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Defense enzyme activities and biochemical variations of *Pelargonium zonale* in response to nanosilver application and dark storage

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Abstract: *Pelargonium* petal abscission and ethylene-induced leaf senescence are major problems that reduce the longevity of plants. This study was carried out to determine the poststorage influence of nanosilver (NS) particle (0, 20, 40, 60, and 80 mg L^{-1}) application during dark storage period on chlorophyll and carotenoid pigments, malondialdehyde (MDA) content, leaf protein and ethylene concentration, petal abscission, membrane stability index (MSI), and enzyme activity including ascorbate peroxidase (APX), peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), and β-glucosidase (β-GLU) in two *Pelargonium* pot plants of the cultivars Flowerfairy and Foxi. Enhanced photosynthetic pigment contents were recorded in leaves of both NS-treated cultivars when compared to the control. In both cultivars, MDA content decreased significantly with the increase of NS concentration up to 60 mg L^{-1}, and then a rapid increase followed at 80 mg L^{-1}. NS treatments markedly decreased petal abscission compared to the control. Enzymes activities were affected differently by various NS concentrations. The highest APX activity was observed at 40 mg L^{-1} NS concentration, but specific activities of SOD and POD increased to a maximum level at 60 mg L^{-1} in both cultivars. CAT activity was pronounced at 20 mg L^{-1} and 40 mg L^{-1} NS treatments in Flowerfairy and Foxi, respectively. Both under control conditions and at the highest NS concentration, β-GLU showed lower activity than other NS levels. It was suggested for the first time that NS extended petal longevity and improved defense enzyme activities of *Pelargonium* pot plants under dark storage conditions.

Key words: Nanosilver, *Pelargonium*, dark storage, antioxidant enzymes, lipid peroxidation, abscission

1. Introduction

Pelargonium zonale is one of the most important bedding ornamental plants, which is grown as a potted plant for its colorful, single or double showy flowers and attractive foliage. Leaf senescence and petal abscission are critical problems that break down cellular components and reduce the longevity and marketability values of many flowering plants (Serek et al., 1998). Moreover, leaf senescence is a complex molecular and physiological action that may result from several factors including phytohormones (e.g., abscisic acid and ethylene) and environmental stresses (e.g., temperature and darkness) (Weaver et al., 1998). Dark-induced senescence happens during commercial shipping and handling of *Pelargonium* species or other members of the family Geraniaceae (Reid et al., 2002). Many investigations suggested that the most important variations observed during plant senescence are chlorophyll degradation, increase in ethylene production, reduction in antioxidant enzyme activity, increase in reactive oxygen species (ROS) levels, and cell membrane damage (Prochazkova and Wilhelmova, 2007). This process has some similarities with natural senescence, which suggests that dark-induced cell death is a result of an active program that include the participation of signaling molecules, transcription factors, and catabolic enzymes (Buchanan-Wollaston et al., 2003). Plants are generally protected against this oxidative stresses by a wide range of radical scavenging systems such as antioxidative enzymes like superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT), as well as nonenzymatic compounds like carotenoids (Cameron and Reid, 2001; Zimmermann and Zentgraf, 2005). Therefore, any treatment that can help diminish ROS levels would be clearly advantageous in improving the plant performance and longevity.

Several approaches using ethylene antagonists such as silver nitrate ($AgNO_3$), silver thiosulfate (STS), and 1-methylcyclopropene (1-MCP) have been investigated over the years for improving quality and longevity of ethylene-sensitive flowers (Serek and Trolle, 2000). However, STS and $AgNO_3$ are heavy metal pollutant substances and were not used commercially due to their

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risks for human health and environment. Although 1-MCP blocks ethylene-induced petal abscission of *Pelargonium*, the effect is impermanent (Cameron and Reid, 2001; Damunupola and Joyce, 2006).

The development of nanotechnology in physiology and biochemistry has expanded the application area of nanomaterials in different fields due to their unique characteristics (e.g., large surface area-to-volume ratio, ability to engineer electron exchange, and highly surface reactive capabilities) (Scrinis and Lyons, 2007). This technology could also open up new approaches in plant sciences and in agricultural research. In recent years, many scientists have studied the effects of nanomaterials on seed germination and plant growth with the aim to promote its use for agricultural productions. Most of these studies are focused on the potential toxicity of nanoparticles in higher plants and both positive and subsequently negative or inconsequential effects were presented. As reported by Lu et al. (2002), treatment of soybean (*Glycine max*) plants with a mixture of nano SiO_2 and TiO_2 increased nitrate reductase activity, stimulated its antioxidant system, and accelerated germination and growth.

It is reported that nano ZnO could be one of the most toxic nanoparticles that retard root growth of examined plants (Lin and Xing, 2007). Most recently it was reported that activity of specific antioxidant enzymes was induced in *Brassica juncea* seedlings treated with silver nanoparticles (Priyadarshini et al., 2012). However, the mechanism of these nanoparticles has not been completely established yet. In the field of floriculture, the use of nanomaterials is also relatively new and needs more research. No previous literature reviews exist about the impact of nanoparticles on the physiology of ornamental plants or their possible effects in the longevity of pot plants, and the mechanisms of delaying senescence and petal abscission are also still unknown. Therefore, the present study was carried out to elucidate the potential effects of nanosilver (NS) particle application on photosynthetic pigments, ethylene production, lipid peroxidation, membrane stability, petal abscission, leaf protein content, and defense enzyme [antioxidant enzymes and β-glucosidase (β-GLU)] activity in 2 *Pelargonium* cultivars during dark storage period.

