The effects of kinetin and IAA on shoot development in *Funaria hygrometrica* and *Ceratodon purpureus*

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Relatively few studies have been devoted to the effects of growth substances on moss development. The influence of auxins on bud formation in the protonema was studied in *Funaria hygrometrica* (Bopp 1953), of auxins and of adenine derivatives (including kinetin) in *Tortella caespitosa* (Gorton & Eakin 1957) and of auxins, kinetin and gibberellic acid in *Pohlia nutans* (Mittra & Allsopp 1959).

In a preliminary study beginning a series of investigations, *Funaria hygrometrica* and *Ceratodon purpureus* were chosen as suitable material for experiments on gametophyte development in mosses (Szweykowska & Maćkowiak 1961). The present investigation was undertaken to prove the effects of auxin and kinetin on bud initiation and shoot development in these species. Attention has been paid to kinetin-auxin interaction which in recent years has been found to be involved in several morphogenetic processes (Skog & Miller 1957, Gorton & Eakin 1957, Wickson & Thimann 1958).

MATERIAL AND METHODS

Two moss species, *Ceratodon purpureus* (Hedw.) Brid. and *Funaria hygrometrica* Hedw., both proved to grow excellently in pure liquid cultures, were used in the experiments.

They were both collected in summer 1960, *Ceratodon* on Ociemny Wierch in the Pieniny Mountains and *Funaria* in Stepnica at Zalew Szczeciński.

The procedure of culture preparation was similar to that described earlier (Szweykowska & Maćkowiak 1962). The culture tubes were filled with 10 ml of liquid Kofler medium (Kofler 1959) and after inoculation grown in light of two white fluorescent tubes and at a temperature 18—22°C. A parallel experimental set consisted of 6 culture tubes.

In order to eliminate as much as possible the influence of active

substances on the quantitative growth of protonema, these substances were not added until the protonema was well developed. Good protonema growth, with as yet no visible shoot buds, was obtained after five weeks. At this time, 2 ml of various kinetin and indoleacetic acid (IAA) solutions were added to the cultures (2 ml of H₂O to control cultures) so as to obtain following combinations: Kinetin at 0.1, 1.0 and 10.0 mg/l; kinetin at the same concentrations with 1.0 mg/l IAA; IAA at 0.1, 1.0 and 10 mg/l; IAA at the same concentrations with 1.0 mg/l kinetin; control with no active substances added.

After five weeks of subsequent growth, the cultures were examined for their gametophore development.

RESULTS

A. *Ceratodon purpureus*

No gametophores were formed in control cultures of *Ceratodon*. Kinetin at 0.1 mg/l induced formation of gametophore buds in the protonema and well developed leafy shoots were present in the cultures. Buds and shoots were more abundant in kinetin at 1.0 mg/l. In kinetin at 10.0 mg/l, very large numbers of buds appeared but their subsequent development into shoots was inhibited to some extent — the shoots were less numerous and smaller.

The addition of 1.0 mg/l IAA to 0.1 mg/l of kinetin resulted in greatly increased bud and shoot number. In 1.0 mg/l kinetin + IAA, the grown shoots were less numerous, pale green and deprived of leaves. No remarkable changes were induced by IAA when added to kinetin at 10.0 mg/l.

IAA alone induced bud formation in all three concentrations used. However, the number of buds was lower than that formed under the influence of kinetin. The buds developed into shoots with some striking

Plate I

Fig. 1. *Ceratodon purpureus*. A young shoot in a medium containing IAA at 1.0 mg/l. (× 300)

Fig. 2. *Ceratodon purpureus* in IAA (1.0 mg/l) medium. A grown leafless shoot with many protonema filaments growing out of it. (× 60)

Fig. 3. *Funaria hygrometrica* in IAA (0.1 mg/l) medium. A shoot-bud with large, elongated cells. (× 300)

Fig. 4. *Funaria hygrometrica* in IAA medium (1.0 mg/l). A young shoot with no leaves formed and filaments growing out of the stem. (× 60)

Plate I

morphological features. They were nearly deprived of leaves in 0.1 mg/l conc. and completely leafless in 1.0 and 10.0 mg/l concentrations. In these two higher IAA concentrations, the plants consisted of pale stems, often with protonema filaments growing out of them (Fig. 1—2).

Kinetin at a constant 1.0 mg/l level added to various IAA concentrations resulted in much more intensive bud formation but the grown shoots were not affected, i.e. they were more or less deprived of leaves.

B. *Funaria hygrometrica*

Funaria formed leafy shoots abundantly in control cultures. Kinetin at 0.1 mg/l stimulated bud formation and leafy shoots were also present.

At 1.0 and 10.0 mg/l concs., masses of buds were formed but their development into leafy shoots was completely inhibited.

When IAA was added to 0.1 mg/l of kinetin, the number of buds was greater but their subsequent growth was inhibited and no grown shoots were present. The cultures were pale, with little chlorophyll inside the cells. No modifications of kinetin effects were induced by IAA when added to the two higher, 1.0 and 10 mg/l kinetin concentrations.

