ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 51, nr 2: 167-172
1982

Regeneration of whole plants of geranium from petioles cultured *in vitro*

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(Received: April 4, 1981)

Abstract

Studies were made on the morphogenetic potential of petioles of *Pelargonium hortorum* (1 variety, 2 clones) and *Pelargonium peltatum* (1 variety, 1 clone) grown on the medium of Murashige and Skoog (MS) supplemented with auxins (IAA, IBA, NAA) and cytokinins (KIN, BAP, zeatin). The most intensive growth of callus of both species was observed on medium supplemented with 0.1 mg/l NAA and 10 mg/l KIN, both under constant illumination and in darkness. Differentiation of callus and regeneration of plants occurred in *P. peltatum* variety "PAC Dresdner Amethyst" and in *P. hortorum* clone 3766/4 on medium containing 1 mg/l BAP, 1 mg/l IAA and exposed to a photoperiod (L:D = 16:8).

INTRODUCTION

The geranium due to its large decorative value and wide application in horticulture is freguently used as an experimental material in *in vitro* cultures. The investigations are mainly performed on vegetative reproduction of various organs. Pillai and Hildebrandt (1968a, 1969a) and Chen and Galston (1967) have shown that it is possible to obtain fully developed plants from fragments of the internodes and pith, and Abo El-Nil and Hildebrandt (1971) and Abo El-Nil et al. (1976) have determined the conditions for organogenesis of

Abbreviations: IAA — indolil-3-acetic acid, NAA — naphthyl-1-acetic acid, IBA — indolilbutyric acid, KIN — 6-furfurylaminopurine, BAP — 6-benzylaminopurine, zeatin — trans-6-(4-hydroxy-3-methylbutyl-2-enyl)aminopurine.

callus formed from anthers. Plants were also obtained from isolated apical shoot meristems on a synthetic medium (Pillai and Hildebrandt 1968a, b, Theiler 1977). Geranium is often heavily infected with viruses and the method of *in vitro* regeneration of plants from petioles enables a rapid propagation of virus-free material. However, there are some difficulties in regenerating plants from petioles cultured *in vitro*. Pillai and Hildebrandt (1968c, 1969b) were able to obtain fully developed plants whereas Klimaszewska (1977) obtained only callus which did not differentiate into organs.

The following work was undertaken to find optimal conditions for vegetative propagation of 2 species of *Pelargonium* from leaves cultured *in vitro*.

MATERIAL AND METHODS

For experiments performed in the winter-spring season of 1980, petioles of the two following species were used:

1. *Pelargonium hortorum*: a) variety "Maryla"; b) clone 3782/4 = cv. WS × cv. Sonnenkind S_1; c) clone 3766/4 = cv. Salomon Irene × cv. Sonnenkind S_1.

2. *Pelargonium peltatum*: a) variety "PAC Dresdner Amethyst"; b) clone 2824/4 = cv. Cattleya × cv. Perła Smolic.

The plants were obtained from the Experimental Station of Plant Culture and Acclimatization in Smolice and from the District Center of Investigation of Cultivated Plants in Śrem.

The isolated petioles were sterilised for 3 minutes in 0.5% mercuric chloride and later washed several times with sterile distilled water. Subsequently, under sterile conditions, petioles were cut with a scalpel into 0.5-cm long fragments and placed horizontally in test tubes. The explants were cultured on Murashige and Skoog (1962) medium (MS) containing 3% sucrose. The medium was supplemented with 0.1 mg/l NAA and 10 mg/l KIN according to Pillai and Hildebrandt (1968c). Other variants of the MS medium with different combinations and concentrations of growth substances were also tested (Table 1). MS medium without growth substances was the control. The cultures were incubated at 22-25°C in a light chamber or in darkness. In order to induce the differentiation of callus the explants were transferred to a 16-hour light and 8-hour dark cycle.

Table 1

Variants of Murashige and Skoog (1962) medium

Auxins	Concentration, mg/l	Cytokinins	Concentration, mg/l
IAA	0.5-1	KIN	0.5-1
IAA	0.5-1	BAP	0.5-1
IAA	0.5-1	zeatin	0.5-1
IBA	0.5-1	KIN	0.5-1
IBA	0.5-1	BAP	0.5-1
IBA	0.5-1	zeatin	0.5-1
NAA	0.5-1	KIN	0.5-1
NAA	0.5-1	BAP	0.5-1
NAA	0.5-1	zeatin	0.5-1

RESULTS

THE FORMATION OF CALLUS TISSUE

The most suitable medium for the induction of morphogenetic processes in explants was MS medium supplemented with 0.1 mg/l NAA and 10 mg/l KIN. Two weeks after isolation the fragments of petioles became swollen and later on began to form callus tissues on both cut surfaces. Light enhanced the formation of callus.

