Modifications in regeneration of *Saintpaulia ionantha* Wendl. leaves, due to morphactin added to in vitro cultures

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Abstract

The paper reports the results of experiments on the course of leaf regeneration in *Saintpaulia ionantha* Wendl. in vitro culture, depending on morphactin IT 3456 doses and its relation with kinetin and auxins (IAA and NAA). Morphactin retards regeneration, abolishes its polarity, inhibits root formation and causes developmental anomalies of buds and leaves. Kinetin and IAA neutralize the retardation of regeneration caused by morphactin, auxin and kinetin do not prevent the anomalies.

INTRODUCTION

The results of up to date studies on the course of regeneration of leaves of *Saintpaulia ionantha* Wendl. cultured in vitro in dependence on the action of exogenous auxins and kinetin, and their interaction (Kukulczanka and Suszyńska, 1972) encouraged further investigations on the influence of morphactin on the course of this process. The experiments were planned with the hope of obtaining a distinct morphogenetic reaction since the effect of this growth regulator on meristematic tissue of *Cymbidium* Sw. was already known (Kukulczanka and Twarda 1973). This plant, like *Saintpaulia ionantha*, exhibits a high regeneration potential. The question remained open whether these reactions would be specific for the species investigated and whether they could be controlled by an appropriate interaction between morphactin, auxins and kinetin. It was attempted to find the solution of this problem by planning adequate experimental combinations.

MATERIAL AND METHODS

The experiments with leaves of *Saintpaulia ionantha* Wendl. var. *alba* (family *Gesneriaceae*) were performed *in vitro*. The methods, technique, plant material and culture conditions were the same as those described in the preceding paper (Kukulczanka and Suszyńska, 1972). The medium of Murashige and Skoog (1962) pH 5.2 to 5.8 was used. In this medium (MR) the doses of morphactin IT 3456 were varied and synthetic auxins were added in the form of α -naphthalene-acetic acid (NAA) or β -indolyl-acetic acid (IAA) as well as kinetin ($C_{10}H_9N_5O$).

Experiment A included three replications at various dates (19.8.1971, 24.8.1971, 25.8.1971) in two groups, MR and MR with 1 ppm kinetin and 1 ppm IAA added. In both groups morphactin IT 3456 doses were varied: 0.01, 0.1 and 1.0 ppm.

Experiment B included four replications at various dates (16.2.1972, 18.2.1972, 29.2.1972 and 8.3.1972) also in two groups: MR and MR with 1 ppm morphactin IT 3456. Moreover, in both series 1 ppm kinetin, 1 ppm IAA and 1 p.p.m NAA were added.

In each replication each combination was represented by 6—15 test tubes with regenerating leaves.

Thirty, 40, 50, 60 and 90 days after inoculation the leaves were inspected to note their growth and regeneration of the adventitious buds. Anatomical slides were prepared. Free-hand tissue sections were digested for 10 min in Javelle water, stained with hematoxylin and embedded in glycerogelatin (Filutowicz and Kuźdowicz, 1951).

RESULTS

The growth and development of *Saintpaulia ionantha* leaves on MR medium occurred similarly as described in the previous paper (Kukulczanka and Suszyńska, 1972). On medium without growth regulators added some of the leaves began to develop adventitious buds

Fig. 1. Influence of morphactin IT 3456 on leaf regeneration in *Saintpaulia ionantha* (experiment A, photographs after two months of leaf growth)
 a — control medium MR; b — MR + 0.01 ppm IT 3456; c — MR + 0.1 ppm IT 3456; d — MR + 1.0 ppm IT 3456

Table 1

Saintpaulia ionantha leaf regeneration delayed by morphactin IT 3456

Combination of medium	% of leaves with adventitious buds			
	Observations after			
	30 days	40 days	50 days	60 days
MR	25	30	75	90
MR+0.01 ppm IT 3456	11	44	66	88
MR+0.1 ppm IT 3456	7	28	54	68
MR+1.0 ppm IT 3456	0	25	71	100
MR+1.0 ppm kin.+1.0 ppm IAA	84	90	100	100
MR+1.0 ppm kin.+1.0 ppm IAA+ +0.01 ppm IT 3456	73	78	94	94
MR+1.0 ppm kin.+1.0 ppm IAA+ +1.0 ppm IT 3456	33	66	80	100
MR+1.0 ppm kin.+1.0 ppm IAA+ +1.0 ppm IT 3456	30	77	100	100

(Table 1). After a further 10 days buds had formed on most of the growing leaves. The first buds formed at the base of the blade and then further along the main rib, always on the upper side of the leaf. Five to seven weeks after inoculation of the leaf the buds developed into leaves forming rosettes (Fig. 1a). The young leaves in the rosettes had a normal shape and were dark green, covered with hairs and their anatomical structure was normal. On the lower side of the leaf blade fine roots appeared.

