EFFECT OF CYTOKININS AND AMINO ACIDS ON MULTIPLICATION OF *PELARGONIUM* CULTIVARS

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ABSTRACT

The effect of two cytokinins (BAP, m-Topolin) tested singly or in combination with a mixture of amino acids (glycine, glutamine, cysteine) on multiplication rate and quality of *Pelargonium* × *hederaefolium* and *Pelargonium* × *hortorum* shoots were investigated. For both *Pelargonium* cultivars, the application of cytokinins greatly increased the production of axillary and adventitious shoots compared with the shoots produced on the medium without growth regulators. In the case of *P.* × *hederaefolium* no significant differences it were found in the number of shoot formed for BAP and m-Topolin treatments. For *P.* × *hortorum* the most effective multiplication of shoots was obtained on the medium supplemented with m-Topolin (1.0 mg l^{-1}). Moreover, in the presence of m-Topolin an improvement of shoot quality of both genotypes has been observed (well developed shoots with soft green leaves). It was particularly apparent for *P.* × *hortorum* shoots.

The addition of amino acids to a cytokinin medium increased slightly the number of formed shoots of *P.* × *hederaefolium* and influenced the shoot quality of both *Pelargonium* cultivars (well developed leaf blades, uniform growth of shoots in clumps, slower process of shoot senescence).

KEY WORDS: *Pelargonium*, multiplication, cytokinins, amino acids, in vitro.

INTRODUCTION

Pelargonium sp. has been a subject of numerous studies to determine the effectiveness of in vitro techniques to produce a large number of pathogen-free plants. A successful initiation of shoots and somatic embryos formation has been widely reported for the variety of *Pelargonium* cultivars (Theiler 1977; Stefaniak and Zenkteler 1982; Reuther 1983; Horn 1988; Gill et al. 1993; Murthy et al. 1996; Robichon et al. 1997), but the knowledge about their futher development is still limited. It is known that *Pelargonium* cultivars have strongly differed in their morphological potency; one genotype easily forms shoots, another one produces only callus instead, which dies after several subcultures (Theiler 1977; Reuther 1983). A high multiplication rate of *Pelargonium* shoots from primary explants has been obtained by Horn (1988) and other authors (Reuther 1983; Corneanu and Corneanu 1991). However, no information has been found about the *Pelargonium* multiplication through a stimulation of shoot branching. Hence, the objective of this study was to evaluate the multiplication potential of *P.* × *hortorum* 'Bargpalais' and *P.* × *hederaefolium* shoots, after 2 years of growing in tissue culture conditions.

On the basis of the previous works it is known, that each genotype of *Pelargonium* needed specific components of the medium. As a rule, the most popular basal media used for various *Pelargonium* genotypes were the MS or LS medium (Theiler 1977; Hammerschlag 1978; Cassells 1979; Stefaniak and Zenkteler 1982; Robichon et al. 1997). For the same genotype an advantageous effect of organic components such as: coconut milk (Abo El-Nil and Hildebrandt 1971), casein hydrolysate (Theiler 1977; Horn 1988), amino acids (Theiler 1977) or myo-inositol (Horn 1988) was observed. The biggest differences concerned the application of growth regulators. One genotype favourized the presence of BAP in the medium, another kinetin, zeatin, 2iP or TDZ added to the medium alone or in combination with an auxin (IBA, IAA, 2,4-D).

The aim of this study was to compare the effects of two cytokinins: m-Topolin and BAP as well the different amino acids on the multiplication and quality of *Pelargonium* shoots in vitro.

Abbreviations

BAP – N^6-benzylaminopurine; m-Topolin – N^6 – (*meta* – hydroxybenzyl) adenine; 2iP – 6 (γ, γ – dimethylallyamino) purine; Ga_3 – gibberellic acid; IBA – 3-indolebutyric acid; NAA – 1-naphtylacetic acid; 2,4-D – 2,4 – dichlophenoxyacetic acid; TDZ – thidiazuron (N-phenyl-N'-1, 2, 3-thiadiazol-5-ylurea); LS – Linsmaier and Skoog medium; MS – Murashige and Skoog medium.

