ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLI — nr 4
1972

Regenerative properties of *Saintpaulia ionantha* Wendl. leaves cultured in vitro

K. KUKUŁCZANKA and G. SUSZYŃSKA

Botanical Garden. The University. Kanonia 6/8. Wrocław, Poland

(Received: March 20, 1972,)

Abstract

The regenerative properties of *Saintpaulia ionantha* leaves were investigated in culture in vitro. The leaves acquire this ability after the end of growth. Restitution regeneration was found to be polar and the sequence in organogenesis of roots and buds was different than on a peat-sand substratum. NAA inhibits growth of isolated leaves and stimulates callus and root development. Kinetin abolishes the polarity of regeneration, stimulates leaf growth, initiates formation of numerous buds and inhibits rhizogenesis. The interaction of kinetin with NAA or IAA in dependence on the order in which these substances are applied stimulates in various extents the growth of isolated leaves and callus, and bud and root formation.

INTRODUCTION

A rieview of up-to-date investigations on leaf regeneration is given by Dore (1965). Among these studies that of Goebel (1903, 1908) may be considered as classical. To the families exhibiting a high ability of leaf regeneration belong *Gesneriaceae*. Goebel studied in detail the species *Achimenes* and *Streptocarpus* belonging to this family, and Zimmerman and Hitchcock (1940) only summarily *Saintpaulia ionantha* Wendl. Goebel (1903) and Isbell (1931a, b) noted a different regenerative ability in various parts of the leaf, restitution regeneration being highest in the basal part. According to Dore in most plants leaf regeneration is of polar character, and according to Wirth (1959) is conditioned by the unidirectional auxin transport.

The present investigations were undertaken to establish the regeneration ability of *Saintpaulia ionantha* Wendl. leaves, the influence of auxins and kinetins and of their interaction on the course of the morphogenetic processes in leaf regeneration.

MATERIAL AND METHODS

The experiments with leaves of *Saintpaulia ionantha* Wendl. var. *alba* of the family *Gesneriaceae* were carried out in vitro. As outset material served sterilised leaf fragments cultured on the agar medium of Murashige and Skoog (1962). On the isolated leaf fragments numerous leaf rosettes regenerated in the course of 1—2 months. From these rosettes leaves 2—5 mm in diameter were taken for experiments with the addition of various synthetic auxin amounts in the form α-naphthaleneacetic acid (NAA) or β-indolylacetic acid (IAA) and kinetin ($C_{10}H_9N_5O$ — Permedra Lublin).

The leaves were cultured in test tubes 7.5 cm long, 13—15 mm in diameter containing 2—2.5 ml of medium without edamine and glycine. The pH of the medium after sterilisation was 5.8—6.2. The following combinations of Mursahige and Skoog's medium (MR) were used: 1 — without any additions (exp. A, B, C, D), and with addition of: 2 — 1 mg kinetin (exp. A, C, D), 3 — 2 mg kinetin (A, C, D), 4 — 0.5 mg NAA (A, C, D), 5 — 1 mg NAA (A, C, D), 6 — 0.5 mg kinetin and 0.5 mg NAA (B), 7 — 0.5 mg kinetin and 1 mg NAA (B), 8 — 0.5 mg kinetin and 2 mg NAA (B), 9 — 1 mg kinetin and 0.5 mg NAA (A, C, D), 10 — 1 mg kinetin and 1 mg NAA (A, C, D), 11 — 2 mg kinetin and 0.5 mg NAA (A, D), 12 — 1 mg kinetin and 0.5 mg IAA (D), 13 — 1 mg kinetin and 1 mg IAA (D), 14 — 1 mg kinetin and 2 mg IAA (D).

Experiment A was started in September 1970 in three replications, experiment B in November 1970 in two replications, experiment C in January 1971 in two replications and experiment D in February 1971 in four replications. In experiments B, C and D the leaves were taken for culture with the petioles, and in experiment A the petioles were cut off and cultured. In each replication the combination comprised 6—15 test tubes with regenerating leaves.

The test tubes were placed in the culture room at 22°—24°C under continuous illumination of 1700 lux.

