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Biochemical, physiological changes and antioxidant responses of cut gladiolus flower 'White Prosperity' induced by nitric oxide

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Key words: anthocyanin, catalase, cut gladiolus flower, enzymatic antioxidant system, nitric oxide (NO), sodium nitroprusside (SNP)

Abstract: Sodium nitroprusside (SNP), as nitric oxide (NO) donor, has been considered by postharvest researchers as one of the best option for slowing the processes controlling senescence in cut flowers. Here, we investigate the role of NO on postharvest physiology and vase life of the *Gladiolus grandiflorus* cv. White Prosperity. Vase life markedly extended by SNP at 150 µM from 3 day to 7.33 day and thus those inducer effects were dose- and time-dependent. SNP at 125 µM interdependent on vase life time period was observed to be the optimal dose for improving of relative fresh weight (RFW), peroxidase (POD), and total monomeric anthocyanin (TMA) in cut flowers. Supplementing vase solution with SNP indicated significant increase in water uptake of cut flowers and consequently protected to decline in RFW due to alleviate water losses stress. SNP was maintained the level of total soluble protein, lipid peroxidation, and POD, whereas it enhanced the level of catalase (CAT) and TMA in flower petals. Summary of our results revealed that SNP exogenous prolongs vase life via maintaining protein degrade, scavenging free radical in term of anthocyanin and enzymes antioxidant, decreasing polyphenol oxidase, inhibiting lipid peroxidation, and improving membrane stability in 'White Prosperity' cut flowers.

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The authors declare no competing interests.

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1. Introduction

Floriculture is an emerging and fast expanding globalized market and subsequently studies on postharvest handling of cut flowers occupy a fundamental position (Gul and Tahir, 2013). Therefore, the postharvest longevity of flowers have a vital importance in evaluating the value of the each horticulture plant. This aspect can be particularly hold good with cut flowers and it is a necessity for extended handling and transportation periods. Cut flowers are greatly perishable, and consequently they have short vase life and also are exposed to early senescence processing, which restricts efficient marketing of economically significant ornamental plants

(Nasibi *et al*., 2014). However, postharvest senescence is a major restriction to the marketing of many species of cut flowers and so much appreciable efforts have been dedicated to developing postharvest treatments to extend the marketing period or increasing postharvest longevity (Vajari and Nalousi, 2013). Sodium nitroprusside (SNP), as donor nitric oxide (NO, is one of the postharvest treatments which recently using from it for improving postharvest life of horticulture crops has exceptionally increased. Postharvest application of SNP has been shown to be effective in extending the postharvest life of a range of flowers, fruits and vegetables when applied as a short term fumigation treatment at low concentrations (Wills *et al*., 2000). NO is a short-lived bioactive molecule, which is considered to function as prooxidant as well as antioxidant in plants. NO molecule is now documented as an important signaling molecule and reported to be involved in various key physiological processes such as plant defense mechanism, abiotic stress resistance, germination, stimulate antioxidant compounds, decrease lipid peroxidation, growth and development of plants etc. (Zhao *et al*., 2004). Furthermore, it was also revealed that plant response to such stress or like drought, high or low temperature, salinity, heavy metals and oxidative stress derived from reactive oxygen species (ROS), is moderate by NO (Mandal and Gupta, 2014). NO is recognized as a biological messenger in plants and it has been proved that NO is effective for increase the vase life of cut flowers because it can be may play role as anit-ethylene synthesized from wounded or non-wounded organ (Abasi, 2014). Liao *et al*. (2009) reported that NO may act as an antagonist of ethylene in cut rose flowers senescence. Optimum SNP levels could postponement the climacteric phase of many tropical fruits and elongate the post-harvest shelf life of a wide range of horticultural crops by inhibiting ripening and senescence (Singh *et al*., 2013).

Gladiolus is one of the four famous cut flowers in the world (Bai *et al*., 2009). Gladiolus cut flowers have extremely used to decorate graves and celebrate major life events in Iran. Likewise, the longevity of cut flowers is one of the main challenges of florists today. First data concerning about the effect of SNP on differential activity of antioxidants and expression of SAGs (senescence associated genes) in relation to vase life of gladiolus cut flowers (*Gladiolus grandiflora* cv. Snow Princess) has been reported by Dwivedi *et al*. (2016). Finding of their study suggested that the application of SNP increases vase life by increasing the scavenging mechanism of reactive oxygen species (ROS) in terms of antioxidants activity, membrane stability and down-regulation of *GgCyP1* gene expression in gladiolus cut flowers. Under condition in plants subjected to SNP, not only the responses of various genotypes or cultivars to SNP may be multi-response, but also the responses rely on dose-and cultivar-dependent, physiological growth state, and environmental factors status. The same trend has been stated by Naing *et al*. (2017) who found that SNP promoted the vase life of the cut gerbera flowers via a delay in the time to stem bending; however, all three gerbera cultivars responded to SNP and the effects were found to be dose- and cultivar-dependent. In the previous study, it has been demonstrated that the SNP dose that was best for one cultivar was not suitable for another; thus, variation in the optimal dose of SNP among cultivars for the enhancement of their vase life could result from differences in their genetic background (Naing *et al*., 2017).

