EFFECT OF *META*-TOPOLIN ON IN VITRO PROPAGATION OF *PELARGONIUM* × *HORTORUM* AND *PELARGONIUM* × *HEDERAEFOLIUM* CULTIVARS

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ABSTRACT

The aim of this study was to develop an efficient in vitro propagation method of *Pelargonium* plant (seven cultivars) using axillary buds and shoot tips as explants. The influence of the time period in which the explants were collected and the influence of the growth regulators (BAP, *meta*-topolin, IBA) were studied.

The explants taken in April had a higher regeneration ability than those isolated in July and September. The most efficient regeneration and axillary multiplication were achieved on the medium supplemented with *meta*-topolin. The application of BAP caused a lower regeneration potency of explants and resulted in a decrease of shoot quality with every subculture. Four of the six cultivars showed growth inhibition after three months of growth on BAP-medium. The highest multiplication rate (2.7-4.7 depending on genotype) and the high quality of shoots were noted on the medium supplemented with *m*T (0.5-1.0 mg l^{-1}). It is also very important to note that *m*T had stimulating effect on organogenesis in *P.* × *hederaefolium* and *P.* × *hortorum* cultivars over the long term. Moreover, *meta*-topolin had no after-effect on the growth and inhibition of rooting. Only one cultivar ('Sofie Cascade') rooted better on control medium without auxin. In case of the other cultivars, IBA added in concentrations of 0.01-0.1 mg l^{-1} had a stimulating effect on root production. The higher level of auxin inhibited root formation, stimulated senescence of shoots and had a negative after-effect on acclimatization in greenhouse conditions.

KEY WORDS: micropropagation, cytokinins, shoot and root formation, *Pelargonium*.

INTRODUCTION

The basic factor for a profitable mass production of *Pelargonium* × *hortorum* and *Pelargonium* × *hederaefolium* cultivars is a healthy starting material. Micropropagation based on axillary shoot growth is a good system for maintaining an in vitro stock of pathogen-free mother plants (Reuther 1988). The regeneration of *Pelargonium* cultures from axillary buds as well shoot tips and meristem explants has been reported by many researches (Hamdorf 1976; Theiler 1977; Reuther 1983, 1988; Cassells and Minas 1983; Cassells and Carney 1987; Horn 1988; Corneanu and Corneanu 1991; Desilets et al. 1993). However, very often regeneration was obtained indirectly by callus, which is a very easy form, especially in *Pelargonium* × *hederaefolium* cultivars. The main problem in the propagation of *Pelargonium* in vitro is fast senescence and loss of regeneration ability in the initiated shoots of various cultivars (Reuther 1988; Paludan 1991; Desilets et al. 1993; Mithila et al. 2001). It is a serious limiting factor in maintaining stock cultures over the long term.

The results of an earlier study showed that natural cytokinin – *meta*-topolin (*m*T) (Strnad et al. 1992; Strnad 1997), had a positive influence on shoot multiplication of *P.* × *hederaefolium* 'Bonete' and *P.* × *hortorum* 'Bergpalais' (Wojtania and Gabryszewska 2001). The objective of this study was to compare the effect of *m*T and BAP (the most widely recommended cytokinins in the propagation of this plant) on initiation of the culture, as well as long-term multiplication and rooting of seven cultivars of *Pelargonium* in vitro.

MATERIAL AND METHODS

The experiments were performed with seven *Pelargonium* cultivars: (*P.* × *hederaefolium* 'Luna', 'Sofie Cascade', 'Beach' and *P.* × *hortorum* 'White Rocky Mountain', 'Jazz Rocky Mountain', 'Grand Prix', 'Bergpalais').