Experiments in light

Explants of *Pelargonium hortorum* clones 3766/4 and *P. peltatum* variety "PAC Dresdner Amethyst" formed a light green and hard callus in the third week of culture. When subculturing to a fresh medium it grew as a firm green coloured callus of a compact structure. The callus of *P. hortorum* clone 3782/4 and *P. peltatum* clone 2824/4 appeared in the fourth week of culture. Initially the callus was fragile and white but after transferring to a fresh medium it became green. It grew less intensively than callus of the above mentioned plants.

Pelargonium hortorum variety "Maryla" during the same time formed a white and fragile callus containing small green centers. After transferring to a fresh medium the green centers did not increase. Callus developed abundantly between the second and third passage. Subsequently it developed much slower and at the same time all calluses became yellowish-white. Unfortunately callus failed to produce roots or shoots. Explants of both geranium species when inoculated on control medium degenerated in the second week of culture.

Experiments in darkness

On the medium described by Pillai and Hildebrandt (1968c) about 40% of all explants died. The remaining ones, regardless of the form, formed an abundent, white brittle callus of a compact structure. This process was delayed by about 5 days in respect to callus formation in light, but the intensity of growth was much higher. After the fifth passage it grew much slower and shortly afterwards became brown and consequently died. The callus of *P. hortorum* clone 3766/4 had the highest rate of viability, but it too died after 8 months of culture in darkness. During the whole period of culture no sings of the formation of shoots or roots were discernible. In the control medium the inoculated petioles degenerated within the first 10 days of culture.

PLANT REGENERATION

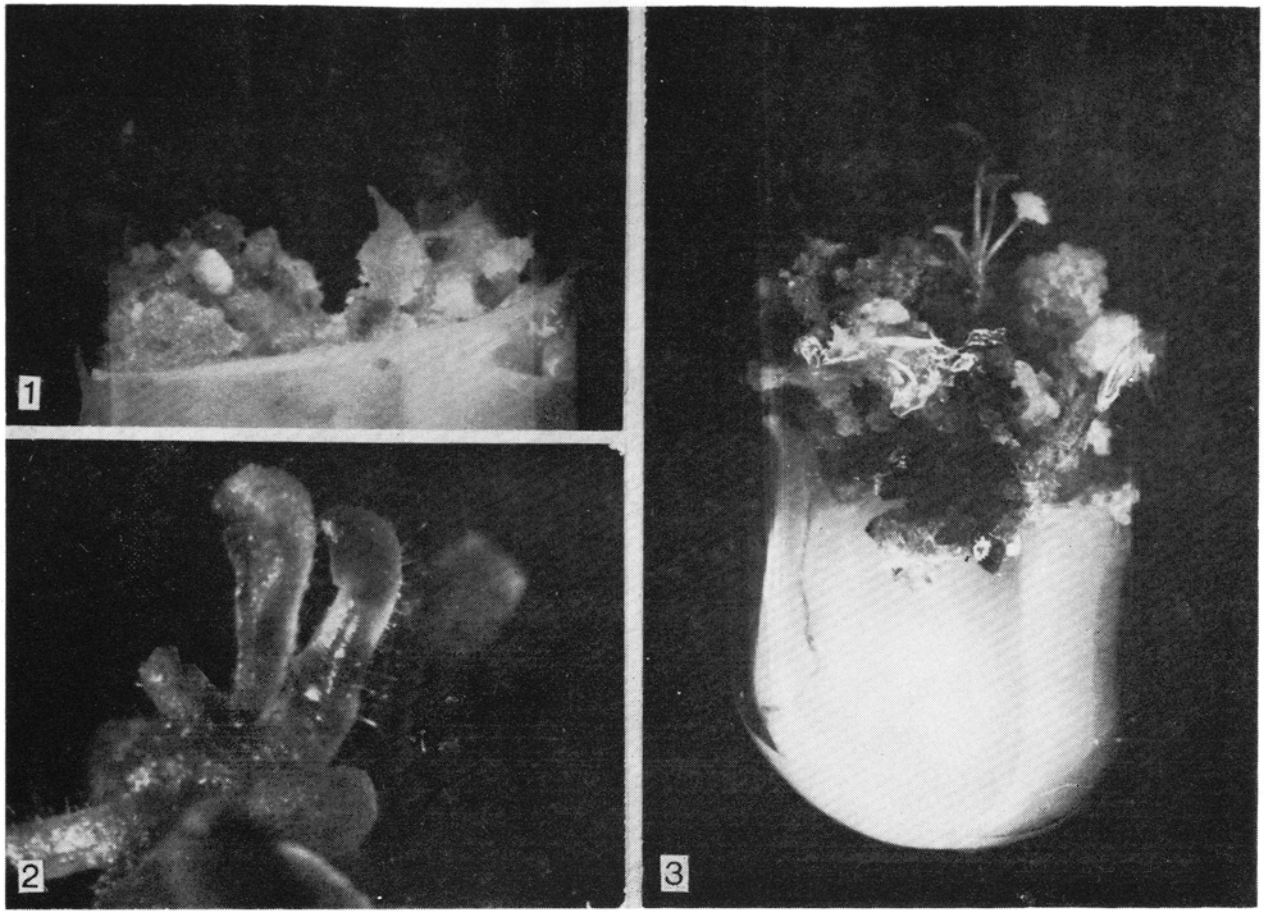
In order to initiate the differentiation of organs the calluses were transferred to media with various concentrations of cytokinins. Also the growth conditions were modified. Half of the inoculated calluses were left in light or in dark as described above and half in a photoperiod L:D = 16:8. The modification of MS medium are described in Table 1. Calluses of *P. hortorum* variety "Maryla" and *P. peltatum* clone 2824/4 did not form organs on any of the media. Roots differentiated only in calluses of *P. hortorum* clone 3782/4 on MS medium containing 1 mg/l IBA and 1 mg/l zeatin. In this medium roots appeared after three weeks, but degenerated soon. Under conditions applied by Pillai and Hildebrandt (1968c) callus tissues of *P. hortorum* clone 3766/4 contained only tiny buds which did not develop further.

