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EFFECT OF MONOCHROMATIC IRRADIATION ON GIBBERELLIN CONTENT AND APICAL BUD OPENING IN ETIOLATED PEAS

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INTRODUCTION

It was shown in a previous paper (Nešković and Konjević, 1974) that various phytochrome – controlled morphogenetic reactions in etiolated pea stems were not equally susceptible to the reversion by far red light. While the changes in gibberellin content, induced by 5 min red light, were not reversible, the inhibition of stem elongation and the opening of apical buds could be reversed by far red to different extents. Although the possible involvement of endogenous gibberellins in photomorphogenetic phenomena has been studied for many years, the causal relationship between the two sets of changes has not been clearly demonstrated. If the effects of light on growth were mediated by gibberellins, then one could expect that gibberellin content and growth reactions would change in parallel, in response to the same light treatment. Therefore, a comparative study of monochromatic light effects was undertaken, in the hope that the results might give some information on the involvement of gibberellins in growth responses.

MATERIAL AND METHODS

Seeds of *Pisum sativum* cv. Alaska were cultivated in darkness, as previously described (Nešković and Konjević, 1974). When the internodes were 10–15 mm long, plants were irradiated and left in darkness for another 24 h. The apical buds were then cut off and their shadowgraphs were made.The angle of bud opening was determined in a manner similar to that used by Klein *et al.* (1956). For each irradiation 10 plants were used and the experiments were repeated at least three times.

Similarly irradiated plants, 100 in each group, were used for gibberellin determination. Extracts were prepared 20 or 120 min after irradiation. The apical buds, including the hook region were separated from the rest of the third internode and the

two parts extracted separately. The methods of extraction, purification of extracts, chromatography and bioassay were essentially same as described previously (Nešković and Konjević, 1974). All extractions were repeated three times. As the results in three experiment were similar, the histograms in this paper represent one set of measurements.

Monochromatic light of different wavelengths was obtained by using appropriate VEB Carl Zeiss (Jena) interference filters. The light source was an incandescent 500 W projector bulb. Plants were held in a light–tight box, the filters were mounted on one side and the plants were irradiated from above by reflecting the light from a tilted mirror. In preliminary experiments the whole set of filters in the range from 425 nm to 725 nm, with intervals of 25 nm, was used and the duration of irradiation was 10 min. Based on these results, only four wavelengths were selected for more detailed study. These were as follows: (a) 450 nm, half band width 10 nm, intensity 0.16 μW cm^{-2} nm^{-1}; (b) 550 nm, half band width 7.5 nm, intensity 0.39 μW cm^{-2} nm^{-1}; (c) 657 nm, half band width 7 nm, intensity 0.74 μW cm^{-2} nm^{-1}; 732 nm, half band width 8.5 nm, intensity 0.51 μW cm^{-2} nm^{-1}. Light energies were measured by using an ISCO Spectroradiometer, at the plant level. The next section describes how the duration of light treatments was chosen.

RESULTS AND DISCUSSION

In preliminary experiments, in which plants were irradiated using the whole set of filters for 10 min, stem elongation, bud opening and gibberellin content were determined. Although these results could not be used to determine the correct action spectra, since light energies were unequal at different wavelengths, they nevertheless showed that the three responses were not equally sensitive to light. The red light of 657 nm was most effective in all cases, but stem elongation was not affected by light shorter than 500 nm, bud opening was sensitive to all treatments, as well as was the gibberellin content. In further work elongation was not measured.

In order to make a comparison between bud opening and gibberellin content, quantitative effects of 450 nm, 550 nm, 657 nm and 732 nm were firstly determined for bud opening. Plants were irradiated with each wavelength of light for 1, 3, 10 and 30 min. From the irradiation/response curves so obtained the duration of light treatments was calculated, which was necessary to produce bud opening of 20°. It was found that 21 min of 450 nm, 8 min of 550 nm, 1 min of 657 nm and 10 min of 730 nm had equal effects, i.e. the angle of bud opening was 20°. Therefore, the same light treatments were applied to the plants before assaying the content of gibberellins.

The gibberellin–like activity, found in the extracts 20 min after irradiation (Fig. 1) was markedly increased only in the internodes, while in apical buds it was significantly lower than in controls. When the plants were extracted 120 min after irradiation (Fig. 2), the gibberellin content was lower after all light treatments in both parts of stem. Fig. 3 summarizes data in a quantitative manner, so that the changes in gibberellins can be evaluated more easily.

