BIOLOGIA PLANTARUM (PRAHA)

12 (4) : 291—296, 1970

Sensitivity of Apple Seed Germination to Light and Some Growth Regulators

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Received November 18, 1969

Abstract. Experiments were aimed at checking whether the non-stratified apple embryos are sensitive to growth factors and light. The effect of growth regulators on light sensitivity was also studied. The stimulating or inhibiting effect of GA_3, benzyladenine and IAA or coumarin on the dynamics of apple embryo germination was demonstrated. Seasonal fluctuations of the effect of the growth factors were noted. The stimulating effect of light on the germination of non-stratified apple embryos was demonstrated. This effect is independent of season and seems to be independent of the effects of growth regulators.

The germination of non-photoblastic seeds depends on the sufficient concentration of the active form of phytochrome (P_{fr}) in the embryo. If this is the case, the light factor is ineffective. As suggested by ROLLIN (1966) rise of the P_{fr} level above that indispensable for the initiation of germination is of no effect.

Light stimulus is not indispensable for the germination of apple seeds. It had been observed, however (LEWAK and SMOLEŃSKA 1968), that during secondary ripening the apple seeds revealed marked sensitivity to light for several days before the stratification was finished. This sensitivity disappears at the end of stratification. The transient sensitivity may be due to the synthesis of phytochrome or non-photochemical transformation of inactive phytochrome P_r into its active P_{fr} form. The accumulation of sufficient amounts of the active form may be the reason for the lack of light sensitivity of apple seeds.

On the other hand, however, there is ample evidence (BADIZADEGAN and CARLSON 1966, 1967, KAMIŃSKI and PIENIĄŻEK 1968, KHAN and HEIT 1967, MILLER 1956, PIENIĄŻEK and GROCHOWSKA 1967, PIENIĄŻEK and RUDNICKI 1967, SIŃSKA and LEWAK 1970) indicating that germination of embryos isolated from non-stratified or not completely stratified apple seeds is regulated by growth factors such as gibberellins, cytokinins or abscisic acid. Other data (EVENARI 1952, IKUMA and THIMANN 1960, KAHN

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et al. 1957, KAHN 1960, KHAN and TOLBERT 1965, KNYPL 1956, MILLER 1956, THOMPSON 1968) indicate a relation between the light sensitivity of the photoblastic seeds and the presence of some growth regulators. Therefore we felt it justified to investigate whether a period of light sensitivity does appear in the isolated apple embryos and whether and to what extent this sensitivity depends on the presence of some growth factors.

Material and Methods

Material

The experiments were carried out on non-stratified apple seeds (Antonovka cv.) 1967 and 1968 collection from the Experimental Station of Institute of Pomology at Sinofęka.

Culture of Embryos

The seeds were soaked in water for 24 h and then the seed cover and endosperm were removed. The embryos were grown in lots of 30 in Petri dishes on filter paper and 5 ml of the solution of the factor studied or 5 ml of distilled water (control) were added. The embryo-containing dishes were placed in light and temperature controlled rooms. Day length- 12 h, temperature 24°, light intensity 10^4 Lx. Night temperature- 20°. The embryos cultivated in darkness were placed in blackened Petri dishes. Three mm length of the embryo radicle was accepted as a criterion of germination.

Expression of Results

Each experiment was run simultaneously on three dishes and was repeated two or three times. The results are expressed as mean values. For some series of experiments the confidence interval was calculated and it did not exceed 0·2 of the stimulation and/or inhibition values.

