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AUXIN-SYNTHESIS INHIBITION BY ABSCISIC ACID, AND ITS REVERSAL BY GIBBERELLIC ACID

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SUMMARY

Abscisic acid inhibits 1) the production of auxin and 2) the reaction to auxin of decapitated *Avena* coleoptiles.

Gibberellic acid, having no direct influence on these processes itself, cancels the first but does not reverse the second effect of abscisic acid.

1. INTRODUCTION

The extensive literature on abscisic acid (ABA) leaves no doubt that this substance is a potent inhibitor of cell elongation. Since indoleacetic acid (IAA) is the most common promoter of this process, much attention has been paid in recent years to the interaction between these growth regulators. The main question was whether these hormones act independently of each other, or in an antagonistic way. The bulk of evidence seems in favour of an independent action (WAREING et al. 1968), and some results of the present investigation, as far as they throw any light on this problem, gave additional support.

A third way of interplay between ABA and IAA has been suggested by authors who studied developmental processes. DÖRFFLING (1963, 1964) mentions the possiblity that ABA blocks the synthesis or the activation of growth substances in a dormant bud. The same idea was suggested by LIBBERT (1958) when he found that a substance for correlative inhibition, present in *Pisum* extracts, inhibited the enzymatical generation of IAA from tryptophane in vitro.

The main result of the present investigation is the experimental support to this possibility of interplay. ABA appeared to carry out its action on two systems. One of them is localized in the tip of the decapitated coleoptile segment, where it inhibited the IAA synthesis. This effect of ABA was cancelled by gibberellic acid (GA). The second site of action is the rest of the segment where it decreased the reaction of the cells to endogenous or exogenous IAA. The action of ABA on that system was not counteracted by GA.

2. MATERIAL AND METHODS

For the study of the development of the capacity of a tissue to synthesize auxin,

the coleoptile seems to give an adequate opportunity. The auxin production of this structure takes place in the extreme tip. When this part is removed by decapitation, a regeneration process is set going in the tip of the stump, the result of which is the initiation of a new auxin-production centre. The regeneration is finished 2 to 3 hours after the decapitation, which appears from the resumption of the growth of the stump. The rate of elongation is a measure of the activity of the new physiological tip.

The auxin synthesis by the new tip can be influenced in various ways (ANKER et al. 1973; ANKER 1973, 1974). The present study is concerned with the effect of synthetic (racemic) ABA and of GA_3 (plus combinations of these hormones) on this function.

To this purpose 12 apical coleoptile segments (19 mm long, 1 mm tip removed) were placed on pins and submerged in the vertical position in aerated solutions of the substances to be studied. The increase in length was measured on shadowgraphs made with phototropically inactive red light at intervals of ½ or 1 hour. The segments had been cut from 88 hours old seedlings, cultivated at 23°C (air humidity: 80–90%) in weak incandescent light, filtered by red selenium glass, and transferred to the dark about 24 hours before sectioning.

3. RESULTS

3.1 The course of the growth rate of coleoptile segments in ABA solutions

The course of the growth rate of coleoptile segments during the first 5 hours following decapitation has been described in previous papers, most recently in ANKER (1974). Owing to the exhaustion of the residual auxin the growth decreases rapidly in the first part of this period. As soon as the internal auxin concentration has fallen below a critical level (ANKER 1973), the distal cells of the coleoptile stump enter upon their new function of auxin production, and as a result of this so-called regeneration of the physiological tip the segments resume their growth.

When ABA was added to the decapitated coleoptiles the same sequence of events was observed, each event going on for the same period of time as in the control segments. As a result of this, the increase of the growth rate started at the same moment as in the control segments without ABA. This is seen from *fig. 1a* and *1b*, for the 0.5 and the 0.25 mg/l solution of ABA.

The course of the graphs demonstrates clearly that the regeneration was not delayed by ABA. Apparently the time required for the formation of the enzymes catalyzing the synthesis of IAA was not prolonged by ABA, although the production of IAA was decreased (see following section). The inhibiting action of ABA, therefore, was different from that observed with sugars (ANKER 1974), since galactose, lactose and raffinose caused a delay of the regeneration. Galactose (0.5%) could even prevent the auxin synthesis, just like IAA, which is the end product (ANKER 1973).

