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KINETIN ANTAGONIZES THE INHIBITION OF AUXIN SYNTHESIS IN THE AVENA COLEOPTILE CAUSED BY GALACTOSE, ABSCISIC ACID OR INDOLEACETIC ACID

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SUMMARY

The degree of inhibition of the auxin production in the tip of a decapitated *Avena* coleoptile, caused by galactose at low concentrations (0.10 and 0.125%) is reduced by kinetin (1 mg/l), but not by gibberellic acid (GA_3, 10 mg/l). At higher galactose concentrations the reduction is insignificant or absent. The inhibiting effect of galactose on the cell elongation due to external auxin is not reversed by kinetin.

Comparable effects of kinetin are observed when the auxin production and the auxin action are inhibited by abscisic acid.

Kinetin also abolishes the repression of the auxin production caused by indoleacetic acid itself, but this effect is limited to very low IAA concentrations (0.01 mg/l). When the inhibition is caused by 0.02 mg/l IAA the effect of kinetin added in concentrations of 1, 5 or 10 mg/l is already absent.

1. INTRODUCTION

In my previous papers on the regeneration of the physiological tip in a decapitated *Avena* coleoptile, it was reported that the synthesis of auxin in the apical cells of the stump is inhibited or postponed by galactose and galactose-containing sugars (ANKER 1974), by abscisic acid (ANKER 1975), and by IAA itself (ANKER 1973). Kinetin, on the other hand, promoted the auxin production in the new tip (ANKER 1977).

In the present experiments the problem to be resolved was whether kinetin also reduces the inhibitions of the auxin production, caused by the above-mentioned substances. This was actually found. The results may be of wider significance e.g. for the understanding of the mechanism of apical dominance (see section 4).

2. MATERIAL AND METHODS

Subapical 19 mm segments of decapitated *Avena* coleoptiles (1 mm removed) were submerged in solutions of the substances, the effect of which on the growth rate was to be studied. The advantage of submersion over application in agar blocks or in paste to segments in air is that the concentration of the supplied substance practically remains constant throughout an experiment (there were only 12 segments in one liter of the solution). This is important when the concentrations are very low as was the case in most of the present experiments.

The segments being gently pressed on metal pins were standing in the vertical position. The solutions were aerated.

Details of the cultivation and of the preparation of the experimental material as well as of the measurements of the growth of the segments have been given in previous papers (ANKER 1973–1977). Adaptations of the procedures are mentioned where appropriate.

All experiments were carried out at 23 °C, in weak red light (incandescent light filtered through selenium glass).

3. RESULTS AND DISCUSSION

3.1. Effect of kinetin and gibberellic acid on the growth of galactose-treated segments

In a previous investigation (ANKER 1974) galactose appeared to be a potent inhibitor of the regeneration of the physiological tip on the stump of a decapitated *Avena* coleoptile. Further analysis of this galactose effect revealed that it not only prevented the synthesis of auxin but also decreased the growth-stimulating effect of externally added auxin. Since kinetin promoted both the auxin production and the auxin action (ANKER 1977) it seemed desirable to investigate whether this substance also counteracts the above-mentioned inhibitions.

The effect of kinetin, added in a concentration of 1 mg/l (see ANKER 1977), depended on the galactose concentration used. At galactose concentrations of 0.10 and 0.125% the growth inhibition was reduced by kinetin, but at higher galactose concentrations (0.15 and 0.25%) the effect was either insignificant or absent (*figs. 1a* and *1b*). The dotted line in these and the following figures always represents the assumed course of the growth rate in pure water, which is based on experience with hundreds of previous experiments (cf. ANKER 1974).

It is evident from *fig. 1* that the inhibition of auxin synthesis by galactose is not permanent. There is an adaptation to the presence of galactose. As to the mechanism of the kinetin action, it is possible that it promotes this adaptation.

