtained by Briggs (1963). Fletcher and Zalik (1964) analysed the content of IAA in *Phaseolus* extracts by means of the *Avena* first internode test and by spectrophotometry; light of all wavelengths decreased the amount of IAA, red light being the most effective. On the contrary, Oppenorth (1942) stated that treatment of etiolated *Avena* coleoptiles with white, blue or orange light induced a synthesis of auxin in the tips, which was evident two to three hours after light treatment. Biebel (1942)

reported that bean hypocotyls after irradiation with visible light longer than 620 nm (15 min daily for 5 days) contained the same amount of auxin per hypocotyl, but on the fresh weight basis the irradiated plants had about 2.2 times more Shen-Miller and Gordon (1966) exposed corn coleoptiles to blue light of first positive phototropic response and found an increase in IAA content, while the amount of two other biologically active substances decreased.

A possible mechanism of the effect of light on auxin content may be exerted through the control of synthesis of phenolic compounds, acting as cofactors or inhibitors of IAA-oxydase activity. Light was found to regulate the synthesis of phenylalanine deaminase, an enzyme which catalyses the conversion of phenylalanine to *trans*-cinnamic acid, the latter being the key substance in the metabolism of other phenolic acids (Zucker, 1965; Engelsma, 1967a, 1967b). Other authors had evidence that red light inhibits the activity of IAA-oxydase (Hillman and Galston, 1957; Dinant and Gaspar, 1967); it is possible that this inhibition is due to the red light enhanced synthesis of a quercetin derivative, acting as an inhibitor of IAA-oxydase (Bottomley et al., 1966), although the authors consider that the relationship between this synthesis and growth is not proved.

There are some recent results showing that the conversion of tryptophan to IAA may also be affected by light. Moore and Shaner (1967) found that cell-free extracts of light-grown pea plants were more active in converting ^{14}C-tryptophan to IAA, than the comparable extracts of dark-grown plants. This work and the work concerning the inhibitors of IAA-oxydase would suggest, that light-grown plants might be expected to contain more IAA than the dark-grown ones.

The present paper deals with the content of indole auxins in pea shoots, as affected by red light. As it was shown previously (Nešković and Burnett, 1966), tryptophan and IAA are the main indole compounds found in pea shoot extracts and the changes in these two substances have now been reported. Work concerning other growth hormones - gibberellins will be reported later on.

MATERIAL AND METHODS

Seeds of *Pisum sativum L.*, var. Alaska were soaked for 6 hours, spread on moist filter paper for 24 hours, after that time selected for uniformity and planted on washed sterile sand in darkness. After 6 days the plants were illuminated for 1 hour with red light of a Philips TL-20W/15 red fluorescent tube, with maximal emission at 660 nm, light intensity at the plant level being 50 μ Wcm^{-2}. After illumination the plants were returned to darkness. 24 or 48 hours later whole stems were harvested, frozen and extracted with cold methanol in the usual manner.

For tryptophan determination it was found suitable to purify the extracts by filtering through short (10 $\times$ 1 cm) cellulose powder columns. 0.4 ml of the extract, an equivalent of 5 plants was put on the top of the

column and eluted with water. The water was evaporated off under reduced pressure, the residue spotted on a silica gel H thin layer and the chromatogram developed in ethylacetate: isopropanol: water, 65 : 24 : 11 (Ballin, 1964). 1 cm zones of silica gel were eluted with 1.25 ml of water, centrifuged and examined for fluorescence by an Aminco-Bowman spectrophotofluorometer. Fractions containing tryptophan, with activation and fluorescence spectra at 280 and 360 nm respectively, were combined and serial dilutions were made for the quantitative determination of tryptophan. The amount of this substance was read from a calibration curve of pure DL-tryptophan.

For IAA determination methanol extracts corresponding to 500 plants were separated into water soluble and ether soluble fractions, and the acid ether soluble fraction obtained by using a DEAE-cellulose column, as described previously (Nešković and Burnett, 1966). The residue of the acid ether soluble fraction was also spotted on a silica gel H thin layer. As the extracts contained many fluorescent substances and a strong growth inhibitor as well, it was found that two successive chromatographic separations were convenient in order to remove the bulk of interferring substances. So the chromatograms were first developed in a nonpolar solvent like benzene: aceton (90 : 10) or chloroform: methanol (93 : 7) (Stahl, 1967). In these solvents IAA does not move from the start, but the inhibitor and some of the fluorescent stuff do. The starting line up to the Rf 0.07 was then eluted with 1 ml of methanol and spotted again on another silica gel H plate, which was developed either in ethylacetate: isopropanol : water (65 : 24 : 11) or methylacetate : isopropanol : ammonia (45 : 35 : 20) (Stahl, 1967). The zones of chromatograms were eluted with 1.25 ml of water, each fraction centrifuged and examined for fluorescence. Fractions showing activation and fluorescence spectra of IAA, at 285 and 365 nm respectively, were combined, a concentrated sucrose-buffer solution added and they were assayed by the *Avena* mesocotyl test (Nitsch and Nitsch, 1956).