Regeneration of *Saintpaulia* leaves on MR medium with the addition of 0.01—1.0 ppm morphactin has a somewhat different course. It is delayed (Table 1) the more the higher the concentration of morphactin. In time, after 50—60 days of culture the number of leaves on which adventitious buds differentiated on medium with morphactin came close to that in the combination without this admixture.

The regeneration process occurred quickest in leaves growing on MR medium with 1 ppm kinetin and 1 ppm IAA added. The admixture of these growth substances partly neutralized the retardation caused by morphactin (Table 1).

Kinetin and IAA addition favoured growth of inocultted *Saintpaulia* leaves, reduced the percentage of necroses to 58% as compared to those on MR medium without growth substances on which 76 per cent was noted. Morphactin is not toxic to leaves so it does not increase the percentage of necroses.

On MR medium with 0.01 ppm morphactin added the leaves grow similarly as in the control combination. Adventitious buds first appear at the base of the blade and then on all its surface (Fig. 1b). The buds are modified, mostly thicker, club-shaped and as they grow they form

Fig. 2. Effect of morphactin IT 3456 on adventitious bud development on *Saintpaulia ionantha* leaves (experiment A and B)
a — normal bud; b-i — modified bud

thickened funnels without any lumen. From some buds modified leaves differentiate. Their blades coalesce into a funnel, and the petiole thickens considerably. Sporadically buds or leaves appear grown together in pairs. The regenerating leaves form no roots.

On MR medium with an admixture of 0.1 ppm morphactin the growth of the leaves is less intensive. Like in the preceding combination, the whole leaf blade forms buds on its upper side and modified leaves (Fig. 1c). The buds and leaves exhibit characteristic modifications (Figs 2 and 3). In some cases young buds develop from the funnel-shaped ones. The leaves do not form normal rosettes, neither do they develop roots.

With the highest morphactin dose (1.0 ppm) the changes in leaf regeneration in *Saintpaulia* are the same as in the combination with 0.1 ppm. Modified buds and leaves develop on both sides of the leaf blade. Many leaves grow in pairs with thick petioles, and the blades

Fig. 3. Influence of morphactin IT 3456 on adventitious leaf formation in *Saintpaulia ionantha* (experiment A and B)
a — normal leaf; b-e — modified leaves

form a flat plate (Fig. 3b—e). On the regenerating leaf (Fig. 1d) numerous young modified leaves are visible with blades rolled a thick brownish edge begin to show necrosis after 2 months of growth. In 3-month observation no root formation was noted.

The regeneration process of *Saintpaulia* leaves is different on MR medium with 1 ppm kinetin and 1 ppm IAA added (Fig. 4a). The leaves grow more intensively than in the control (MR) combination and are thicker (larger increment of parenchymatous tissue). Buds differentiate earlier on the entire blade surface on its upper side, sometimes from the petiole base on the lower part of the blade. Young leaves form from minute buds (0.5—1 mm). They develop intensively in the course of 3 months and their blades reach a diameter of 8 mm. The leaves form

Fig. 4. Influence of morphactin IT 3456 and growth regulators (kinetin and IAA) on leaf regeneration in *Saintpaulia ionantha* (experiment A, photos after 2 mos. of leaf growth)

a — MR + 1 ppm kin. + 1.0 ppm IAA; b — MR + 1 ppm kin. + 1.0 ppm IAA + 0.01 ppm IT 3456; c — MR + 1 ppm kin. + 1.0 ppm IAA + 0.1 ppm IT 3456; d — MR 5.1 ppm kin. + 1.0 ppm IAA + 1.0 ppm IT 3456

rosettes, they are dark green and pubescent of normal shape and anatomical structure, only the petioles are somewhat thickened. Sometimes their thick petioles coalesce but the blades remain free and do not form funnels. On the lower side of the inoculated leaf, roots differentiate reaching a length of 1.5 cm and form root hairs.