A few interesting observations can be pointed out, based on the above data. Apparently, the axial parts of the third internodes react differently from the plumules in respect to endogenous gibberellin changes after irradiation. It appears that the increase in gibberellins after 20 min occurs only in the internodes, while the decrease in gibberellins

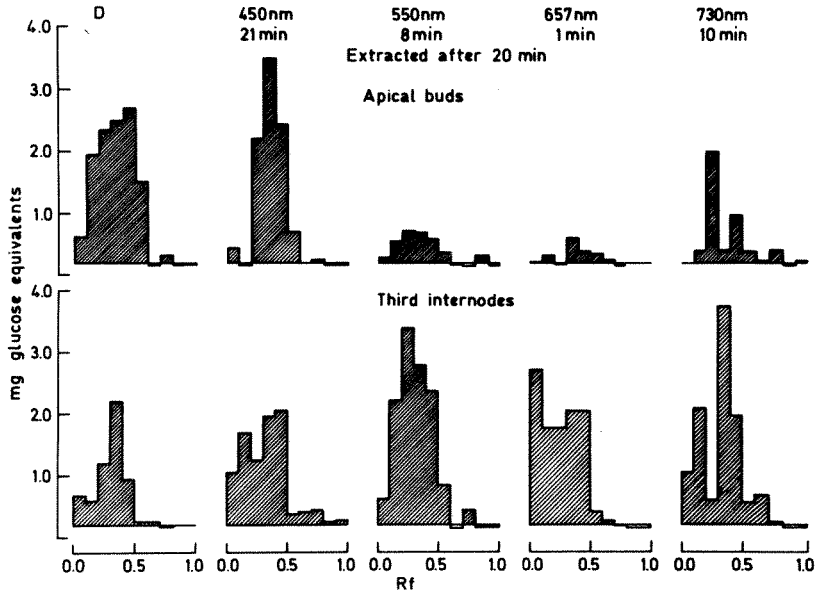

Fig. 1. – The content of gibberelin–like substances in apical buds and third internodes of etiolated peas, 20 min after irradiation. The extracts were chromatographed in TLC, on silica gel G layers, developed in benzene–acetic acid–water (8:3:5), upper phase, assayed by barley endosperm test.

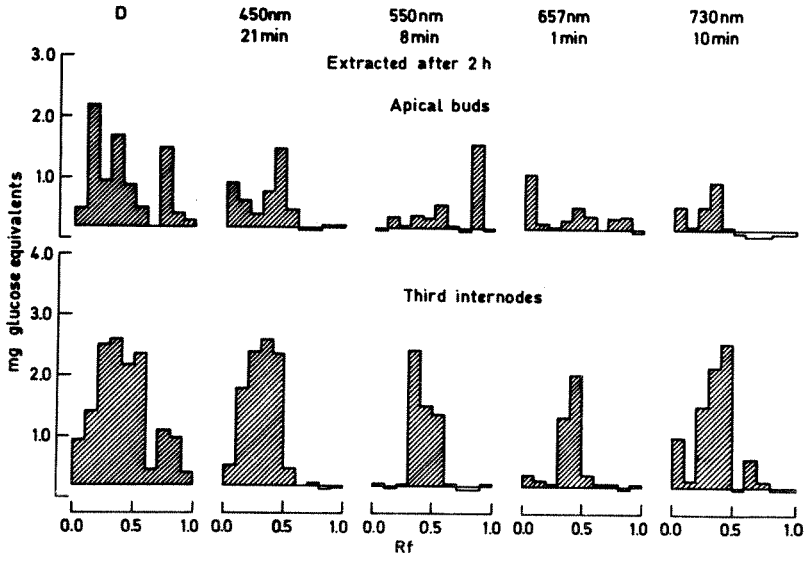

Fig. 2. – The content of gibberellin–like substances, obtained 2 h after irradiation. Other details same as in Fig. 1.

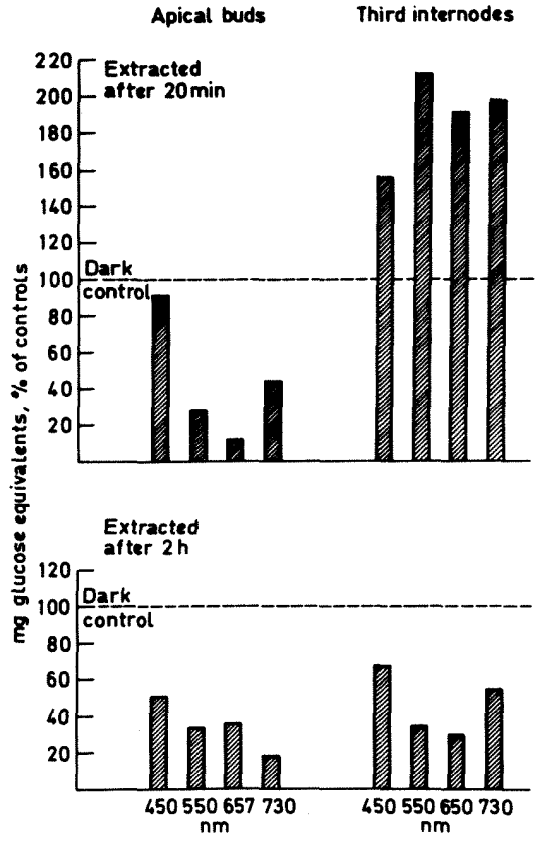

Fig. 3. – Total content of gibberellin–like substances in pea internodes and apical buds, as calculated from the Figures 1 and 2.