A full regeneration was only observed in *P. hortorum* clone 3766/4 and *P. peltatum* variety "PAC Dresdner Amethyst". In calluses of both forms numerous green centers of cells appeared when the medium was supplemented with 1 mg/l BAP and 1 mg/l IAA. These centers were observed in about 40% of the passaged calluses. In the fourth week of culture buds began to differentiate and after two more weeks grew out from the tissue (Figs. 1, 2). The buds after transferring onto fresh medium grew further and developed into tiny plants with roots (Figs. 3, 4). After 9 more weeks of culture, young plantlets of *P. hortorum* clone 3766/4 and *P. peltatum* variety "PAC Dresdner Amethyst" were transferred to a hydroponic culture and than to soil (Fig. 5).

DISCUSSION

In our work wee found that induction of callus formation occurred only on medium containing the growth substances. In the case of all explants of *P. hortorum* and *P. peltatum* the most intensive growth of

PLATE I

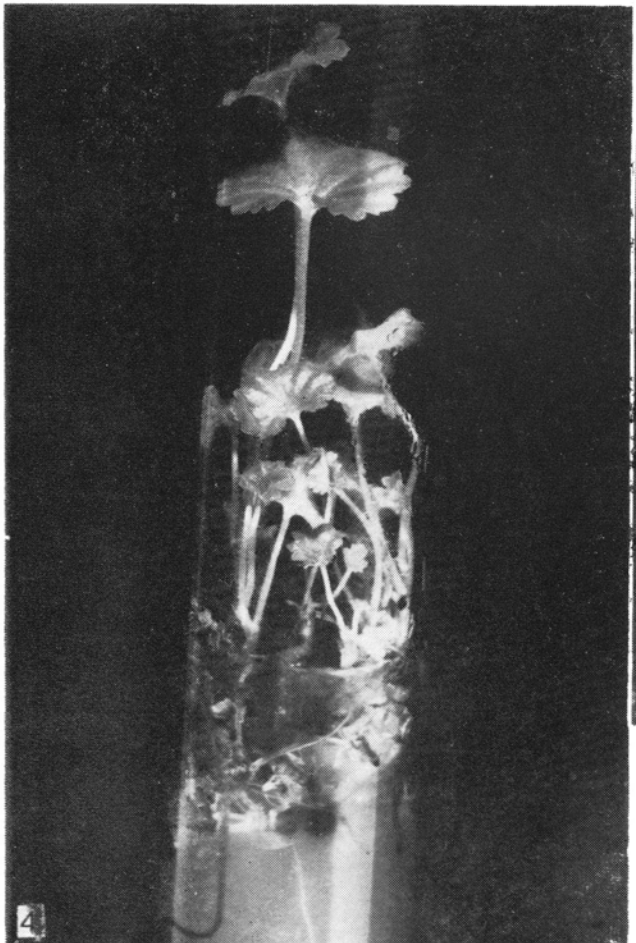


Stages of regeneration of petioles of geranium cultured on MS medium supplemented with 1 mg/l BAP and 1 mg/l IAA under photoperiod L:D = 16:18

Fig. 1. Callus of *P. peltatum* variety "PAC Dresdner Amethyst" after four weeks of culture. 2 ×

Fig. 2. Buds of *P. peltatum* variety "PAC Dresdner Amethyst" growing out from the callus after 6 weeks of culture. 30 ×

Fig. 3. Shoots of *P. peltatum* variety "PAC Dresdner Amethyst" in the tenth week of culture. 2 ×