Observations of growth and regeneration were continued for a period of three months. After 4—6 weeks microscopic slides were prepared from the cultured leaves and regenerating adventitious buds. The slides were prepared by the paraffin method. The material was fixed with Kraft's reagent and stained with alkaline fuchsin or light fast green.

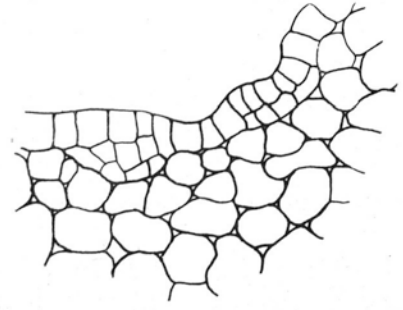
RESULTS

The experiments demonstrated that petioles separated from the leaves have no regenerative properties and undergo complete necrosis on Murashige and Skoog's medium. Necrosis occurs faster than in the case of leaves. Leaf necrosis occurred in the experiment in 50—60 per cent.

Development of a leaf isolated from a rosette obtained uned sterile conditions is manifested by growth a few days after transfer to the new medium. Growth lasts about three weeks and ends when the leaf reaches the size of the test tube diameter. When growth ceases, new structures appear on the leaf surface in dependence on the growth regulators in the medium. These structures may be adventitious buds, callus, roots, or two three of these structures simultaneously. In all exeriments the results in the same combination were the same.

On MR medium without auxin and kinetin added the leaves after the end of growth begin to develop on the base of the laminae and of the petioles adventitious buds from which leaf rosettes form further. The rosettes appear 4—8 weeks after starting the experiment and thin roots develop subsequently.

Regeneration of *Saintpaulia* leaves on MR medium has a somewhat different course when kinetin is added in the amount of 1 and 2 mg/l. of medium. The leaves thicken considerably during growth and form adventitious buds on the whole upper and lower surfaces of the lamina and on the petiole. On the lower leaf surface the buds are less numerous.



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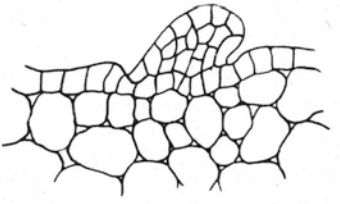

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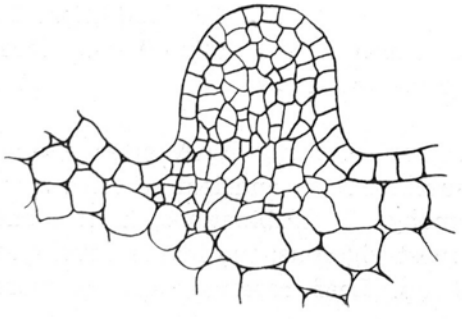

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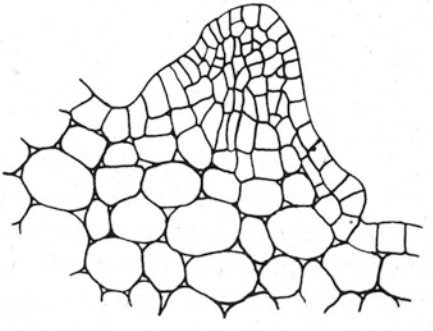

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Figs. 1—4. Successive stages of adventitious bud development from epidermis of *Saintpaulia ionantha* Wendl. leaves

Most adventitious buds form along the main rib. The new leaves developing from the adventitious buds are large, ca. 1 cm long, thick, dark green, rolled up trumpet-like with thick petioles. Rosette growth is more intensive in the combination receiving 2 mg kinetin than at the lower dose. In the course of 3-month observation the leaves and adventitious rosettes did not form roots.

Leaves on MR medium with NAA added in the amount of 0.5 and 1 mg/l. of medium, showed only slight growth or complete growth inhibition. Three to four weeks after starting the experiment callus began to advance from the blade base and rapidly covered the entire leaf surface. After the next month, roots differentiated from the callus. On medium with 1 mg NAA adventitious buds do not form, but when 0.5 mg NAA is added minute leaf rosettes form, they do not, however, continue to grow.