2. Materials and methods

2.1. Plant materials and dark storage conditions

Two *Pelargonium zonale* cultivars, Flowerfairy and Foxi, were obtained from a commercial institute (Sharifi Flowers), in Pakdasht, Tehran, Iran. Thereafter, plant cuttings were taken by cutting the stems (4–5 cm in height with 1–2 leaves) of source plants and were planted in pots containing soil, cocopeat, and peat (0.5:1:1) at the rate of 1 cutting per pot. Subsequently, cuttings were grown in the greenhouse at the University of Guilan, Rasht, Iran, under normal environmental conditions to produce stock plants: 25 °C day/17 °C night temperatures, natural light (16 h light, 8 h dark), and 75% relative humidity (RH). Two-month-old uniform plants (at blooming stage) were then stored in a dark chamber at 20 ± 2 °C and 65% RH for 5 days to simulate suboptimal transport conditions.

2.2. NS particle characterization and treatments

NS particles were purchased from the Iranian Nanomaterials Pioneers Company, NANOSANY (Mashhad, Iran). The size of NS particles was estimated to be 10–20 nm in diameter, of metal basis and spherical. A transmission electron microscopy image of the NS particles is shown in Figure 1. Deionized water was used to prepare 0, 20, 40, 60, and 80 mg L^{-1} NS solutions. NS was applied to plants on the first day of darkness in the dark chamber. Control plants were only treated with deionized water. Whole plant foliage (both sides of the leaves, and stems) was sprayed with equal amounts of 50 mL of aqueous solution of NS by hand atomizer. All measurements were made at the end of 5 days of dark storage period.

2.3. Pigment measurements

For chlorophyll (Chl) and carotenoid extractions, 0.5 g of fresh leaf was ground in 0.5 mL of acetone (80% V/V). The absorption was read at 645, 663, and 470 nm for Chl *a*, Chl *b*, and carotenoid values in a spectrophotometer (PG Instrument LTD T80+UV/VIS), respectively. Measurements were performed on 20 leaves per treatment (n = 20). Pigment contents were calculated from the following equations, as described by Lichtenthaler (1987).

Chl *a* (mg g^{-1} FW) = $11.75 \times A_{663} - 2.35 \times A_{645}$

Chl *b* (mg g^{-1} FW) = $18.61 \times A_{645} - 3.96 \times A_{663}$

Carotenoids (mg g^{-1} FW) = $4.69 \times A_{470} - 0.268 \times (20.2 \times A_{645} + 8.02 \times A_{663})$

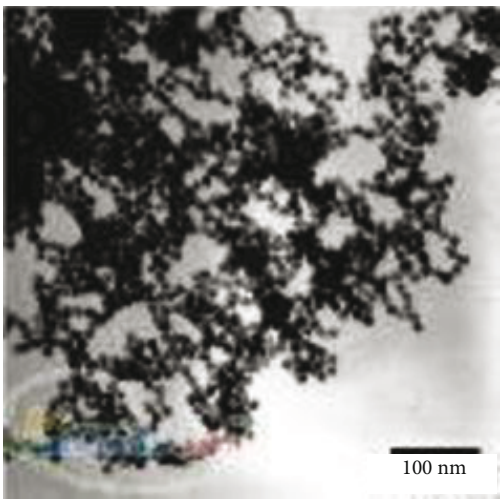

Figure 1. Transmission electron microscopy image of spherical NS particles. Distribution of NS particles size was estimated to be 10–20 nm (scale bar = 100 nm).

2.4. Lipid peroxidation

The level of lipid peroxidation was determined according to Heath and Packer (1968). Leaf fresh tissues (0.5 g) were ground with 10% trichloroacetic acid and centrifuged at 10,000 × *g* for 10 min. Two milliliters of supernatant was added with 2 mL of 0.6% thiobarbituric acid. The mixture was heated at 95 °C for 30 min, then cooled on ice and centrifuged at 4000 × *g* for 20 min. Thereafter, the supernatant absorbance was read at 532 nm. MDA content was obtained according to its extinction coefficient of 155 mM^{-1} cm^{-1}.

2.5. Petal abscission

The petal abscission was calculated as the number of petals that were shed at the end of 5 days of darkness divided by the total number of petals at the start of the dark storage period, expressed as percentage (Ascough et al., 2008).

2.6. Determination of ethylene production

Three flowers were detached from 3 plant samples of each treatment in the same stage of flower development and were then incubated in a 25-mL sealed jar at room temperature (22–26 °C) for 2 h. Next, 1 mL of gas samples was taken from the headspace with a hypodermic syringe for ethylene analysis by an Agilent GC-14A gas chromatograph using a flame ionization detector column (150 × 0.4 cm); detector temperatures were 50 °C and 350 °C, respectively, and the carrier gas was nitrogen at a flow rate of 30 mL min^{-1}. Ethylene production values were expressed as nL g^{-1} h^{-1} FW (Ferrante et al., 2002).

