

DORMANCY IN FRUITS OF *TILIA PLATYPHYLLOS* SCOP. III. A POSSIBLE ROLE OF IAA AND CYTOKININS IN BREAKING DORMANCY

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Abstract

We have performed investigations relating to the possible role of IAA and cytokinins, besides gibberellins, interaction with ABA, in breaking the dormancy of *T. platyphyllos* seeds. For the investigations we have used excised embryos and GA_3 , IAA and kinetin solutions of various concentrations, as well as ABA solutions of 10^{-4} M and 10^{-5} M concentrations. It is shown by the results of our model experiments that in case of a low (10^{-5} M) ABA concentration both IAA and kinetin are effective in stimulating germination and reversing the inhibition of radicle and hypocotyl. The inhibition of chlorophyll-synthesis, however, cannot be reversed by any of the promotors applied. In the case of a higher (10^{-4} M) ABA concentration, only GA_3 is effective in stimulating germination. We have investigated also the joint effect of the promotors applied in the case of 10^{-4} M ABA treatment and established that both kinetin and IAA reinforce the antagonistic effect of GA_3 . This synergy between GA_3 and IAA is greatest in the case of a lower (10^{-7} M) IAA concentration, and between GA_3 and kinetin in the case of a higher (10^{-3} M) kinetin concentration. Our results achieved with excised embryos are also reinforced by our investigations carried out with intact seeds. In case of applying all three promotors jointly the germination percentage may rise to 100 but the length of the radicle and hypocotyl does not reach that of the control. The promotors applied cannot quite reverse (in the case of optimum concentration conditions, too) the ABA induced inhibition of radicle and hypocotyl growth.

Introduction

The dormant fruits and seeds of *T. platyphyllos* require a chilling period at 5 °C breaking dormancy. The isolated embryos, however, germinate quickly on a wet filter paper at 20 °C, without any primary treatment (NAGY and SZALAI, 1972). In the course of our earlier investigations we established that the seed of *T. platyphyllos* in dormancy contained a considerable amount of abscisic acid. This inhibitor-substance content, however, decreases in the course of stratification. At the same time, an increase in the amount of the endogenous gibberellins can be observed at the end of stratification (SZALAI and NAGY, 1974). The change in hormonal equilibrium has, therefore, a role which is probably important in breaking dormancy. As the exogenous gibberellin acid does not cease dormancy at room-temperature, its germination-stimulating effect can only be effective at 5 °C (NAGY and SZALAI, 1972); the question has been raised that, apart from gibberellins, cytokinins and indole-acetic acid may have played an important part in the interaction, as well. We have carried out the investigation of a possible part of ABA and other growth regulating substances by using excised embryos.

Materials and Methods

We got the material for investigation from the Woods and Forests Management of County Csongrád. It originated, therefore, from trees in a closed stand. We removed the pericarpium from the fruits, scarified the seeds with concentrated sulphuric acid and let them swell for 36 hours. Then we excised the embryos. Our experiments were set up under half-sterile conditions, with White's nutritive solution, namely on a filter paper wetted with White's nutritive solution containing the test solution of the required concentration, in a Petri dish.

For the investigations we used the following concentrations:

- 10^{-4} M and 10^{-5} M abscisic acid (ABA) (Sigma, USA)
- 10^{-3} M— 10^{-7} M gibberellic acid (GA_3) (Phylaxia, Budapest)
- 10^{-3} M— 10^{-7} M indole-acetic acid (IAA) (E. Merck AG, Darmstadt)
- 10^{-3} M— 10^{-7} M kinetin (KIN) (Fluka AG, Buchs SG.)

The Petri dishes containing the excised embryos and illuminated with 6000 Lux were incubated at 20 °C. The results were evaluated on the tenth day.

We regarded as germination the first geotropic curvature of the radicle (WAREING and WEBB, 1972). The length of the hypocotyl and radicle was measured in a photoenlarger. The chlorophyll content of the leaves of seedlings was determined photometrically using the method of WINTERMANS and MONS (1965).