after 120 min is evident in all tissues. One could envisage two possible explanations. Since the increase in gibberellin content was shown to be transient, followed by a quick decrease (Nešković and Konjević, 1974), perhaps these events proceed more rapidly in the plumules, than in the internodes. Alternatively, it is possible that the gibberellins are translocated to the internodes within 20 min following irradiation, where they become eventually inactivated. A higher uptake of labelled GA_3 from the stem base in irradiated plants was noticed in earlier work (Nešković *et al.*, 1974). It is also reported that red light irradiation of pea epicotyls causes a rapid translocation of sucrose from the base of the stem to the buds (Goren and Galston, 1966). Therefore, the possibility that the gibberellin content in the internode tissue rises after irradiation as a consequence of higher translocation rate should also be taken into account.

It should be noticed that blue light (450 nm) is relatively ineffective in gibberellin changes, while it affects more strongly bud opening. The rather strong effect of green (550 nm) light on gibberellin content is unexpected. It seems that in future work the possible effect of „safety" light should not be disregarded.

Finally, it is clear that the same light treatments are not equally effective in causing bud opening and gibberellin changes. While at all wavelengths of light bud opening was comparable (20°), the gibberellin content was variable. Therefore, a „parallel variation" (Jacobs, 1959) of the two processes has not been established. This may be taken as an indirect evidence that different secondary pathways may be involved in the two reaction chains. There is no evidence in the present experiments that the opening of apical buds could be mediated by light–induced gibberellin changes.

SUMMARY

Etiolated peas *(Pisum sativum* cv. Alaska) were irradiated with light of 450 nm, 550 nm, 657 nm and 732 nm. The effect of light on bud opening and gibberellin content was determined. The duration of light treatments was variable, but adjusted for each wavelength to the period necessary to produce bud opening of 20°. Irradiations caused changes in the content of endogenous gibberellins. Since the gibberellin content in the internodes and plumules was not equal after all light treatments, it is assumed that bud opening and metabolism of gibberellins are not two causally related phenomena.

REFERENCES

Goren, R., Galston, A.W. (1966): Control by phytochrome of ^{14}C–sucrose incorporation into buds of etiolated pea seedlings. – Plant Physiol. 41: 1055–1064.

Jacobs, W. P., (1959): What substance normally controls a given biological process? I. Formulation of some rules. – Dev. Biol. 1: 527–533.

Klein, W.H., Withrow, R.B., Elstad, V.B. (1956): Response of the hypocotyl hook of bean seedlings to radiant energy and other factors. – Plant Physiol. 31: 289–294.

Nešković, M., Konjević, R. (1974): The non–reversible effects of red and far red light on the content of gibberellin–like substances in pea internodes. – J. Exper. Bot. 25: 733–739.

Nešković, M., Konjević, R., Ćulafić, Lj. (1974): Changes in the metabolism of gibberellins induced by light. In: K. Schreiber, H.R. Schutte and G. Sembdner (Eds.): „Biochemistry and Chemistry of Plant Growth Regulators", Halle, pp. 91–97.

Rezime

GORDANA NAUNOVIĆ i MIRJANA NEŠKOVIĆ

EFEKAT MONOHROMATSKE SVETLOSTI NA KOLIČINU ENDOGENIH GIBERELINA I OTVARANJE APIKALNOG PUPOLJKA ETIOLIRANOG STABLA GRAŠKA

Etiolirane biljke graška *(Pisum sativum* cv. Aljaska) su osvetljavane svetlošću od 450 nm, 550 nm, 657 nm i 732 nm. Meren je efekat svetlosti na otvaranje pupoljka i količinu giberelina. Dužina osvetljavanja je bila različita za svetlosti raznih talasnih dužina, ali je bila podešena tako da u svim slučajevima izazove otvaranje pupoljka od 20°. Osvetljavanje je izazvalo promene u sadržaju endogenih giberelina. Pošto količina giberelina nije bila ista posle svih svetlosnih tretmana, može se pretpostaviti da otvaranje pupoljka i promene u količini giberelina nisu kauzalno povezane pojave.