2.7. Membrane stability index

Fresh leaf samples (0.1 g) were divided into pieces of 0.3 cm in length and placed in vials containing 10 mL of distilled water. The vials were put in a laboratory water bath (30 °C) for 30 min and the first electrical conductivity was recorded (C_1). The other samples were heated (100 °C) for 10 min to liberate all electrolytes, then cooled, and the second electrical conductivity was recorded (C_2). The mean of 4 readings per leaf from 3 leaves per treatment were measured. The membrane stability index (MIS) was calculated by the following equation (Sairam, 2003): $MSI = [1 - (C_1 / C_2)] \times 100$.

2.8. Leaf protein analysis

Fresh leaf samples (0.5 g) were ground with liquid nitrogen, and then 0.3 mL of extraction buffer (0.1 M, pH 8.1, 10 mM, 5 mM EDTA) was added and the mixture was centrifuged for 10 min at 14,000 × *g* at 4 °C. Total protein was determined as described by Bradford (1976).

2.9. Antioxidant enzyme assay

Fresh leaf sample (0.5 g) was placed into liquid nitrogen and then homogenized with a prechilled mortar and pestle under ice-cold conditions in 4 mL of 50 mM potassium phosphate buffer (pH 7.0), adding 1 mM EDTA. The homogenate was centrifuged at 14,000 × *g* at 4 °C for 20 min. The supernatant was stored at –20 °C and used for determination of enzyme activities.

2.9.1. APX activity

The activity of APX (EC 1.11.1.11) was determined following the method of Jimenez et al. (1997) with minor modification. Soluble protein was extracted by homogenizing the powder in 2 mL of 50 mM sodium phosphate buffer (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid, 250 mM H_2O_2, and enzyme extract equivalent to 50 µL of protein. The enzyme activity was recorded as decrease in absorbance at 290 nm for 1 min and the amount of ascorbate oxidized was obtained from the extinction coefficient of 2.8 mM^{-1} cm^{-1}.

2.9.2. SOD activity

SOD (EC 1.15.1.1) activity was based on the method of Beauchamp and Fridovich (1971). The required cocktail for SOD activity estimation was prepared by mixing 25 mL of sodium phosphate buffer (pH 6.8), 1 mL of nitro-blue tetrazolium (NBT; 75 µM), 1.5 mL of methionine (13 mM), 1.5 mL of 0.1 mM EDTA, and 1 mL of 0.002 mM riboflavin. The reaction mixture was illuminated for 15 min using 2 fluorescent lamps (15 W). The tube without enzyme, which was kept in the light, was regarded as the control, while the tube with enzyme extract that was kept in the dark was considered as the blank. SOD activity was the difference in NBT reduction recorded at 560 nm in light with and without enzyme extract. One unit of SOD activity was taken as that amount of enzyme required to inhibit 50% initial reduction of NBT under light.

2.9.3. POD activity

POD (EC 1.11.1.7) activity with guaiacol as the substrate was determined by the method of Lee and Kim (1994) with some modifications. The 1-mL assay mixture contained 40 mM phosphate buffer (pH 6.8), 15 mM guaiacol, and 5 mM H_2O_2. The reaction was started by the addition of H_2O_2 and the increase in absorbance at 470 nm was measured for 1 min. POD activity was measured as per its extinction coefficient (26.6 mM^{-1} cm^{-1}).

2.9.4. CAT activity

CAT (EC 1.11.1.6) activity was assayed by measuring the disappearance of H_2O_2 as described by Aebi (1984). The reaction mixture contained 50 mM potassium phosphate buffer (pH 6.8), 1 mM EDTA, 15 mM H_2O_2, and 20 µL of enzyme extract. CAT activity was measured by the decrease in absorbance at 240 nm for 1 min.

2.9.5. β-GLU activity

The β-GLU (EC 3.2.1.21) activity was determined by a method described by Del Campillo and Shannon (1982) with some modifications. Fresh leaf (0.5 g) was homogenized with 1 mL of 100 mM citrate buffer adjusted to pH 5.0 with KOH. Then homogenate was centrifuged at 10,000 × *g* at 4 °C for 15 min. The β-GLU activity was

measured using p-nitrophenyl-β-D-glucopyranoside as the substrate. A total volume of 220 µL contained 50 µL of extract, 45 µL of substrate, and 75 µL of extraction buffer. The reaction mixture was stopped by the addition of 600 µL of 1 M Na_2CO_3 after holding it at 35 °C for 30 min. The p-nitrophenol liberated in the reaction was quantified spectrometrically at 405 nm. A p-nitrophenol calibration curve had earlier been prepared and was calculated using the molar extinction coefficient ε = 18300 ΔAbs × 1 × (mol cm)$^{-1}$.

