EFFECTS OF PHYTOHORMONES AND DARK STORAGE ON POSTHARVEST QUALITY OF *PELARGONIUM* CUTTINGS

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ABSTRACT
Maintenance of the leaf green colour, capacity to initiate roots and continued growth of initiated roots are important quality attributes in *Pelargonium* cuttings. Leaf senescence is a common problem in *Pelargonium* and leads to high postharvest losses. Ethylene, abscisic acid (ABA) and darkness have been implicated in promoting senescence, while cytokinins retard it. The effects of post-harvest treatments with ethylene, Thidiazuron (TDZ), ABA or dark storage were investigated to determine a potential commercial approach for improving postharvest quality of *Pelargonium* cuttings. Experiments were conducted in a completely randomized design, with two replications. The data obtained were subjected to a single factor analysis of variance using Statistical Analysis System programme. Storing cuttings in the dark for 4 days and/or treating them with 2 µL/L ethylene or 100 µM ABA hastened the onset of visible leaf yellowing in 'Fire', 'Ganymed' and 'Katinka'. Conversely, 5 µM TDZ markedly delayed the onset of leaf senescence in all the cultivars. Thidiazuron increased leaf hue values (greenness), while ethylene, ABA or dark storage decreased leaf hue (hastened yellowing). Exposing *Pelargonium* cuttings to ethylene reduced root fresh weight in 'Katinka', but had no effect on root dry weight and root water content. Storing *Pelargonium* cuttings for 4 days in the dark reduced root fresh weight, root dry weight and water content in 'Katinka', but this was not apparent in other cultivars. Applying, 100 µM ABA reduced root fresh weight, root dry weight and water content in all cultivars. Thidiazuron severely inhibited adventitious root formation in all cultivars. The inhibitory effect was overcome by inclusion of indole-3-butyric acid (IBA) in the nutrient solutions. Thidiazuron counteracted the deleterious effects of dark storage, ethylene and ABA by delaying the onset of leaf yellowing in *Pelargonium* cuttings during storage and/or shipment. Thidiazuron should be applied to *Pelargonium* cuttings as a postharvest treatment to prevent subsequent leaf yellowing. Cuttings should not be stored under darkness or water stress to prevent accumulation of ethylene and ABA. After TDZ

treatment, transportation and storage, *Pelargonium* cuttings should be dipped in 4 µL/L IBA to induce root development and enhance quality.

Key words: Abscisic acid, Ethylene, *Pelargonium zonale*, Rooting, Storage, Thidiazuron

INTRODUCTION

European and North American growers depend on imports of *Pelargonium* cuttings and other tropical foliage plants from Africa (Serek et al., 1998). Africa is suited for the production of cuttings because of presence of favourable climate throughout the year, cheap labour and readily available land. *Pelargonium* cuttings are subsequently rooted and finished in importing countries (Serek et al., 1998). The delivery process from Africa to Europe can take between 4 to 7 days. However, during shipment, cuttings are exposed to adverse conditions such as ethylene, water stress and darkness (Purer and Mayak, 1989), which induce leaf senescence (Behrens, 1988).

Absence of senescence symptoms in the leaves, capacity to initiate roots and continued growth of initiated roots in *Pelargonium* cuttings are important quality attributes (Purer and Mayak, 1988). Cuttings of high quality are essential in the highly competitive market. This is because senescing leaves turn yellow, thus reducing acceptability of cuttings to customers and they become more prone to infection by diseases such as *Botrytis*. This in turn affects the rate of development and survival of new plants that take longer to get established and start vigorous growth (Purer and Mayak, 1988). Furthermore, application of fungicides and careful handling measures tend to increase cost of production and are, therefore, undesirable in the competitive world market (Purer and Mayak, 1988).

Leaf senescence is a complex physiological process that may result from one or several inducers and is thought to be under control of phytohormones (Weaver et al., 1998). Ethylene, ABA and darkness have been implicated as important factors in promoting leaf senescence (Purer and Mayak, 1989). Senescing leaves are readily recognisable by a characteristic yellowing, starting at the veins and extending outwards, resulting in a loss of chlorophyll (Quirino et al., 2000). This in turn leads to a rapid decline in photosynthesis, as chlorophyll is lost. Consequently, rooting of the cuttings is delayed (Purer and Mayak, 1989). Smart (1994) proposed that the decrease in photosynthesis below a certain threshold level might function as a signal to induce senescence.

