MODE OF ACTION OF MORPHACTIN IN EPIPHYLLOUS BUDS OF *BRYOPHYLLUM TUBIFLORUM*

K. K. NANDA, A. N. PUROHIT and ARUN PRABHA

Panjab University Botany Department, Chandigarh-14, India

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SUMMARY

This paper deals with the effect of morphactin (IT 3233), IAA, kinetin, GA_3 and thymidine singly and in combination with each other on internodal elongation and root growth of excised epiphyllous buds of *Bryophyllum tubiflorum* under light and dark conditions.

Morphactin and kinetin, alone or together, inhibited internodal as well as root growth, the effect being more marked in light than in dark.

GA_3 reversed morphactin induced inhibition of internodal elongation but not of root growth, suggesting that morphactin and GA_3 effects on internodal and root growth are not mediated through common sites of action. Morphactin effect is neither mediated through auxin metabolism nor through lack of thymidine incorporation in DNA synthesis as both IAA and thymidine failed to reverse the inhibition caused by it. It is proposed that morphactin may cause either loss of sensitivity of plant tissue or may enter into a complex with endogenous gibberellin-like substances resulting in inhibition of growth.

INTRODUCTION

Morphactins, derivatives of fluorene-9-carboxylic acid, retard plant growth, break apical dominance (Mann *et al.*, 1966; Ziegler *et al.*, 1966), and disturb phototropism of shoot and geotropism of root (Khan, 1967). Schott and Schraudolf (1967) showed that morphactins and kinetin are similar in their effects on the regeneration of *Begonia* leaf-discs. Ziegler *et al.* (1966) found that GA_3 overcame the inhibitory effect of morphactins and concluded that they are gibberellin antagonists. Wickson and Thimann (1958) and Sachs and Thimann (1967) demonstrated that apical dominance is controlled by a balance between cytokinins and auxins. According to Wain (1958) auxin-like properties of fluorene-9-carboxylic acid are suggestive

that morphactin effect may be mediated through auxin metabolism within the plant. The preceding survey reveals that the role of morphactin in plant metabolism is least understood. Therefore, it was considered of interest to study the effect of morphactin, singly and in combination with GA_3, auxin, kinetin and thymidine under varying light conditions on the excised epiphyllous buds of *Bryophyllum* with a view to elucidating the mechanism of its action. The results of this investigation are presented in this paper.

Materials and Methods

Epiphyllous buds of *Bryophyllum tubiflorum* collected from plants grown under continuous illumination (LD) were floated on 5 ml of test solutions in Petri-dishes (5 cm dia.). These were divided into two groups. While one group was exposed to continuous light (CL) supplied from an incandescent bulb at an intensity of 1,000 lux, the other was kept in the dark (CD). After 24 hours, the buds were transferred to distilled water, 30 ml in each Petri-dish and were continued under the same light condition subsequently at a temperature of $31 \pm 1°C$. The details of the nature and concentration of test solutions that were used are given separately in each experiment.

Observations on the length of the first internode and the number and length of roots produced were recorded on 10 buds sampled at random after five days.

Results

Experiment 1

This experiment was designed to study the effect of different concentrations of morphactin, 2-butyl-9-hydroxy fluorene-9 carboxylate (IT 3233), on the elongation of first internode and number and length of roots. The results together with the concentrations of morphactin used are summarized in Table I.

Morphactin inhibited the length of first internode under both light conditions, the inhibitory effect increasing with concentration and being more marked in light than in dark. Morphactin also inhibited the number and length of roots, the effect again being more marked in light (Table I).

Experiment 2

This experiment was conducted to study the effect of morphactin singly and in combination with IAA, GA_3 and kinetin. The results in Table II show that both morphactin

Table I. Effect of morphactin (IT 3233) on the length of first internode and root, and the number of roots produced on epiphyllous buds of B. tubiflorum *under continuous light (CL) and continuous darkness (CD) observed five days after the treatment. Initial internodal length: 0·5±0·1 mm. Plus and minus values show the standard deviations of the mean of 10 replications*

Morphactin concentration mg/L	Length of internodes (mm)		No. of roots per bud		Length of root (mm)	
	CL	CD	CL	CD	CL	CD
0	3·0±0·30	9·3±0·40	2·2±0·20	3·6±0·20	7·5±0·70	9·0±0·50
0·1	1·1±0·06	7·4±0·20	1·9±0·10	3·1±0·10	6·1±0·40	9·5±0·30
1·0	1·0±0·00	5·1±0·40	1·7±0·20	2·6±0·20	5·8±0·50	6·0±0·40
10·0	1·0±0·00	4·9±0·20	1·5±0·20	2·4±0·20	3·8±0·80	6·7±0·30

