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ANTAGONISTIC EFFECTS OF HORMONES ON THE AUXIN PRODUCTION IN AVENA COLEOPTILES

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SUMMARY

It was found in a further analysis of the regeneration of the physiological tip in decapitated *Avena* coleoptiles, that both kinetin and gibberellic acid (GA_3) completely antagonized the inhibition of the auxin synthesis in the new tip caused by 0.01 mg/l indoleacetic acid (IAA). To antagonize inhibitions caused by 0.02, 0.03 or 0.04 mg/l IAA, kinetin and GA_3 had to be added in combination, but the effect of 0.05 mg/l IAA could no longer be neutralized by these hormones. Phenylbutyric acid did not affect tip regeneration.

The results are briefly discussed in connection with the regulation of breaking apical dominance, and with the limited sensitivity of the *Avena* straight-growth test.

1. INTRODUCTION

In previous investigations on this subject it was found that the synthesis of auxin in the apical cells of a decapitated coleoptile could be influenced artificially by addition of different plant hormones (ANKER 1973, 1975, 1977, 1979). It was inhibited by abscisic acid (ABA), but the strongest inhibitor was IAA itself. Kinetin stimulated the synthesis, and it also reversed the inhibitions caused by IAA and ABA, or by galactose (ANKER 1974).

These results were confirmed by KOCHBA et al. (1978) in a series of investigations on the effects of the same hormones and of galactose on the auxin synthesis in *Citrus* tissue cultures.

Similar hormone antagonisms were reported by several authors studying the mechanism of apical dominance and of bud release (see discussion). Since auxin production is one of the first events observable in released buds, the present results could be of interest to the study of the physiological basis of branching.

2. MATERIAL AND METHODS

The experimental material was taken from five days'old *Avena* seedlings cultivated at 23 °C and 80% humidity. Weak red light from a 40 W bulb, filtered through red selenium glass was used to inhibit mesocotyl growth of the seedlings. On the fourth day of cultivation the light was switched off in order to prevent untimely breaking through of the primary leaves. The experiments were done on the fifth day in the same red light.

All measurements were done with 19 mm apical segments of the coleoptiles from which a tip of exactly 1 mm length had been cut off before. The segments

were gently pressed on metal pins, and they were standing vertically for the duration of the experiment in aerated solutions of the substance(s), the effect of which on the rate of elongation was to be studied. This procedure has the advantage over application in agar or in paste, that the hormone concentration remains constant throughout an experiment, which is important when the hormones are added in very low concentrations. There were only 12 segments in one liter of the solution.

The segments were shadowgraphed at intervals on Gevaert Rapid Document Paper, which is sensitive to the phototropically inactive red light used for this purpose. The photographic paper was kept in a plate holder by way of a glass negative having a lattice of square millimeters, which, being co-photographed, much facilitated the measurements of the shadowgraphed segments.

Further experimental details are given along with the description of the particular experiments.

3. RESULTS

As has been said in the introduction, the auxin production by the apical cells of a decapitated coleoptile (the regeneration of the physiological tip) can be influenced by several hormones, but the most potent inhibitor of the synthesis proved to be IAA itself. When present in the concentration of only 0.01 mg/l it inhibited the regeneration completely. It further appeared that kinetin (1 mg/l) prevented the repression of the auxin production, however, surprisingly enough, this antagonistic effect of kinetin was restricted to this very low IAA concentration. If the inhibition had been caused by 0.02 mg/l IAA, it could not be reversed by kinetin, not even if the latter substance was added in the very high concentrations of 5 and 10 mg/l. From the results of the present experiments it is seen that the inhibition caused by 0.02 mg/l IAA could be overcome if kinetin was given in combination with 10 mg/l GA_3 (*fig. 1*).

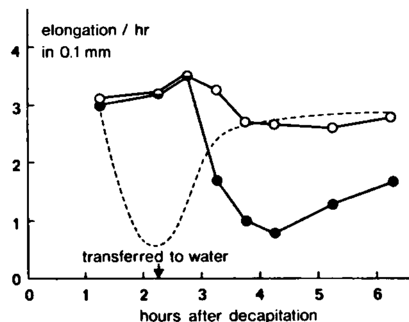


Fig. 1. The regeneration of the physiological tip is inhibited in the presence of 0.02 mg/l IAA. With 1 mg/l kinetin plus 10 mg/l GA_3 the inhibition by IAA is abolished. •——• IAA alone, o——o IAA plus kinetin plus GA_3, ... elongation in water (see text).