Leaf yellowing and chlorophyll degradation can be reduced or eliminated in a wide range of species by application of cytokinins (Thimann, 1980). Cytokinins are powerful inhibitors of leaf senescence (Gan and Amasino, 1996). Pulsing with benzyladenine (BA) effectively reduced leaf yellowing and improved the postharvest vase life of *Alstroemeria* (Mutui et al., 2004) and other plant species (Halevy and Mayak, 1981). Recently, Thidiazuron (TDZ), a substituted phenyl urea with powerful cytokinin-like activity, has been reported to be very effective in preventing leaf yellowing and retarding chlorophyll degradation in *Pelargonium* cuttings (Mutui et al., 2005), *Alstroemeria* cut flowers (Ferrante et al., 2002), cut tulips and cut chrysanthemum (Ferrante et al., 2003). But, TDZ treatment inhibited rooting in *Pelargonium* cuttings (Mutui et al., 2005), thus limiting its practical use. Therefore, the aim of the present study was to investigate whether TDZ could be used to prevent leaf yellowing in *Pelargonium* cuttings emanating from dark storage, ABA and ethylene as stress-response agents. Indole-3-butyric acid (IBA)-treatment was added into nutrient solutions to alleviate the rooting inhibition effect of TDZ.

MATERIALS AND METHODS

Plant Materials

Three new *Pelargonium zonale* cultivars ('Fire', 'Katinka' and 'Ganymed') were obtained from a commercial breeder (Selecta Klemm GmbH & Co. KG, Stuttgart, Germany). They were rooted in a commercially produced soil (Einheitserde, Werkverband EV, Germany) and re-potted into 14 cm diameter pots 4 weeks later. They were subsequently grown in a greenhouse at the University of Hannover under the following conditions: 22^{o}C day/20^{o}C night temperatures with 16 h supplementary irradiance of 100 $\mu moles \cdot m^{-2} \cdot s^{-1}$ from SON-T lamps (Osram, 400W, Philips, The Netherlands) between October and December to produce stock plants that provided experimental materials. An automatic fertigation system was used to apply (w/w): 0.75% Wuxal^{R} Super fertiliser solution (8% N, 8% P_2O_5, 6% K_2O, 0.01% B, 0.007% Cu, 0.015% Fe, 0.013% Mn, 0.001% Mo, 0.005% Zn; Wilhelm Haug GmbH & Co. KG, Ammerbuch-Pfäffingen, Germany) to the plants 1 to 3 times per week, depending on the prevailing weather conditions.

Harvesting and Evaluation of Cuttings

Terminal cuttings were harvested with sterilised knife after 19 weeks growth, leaving the first two leaves of the axillary shoot on the stock-plant. The cuttings were at most 6 cm long and had four leaves of which at least one was fully developed. After harvesting, cuttings were transferred immediately to an interior environment room (IE) maintained at 21^{o}C±1^{o}C and 60% RH with continuous light from cool-white fluorescent tubes (20 $\mu moles \cdot m^{-2} \cdot s^{-1}$).

They were exposed to various treatments as described below. The air in the IE was ethylene-free. Evaluation of the cuttings was done 4 days after the application of treatments. Leaf samples were taken from the cuttings for colour determination before they were rooted under hydroponics in greenhouses to evaluate their rooting potential.

Dark Storage

Cuttings were placed in polyethylene bags and the top of the bag was tightly sealed with a band. They were then packed randomly into boxes and stored at 21^{o}C±1^{o}C in the dark for 4 days to simulate transport conditions.

Ethylene Sensitivity

Cuttings were placed in sealed glass chambers. Ethylene gas was injected with a hypodermic syringe to give 0, 0.5, 1 and 2 µL/L. This procedure was repeated daily after 1 hour ventilation of the glass chambers for 4 days. Ethylene concentrations inside all the chambers were monitored using a portable digital gas chromatograph (GC Voyager FFKG312, Perkin-Elmer, Markham, Ontario, Canada) equipped with a photo-ionisation detector. The carrier gas was N_2 at 40 ml per minute, the injection pressure was 69 kPa, the oven temperature was 60^{o}C and the column temperature was 60^{o}C. Control treatment cuttings were kept sealed in an identical glass chamber, but without ethylene.