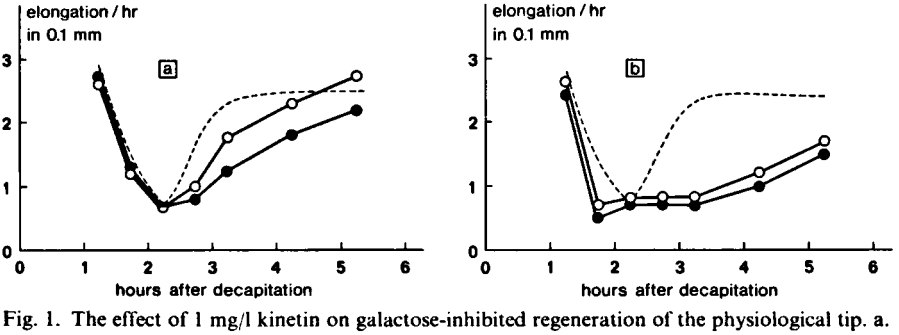

Fig. 1. The effect of 1 mg/l kinetin on galactose-inhibited regeneration of the physiological tip. a. 0.1% galactose, b. 0.25% galactose. ●——● growth in galactose (control), ○——○ growth in galactose plus kinetin, - - - - growth in water (see text).

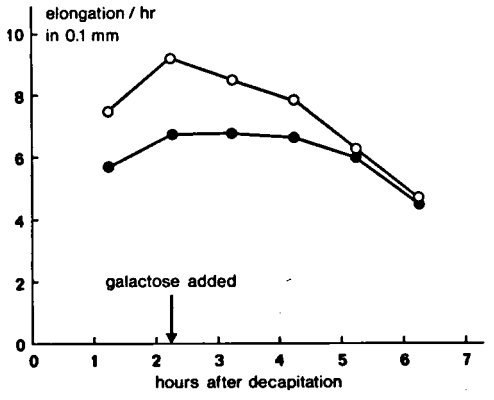

Fig. 2. The effect of galactose (0.1%) on segment growth in a 0.1 mg/l IAA solution, in the presence (O——O) or absence (●——●) of 1 mg/kinetin.

Based on previous information (ANKER 1977), other mechanisms of kinetin action should be considered as well.

One of these was tested. It was found in a previous investigation (ANKER 1974) that galactose not only reduced the auxin production but that it also decreased the reaction of the segments to externally supplied IAA. The effects of kinetin on the growth, illustrated in *fig. 1* could, therefore, in theory be due to a reduction of a galactose-induced inhibition of the auxin action. The results of the following type of experiment demonstrates that this was not true (*fig. 2*).

One set of 12 segments was submerged in a 0.1 mg/l IAA solution, the other set in a solution containing 0.1 mg/l IAA plus 1 mg/l kinetin. Two hours later galactose was added to both solutions. The final concentration of galactose was equal to that used in the experiment of *fig. 1*, being 0.1%. The first effect of galactose was a decrease of the growth stimulation caused by kinetin. At about three hours after the addition of galactose the difference of the growth rate had completely disappeared. In the remaining part of the experiment the presence of kinetin made no longer any difference. This result points out that a reduction of the inhibiting effect of galactose on auxin action by kinetin may be excluded as an explanation of the results shown in *fig. 1*.

At an earlier occasion it was found that gibberellic acid (GA_3) had no direct stimulating effect on the auxin production of decapitated coleoptiles. An indirect stimulating effect was observed since it reversed part of the inhibition of the auxin production caused by abscisic acid (ABA).

From *fig. 3* it is obvious that a similar effect of GA_3 was not observed when galactose was the inhibitor of the auxin production. The growth retarding effect of 0.1% galactose was not removed by 10 mg/l GA.

3.2. Effects of kinetin on ABA-treated segments

The effects of ABA on the regeneration of the physiological tip have been described in ANKER (1975). It was found that ABA never delayed the regeneration

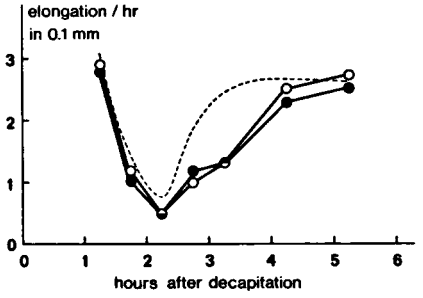

Fig. 3. The effect of 10 mg/l GA_3 on the inhibition of the regeneration of the physiological tip by galactose (0.1%). ●——● growth in galactose (control), O——O growth in galactose plus GA_3, ---- growth in water (see text).

but that the activity of the new tip was impaired. The influence of the presence of kinetin in this situation is illustrated in *fig. 4*. It stimulated the growth of the segments after the regeneration but the inhibiting effect of ABA was only partly abolished.