For the convenience of the reader who is not familiar with the previous papers of this series, the following explanation seems desirable. At time zero, immediately after the decapitation, the segments were submerged either in the 0.02 mg/l IAA solution (the control experiment) or in the solution containing the same amount of IAA together with 1 mg/l kinetin plus 10 mg/l GA_3. In water without hormones, the rate of elongation would have followed the dotted line, an assumption which is based on experience from hundreds of previous experiments. In water the rate of elongation would have decreased to almost zero, followed by an increase in the third hour after decapitation as a consequence of the auxin produced in the tip of the segment. Due to this auxin production the rate of elongation remains at a constant level for several hours.

The course of the growth of the segments in the control experiments is explained by the initial presence and the subsequent removal ($2\frac{1}{4}$ hours after the decapitation) of IAA. The transfer of the segments from the IAA solution to water was followed, after half an hour, by a rapid decrease of the elongation rate to the same, very low, level attained by the segments in water. This proves that the presence of IAA in the medium had prevented the segments from regenerating a physiological tip in the preceding period of time. Two hours after the transfer of the segments from the IAA solution to water, the growth was resumed, due to the beginning auxin production. It will be noticed that the interval between decapitation and tip regeneration in segments submerged in water was also two hours.

The course of the rate of elongation of the segments, initially immersed in IAA plus kinetin plus GA_3, and then transferred to water, differed considerably from that of the control segments. There was only a small decrease of the elongation rate observable after the transfer to water. During the final hours of the experiment the increase in length per hour was equal to that of segments having completed tip regeneration in water. From this result it is concluded that the regeneration had already taken place in the period preceding the transfer to water in spite of the presence if IAA.

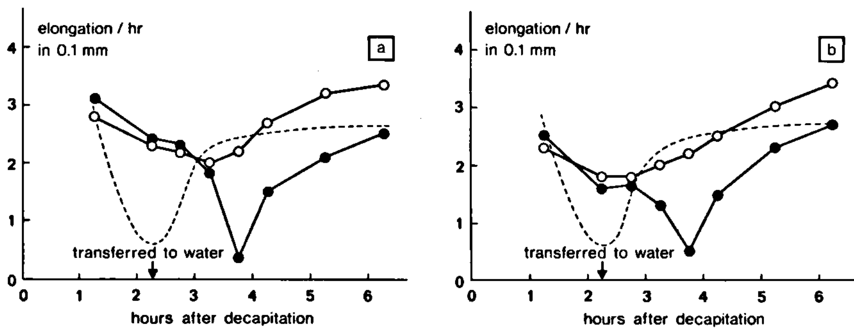
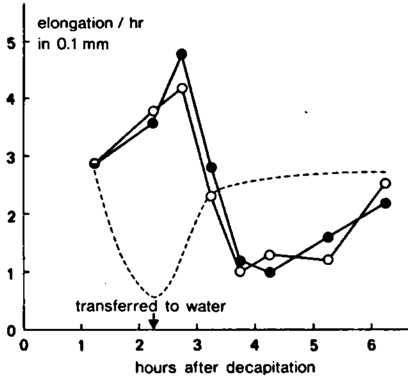


Fig. 2. The inhibition of the regeneration of the physiological tip, caused by 0.01 mg/l IAA is antagonized by GA_3. ●——● IAA alone, o——o IAA plus GA_3, ... elongation in water. a. 10 mg/l GA_3, b. 15 mg/l GA_3.



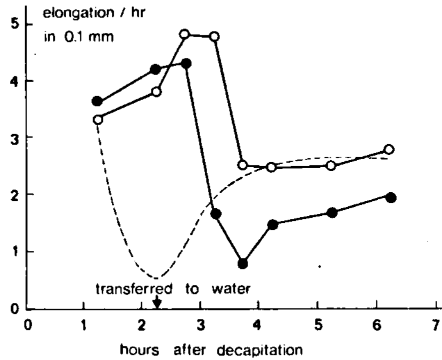
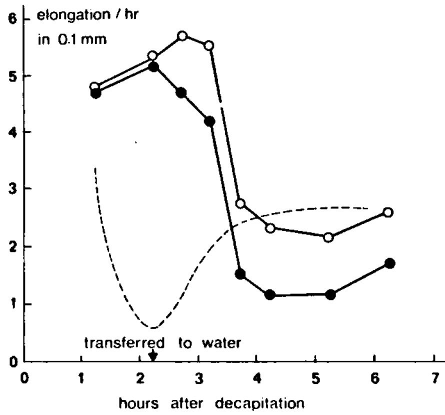


Fig. 3. The inhibition of the regeneration of the physiological tip, caused by 0.02 mg/l IAA is not neutralized by 10 mg/l GA_3. ●——● IAA alone, o——o IAA plus GA_3, ... elongation in water.