TDZ, ABA and IBA Treatments

Thidiazuron (Sigma-Aldrich GmBH, Munich, Germany), ABA (Precision Biochemicals, Cologne, Germany) and IBA (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 wetting agent was used to prepare 5, 10 and 20 µM TDZ and 25, 50 and 100 µM ABA solutions. The foliage on cuttings was immersed completely in a TDZ or ABA solution for 1 minute. 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 minutes. Aliquots of IBA stock solution were dissolved in 10 L nutrient solutions (as described below) to make fresh 4, 8 and 12 µL/L IBA solutions at the beginning of each experiment. Control cuttings were placed in nutrient solutions without IBA.

Colour Measurement

Representative colour measurements were performed in triplicate on the surface of individual leaves using a Minolta Chroma Meter (Model CR-300, Minolta, Osaka, Japan). This tri-stimulus colour analyser consists of a head

with an 8 mm-diameter measuring area, diffuse illumination and a 0^o viewing angle. It was initially calibrated with a white tile and checked between measurements. Three measurements were taken on the left, right and centre of each leaf blade at the start of the experiment. Readings were taken 4 days after dark storage, TDZ, ethylene or ABA treatment. The three parameters of brightness (L^*), red-to-green scale (a^*) and yellow-to-blue scale (b^*) were recorded. Hue was calculated using the formula: Hue = arc tan (b/a). Low hue values indicated that the leaves had turned yellow due to senescence. High hue values indicated that the leaves remained green.

Rooting of Cuttings

Rooting was done in a greenhouse under the following conditions: $24^oC\pm 1^oC$ inside the rooting chamber, 97± 1% RH, and supplementary irradiance from SON-T lamps (Osram, 400W) at 60 $\mu moles \cdot m^{-2} \cdot s^{-1}$. The cuttings were placed in grey Styropor^{TM} plates and floated on nutrient solution in 10 L containers. Containers were covered with non-transparent white polyethylene which, together with the grey Styropor^{TM} plates, substantially reduced the amount of light reaching the base of the cuttings. The nutrient solution was aerated continuously to prevent oxygen depletion. The composition of the nutrient solution was as follows: (mg/L salt): NH_4NO_3, 12; K_2PO_4, 162.8; $MgSO_4$, 71.2; KNO_3, 174; $Mg(NO_3)_2$, 487; FeEDTA, 12; MnSO4, 1.9; $ZnSO_4$, 2.4; $CuSO_4$, 0.36; H_3BO_3, 1.9; $NaMoO_4$, 0.16; NaCl, 25.5; and $Ca(NO_3)_2$, 861. Propagation vessels were placed under a white polyethylene tent to increase humidity. The rooting period was 4 weeks.

Rooting Parameters

The roots were excised from the base of the cuttings using scalpel blades. They were weighed using a balance ELE (Sartorious GmbH, Göttigen, Germany) to obtain fresh weight. Root fresh weight is a function of dry matter accumulation emanating from the growth of roots. The freshly the roots are, the more nutrients they absorb from the nutrient solution. The root samples were then wrapped in aluminium foil, oven-dried at 66°C for 72 hours to a constant weight using incubator (Memmert GmbH, Schwabach, Germany), cooled in a desiccator for 30 minutes and weighed for dry weight. Root water content was determined by subtracting root dry weights from their corresponding fresh weights. Root water content is a measure of freshness of roots.

Experimental Design and Data Analysis

Experiments were conducted in a completely randomised design, replicated twice with four cuttings per replication. Data were subjected to a single factor analysis of variance using the general linear model (Proc GLM) of the

Statistical Analysis System (SAS, 2002) computer programme. The cultivars were analysed separately because they are independent from one another and to demonstrate wide applicability of the treatment effects. In addition, whether a cultivar has high chlorophyll or hue value is not of value. Multiple comparisons among treatment means was done using the Least Significant Difference (LSD) or Student's *t* test at $P = 0.05$.

RESULTS

Effects on Leaf Colour

Short-term dark storage for 4 days hastened the onset of visible leaf yellowing, as evidenced by a decrease in leaf hue in 'Fire', 'Katinka' and 'Ganymed' (Figure 1A).

