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SIMULTANEOUS EFFECTS OF INTERMITTENT LIGHT AND GIBBERELLIC ACID IN THE GROWTH OF GERMINATING EMBRYOS OF AVENA SATIVA

INTRODUCTION

The influence of light on the growth of young seedling organs in *Avena sativa* has been widely investigated. The response of etiolated coleoptiles and internodes to light treatment depends on the age of plants during the exposure (Thomson, 1950, 1951). During the first two days after germination light inhibits the elongation of the first internode and stimulates the elongation of the coleoptile. Later irradiation reduces the lenght of both organs. Early work showed that red light of 660 nm is most effective in these reactions (Johnston *at al.*, 1937).Later it was established that the active pigment was phitochrome, since the effect of red light could be reversed by far red (Hopkins and Hillman, 1965; De Lint, 1957; Loercher, 1966). Blaauw *at al.* (168a) showed that the red — far red reversibility depends on the total quantity of energy supplied.

There are many reports showing that phytochrome — regulated growht may also be controlled by gibberellins (Black and Vlitos, 1972). Besides, the first internode of oats is responsive to GA_3 (Ng and Audus, 1955). As far as the phenomenon of stem elongation is concerned, it seems as if light and gibberellins had mutually antagonistic effects (Lockhart, 1956). Hower, the biochemical mechanism of this interaction is much more complex and has not yet been elucidated (Black and Vlitos, 1972).

We have demonstrated earlier that the inhibition and stimulation of growht can both be increased by intermittent light. The effect of intermittency as a controling factor is remarkable and can provide new information, derived from the possible variations in parameters of the intermittent light input (Damjanović *at al.*, 1972). Therefore, the present paper describes the results on the interaction of intermittent and continuous light with GA_3 in the control of first internode growth.

MATERIAL AND METHODS

Embryos of oat (*Avena sativa* L., c. v. Golden Rain) 18 h old, were used in all experiments. The seeds were first dehusked, then soaked in water for two hours, in darkness, at room temperature. They were then sown, with their embryos oriented upwardes, on wet filter paper in Petri dishes. The Petri dishes were left in a dark room at 25°C for 18 h, when the embryos reached about 4 mm. Whole embryos with scutellum were dissected out by means of a razor blade and put on wet filter paper, until the desired number was prepared. In this way the embryos were actually washed out for an hour approximately, and then a number of them irradiated. After irradiation, lots of five embryos were ranged on a strip of Whatman No. I filter paper, and put into test-tubes (20 X 200 mm), containing 0.6 ml of water or GA_3 solution in the bottom. The tubes were stoppered with aluminium foils and mounted in a roller drum, in a quer position, slightly divergent from horizontal, and rotated at 1 r.p.m. The apparatus was held in darkness, at 25°C. After 24 hours, the embryos were taken out of the test-tubes, their shadowgraphs, magnified four times, were made and the lenght of the first internodes was measured with the precision of 0.5 mm.

Plants were treated with saturating red light obtained from a red fluorscent tube (Philips, TL 20 W/15), filtered through a 3 mm plexiglass filter (Röhm and Haas, N° 501). Light intensity was 1,5 $\mu W\ sec^{-1}\ cm^{-2}$. One third of the plants was irradiated continuously for 40 sec, and the other two thirds were exposed to 40 sec of intermittent light. This regime was provided from the same source by means of a rotating chopper. The frequency was 3.75 Hz and light-dark period 1:1.

The experiments were repeated 16 times, and the mean value for all experiments was calculated. In each experiment, every treatment had five replicates, containing five embryos. Therefore, the mean values of 25 embryos were calculated for each experiment.

RESULTS AND DISCUSSION

The results obtained show that light inhibits the lenght of first internodes. GA_3 in concentrations of 0.1, 1.0 and 10.0 $\mu g\ ml^{-1}$ has a stimulative effect, while lower concentrations (10^{-4} — 10^{-2} $\mu g\ ml^{-1}$) are ineffective. GA_3 can reverse completely the light inhibition, as irradiated and GA_3 — treated internodes are longer than dark-grown controls. However, they do not reach the lenght of GA_3 — treated dark-grown plants (Fig. 1). The curves representing the length of internodes as a function of GA_3 concentration are parallel, which suggests the relative independence of the effects of light and GA_3. The parallel trend of the two curves is better expressed when the growth is approximated by the method of linear regression (Fig. 2). Three functions obtained are:

$Y = 0.29x + 2.91$ for continuous light
$Y = 0.27x + 3.34$ for intermittent light
$Y = 0.29x + 3.34$ for darknes