The effects of the growth factors *i. e.* gibberellin (GA), benzyladenine (BA), β-indolylacetic acid (IAA) and coumarin on the dynamics of germination of apple embryos are most visible within the first 10—12 days of culture. Therefore the dynamics of germination may be expressed as a total amount (Σ) of percentage of the germinating embryos within the first 10 days of experiment (Σ_{10}), as suggested by TIMSON (1965). Thus $\Sigma_{10} = 1\,000$ means that all embryos germinate on the first day of experiment, $\Sigma_{10} = 100$ — that all embryos germinate on the last day. Most of the growth factors studied do stimulate the germination. Their effect on the embryos was expressed by the stimulation value $S = 1 - {}^{k}/_{x}$ where $x = \Sigma_{10}$ of the embryos germinating in the presence of the factor studied and $k = \Sigma_{10}$ of the germinating control seeds (cultured on water). In the case of coumarin which is a growth inhibitor, its effect was expressed by the inhibition value $I = 1 - {}^{x}/_{k}$.

Results

The stimulation (S) or inhibition (I) value as a function of various growth factor concentrations is presented in Fig. 1. As seen in Fig. 1, the effect of

IAA is much smaller than that of GA or BA. The maximum values of IAA stimulation do not exceed 0·4 while for the other growth factors they reach as high as 0·8. In the series of experiments run from October till February (Fig. 1 left) the highest stimulation values were those of gibberellin, while in the series run March—June (Fig. 1 right) the effect of gibberellin is much smaller than that of benzyladenine. Similar seasonal fluctuations are evident in the effect of IAA and coumarin. The IAA stimulation and coumarin inhibition values reach 0·4 during the period October—February while in the spring they are within the confidence interval (Fig. 1). No differences between apple embryo sensitivity to growth factors studied for the seeds of 1967 and 1968 collection were observed.

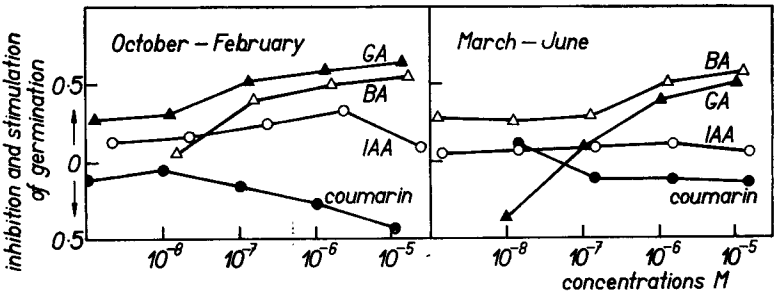

Fig. 1. The effect of gibberellin (GA), benzyladenine (BA), β-indolylacetic acid (IAA) and coumarin on the dynamics of germination of the non-stratified apple embryos.

In the series of experiments dealing with apple embryo culture in darkness and simultaneous culture with 12 h daily exposure to light the marked stimulating effect of light was noted. The effect of stimulation is visible already on the 4-th day of culture i. e. the first day of germination. The stimulation value is independent of the season. The Σ_{10} values for embryos kept in darkness and those exposed to light are presented in Table 1. The light stimulation value (S_l) is expressed in the same way as for the growth factors. As seen in Table 1 the light stimulation value S_l is similar to the maximum value of gibberellin or benzyladenine stimulation and is practically independent of season.

TABLE 1

THE EFFECT OF LIGHT on germination of the non-stratified apple embryos

a Date	b Σ_{10} in the dark	c Σ_{10} on light	d $S_l = 1 - b/c$
October — — February	18·8	51·1	0·63
March — June	53·2	125·7	0·58

The experiments concerning the effect of growth factors on the light stimulation value were run in two parallel series, one of them in darkness,

the other in 12 h photoperiod. Various concentrations of the growth regulators studied were added respectively to each of the series. The results (mean values) for some concentrations of GA, BA and coumarin are presented in Table 2.