2.10. Statistical analysis

Data analyses were performed based on a completely randomized design with a factorial experiment, which was replicated 3 times. Data were subjected to analysis of variance (ANOVA) with the general linear model (Proc GLM) using computer SAS software, version 9.1 (CoHort Software). Multiple comparisons among treatment means was done by Tukey's honestly significant difference (HSD) test at P = 0.05.

3. Results

3.1. Photosynthetic pigments

Significant differences (P < 0.05) in photosynthetic pigments were found between 2 cultivars under the employed NS treatments (Table 1). Chlorophyll *a*, *b*, and total (*a*+*b*) contents had an upward trend with increasing NS concentrations up to 40 mg L^{-1} (in Flowerfairy) and 60 mg L^{-1} (in Foxi), and then dropped down with an increase of NS treatment to 80 mg L^{-1} in both cultivars. Reduction percentages of chlorophyll *a* and *b* in control plants after 5 days of darkness from initial levels (data not shown) were 41.7% and 33.3% (in Foxi) and 42.3% and 50% (in Flowerfairy), respectively. Furthermore, it was also found that the minimum ratio between chlorophyll *a* and *b* (*a*/*b*) was calculated at 0 and 80 mg L^{-1} NS concentration in Foxi and Flowerfairy, respectively. The carotenoid contents in Foxi and Flowerfairy depended on NS concentration and increased significantly (21.89% and 18.36% of the control, respectively) at 60 mg L^{-1} NS.

3.2. Lipid peroxidation

Lipid peroxidation level in the leaves of the 2 cultivars was measured as the content of MDA. Marked difference of MDA content was observed between cultivars (Table 2). The trend in change of MDA content was also different among the NS treatments. MDA content of Foxi was significantly (P < 0.05) lower than that of Flowerfairy under the same employed NS treatments. In both cultivars, the MDA content decreased significantly with the increase of NS concentration up to 60 mg L^{-1} and then a rapid increase followed at 80 mg L^{-1}. On the other hand, maximum decline of 17.34% and 19.97% in MDA content was recorded at 60 mg L^{-1} NS treatment as compared to the control for Foxi and Flowerfairy, respectively.

Table 1. Variations of chlorophyll (Chl *a*, Chl *b*, Chl *a*+*b*, and Chl *a*/*b*) and carotenoid contents in 2 *Pelargonium* cultivars, Foxi and Flowerfairy, under different nanosilver particles concentrations. Values represent mean ± SD.

Cultivars	Nanosilver (mg L^{-1})	Plant pigments (mg g FW^{-1})				
		Chl *a*	Chl *b*	(Chl)*a*+*b*	(Chl)*a*/*b*	Carotenoids
Foxi	0	0.014 ± 0.0045^{a}	0.019 ± 0.0042^{bc}	0.033 ± 0.0046^{b}	0.73 ± 0.0001^{a}	1.32 ± 0.03^{b}
	20	0.037 ± 0.0016^{d}	0.024 ± 0.0024^{cd}	0.061 ± 0.001^{d}	1.54 ± 0.001^{e}	1.47 ± 0.05^{d}
	40	0.038 ± 0.0028^{d}	0.033 ± 0.0027^{e}	0.071 ± 0.001^{e}	1.15 ± 0.003^{c}	1.58 ± 0.07^{e}
	60	0.076 ± 0.0014^{e}	0.034 ± 0.0013^{e}	0.11 ± 0.001^{f}	2.23 ± 0.002^{g}	1.69 ± 0.01^{f}
	80	0.032 ± 0.0039^{c}	0.016 ± 0.0035^{ab}	0.048 ± 0.001^{c}	2.0 ± 0.013^{f}	1.31 ± 0.04^{b}
Flowerfairy	0	0.015 ± 0.0038^{a}	0.010 ± 0.0016^{a}	0.025 ± 0.002^{a}	1.5 ± 0.001^{e}	1.2 ± 0.022^{a}
	20	0.034 ± 0.006^{c}	0.026 ± 0.004^{cd}	0.06 ± 0.005^{d}	1.3 ± 0.005^{d}	1.22 ± 0.04^{a}
	40	0.039 ± 0.0032^{d}	0.034 ± 0.0037^{e}	0.073 ± 0.004^{e}	1.14 ± 0.036^{c}	1.36 ± 0.024^{bc}
	60	0.028 ± 0.004^{b}	0.021 ± 0.0055^{cd}	0.049 ± 0.003^{c}	1.33 ± 0.001^{d}	1.47 ± 0.07^{d}
	80	0.012 ± 0.005^{a}	0.011 ± 0.006^{a}	0.033 ± 0.005^{b}	1.09 ± 0.003^{b}	1.31 ± 0.014^{b}

Treatments sharing the same letter(s) do not show significant differences at 5% probability level.

Table 2. Variations of malondialdehyde (MDA) content, ethylene production, membrane stability index (MSI), total leaf protein content, and abscission percentage in 2 *Pelargonium* cultivars, Foxi and Flowerfairy, under different nanosilver concentrations. Values represent mean ± SD.