Saintpaulia leaves on MR medium with 1 mg kinetin and 0.5 mg NAA or with 0.5 mg kinetin and 0.5 mg NAA added grow well and thicken. After one month they simultaneously form callus and buds, and somewhat later also roots. From these adventitious buds leaf rosettes develop rather quickly in racemose clusters on the entire blade surface and the petiole. A vigorous development of adventitious leaves was also observed, they did not, however, reach the size of those on the medium with only kinetin added.

On MR medium with 1 mg kinetin and 1 mg NAA or 0.5 mg kinetin and 1 mg NAA/l. of medium the leaves grow and regenerate like those in the two preceding combinations, however, a more intensive development of callus and roots, and weaker growth of buds and rosettes is observed.

In the combination in which 0.5 mg kinetin and 2 mg NAA were added per 1 l. of MR medium, development of leaves is similar to that discussed in the four preceding combinations, only a more intensive callus and root development, and weaker bud growth were observed.

When 2 mg kinetin and 1 mg NAA are added to MR, the leaves grew more vigorously than upon 1 mg kinetin and 1 mg NAA addition. In this combination leaf rosettes developed also on the entire leaf surface, and callus and root development was somewhat more intensive than that of rosettes.

Leaves on MR with 2 mg kinetin and 0.5 mg NAA after ending growth develop callus on the entire surface, roots and leaf rosettes from the previously formed buds. From among all combinations, in this one the development of leaf rosettes was most vigorous; the leaves developed normally, did not thicken and roll up. Leaf rosettes develop more intensively here than callus and roots.

In contrast to the leaves regenerating on medium with NAA those on MR with 1 mg and 0.5 mg IAA or 1 mg kinetin and 1 mg IAA added,

after ending growth develop numerous adventitious buds, and further leaf rosettes, but no roots, and form very little callus tissue at the 0.5 mg IAA dose, and somewhat more at 1 mg IAA.

When the IAA dose is increased to 2 mg in the MR with simultaneous addition of 1 mg kinetin, the leaves after ending growth produce numerous rosettes, however, somewhat less than at lower IAA doses. Callus also forms on the leaves and roots develop. Their growth, however, is weaker than on leaves regenerating on medium with NAA.

Microscopic preparations of young leaves ca. 12 mm in diameter regenerated in vitro culture show a quite normal anatomical structure. Their adventitious buds were found to form from single cells of groups of leaf epidermis cells. Owing to numerous cell divisions with unidirectional cell wall formation and considerable increase in the number of cells on a small area, the epidermis cells budge out. These protuberances give rise to adventitious buds, and further cell divisions lead to an increase of the number of cells which rather soon begin to differentiate to epidermal, parenchyma and vascular cells. (Figs 1—4).

DISCUSSION

The leaves of *Saintpaulia ionantha* Wendl. when cultured in vitro show an ability of restitutive regeneration only after having reached their final size determined by the test tube diameter (in normal culture also only those which have ended growth). These leaves exhibit also polar regeneration, the main activity being localised at the base of the lamina. When cultured in vitro the leaves have a different sequence of regenerating organs, they first form adventitious buds and then roots. On the other hand, according to Goebel (1903, 1908), Isbell (1931a) and Dore (1965), regenerating leaves first form roots or callus, and only later adventitious shoots. This would be proof of disturbance of hormonal regulation in leaves cultured in vitro. Isolated petioles of *Saintpaulia ionantha* do not show any regenerative properties, neither do those of *Ipomena batatas* (Isbell 1931a).

On *Saintpaulia ionantha* leaves in vitro adventitious buds arise from single epidermis cells or from their groups. The latter situation is according to Nultsh (1968) the result of abolition of correlative inhibitions of the already differentiated epidermis cells and their return to embryonal state.

The reactions of *Saintpaulia ionantha* leaves regenerating in vitro to NAA and IAA are similar to those of *Begonia rex* leaves (Wirth 1959). The leaves of *Begonia* sp., however, form roots directly under the influence of NAA (Schraudolf and Reinert 1959).