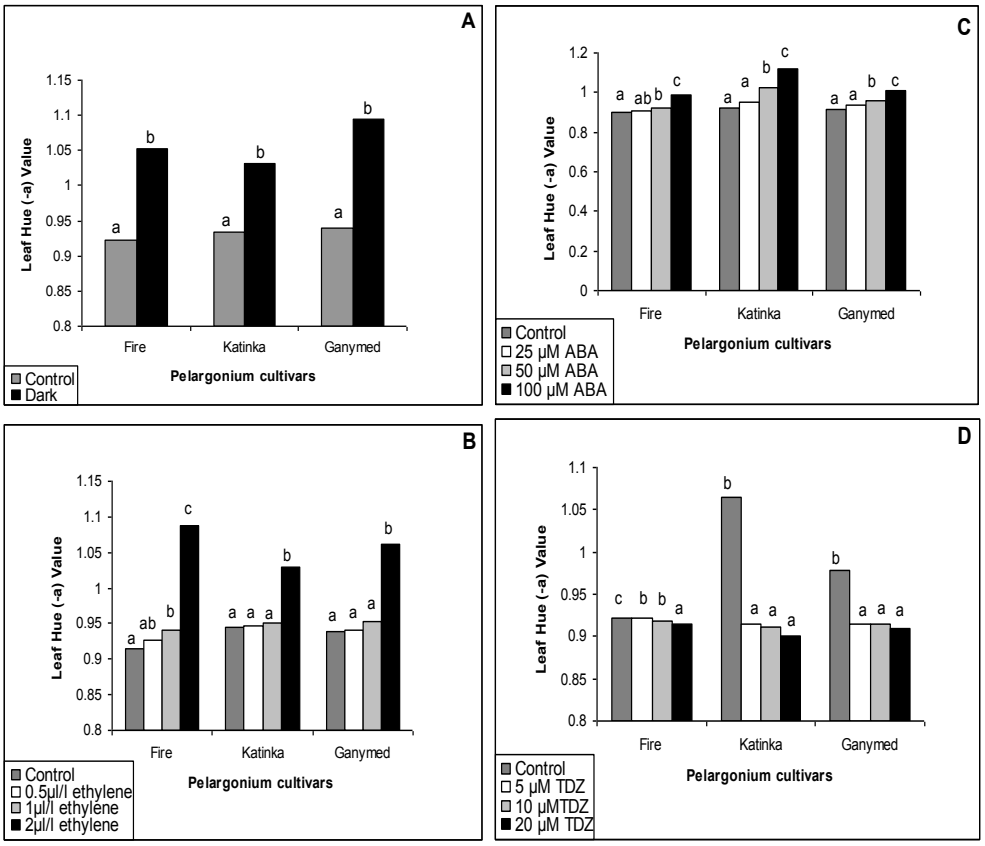

Figure 1. Effects of dark storage (A), continued exposure to ethylene (B), application of ABA (C) and TDZ (D) for 4 days on leaf hue values. Means followed by the same letter(s) within the cultivar are not significantly different. $LSD_{0.05}$ values for 'Fire', 'Katinka' and 'Ganymed' are: (A) 0.02, 0.01, 0.03 (B) 0.02, 0.02, 0.01 (C) 0.01, 0.03, 0.02 (D) 0.02, 0.02 and 0.01, respectively.

Likewise, treating the leaves of *Pelargonium* cuttings with 2 µL/L ethylene for 4 days decreased leaf hue of all the three cultivars (Figure 1B). However, 0.5 µL/L ethylene had no effect on leaf hue for all the cultivars (Figure 1B). Applying 50 µM or 100 µM ABA decreased leaf hue after 4 days in all cultivars, but 25 µM ABA had no significant effect (Figure 1C). Conversely, treating *Pelargonium* cuttings with 5 µM TDZ increased leaf hue for all three cultivars (Figure 1D). There were no differences in hue values between TDZ levels in 'Katinka' and 'Ganymed', except in 'Fire' where 5 and 10 µM were less effective than 20 µM. TDZ at 20 µM was the most effective in increasing leaf hue for all the cultivars (Figure 1D).

Effects on Root Formation

Storing *Pelargonium* cuttings in the dark for 4 days reduced root fresh weight, dry weight and root water content in 'Katinka', but this effect was not apparent in 'Fire' and 'Ganymed' (Table 1).