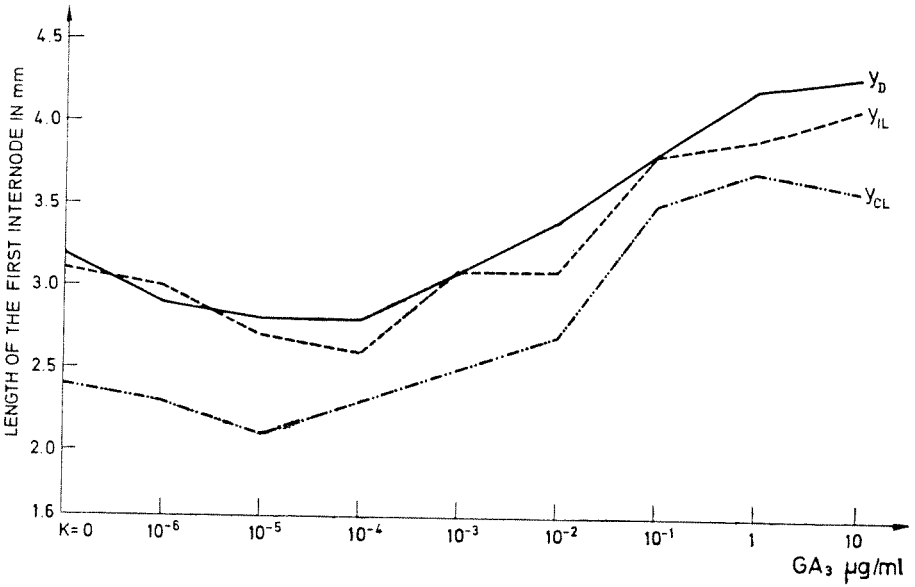

Fig. 1. — Length of the first internode as influenced by GA_3 concentration and light. (————) = darkness; (— — — —) = intermittent light; (— • • — • • —) = continuous light.

Dužina prve internodije u funkciji koncentracije GA_3 i svetlosti. (————) = u mraku; (— — — —) = na intermitentnoj svetlosti; (— • • — • • —) = na kontinuelnoj svetlosti.

The straight lines for continuous light and darkness have the same coefficient of direction and therefore are parallel. One can assume that the straight line for continuous light is derived from that for darkness by the translation of the latter for n = 0.48 downwards. The coefficient of direction for intermittent light is approximately the same as the coefficient of direction for former two.

Fig. 2. further shows that the effect of intermittenat light (that is in the case when only 50% of total light is received) is significantly closer to the effect of darkness, than to the effect of continuous light. This effect, being neither the increase of light, nor of dark effects, may be only interpreted as the summary of two different mechanisms, one of them functioning in darkness, the other one in light. At the given frequency and modulation, the dark process is dominant.

It can be expected that further investigation applying different irradiation frequencies and variations will provide new information on the characteristics (paramerers) of the processes. The observed difference in the coefficient of direction cannot be definitely interpreted,

until the data concerning various conditions of intermittency are available.

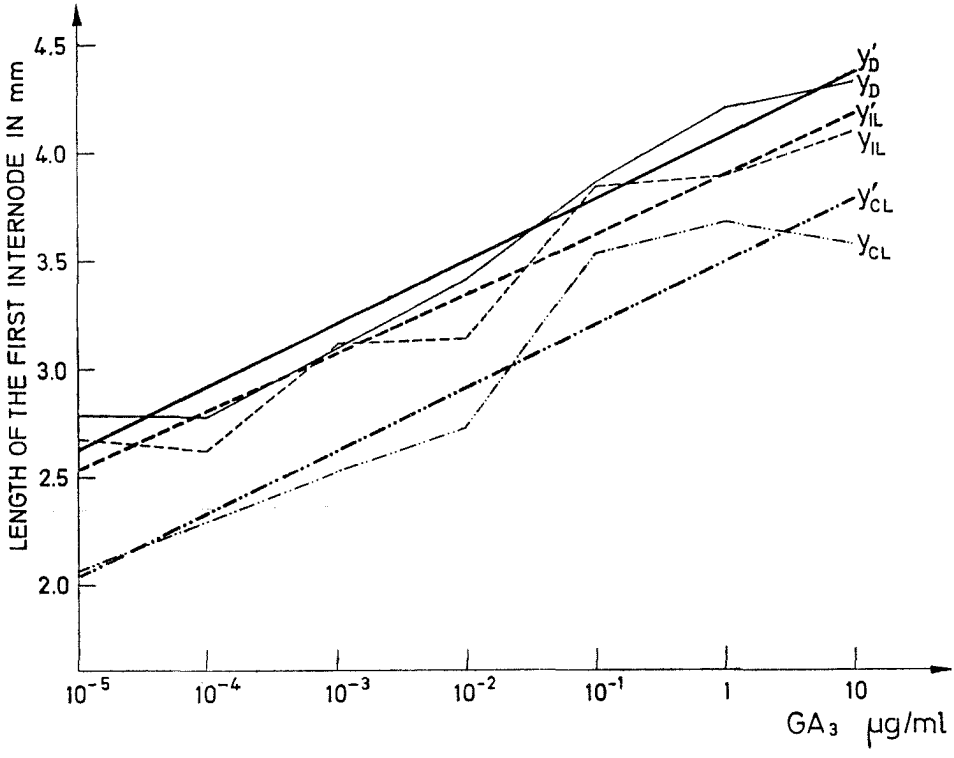

Fig. 2. — Same as Fig. 1., represented as an approximation by the method of linear regression. Yd, Yd' = darkness; Yil, Yil' = intermittent light; Ycl, Ycl' = continuous light; Yd', Yil', Ycl' = straight lines obtained by linear regression. Eksperimentalne krive predstavljene aproksimacijom metodom linearne regresie. Yd, Yd' = u mraku; Yil, Yil' = na intermitentnoj svetlotsi; Ycl, Ycl' = na kontinuelnoj svetlosti; Yd', Yil', Ycl' = prave linije dobijene linearnom regresijom.