TABLE 2

THE EFFECT OF GA, BA and coumarin on germination of non stratified apple embryos grown on light or in darkness

a Growth regulator (in M)	b $S = 1 - {}^{k}/_{x}$	c $I = 1 - {}^{x}/_{k}$	d Σ_{10} in the dark	e Σ_{10} on light	f $S_1 = 1 - {}^{d}/_{e}$
Water			36·0	88·4	0·60
GA 2×10^{-6}	0·70		43·5	292·0	0·85
BA 5×10^{-6}					
Oct. — Febr.	0·55		55·3	196·0	0·72
March — June	0·65		47·1	251·6	0·81
Coumarin 10^{-6}		0·27	28·4	64·5	0·56

As seen in Table 2, the stimulating effect of light is to some extent interposed on the effect of growth factor used. The comparison between germination in presence of water and in the presence of respective growth regulators both in darkness and light (Table 2, col. d and e) indicates a manyfold increase of the number of embryos germinating in light in the presence of GA or BA.

The means of expressing the germination sensitivity to light and chemical factors as accepted here allows to demonstrate the growth regulators studied to be only of slight effect on the light sensitivity. The results presented in Table 2 (col. f) do not prove any additivity of the light and chemical stimulation (compare water control with the value presented in col. b and c). The light stimulation of the apple embryo germination seems to be independent of the effect due to the growth regulators studied.

Discussion

The sensitivity of apple embryos to light demonstrated in our experiments is a consequence of the previously observed (LEWAK and SMOLEŃSKA 1968) phytochrome activity in apple seeds submitted to secondary ripening. It supplies one more proof that the main mechanisms regulating the development of isolated apple embryos and the normal development of seeds submitted to stratification are the same.

The fact that benzyladenine, gibberellin and coumarin are of no effect on the embryo sensitivity to light may indicate a complete independence of the chemical and light factors initiating apple seed germination. On the other hand, however, there is some evidence (BERRIE *et al.* 1968, EVENARI 1952, KAHN 1960, KAHN *et al.* 1957, THOMSON 1968) suggesting active role of gibberellins and cytokinins in the apperance or persistence of seed sensitivity to light. The administration of some growth factors replaces the ligth

exposure in lettuce (KAHN *et al.* 1957) or strawberry (THOMPSON 1968) seeds. Coumarin caused an effect opposite to that of light. The inhibitory effect of coumarin was nivelated by light exposure (KNYPL 1966, THOMPSON 1968).

Attention should be drawn however to the fact that the experiments reported concern photoblastic seeds. The germination of apple embryos is markedly stimulated by light. This factor, however, is not necessary for their germination. Therefore a possibility of different mechanisms regulating the germination of apple embryos and typical photoblastic seeds may be assumed.

As suggested by KAHN (1960) the germination of the seeds may be stimulated by the growth regulators in two parallel ways: (i) by a mechanism related to the effect of light and (ii) by a mechanism completly independent of the light stimulus. According to this idea the stimulation of apple seed germination by the growth factors is regulated by a single mechanism and is independent of light.

The seasonal fluctuations of the sensitivity of germination for different growth factors observed here will be the subject of further studies. These fluctuations seem to be particularly interesting in view of the inexplicable fact that germination is inhibited by the lowest concentration of GA during the March—June period.

This work was partly supported by Grant F.G. — Po- 236 from the U.S. Department of Agriculture. Agricultural Research Service, according to Public Law 480.

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ST. LEWAK, KRYSTYNA BIAŁEK, IRENA SIŃSKA, Botanický ústav Varšavské university, Polsko: **Studium citlivosti ke světlu a růstovým regulátorům při klíčení semene jabloně.** — Biol. Plant. **12** : 291—296, 1970.

Pokusy byly zaměřeny na prověření citlivosti nestratifikovaných embryí jabloní k růstovým faktorům a ke světlu. Rovněž byl studován účinek růstových regulátorů v závislosti na světle. Výsledky prokázaly stimulační nebo inhibiční účinek GA_3, benzyladeninu a IAA nebo kumarinu na dynamiku klíčení embryí jabloní. V souvislosti s ročním obdobím bylo potvrzeno kolísání účinku růstových faktorů. Dále byl prokázán stimulační účinek světla na klíčení nestratifikovaných embryí jabloní. Tento účinek není závislý na ročním období a patrně ani na účincích růstových regulátorů.