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Effect of meta-topolin on leaf senescence and rooting in *Pelargonium* × *hortorum* cuttings

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ABSTRACT

Pelargonium (*Pelargonium* x *hortorum*) is grown as potted or bedding plants for their colourful, showy flowers and scented foliage. Absence of senescence symptoms in the leaves of *Pelargonium* cuttings, their capacity to initiate roots and continued growth of initiated roots is an important quality attribute. The effects of postharvest treatments with meta-topolin (mT) and thidiazuron (TDZ) to 'Katinka' *Pelargonium* cuttings were investigated. Leaves treated with mT or TDZ for 5 d had higher leaf chlorophyll contents than untreated controls. Exposing cuttings to mT had no effect on the rooting proportion (%) and average root diameter. Similarly, 0.05 mM mT had no effect on number of roots per cutting. However, mT slightly reduced root length, root surface area and total volume of the roots. TDZ severely inhibited adventitious root formation, thus it reduced all the root parameters investigated. In conclusion, mT is very active in retarding leaf senescence, and combined with the observed ease of rooting of cuttings after mT treatment, this treatment is a suitable alternative to TDZ in delaying the onset of leaf yellowing in ornamental crops.

1. Introduction

Pelargonium (*Pelargonium zonale* L.) hybrids are grown as potted or bedding plants for their colourful, showy flowers and scented foliage and are very popular in Europe and North America (Serek et al., 1998). European growers import *Pelargonium* cuttings from Africa, Latin America, the Canary Islands or the Middle East since these regions have favourable climate, ready supply of cheap labour and land as compared to Europe (Serek et al., 1998). During shipment, *Pelargonium* cuttings are exposed to adverse conditions such as ethylene, water stress and darkness, which induce leaf senescence.

Senescence in ornamental plants appears as leaf yellowing due to chlorophyll loss. This is a significant problem in *Pelargonium* (Mutui et al., 2005), roses (Bogaert et al., 2006) *Alstroemeria* (Mutui et al., 2006) and lilies (Han, 1997). Senescing leaves exhibit a characteristic yellowing, starting at the veins and extending outwards (Quirino et al., 2000). Absence of senescence symptoms in the leaves of *Pelargonium* cuttings, their capacity to initiate roots, and continued growth of initiated roots are important quality attributes (Purer and Mayak, 1988). After senescence, cuttings show reduced rooting percentages and root numbers. Thus, the ideal *Pelargonium* cuttings should root easily, and have many long roots with high surface area and volume.

Cytokinins are potent anti-senescence hormones and they play an important role in delaying the onset of leaf senescence (Thimann, 1980). Thidiazuron (TDZ), a substituted phenyl urea with powerful cytokinin-like activity has been shown to be very effective in preventing leaf yellowing and retarding chlorophyll degradation, but it inhibited rooting in *Pelargonium* cuttings (Mutui et al., 2005). Strnad et al. (1997) discovered a new family of endogenous aromatic cytokinins which was named meta-topolin (mT), 6-[3-hydroxylbenzylamino] purine. Methoxy and hydroxyl derivatives of 6-benzylaminopurine (BAP) were shown to have a strong cytokinin activity in various bioassays (Holub et al., 1998). The activity of the mT in delaying chlorophyll degradation in excised wheat leaves was double that of BAP (Tarkowská et al., 2003). Also, applying mT exogenously increased chlorophyll contents in wheat leaf segments (Palavan-Ünsal et al., 2002, 2004).

The use of mT has been reported to increase shoot multiplication, maintain histogenic stability, improve rooting efficiency and subsequently reduce production costs in *in vitro* systems (Werbrouck et al., 1996; Bogaert et al., 2006). Consequently, mT has been advocated as a suitable alternative to BAP in micropropagation (Werbrouck et al., 1996; Bogaert et al., 2006; Bairu et al., 2007). This is because mT had the best anti-senescing effects in *in vitro* propagated roses compared to 2.5 μM of BAP, meta-methoxytopolin (MemT), 6-(3-methoxybenzylamino)-purine-9-riboside (MemTR) or 6-(3-fluorobenzylamino)-purine-9-riboside

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(FmTR) (Bogaert et al., 2006). In contrast to BAP or TDZ, which inhibit root formation, mT lacked *in vitro* root inhibition activity and plants treated with it rooted readily post *in vitro*. Furthermore, the abnormality index, which is the ratio of abnormal (hyperhydric shoots and shoots with shoot-tip necrosis) to normal shoots in all the mT treatments used in *in vitro* propagation of *Barleria greenii* was much lower than that recorded at the lowest BAP concentration (Amoo et al., 2011). Abnormality index is a measure of the toxic nature of a cytokinin (Bairu et al., 2007). The objective of this study was to investigate the effects of mT in retarding leaf senescence and improving postharvest quality of *Pelargonium* cuttings.