SUMMARY

Isolated 18h-old embryos of *Avena sativa*, were irradiated with red light continuously for 40 sec, or intermittently for the same period of time, light — dark periods being 1:1. First internodes of irradiated plants are inhibited. When irradiated plants are treated with GA_3, their internodes are longer that irradiated controls, but they do not reach the length of dark-grown GA_3-treated plants. The effect of intermittent light is closer to the effect of darkness, than to the effect of light. It is concluded that this effect points to the existence of two separate mechanisms in light and darkness respectively. At the frequency and modulation used, the dark process is dominant.

REFERENCES

Black, M. and A. J. Vlitos, (1972): Possible Interrelationships between Phytochrome and Plant Hormones. — Phytochrom, pp. 517, Acad. Press, New York.

Blaauw, O. H., G. Blaauw — Jansen, Leeuwen, W. J., (1968a): An irreversible red light-induced growth response in *Avena*. — Planta, *82*:87—104.

Damjanović, Z., G. Vujaklija., M. Nešković (1972): Stimulation and inhibition of organ growth of *Avena sativa* by continual and intermittent light. — Phytopysiologia 1 (in press).

Hopkins, G., and W. S. Hillman (1965): Response of excised *Avena* coleoptile segments to red far red light. — Planta, *65:* 157—166.

Lint P. J. A. L., De (1957): Double action of near infrared in lenght growth of the *Avena* coleoptile. — Med. Landbouwhogesch. Wageningen, *57*, (10).

Johnston, E. S. (1937): Growth of *Avena* coleoptile and first internode in different wavelegth bands of the visible spectrum. — Smithson. Instit. Miscell, Coll., *96*:1—19.

Lockhart, J. A. (1965): Reversal of the light inhibition of pea stem growth by the gibberllins. — Proc. Natl. Acad. Sci. Wash., *42*:841—848.

Loercher, L. (1966): Phytochrome changes correlated to mesocotyl inhibition in etiolated *Avena* seedlings. — Plant Physiol., *41*: 932—937.

Ng, E. K. and L. J. Audus (1965): Growth-regulator interactions in the growth of the shoot system of *Avena sativa* seedlings. II The growth of the first leaf and the coleoptile. — J. exp. Botany, *16*:107—127.

Thomson, B. F. (1950): The effect of light on the rate of development of *Avena* seedlings. — Amer. J. Bot., *37*:284—291.

Thomson, B. F. (1951): The relation between age at time of exposure and response of parts of the *Avena* seeddlings to light. — Amer. J. Bot., *38*:635—638.

Rezime

GORDANA VUJAKLIJA i ZVONIMIR DAMJANOVIĆ

SIMULTANI EFEKTI INTERMITENTNOG OSVETLJAVANJA I GIBERELNE KISELINE U RASTENJU KLIJALIH EMBRIONA AVENA SATIVA

Embrioni ovsa, varijetet »*Zlatna kaša*«, 18h po zasejavanju, izlagani su crvenoj zasićujućoj svetlosti u trajanju od 40 sekundi, jačine 1,5 μW sec sm^{-2}. Biljke su zatim stavljane u različite konc. rastvora GA_3 (od 1 do 10^{-4}μg ml^{-1}). Nakon sledećih 24h merena je dužina prve internodije.

Prva internodija biljaka, koje su osvetljavanne kontinuelnom svetlošću, a zatim tretirane giberelinom je kraća od internodije biljaka u mraku, tretiranih giberelinom Njihove dužine predstavljene u funkciji koncentracije GA_3 daju krive, koje teku manje više paralelno, na osnovu čega se može predpostaviti relativna nezavisnost uticaja giberelne kiseline i svetlosti. Giberelna kiselina u funkciji koncentracije povećava prirast, kako u mraku, tako i na svetlosti. Osim toga, primećeno je da postoji aditivno dejstvo svetlosti i hormona.

Intermitentno osvetljavanje je obezbeđeno iz istog svetlosnog izvora uz korišćenje rotirajućeg čopera, sa frekvencijom od 3.75 Hz, pri odnosu svetlog i tamnog perioda 1:1.

Efekat intermitentnog osvetljavanja je značajno bliži efektu mraka, nego efektu svetlosti. Ovakav efekat koji ne znači ni povećavanje svetlosnog efekta, niti povećavanje efekta mraka, može se tumačiti samo kao sumarno dejstvo dva različita mehanizma, od kojih jedan funkcioniše na svetlosti, a drugi u mraku. Pri datoj frekvenciji i modulaciji preovlađuje tamni proces.