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Dormancy Regulation by Morphactin in Aerial Tubers of *Begonia evansiana*

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Summary. The sprouting of aerial tubers of *Begonia evansiana* was promoted by treatment with morphactin. As with cytokinins, the promotion of sprouting occurred in both the immature and mature tubers. Unlike cytokinins, however, morphactin did not stimulate tuber enlargement. The sprout-inhibiting action of applied gibberellin (GA) was overcome by morphactin. The possible mechanism of the inhibitory action of GA is discussed in relation to apical dominance.

Introduction

Although morphactins, fluorene-9-carboxylic acid and its derivatives, are well known to influence plant growth (see Schneider, 1970), their mode of action remains quite obscure. However, morphactin, like cytokinins, enhances the growth of lateral buds by braeking apical dominance (Tognoni *et al.*, 1967; Nanda *et al.*, 1970), and induces the regeneration of adventitious buds (Schott and Schraudolf, 1967).

We have suggested that apical dominance may be involved partially in the regulation of dormancy of aerial tubers in *Begonia evansiana*, based on the following results (Esashi *et al.*, 1971): kinetin (Kin) and benzyladenine (BA) stimulate photo-sprouting of immature tubers and cause sprouting of mature tubers. The endogenous level of cytokinin in the tubers increases with prolonged chilling, resulting in complete release of their dormancy. During tuber development, the content of indole-3-acetic-acid (IAA) rose, generally enhancing apical dominance; conversely, chilling decreased the IAA content.

In addition, the dormancy of aerial tubers of this plant is known to be induced and prolonged by applied GA (Nagao and Mitsui, 1959; Okagami and Nagao, unpubl.). Nagao and Okagami (1966) found that CCC (cycocel), an inhibitor of GA biosynthesis (see Lang, 1970), prevents the tuber from entering a dormant state, thus suggesting a possible role of endogenous GAs in controlling tuber dormancy in this plant. Similar effects of GA and CCC in dormancy regulation are found in the bulbils of some species of the genus *Dioscorea* (Okagami and Nagao, 1971).

These facts suggest that, if morphactin is able to overcome the action of GA in some manner, it may prevent immature tubers from entering into the dormant state, and may cause the sprouting of mature tubers even in the prescence of applied GA.

Materials and Methods

Immature and mature aerial tubers of *Begonia evansiana* Andr. were used as experimental material. Immature tubers at tuber stage 5 to 6 (Esashi, 1960) were obtained from plants which had been under short days in the experimental field from late September to early Octover, or in a greenhouse. Mature, half-dormant tubers, which had been subjected to a short chilling under natural conditions, were harvested from the same field in late November. 60–70 immature tubers or 80–90 mature tubers per treatment, selected for uniformity, were placed on a thin layer of absorbent cotton moistened with distilled water or various reagent solutions, in a Petri dish of 6 or 9 cm diameter. White light was supplied from fluorescent tubes of the natural-daylight type at an intensity of 1000 lux. After treatment, the tubers were washed with water several times and were then incubated for sprouting at 25° C. Morphactin (Methyl-2-chloro-9-hydroxyfluorene-(9)-carboxylate) was supplied by E. Merck Co. (Japan).

Results

Effect of Morphactin on the Sprouting of Immature Tubers. In the experiment shown in Fig. 1, the naturally harvested immature aerial tubers were pre-treated with morphactin solution (1×10^{-4}M) or water as control under continuous illumination at 15° or 25° C for 20 days, and were then kept under the same light at 25°. At 25°, some of morphactin-treated tubers began to sprout within 20 days (*cf.* Esashi, 1969), and

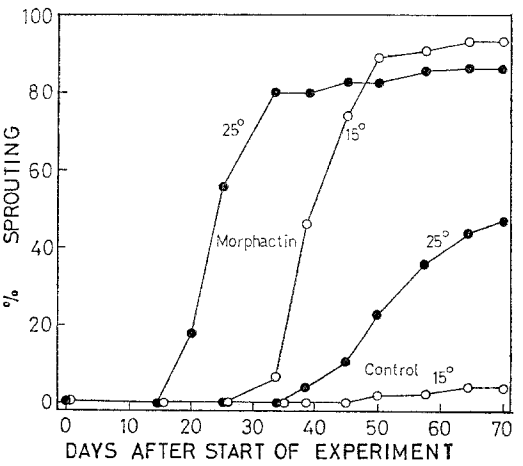

Fig. 1. Photo-sprouting of immature aerial tubers with 1×10^{-4} M morphactin at 15° or 25° C from 0 to 20 days, and then washed and transferred to, or maintained at, 25° C

Table 1. Effects of morphactin, at 5° and 25° C, on the sprouting of mature half-dormant aerial tubers

Temperature (°C)	Sprouting (%)		
	Morphactin concentration (M)		
	0	3×10^{-4}	1×10^{-3}
5	44.1	55.5	51.3
25	11.4	77.2	66.6

Half-dormant mature tubers were treated with the various concentrations of morphactin at 5° C or 25° C for 30 days, and were then washed and kept at 25° C for 52 days.