Cultivars	Nanosilver (mg L^{-1})	Physiological traits				
		MDA (nmol g^{-1} FW)	Ethylene (nL h^{-1} g^{-1} FW)	MSI (%)	Protein (mg g^{-1} FW)	Abscission (%)
Foxi	0	13.32 ± 0.25^{d}	0.88 ± 0.045^{c}	28 ± 1.54^{a}	21.23 ± 1.52^{c}	21.23 ± 1.52^{c}
	20	12.74 ± 0.16^{c}	0.85 ± 0.045^{c}	36.3 ± 1.65^{c}	25.68 ± 1.32^{e}	25.68 ± 1.32^{e}
	40	12.62 ± 0.22^{c}	0.76 ± 0.031^{b}	42 ± 1.54^{d}	25.91 ± 1.76^{e}	25.91 ± 1.76^{e}
	60	11.01 ± 0.16^{a}	0.66 ± 0.033^{a}	43.6 ± 1.48^{e}	28.64 ± 1.54^{f}	28.64 ± 1.54^{f}
	80	13.85 ± 0.14^{d}	0.86 ± 0.052^{c}	32 ± 1.56^{b}	17.32 ± 0.85^{a}	17.32 ± 0.85^{a}
Flowerfairy	0	15.12 ± 0.24^{f}	1.14 ± 0.064^{e}	31.4 ± 1.42^{b}	16.32 ± 1.45^{a}	16.32 ± 1.45^{a}
	20	13.28 ± 0.12^{d}	1.01 ± 0.083^{d}	40.5 ± 1.66^{d}	21.25 ± 0.76^{c}	21.25 ± 0.76^{c}
	40	13.31 ± 0.11^{d}	0.87 ± 0.054^{c}	48 ± 1.94^{f}	22.94 ± 0.76^{cd}	22.94 ± 0.76^{cd}
	60	12.10 ± 0.15^{b}	0.60 ± 0.098^{a}	49.1 ± 1.53^{f}	27.44 ± 0.65^{f}	27.44 ± 0.65^{f}
	80	14.93 ± 0.17^{e}	1.07 ± 0.069^{d}	37.6 ± 1.97^{c}	18.21 ± 0.68^{ab}	18.21 ± 0.68^{ab}

Treatments sharing the same letter(s) do not show significant differences at 5% probability level.

3.3. Changes in ethylene production

Table 2 also show the change in ethylene production from petals of 2 *Pelargonium* cultivars under various applied NS concentrations. The rate of ethylene production of Flowerfairy was always higher than that in Foxi in all NS treatments except at 60 mg L^{-1}, where no significant change was found between cultivars. The highest (1.14 nL h^{-1} g^{-1} FW) and the lowest (0.6 nL h^{-1} g^{-1} FW) ethylene formation rates were recorded in Flowerfairy with the control treatment and 60 mg L^{-1} NS treatment, respectively.

3.4. Petal abscission

Significant ($P < 0.05$) difference in petal abscission percentage was found between the 2 cultivars and also among the employed NS levels (Table 2). Petal abscission decreased drastically by about 44% and 52% in Flowerfairy and Foxi NS treated with 60 mg L^{-1} as compared to the control plants, respectively. In the lowest NS treatment of 20 mg L^{-1}, petal abscission was not significantly pronounced between cultivars; however, it was higher in Flowerfairy than Foxi. Interestingly, even at 80 mg L^{-1} NS treatment, the percentage of petal abscission was lower than the control in the Foxi cultivar.

3.5. Membrane stability index

The NS-treated Foxi and Flowerfairy plants showed higher MSI percentages than the untreated control (Table 2). Moreover, a maximum decline of 36% in MSI was recorded at 60 mg L^{-1} NS concentration in cultivars as compared to the control, evidence of the protective role of NS application at appropriate concentrations against membrane damage.

3.6. Total leaf proteins

The leaf protein content of the cultivars also showed a varying pattern with different NS concentrations (Table 2). It increased steadily with increasing NS levels until 60 mg L^{-1} and then followed a rapid decrease. Leaf protein improvement under NS application was more pronounced in Foxi than in Flowerfairy up to 40 mg L^{-1}. However, no significant changes were observed at higher NS concentrations between cultivars. Maximum and minimum (28.64 and 16.32 mg g^{-1} FW) protein contents were obtained for Foxi and Flowerfairy at 60 and 0 mg L^{-1} NS, respectively.

3.7. Antioxidant enzymes

After 5 days of dark storage, marked difference in the activity of APX was found between the 2 *Pelargonium* cultivars under NS concentrations (Figure 2). The highest APX activity (1.5 and 1.2 μmol ASA min^{-1} mg^{-1} protein) was observed at 40 mg L^{-1} NS for Foxi and Flowerfairy, respectively. However, APX activity variations under control and 80 mg L^{-1} NS treatments were not significant between cultivars. In both cultivars, specific activity of SOD increased continuously with increasing NS concentrations up to 60 mg L^{-1} and thereafter declined. However, the decrease of SOD activity under the highest NS treatment was not significantly lower than that of the control in the cultivars (Figure 3). The change trend of POD activity