As reported by Gautheret (1959), NAA had a stronger influence on callus and root formation by *Saintpaulia* leaves than had IAA. Essen-

tial for the course of organogenesis in restitution regeneration of *Saintpaulia* leaves are, on the one hand, the culture conditions, and on the other, the amounts of IAA and NAA applied, since Zimmerman and Hitchoock (1940) also induced direct rhizogenesis in *Saintpaulia* leaves. It is possible that also in in vitro culture *Saintpaulia* leaves would be capable of direct rhizogenesis under the influence of IAA in the absence of kinetin, like the leaves of *Atropa belladonna* in the experiments of Zenkteler (1971). The *A. belladonna* leaves form buds under the influence of kinetin and IAA, and, if they are fragmented, first callus and then buds. The leaves of *Saintpaulia ionantha*, on the contrary, produce simultaneously buds, callus and roots. These differences may be explained by a different nature of these two species or by a different kinetin and IAA sequence.

Independently of the doses applied, kinetin abolished the polarity of regeneration of *Saintpaulia* leaves in all combinations and inhibited root growth. The same has been observed in regenerating *Begonia* sp. leaves by Schraudolf and Reinert (1959).

CONCLUSIONS

1. Isolated *Saintpaulia ionantha* Wendl. leaves cultured in vitro are capable of restitutive regeneration only after having accomplished growth. The highest regeneration ability is localised at the base of the lamina. Cut off petioles show no regenerative properties.

2. *Saintpaulia ionantha* leaves in vitro show a different sequence of regenerating organs than on a peat-sand substratum, they first form adventitious buds from secondarily activated epidermis cells, and thereafter roots from vascular cells.

3. NAA in the amonut of 1 mg/l. of medium inhibits growth of isolated leaves inducing at the same time callus and root formation. In a dose of 0.5 mg/l. NAA causes formation of scarse buds.

4. Kinetin in 1 and 2 mg/l. doses stimulates growth and causes thickening of isolated leaves and formation of scarse buds developing to leaf rosettes on the entire leaf surface, it inhibits, however, rhizogenesis.

5. The interaction of kinetin with NAA or IAA produces in the regenerating leaves:

a) growth and thickening in all combinations in which kinetin is applied with NAA or IAA;

b) callus, root and bud formation on the entire leaf surface in all combinations with kinetin and IAA and in the combination with 1 mg kinetin and 2 mg IAA/l.

c) callus and bud formation on the entire leaf surface in combinations with 1 mg kinetin and 0.5 or 1 mg IAA/l.

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Zdolności regeneracyjne liści Saintpaulia ionantha Wendl. w kulturach in vitro

Streszczenie

Izolowane fragmenty liści *Saintpaulia ionantha* Wendl. var. alba hodowane in vitro na pożywce Murashige i Skooga wytwarzały rozetki liściowe. Z rozetek tych pobierano liście do doświadczeń ze zróżnicowanym dodatkiem NAA, IAA i kinetyny (14 kombinacji). Liście *Saintpaulia* w kulturze in vitro wykazują zdolność regeneracji restytucyjnej dopiero po zakończeniu wzrostu, przy czym ich główny potencjał regeneracyjny zlokalizowany jest u nasady blaszki liściowej, natomiast odcięte ogonki liściowe nie mają zdolności regeneracji. W kulturze in vitro izolowane liście najpierw tworzą pączki przybyszowe z wtórnie uczynnionych komórek epidermy, a następnie korzenie z komórek przewodzących. Pod działaniem NAA w ilości 2 mg/l następuje zahamowanie wzrostu izolowanych liści, a jednocześnie powstaje kalus i korzenie. NAA w ilości 0,5 mg/l podobnie hamuje wzrost liścia, stymuluje rozwój kalusa, korzeni i nielicznych pączków. Kinetyna w ilości 1 i 2 mg/l wywołuje wzrost i grubienie liści, wytwarzanie przez nie dużej liczby pączków, a z nich rozetek liściowych na całej powierzchni liści oraz zahamowanie rizogenezy. We wszystkich kombinacjach współdziałanie kinetyny z NAA lub z IAA powoduje wzrost i grubienie regenerujących liści. Kinetyna w ilości 1 mg/l z NAA w różnej ilości lub z 2 mg/l IAA stymuluje tworzenie się kalusa, korzeni i pączków na całej powierzchni liści. Kinetyna w ilości 1 mg/l z 0,5 i 1 mg IAA wywołuje tworzenie się kalusa i pączków na całej powierzchni liści, natomiast nie wywołuje rizogenezy.

Ogród Botaniczny,
Instytut Botaniki i Biochemii,
Uniwersytet Wrocławski