Exposing *Pelargonium* cuttings to ethylene for 4 days reduced root fresh weight, dry weight and root water content in 'Katinka' (Table 2). However, ethylene had no effect on all the root parameters measured in 'Fire' and 'Ganymed' (Table 2).

Treating *Pelargonium* cuttings with 50 µM or 100 µM ABA for 4 days reduced root fresh weight, dry weight and root water content in 'Fire' and 'Katinka' (Table 3). However, only 100 µM ABA was effective in reducing root fresh weight, dry weight and root water content in 'Ganymed' (Table 3).

Table 1. Effect of dark storage for four days on root induction and growth in cuttings of three *Pelargonium* cultivars

Cultivar	Treatment	Root fresh weight (mg)	Root dry weight (mg)	Root water content (mg)
'Fire'	Control	2072.9a	83.1a	1989.8a
	Stored	928.4a	38.9a	889.5a
	$LSD_{0.05}$	NS	NS	NS
'Katinka'	Control	954.0a	48.0a	906.0a
	Stored	368.1b	18.3b	349.9b
	$LSD_{0.05}$	78.8	25.7	53.9
'Ganymed'	Control	924.8a	39.6a	885.1a
	Stored	1264.1a	46.3a	1217.9a
	$LSD_{0.05}$	NS	NS	NS

For each cultivar, means followed by the same letter(s) within columns are not significantly different at P=0.05. NS = Not significant

Table 2. Effect of exposure to ethylene for four days on root induction and growth in cuttings of three *Pelargonium* cultivars

Cultivar	Treatment ethylene (µL/L)	Root fresh weight (mg)	Root dry weight (mg)	Root water content (mg)
'Fire'	Control	323.8a	37.5a	286.3a
	0.5	243.8a	36.3a	207.8a
	1.0	376.3a	51.6a	324.7a
	2.0	341.3a	42.8a	298.6a
	$LSD_{0.05}$	NS	NS	NS
'Katinka'	Control	882.5a	32.8a	849.8a
	0.5	263.5b	19.5b	244.0b
	1.0	422.5b	21.3b	401.3b
	2.0	108.3b	14.1b	94.2b
	$LSD_{0.05}$	82.6	2.5	2.6
'Ganymed'	Control	497.50a	47.8a	449.8a
	0.5	256.30a	30.5a	225.8a
	1.0	350.00a	41.5a	308.5a
	2.0	281.30a	35.8a	245.6a
	$LSD_{0.05}$	NS	NS	NS

For each cultivar, means followed by the same letter(s) within columns are not significantly different at P=0.05. NS = Not significant

Table 3. Effect of ABA for four days on root induction and growth in cuttings of three *Pelargonium* cultivars

Cultivar	Treatment (ABA, µM)	Root fresh weight (mg)	Root dry weight (mg)	Root water content (mg)
'Fire'	Control	871.3a	36.5a	834.8a
	25	711.1ab	28.8ab	682.2ab
	50	521.1b	24.3b	496.8b
	100	502.5b	26.5b	476.0b
	$LSD_{0.05}$	342.6	9.96	334.5
'Katinka'	Control	329.8a	16.3a	313.4a
	25	194.5b	10.6b	184.0b
	50	183.2b	11.0b	172.2b
	100	135.0b	9.3b	125.8b
	$LSD_{0.05}$	85.6	5.2	83.3
'Ganymed'	Control	1080.3a	46.2a	1034.1a
	25	748.0ab	35.9ab	712.2ab
	50	645.1ab	29.3ab	615.9ab
	100	468.7b	20.0b	448.7b
	$LSD_{0.05}$	517.5	17.9	502.0

For each cultivar, means followed by the same letter(s) within columns are not significantly different at P=0.05.

Thidiazuron severely inhibited root formation at the stem base of the cuttings. This resulted in only 1% to 2% rooting in all the three cultivars (data not presented). Treating cuttings of 'Katinka' with 5 µM TDZ, followed by addition of 4 µL/L IBA in nutreint solutions at the beginning of the experiment, increased root fresh weight (Table 4). In addition, 'Fire' and 'Ganymed' showed a similar trend with respect to root fresh weight (Table 4). Treating *Pelargonium* cuttings with TDZ followed by application of 4 µL/L IBA in nutrient solutions had no effect on root dry weight and root water content in all cultivars (Table 4). Generally, high (8 and 12 µL/L) IBA levels were not different from each other for all cultivars (Table 4).