Fig. 4. The inhibition of the regeneration of the physiological tip, caused by 0.03 mg/l IAA is antagonized by 1 mg/l kinetin plus 10 mg/l GA_3. ●——● IAA alone, o——o IAA plus GA_3, ... elongation in water.



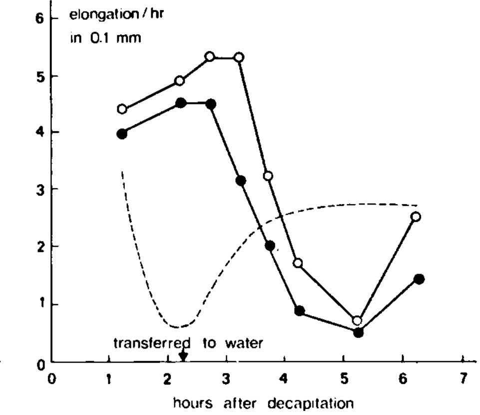


Fig. 5. The inhibition of the regeneration of the physiological tip, caused by 0.04 mg/l IAA is reversed by 1 mg/l kinetin plus 10 mg/l GA_3. ●——● IAA alone, o——o IAA plus kinetin plus GA_3, ... elongation in water.

Fig. 6. The inhibition of the regeneration of the physiological tip, caused by 0.05 mg/l IAA is not revertible by 1 mg/l kinetin plus 10 mg/l GA_3. ●——● IAA alone, o——o IAA plus kinetin plus GA_3, ... elongation in water.

In supplementary experiments it was found that GA_3 alone, like kinetin alone, could antagonize the effect of 0.01 mg/1 IAA, but it could not do so if the IAA concentration was doubled (*figs. 2* and *3*). All these experiments were repeated two or three times. Apart from small differences, due to uncontrollable variations of the experimental material, the results were identical: the inhibition of the regeneration of the physiological tip by 0.02 mg/l IAA could be checked if kinetin and GA_3 were jointly added to the medium.

The next step was to investigate whether this combination of kinetin plus GA_3 was also able to antagonize the effect of IAA if the concentration of the latter was increased. To this purpose IAA was added in the following experiments in the concentrations of 0.03, 0.04, and 0.05 mg/l. The results are given in the *figs. 4, 5* and *6*. One observes that, indeed, the effects of higher concentrations of IAA could be neutralized, however, a second limit was found, lying between the 0.04 and the 0.05 mg/l concentration: the effect of 0.05 mg/l IAA could not be counterbalanced. These results, again, were fully reproducible in duplicate experiments. A further increase of the kinetin and the GA_3 concentrations above these, already unnaturally high levels was deemed of no use.

4. DISCUSSION

It has been demonstrated by the present results, and by identical results obtained by KOCHBA et al. (1978) with tissue cultures, that the auxin production in plants can be antagonistically influenced by hormones. As will be seen from the data cited below, the same hormone antagonisms were found in research on the regulation of apical dominance. Since auxin production is one of the first events observable in released buds, the present results suggest that auxin metabolism in the buds is the target process of the antagonistic actions.

This possibility was already mentioned by PHILLIPS (1969), who studied apical dominance in some tall and dwarf varieties of pea and bean, where the inhibiting effect of IAA on bud growth was antagonized by GA_3. Based on a series of ten articles in which effects of exogenous GA_3 on auxin synthesis were reported, he suggested 'that gibberellins influence apical dominance in intact plants indirectly through such effects on auxin metabolism'. This suggestion is supported by the present demonstration that GA_3 reversed the inhibition of auxin production caused by IAA.

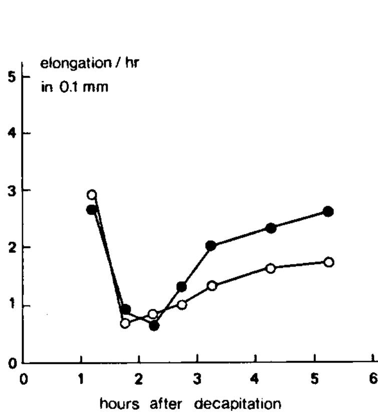
In the experiments of WICKSON & THIMANN (1958, 1960) on bud release in isolated pea stem segments, the inhibition caused by IAA was antagonized by kinetin. The buds were released if the ratio auxin: kinetin was not far from 1:1, though it varied with the conditions. In our experiments the inhibiting effect of IAA (0.01 mg/1) was neutralized by 1 mg/1 kinetin, but even a tenfold increase of the kinetin concentration could not antagonize the effect of 0.02 mg/l IAA. Neither GA_3 alone was able to do this, however, when added in combination they could even reverse the action of 0.04 mg/l IAA.