Hence, whether SNP participates in improving of cut flowers of White Prosperity cultivar has not been yet reconnoitered. However, the purpose of the present study was to evaluate the effect induced by nitric oxide donor namely, SNP, on the enzymatic antioxidant activity, biochemical and physiological processes of cut gladiolus (*Gladiolus grandiflorus* cv. White Prosperity) flowers in order to extend their vase life and postharvest shelf-life.

2. Materials and Methods

Plant material and SNP treatments

Cut flowers used in the experiment were *G. grandiflorus* cv. White Prosperity. Cut gladiolus flowers were obtained from a commercial grower presented in Mahallat city, as famous central commercial production of ornamental plant, in Iran at normal harvest maturity and transferred immediately to laboratory of the Postharvest Physiology and Technology Research, Faculty of Agriculture and Natural Resources, Hormozgan University at Jun, 2017 and the experiments were established on the same day. Flowers stems ends were recut under tap water to eliminate air emboli, to inhibit vascular blockage, and to trim to a uniform length of 70 cm. Stock solutions of SNP (Enzo Life Sciences) were prepared following the manufacturer's instructions. Uniform cut flowers

were placed in holding solutions, that containing of SNP, Na_2 [Fe (CN) 5 NO]. $2H_2O$ (Sigma-Aldrich), as NO donor (0, 25, 50, 75, 100, 125 and 150 µM) plus 3% sucrose as carbohydrate supplement. For control set, flowers were dipped in distilled water plus 3% sucrose. Finally, the flowers stems were placed in 500 ml bottles with 250 ml of each mentioned solutions containing different concentrations of the SNP solutions + 3% sucrose and they were maintained at a temperature of 23±3°C, 60±5% relative humidity and under a 12 h photoperiod using cool-white fluorescent lamps (24 µmol m^{-2} s^{-1} irradiance) during experimental period. There were three bottles (21 flowers) per treatment and the experiment was done seven treatments. To escape from photodegradation of SNP (release of a nitrosyl ligand and a cyanide ion), the bottles were shielded with black nylons. SNP treatment was applied as a continuous treatment and flower stems were kept in solutions till the end of vase life.

Vase life and water uptake

The vase life was determined based on wilting of more than one-third of the petals of flower and vase life termination of each floret was considered as soon as the first symptom of wilting was observed. Indeed, it was defined as the number of days in vase life required for one-third of the florets of each spike to lose its ornamental value (lost turgor and wilted). Water uptake was measured by periodically weighting the vase of a control bottle without cut flowers and bottles containing flowers. Finally, vase water uptake was determined using the formula (Rezvanypour and Osfoori, 2011):

$$\text{Water uptake (ml day}^{-1}\text{ g}^{-1}\text{ fresh weight)} = (St^{-1}\text{-}St)/Wt$$

Where St= solution weight (g) at = days 1, 4, 8 and S_{t-1}= solution weight (g) on the preceding day, and Wt = fresh weight of the cut flower (g) on t days.

Number of opened, unopened florets and relative fresh weight

On each spike, the number of opened and unopened florets was recorded from the beginning of the experiment to until 20 days after SNP treatments. The fresh weight was measured every 4 days and relative fresh weight (RFW) of cut flowers was calculated by the following equation:

$$RFW\ (\%) = (Wt/W_{t=1}) \times 100$$

where Wt = weight of cut flowers (g) at t = days 1, 4, 8 and $W_{t=1}$ = the initial fresh weight of the same cut flower (g) on day 1 (Rezvanypour and Osfoori, 2011).

Antioxidant Enzyme assays

Antioxidant enzyme activities were determined in the third floret from the base of spike at three time points (days 1, 4, and 8). The 100 mg of floret tissue from controls and SNP treatments were removed, were homogenized with mortar and pestle in 1 mL 50 mM EPPS buffer (pH 7.8) containing 0.2 mM EDTA and 2% PVP, and were ice-covered for the analysis of antioxidant activity. The homogenates were centrifuged at 4°C for 20 min at 12 000×g and the obtaining supernatants were used to evaluate of antioxidant enzyme activities. Catalase (CAT) activity was assayed as described by Chance and Mahly (1995) as follows: the assay reaction mixture of CAT contained 50 mM phosphate buffer (pH 7.8), 15 mM H_2O_2, and crude enzyme. The decomposition of H_2O_2 was followed at 240 nm (E = 39.4 mM^{-1} cm^{-1}). Absorbance values were quantified using standard curve generated from known concentrations of H_2O_2. For the measurement of peroxidase (POD) activity, the reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM guaiacol, 5 mM H_2O_2 and enzyme. The reaction was started by adding 300 µl of H_2O_2 (0.03%). The POD activity was determined by the increase in absorbance at 470 nm due to guaiacol oxidation (E = 26.6 mM^{-1} cm^{-1}) (Chance and Mahly, 1995). The polyphenol oxidase (PPO) activity was assayed in 2.8 mL of reaction mixture comprised 2.5 mL of 50 mM potassium phosphate buffer (pH 7.8), 0.3 mL substrate containing 0.2 mL pyrogallol and 0.1 mL crude enzyme (Kar and Mishra, 1976). The reaction mixture was mixed and the PPO activity was determined in absorbance at 420 nm (6.2 mM^{-1} cm^{-1}). It's to be remembered that the blank cuvette consisted of 3.0 mL potassium phosphate buffer (pH 7.8). The results of antioxidant enzymes activitie was expressed as units (U) per mg FW.