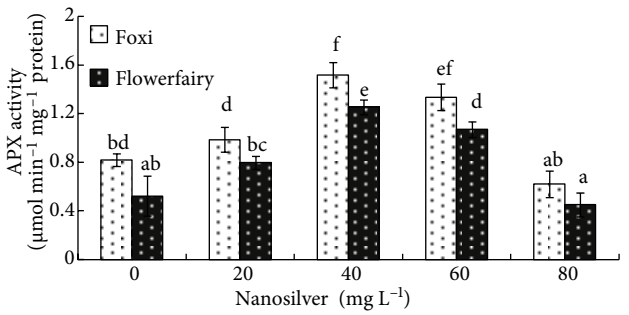

Figure 2. Ascorbate peroxidase (APX) activity of 2 *Pelargonium* cultivars, Foxi and Flowerfairy, in response to different nanosilver concentrations after 5 days of dark storage. The error bars represent the standard deviation for 3 replications. Different letters indicate significant differences in each treatment as determined by Tukey's HSD test at P = 0.05.

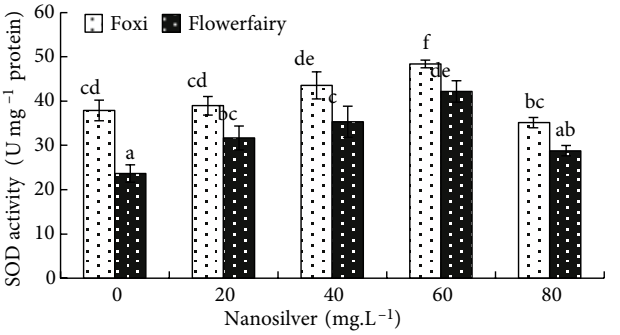

Figure 3. Superoxide dismutase (SOD) activity of 2 *Pelargonium* cultivars, Foxi and Flowerfairy, in response to different nanosilver concentrations after 5 days of dark storage. The error bars represent the standard deviation for 3 replications. Different letters indicate significant differences in each treatment as determined by Tukey's HSD test at P = 0.05.

was similar to that of SOD in the cultivars (Figure 4). At 60 mg L^{-1} NS treatment, the POD activity increased to maximum levels and then decreased with increase of NS concentration. However, the decrease of POD activity in Foxi was more pronounced than in Flowerfairy. Significant ($P < 0.05$) and different variations of CAT activity were observed between cultivars under various NS treatments (Figure 5). CAT activity was noticed and pronounced in NS-treated Flowerfairy and Foxi plants at 20 and 40 mg L^{-1}, respectively. Under higher NS treatment, a decrease of the CAT activity was observed in the 2 cultivars. The β-GLU activity in NS (40 and 60 mg L^{-1})-treated Foxi plants was significantly higher compared to the other levels of NS treatments (Figure 6), whereas in Flowerfairy, the highest activity of β-GLU was observed at 20 mg L^{-1} NS (twice the control), and thereafter it decreased with increase of NS concentration. Both under control conditions and at the highest NS concentration, β-GLU showed lower activity than at other NS levels for the 2 cultivars.

4. Discussion

Application of nanotechnology is now widely distributed throughout life, and especially in agricultural systems. NS particles, because of their physicochemical characteristics, are among the potential candidates for modulating the redox status and changing the growth, performance, and quality of plants (Mukherjee and Mahapatra, 2009).

The current study confirmed that NS application could improve pigment status of *Pelargonium zonale* plants under dark storage, which reflects the delay of senescence.

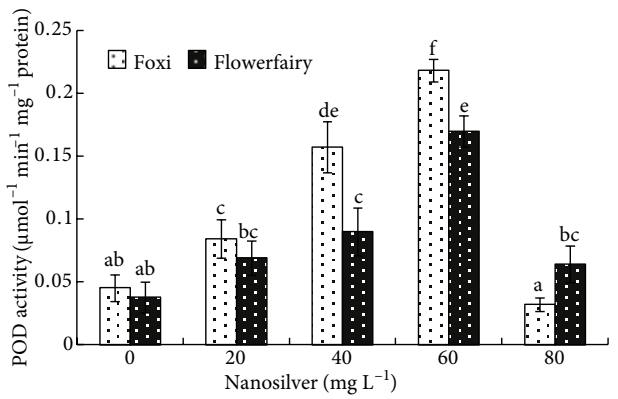

Figure 4. Peroxidase (POD) activity of 2 *Pelargonium* cultivars, Foxi and Flowerfairy, in response to different nanosilver concentrations after 5 days of dark storage. The error bars represent the standard deviation for 3 replications. Different letters indicate significant differences in each treatment as determined by Tukey's HSD test at P = 0.05.

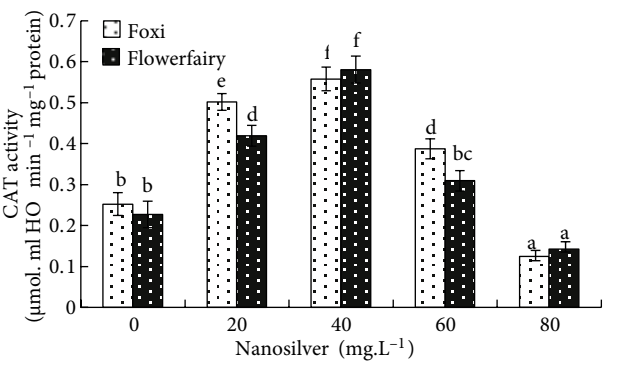

Figure 5. Catalase (CAT) activity of 2 *Pelargonium* cultivars, Foxi and Flowerfairy, in response to different nanosilver concentrations after 5 days of dark storage. The error bars represent the standard deviation for 3 replications. Different letters indicate significant differences in each treatment as determined by Tukey's HSD test at P = 0.05.