IAA supplied alone decreased the number of buds in *Funaria* cultures in which, as was already pointed out, buds were easily formed in the absence of any active substances. In 0.1 mg/l IAA, gametophores were present in small numbers, the leaves were poorly developed or absent, numerous protonema filaments grew out of the gametophore stems, the cells were poor in chlorophyll. The cells in the buds were much elongated, which is an effect opposite to that of kinetin under influence of which the cells expand isodiametrically and the buds have a moruloid appearance (Fig. 3). In 1.0 mg/l IAA, only few gametophores formed, with protonema filaments growing out of leafless and colourless stems (Fig. 4). The cultures supplied with IAA at 10.0 mg/l consisted of poor, degenerating, colourless protonema with no buds formed.

Kinetin at a 1.0 mg/l level added to 0.1 and 1.0 mg/l IAA increased greatly the number of buds but inhibited the development of shoots. In 10.0 mg/l IAA + kinetin, the cultures were pale green but some disorganized buds were formed.

DISCUSSION

In respect to moss morphogenesis, kinetin was found by Gorton & Eakin (1957) and Mitra & Allsopp (1959) to be a substance stimulating bud formation and inhibiting further development of buds

into leafy shoots. The results of the present investigation have shown that the inhibiting effect of kinetin on bud development depends upon concentration. Kinetin at 0.1 mg/l stimulated bud formation in *Funaria* and at 0.1 and 1.0 mg/l induced it in *Ceratodon* without any inhibition of leafy shoots formation. However, the inhibitory effect was distinct at higher concentrations, i.e. at 1.0 and 10.0 mg/l in *Funaria* and at 10.0 mg/l in *Ceratodon*.

According to Bopp (1953) and Gorton & Eakin, auxin at lower concentrations stimulates and at higher ones inhibits bud initiation. Mitra & Allsopp using rather high concentrations, 0.1 — 10.0 mg/l, did not find any stimulatory effects of auxin on bud formation. Evidently, similarly as in many other processes, the auxin effect highly depends upon concentration used. In the present experiments, the concentration range 0.1 — 10.0 mg/l of IAA inhibited bud formation in *Funaria*. It was, however, striking that all IAA concentrations used induced bud formation in *Ceratodon purpureus*. This is a further interesting feature of *Ceratodon purpureus* (Szweykowska & Maćkowiak 1962, Szweykowska 1962) because such high auxin concentrations, as e.g. 10.0 mg/l, were never found to promote bud formation.

Gorton & Eakin and Mitra & Allsopp found an inhibiting effect of auxin on the development of buds into leafy shoots. It was not confirmed in the present investigation for both *Funaria* and *Ceratodon*. There was no inhibition of bud development in auxin media. However, the gametophores grew in an abnormal manner, resulting in shoots with deficient leaf development, often consisting of a leafless stem only.

The elongating effect of auxins on protonema cells has been pointed out by Gorton & Eakin. A similar effect of IAA on the bud cells of *Funaria* was found in the present investigation. Chlorosis observations in moss cells under the influence of auxin is in agreement with those of previous authors.

Since Skoog and Miller experiments on the influence of kinetin and IAA on the organogenesis in tobacco callus culture, interest has been paid to kinetin-IAA interaction in various physiological processes (Gorton & Eakin 1957, Wickson & Thimann 1958). Gorton & Eakin found that kinetin can reverse the inhibition of bud formation by auxin and suggested a competitive character of this reversal comparing the effects of adenine and adenine derivatives to those of anti-auxins. Skoog & Miller (1957) rather precluded a simple competitive interaction of IAA and kinetin and pointed out that both substances are required for regulation of organ formation. Some results obtained with *Funaria* are similar to those of Gorton

& Eakin established for *Tortella*, i.e. the IAA inhibition of bud formation could be overcome by a kinetin treatment. However, in some instances, the kinetin effect was increased by the addition of IAA, e.g. the effect of 0.1 mg/l kinetin + 1 mg/l IAA was similar to that of 1.0 mg/l kinetin alone in both *Funaria* and *Ceratodon*. This is evidence of a cooperation rather than competition. Secondly, the bud inducing effect of a wide concentration range of IAA in *Ceratodon* supports views of Skoog and Miller that both kinetin and IAA are required for organ formation.

SUMMARY

The effects of various concentrations of IAA and kinetin, supplied separately and together, on bud initiation and shoot development in the protonema were studied in two moss species, *Ceratodon purpureus* and *Funaria hygrometrica*. The growth substances were added to well developed protonemas in pure liquid cultures, at a time when the gametophore buds had not appeared yet.

Kinetin stimulated strongly bud initiation and at higher concentrations inhibited bud development into leafy shoots. IAA inhibited bud initiation in *Funaria* and induced it in *Ceratodon*. Morphological abnormalities were induced in gametophores grown in IAA media: the shoots grew well in length but were more or less deprived of leaves and consisted of pale green stems with protonema filaments growing out of them.

In most IAA + kinetin combinations, IAA increased the effect of kinetin which is evidence of a cooperation rather than competition between the two growth substances.

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(Entered: 30.4.1962)

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