RESULTS

At the time of irradiation, etiolated pea plants had two internodes, the third one just beginning to develop. The treatment with red light caused a redistribution of growth between the second and the third internodes, the second internode being inhibited, the third one stimulated. After 48 hours the inhibition and the stimulation were 66% and 198% respectively, if the length of the corresponding internodes of dark-grown plants is taken as 100%. The total stem length was not significantly affected.

The amount of tryptophan was determined according to its fluorescence intensity, as compared to the fluorescence intensity of known concentrations of DL-tryptophan. The serial dilutions of tryptophan eluted from the chromatograms showed that the eluate had to be diluted ten times in order to obtain a fluorescence value lying on the linear part of

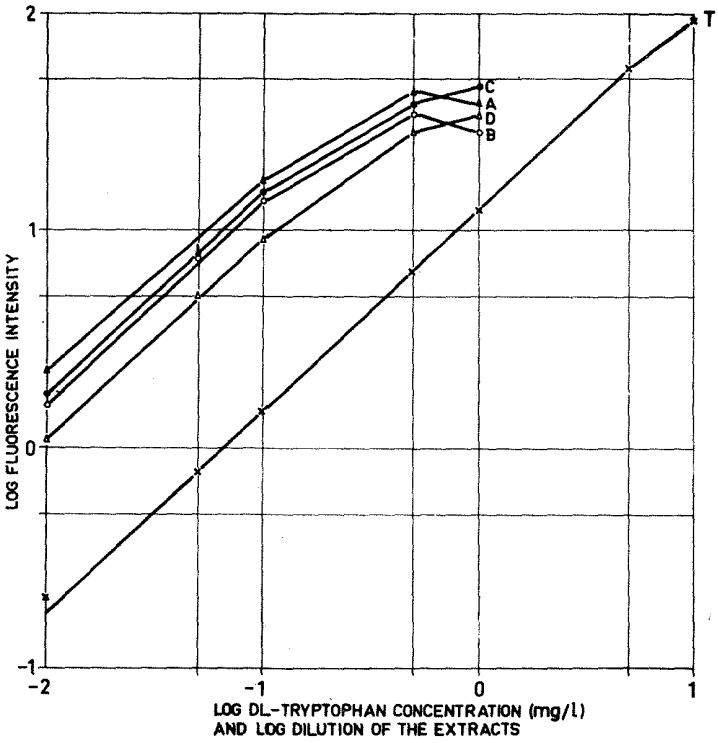

Fig. 1. Quantitative determination of tryptophan content in shoot extracts Abscissa: log concentration of DL-tryptophan and extracts; extracts at point O without dilution. T = DL-tryptophan; A = dark-grown plants, 24 hrs after treatment; B = irradiated plants after 24 hrs; C = dark-grown plant, 48 hrs after treatment; D = irradiated plants after 48 hrs; each curve is the mean of 4 experiments.

For determination of tryptophan content in A, B, C and D, the concentrations calculated at point -1, lying on the linear part of the curves, were multiplied by 10 in order to obtain the correct value of the undiluted extracts; as each extract consisted of a total of 4 or 5 ml, and for the measurement only 1 ml was used, the final amount of tryptophan per 5 plants was calculated by multiplyng the obtained values by 4 (B, C and D) or 5 (A). For all measurements activation wavelength was 230 nm, fluorescence wavelength 360 nm.

Table 1

Tryptophan content in pea shoot extracts in μg per 5 plants; each value is the mean of 4 replicates

	Time after light treatment: 24 hrs	48 hrs
Dark-grown plants	62.50	54.74
Irradiated plants	45.30	31.10

the calibration curve. The method used for the calculation is explained in Fig. 1. As can be seen from Table 1, irradiation with red light caused a decrease of tryptophan content in pea shoot extracts.