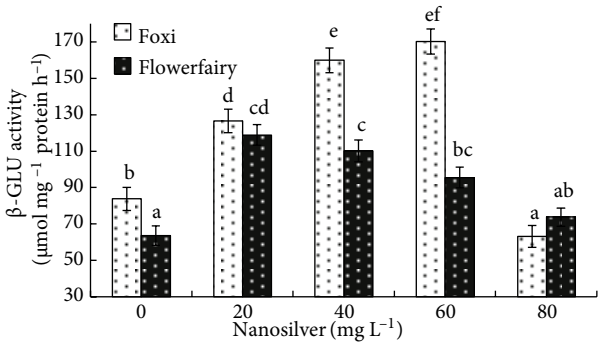

Figure 6. β-Glucosidase (β-GLU) activity of 2 *Pelargonium* cultivars, Foxi and Flowerfairy, in response to different nanosilver concentrations after 5 days of dark storage. The error bars represent the standard deviation for 3 replications. Different letters indicate significant differences in each treatment as determined by Tukey's HSD test at P = 0.05.

Additionally, the minimum ratio between chlorophyll *a* and *b* under 0 and 80 mg L^{-1} NS concentrations in both cultivars indicate that chlorophyll *b* was the better improved pigment under such conditions. In our study, plant exposure to the highest NS concentration resulted in reduction in the content of pigments when compared with the other NS treatments (Table 1). However, the extent of decrease in photosynthetic pigments was not lower than that of the control. It is well established that chlorophyll *a* breaks down during senescence; however, it is supposed that chlorophyll *b* was converted to chlorophyll *a* before bleaching or degradation (Matile et al., 1996). It has been recently reported that NS treatments increase the chlorophyll contents (40% in chlorophyll *a* and 25% in total chlorophyll) of *Brassica juncea* seedlings at 100 mg L^{-1} NS concentration (Priyadarshini et al., 2012). The same authors also stated that improved quantum efficiency in the leaves of NS-treated seedlings positively correlates with higher chlorophyll content. Research on the influence of magnetic nanoparticles (coated with tetramethyl ammonium hydroxide) in maize crops revealed that chlorophyll *a* increased at low ferrofluid concentrations, while a reverse effect was observed with increasing concentrations of solution (Racuciu and Creanga, 2006).

MDA accumulation has been continually explained as a suitable biomarker for lipid peroxidation (Debasis et al., 2007). The level of this compound in plant cells increases under dark-induced senescence and stress situations. This increase in lipid peroxidation was supported by the high degree of membrane damage expressed as a decrease in MSI (Debasis et al., 2007). In our present experiment, a steady decrease in MDA content of *Pelargonium* cultivars under particular NS treatments indicates a gradual loss of MSI compared to the control plants (Table 2). The decline in MSI of *Pelargonium* cultivars was alleviated by treatment with NS at certain concentrations, which was associated with an increase in protein content and defense enzyme activities (Smirnoff, 1993). It was shown that NS-treated plants encourage an efficient cellular electron exchange mechanism, which slows down electron leakage and consequently reduces the ROS production and MDA content (Lu et al., 2002). Most recently it was shown that nanoparticles (TiO_2) not only induced oxidative damage but also alleviated membrane damage indexes (electrolyte leakage) under cold stress treatment in chickpea genotypes. The same study found that physiological indexes were positively affected by nanoparticle treatments during thermal treatments (Mohammadi et al., 2013).

Pelargonium is one the ethylene-sensitive flowering plants (Woltering, 1987). Leaf yellowing, leaf drop, and petal abscission are major senescence indications caused by ethylene (Serek et al., 1998). It is recognized that inhibition of ethylene action or synthesis prolongs senescence in many plant species (Abeles et al., 1992). It is also well known that application of Ag^{+} can replace Cu^{2+} from the receptor proteins, which causes a reverse effect on ethylene action in plant tissues due to the critical function of Cu^{2+} in ethylene binding upon receptors (Strader et al., 2009). Furthermore, NS efficiency in our experiment, especially on petal abscission, could be due to greater ion liberation from the nanoparticles, which is consistent with previously reported results by Harris and Bali (2008).

