

GEOTROPISM AND TIP REGENERATION OF THE AVENA COLEOPTILE IN THE PRESENCE OF GIBBERELIC ACID

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SUMMARY

The apical section (18 mm long) of a decapitated (1 mm removed) *Avena* coleoptile does not curve geotropically in water nor in a gibberellic acid solution.

Gibberellic acid stimulates the geotropic curvature of similar sections carried out in indoleacetic and naphthylacetic acid solutions of a suboptimal concentration for the geotropism, but it inhibits the curvature if indoleacetic acid is supplied in a supraoptimal concentration. The inhibition requires much more gibberellic acid than is needed for the promotion.

These results, together with data and suggestions from the literature, motivate an investigation into the possibility of a competitive synergism between gibberellic acid and auxins in the process of auxin transport.

The regeneration of the physiological tip is not accelerated by gibberellic acid, but the geotropic curvature, made possible by the auxin production in the new tip, is increased.

1. INTRODUCTION

The aim of the present investigation was not so much to study the geotropism of a decapitated coleoptile as to use this auxin concentration-sensitive process as a test of possible effects of gibberellic acid on the uptake, the transport and the metabolism of auxins.

On previous occasions (ANKER 1954, 1956) it appeared that decapitated *Avena* coleoptiles, when placed in water horizontally, show hardly any geotropic curvature during the first hours after decapitation. Addition of auxins to the water in the appropriate concentrations, however, restores their ability to react completely. It further appeared that small variations of the auxin concentration will cause large variations of the rate of curvature. The graph relating the auxin concentration to the rate of curvature shows a maximum at about 75 gamma indoleacetic acid per liter, and declines steeply on both sides of that value. In media containing less than 40, and more than 400 gamma per liter indoleacetic acid the geotropic reaction of decapitated coleoptiles is very slow.

This high sensitivity of the geotropic reaction to the auxin concentration seemed to provide a good assay for examining possible interactions of gibberellic acid (GA) with indoleacetic acid (IAA) and with naphthylacetic acid (NAA). The experiments were made with apical sections of the *Avena* coleoptile, the tips of which had been removed beforehand. By using so simple an object, one has the advantage that stimulations of the natural auxin production in the tip, or of the mobilisation and the transport of a food factor or an auxin precursor from

the seed by addition of GA, can be ruled out when the results are evaluated.

GA appeared to decrease the amounts of IAA or NAA needed for the regulation of the geotropic reaction. The sections did not curve, however, in a GA solution without auxin. The regeneration of the physiological tip was not advanced by GA.

2. METHODS

Since a detailed description of the methods and of the apparatus used has been given elsewhere (ANKER 1954, 1956), the experimental procedure will be dealt with here in outline only.

The experiments were carried out with the apical section of 4 days' old coleoptiles from which a tip of exactly 1 mm had been cut off. Twelve of these sections, each being 18 mm long, were used in one treatment. They remained for 95 minutes in the solution to be tested, 20 minutes in the vertical and 75 minutes in the horizontal position. As coleoptiles are but rarely perfectly straight, a shadowgraph was also made before the change in the position. The initial curvature, when present, was added to or subtracted from the final curvature measured on the shadowgraph made at the end of the period of horizontal exposition.

Care was taken that the geotropic curvature was carried out in the plane through the vascular bundles, a precaution which, literally, greatly enhances uniformity.

The pH of the solutions, which were aerated with purified air, was always 6.9. Except for the last 24 hours of darkness preceding the decapitation, red light was admitted throughout the cultivation of the seedlings and during the experiments. The light came from an incandescent lamp and was filtered by red selenium glass, the transmission of which has been given in BLAAUW & BLAAUW-JANSEN (1964). The room temperature was kept at 23°C and the air humidity varied between 85 and 95%. Where necessary, the standard error of the mean has been drawn in the diagrams.

3. RESULTS

The research was started with experiments on the influence of GA on the auxin requirement for the geotropic response of decapitated coleoptiles with IAA as the applied auxin. The results are presented in *fig. 1*. It appears that GA, used in the concentration of 1 mg per liter will cause a shift in the curve, relating section curvature to IAA concentration, to the lower concentrations of IAA. Thus it promotes the geotropic reaction of the sections at low IAA concentrations, but it inhibits the development of the curvature at somewhat higher concentrations of IAA.

The results of our experiments to determine roughly the lowest GA concentration necessary to obtain the positive as well as the negative effects just mentioned are given in the *figures 2 and 3*.