Abbreviations
BAP – 6-benzylaminopurine; IBA – 3-indolebutyric acid; *m*T (*meta*-topolin) – 6-(3-hydroxybenzylamino)purine; MS – Murashige and Skoog medium; 2iP – isopentyladenine

In vitro cultures of shoots were established, multiplicated and rooted on MS media (Murashige and Skoog 1962) supplemented with 30 g l^{-1} sucrose, 0.1 g l^{-1} myo-inositol and solidified with LAB-AGAR (5.5 g l^{-1}). The pH of the medium was adjused to 5.6 before autoclaving. The shoots were subcultured at 3-4 weeks' intervals into fresh medium in an Erlenmeyer flask and kept at the temperature of 20°C, under a 16 h photoperiod provided by cool-white fluorescent lamps at 40 µmol $m^{-2}s^{-1}$ (Philips TLD 36W/95).

Culture establishment

Shoot-tips and axillary buds collected from *Pelargonium* plants grown in the greenhouse were used for culture initiation. The explants were excised in April, July and September and after sterilization (3% Chloramine T and 0.1% $HgCl_2$) were placed in the MS medium containing the cytokinin: BAP (0.1 mg l^{-1}) or *meta*-topolin (0.2 mg l^{-1}) and IBA at concentration of 0.02 mg l^{-1} (Wojtania and Gabryszewska 2004). One individual explant was placed in each 50 ml Erlenmeyer flask. At least 60 replications were used. The number of buds which developed into shoots was estimated after four weeks.

Shoot multiplication

The initiated *Pelargonium* shoots were then transferred to the medium supplemented with BAP (0.5 mg l^{-1}) or *meta*-topolin (0.5; 1.0 mg l^{-1}). Application of cytokinins in different concentrations resulted from their different activity noted for other genotypes (Werbrouck et al. 1996; Podwyszyńska et al. 2000) and *Pelargonium* cultivars (Wojtania and Gabryszewska 2001).

The shoots were subcultured every four weeks. The number of shoots was determined after 4, 8, 16, 20 and 24 weeks of the culture. The number of leaves and length of shoots (mm) were also measured of the explants grown on media without growth regulators (control) and supplemented with *m*T. The experiment was repeated three times. One experimental treatment was represented by 30 explants (6×5 explants per Erlenmeyer flask). Data were subjected to analysis of variance and the means were compared by Duncan's test at significance level P=0.05. Analysis of variance and differences between mean values were determined separately for each cultivar.

Rooting and acclimatization

The after-effect of the *m*T on the rooting of seven *Pelargonium* cultivars shoots was evaluated. The single shoots (>10 mm) were rooted on MS medium without growth regulators or supplemented with IBA at different concentrations (0.01; 0.1; 0.2; 0.5 mg l^{-1}). The number of rooted shoots was determined after three weeks. The experiment was repeated twice. One experimental treatment was represented by 30 explants (6×5 explants per Erlenmeyer flask). The rooted microcuttings were planted in a growing substrate composed of peat and perlite (1:4), supplemented with complete fertilizer (Azofoska) at 0.5 g l^{-1} and in relatively high humidity.