Table 4. Effect of treating cuttings with 5 µM TDZ followed by application of IBA in rooting solution for 28 days on root induction and growth for three *Pelargonium* cultivars

Cultivar	Treatment	Root fresh weight (mg)	Root dry weight (mg)	Root water content (mg)
'Fire'	DI Water	603.4ab	5.7b	94.3a
	5 µM TDZ + 4 µL/L IBA	889.6a	5.4b	94.6a
	5 µM TDZ + 8 µL/L IBA	576.2ab	6.5ab	93.5ab
	5 µM TDZ + 12 µL/L IBA	298.2b	6.0ab	94.0ab
	Critical t value	2.1	2.1	2.1
'Katinka'	DI Water	457.0b	5.0a	95.0a
	5 µM TDZ + 4 µL/L IBA	860.4a	4.8a	95.2a
	5 µM TDZ + 8 µL/L IBA	554.6ab	5.4a	94.6a
	5 µM TDZ + 12 µL/L IBA	704.3ab	5.0a	95.0a
	Critical t value	2.1	NS	NS
'Ganymed'	DI Water	480.3a	4.6a	95.5a
	5 µM TDZ + 4 µL/L IBA	592.3a	5.0a	95.1a
	5 µM TDZ + 8 µL/L IBA	390.0a	4.4a	95.6a
	5 µM TDZ + 12 µL/L IBA	460.7a	4.9a	95.1a
	Critical t value	NS	NS	NS

For each cultivar, means followed by the same letter(s) within columns are not significantly different at P=0.05. NS = Not significant

DISCUSSION

Application of ethylene, ABA or dark storage accelerated senescence in the leaves of *Pelargonium* cuttings. These treatments decreased leaf hue which is used to quantify a decrease in green colour of the leaves as they turn yellow (Steet and Tong, 1996). Ethylene has been shown to induce premature leaf yellowing in many plants as a result of accelerated chlorophyll degradation (Purer and Mayak, 1989). Application of ethylene action inhibitor (1-MCP) retarded storage-induced leaf yellowing in zonal *Pelargonium* (Serek et al., 1998), implying ethylene action is involved in storage-induced leaf senescence. Also, storing cuttings shortly after harvest leads to water stress (Schatz, 1982) and accumulation of wound-ethylene in the packing material

(Kadner et al., 2000), which promotes senescence as evidenced by leaf chlorosis (Schatz, 1982). Moreover, during storage cuttings are exposed to darkness and low humidity that causes chlorosis (Behrens, 1988). Darkness stimulates senescence of green tissues (Thimann, 1980). Conversely, Zacarias and Reid (1990) found dark-induced leaf yellowing did not require the action of ethylene. Moreover, the levels of ABA increases in water-stressed plants leading to leaf chlorosis (Aharoni et al., 1977), thus showing interaction of hormones in regulation of leaf senescence.

Leaves treated with TDZ remained green while those of untreated controls turned yellow, thus they increased leaf hue. Cytokinins reduce leaf yellowing in ornamental plants (Richmond and Lang, 1957; Mutui et al., 2004) because they are involved in chlorophyll biosynthesis (Zavaleta-Mancera et al., 1999). Cytokinins activate NADH protochlophyllide reductase, an enzyme involved in chlorophyll biosynthesis, and reduces chlorophyll degradation (Zavaleta-Mancera et al., 1999). Present results are supported by Ferrante et al. (2002) and Mutui et al. (2004) in that TDZ and BA, respectively, prevented leaf yellowing in *Alstroemeria* cut flowers by inhibiting chlorophyll degradation.

All ethylene levels reduced root fresh weights, dry weight and root water content when compared to the control in 'Katinka' (Table 2). This outcome could be attributed to stress-response mechanisms induced by high levels of ethylene. Based on studies with ethylene and ethylene inhibitors, Jusaitis (1986) suggested that low (10-fold basal ethylene) concentrations of ethylene are required for rooting, while high (1000-fold basal ethylene) concentrations have an inhibitory effect; observations which are supported by the present results. Application of 50 or 100 µM ABA reduced the ability of *Pelargonium* cuttings to continue growth of induced roots (Table 3). ABA can inhibit ethylene production from various organs in a range of species (Spollen et al., 2000). In the present study, exogenous ABA may have restricted ethylene production (Spollen et al., 2000), which in turn decreased the incidence of *Pelargonium* root growth.