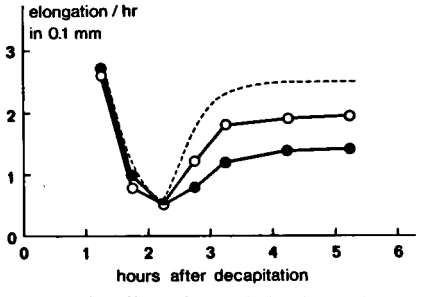

Fig. 4. The effect of 1 mg/l kinetin on the inhibition of the regeneration of the physiological tip by ABA (1 mg/l). ●——● growth in ABA, O——O growth in ABA plus kinetin, ---- growth in water (see text).

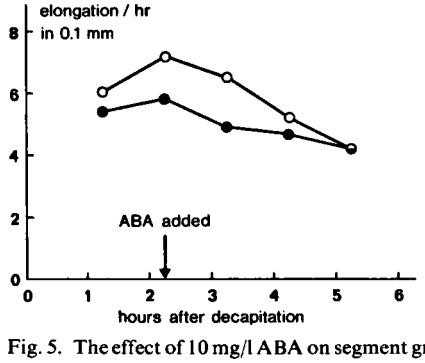

Fig. 5. The effect of 10 mg/l ABA on segment growth in a 0.1 mg/l IAA solution, in the presence (O——O) or absence (●——●) of 1 mg/l kinetin.

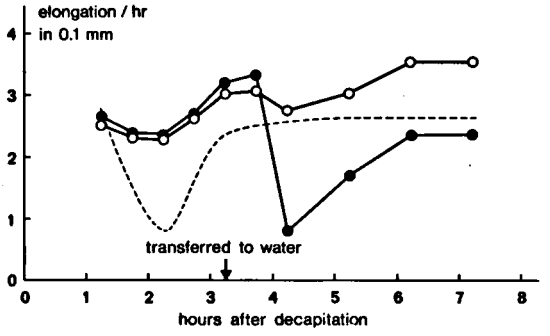

Fig. 6. The regeneration of the physiological tip is inhibited in the presence of 0.01 mg/l IAA. With 1 mg/l kinetin the inhibition by IAA is abolished. ●——● IAA alone, O——O IAA plus kinetin, ---- growth in water (see text).

In order to investigate the mechanism of the effect of kinetin in this case, similar supplementary experiments were done as described in the previous section. When the growth of the segments was controlled by externally added IAA plus ABA, kinetin did not relieve the inhibiting effect of ABA. This is illustrated in *fig. 5*. Hence it may be concluded that the effect of kinetin is limited to an incomplete reversal of the inhibiting influence of ABA on the auxin production of the segments.

3.3. Effects of kinetin on IAA-inhibited auxin synthesis

The strongest inhibitor of the auxin production in the apical cells of a decapitated coleoptile was shown to be IAA itself (ANKER 1973). The regeneration of the physiological tip was already completely inhibited when it was added in the concentration of 0.01 mg/l. The present results will show that this suppression can be completely removed with kinetin.

The experiments ran as follows. The segments were submerged in a solution of 0.01 mg/l IAA plus 1 mg/l kinetin. Control segments were submerged in the same IAA solution without kinetin. After a three hours' stay in these solutions all segments were transferred to water. The course of the growth rates of the kinetin-treated and the control segments is shown in *fig. 6*.

As was to be expected the transfer to water was not immediately followed by a decrease of the growth rate, but one hour later the growth of the control segments had decreased to the low level to which it is always reduced just before the resumption of the growth as a consequence of the auxin production in the regenerating tip. The dotted line in *fig. 6* represents the course of the growth in water without IAA. During the initial three hours of this experiment no regeneration of the physiological tip had taken place in the control experiments in the presence of these small amounts of IAA in the medium. But more than one hour after the removal of IAA from the external medium, the decline of the growth was followed by a rise caused by the auxin production in the tip.