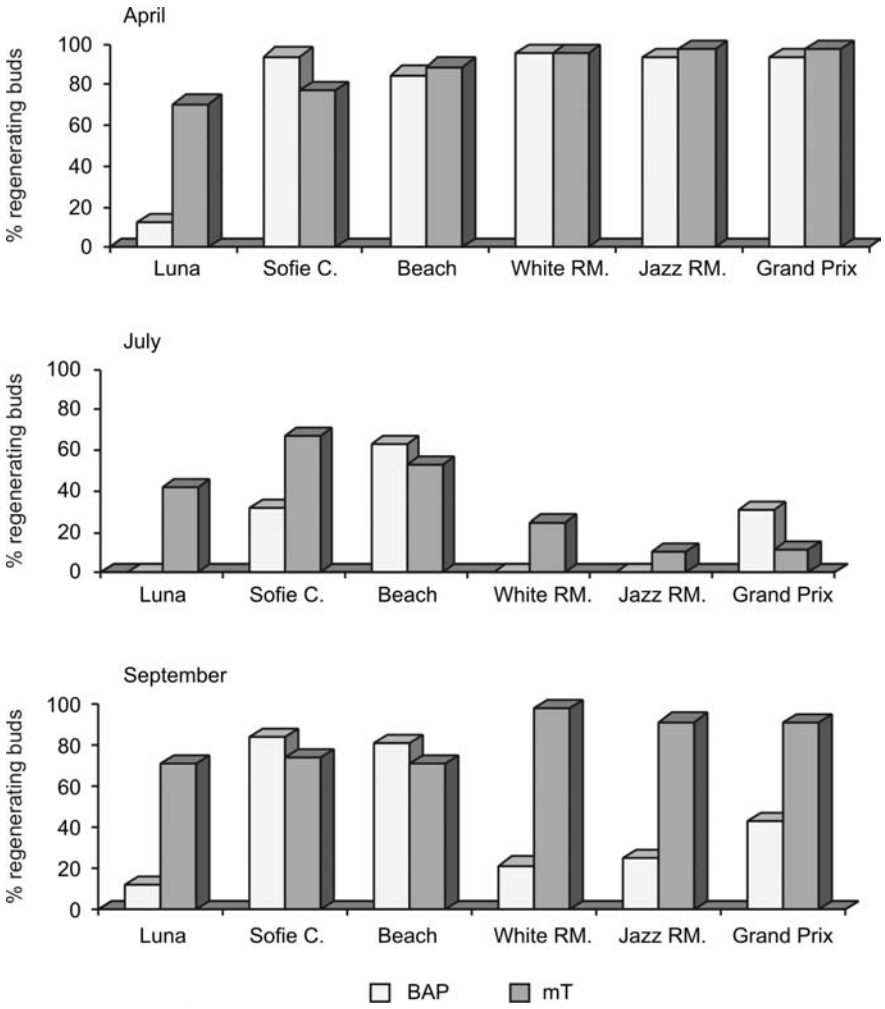

Fig. 1. The effect of type of cytokinin and the season in which explants were collected on the number (%) of buds which developed into single shoots of *P.* × *hederaefolium* ('Luna', 'Sofie-Cascade', 'Beach') and *P.* × *hortorum* ('White Rocky Mountain', 'Jazz Rocky Mountain','Grand Prix'). BAP (0.1 mg l^{-1}) or *m*T (0.2 mg l^{-1}) were added together with IBA (0.02 mg l^{-1}).

RESULTS AND DISCUSSION

Culture establishment

On MS medium supplemented with *meta*-topolin (*m*T) (0.2 mg l^{-1}), as well as BAP (0.1 mg l^{-1}) added together with IBA (0.02 mg l^{-1}), shoot tips and axillary buds of all *Pelargonium* cultivars developed into a single shoot. The efficiency of this process depended on type of the cytokinin, the term of explants collecting and genotype (Fig. 1). According to genotype the shoots developed in 2-4 weeks. The best initiation of *Pelargonium* cultures was observed in April (Fig. 1), when mother plant showed intensive vegetative growth. Similar results were obtained by Corneanu and Corneanu (1991) in *P.* × *hortorum* and *P.* × *domesticum*. In the present study there were no significant differences between cytokinin treatments in growth and development of buds in most *Pelargonium* cultivars (except for 'Luna') when explants were collected in the spring. Recorded were 85-97,8% of regenerating explants in the presence of BAP and 77,5-100% on the *m*T-medium (Fig. 1). In 'Luna' (recalcitrant in vitro), the use of *m*T resulted in a six-fold increase in number of buds which developed into shoots as compared with the BAP treatment. Moreover, application of *m*T enhanced, approximate three-fold the regeneration frequency of some *P.* × *hortorum* cultivars in July and two-fold, when explants were excised in September (Fig. 1). A lower regeneration ability of shoot buds in July and September could be correlated with changes in synthesis, transport, interaction and balance between endogenous phytohormones, as well as with changes in sensitivity of the cell to the exogenous cytokinin (Gaspar et al. 2003; del Pozo et al. 2005). In the case of *P.* × *hederaefolium* 'Luna', which showed only a little or no organogenic potency in the presence of BAP (depending on the term of explants' collecting), *m*T was able to stimulate the growth of shoots. In previous reports (Theiler 1977; Reuther 1983), using such cytokinins as BAP, 2iP and kinetin indicated that the recalcitrant genotype of *Pelargonium* developed only non organogenic callus and finally died. The present study showed that among the tested cytokinins only *m*T influenced the high quality of initiated shoots (well-developed, without vitrification). This is very important for further growth and development of *Pelargonium* in vitro.