Anthocyanin content assay

Petals were cut from controls and SNP treatments of at three time points (days 1, 4, and 8) and were frozen for the analysis of total monomeric anthocyanin (TMA). TMA content in petals extract was determined by the pH-differential method based on two buffer system described previously by Giusti and Wrolstad (2005). To measure the absorbance at pH 1.0 and 4.5, the samples were diluted 2 times with pH 1.0 potassium chloride buffer (0.025 M) and pH 4.5 sodium acetate buffer (0.4 M), respectively. Therefore, the TMA content analyses of prepared mixtures were performed following the methods of Giusti and Wrolstad (2005).

Lipid peroxidation

The level of lipid peroxidation in petals tissue was measured by determination of malondialdehyde (MDA), which is recognized to be breakdown products of lipid peroxidation, at the end of time points (days 8). The MDA content was determined with the thiobarbituric acid (TBA) reaction. Temporarily, 0.2 g of sample tissue was homogenized in 5 ml 0.1% TCA. The homogenate was centrifuged at 10000 g for 5 min. 4 ml of 20% TCA containing 0.5% TBA were added to 1 ml aliquot of the obtained supernatant. The mixture was heated at 95 ºC for 15 min and cooled immediately on ice. The absorbance was measured at 532 nm by a spectrophotometer. The value for the non-specific absorption at 600 nm was subtracted from the above value. The level of lipid peroxidation was expressed as mmol of MDA formed using an extinction coefficient of 155 $mmol^{-1}$ cm^{-1} (Heath and Packer, 1968).

Total soluble proteins

Total soluble proteins (TSP) content of petals at the three time point (days 1, 4, and 8) was determined according to the method of Bradford (1976) using Bovine serum albumin as standard.

Statistical analysis

The experiment was carried out in completely randomized design (CRD) with three replications. Three flowers stems were used for each replication and thus, the experiment was done with seven treatments and three replication per treatment. The non-normalize date of the total soluble protein, POD enzyme activity and RFW of cut flowers were normalized with kurtosis and skewness test; so, their transformed date used for analyzing. Data were statistically analyzed using analysis of variance (ANOVA) in SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). Correlations among the evaluated parameters were analyzed using Pearson's correlations (p<0.05 and p<0.01). Mean comparisons to identify significant differences between treatments were performed using Least Significant Difference (LSD) at the p<0.01 or 0.01 level of probability.

3. Results

Vase Life, RFW and water uptake

Application of SNP markedly enhanced the time to vase life for White Prosperity cultivar (p < 0.01). Results showed that the bottle solution containing SNP + sucrose, significantly increased the vase life of cut flowers compared to the control solution (distilled water), as maximum vase life with higher concentration of SNP treatments was verified near the end of storage (Table 1). However, it positive impacts on increasing vase life was dose-dependent: 150 and 125 µM were displayed to be the best concentration for vase life (7.33 and 5.66 days) of 'White Prosperity', respectively, whereas the other concentrations lower than the 125 µM did not markedly influenced vase life as compared to control (p<0.01). Generally, based on the results of vase life, 'White Prosperity' exhibited a longer vase life (4.33 days) when exposed to 150 µM NO as compared to control (3 days). The prolonged vase life in SNP-treated cut flowers were approximately associated with increasing in floral opening of cut flower by 150 µM treatment (Table 1). A direct significant relationship was detected between SNP and floral opening (%); however, increasing in SNP concentration resulted in increasing in floral opening percentage. Statistically, the floral abscission and un-opened flower did not affected by SNP treatments as compared to control (p<0.01).

Table 1 - Effect of different concentrations of sodium nitroprusside (as nitric oxide donor) on vase life, flower opening and floral abscission in cut Gladiolus flowers (*Gladiolus grandiflorus* cv. White Prosperity)

Treatments	Vase Life (days)	Full-opened flower (%)	Un-opened flower (%)	Floral abscission (%)
Sodium nitroprusside 0 µM	3.0 c	58.60 b	19.21 a	24.39 a
Sodium nitroprusside 25µM	3.0 c	60.33 ab	15.51 a	22.99 a
Sodium nitroprusside 50 µM	3.33 c	69.83 ab	14.83 a	18.02 a
Sodium nitroprusside 75 µM	4.0 bc	66.92 ab	15.87 a	17.19 a
Sodium nitroprusside 100 µM	3.33 c	72.38 ab	10.52 a	19.72 a
Sodium nitroprusside 125 µM	5.66 ab	74.81 ab	6.38 a	18.79 a
Sodium nitroprusside 150 µM	7.33 a	80.25 a	6.52 a	13.21 a

Values followed by the same letter within a column indicate they are not significantly different (p< 0.01) by Least Significant Difference (LSD).