Experimental results

1. Effects of plant growth regulating substances on germination of embryos

The embryos coming from seeds in dormancy were kept in White's nutritive solution containing 10^{-5} M ABA. That is the smallest concentration on the application of which every embryo remains in dormancy. The effect of GA_3 , kinetin, and IAA in various concentrations, on the germination of the embryos treated with 10^{-5} M ABA is shown in Fig. 1.

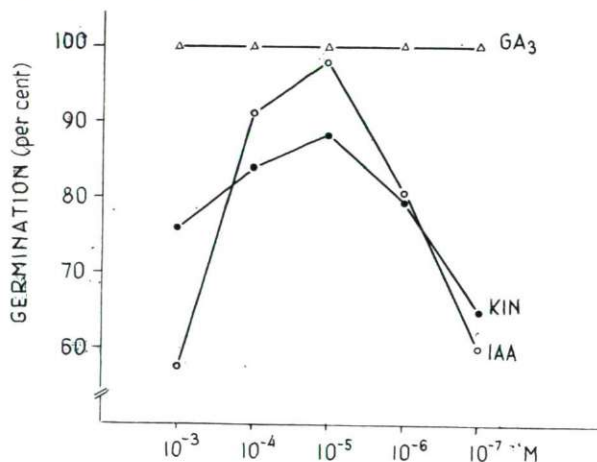


Fig. 1. Germination of 10^{-5} M ABA-treated *T. platyphyllos* embryos, in the presence of GA_3 , IAA, and kinetin of various concentrations. The embryos were incubated at 20 °C, in light (6000 Lux), for 10 days.

As demonstrated by the results, any concentration of GA_3 applied fully reverses the ABA-induced inhibition of germination. In the case of kinetin and IAA, some antagonism may be observed, as well, showing its maximum effect in equimolar concentration.

2. Effects of plant growth regulating substances on seedling growth

The results connected with the growth of seedlings are shown Fig. 2.

It is quite clear from the data that the growth-stimulators applied not only reverse the ABA induced inhibition of the growth of radicula but their stimulating effect ma-

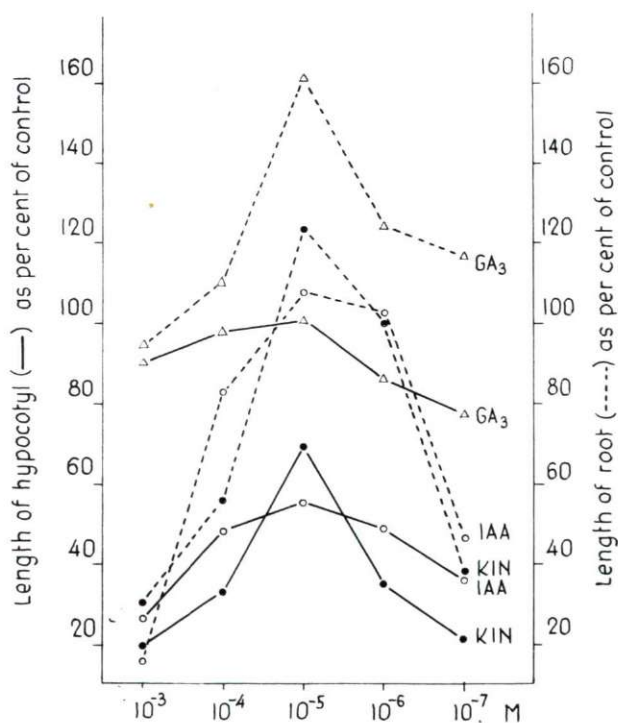


Fig. 2. Effects of GA_3 , IAA, and kinetin of various concentrations on the growth of radicula and hypocotyl of *T. platyphyllos* seedlings, in the case of 10^{-6} M ABA treatment.

nifests itself, as well. This stimulating effect is most considerable in the case of GA_3 . In the case of the hypocotyl, however, the situation is different. The inhibition of the hypocotyl growth can be fully reversed only by GA_3 . Some growth can be observed in kinetin and IAA treatments, too, but in these cases the length of hypocotyl falls far behind that of the control.