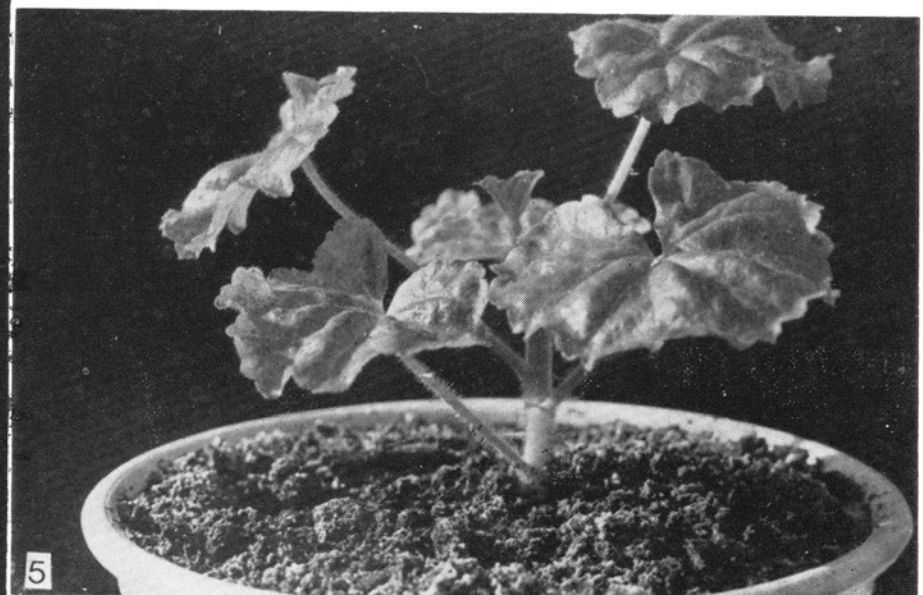


Fig. 4. Regenerated plants of *P. hortorum* clone 3766/4 after thirteen weeks since transferring the callus on MS medium containing 1 mg/l BAP, 1 mg/l IAA under a photoperiod L:D = 16:8. 1.5 ×

Fig. 5. Regenerated plant of *P. peltatum* variety "PAC Dresdner Amethyst" after tranfer to a pot in the fifteenth week of culture. Natural size

callus took place when MS medium contained 0.1 mg/l NAA and 10 mg/l KIN. Explants of both species formed callus tissue both under constant illumination and in darkness. However, the percentage of degenerating explants was higher in the darkness. It was found that darkness had a favourable effect on the production of callus. A similar results were obtained by Klimaszewska (1977). In spite of the identical culture conditions the growth of calluses differed among groups of plants belonging to the same species. Therefore, it seems that the morphological potentials of those plants are also different.

Either constant light or darkness were found to favour callus differentiation, in spite of application of different modifications of MS medium. Only after introduction of photoperiod callus differentiated and consequently plants developed. Pillai and Hildebrandt (1968c) considered that a suitable photoperiod was indispensable for the initiation of organogenesis, however, this was not confirmed by Klimaszewska (1977). In our experiments we found that the medium used by Pillai and Hildebrandt (1968c) only slightly induced the process of organogenesis in the investigated plants of geranium. A high percentage of regenerated plants was observed after supplementing the medium with 1 mg/l BAP and 1 mg/l IAA and after introduction of a photoperiod. Within *P. hortorum* the highest regenerative potential occurred in petioles of clone 3766/4 which callus produced a large number of plants, while clone 3782/4 regenerated only roots. In *P. peltatum* only callus of the variety "PAC Dresdner Amethyst" formed roots and shoots from which plants were developing. Petioles of the remaining forms of both geranium species regardless of the medium and culture conditions only formed callus tissue. In neither of the investigated forms shoots or roots developed.

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Regeneracja pelargonii z ogonka liściowego w warunkach kultur in vitro

Streszczenie

Badano zdolności regeneracyjne izolowanych ogonków liściowych *Pelargonium hortorum* (1 odmiana, 2 klony) i *P. peltatum* (1 odmiana, 1 klon) hodowanych na pożywce Murashige i Skooga (MS) z dodatkiem auksyn (IAA, IBA, NAA) i cytokinin (KIN, BAP, ZEA). Najintensywniejszy wzrost tkanki kalusowej obu gatunków stwierdzono na pożywce z dodatkiem 0,1 mg/l NAA i 10 mg/l KIN, zarówno w świetle ciągłym, jak i w ciemności. Różnicowanie kalusa i regenerację roślin uzyskano u *P. peltatum* odmiana „PAC Dresdner Amethyst" i *P. hortorum* klon nr 3766/4 na pożywce zawierającej 1 mg/l BAP, 1 mg/l IAA i po wprowadzeniu fotoperiodu (L:D = 16:8).