Storing cuttings for four days in the dark reduced all the root parameters investigated (Table 1). Our results suggest that the amount of ethylene released by zonal *Pelargonium* cuttings enclosed in un-perforated polyethylene bags (Kadner et al., 2000) could have exceeded the threshold level required for optimal root growth (Jusaitis, 1986). Also, ethylene emanating from water-stressed plant tissues could accumulate in packing materials and promote senescence (Schatz, 1982). In agreement with our

results, Serek et al. (1998) reported that short-term storage inhibited rooting of *Pelargonium* cuttings.

Thidiazuron inhibited root induction in our study since endogenous cytokinins at normal physiological concentrations inhibit root growth. This result may be explained in that TDZ is very stable in *Pelargonium* leaves, leading to inhibition of root initiation. Mok and Mok (1985) found that [^{14}C]-TDZ was not substantially broken down in *Phaseolus lunatus* callus tissue over 33 days. Mok and Mok (1985) concluded that TDZ itself and not its catabolites, was stimulating the physiological responses. Mutui et al. (2005) reported that TDZ inhibited rooting in *Pelargonium* cuttings. Furthermore, Sakai et al. (2001) reported that in *Arabidopsis*, a weak allele of a cytokinin receptor mutant and loss-of-function allele of a cytokinin-signaling element both have longer roots than the wild type. These findings suggest that endogenous cytokinins may negatively regulate root elongation.

Auxins are known to induce ethylene biosynthesis (Kawase, 1971), and application of 4 µL/L IBA restored the rooting abilities of *Pelargonium* cuttings pre-treated with TDZ (Table 4). This remedy suggests that auxins play a central role in adventitious root formation. Kawase (1971) suggested that ethylene, induced by auxins, is responsible for the root-promoting activity of auxins, possibly explaining our data. Reports of the variable rooting response of many plants to ethylene compared with ubiquitous reports of auxin-stimulated rooting have suggested that ethylene is less often a limiting factor or is less directly involved in the rooting process than auxins (Mudge, 1988). Overall, the promotive effect of auxins on adventitious rooting is influenced by ethylene responsiveness (Clark et al., 1999).

CONCLUSIONS AND RECOMMENDATIONS

Exogenous application of ethylene, ABA and dark storage accelerated leaf senescence and reduced the capacity of induced roots to continue growth in *Pelargonium* cuttings. In contrast, TDZ delayed leaf senescence but severely inhibited root formation. ABA and dark storage appeared to interact in stimulating ethylene biosynthesis in plant tissues. Therefore, the current results support the view that ethylene plays an important role in the process of root initiation and root growth in *Pelargonium* cuttings. It is recommended that TDZ should be applied to *Pelargonium* cuttings as a postharvest treatment to prevent subsequent leaf yellowing. Cuttings should not be stored in the dark or under water stress to prevent production of ethylene and accumulation of ABA, respectively, because they are implicated in leaf chlorosis. After transportation and storage, *Pelargonium*

cuttings should be dipped in a rooting hormone, containing 4 µL/L IBA to induce root development and enhance quality.

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REFERENCES

Aharoni, W., A. Blumenfold and A. E. Richmond. 1977. Hormonal activity in detached Lettuce leaves as affected by water content. Planta 59: 1169-1173.

Behrens, V. 1988. Storage of unrooted cuttings. *In*: Davies, T., B.E. Haissing and N. Sankhla. (Eds.). Adventitious root formation in cuttings. Vol. 2, Dioscorides Press, Oregon, USA.

Clark, D.G., E.K. Gubrium, J.E. Barrett, T.A. Nell and H.J. Klee. 1999. Root formation in ethylene-insensitive plants. Plant Physiology 121:53-59.

Ferrante, A., D.A. Hunter, W.P. Hackett and M.S. Reid. 2002. Thidiazuron-a potent inhibitor of leaf senescence in Alstroemeria. Postharvest Biology and Technology 25:333-338.

Ferrante, A., F. Tognoni, A. Mensuali-Sodi and G. Serra. 2003. Treatment with thidiazuron for preventing leaf yellowing in cut tulips and chrysanthemum. Acta Horticulturae 624:357-363.

Gan, S. and R.M Amasino. 1996. Cytokinins in plant senescence: from spray and pray to clone and play. Bioessays 18:557-565.