On medium with 1 ppm kinetin, 1 ppm IAA and 0.01 ppm morphactin the leaves grow intensively (Fig. 4b). On both their surfaces modified buds and leaves develop (Figs 2 and 3). The buds and leaves are large and thicker than when morphactin alone is added. Sometimes secondary buds develop from the funnel-shaped ones (Fig. 4b). Normal leaves do not develop in rosettes neither do roots form.

Saintpaulia leaves grafted on MR medium with 1 ppm kinetin, 1 ppm IAA and 0.1 ppm morphactin grow less intensively as compared with those in the combination with kinetin and IAA but without morphactin. The entire leaf blade is densely covered with adventitious buds which show modifications typical for morphactin treatment (Figs 2 and 4c). Small, poorly developed whitish buds are more numerous here than at lower morphactin doses. Less green leaves with blades rolled into funnels are noted, but all of them are thickened and single. Roots do not form.

Addition to MR medium of 1 ppm kinetin, 1 ppm IAA and 1 ppm morphactin causes the typical previously described changes in the course of regeneration. Fig. 4d shows buds and the shape of thickened funnels.

In experiment B with 1 ppm NAA added to the MR medium the growing leaves are greatly thickened and from the petiole a thick layer of callus (Fig. 5) overgrows the lower side of the blade (Fig. 6a). On the

Fig. 5. Cross section of petiole of regenerating *Saintpaulia ionantha* leaf
a — control medium MR; b — MR + 1 ppm NAA; WL — phloem-xylem bundle; K — callus

Fig. 6. Longitudinal section through regenerating *Saintpaulia ionantha* leaf
a — MR + 1 ppm NAA; b — MR + 1 ppm NAA + 1 ppm IT 3456
BL — leaf blade with adventitious buds; WL — phloem-xylem bundle; K — callus

Fig. 7. Longitudinal section through regenerating *Saintpaulia ionantha* leaf

a — medium MR; b — MR + 1 ppm IAA + 1 ppm IT 3456

E — epidermis; M — parenchyma cells; P — precambium; C — dividing epidermal cells from which, under the influence of morphactin, a secondary adventitious bud is formed

upper side normal adventitious buds form, beginning from the base. The young leaves are arranged in rosettes. The leaf petioles are thin and long. Nine weeks after inoculation, on the lower side of the leaves numerous roots and root hairs differentiate from the callus.

Addition of 1 ppm morphactin to the MR medium with 1 ppm NAA favours callus development. The latter overgrows with a thick layer the lower side of the blade and its edges (Fig. 6b). The leaves are also considerably thickened and roots differentiate from the callus. On the upper side of the blade numerous buds develop with modifications typical for morphactin.

On MR medium with 1 ppm IAA, regeneration of *Saintpaulia* leaves occurs similarly as on MR medium without growth regulators. Only more numerous roots are formed and no callus develops. Addition of 1 ppm morphactin to this medium produces modifications in the buds and adventitious leaves. Less numerous leaves are formed, plate-like or funnel-shaped. Buds cover the entire leaf surface. The buds and leaves are less thickened, they form thinner funnels than on MR medium with morphactin kinetin and IAA. The buds are modified (Figs 2 and 7b). At the base of the petiole a small amount of callus appears from which roots differentiate.

Kinetin added in the amount of 1 ppm to the MR medium causes a considerable thickening of the leaves. Adventitious buds form more numerous than on MR medium with IAA or NAA, whereas callus does not form nor roots.

On MR medium with 1 ppm kinetin and 1 ppm morphactin the growth of the leaves resembles that on the medium with kinetin alone added. On the entire blade surface numerous buds form. They are modified, on the entire upper surface and also on the lower one at the base of the

blade. New leaves developing from the adventitious buds grow intensively, they are thick, dark green with normal anatomical structure, sometimes rolled up, trumpet-like and they show a tendency to coalesce by their thick petioles. On medium with kinetin, leaf rosettes are much more greatly thickened and have the shape of thick funnels with a broad whitish or brown edge (Fig. 3). From some funnels secondary buds develop, but few leaves appear. The inoculated leaves do not form callus or roots.

It was found in microscopic preparations that the adventitious buds form as the result of cell division in the leaf epidermis. As the cells divide a convexity appears which grows to form a bud differentiating early into epidermal, parenchymatous and phloem-xylem tissues. Then leaves differentiate from the bud, arranged in rosettes. Under the influence of morphactin the development of the adventitious buds takes a different course. The buds grow intensively to a size of 3—6 mm and frequently leaves do not differentiate from them. In such buds a considerable increment of parenchymatous tissue (Fig. 7) and differentiating precambium is observed. The epidermis cells at the tips of the funnel-shaped buds may divide yielding new buds (Fig. 7).