3. Effects of plant growth regulating substances on the chlorophyll content of seedlings

The ABA induced inhibition of chlorophyll synthesis cannot be fully reversed by any of the promotors applied (Fig. 3).

The maximum effect is shown here, too, by the equimolar concentrations. The inhibition of chlorophyll synthesis can be reversed by GA_3 in 65.3 per cent, by kinetin in 58.9 per cent, and by IAA in 41.0 per cent.

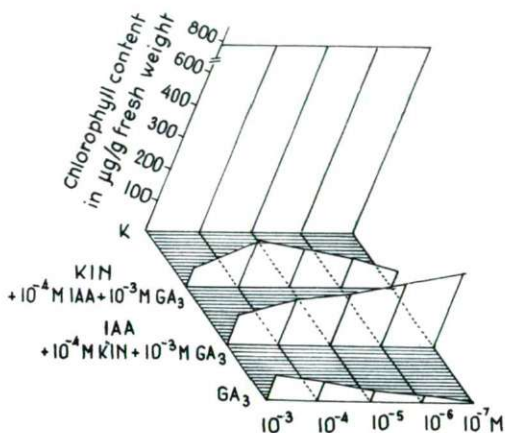


Fig. 3. Effects of GA_3 , IAA, and kinetin of various concentrations on the chlorophyll content of *T. platyphyllos* seedlings, in the case of 10^{-5} M ABA treatment.

4. Joint effects of GA_3 , kinetin, and IAA on dormant embryos

If we suppose, on the basis of the results achieved so far, that cytokinins and IAA, in interaction with ABA, may have had a part in breaking dormancy, then we have to take into consideration the fact that these growth regulators are present in the seed jointly, and we must, therefore, investigate their joint effect, as well.

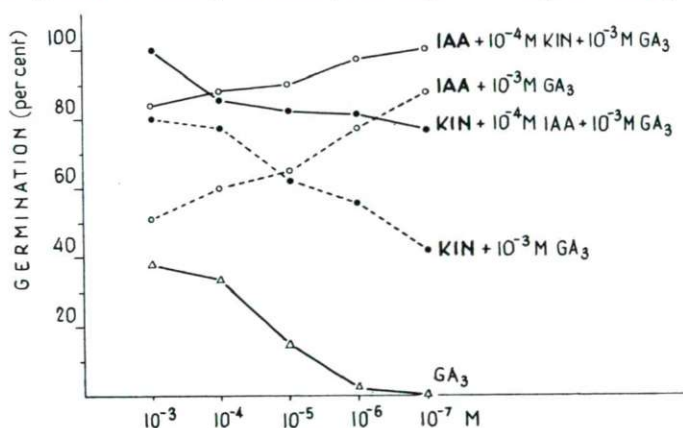


Fig. 4. Joint effects of GA_3 , IAA, and kinetin on the germination of *T. platyphyllos* embryos, in the case of 10^{-4} M ABA treatment.

The antagonistic effect of kinetin and IAA on stimulating the germination of the dormant embryos can only be observed in the case of a 10^{-5} M concentration and no longer in the case of a $5 \cdot 10^{-5}$ M concentration, and at a 10^{-4} M ABA concentration the germination-stimulating effect of GA_3 is also below 40 per cent. In investigating the joint effect of promoters, we used 10^{-3} M GA_3 , 10^{-4} M IAA, and 10^{-3} M— 10^{-7} M kinetin concentrations, respectively 10^{-3} M GA_3 , 10^{-4} M kinetin, and 10^{-3} M— 10^{-7} M IAA concentrations. Their effect on the germination of dormant embryos is shown in Fig. 4.

On applying a 10^{-4} M ABA concentration, GA_3 in itself results in a germination lower than 40 per cent, even in a 10^{-3} M concentration. It is shown by the results that in the joint presence of GA_3 , IAA, and kinetin the antagonism is stronger. In reversing the ABA induced inhibition of germination, in these cases, the higher kinetin and lower IAA concentrations are effective. As regards a tendency, a similar result was also obtained in the cases where we used kinetin without IAA, resp. IAA without kinetin, in addition to GA_3 (Fig. 4, dotted line). Although the joint application of these promoters may in certain cases result in the full reversal of the inhibition of germination, the seedlings do not approach that of the control in size (Fig. 5).