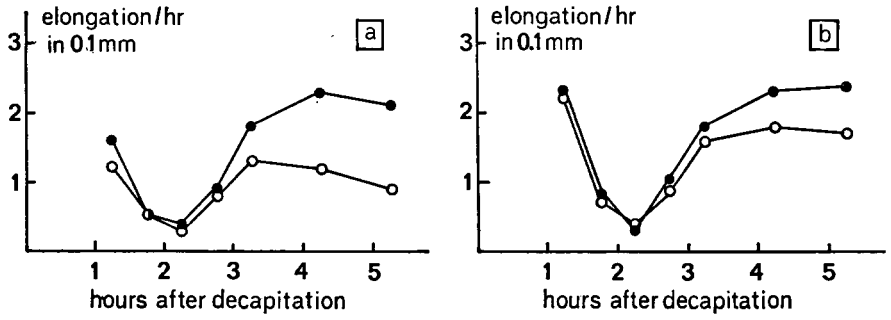

Fig. 1. The effect of ABA on the resumption of the growth after the regeneration of the physiological tip, a) in the 0.5 mg/l, and b) in the 0.25 mg/l concentration. ●—● course of the growth rate in water; ○—○ idem in the ABA solution.

3.2. The inhibition of the growth of coleoptile segments as a function of the ABA concentration

When IAA was absent from the medium, the growth of the coleoptiles occurring after the regeneration was considerably inhibited by ABA. Maximum inhibition was attained already at the ABA concentration of 1 mg/l. Increasing the strength of the ABA solution to 4 mg/l did not cause a significantly stronger inhibition. The maximum reduction of the growth rate amounted to 50–55% (*fig. 2*, curve A). See also *fig. 1a* and *1b*.

The same dose-response curve holds good for the ABA action in the presence

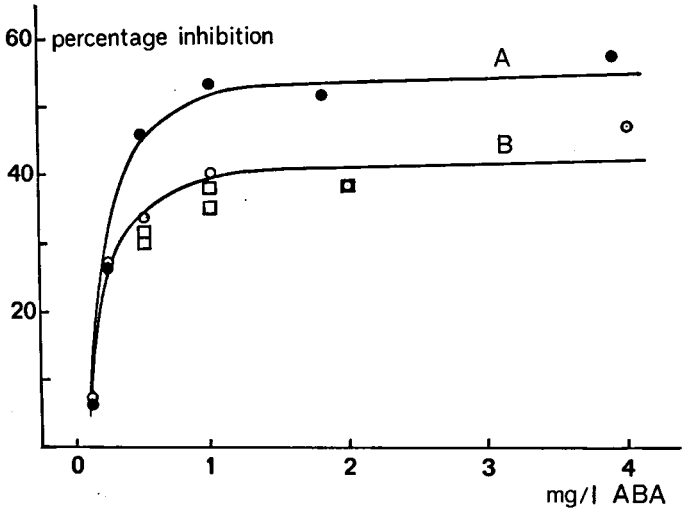

Fig. 2. The relation between the percentage of inhibition of the growth rate and the ABA concentration; ● ABA alone (curve A); ○ ABA plus 0.05 mg/l IAA (curve B); □ ABA plus 10 mg/l GA.