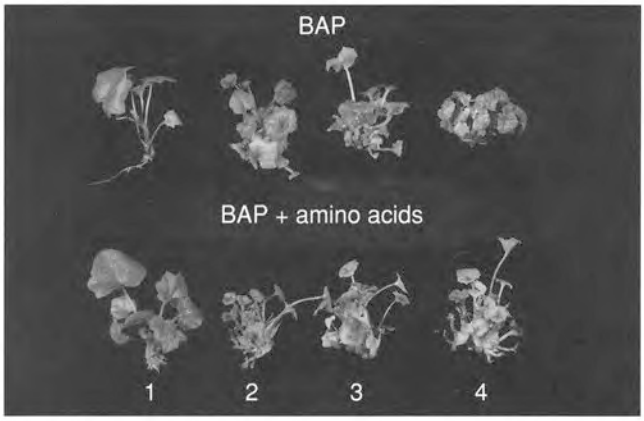

Fig. 1. The growth of *P.* × *hederaefolium* shoots on MS medium containing different concentrations of BAP or BAP in combination with the mixture of amino acids (glycine, glutamine, cysteine – each in a concentration of 10 mg l^{-1}); 1 – control, 2 – 0.25 mg l^{-1}, 3 – 0.5 mg l^{-1}, 4 – 1.0 mg l^{-1} of BAP.

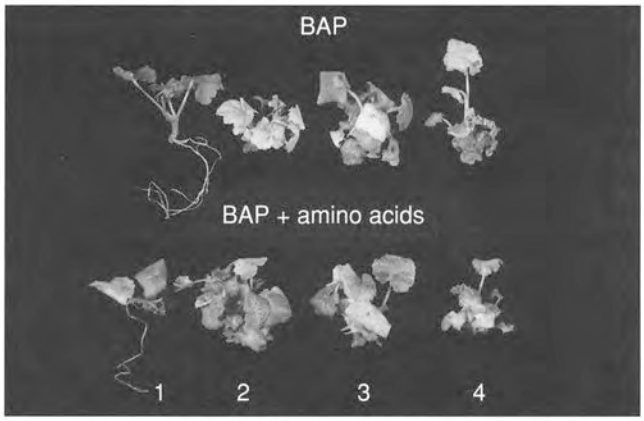

Fig. 3. The growth of *P.* × *hortorum* shoots on MS medium containing different concentrations of BAP or BAP in combination with the mixture of amino acids (glycine, glutamine, cysteine – each in a concentration of 10 mg l^{-1}); 1 – control, 2 – 0.25 mg l^{-1}, 3 – 0.5 mg l^{-1}, 4 – 1.0 mg l^{-1} of BAP.

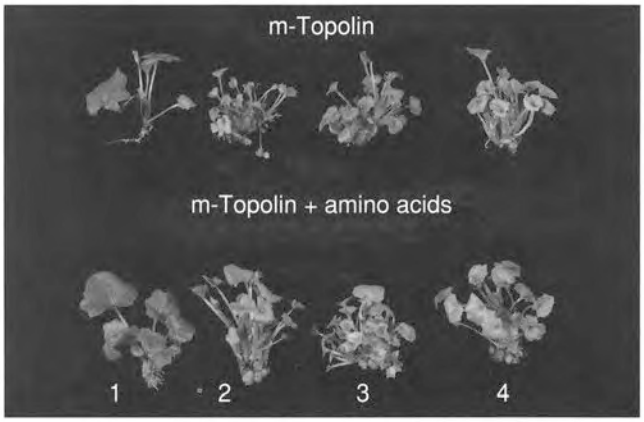

Fig. 2. The growth of *P.* × *hederaefolium* shoots on MS medium containing different concentrations of m-Topolin or m-Topolin in combination with the mixture of amino acids (glycine, glutamine, cysteine – each in a concentration of 10 mg l^{-1}); 1 – control, 2 – 0.5 mg l^{-1}, 3 – 1.0 mg l^{-1}, 4 – 1.5 mg l^{-1} of m-Topolin.