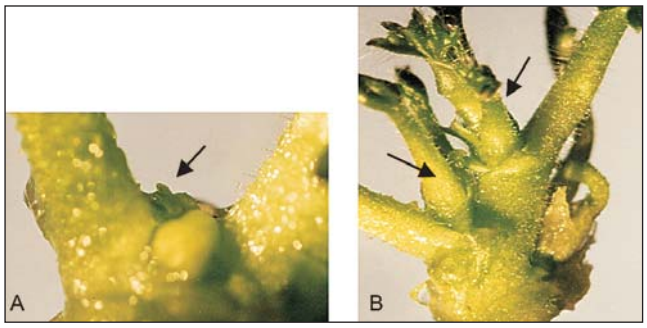

Fig. 2. The activity of axillary buds in *P.* × *hederaefolium* 'Beach' after three weeks of growth on the medium containing: A – BAP (0.5 mg l^{-1}), B – *m*T. 1.0 mg l^{-1}). Developing buds were marked with an arrow.

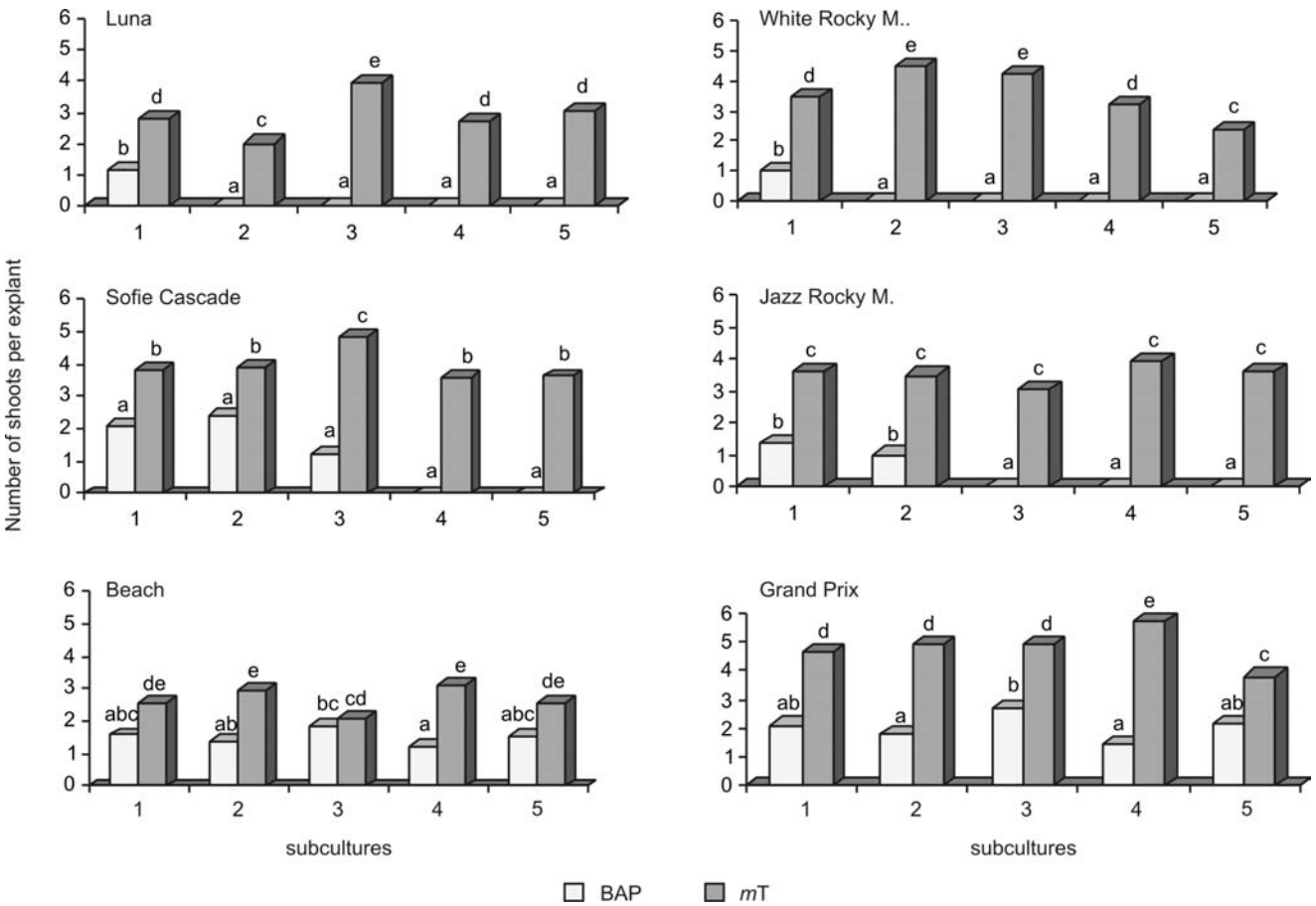

Fig. 3. The survival and multiplication rate of the different cultivars of *P.* × *hederaefolium* and *P.* × *hortorum* through five subcultures. Means of each cultivar assigned by the same letter do not differ significantly (P=0.05) with Duncan's test.