As shown in figure 1A, RFW (data normalized; 4.6 is equal to 100%) in cut gladiolus flowers of control gradually was declined during the vase life period, while the decline in RFW was no observed by SNP treatments throughout the vase life. It is notable that the RFW of the SNP treatments solutions except to 125 µM at three point time did not significantly difference with those in control solutions at initial point time (day 1). So, the presence of SNP in vase solutions displayed a protective role to the inhibition of RFW decline in vase life period, even when the vase life of cut flowers ended. Only SNP with concentration 125 µM (5.60±0.08) in vase solution prolonged the RFW 22.55% higher than other concentrations on day 4 or day 8 in comparison to those in controls at initial time (Fig. 1A).

Senescence is a process characterized by water loss and desiccation of plant tissues. During vase life period, water uptake gradually was declined in both some of the SNP treatments (25, 50, and 75 µM) and control cut flowers (Fig. 1B). Generally, the water uptake with SNP concentration 100, 125, 150 µM were higher than those under control condition, respectively (p<0.01). At the initial point time (day 1) of vase life, the White Prosperity showed a rapid response to high SNP concentrations for promoting water uptake; however, the water loss was not observed during its vase life period. The vase solutions containing SNP at concentration 125, 150 µM significantly increased water uptake (12.33±0.56 and 12.69±1.51 ml day^{-1} FW) compared to the control (9.16±0.81 ml day^{-1} FW) resulted in 34.60% and 38.53% increase in vase solution uptake on day 1, respectively; however, they were also conserved the same manner on day 4 or day 8. In contrast, the low concentration did not sufficiently play protective role to inhibit water losses on 4 days, which was also detected that the 25 and 50 µM accelerated water losses, even faster than controls, especially on 8 days.

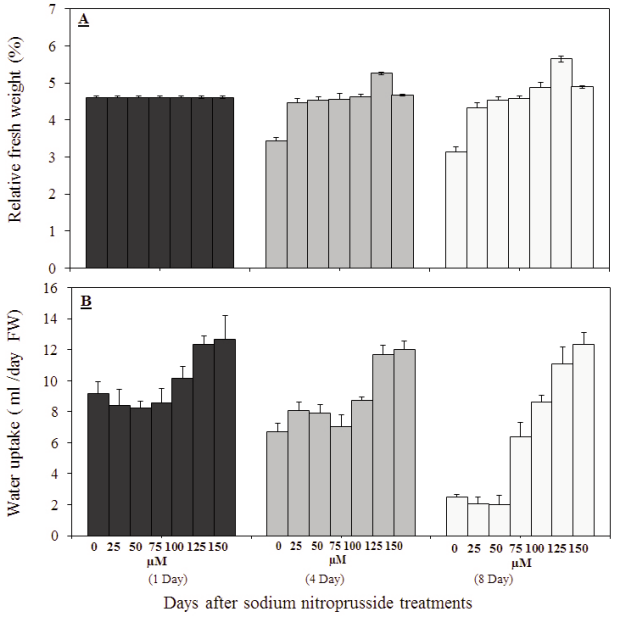

Fig. 1 - The effects of different concentrations of sodium nitroprusside (as nitric oxide donor) on physiological changes of *Gladiolus grandiflorus* cv. White Prosperity cut flowers during vase life. Data are means of three replications. Vertical bars indicate standard deviation.

Lipid peroxidation

The data, belong to MDA concentration of flowers petals representing the level of lipid peroxidation is revealed in figure 2A. Measurement of MDA demonstrated that vase solutions containing 125 and 150 µM SNP significantly decreased MDA production in comparison to control (p<0.01). Overall, it was predictable that White Prosperity without treatment (control) showed higher MDA concentration than the SNP treatments. Results found inversely correlation between lipid peroxidation and higher SNP concentration. At any specified level of SNP concentration, the production of lipid peroxidation product was lesser in treatments at the end experiment (8 days) comparison to control. Thus, the vase solutions having

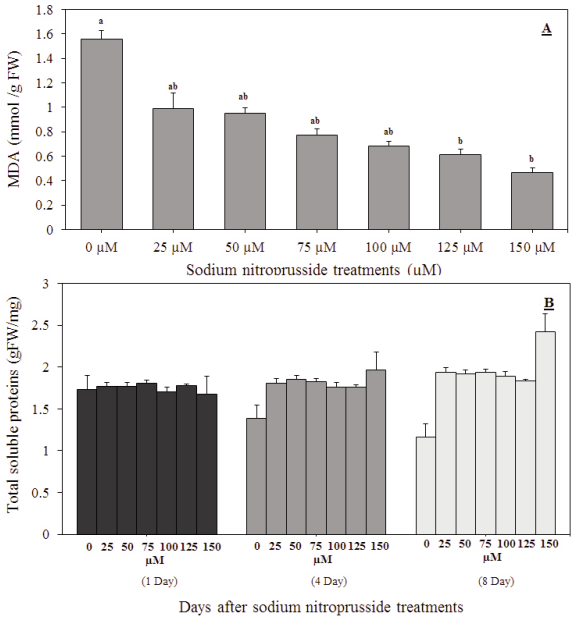

Fig. 2 - Effect of different concentrations of sodium nitroprusside (as nitric oxide donor) on Malondialdahyde (MDA), as an indicator of lipid peroxidation, total soluble protein in *Gladiolus grandiflorus* cv. White Prosperity cut flowers during vase life. Vertical bars with the same letters did not show significantly different using LSD method at P<0.01 significant level.