Table II. Effect of 10 mg/L of morphactin (IT 3233), GA_3, IAA and kinetin (K) separately and in combination on the length of the first internode, and the number and the length of roots produced on epiphyllous buds of B. tubiflorum *under continuous light (CL) and continuous darkness (CD) observed five days after the treatment. Initial internodal length: 0·5±0·1 mm. Int.=Root initials*

Treatment	Length of internode (mm)		No. of roots per bud		Length of root (mm)	
	CL	CD	CL	CD	CL	CD
Control (Water)	2·4±0·10	6·0±0·30	2·3±0·10	3·2±0·20	9·3±0·50	8·0±0·50
Morphactin	1·0±0·00	2·7±0·10	2·1±0·10	1·9±0·10	5·4±0·20	5·0±0·50
GA_3	5·5±0·10	8·6±0·20	2·3±0·10	2·0±0·10	10·1±0·40	10·7±0·80
IAA	2·9±0·06	6·1±0·20	3·1±0·30	3·1±0·16	9·1±0·60	9·4±0·50
K	1·0±0·10	0·9±0·03	0·2±0·10	0	Int.	—
Morphactin + GA_3	3·4±0·10	4·0±0·20	1·9±0·10	2·2±0·10	4·7±0·30	5·4±0·40
Morphactin + IAA	1·1±0·10	3·1±0·06	2·0±0·00	1·9±0·16	3·7±0·30	5·5±0·40
Morphactin + K	0·7±0·06	1·2±0·06	1·1±0·30	1·6±0·20	0·2±0·01	0·5±0·10
K + GA_3	1·5±0·03	3·2±0·10	0	0	—	—
K + IAA	1·0±0·00	1·3±0·10	0	0	—	—

Table III. Effect of 10 mg/L of morphactin, GA_3, thymidine (Th.) and 5-FDU separately and in combination on the length of first internode and number and length of roots produced on epiphyllous buds of B. tubiflorum *under continuous light (CL) and continuous darkness (CD) observed five days after the treatment. Initial length of internode: 0·5±0·1 mm. Int.=Root initials*

Treatment	Length of internode (mm)		No. of roots per bud		Length of root (mm)	
	CL	CD	CL	CD	CL	CD
Control (Water)	2·2±0·10	5·8±0·20	3·2±0·10	3·3±0·30	7·8 ±0·50	11·7 ±0·40
Morphactin	1·0±0·03	3·5±0·10	2·0±0·10	2·3±0·20	6·2 ±0·30	6·5 ±0·20
GA_3	6·0±0·20	7·0±0·10	2·0±0·10	2·4±0·20	11·3 ±0·50	12·6 ±0·60
Th.	2·8±0·10	5·5±0·20	2·8±0·20	3·0±0·20	10·0 ±0·40	11·5 ±0·30
5-FDU	0·5±0·00	1·0±0·00	2·1±0·10	1·3±0·20	0·5 ±0·10	0·7 ±0·20
Morph. + GA_3	3·1±0·10	4·2±0·10	2·2±0·10	2·2±0·20	5·1 ±0·30	9·3 ±0·40
Morph. + Th.	1·0±0·00	2·7±0·10	2·1±0·16	2·2±0·10	5·5 ±0·40	7·0 ±0·40
Morph. + 5-FDU	0·5±0·00	1·0±0·00	0·7±0·20	1·3±0·30	Int.	0·5 ±0·10
GA_3 + Th.	6·3±0·20	8·3±0·20	2·5±0·16	2·6±0·10	10·4 ±0·50	13·0 ±0·60
GA_3 + 5-FDU	0·5±0·00	0·8±0·06	0·7±0·10	1·3±0·30	0·20±0·10	0·60±0·20
Th. + 5-FDU	2·0±0·03	6·4±0·40	2·6±0·20	2·5±0·20	8·7 ±0·60	9·7 ±0·40
Morph. + Th. + GA_3	3·2±0·10	3·2±0·10	1·6±0·20	2·0±0·00	6·2 ±0·70	7·3 ±0·20
GA_3 + Th. + 5-FDU	5·2±0·30	5·0±0·20	2·0±0·20	2·4±0·10	9·9 ±0·60	11·0 ±0·50

and kinetin, used alone, reduced the length of the first internode as well as the number and length of roots, the effect being more marked with the latter. GA_3 stimulated both internodal and root elongation but did not affect the root number in light and reduced it in dark. The effect of IAA was not marked although it caused slight stimulation of internodal elongation and number of roots produced in light and root length in dark.