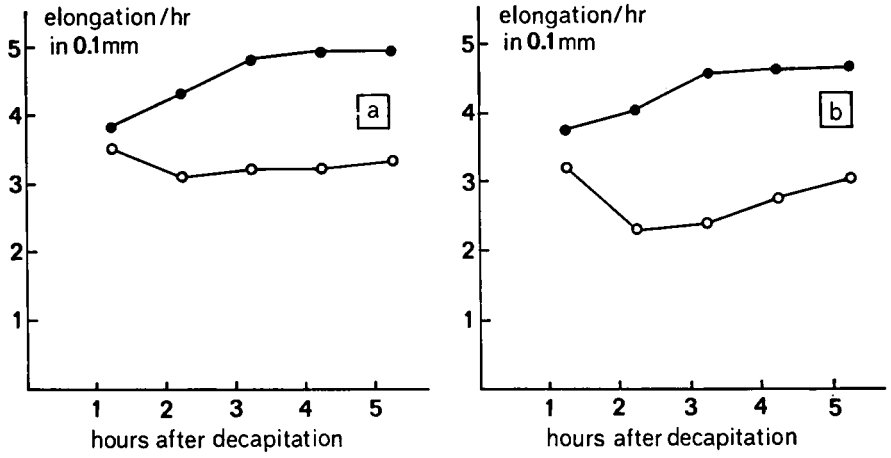

Fig. 3. The effect of ABA on the growth of segments in 0.05 mg/l IAA; a) 0.5 mg/l ABA and b) 1.0 mg/l ABA. ●—● course of the growth rate in 0.05 mg/l IAA alone; ○—○ idem in 0.05 mg/l IAA plus ABA.

of IAA. For this series of experiments the concentration of 0.05 mg/l IAA was chosen because it is not far from the natural IAA concentration in coleoptiles cultivated under the present circumstances. In that case the maximum inhibition was 40–45% (*fig. 2*, curve B).

Fig. 3a is a diagram of one of these experiments. It is seen that the rate of growth in the control segments was not yet constant in the first hours. The upward slope of the curve is connected with the slow permeation of IAA through the cuticle (ANKER 1971), so that in this diluted IAA solution the segments almost completely depended on the IAA molecules that came in via the apical cut surface. For the calculation of the percentage of inhibition caused by ABA, therefore, only the last three hours of each experiment were considered. In *fig. 3b* a second experiment has been diagrammed in which ABA was applied in a higher concentration.

It appeared, furthermore, that the concentration of IAA was of no influence on the percentage of inhibition caused by an ABA solution of a given strength. Even at IAA concentrations as high as 0.5 and 1.0 mg/l, a 1 mg/l ABA solution caused the same inhibition of about 40% (36 and 37% resp.). That a high dosis of IAA could not overcome the inhibition caused by ABA supports the idea of Wareing et al. that the interaction between these hormones is not of a competitive nature.

The above results confirm the observations of the authors just-mentioned and of DÖRFFLING & BÖTTGER (1968), all experimenting with Avena coleoptile sections, in that the maximum inhibition of the growth is attained at the 1 mg/l concentration of ABA.

Another similarity with the results of Wareing et al. is the excess inhibition

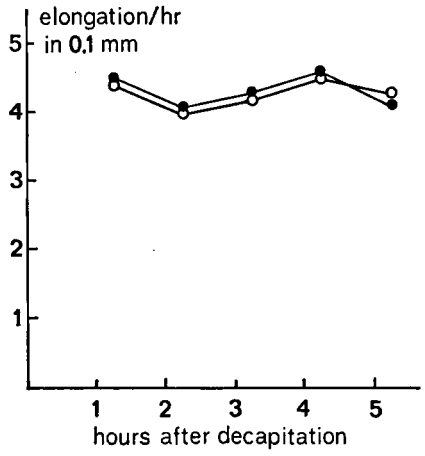

Fig. 4. GA does not relieve the ABA-induced inhibition of the growth rate of segments submersed in IAA solutions. ●—● 0.5 mg/l IAA plus 0.5 mg/l ABA; ○—○ 0.5 mg/l IAA plus 0.5 mg/l ABA plus 10 mg/l GA.

of growth by ABA in the absence of IAA. An explanation of the quantitative difference of the ABA effect in different experimental systems will be given after the description of the results of experiments in which ABA was added in combination with GA.

3.3. Effect of GA_3 on the growth inhibition caused by ABA

3.3.1. In the presence of IAA in the medium

As the regeneration of the physiological tip is prevented by the presence of IAA in the medium (ANKER 1973), the growth is entirely determined by the response of the cells to the added auxin. In this experimental condition the inhibition of the growth caused by ABA, was not relieved by GA (*fig. 4*). Since it is known that coleoptiles are rather insensitive to the latter hormone, GA was always supplied at the relatively high concentration of 10 mg/l.