Halevy, A.H. and S. Mayak. 1981. Senescence and post harvest physiology of cut flowers. II. Horticulture Reviews 3:59-143.

Jusaitis, M. 1986. Rooting response of Mung bean cuttings to 1-aminocyclopropane-1-carboxylic acid and inhibitors of ethylene biosynthesis. Scientia Horticulturae 29:77-85.

Kadner, R., U. Drüege and F. Kühnemann. 2000. Ethylenemission von Pelargonien-stecklingen während der Lagerung bei unterschiedlichen Temperauren. Gartenbauwissenschaft 65:272-279.

Kawase, M. 1971. Causes of centrifugal root promotion. Physiologia Plantarum 23:159-170.

Mok, M.C. and D.W.S. Mok. 1985. The metabolism of [^{14}C]-Thidiazuron in callus tissues of *Phaseolus lunatus.* Physiologia Plantarum 65:427-432.

Mudge, K.W. 1988. Effect of ethylene on rooting, p. 150-161. *In*: Davies, T., B. E. Haissing and N. Sankhla. (Eds.). Adventitious root formation in cuttings. Volume 2. Dioscorides Press, Oregon, USA.

Mutui, T.M., V.E. Emongor and M.J. Hutchinson. 2004. Influence of plant growth regulators on postharvest characteristics of *Alstroemeria* cut flowers. Discovery and Innovations 16:195-201.

Mutui, T.M., H. Mibus and M. Serek. 2005. Effects of thidiazuron, ethylene, abscisic acid and dark storage on leaf yellowing and rooting of *Pelargonium* cuttings. Journal of Horticultural Science and Biotechnology 80:543-550.

Purer, O. and S. Mayak. 1988. Postharvest treatments for improvement of the quality of Pelargonium cuttings. Acta Horticulturae 226:591-595.

Purer, O. and S. Mayak. 1989. Pelargonium cuttings-Effect of growth regulators. Acta Horticulturae 261:347-354.

Quirino, B.F., Y.S. Noh, E. Himelblau and R. M. Amasino. 2000. Molecular aspects of leaf senescence. Trends in Plant Science 5:278-282.

Sakai, H., T. Honma, T. Aoyama, S. Sato, T. Kato, S. Tabata and A. Oka. 2001. *Arabidopsis* ARR1 is a transcription factor for genes immediately responsive to cytokinins. Science 294: 1519-1521.

SAS. 2002. SAS/STAT User's Guide for Personal Computers, Version 8. SAS Institute, NC, USA.

Schatz, Y. 1982. Storage and shipment of *Pelargonium* cuttings: The problems and some solutions. M. Sc. Thesis, Hebrew University of Jerusalem, Rehovot, Israel.

Serek, M., A. Prabucki, E.C. Sisler and A.S. Andersen. 1998. Inhibitors of ethylene action affect final quality and rooting of cuttings before and after storage. HortScience 33:153-155.

Smart, C.M. 1994. Gene expression during leaf senescence. New Phytologist 126:419-448.

Spollen, W.G., M.E. LeNoble, T.D. Samuels, N. Bernstein and R.E. Sharp. 2000. Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. Physiologia Plantarum 122:967-976.

Steet, J.A. and C.H. Tong. 1996. Degradation kinetics of green colour and chlorophyll in peas by colorimetry and HPLC. Journal of Food Science 61:924-927.

Thimann, K.V. 1980. The senescence of leaves, p. 85-115. *In*: Thimann, K. V. (Ed.). Senescence in Plants. CRC Press, Boca Raton, Florida, USA.

Weaver, L.M., S. Gan, B. Quirino and R.M. Amasino. 1998. A comparison of the expression patterns of several senescence associated genes in response to stress and hormone treatment. Plant Molecular Biology 37:455-469.

Zacarias, L. and M.S. Reid. 1990. Role of growth regulators in the senescence of *Arabidopsis thaliana* leaves. Physiologia Plantarum 80:549-554.

Zavaleta-Mancera, H.A., K.A. Franklin, H.J. Ougham, H. Thomas and I.M. Scott. 1999. Regreening of senescent Nicotiana leaves I. Reappearance of NADPH-protochlorophyllide oxidoreductase and light-harvesting chlorophyll a/b-binding protein. Journal of Experimental Botany 50:1677-1682.