Table 2. Effects of morphactin, Kin, BA and GA_3 on the photo-sprouting and enlargement of immature tubers

Treatment		Condition	Sprouting (%)	Tuber enlargement (%)
Control		Dark	11.3	100
		Light	46.4	100
Morphactin	3×10^{-5} M	Dark	50.3	99.1
		Light	74.3	98.3
	1×10^{-4} M	Dark	62.3	97.5
		Light	87.9	90.5
Kin	1×10^{-4} M	Dark	47.6	107.5
		Light	68.8	115.8
BA	3×10^{-5} M	Dark	74.2	146.6
		Light	82.7	161.0
	1×10^{-4} M	Dark	58.3	159.3
		Light	98.0	179.7
GA_3	1×10^{-4} M	Dark	0	102.6
		Light	0	99.4

Immature aerial tubers were treated by each reagent under the light or in the dark at 25° C for 20 days. Tuber enlargement was determined as increment in fresh weight (in percent). For the observation of photo-sprouting, the tubers were incubated in the light for an additional 42 days.

their photo-sprouting percentage reached about 80% before the onset of photo-sprouting of controls tubers. Such a promotive effect of morphactin was found in the tubers treated initially at 15° C.

Effect of Morphactin on the Sprouting of Mature, Half-Dormant Tubers. In the experiment shown in Table 1, mature, half-dormant tubers were

Table 3. Interaction of morphactin and GA_3 in dormancy regulation of mature, half-dormant aerial tubers

GA_3	Sprouting (%)		
	Morphactin		
	0	1×10^{-4} M	3×10^{-4} M
0	28.5	68.4	74.4
1×10^{-4} M	14.4	47.8	73.2

pre-treated with water or with 1×10^{-4} and 3×10^{-4}M of morphactin at either 5° or 25° C in the dark for 30 days. They were then kept at 25° for 52 days in the dark. Morphactin very effectively increased the sprouting of mature tubers at high temperature. Much less effect was seen at a temperature (5° C) favorable for release from dormancy. However, the sprouts from morphactin-treated tubers did not elongate. Interestingly, there was no protrusion of roots from tubers pre-treated with morphactin.

Effects of Morphactin, Kin, BA and GA on the Sprouting and Enlargement of Immature Tubers. When immature tubers were treated with various growth regulators for 20 days at 25° C in the light or in the dark, BA and Kin brought about both photo-sprouting and tuber enlargement, while morphactin promoted photo-sprouting but was somewhat inhibitory to tuber enlargement (Table 2). GA completely suppressed photo-sprouting of immature tubers but did not influence tuber enlargement (Table 2).

Effect of Morphactin on the Sprouting of Mature, Half-Dormant Tubers Pre-Treated with GA. Mature, half-dormant tubers were immersed in water or a 1×10^{-4}M GA_3 solution for 30 min. They were then washed with water, incubated in water or 1×10^{-4} or 3×10^{-4}M morphactin at 25° C for 25 days, and assayed for sprouting after incubation for an additional 42 days at 25°. As can be seen in Table 3, GA_3-induced inhibition was almost completely removed by high concentration of morphactin applied subsequently.

Discussion

Sankhla and Sankhla (1967) reported that morphactin inhibits lettuce-seed germination. In the aerial tubers of *B. evansiana*, however, morphactin caused their sprouting independent of their physiological state (Table 1, Fig. 1). That is, morphactin not only blocked the onset of dormancy in immature aerial tubers at 15° C, a temperature favorable for entrance into dormancy (Esashi, 1969), but hastened the release from dormancy of mature tubers even at 25° C, a temperature favorable for retention of the

dormant state (Esashi, 1969). In Table 3, moreover, the inhibitory action of GA_3 on the sprouting was completely removed by 3×10^{-4}M morphactin. These results are consistent with the possibility that morphactin brings about sprouting in this plant by antagonizing in some manner the action of GAs. Ziegler *et al.* (1966) concluded that morphactin is a competitive GA antagonist on the basis that morphactin reduced growth stimulation by GA in CCC-treated pea seedlings.

As shown in Table 2, morphactin promoted, while GA inhibited, the photo-sprouting of immature aerial tubers; however, morphactin also inhibited tuber enlargement, which was not affected by applied GA_3. This supports the view that, at least with regard to tuber enlargement, morphactin has a mode of action independent from GA (Mann *et al.*, 1966; Schott and Schraudorf, 1967; Krelle and Libbert, 1967; Tognoni *et al.*, 1967; Nanda *et al.*, 1970).

According to Cho (1970), GA_3, in the presence of IAA, inhibited the growth of aseptically cultured apical buds isolated from aerial tubers of *B. evansiana*, regardless of their developmental stage. Similary, growth of lateral buds in pea seedlings is inhibited by GAs applied together with IAA (Scott *et al.*, 1967). As previoulsy shown (Esashi *et al.*, 1971), the dormant aerial tubers of *B. evansiana* contain IAA. Cytokinins, known to reduce apical dominance, cause them to sprout (Table 2), in addition to stimulating tuber enlargement (Esashi and Leopold, 1968). Like cytokinins (Esashi *et al.*, 1971), morphactin causes the sprouting even of mature tubers (Table 1), suggesting that morphactin, too, may act on the regulation of dormancy through a mechanism involved in the regulation of apical dominance. That mechanism presumably differs from the mechanism by which cytokinins act, because of the lack of effect of morphactins on tuber enlargement (Table 2). Thus, one possible action of endogenous GAs in the regulation of dormancy in *Begonia* tubers may be to inactivate the apical meristem of the tuberous buds just as, in the control of lateral bud growth, GAs act together with auxin on the maintenance of apical dominance. However, the dormancy of *Begonia* tubers is not bloken completely by morphactin since sprouts from morphactin-treated tubers do not elongate.

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