Acta Bot. Neerl. 26(5), October 1977, p. 369–375.

AN ANALYSIS OF KINETIN EFFECTS ON COLEOPTILE GROWTH

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SUMMARY

From quantitative differences of kinetin effects on the growth rate of intact and decapitated coleoptiles under various experimental conditions it is inferred that kinetin itself has no growth-promoting activity, but that it stimulates both auxin action and auxin production, and that it protects the auxin requiring mechanism of cell growth from being damaged by excessive auxin.

1. INTRODUCTION

Kinetin has often been shown to stimulate the growth of coleoptiles and of other organs, but the mechanism of its action is yet unsolved. When added in combination with indoleacetic acid, kinetin increased the response to this auxin (SCHRANK 1958; WRIGHT 1968; HEMBERG 1972; HEMBERG & LARSSON 1972). Other results suggested that kinetin may also increase the production of auxin (JORDAN & SKOOG 1971; HEMBERG 1972; ELKINAY & HEMBERG 1974). It is difficult to demonstrate these two actions separately.

In the present investigation an attempt has been made to demonstrate the second action of kinetin by studying its influence on the regeneration of the physiological tip with decapitated *Avena* coleoptiles. During the regeneration the distal cells develop the capacity to produce auxin, thus enabling the stump to resume the growth.

The observed positive effect of kinetin on the resumed growth could not, however, be attributed with some confidence to increased auxin *production* until information was obtained on the effect of kinetin on the auxin *action*. From quantitative differences of the response to kinetin, obtained in a variety of experimental conditions, the conclusion is finally drawn that besides other effects, increased auxin production is a genuine aspect of kinetin action.

2. MATERIAL AND METHODS

The details of the method have been described in ANKER (1954). The general procedure is that 12 apical coleoptile segments, taken from 88 hours old seedlings, are pushed on pins and submerged in the vertical position in one liter of an aerated solution of the substance the effect of which on the growth is to

be studied. The length of the segments is 19 mm, and in case of decapitation exactly 1 mm is cut off.

The seedlings are cultivated and the experiments are done in weak incandescent light, filtered by red selenium glass. Throughout the last 24 hours of the cultivation period the seedlings are in the dark in order to prevent early break through of the primary leaves, and to delay senescence of the coleoptiles.

During the experimental period (5–6 hours) shadowgraphs are made of the segments at set times with phototropically inactive red light.

The advantage of submerging the segments in solutions of the substances to be tested over supplying them in agar blocks or in paste to coleoptiles in air is that the concentration of the added substance remains practically constant (only 12 segments in 1 liter) which is significant in the case of very low concentrations.

The cultivation of the material and the performance of the experiments are done in a room kept at 23 °C and at a relative humidity of 80–90 %.

3. RESULTS AND DISCUSSION

3.1 Kinetin and tip regeneration

From early work in this laboratory (DOLK 1926; WENT 1928) we know that the resumption of the growth of the stump of the coleoptile, beginning about 2 to 3 hours after the decapitation, is caused by auxin synthesis in the apical cells. In previous investigations it appeared impossible to put forward the growth resumption, neither with sugars nor by addition of precursors of the auxin (IAA) to be synthesised nor by gibberellic acid (ANKER 1973, 1974, 1975 and ANKER, DE BRUYN & WIERCX 1973). The unalterable length of the period between decapitation and regeneration is at least partly determined by the rate of disappearance of the residual auxin from the distal cells of the stump since the presence of IAA, even in very low concentration completely inhibits auxin synthesis in the stump (ANKER 1973).

Even kinetin (*fig. 1*) did not shorten this period. In none of the 12 experiments done to this purpose with concentrations of 0.05, 0.1, 1.0, 2.5, 5.0, and 7.5 mg/l the regeneration was put forward. Kinetin was further unable to stimulate the growth during the period of auxin exhaustion. Not until the growth had been resumed, owing to auxin synthesis, the presence of kinetin became noticeable as a strong stimulation of the growth rate. The increase varied with the concentration (*table 1*). Distinct effects were still observable with the 0.05 and the 0.1 mg/l concentrations. The saturation concentration was about 1 mg/l.

The principal purpose of this investigation was to try to give an answer to the question whether these considerable kinetin effects on the growth are due to an increased auxin action or to an increased auxin production or to both.

The first thing to be done in connection with this question was to compare these growth stimulations with those observed in coleoptiles that have no proper auxin synthesis.