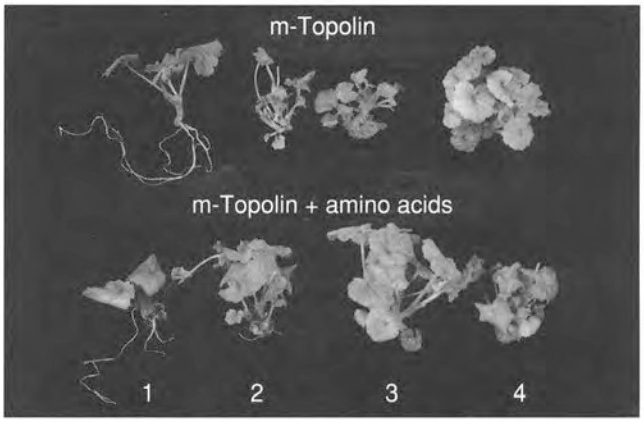

Fig. 4. The growth of *P.* × *hortorum* shoots on MS medium containing different concentrations of m-Topolin or m-Topolin in combination with the mixture of amino acids (glycine, glutamine, cysteine – each in a concentration of 10 mg l^{-1}); 1 – control, 2 – 0.5 mg l^{-1}, 3 – 1.0 mg l^{-1}, 4 – 1.5 mg l^{-1} of m-Topolin.

MATERIAL AND METHODS

The experiment was carried out on single shoots of *P.* × *hederaefolium* 'Bonete' and *P.* × *hortorum* 'Bargpalais' obtained from axillary buds derived from the plants growing in a greenhouse. The cultures of the shoots grew for 2 years on the modified MS (1962) medium containing 170 mg l^{-1} NaH_2PO_4, 100 mg l^{-1} myo-inositol, 1 mg l^{-1} thiamine, 1 mg l^{-1} pyridoxine, 1 mg l^{-1} nicotinic acid, 3% sucrose, 0.6% agar (Sigma) and different growth regulators (2iP, BAP, GA_3). The pH of the medium was adjused to 5.6 before autoclaving. This basal medium was used in all experiments. The shoots were subcultured at 3-4 week intervals into the fresh MS medium in Erlenmeyer flask (100ml) and kept at the temperature of 23-25°C, under 16h photoperiod provided by cool-white fluorescent lamps at 80 µmol m^{-2} s^{-1} (Philips TLD 36W/95).

In order to obtain a stimulation of shoot branching, single *Pelargonium* shoots were placed on the medium supplemented with BAP (0.25; 0.5; 1.0 mg l^{-1}) or m-Topolin (0.5; 1.0; 1.5 mg l^{-1}) added separately or in combination with the mixture of amino acids: glycine, glutamine, cysteine (each in the concentration of 10 mg l^{-1}). An addition the shoots were treated with the mixture of amino acids only. Explants growing on the medium without growth regulators were the control.

The experiment was repeated three times. One experimental treatment was represented by 25 explants (5 × 5 explants per Erlenmeyer flask). The number of axillary shoots, number of leaves, length of shoots (mm) and fresh weight of shoots (mg) were determined after 4 weeks. The results of the experiment were interpreted statistically and subjected to analysis of variance. Duncan's test was used to estimate the differences between means at P=0.05.

RESULTS AND DISCUSSION

In both *Pelargonium* species used in the experiment application of cytokinins greatly increased the axillary and adventitious shoot formation compared with the medium without growth regulators or supplemented with amino acid only. In the case of *P.* × *hederaefolium* no statistical differences in the number of shoots produced in the presence of BAP or m-Topolin were found (Table 1). However, the type of cytokinin greatly influenced the

TABLE 1. The influence of BAP, m-Topolin and amino acids on growth and development of shoots of *Pelargonium* × *hederaefolium* cv. Bonete in vitro.

Growth regulators [mg l^{-1}]	Fresh weight of shoots [mg]	Mean number of shoots	Mean length of shoot [mm]	Mean number of leaves per shoot
Control *	235.7 a	1.1 a	34.1 b	5.2 d
0.25 BAP	375.8 abc	3.1 bc	19.6 a	3.3 a
0.5 BAP	490.3 bcd	3.1 bc	16.6 a	4.1 abc
1.0 BAP	639.2 de	3.2 bc	15.5 a	4.3 bcd
0.5 Top. **	407.7 a-d	3.5 c	22.1 a	3.7 ab
1.0 Top.	489.3 bcd	3.1 bc	20.0 a	4.1 abc
1.5 Top.	518.3 bcd	3.4 c	16.6 a	4.1 abc
Amino acids***	282.8 ab	1.4 ab	29.8 b	4.9 cd
0.25 BAP + amino acids	424.5 a-d	3. 9 c	19.9 a	3.6 ab
0.5 BAP + amino acids	592.7 cde	3.4 c	16.8 a	4.1 abc
1.0 BAP + amino acids	795.5 e	3.4 c	18.5 a	4.1 abc
0.5 Top. + amino acids	524.8 cd	3.7 c	22.5 a	3.5 ab
1.0 Top. + amino acids	507.7 bcd	3.9 c	20.0 a	4.2 abc
1.5 Top. + amino acids	792.2 e	4.8 c	19.4 a	4.3 bcd