SNP at concentration 125, 150 µM significantly declined the lipid peroxidation of cell membrane (0.463±0.04 and 0.61±0.04 mmol g^{-1} FW) compared to the control (1.55±0.07 mmol g^{-1} FW) resulted in 70.13% and 60.65% decline in product induction of lipid peroxidation, MDA, on days 8 prior to senescence appearance in cut flowers.

Total soluble proteins

The chemical analysis for TSP of the flower petals exhibited that SNP significantly increased the TSP during vase life period in comparison with control (p<0.01) (Fig. 2B). The protein degradation of flower petals in control was higher than SNP treatments; however, the total soluble protein gradually was declined in control across days. Thus, not only SNP lead to help to the inhibition of protein degradation in flowers petals on day 4 or day 8, but also it caused in delaying the senescence of gladiolus flowers. So, all of the SNP treatments except to 150 µM displayed a protective or maintain role for protein degradation in cut flowers. Overall, only increase in TSP was observed with 150 µM SNP and also was recorded highest TSP for its on day 8; however, the protein degradation did not occurred by 150 µM SNP treatment during vase life period. Furthermore, the prolong vase life of White Prosperity flowers can be strongly associated with increasing in TSP and inhibiting from its degradation in flower petals during vase life.

Enzymatic antioxidant and non-enzymatic antioxidant activities

ANOVA analysis with mean comparison showed that antioxidant enzymes and non-enzymatic antioxidant activities in flower petals differed significantly between control and treatments in White Prosperity (p<0.01). As expected, the PPO activity (U/mg FW) was continually increased in control during vase life, which this tendency was also approximately found for 25 µM (Fig. 3A). The low concentration from 25 to 75 µM did not sufficiently decrease the PPO activity in flower petals comparison to control (p<0.01). So, the decrease in PPO activity was observed by 100, 125, and 150 µM treatments on day 4 or day 8, respectively; however, increasing SNP concentration in vase solution resulted in markedly decreasing PPO activity in comparison to controls at initial time of vase life (p<0.01). It can be predictable that the positive effect of SNP on maintaining or decreasing PPO activity was high dose-dependent. It is now well recognized that high PPO activity accelerate to senescence and to induce browning in plant tissues. Generally, the high concentrations of SNP to White Prosperity cut flowers, check the activity of PPO enzyme, lead to help in delaying the senescence of gladiolus flower via preventing the PPO activity compared to control (p<0.01). As shown in figure 3B, the POD activity (U/mg FW) significantly decreased in control flowers throughout vase life, while the CAT activity (U/mg FW) in control flowers displayed a constant tendency at all of the 3 point time of vase life (Fig. 3C) comparison to SNP treatments(p<0.01). It is appears that all of the treatments except to 125 µM significantly played a protective role to conserve the decrease of POD activity during vase life (p<0.01). The highest POD activity obtained by 125 µM on 8 days, according to LSD test at p<0.01. However, the positive effect of SNP on POD activity was dose- and time-dependent: 125 µM was observed to be the optimal concentration for POD activity on day 4. Thus, in White Prosperity, low concentrations did not adequately increase POD activity, which was also found for concentrations higher than the optimal levels (Fig. 3B). In concerning about CAT activity, the positive relation was detected between CAT activity and SNP treatments; however, increasing in SNP concentration resulted in increasing CAT activity, especially on day 4 or day 8, compared to control (p<0.01) (Fig. 3C). The results of the present study indicated that with more addition SNP concentration by 100 to 150 µM into vase solution was lead to positively increase in CAT activity at each of three time points during vase life.

The results of LSD test (p<0.01) indicated that TMA degradation was gradually happened in control flowers during vase life (Fig. 3D). The SNP treatments not only significantly prevented from the TMA degradation but also they were greatly enhanced the TMA production over 8 days, compared to control (p<0.01). At the during vase life, the low concentrations of SNP demonstrated a protective role to inhibit TMA degradation in flower petals in comparison to control (p<0.01). The furthest increase in the TMA production was archived for 125 µM (0.273 ± 0.037 mg l^{-1}), and 150 µM (0.193±0.015 mg l^{-1}) with a significant difference compared to control, respectively. Hence, improved TMA in flower petals likely to POD activity was dose- and time-dependent: 125 µM was observed to be the optimal concentration for TMA content. Thus, in White Prosperity, low concentrations did not adequately increase TMA content, which was also detected for concentrations higher than the optimal levels on day 4 or day 8.