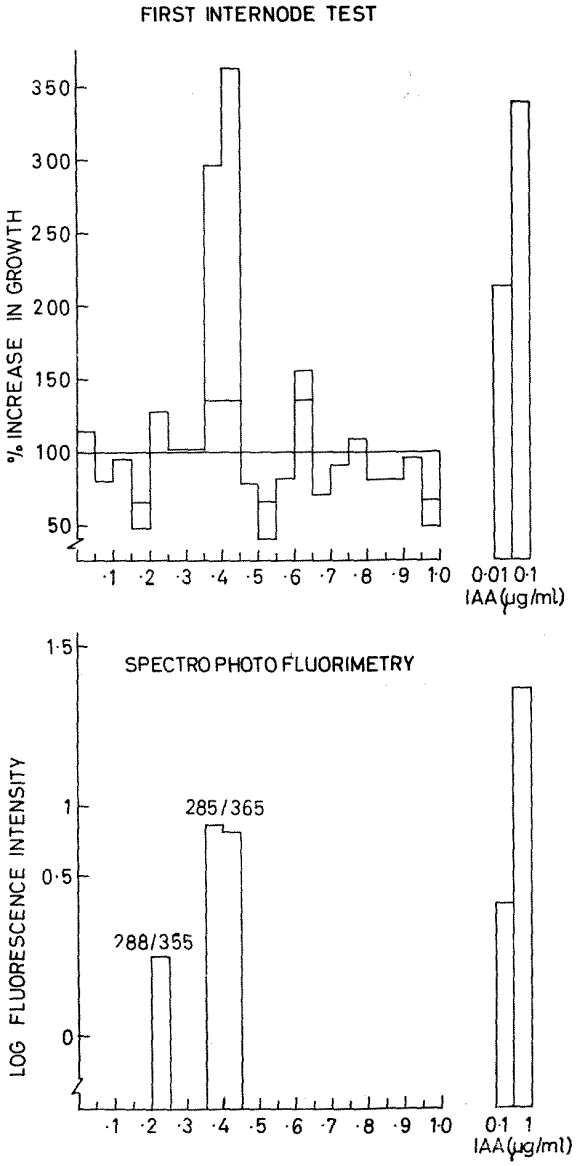

Fig. 2. Histogram showing the fluorescence intensity (below) and biological activity (above) of the acid ether soluble fraction of the extracts; thin layer chromatograms developed in methylacetate: isopropanol. ammonia.

It was not possible to use the fluorescence measurements for the quantitative determination of IAA. Acid ether soluble fraction of the extracts contained several fluorescent compounds and even the two chromatographic separations with different solvents failed to eliminate completely other substances from the IAA zone. The activation and fluorescence peaks of IAA at 285 and 365 nm were distinguishable and sharp, but there was a fluorescent background spread all over a wide area, which made a precise quantitative determination of IAA very unreliable. So the fluorometry was used as a qualitative proof of the identity of IAA, while the estimation of its content had to be done by bioassay only. With regard to the known variability of the bioassays, a calibration curve for synthetic IAA was made along with each experiment and the amount of IAA was read from the corresponding curve. Fig. 2 is presented as the example of the results obtained and shows the correspondence of fluorescence and growth activity. Table 2 shows the results of IAA determination in dark-grown and irradiated plants.

Table 2

IAA content in extracts corresponding to 500 plants, measured by the Avena first internode test

	% increase in growth	µg IAA
Dark-grown plants	346.6	0.60
Irradiated plants		
After 24 hrs	382.2	0.80
After 48 hrs	424.4	1.04

As can be seen, a slight increase in IAA content occurred after illumination with red light. The experiment presented in Table 2 was repeated several times and the relative amounts of IAA in dark-grown and irradiated plants were always similar, the latter containing nearly twice as much IAA, as the dark-grown ones. The absolute values for IAA, however, were more variable in different assays.

DISCUSSION

It has been shown that a short red light treatment of etiolated pea plants caused changes in the content of growth substances extracted from the shoots. The amount of tryptophan was found to be considerably lower. Although tryptophan is regarded as a precursor of IAA, it is not likely that such a large quantity of tryptophan would enter the pathway of IAA synthesis. Rai and Laloraya (1967) have recently shown that light causes a higher protein/soluble N ratio in lettuce hypocotyls. It is possible that in peas too the decrease of tryptophan content in the light may be explained by its incorporation into new proteins. This hypothesis should be verified by estimating the changes in other free amino acids.

The results of IAA determination in our experiments are in agreement with those of other authors, who found an increase of auxin in the light. It is certainly a disadvantage that the quantitative determination of IAA was not possible with the precise fluorometric method. However, the identity of IAA is not in doubt and the slight relative increase shown in Table 2 was confirmed several times in bioassays. For the reasons mentionned above, it is not possible to compare our results to the results of other authors. Fletcher and Zalik (1964), who identified IAA by UV absorption, have found a decrease of IAA after irradiation. It seems that the metabolism of indole compounds in *Phaseolus* is different from that in *Pisum*, since they found a several hundred times greater amount of free IAA in their material. Moreover, they applied 8 hours of continuous light instead of 1, which also may account for the difference in results.

The fact that we have found an increase in one substance and a decrease in another, points to the necessity of precise identification of the substances in such studies. If this is not the case, the results may reflect the sum of different light effects.

Obviously it is not possible as yet to say whether the different content of IAA found in dark-grown and irradiated plants has any direct relationship to the morphological phenomena caused by light. As the light treatment affects the second and the third internodes in different ways, it seems that the extraction of the two internodes separately from each other may be of interest. The interaction with other growth hormones must also be considered. Some work on gibberellin determination has already been done and will be reported in a subsequent paper.