GA_3 reversed the effect of morphactin on internodal elongation but not that on the number and length of roots. However, internodal length in morphactin + GA_3 was less than that in GA_3 alone. It is interesting to note that while GA_3 tended to reverse to some extent the inhibitory effect of kinetin on internodal elongation, it caused more inhibition of root number and their elongation. IAA did not reverse the inhibitory effect of either morphactin or kinetin. The inhibition caused by morphactin increased when it was used in combination with kinetin.

Experiment 3

This experiment was conducted to study the effect of morphactin singly and in combination with GA_3, thymidine and 5-FDU (5-fluorodeoxyuridine).

Table III shows that morphactin and 5-FDU singly or in combination with each other, inhibited internodal and root elongation as well as the number of roots. GA_3 stimulated internodal and root elongation under both light conditions, while thymidine stimulated it only in light. The effect of thymidine with GA_3 on internodal elongation was additive. It is interesting to note that while thymidine reversed more or less completely the inhibitory effect of 5-FDU regardless of the presence of GA_3, it did not reverse the inhibitory effect of morphactin. On the other hand GA_3 that reversed the morphactin induced inhibition failed to reverse the inhibition caused by 5-FDU.

These experiments were repeated three times and the trends of results were similar.

Discussion

The results presented in this paper demonstrate that both kinetin and morphactin inhibit internodal elongation as well as root growth. Therefore, the two regulators closely resemble in their effect on epiphyllous buds of *Bryophyllum tubiflorum*. Resemblance in the effectiveness of these two regulators has also been shown earlier in the regeneration of *Begonia* leaf-discs (Schott and Schraudolf, 1967).

GA_3 reversed the internodal inhibition regardless of whether it was caused by kinetin or morphactin. It is, however, interesting to note that the reversal effect of GA_3 on morphactin-caused inhibition was more pronounced in light than in dark, while that of kinetin was more in dark than in light. Both morphactin and kinetin may, therefore, be considered as GA_3 antagonists. The reversal of morphactin induced inhibition of internodal elongation has also been reported by Mann *et al.* (1966) in *Citrus* and by Ziegler *et al.* (1966) in dwarf pea seedlings.

An interesting point that emerges from this investigation is that while GA_3 reversed the inhibition of internodal elongation, it failed to reverse the inhibition of root growth regardless of whether it was caused by kinetin or morphactin. Khan (1967) also showed that GA_3 overcomes the inhibition of internodal elongation but it does not reverse the morphactin effect on photo- and geo-tropism. Tognoni *et al.* (1967) also showed that GA_3 failed to counteract the inhibitory effect of morphactin on dwarf-peas and dwarf-corn. All these results suggest that GA_3 and morphactin effects on internodal elongation and root growth are probably mediated through different sites of action.

Ziegler *et al.* (1966) showed that morphactins neither inhibited the biosynthesis nor destroyed or inactivated endogenous gibberellin. These also did not block the physiological response (amylase activity) mediated by GA_3 (Tognoni *et al.*, 1967) and, therefore, considered that morphactin effect may be mediated through their influence on auxin metabolism within the plant resulting in impairment of normal hormonal control. Our results do not substantiate this postulate because if it were so the exogenously applied IAA in Experiment 2 (Table II) should have overcome this impairment of hormonal control and, therefore, should have caused reversal of morphactin caused inhibition. As this does not happen, it may not be unreasonable to conclude that morphactin effect is not mediated through its effect on auxin metabolism in the plants.

The competitive action of morphactin and GA_3 on internodal elongation may, therefore, be explained by assuming that either (i) morphactins form a complex with endogenous gibberellin-like substances thereby inactivating them; or (ii) these reduce the sensitivity of tissues to endogenous gibberellin; or (iii) these cause inhibition of DNA synthesis by preventing the incorporation of thymidine that is stimulated by GA_3 (Nitsan and Lang, 1966). The results of experiment 3 (Table III) show that while thymidine reversed the 5-FDU induced inhibition it failed to reverse the morphactin effect. The possibility of morphactin effect due to lack of thymidine incorporation is,

therefore, ruled out. The more pronounced inhibition of internodal elongation by morphactin in light is suggestive that its effect may be a consequence of reduced sensitivity of tissues to endogenous gibberellin-like substances.

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