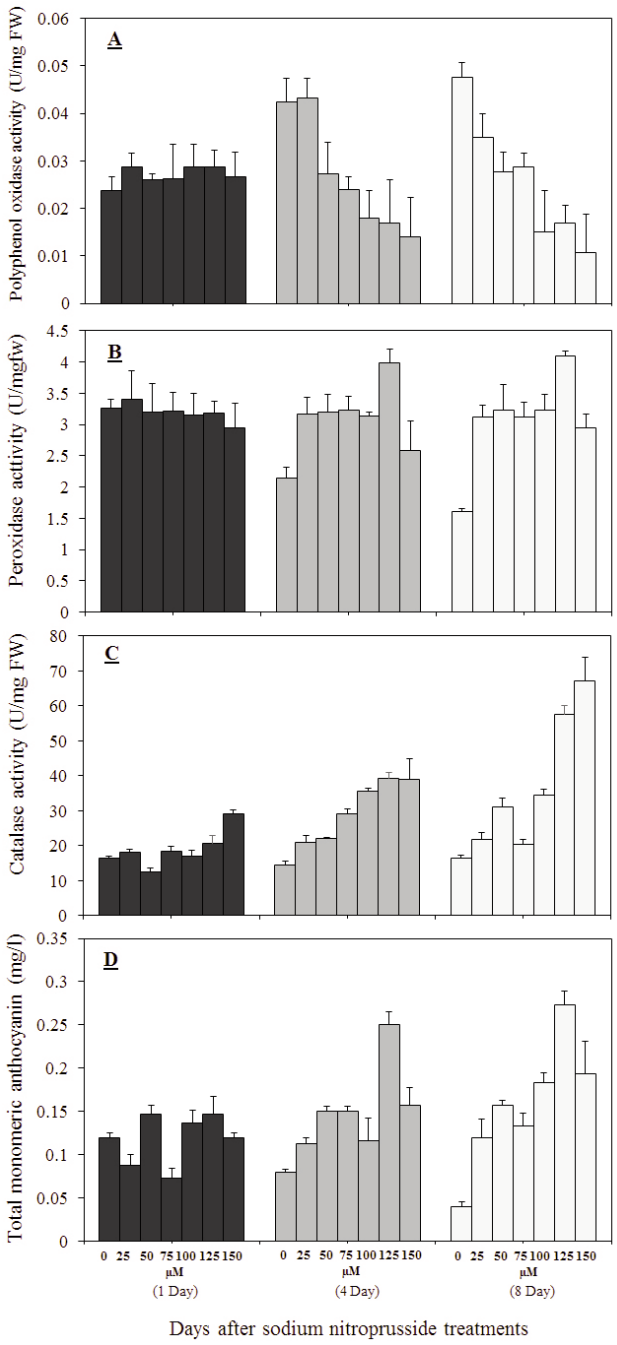

Fig. 3 - Evaluating the effects of different concentrations of sodium nitroprusside (as nitric oxide donor) on enzymatic and non-enzymatic antioxidant system changes during vase life period of *Gladiolus grandiflorus* cut flowers. Vertical bars indicate standard deviation.

Pearson correlation analysis reveals interactions between physiological, biochemical and antioxidant system related traits

In order to arrange for an overview of the associations between physiological, biochemical traits, and antioxidant system activity, the Pearson correlation test used for analyzing and thus was investigated all of the significant associations, as presented in Table 2. From this analysis 23 positive and 11 negative significant correlations was achieved. Among them, some correlations were expected, such as the positive and negative correlations observed between antioxidant system activity, for example, CAT activity and TMA content (r= 0.89, p<0.01), and PPO activity and TMA content (r= -0.95, p<0.01) on days 8, respectively. With regard to physiological traits, the results of paired linear correlation indicated that RFW was positively correlated with CAT (r= 0.85, p<0.05 on days 4), and POD activity (r= 0.86, p<0.05 on days 4 and r= 0.81, p<0.05 on days 8), and TMA content (r= 0.79, p<0.05 on days 8), while was negatively correlated with PPO activity on day 4 (r= -0.80, p<0.05) and day 8 (r= -0.87, p<0.05) of the White Prosperity vase life. Also, the Pearson correlation of water uptake with CAT activity (r= 0.80, p<0.05 on days 1; r= 0.82, p<0.05 on days 4; r= 0.86, p<0.05 on days 8) and with TMA content (r= 0.81, p<0.05 on days 8) was positive significant, whereas displayed a negative significant with PPO activity (r= -0.79, p<0.05 on days 4 and r= -0.85, p<0.05 on days 8) (Table 2). However, suggesting that the SNP treatment is a key inhibitor to water loss and an inducer to antioxidant system for delaying the senescence of gladiolus flowers during vase life, especially on 4 and days 8.

Total soluble protein had a positive correlation with TMA content and a negative correlation with PPO activity. TMA indicated a positive correlation with water uptake, RFW, total soluble protein, and CAT activity and a negative correlation with PPO and POD activity. CAT activity was positively correlated with physiological traits, TMA and negatively correlated with PPO and POD activity. However, according to the results of Pearson correlation, suggesting that SNP might be an important protective or inducer involved in the physiological, biochemical process and antioxidant system in White Prosperity vase life that can be alleviate to water loss, RFW, and to browning process, which lead to early senescence appearance.