The growth of the kinetin-treated segments, on the other hand, did not show

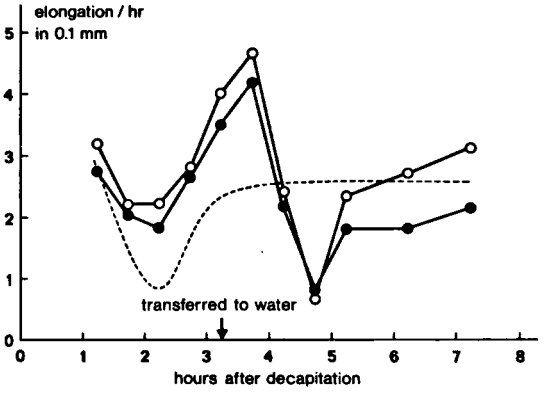

Fig. 7. The inhibition of the regeneration of the physiological tip by 0.02 mg/l IAA is not abolished by 1 mg/l kinetin. ●——● segments during the first three hours in IAA, O——O segments during the first three hour in IAA plus kinetin, ---- growth of segments in water.

this decline after the transfer to water. Kinetin apparently had antagonized the inhibiting action of IAA.

If, however, IAA was added in a slightly higher concentration (0.02 mg/l), its action was not abolished by kinetin (*fig. 7*). Because of this surprising result these experiments were repeated several times. In all of them kinetin abolished the inhibition caused by 0.01 mg/l IAA, but it failed to do so at the 0.02 mg/l IAA concentration.

The possibility was investigated that at the higher IAA concentration higher kinetin concentrations were needed to remove the inhibition. But, as is seen from *fig. 8*, even with the high kinetin concentrations of 5 or 10 mg/l the suppression of the regeneration was not removed if it was caused by 0.02 mg/l IAA.

4. RELATED RESEARCH

4.1. Somatic embryogenesis in *Citrus* tissue cultures

The effects of sugars and of hormones on the synthesis of auxin, described above

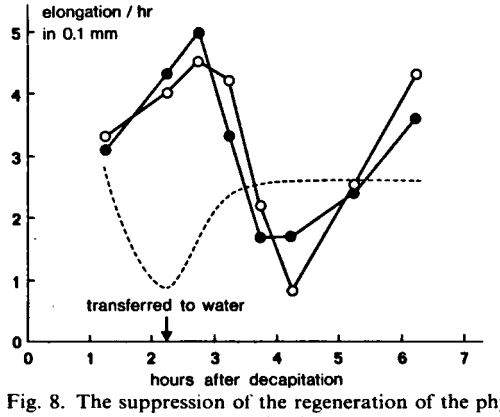

Fig. 8. The suppression of the regeneration of the physiological tip by 0.02 mg/l IAA cannot be neutralized by 5 or 10 mg/l kinetin. ●——● segments during the first two hours in IAA plus 10 mg/l kinetin, O——O segments during the first two hours in IAA plus 5 mg/l kinetin, ---- growth of segments in water.

and in previous papers, are not restricted to the *Avena* coleoptile. The same effects were observed in tissue cultures of *Citrus*. In the so-called "habituated" tissues, which are auxin-independent for their growth because they produce it themselves, no somatic embryogenesis can take place unless the auxin synthesis is artificially suppressed. KOCHBA et al. (1978a and b) examined whether the substances active in auxin-synthesis suppression in the coleoptile, could also be used as stimulants of embryogenesis in habituated tissues. This was actually the case: with galactose and abscisic acid the habituated tissues for the first time produced embryos. Preliminary experiments with precursors of IAA indicated that galactose possibly inhibited conversion of indoleacetaldehyde to IAA.

GA, which abolishes the ABA-induced suppression of IAA production in coleoptiles also antagonized the effect of ABA on embryogenesis.

Cytokinins, lastly, having a stimulative effect on the auxin production of the coleoptile, caused a decrease of embryogenesis in *Citrus* (KOCHBA & SPIEGEL-ROY 1977).

4.2. Apical dominance

Perhaps part of the present results – the antagonism of IAA and kinetin on the auxin production in the coleoptile – might contribute to the explanation of their antagonism in apical dominance. Since auxin production is one of the first events observable in released buds, the possibility exists that the ability to synthesize IAA is the critical step for bud release. WICKSON & THIMANN (1958, 1960) found that kinetin released the inhibition of lateral bud growth on isolated pea stem segments caused by IAA.

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