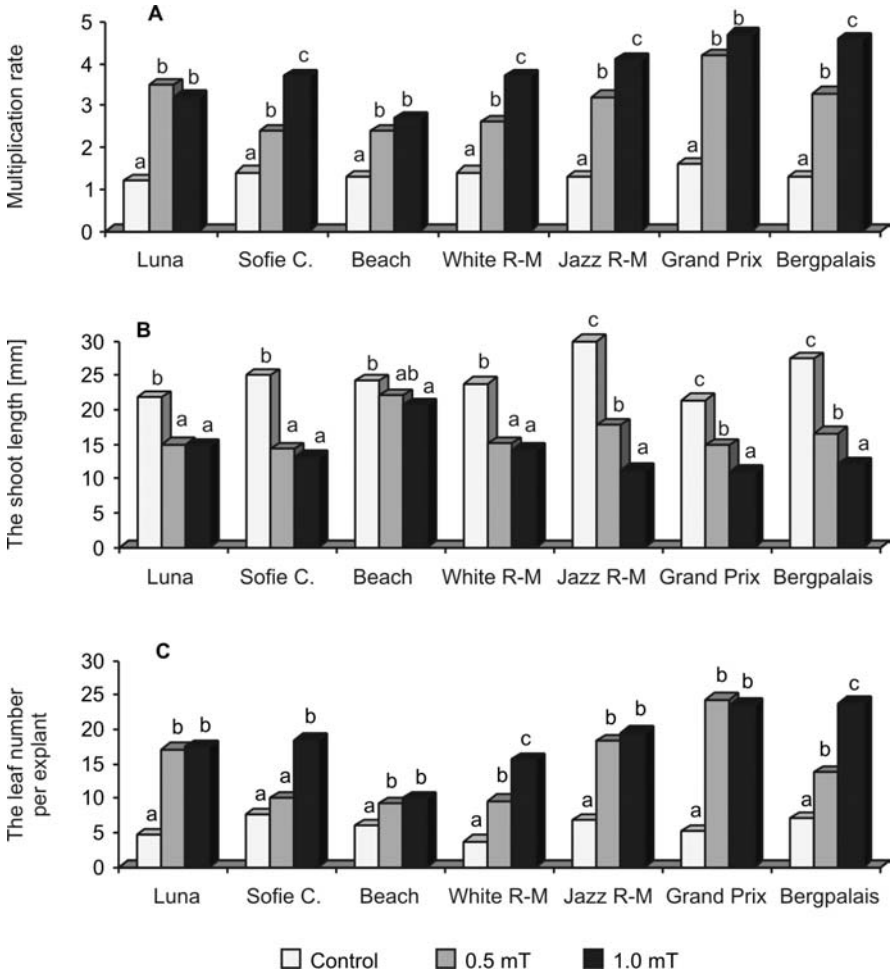

Fig. 4. The effect of different concentration of *meta*-topolin on the multiplication rate (A), the length of shoots (B) and the number of leaves (C) in 3 genotypes of *P.* × *hederaefolium* ('Luna', 'Sofie-Cascade', 'Beach') and 4 genotypes of *P.* × *hortorum* P ('White Rocky Mountain', 'Jazz Rocky Mountain', 'Grand Prix', 'Bergpalais'). Means of each cultivar assigned by the same letter do not differ significantly (P=0.05) with Duncan's test.

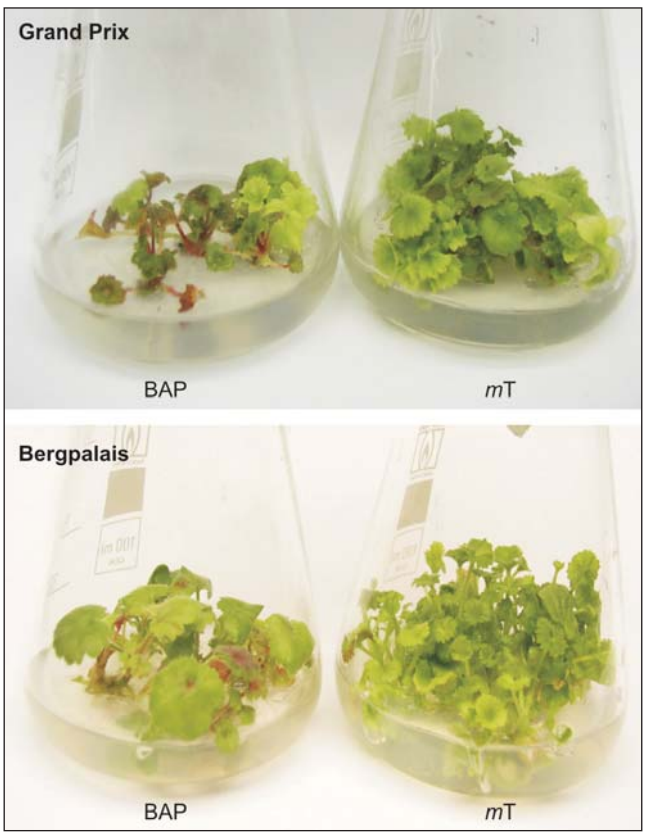

Fig. 5. The shoots of two *Pelargonium* cultivars after three weeks of growth on MS medium containing BAP or *m*T.

Shoot multiplication

The established, single shoots of different *Pelargonium* cultivars were transferred to a MS medium containing BAP (0.5 mg l^{-1}) or *m*T (0.5; 1.0 mg l^{-1}) for shoot multiplication. There were clear differences between the cytokinin treatments. Adventitious multiplication was found in the presence of BAP. The axillary buds did not develop into shoots (Fig. 2). Very often, the adventitious shoots were vitrified and deformed, the callus (intensively formed at the base of the shoots) had a tendency to browning and this kind of culture finally died. In 'Luna' BAP affected senescence of cultures after the first subculture, for 'Jazz Rocky Mountain' after the second subculture and for 'Sofie Cascade' after the third subculture (Fig. 3). In the case of genotypes with red flowers ('Beach' and 'Grand Prix'), BAP stimulated adventitious shoot formation over the long term, but the multiplication rate was lower than those obtained on *m*T-medium; the average 1,0-1,9 shoots/explant (Fig. 3).