* MS medium without growth regulators
** m-Topolin
*** mixture of three amino acids (glycine, glutamine, cysteine)

TABLE 1. The influence of BAP, m-Topolin and amino acids on growth and development of shoots of *Pelargonium* × *hederaefolium* cv. Bonete in vitro.

Growth regulators [mg l^{-1}]	Fresh weight of shoots [mg]	Mean number of shoots	Mean length of shoot [mm]	Mean number of leaves per shoot
Control *	449.8 a	1.1 a	35.1 b	3.9 ab
0.25 BAP	526.7 ab	1.5 a	26.6 a	3.1 a
0.5 BAP	902.5 bc	2.6 c	23.7 a	3.4 ab
1.0 BAP	948.7 bc	2.9 cd	22.2 a	3.3 a
0.5 Top. **	766.3 abc	2.7 cd	26.3 a	3.7 ab
1.0 Top.	1076.3 c	3.6 d	24.5 a	3.9 ab
1.5 Top.	920.5 bc	2.8 cd	23.7 a	4.3 b
Amino acids***	369.0 a	1.0 a	36.5 b	3.0 a
0.25 BAP + amino acids	675.0 abc	1.6 ab	26.6 a	3.2 a
0.5 BAP + amino acids	980.7 bc	2.7 cd	23.0 a	3.0 a
1.0 BAP + amino acids	1065.0 c	2.7 bc	22.1 a	3.6 ab
0.5 Top. + amino acids	680.3 abc	2.4 bc	26.7 a	3.1 a
1.0 Top. + amino acids	957.0 bc	2.6 c	25.8 a	3.6 ab
1.5 Top. + amino acids	1025.7 c	3.2 cd	22.7 a	3.6 ab

* see Table 1

shoot quality. Well developed shoots without any deformation and with healthy green leaves were obtained on the medium supplemented with m-Topolin (Fig. 2). For *P.* × *hortorum,* the application of m-Topolin caused a higher shoot production (Table 2) and a perceptible improvement in foliage colour and their proper development (Fig. 4). Most effective in stimulation of axillary and adventitious shoot formation was m-Topolin at concentration of 1.0 mg l^{-1}, giving 3. 6 shoots per explant. The lowest multiplication rate of *P.* × *hortorum* shoots (1.5) was noted at the lowest concentration of BAP. These differences were shown to be statistically significant. Moreover, the shoots growing on the medium with BAP had light green leaves with a tendency to senescence (Fig. 3). Sporadically, the sign of anthocyanin was also noted. Generally, the application of cytokinins had an inhibitory effect on the elongation of *Pelargonium* shoots and resulted also in decreasing the number of leaves per shoot formation (particularly apparent for *P.* × *hederaefolium*) compared with the control medium without growth regulators and supplemented with amino acids only (Tables 1 and 2). The effect of m-Topolin in comparison with BAP was earlier examined on in vitro multiplied and rooted *Spathiphyllum floribundum* shoots (Werbrouck et al. 1996). These authors observed that the multiplication rate of shoots on the medium with m-Topolin was similar to that obtained in the presence of BAP, when the concentration of m-Topolin was twice as high than BAP. Moreover, the shoots derived from the m-Topolin medium were longer, with well developed dark green leaves, and rooted easier. Similar effects were noted by Podwyszyńska et al. (2000) for *Magnolia* and *Coccoloba*, whereas for *Rosa* m-Topolin had a rather negative effect. In the case of *Pelargonium* used in our experiment it seems very important, that m-Topolin resulted in good growth of shoots belonging to two different group: hortorum and peltatum.