In the experiment of *fig. 4*, GA was added simultaneously with IAA and ABA. The course of the growth in GA-treated and control sections being almost identical, the conclusion is justified that the inhibition by ABA was not in the least relieved by GA.

In a slightly different experiment in which the same quantity of GA was given 3 hours later the same result was obtained. In that case the control segments were submersed in an IAA solution without ABA so that the additional observation could be done that GA also failed to stimulate the growth induced by IAA alone *(fig. 5)*.

Fig. 5, furthermore, illustrates the information given in the previous section, that the percentage of inhibition by ABA in a given concentration is independent of the IAA concentration. In both experiments (compare *fig. 2* with *fig. 5*) the 0.5 mg/l ABA solution caused an inhibition of the growth of about 30%, whereas the concentration of IAA was twice as high in the experiment of *fig. 5*.

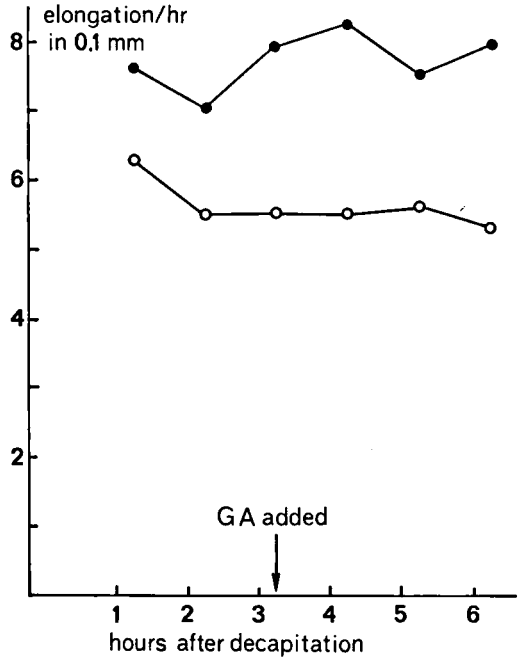

Fig. 5. GA does not relieve the ABA-induced inhibition of the growth rate of segments submersed in IAA solutions, nor does it stimulate the growth in the presence of IAA alone. At the arrow GA was added. ●—● 0.1 mg/l IAA; ○—○ 0.1 mg/l IAA plus 0.5 mg/l ABA.

3.3.2. In the absence of IAA from the medium

In a medium without IAA the elongation is dependent on 2 activities of the segments, 1) the auxin production by the physiological tip, and 2) the response of the cells to the auxin produced. That in the absence of IAA the inhibiting effect of ABA was stronger (see *fig. 2*) suggests the possibility that both activities of the segments are susceptible to the inhibitor. It is seen from *fig. 6* that in an IAA-free medium the inhibition, caused by ABA, was partially relieved by GA.

The remaining inhibition was almost exactly of the same magnitude as that caused by ABA in an IAA-containing medium. This is indicated by the squares in *fig. 2*.

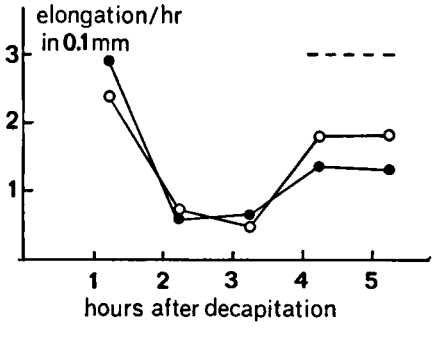

Fig. 6. GA partly relieves the ABA-induced inhibition of growth of segments submersed in a medium without IAA. ●—● 1 mg/l ABA; ○—○ 1 mg/l ABA plus 10 mg/l GA. Dotted line: growth rate of segments in water.

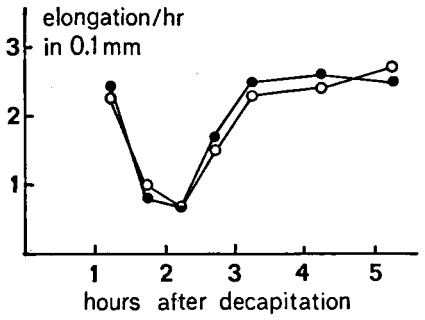

Fig. 7. Absence of any direct effect of GA on the regeneration of the physiological tip. ●—● segment growth in water; ○—○ idem in 10 mg/l GA.