4. Discussion and Conclusions

The postharvest longevity of cut flower has a critical importance in determining the value of crop. Recently, SNP, a NO donor known to be a signal molecule involved in biotic and abiotic stress tolerance, has been increasingly used to extend the vase life of

Table 2 - Pearson correlation between physiological and biochemical characteristics of Gladiolus grandiflorus cv. White Prosperity cut flowers affected by sodium nitroprusside during vase life period

Traits	WU1	WU2	WU3	RFW2	RFW3	TSP1	TSP2	TSP3	PPO1	PPO2	PPO3	POD1	POD2	POD3	CAT1	CAT2	CAT3	TMA1	TMA2	TMA3
WU1	1	0.932 **	0.919 **	0.499 NS	0.595 NS	-0.499 NS	0.261 NS	0.408 NS	0.288 NS	-0.584 NS	-0.713 NS	-0.741 NS	0.152 NS	0.152 NS	0.803 *	0.746 NS	0.927 **	0.421 NS	0.655 NS	0.732 NS
WU2		1	0.849 *	0.698 NS	0.753 NS	-0.383 NS	0.517 NS	0.616 NS	0.48 NS	-0.579 NS	-0.801*	-0.666 NS	0.347 NS	0.347 NS	0.752 NS	0.829 *	0.981 **	0.434 NS	0.752 NS	0.854 *
WU3			1	0.627 NS	0.722 NS	-0.388 NS	0.430 NS	0.553 NS	0.40 NS	-0.791*	-0.850*	-0.787*	0.271 NS	0.271 NS	0.780 *	0.871 *	0.862 *	0.25 NS	0.697 NS	0.811 *
RFW2				1	0.988 **	0.208 NS	0.747 NS	0.687 NS	0.802 *	-0.575 NS	-0.809*	-0.295 NS	0.866 *	0.866 *	0.296 NS	0.854 *	0.656 NS	0.233 NS	0.534 NS	0.745 NS
RFW3					1	0.100 NS	0.713 NS	0.677 NS	0.774 *	-0.663 NS	-0.872*	-0.406	0.817 *	0.817 *	0.352 NS	0.892 **	0.724 NS	0.311 NS	0.565 NS	0.794 *
TSP1						1	-0.009 NS	-0.208 NS	0.071 NS	0.36 NS	0.342 NS	0.615 NS	0.585 NS	0.585 NS	-0.514 NS	-0.215 NS	-0.436 NS	-0.333 NS	-0.319 NS	-0.405 NS
TSP2							1	0.971 **	0.558 NS	-0.64 NS	-0.75 NS	-0.432 NS	0.445 NS	0.445 NS	0.405 NS	0.685 NS	0.543 NS	-0.062 NS	0.771 *	0.820 *
TSP3								1	0.526 NS	-0.693 NS	-0799*	-0.561 NS	0.299 NS	0.299 NS	0.591 NS	0.751 NS	0.642 NS	-0.073 NS	0.851 *	0.889 **
PPO1									1	-0.272 NS	-0.615 NS	0.056 NS	0.747 NS	0.747 NS	0.16 NS	0.775 *	0.365 NS	0.068 NS	0.154 NS	0.507 NS
PPO2										1	0.897**	0.868*	-0.21 NS	-0.21 NS	-0.486 NS	-0.7 NS	-0.682 NS	-0.286 NS	-0.734 NS	-0.847*
PPO3											1	0.739 NS	-0.45 NS	-0.45 NS	-0.556 NS	-0.910**	-0.832*	-0.36 NS	-0.742 NS	-0.956**
POD1												1	0.152 NS	0.152 NS	-0.688 NS	-0.532 NS	-0.786*	-0.363 NS	-0.817*	-0.791*
POD2													1	1.000**	-0.14 NS	0.548 NS	0.269 NS	0.185 NS	0.086 NS	0.318 NS
POD3														1	-0.14 NS	0.548 NS	0.269 NS	0.185 NS	0.086 NS	0.318 NS
CAT1															1	0.661 NS	0.749 NS	-0.124 NS	0.779 *	0.682 NS
CAT2																1	0.786*	0.125 NS	0.646 NS	0.862 *
CAT3																	1	0.482 NS	0.823 *	0.894 **
TMA1																		1	0.084 NS	0.3 NS
TMA2																			1	0.887 **
TMA3																				1

WU= water uptake; RFW= relative fresh weight; TSP= total soluble protein; PPO= polyphenol oxidase activity; POD= peroxidase activity; CAT= catalase activity; TMA= total monomeric anthocyanin, the 1, 2, and 3 representing vase life time for each variable on day 1, day 4, and day 8.
NS, *, ** non-significant, correlation is significant at the 0.05 and the 0.01level, respectively. RFW1 has no computed because at least one of the variables was constant.

cut flowers, such as rose, gladiolus, and carnation (Naing *et al*., 2017). First data concerning about application exogenous SNP to improve vase life of G. *grandiflora* cv. Snow Princess cut flower has been reported by Dwivedi *et al*. (2016). It is generally accepted that different genotypes or cultivars might indicate different physiological or biochemical responses to exogenous SNP, which is the effects induced by it may be rely on dose- and cultivar-dependent. Some published evidences supports NO acting as a negative regulator during leaf senescence, but also there is opposite result in this regard; NO enhances flower abscission and senescence in cut racemes of *Lupinus havardii* Wats (Sankhla *et al*., 2003; Guo and Crawford, 2005). Thus, the properly effects of SNP on enhancing physiological and biochemical processes for one cultivar, may not be suitable for another, which is due to differences in their genetic background. This aspect has also been confirmed by Naing *et al*. (2017), who found that SNP dose that was best for one cultivar of Gerbera cut flower was not suitable for another; thus, variation in the optimal dose of SNP among cultivars for the enhancement of their vase life could result from differences in their genetic background. Hence, whether SNP participate in improving of cut flowers of White Prosperity cultivar has not been yet reconnoitered.