The present study showed that in contrast to BAP, in the presence of *m*T it was possible to obtain axillary multiplication of all *Pelargonium* cultivars (Fig. 2 and 3), even for the next few years (unpublished data). Moreover, it was observed that the effectiveness of shoot formation depended on the cultivar and varied between subcultures (Fig. 4). The highest multiplication rate (average 4.8 shoots/eksplant) was obtained in *P.* × *hortorum* 'Grand Prix' by using 1.0 mg $^{-1}$ *m*T and the lowest (2.7 shoots/explants) in *P.* × *hederaefolium* 'Beach' (Fig. 4). A similar concentration of *m*T was optimal for effective multiplication of shoots in

other plants, such as *Musa* (Escalona et al. 2003), *Aloe polyphylla* (Bairu et al. 2007), *Magnolia* × *soulangeana*, *Coccoloba uvifera* i *Actinidia chinensis* (Podwyszyńska et al. 2000). As Werbrouck et al. (1996) and Podwyszyńska et al. (2000) reported, some plants needed a two-fold higher concentration of *m*T than BAP to obtain a similar number of shoots. In *Beta vulgaris* cultures, *m*T was more active than zeatin (Kubaláková and Strnad 1992). The present study showed also that an increased concentration of *m*T (0.5-1.0 mg $^{-1}$) enhanced the number of leaves per explants and decreased the length of shoots (Fig. 4). For the same genotype ('Luna', 'Grand Prix') the first symptoms of vitrification were also observed. A further increase in the *m*T level up to 1.5 mg l^{-1} stimulated shoot formation, but simultaneously a rapid decrease of shoot quality was noted (vitrification, deformation and occasionally fasciation of shoots) (data not presented).

Similar to *Beta vulgaris*, *Musa* AAB, *Malus* × *domestica* and *Aloe polyphylla* (Kubalákowa and Strnad 1992; Escalona et al. 2003; Dobránszki et al. 2002; Bairu et al. 2007) in *Pelargonium*, *m*T resulted in higher shoot quality than the other cytokinin. As showed in this study, on *m*T-medium all *Pelargonium* cultivars produced juvenile, well developed shoots, with high chlorophyll content (Fig. 5). Moreover, *m*T was able to prevent the senescence of *Pelargonium* shoots. The inhibition of senescence by *m*T was previously observed in the wheat chlorophyll retention bioassay (Holub et al. 1998; Palavan-Ünsal et al. 2004), radish cotyledons (Cað and Palavan-Ünsal 2003) and potato 'Kennebec' cultures as well *Zantedeschia aethiopica* fruit (Baroja-Fernandez et al. 2002). It is known that formation of not organonogenic callus and fast senescence of cultures were the major factors, which limited obtaining the long-term multiplication of *Pelargonium* shoots in vitro (Desilets et al. 1993; Mithila et al. 2001). It is also very important that *m*T had a stimulating effect on shoot formation for *P.* × *hederaefolium*, as well as *P.* × *hortorum* cultivars. They often exhibited variety-specific differences in regeneration potential and required specific growth regulators (Hamdorf 1976; Theiler 1977; Reuther 1983).

Werbrouck et al. (1996) demonstrated that the difference between BAP and *m*T on cultures of *Spathiphyllum floribundum* resulted from slight structural differences. It is known that unlike BAP, *m*T is translocated faster in plant tissue, which prevents its localized accumulation (Kaminek et al. 1987). Current knowledge suggests that responses to cytokinin are mediated by a family of receptors, which regulate a signal-transduction pathway (Heyl and Schmülling 2003). As reported by Mok et al. (2005), BAP and *m*T have a different affinity in receptor recognition. The authors found that *m*T like *trans*-zeatin interacts with the *Arabidopsis* AHK4 receptor while BAP and TDZ with the maize ZmHK1 receptor.