The positive effect of GA on ABA-inhibited growth is more directly shown by the results of another experiment in which the growth of control segments in a 1 mg/l ABA solution was compared with that of segments submersed in the same ABA solution plus 10 mg/l GA (*fig. 6*). The relief of the inhibition by GA is clearly visible in the second part of the experiment i.e. after the regeneration of the physiological tip. From experience, obtained with scores of experiments, it may be assumed that the growth of segments in water would have had the level of the dotted line. If this assumption be correct, the percentages of inhibition were approximately 50–55% and 40–45%. This means that in the presence of GA the percentage of the inhibition caused by ABA is equal to that caused in a medium containing IAA.

From the combined results reported in this paper the conclusion has been drawn that ABA inhibits both the production of IAA and the reaction of the cells to IAA, and that GA cancels only the former effect.

This interpretation cannot be invalidated by the objection that GA itself might stimulate IAA synthesis since any effect of GA on the regeneration was absent (*fig. 7*), a fact already established earlier with a different technique (ANKER 1967).

4. GENERAL DISCUSSION

The above observations confirm the results of comparable investigations done by other authors. MILBORROW (1966) has found with the oat mesocotyl that GA failed to relieve the inhibition caused by dormin when the medium contained IAA. Wareing et al., experimenting with oat coleoptile sections, could partially overcome the inhibition, caused by ABA, with GA, provided that IAA was absent from the medium. Apparently ABA acts on 2 systems, the first of which is present in the tip of the segments and is responsible for the synthesis of IAA. The second system is present in the rest of the segments and is functioning in the reaction to auxin. Although it was not distinctly stated by these authors, Milborrow was studying the effect of ABA on the second system, whereas Wareing et al. were also dealing with the first one.

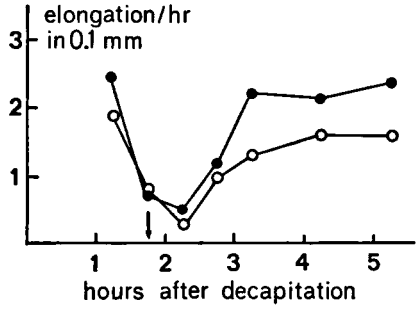

Fig. 8. ABA has a long after-effect. At the arrow the segments were transferred from the ABA solution to water. ●—● growth in water; ○—○ idem in 1 mg/l ABA, subsequently in water.

As the present paper is part of a study on the regeneration of the physiological tip, the discussion will be limited to the first system.

The way of inhibiting the regeneration of the physiological tip by ABA is clearly different from that found with IAA and galactose (ANKER 1973, 1974). The latter substances completely inhibited the auxin synthesis. Apparently their presence in the medium simulates the presence of the natural tip, as in both cases the subapical cells are prevented from producing auxin, and as there is a striking equality between the length of the period between the transfer of the segments from an IAA or a galactose solution to water and the regeneration on the one hand, and the duration of the interval between decapitation and the regeneration on the other hand, in both cases being 2 to 3 hours.

Comparison of the action of ABA with these effects justifies the conclusion that its presence in the medium certainly does not simulate the presence of the natural tip. In the first place because the regeneration was inhibited only partially, and in the second place because not even a small delay of the regeneration was visible. The IAA synthesis was not blocked by ABA, it was only reduced.

Still another characteristic of the effect of ABA, which has not yet been mentioned and which is illustrated with *fig. 8*, was that the transfer of the segments from an ABA solution to water was not followed by a complete recovery of the growth after 2 to 3 hours, as was observed in the case of IAA and galactose. ABA had a long after-effect.

Providing that these effects of ABA and GA are not limited to the *Avena* coleoptile, they underline the importance of the idea, already expressed by other authors, that the regulation of plant processes by hormones can be achieved by a mutual influence on each other's metabolism. Theoretically, this influence can be positive or negative, direct or indirect. The positive influence of GA on the synthesis of IAA, reported in this paper, is an example of an indirect effect, since its action was limited to cancelling the negative effect of ABA on this process.

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