SUMMARY

Etiolated Alaska pea plants were irradiated on the 6th day with 1 hour red light. 24 and 48 hours later the contents of tryptophan and IAA were determined in shoot extracts. It was found that the amount of tryptophan is decreased to about half the value found in dark-grown plants. The content of IAA, on the contrary, is slightly increased after irradiation.

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LITERATURE

Biebel, J. P. (1942): Some effect of radiant energy in relation to etiolation. Plant Physiol. 17 : 377—396.

Blaauw-Jansen, G. (1959): The influence of red and far-red light on growth and phototropism of the *Avena* seedling. Acta bot. néerl. 8 : 1—39.

Bottomley, W., H. Smith and A. W. Galston (1966): Flavonoid complexes in *Pisum sativum*. III. The effect of light on the synthesis of kaempferol and quercetin complexes. Phytochemistry, 5 : 117—123.

Briggs, W. R. (1963): Red light, auxin relationships, and the phototropic responses of corn and oat coleoptiles. Amer. Jour. Bot. 50 : 196—207.

Dinant, M. and T. Gaspar (1967): Acide ß-indolylacétique-oxydase, péroxydase, catalase, phénoloxydase et effecteurs naturels chez *Phaseolus vulgaris* cultivé à l'obscurité et à la lumière. Bull. Soc. Roy. Bot. Belg. 100 : 73—94.

Engelsma, G. (1967a): Photoinduction of phenylalanine deaminase in gherkin seedings. I. Effect of blue light. Planta, 75 : 207—219.

Engelsma, G. (1967b): Photoinduction of phenylalanine deaminase in gherkin seedlings. I. Effect of blue light. Planta, 75 : 207—219.

Fletcher, R. A. and S. Zalik (1964): Effect of light quality on growth and free indoleacetic acid content in *Phaseolus vulgaris*. Plant Physiol. 39 : 328—333.

Hillman, W. S. and A. W. Galston (1957): Inductive control of indoleacetic acid oxydase activity by red and near infrared light. Plant Physiol. 32 : 129—135.

Hillman, W. S. and A. W. Galston (1961): The effect of external factors on auxin content. In: Encyclopedia of Plant Physiology, Vol. 14 : 683—702.

Moore, T. C. and C. A. Shaner (1967): Biosynthesis of indoleacetic acid from tryptophan- ^{14}C in cell-free extracts of pea shoot tips. Plant Physiol. 42 : 1787—1796.

Nešković M. and D. Burnett (1966): Identification of tryptophan and indoleacetic acid in pea shoot extracts. Arhiv biol. nauka, 18 : 221—227.

Nitsch, J. P. and C. Nitsch (1956): Studies on the growth of coleoptile and first internode sections. A new, sensitive, straight-growth test for auxins. Plant Physiol. 31 : 94—111.

Overbeek, J. van (1936): Growth hormone and mesocotyl growth. Rec. Trav. bot. néerl. 33 : 333—340.

Oppenoorth, W. F. F. Jr. (1942): On the role of auxin in phototropism and light-growth reactions of *Avena* — coleoptiles. Rec. Trav. bot. néerl. 38 : 287—372.

Rai, V. K. and M. M. Laloraya (1967): Correlative studies on plant growth and metabolism. II. Effect of light and of gibberellic acid on the changes in protein and soluble nitrogen in lettuce seedlings. Plant Physiol. 42 : 440—444.

Shen-Miller J. and S. A. Gordon (1966): Hormonal relations in the phototropic response. IV. Light induced changes of the endogenous auxins in the coleoptile. Plant Physiol. 41 : 831—841.

Stahl, E. (1967): Dünnschicht — Chromatographie, 2 Aufl., Springer Verl.

Zucker, M. (1965): Induction of phenylalanine deaminase by light and its relation to chlorogenic acid synthesis in potato tuber tissue. Plant Physiol. 40 : 779—784.

Rezime

MIRJANA NEŠKOVIĆ i LJUBINKA ĆULAFIĆ

UTICAJ SVETLOSTI NA KOLIČINU HORMONA RASTENJA U STABLU GRAŠKA. I. UTICAJ CRVENE SVETLOSTI NA INDOLNE AUKSINE DOBIJENE EKSTRAKCIJOM

Etiolirane mlade biljke graška »Aljaska« su osvetljavane šestog dana crvenom svetlošću u toku jednog sata. Posle 24 ili 48 časova biljke su ekstrahovane i merena je količina triptofana i indolsirćetne kiseline u ekstraktima. Nađeno je da se količina triptofana smanjuje na približno polovinu u poređenju sa količinom koju sadrže zamračene biljke. Nasuprot tome, količina indolsirćetne kiseline se nešto povećava posle osvetljenja.