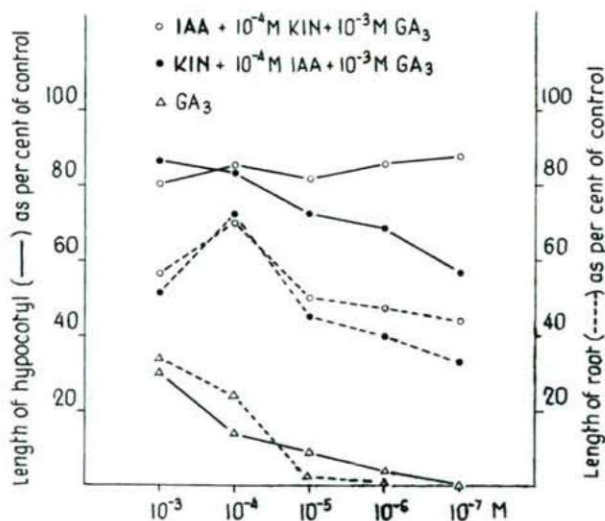


Fig. 5. Joint effects of GA_3 , IAA, and kinetin on the growth of *T. platyphyllos* seedlings, in the case of 10^{-4} M ABA treatment.

The promoters applied can to a lesser extent reverse the inhibition of the growth of radicle than that of hypocotyl growth, but in any case they strengthen the antagonistic effect of GA_3 . The effect of IAA on the reversal of hypocotyl-inhibition is stronger if the concentration decreases, but the effect of kinetin, however, is stronger if the concentration increases. In reversing the inhibition of root-growth the antagonism is strongest in the case of the equimolar concentration of IAA and kinetin.

In the chlorophyll content of seedlings, too, a striking difference appears between plants treated with only GA_3 , respectively with GA_3 , IAA, and kinetin jointly (Fig. 6).

In the case of GA_3 -treatment, there is a minimum degree of turning green. The inhibition of chlorophyll synthesis is not fully reserved even by a joint application of promoters. Most effective is a 10^{-7} M concentration of IAA in the presence of 10^{-3} M GA_3 and 10^{-4} M kinetin (30 per cent). Kinetin is most effective in a 10^{-4} M concentration in the presence of 10^{-3} M GA_3 , and 10^{-4} M IAA (17 per cent).

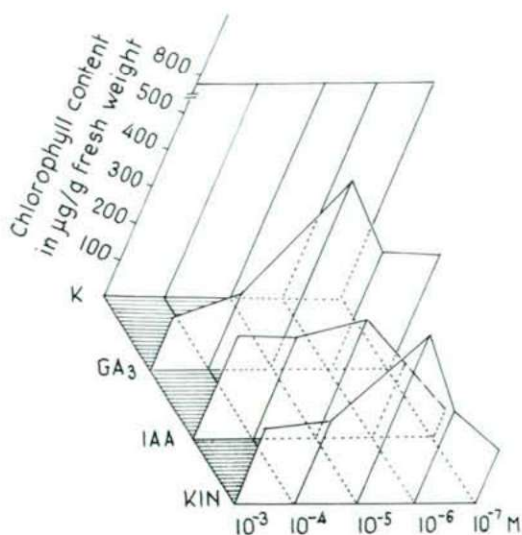


Fig. 6. Joint effects of GA_3 , IAA, and kinetin on the chlorophyll content of *T. platyphyllos* seedlings, in the case of 10^{-4} M ABA treatment.

5. Effects of plant growth regulating substances on germination of intact seeds

We used a $3 \cdot 10^{-4}$ M GA_3 solution for treating intact seeds, because in our previous investigations (NAGY and SZALAI, 1972) this had proved to be the most effective concentration. We applied IAA and kinetin, too, in a $3 \cdot 10^{-4}$ M concentration as our investigations carried out with embryos had shown that synergy is strongest in an equimolar concentration. The treatment was performed at $+20^\circ\text{C}$ and $+5^\circ\text{C}$. The results, given in germination percentage, are contained in Table 1.