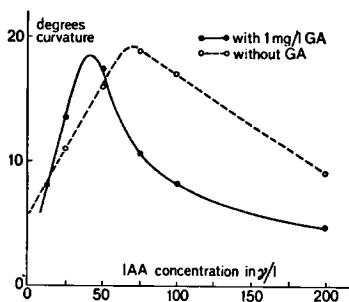


Fig. 1. The effect of gibberellic acid on the indoleacetic acid requirement for the geotropic reaction of decapitated *Avena* coleoptiles.

Fig. 2. The stimulation of the geotropic reaction of decapitated *Avena* coleoptiles in a suboptimal indoleacetic acid solution by gibberellic acid, added in a wide range of concentrations.

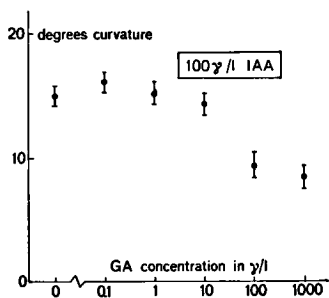
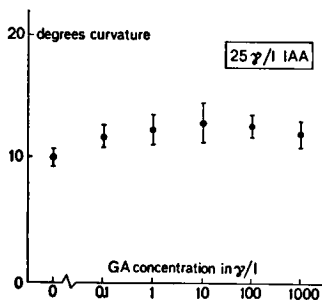


Fig. 3. The inhibition of the geotropic reaction of decapitated *Avena* coleoptiles in a supraoptimal indoleacetic acid solution by gibberellic acid, added in a wide range of concentrations.

It is seen that the promotion of the rate of curvature by GA at the suboptimal IAA concentration of 25 gamma per liter is already obvious when it is added in the concentration of only 0.1 gamma per liter, but that for the inhibition of the curvature at the supraoptimal IAA concentration of 100 gamma per liter, a GA concentration of at least 100 gamma per liter is necessary.

The action of GA appeared not to be limited to the condition that the natural IAA is the regulator of the geotropic reaction. It produced similar effects when the auxin analogue NAA was applied. This is shown in *fig. 4*.

During a current study of the effect of various substances on the regeneration of the physiological tip, which will be published later, a few experiments were performed with GA. To decide whether a given substance is active in speeding up the process of auxin production in the tip of the stump, we determined the moment at which horizontal decapitated sections start to curve in a solution of this substance. GA was not capable of promoting the regeneration. However, once the regeneration was complete, it accelerated the curvature starting after the formation of auxin in the tip of the stump (*fig. 5*).

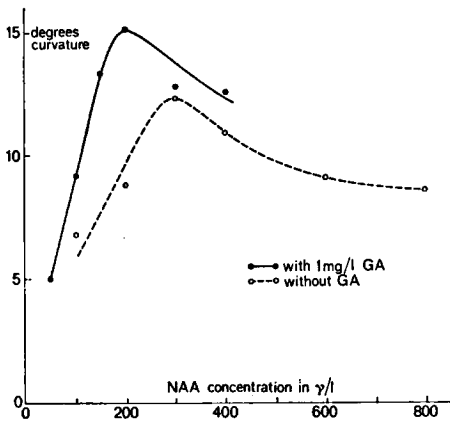


Fig. 4. The effect of gibberellic acid on the naphthylacetic acid requirement for the geotropic reaction of decapitated *Avena* coleoptiles.

Particular attention may be drawn to the fact that GA by itself was not capable to control the geotropic reaction (fig. 5). During the period preceding the regeneration of the tip the coleoptile sections did not show the slightest curvature in the GA solution above that produced in water. This period was certainly long enough for an effect to become visible, since the effect of GA produced in the experiment of fig. 1 was measurable within about 30 minutes after the transfer to the horizontal position. The very small curvatures appearing previous to the regeneration of the tip, even in water, are due to the residual auxin in the stump. The greater part of this auxin will already have been used up during the first period following decapitation, before the sections were put horizontal.

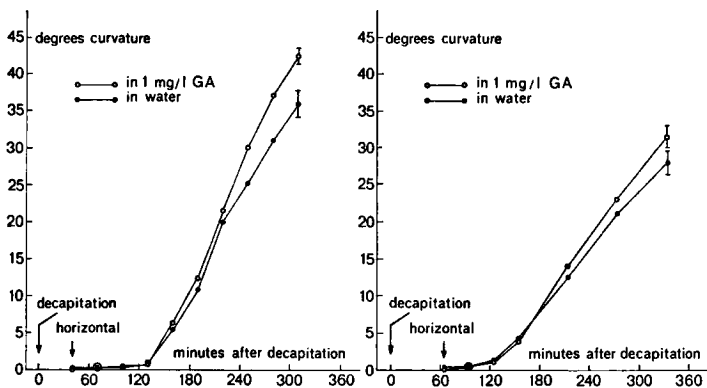


Fig. 5. Gibberellic acid by itself does not enable decapitated *Avena* coleoptiles to curve geotropically; it does not advance the regeneration of the physiological tip; it stimulates the geotropic curvature effected by the auxin produced in the new tip.