Dark stress, similar to other environmental factors, causes oxidative damage in plant cells by production of ROS such as O^{-2}, H_2O_2, and OH^{-} (Winterbourn, 1982). It was proven that dark storage increases the plant petal abscission and leaf senescence, which is described by protein loss (Brady, 1988). There is some evidence that both synthesis reduction and proteolysis enhancement are the reasons for the protein reduction observed during senescence (Droillard et al., 1992). In our current study, the control and the highest NS concentration-treated plants showed a significant ($P < 0.05$) increase in MDA and ethylene content (Table 2). On the contrary, a significantly lower MDA and ethylene content along with a higher MSI value was observed at 60 mg L^{-1} NS concentration in both cultivars, which indicated that a better protective mechanism might exist in NS application. Recently, Priyadarshini et al. (2012) reported that NS particles decreased H_2O_2 production and increased the efficiency of redox reactions. They also reported that higher concentrations of NS enhanced the activity of H_2O_2-metabolizing enzymes. In our research, activity of antioxidant enzymes (APX, SOD, POD, and CAT) and β-GLU (as a glycoside hydrolase enzyme) were affected differently in 2 cultivars under various employed NS treatments. Enhanced SOD activity of leaves under NS treatments may be interpreted as a

direct response to augmented O^{-2} formation since SOD is an enzyme that catalyzes the conversion of the O^{-2} to O_2 and H_2O_2 (Hafis et al., 2011). Furthermore, reduced SOD activity in both cultivars under the control and the highest NS dose treatments may reflect the low ROS scavenging capacity and increased damage to the plant parts (petal abscission) and structure (protein). Our results show that certain NS concentration treatments upregulated the activity of POD in *Pelargonium* cultivars. This might be an important protection mechanism in plants against the excessive increase of H_2O_2 during dark-induced stress. It was previously suggested that the overexpression of SOD, if accompanied by increment of H_2O_2 scavenging mechanisms like POD and CAT, be considered as a strategy to cope with oxidative damage (Kohler et al., 2006). The SOD and POD change trend for both cultivars was similar and concurrent decrease and increase were observed, which may be due to their co-regulation, in agreement with the findings of Shigeoka et al. (2002) and Ghorbanpour et al. (2013). It was reported by Krishnaraj et al. (2012) that high levels of CAT and POD activity were recorded from leaf samples of plants subjected to NS treatment, implying less ROS formation and resulting in less toxicity to the plants. Lei et al. (2008) stated that nanoparticles (TiO_2) decreased oxidative damage in spinach chloroplast by increasing APX, SOD, POD, and CAT activity. It was suggested that combined reduction of APX, SOD, and CAT activity resulted in high generation of intercellular ROS concentrations, which may directly or indirectly be involved in the lipid peroxidation, senescence, and cell death of plant florets (Debasis et al., 2007). It can be noted that NS releases Ag^+ ions, which interact with cytoplasmic organelles and nucleic acids to inhibit respiratory enzymes and interfere with cellular functions such as membrane leakage (Lu et al., 2010). In the current work, the β-GLU enzyme response to NS application in 2 *Pelargonium* cultivars was completely different (Figure 6). In plants, β-GLU could deliver many important functions, including bioactivation of defense compounds, cell wall degradation, activation of phytohormones, and lignification and abscisic acid liberation (Suzuki et al., 2006). These defense compounds are regarded as protective mechanisms against the toxicity of the plant's own chemical defense system, to add to solubility and to facilitate storage (Lee et al., 2006).

Yin et al. (2011) mentioned that increasing NS concentrations caused a decrease in plant root growth, which indicated an increase in phytotoxicity of NS particles. Phytotoxicity of NS particles in living organisms was also reported (Çiftçi et al., 2013). Interestingly, in our experiment, NS-treated *Pelargonium* plants at low to moderate concentrations caused higher levels of defense enzyme activity than in the control untreated plants. Meanwhile, the highest NS concentration caused no positive variations when compared to the control, indicating the potential toxicity of NS particles with this adverse effect. In our research, the decrease in antioxidant enzyme activity observed in control plants may be directly attributed to ethylene production (Table 2) or may be indirectly associated with leaf senescence and pigment reduction (Table 1). Different physiological and biochemical responses of 2 *Pelargonium* cultivars were also observed in the various employed NS treatments under dark storage conditions. Some reports showed that activity of antioxidant enzymes under different stress conditions were relatively higher in tolerant species than in sensitive ones (Bor et al., 2003). The current findings clearly indicate that poststorage performances of *Pelargonium* pot plants were both positively and negatively affected by NS treatments via their biochemical and enzymatic variations. Decrease in ethylene production and enhancement of leaf protein content confirmed the improvement in petal longevity and in antioxidant status of certain NS-treated plants.

In conclusion, from the substantial differences of antioxidant enzyme activity and biochemical responses in the 2 examined *Pelargonium* cultivars under different NS treatments during a dark storage period, it can be concluded that using appropriate NS particle concentrations to maintain and enhance the photosynthetic pigments, reduce ethylene production and petal abscission, and improve protein and defense enzyme status under dark storage conditions could be a new strategy for improving tolerance in *Pelargonium* pot plants during shipment situations. Optimized use of NS particles could also modulate 'stress ethylene' in plants, especially in storage-sensitive cultivars. It should be noted that the Foxi cultivar could be introduced as a better species for transport conditions or ethylene exposure than Flowerfairy. Our findings suggest for the first time that NS application improved petal longevity and defense enzyme status of *Pelargonium* pot plants under dark stress conditions.

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