A similar joint action of kinetin and GA_3 was reported for the promotion of lateral bud growth in tomato, by CATALANO & HILL (1969).

WOOLLEY & WAREING (1972) suggested that the role of cytokinins in apical dominance might be to overcome inhibitory substances such as ABA in axillary buds. There are no suggestions in the literature on the mechanism of the cytokinin-ABA antagonism in relation to apical dominance, but in view of the present results the target process of their action, again, could be the auxin metabolism in the buds, since in coleoptiles the inhibiting action of ABA on auxin production was reduced by kinetin, and also by GA_3 (ANKER 1975, 1979).

It seemed of interest to include gamma-phenylbutyric acid in this investigation. This substance with little auxin activity and showing competitive antagonism to auxin action was found by VAN OVERBEEK (1938) to prevent the formation of auxin in the bud if it was added in the high concentration of 2‰. As is seen from *fig. 7* gamma-phenylbutyric acid (10 mg/l) did not delay the regeneration of the tip, but the elongation of the segments, occurring after the regeneration of the tip was considerably slower than that of the controls in water. This effect could theoretically be due to a reduced auxin production by the new tip, but the observation that the percentage of growth reduction was the same when it was added to segments externally supplied with 0.05 mg/l IAA points to the competitive antagonism to auxin just mentioned (*fig. 8*).

The absence of any influence on the regeneration of the physiological tip is in agreement with observations made earlier with other auxin analogues. Neither naphthaleneacetic acid nor 2,4-dichlorophenoxyacetic acid did repress the auxin synthesis in decapitated coleoptiles (ANKER 1973). WICKSON & THIMANN (1958, 1960), on the other hand, found that both substances were even more potent inhibitors of lateral bud growth than IAA was. A possible cause of the inactivity



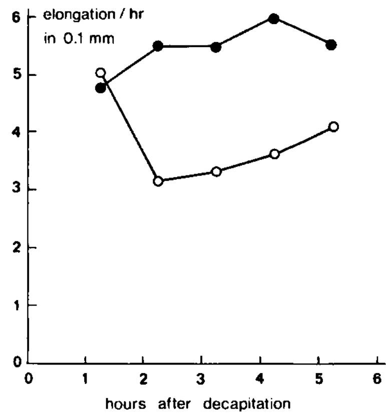


Fig. 7. The regeneration of the physiological tip is not delayed by gamma-phenylbutyric acid; the elongation rate is decreased after the regeneration. ●——● elongation in water, ○——○ elongation in gamma-phenylbutyric acid (10 mg/l).

Fig. 8. Reduction of the elongation rate of segments submersed in 0.05 mg/l IAA by 10 mg/l gamma-phenylbutyric acid. ●——● elongation in IAA, ○——○ elongation in IAA plus gamma-phenylbutyric acid.

of these herbicides could be that *Avena* is a monocotyledonous plant and that much higher concentrations would have been required to obtain the inhibition.

The present results could also be relevant to the explanation of a completely different phenomenon to wit the cause of the lower limit of sensitivity of the Avena straight growth test.

It has been observed by several authors (BARLOW et al. 1957; BLAAUW-JANSEN 1959, 1962) that coleoptile sections extend less in very dilute IAA solutions than they do in water. This was particularly evident if the experiments were extended over more than five hours (BARLOW et al. 1957). This fact has never been considered in connection with another repeatedly observed phenomenon, that even small coleoptile sections (1 or 2 mm long) have the ability to develop the faculty to produce auxin for a period of many hours (VAN OVERBEEK 1941; WENT 1942; BENNET-CLARK & KEFFORD 1954).

With the present observations in mind that even very dilute IAA solutions (0.01 mg/l) inhibit the auxin production in isolated segments, the explanation of the faster elongation in water would be that the actual provision of the growing cells with auxin – as a consequence of their own synthesis – is better in water than in the diluted IAA solutions. This explanation is based on the dual effect of IAA. When the concentration of the added IAA is so low that its growth-stimulating activity no longer compensates for its repression of the internal auxin production, the lower limit of sensitivity of the *Avena* straight-growth test has been attained.

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