4. DISCUSSION

In search of an explanation of the reduced auxin demand of decapitated coleoptiles for the geotropic reaction in the presence of GA, all those well-known actions of GA that require more time to become apparent than was available in the present experiments can be left out of consideration. In addition, the removal of the tip, and the absence of the seed preclude influences on the normal auxin production and on the mobilisation of auxin precursors or of food factors in the seed.

It is evident that GA was incapable of regulating the geotropic reaction of the sections in the absence of exogenous or endogenous auxin, which indicates that the action is an indirect, auxin-mediated one. This result confirms the observations of many investigators who studied the conditions under which GA is active on the extension growth (surveyed by PALEG 1965).

GA increases the activity of a given auxin solution. This is the reason why it promotes the curvature at suboptimal, and why it is inhibitive at supraoptimal IAA concentrations for the geotropic reaction. The sign of the GA effect seems therefore to have no theoretical significance.

It is essential to bear in mind that the optimal concentrations for growth and geotropism are not the same (ANKER 1956). The supraoptimal concentrations used in the present experiments were still infraoptimal for the straight growth. These concentrations were therefore not unphysiologically high. By increasing the concentration above the optimal one, only the difference between the rates of growth of upper and lower side of the sections became gradually less. Even at the highest concentrations used the sections were not flooded with auxin, since ANKER (1956) and DE WIT (1957), employing the same technique, have demonstrated that in the course of these relatively short-lasting experiments no measurable amounts of auxin entered the sections via the cuticle from solutions of similar concentrations. Hence it may be assumed that the auxin supply was controlled by the rate of uptake via the apical cut surface and/or by the intensity of the auxin transport. Stimulation of these functions by GA could provide an explanation of the shift in the optimum. This explanation is not without some foundation, since improvement of the transport of radiocarbon from carboxyl-labelled IAA by GA has already been shown by PILET (1965) with sections of *Lens culinaris*, and JACOBS & CASE (1965) found that in the *Pisum* shoot "gibberellic acid caused more indoleacetic acid to be present and effective far from the site of application". This effect of GA should be confirmed with *Avena*.

Using the *Avena* coleoptile, SÆBØ (1960) studied the effect of GA on the curvature test. He found that the amount of GA necessary to increase the effect of a given auxin solution depended on the concentration of the auxin in the medium. He was tempted to assume that the postulated synergism between GA and IAA is of a competitive nature. A similar phenomenon has been observed in the present investigation. However, more GA-IAA combinations should be tried if we are to be able to confirm the competitive nature of the synergism.

A synergism of GA with auxin analogues has also been reported by BRIAN & HEMMING (1961). In their experiments GA showed synergism with NAA and

2,4-D in the promoting of the extension growth of pea stems, whereas GA alone had little or no effect, as it did neither in our experiments. According to the authors this synergism with auxin analogues throws "considerable doubt on the theory, that GA inhibits metabolic destruction of IAA". In fact, it indicates a less specific action of GA, which in this connection might comprise the inhibition of the removal of auxin from the transport system, thus inhibiting the immobilisation.

GA did not accelerate the formation of the physiological tip in the stump of the decapitated coleoptile. This means that the auxin production did not start earlier. The slightly increased rate of curvature after the regeneration was completed, could be due to an intensification of the auxin production by GA, because, according to WENT (1942), the new tip produces amounts of auxin to 25 percent of the production of the normal tip. This auxin supply is certainly insufficient, even for optimal geotropic response. An alternative explanation is that the auxin, produced in equal amounts, is better utilized in the presence of GA, through the mechanism proposed for the synergistic action with exogenous auxins.

A happy circumstance was that the absence of the seed did not hinder the regeneration of the tip, and that the newly formed auxin did not diffuse from the section into the solution. This might have been worse considering the results of SKOOG (1937) and of GORTER (1927) respectively. Apparently the sections contain enough auxin precursor themselves, and, as for the limited leakage, this may be due to the well-known polar transport of auxin in the basal direction.

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