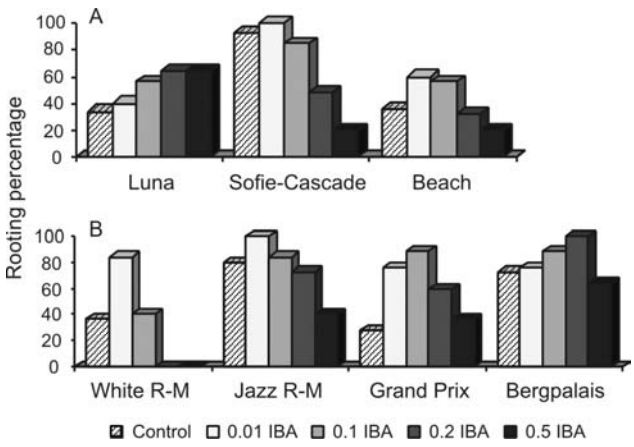

Fig. 6. The in vitro rooting of *Pelargonium* on control medium (without growth regulators) or after treatment with different IBA concentrations. A – *P.* × *hederaefolium* cultivars; B – *P.* × *hortorum* cultivars. Means of each cultivar assigned by the same letter do not differ significantly (P=0.05) with Duncan's test.

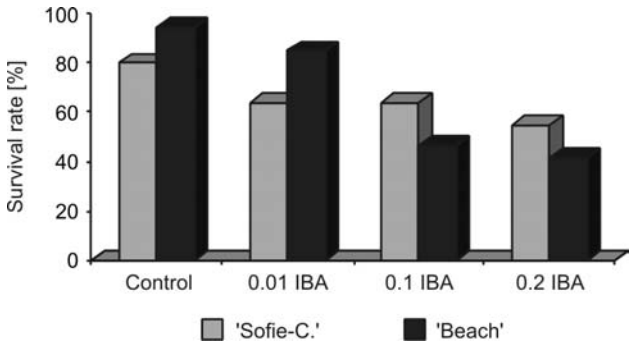

Fig. 7. The after-effect of IBA on survival rate of *Pelargonium* microcutting in greenhouse conditions.

Rooting of shoots

It is known that root formation in *Pelargonium* plants in vitro depends on the quality of the shoots. As reported by Reuther (1983) and Corneanu and Corneanu (1991) only well-developed shoots, longer than 1.0 cm, were easy to root. The present study showed that the shoots of all the *Pelargonium* cultivars derived from the *m*T-medium, produced roots on the control medium (without growth regulators) as well in the presence of IBA (Fig. 6). The ability to root depended on the genotype. The lowest rooting percentage (56%) was noted for *P.* × *hederaefolium* 'Beach' (Fig. 6). 'Sofie Cascade' rooted better on the control medium than on those supplemented with auxin. In other cultivars, IBA stimulated the root formation, but only at low concentrations (0.01-0.1 mg l^{-1}). A high level of auxin, inhibited root formation, stimulated senescence of shoots and had a negative after-effect on acclimatisation in greenhouse conditions (Fig. 7).

CONCLUSIONS

The results of the present study indicated that *m*T plays an important role in morphogenesis of *Pelargonium* in vitro. This cytokinin has a positive influence both on growth and development of shoots and their quality. The use of *m*T makes possible the initiation of cultures of recalcitrant cultivars in the wider range of deadlines as compared with BAP, axillary multiplication of over the long term and further rooting of shoots. Its positive influence on *P.* × *hederaefolium* as well as *P.* × *hortorum* is also very important. The above results suggest that *m*T can be very useful in micropropagation of various *Pelargonium* cultivars.

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