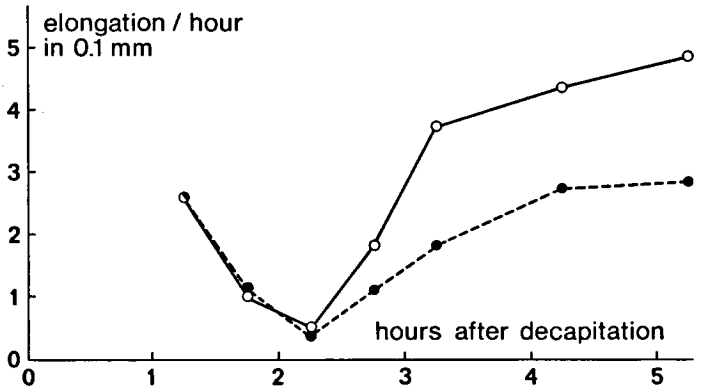

Fig. 1. The effect of 2.5 mg/l kinetin on the growth of decapitated coleoptile segments. ●---● growth in water (controls), o—o growth in the kinetin solution.

Table 1. Growth stimulation by kinetin after the completion of the regeneration of the physiological tip.

kinetin concentration in mg/l	elongation/hour in 0.1 mm		% stimulation
	in water	in kinetin	
0.05	27	32	19
0.1	21	27	29
1.0	21	35	67
2.5	24	43	79
5.0	25	43	72
7.5	27	47	74

3.2 Growth stimulation by kinetin in decapitated coleoptiles submerged in a 0.01 mg/l IAA solution

The IAA concentration of 0.01 mg/l was selected for two reasons, 1) it causes a growth rate equal to that of coleoptiles after the regeneration is completed so that percentages of stimulation may be compared, and 2) because it is sufficient to repress the regeneration of the physiological tip (ANKER 1973).

Comparison of the data from the *tables 1* and *2* demonstrates that the growth regulated by the physiological tip is increased about twice as much as that of coleoptiles submerged in IAA. Since in this region of concentrations the growth rate is proportional to the auxin concentration (see next section) this comparison suggests that the auxin production of the coleoptile segments submerged in water was increased by kinetin. Since the evidence in favour of this suggestion is derived from differences in growth rate, it is impossible to draw quantitative conclusions about the supposed increases of the internal auxin concentration.

It seemed of interest also to examine in this connection the effect of kinetin on the auxin production by the natural tip.

Table 2. Growth stimulation by kinetin of coleoptiles submerged in a 0.01 mg/l IAA solution which prevents proper auxin production.

kinetin concentration in mg/l	elongation/hour in 0.1 mm		% stimulation
	in IAA	in IAA + kinetin	
1.0	25	33	32
2.5	34	41	21
5.0	34	45	32
7.5	22	30	36

3.3 Growth stimulation of intact coleoptiles by kinetin

In previous investigations it was found that the auxin production by the natural tip of the *Avena* coleoptile is not sufficient for a maximum rate of elongation (ANKER 1971). This was confirmed by the present results since added IAA could more than double the growth rate of intact coleoptiles (*fig. 2A*). Both in the presence and in the absence of IAA, kinetin caused a growth excess. Because of the great differences in growth rate between the control coleoptiles in water and those submerged in the IAA solution it is not feasable in this case to express the kinetin effects in percentages of stimulation. The additional growth, due to kinetin treatment, when expressed in 0.1 mm/hour, amounted to 15 in the absence of external IAA, while in the presence of IAA only 8 units were measured on an average. These numbers approximate those found with the decapitated coleoptiles, being 18 and 9 respectively (*tables 1* and *2*). These equalities suggest the possibility that in the natural tip too the auxin production was stimulated by kinetin and that applied IAA also repressed the natural auxin production.

3.4 Kinetin effects on the growth of decapitated coleoptiles regulated by IAA in infra-optimum, optimum and supra-optimum concentrations

The following experiments were designed to examine the nature of the increased auxin action caused by kinetin. Kinetin was added in the saturation concentration of 1.0 mg/l together with IAA in a variety of concentrations ranging from infra-optimum to supra-optimum for the growth (*fig. 2B*).

In the region of the low concentrations the increase of the growth per hour was practically independent of the IAA concentration used. Even at the optimum IAA concentration the stimulation was not significantly different. These results suggest that the increased growth due to kinetin was not accomplished by an increase of the amount of active IAA molecules, for instance by freeing them from the bound state. This is further supported by the fact that kinetin relieved the inhibition of the growth rate at supra-optimum IAA concentrations, rather than causing an even stronger inhibition.