2. Materials and methods

2.1. Plant material

A new *Pelargonium* × *hortorum* cultivar, 'Katinka', was obtained from Selecta Klemm GmbH & Co., KG, Stuttgart, Germany. It was rooted in a commercially produced soil (Einheitserde, Werkverband E.V., Germany) and re-potted into 14 cm diameter pots 4 weeks later. The cuttings were subsequently grown in an experimental greenhouse at the Leibniz University of Hannover, Germany, under the following conditions: 22 °C day/20 °C night temperatures with 16 h supplementary irradiance of 100 μmol m^{-2} s^{-1} from SON-T lamps (Osram, 400 W, Philips, The Netherlands) to produce stock plants that provided experimental materials. An automatic fertigation system was used to apply (w/w): 0.75% Wuxal® Super fertiliser (8% N, 8% P_2O_5, 6% K_2O, 0.01% B, 0.007% Cu, 0.015% Fe, 0.013% Mn, 0.001% Mo, and 0.005% Zn; Wilhelm Haug GmbH & Co. KG, Ammerbuch-Pfäffingen, Germany) to the plants 1–3 times/week, depending on the prevailing weather conditions.

2.2. Harvesting and evaluation of cuttings

Terminal cuttings were harvested and handled according to Mutui et al. (2010). The cuttings were exposed to various treatments as described below. They were then rooted under hydroponics in the greenhouse to evaluate their rooting potential according to Mutui et al. (2010).

2.3. Meta-topolin (mT) and thidiazuron (TDZ) treatment

mT (Duchefa, Haarlem, The Netherlands) and TDZ (Duchefa, Haarlem, The Netherlands) were dissolved in 1 M KOH to prepare separate stock solutions. Deionised water containing 0.2% (v/v) Tween 20 (Duchefa) as a wetting agent was used to separately prepare 0.05, 0.1 and 0.2 mM mT solutions and 5 μM TDZ solutions. In our previous study, we tested 5, 10 and 20 μM TDZ solutions (Mutui et al., 2005) and we found 5 μM TDZ to be effective in retarding chlorophyll degradation but it severely inhibited root formation, hence it was used as a positive control. The foliage on cuttings was immersed completely in a mT or TDZ solution for 1 min. Care was taken that no solution reached the stem base. Control cuttings were immersed in deionised water containing 0.2% (v/v) Tween 20. After treatment, the cuttings were laid on absorbent paper to dry for 30 min.

2.4. Chlorophyll determination

Three 8 mm-diameter discs were excised from the left, centre and right of each leaf blade without petiole using a cork borer. Chlorophyll contents were analysed according to Lichtenthaler (1987). Extraction was in 80% (v/v) ethanol at 75 °C for 10 min. Absorption was measured using a SmartSpec™ 3000 Spectrophotometer (BioRad, CA, USA) at 647, 664 and 700 nm. Chlorophyll content was then calculated using the equation:

$$\text{Chlorophyll a + b} = 5.24(A664 - A700) + 22(A647 - A700)$$

where A is the absorbance at 647 nm, 664 nm or 700 nm. The results were expressed as mg chlorophyll cm^{-2} leaf area.

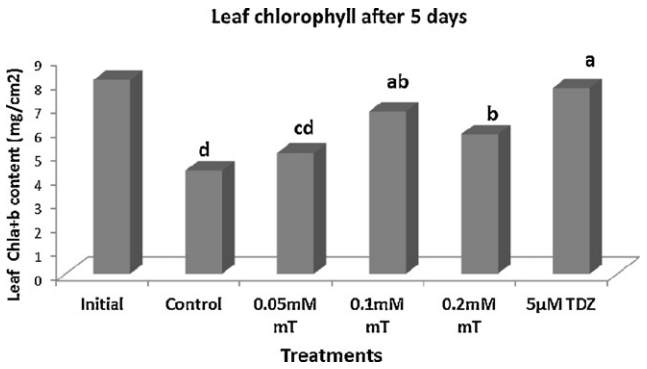

Fig. 1. Effect of application of meta-topolin (mT) and thidiazuron (TDZ) on total leaf chlorophyll a + b content in 'Katinka' *Pelargonium* cuttings. Initial chlorophyll content was determined at the beginning of the experiment before application of the treatments to show effect of treatments on chlorophyll degradation. Means of treatments and control (not initial reading) were separated by Tukey's Honest Significant Difference (HSD), $P = 0.05$. Means followed by the same letter(s) within columns are not significantly different.

2.5. Rooting procedure

Rooting of cuttings was done in a greenhouse according to Mutui et al. (2010). The rooting period was 4 weeks.

2.6. Rooting parameters

The proportion (%) of rooted cuttings was determined by counting the number of rooted cuttings against the initial number that was placed in the rooting solution. The roots were excised from the base of the cuttings using scalpel blades and placed in plastic cans containing tap water. The total number of root, root length (cm), root surface area (cm^2), root volume (cm^3) and average root diameter (mm) were determined using a root scanner (Epson GT-10,000 Scanner TWAIN Pro 2.10).

2.7. Statistical analysis

Experiments were conducted in a completely randomised design, replicated three times with five cuttings per replication. Data were subjected to analysis of variance (ANOVA) using the general linear model (Proc GLM) of the Statistical Analysis System (SAS, 2002) computer programme. Multiple comparisons among treatment means was done using the protected Tukey's Honest Significant Difference (HSD) at $P = 0.05$.

3. Results

3.1. Leaf chlorophyll content

Chlorophyll contents of *Pelargonium* leaves treated for 5 d with 0.1 or 0.2 mM mT were higher than untreated controls (Fig. 1). However, 0.05 mM mT was not different from the untreated control with respect to leaf chlorophyll content (Fig. 1). TDZ at 5 μM had the highest leaf chlorophyll content and it was not different from 0.1 mM mT (Fig. 1).