DISCUSSION

Morphactine disturbs the polarity in *Saintpaulia ionantha* leaf regeneration in cultures *in vitro* similarly as does kinetin (Kukuczanka and Suszyńska, 1972). A similar reaction to morphactin has been described by Schraudolf and Schott (1969) in the leaves of *Begonia rex*. Polarity disturbances are associated with retardation of regeneration. These changes are partly neutralized by kinetin and IAA. It may be concluded therefrom that morphactin causes disturbances in natural hormonal regulation. This is also supported by the fact that IAA seems to unblock the inhibition of root initiation due to morphactin. The present experiments confirmed the results of Alleweldt (1969) concerning the stimulating action of morphactin on callus formation. In the present study this was most pronounced in interaction with NAA. This interaction distinguish the species specificity of leaf regeneration in *Saintpaulia ionantha*.

From among the so far described developmental anomalies of leaves due to morphactin in various plant species (Haccius, 1969; Scheider, 1969; Denffer, Fricke and Ring, 1969) the phenomenon of lack of leaf differentiation from buds and of formation of secondary buds on already modified ones has been for the first time observed in *Saintpaulia ionantha* in the present study. This kind of anomaly confirms the opinion of Denffer et al. (1969) that the changes caused

by morphactin are the more extensive and pronounced the younger are the development stages of the organ exposed to its action. This has been verified by experiments with a radioactive ^{14}C -morphactin isotope indicating its transport mainly to meristematic tissues (quoted by Pieńiążek and Saniewski, 1969).

CONCLUSIONS

1. Morphactin IT 3456 in 0.01, 0.1 and 1.0 ppm doses retards regeneration of adventitious buds, inhibits root formation, abolishes the polarity of leaf regeneration in *Saintpaulia ionantha*.

2. IAA and kinetin partly neutralize the delaying effect of morphactin on *Saintpaulia* leaves regeneration, and IAA with NAA neutralize the inhibition of root formation by the same substance.

3. The stimulation of callus development on *Saintpaulia* leaves produced by NAA is markedly enhanced by morphactin.

4. Morphactin produces specific developmental anomalies of the regenerating buds and leaves of *Saintpaulia ionantha*, namely, the appearance of undifferentiated club-shaped or funnel-shaped buds deprived of leaf primordia or of buds on the tips of which secondary buds or leaves grow (Fig. 3), and causes the formation of leaves thickened, funnel-shaped, plate-like or grown together in pairs (Fig. 3).

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*Modyfikacje regeneracji liści Saintpaulia ionantha Wendl.
wywołane morfaktyną w kulturach in vitro*

Streszczenie

W przeprowadzonych poprzednio doświadczeniach badano w kulturze *in vitro* przebieg regeneracji liści *Saintpaulia ionantha* Wendl. w zależności od działania syntetycznych auksyn i kinetyny (Kukułczanka i Suszyńska 1972), w niniejszej pracy natomiast zbadano wpływ morfaktyny IT 3456 na ten proces i jej współdziałanie z kinetyną i auktydami (IAA i NAA). Przeprowadzono dwa doświadczenia w 16 kombinacjach.

Stwierdzono, iż morfaktyna IT 3456 w dawkach 0,01, 0,1 i 1,0 mg/l pożywki, opóźnia regenerację pączków przybyszowych (tabela 1), hamuje tworzenie się korzeni, znosi polarność regeneracji liści *Saintpaulia*. IAA i kinetyna częściowo niwelują efekt opóźniania regeneracji liści wywołany morfaktyną (tabela 1) a IAA i NAA niwelują zahamowanie wytwarzania korzeni. Stymulację rozwoju kalusa na liściach *Saintpaulia* wywołaną przez NAA, morfaktyna wyraźnie potęguje (ryc. 5 i 6).

Morfaktyna wywołuje swoiste anomalie rozwojowe regenerujących się pączków i liści u *Saintpaulia ionantha* (ryc. 1,2,3,i 4), a mianowicie powstawanie niezróżnicowanych pączków buławkowatych, lejkowatych bez zawiązków liściowych lub pączków, na których szczycie wyrastają wtórne pączki (ryc. 2). Ponadto wywołuje powstawanie zgrubiałych liści lejkowatych, tarczowatych lub zrośniętych po dwa (ryc. 3).