To explain the combined results reported in this section it is suggested that kinetin not only favourably influenced the IAA requiring mechanism of the

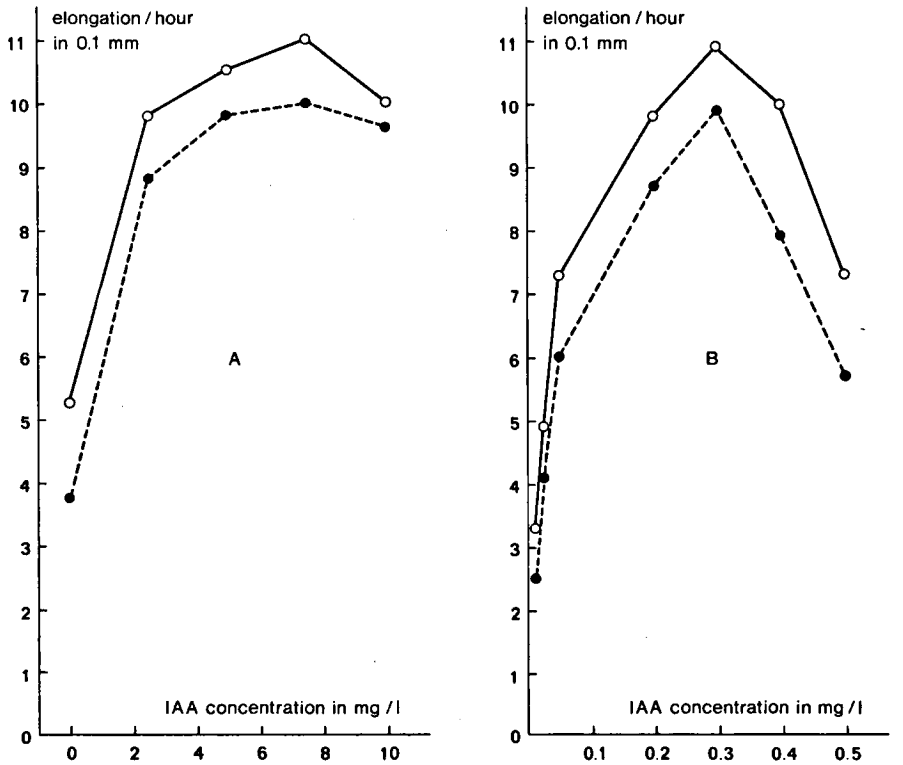

Fig. 2. The effect of 1 mg/l kinetin on the growth at various IAA concentrations. A of intact, B of decapitated coleoptile segments. ●---● in IAA, o——o in IAA plus kinetin.

growing cells but also reduced the damage done by IAA at the supra-optimum concentrations.

4. RECAPITULATIONS AND FURTHER DISCUSSION

The effect of kinetin on the growth of *Avena* coleoptiles has been investigated with experimental material the growth of which was dependent on:

1. the natural tip,
2. the natural tip plus exogenous auxin,
3. the regenerated physiological tip,
4. exogenous auxin in infra-optimum concentration,
5. exogenous auxin in optimum concentration,
6. exogenous auxin in supra-optimum concentration,
7. auxin depletion in the course of the experiments.

In variation (7) where auxin was rapidly used in the first part of the experiments (*fig. 1*), kinetin was not able to influence the reduced growth. This

means that kinetin itself is not a growth promoting substance. Kinetin promotes IAA dependent growth in one way or another.

In all other variations where IAA was present kinetin increased the growth rate but the effects were quantitatively different. The strongest stimulations were found in the variations (1), (3), and (6), the excess effect being ascribed here to an increase of the auxin production (1,3) or to a protection of the protoplasm against the damaging effects of the high IAA concentrations (6).

In the remaining variations where the growth was regulated by exogenous auxin (probably this is also true in (2)) kinetin increased the rate of growth to a degree which seemed independent of the concentration of the applied IAA. In these cases the effects of kinetin are vaguely interpretated as a favourable influence on the auxin requiring mechanism of the cell.

So it appeared that in none of the experimental variations kinetin had a negative effect on the growth rate. This outcome is contrary to part of the results of HEMBERG and LARSSON (1972). They found that 5.4 mg/l kinetin reduced the growth of *Avena* coleoptile sections in 1.75 mg/l IAA in experiments lasting 16–20 hours. This inhibition is explained by the assumption that kinetin caused an increased absorption of the exogenous auxin, the concentration of which was already supra-optimum. A possible cause of this opposite result at the supra-optimum IAA concentrations may be the differences of the IAA and of kinetin concentrations which were higher than in comparable experiments of the present investigation. The main cause presumably, is the great length of their experiments. The long exposure to these unnatural conditions might have damaged the tissues to such an extent that an initially favourable factor becomes inhibitory in the long run.

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