As shown by the data of the Table, a definite synergism may be seen, in the case of intact seeds, as well, between GA_3 and IAA, as well as between GA_3 and kinetin, in stimulating germination. Similarly as in our investigations carried out with excised embryos, the higher germination percentage was found, here too by applying GA_3 and IAA jointly. That effect, however, manifested itself only at low temperature.

Table 1. Effects of GA₃, IAA, and kinetin on the germination of intact *T. platyphyllos* seeds. The beginning of the experiment was on April 10, 1975.

Treatment	3 · 10 ⁻⁴ M GA ₃		3 · 10 ⁻⁴ M KIN		3 · 10 ⁻⁴ M IES		3 · 10 ⁻⁴ M GA ₃ + 3 · 10 ⁻⁴ M KIN		3 · 10 ⁻⁴ M GA ₃ + 3 · 10 ⁻⁴ M IES		Control	
	+ 20 °C	+ 5 °C	+ 20 °C	+ 5 °C	+ 20 °C	+ 5 °C	+ 20 °C	+ 5 °C	+ 20 °C	+ 5 °C	+ 20 °C	+ 5 °C
Temperature												
VII. 4.	—	—	—	—	—	—	—	—	—	—	—	—
VIII. 4.	—	—	—	—	—	—	—	—	—	—	—	—
VIII. 20.	—	1	—	—	—	—	—	2	—	1	—	—
VIII. 30.	—	6	—	—	—	—	—	10	—	8	—	—
IX. 10.	—	18	—	—	—	—	—	33	—	31	—	—
IX. 5.	—	26	—	1	—	—	—	42	—	89	—	—

Discussion

By means of these investigations we have tried to answer the question whether in breaking the dormancy of *T. platyphyllos* seeds auxins and cytokinins whether may play a part, apart from the endogenous gibberellins, or from among the promoters only gibberellins bear a part in it.

There are several data concerning the physiological antagonism between GA_3 , IAA, kinetin, and ABA. The results are however, different depending on the object to which they are applied.

In a number of cases, a treatment with exogenous gibberellin acid could be substituted successfully for one requiring stratification (FRANKLAND and WAREING, 1966; AMEN, 1968; BASKIN and BASKIN, 1970). In the case of *T. platyphyllos* seeds, on the other hand, the exogenous GA_3 does not replace the stratification required (NAGY and SZALAI, 1972). Gibberellin was not effective in stimulating the germination of *Acer* seeds, either (WEBB et al., 1973). Gibberellin did not promote the ABA-inhibited germination of lettuce seeds (SANKHLA and SANKHLA, 1968) or that of isolated lettuce embryos, either (BLACK et al., 1974), but kinetin was effective. SONDHEIMER et al. (1968) reversed the ABA induced inhibition of the germination of the isolated *Fraxinus* embryos by combining gibberellin and kinetin.

The antagonism between ABA and IAA can be observed in some tests, as well (WAREING et al., 1968; WAREING and RYBACK, 1970). SANKHLA and SANKHLA (1968), on the other hand, found IAA ineffective on the ABA-inhibited germination of lettuce seeds, as well as SONDHEIMER and GALSTON (1966) in the case of *Fraxinus ornus* and *Fraxinus americana* embryos.

On the basis of our results we suppose that in ceasing the dormancy of *T. platyphyllos* seeds, and in changing the hormonal equilibrium, the increase in gibberellins produced or activated during stratification is most decisive but in forming the promotor level, apart from gibberellins, IAA and cytokinins, which strengthen the antagonistic effect of gibberellins against ABA, may have played a considerable part, as well.

Our model experiment refers also to the fact that in changing the hormonal equilibrium the increase in the amount of promoters is not enough. Under natural conditions, a decrease in ABA content must follow, as well, because in the case of a high ABA content the promoters that can be taken into account cannot fully reverse the inhibition the growth of radicle and hypocotyl, even in case of optimum concentration circumstances. The failure of the exogenous hormone treatment at room temperature can probably be explained by that fact that a decrease in ABA content only comes about at a low temperature.

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