It is known, that even cultivars of the same species require a specific regulator treatments. This fact considerably hindered the elaboration of a universal method of propagation of *Pelargonium* in vitro. Furthermore, our

investigation demonstrated, that the cultures of *Pelargonium* shoots maintained their capacity for morphogenesis after 2 years of growing in tissue culture conditions. As a rule, the preliminary studies were limited to obtain the regeneration and multiplication of *Pelargonium* shoots from preliminary explants and the authors observed a tendency to decrease the organogenetic potency with every subculture (Theiler 1977; Reuther 1983).

As mentioned above, on medium supplemented with amino acids only (mixture of glycine, glutamine, cysteine) no shoot production was observed. The addition of these amino acids increased slightly the number of formed shoots of *P.* × *hederaefolium* at each BAP and m-Topolin level, with the highest number (4.8) in presence of 1.5 mg l^{-1} m-Topolin (Table 2). A positive effect of amino acids was observed in the production of fresh weigh of *P.* × *hederaefolium* and *P.* × *hortorum* shoots when they were combined with the highest concentration of BAP or m-Topolin (Tables 1 and 2). Moreover, the shoots of both genotypes grown on the medium with m-Topolin and amino acids tended to have larger leaf blades, more uniform growth of shoots in clumps and a slower process of shoot senescence than those grown on medium containing m-Topolin only (Figs 2 and 4). According to several authors (Wetherell and Dougall 1976; Stuart and Strickland 1984; Armstrong and Green 1985), amino acids are an important source of nitrogen in plant metabolism and can be used either as a sole nitrogen source, as the sole reduced nitrogen source or as a supplement to the regeneration medium. Combinations of amino acids are known to increase the number and quality of somatic embryos formed of *Medicago sativa* (Stuard and Strickland 1984), *Zea mays* (Armstrong and Green 1985), and also influenced the maintenance of embryogenic potency of wheat cultures for a long time without decreasing their regeneration ability (Borelli et al. 1991). In the case of *P.* × *hortorum* and *P.* × *domesticum*, improvement of somatic embryos formation was noted in the presence of glutamine (5-30 mM) or proline (10-100 mM) (Marsolais et al. 1991; Gill et al. 1993). Yet, in available literature no information was found concerning the influence of amino acids in the multiplication stage of geranium shoot cultures. It seems to be clear from this study that the addition of the mixture of amino acids to the multiplication medium greatly increased the quality of *P.* × *hortorum* and *P.* × *hederaefolium* shoots. Little effect of the amino acids on shoot branching could result from a too low concentration of these compounds used in the experiment. Hence, further studies are nedeed to test the influence of different amino acids in wide range of concentrations on the growth and development of *Pelargonium* shoots.

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WPŁYW CYTOKININ I AMINOKWASÓW NA NAMNAŻANIE PĘDÓW *PELARGONIUM* × *HEDERAEFOLIUM* BONETE I *PELARGONIUM* × *HORTORUM* BARGPALAIS IN VITRO

STRESZCZENIE

Porównywano wpływ cytokinin (BAP i m-Topolin) podanych osobno lub w połączeniu z mieszaniną aminokwasów (glicyna, glutamina, cysteina) na namnażanie i jakość pędów *Pelargonium* × *hederaefolium* i *Pelargonium* × *hortorum* in vitro. Dla obydwu gatunków pelargonii cytokininy istotnie wpływały na tworzenie się pędów przybyszowych i na rozwój pąków kątowych. W przypadku *P.* × *hederaefolium* rodzaj i stężenie zastosowanych cytokinin nie powodowały istotnych różnic w ilości formujących się pędów, natomiast dla *P.* × *hortorum* bardziej intensywne namnażania pędów otrzymano w obecności m-Topolin. Ponadto dla obydwu gatunków pelargonii m-Topolin wpływała korzystnie na jakość pędów (intensywnie zielone prawidłowo wykształcone, bez deformacji). Dodanie aminokwasów do pożywki z cytokininą (zarówno BAP jak i m-Topolin) powodowało nieznaczny wzrost współczynnika namnażania pędów *P.* × *hederaefolium*. Ponadto obydwa gatunki pelargonii rosnące na pożywce zawierającej m-Topolin i aminokwasy charakteryzowały się większymi blaszkami liściowymi i bardziej wyrównanym wzrostem pędów w zespole namnożonych pędów w porównaniu z pędami rosnącymi w obecności samej m-Topolin.

SŁOWA KLUCZOWE: *Pelargonium*, namnażanie, cytokininy, aminokwasy, in vitro.