Therefore, in the current study, we investigated the role of SNP in the enhancement of physiological, biochemical responses, and antioxidant activity to extend vase life of *Gladiolus grandiflorus* cv. White Prosperity cut flower. Cut flower senescence is linked to a sequence of highly regulated physiological and biochemical processes such as degradation of proteins, DNA content, peroxidation lipids and membrane leakage, degradation of macromolecules, cellular decompartmentalization, floral abscission, color change, leaf yellowing, and weight loss (Buchanan-Wollaston *et al*., 2003; Nasibi *et al*., 2014). In this study, results of our findings revealed that the physiological, biochemical, and antioxidant activity induced by SNP in White Prosperity cultivar were more different than those induced in Snow Princess cultivar, a previous study by Dwivedi *et al*. (2016), which it may be due to differences in their genetic background. Hence, in present study, SNP was significantly promoted the vase life of 'White Prosperity' cut flowers through help to delay the senescence appearance and desiccation on tissue or organ level; however, it effects were discovered to be dose- and time-dependent. Vase life positively associated with

RFW, water uptake, TSP content, TMA, enzyme antioxidant activity and lipid peroxidation. At the start of vase life, there was a noticeably increase and then a constant tendency in water uptake of White Prosperity cut flowers during their vase life, which suggested that SNP might have a protective role in cut flowers against water losses stress (Fig. 1B). The rapid increase in initial water uptake was dose-and time-dependent, while increase in RFW was more dose-dependent (Fig. 1A). The 125 µM concentration was observed to be the optimal concentration for increasing in RFW. The RFW increase obtained in White Prosperity is in isagreement with results pronounced by Dwivedi *et al*. (2016) in Snow Princess. The inhibition or improvement in RFW across days under NO condition is probbly attributed to the excessive potential of water uptake, leading to a stability or promote in cell turgidity pressure, which restricts burning from reserved carbohydrates in respiration and limits fresh weight reduction. An association of improved water uptake and inhibited fresh weight reduction has been reported by Vajari and Nalousi (2013) in carnation and Naing *et al*. (2017) in gerbera cut flower. Overall, the 'White Prosperity' in SNP (150 µM) had prolonged vase life over control, which was strongly associated with increased water uptake and improved RFW.

The damage to the plant cell's biomembrane liable to senescence process, decrease in the ratio of unsaturated fatty acids, change mobility of the cell membrane, and generate free radicals are resulted in an increase in the concentration of Malondialdahyde (MDA), which is an indicator of lipid peroxidation and of injury to the plant cell membrane (Chen, 2009). So, the higher membrane stability plays a key role in inhibiting leakage of electrolytes, sugars, pigment, solute leakage, and also lipid peroxidation as well as in delay senescence during gladiolus cut flowers postharvest (Ezhilmathi *et al*., 2007; Ghadakchiasl *et al*., 2017). Our results showed that the change in membrane stability and lipid peroxidation occurrence resulting from the MDA production were alleviated by SNP concentrations (150, 125 µM) on day 7 after treatment and therefore protected and reduced White Prosperity MDA production in cell membrane (Fig. 2A). These results are in agreement with those reported earlier by Mansouri (2012), who suggested that SNP prolonged the vase life of chrysanthemum flowers, which was accompanied by decreasing in the electrolyte leakage, levels of MDA and lipid peroxidation. Indeed, the role of NO in prevention of lipid peroxidation is related to the ability of NO to react with lipid alcoxyl (LO•) and lipid peroxyl (LOO•) radicals and stop the chain of peroxidation in a direct fashion (Beligni and Lamatina, 1999). The role of SNP in reducing membrane lipid peroxidation has previously been stated by Liao *et al*. (2012) and Dwivedi *et al*. (2016).

Many researchers have been shown that protein degradation and also shortage protein due to consumption it instead of soluble carbohydrate during senescence process for respiration in petals are the most important causes for shortening cut flowers vase life (Rezvanypour and Osfoori, 2011). In addition, SNP significantly maintained the TSP degradation in cut flowers petals, while in absence SNP increased the TSP degradation to a greater rate than SNP treatments on day 4 and day 8 (Fig. 2B). The TSP measured in vase solution supplemented by 150 µM was distinctly higher than those placed in controls on day 8. The proteins are the basic components of all cell activities, their reduction degrades enzymes and causes higher production of free radicals, as well as reducing protein synthesis (Saed-Moucheshi *et al*., 2014). Therefore, it was clear that the proteins degradation during vase life significantly inhibited by SNP supplements in the vase life of 'White Prosperity'. The increase or protect of TSP degradation by SNP application in strawberry (Ghadachiasl *et al*., 2017) and in peanuts (Verma *et al*., 2010) has also been claimed.

Earlier studies have been confirmed that SNP may either be directly scavenging ROS and thus decreasing lipid peroxidation, or it may be modulating the activity of antioxidant system (Beligni and Lamatina, 1999; Saed-Moucheshi *et al*., 2014). Various studies have demonstrated that the vase life of cut flowers is modulated by antioxidant enzymes and non- enzymatic antioxidant activities (Vajari and Nalousi, 2013). Thus, supplemented vase solutions with SNP stimulated a higher enzymatic or non-enzymatic antioxidant activity in flower petals during vase life period. The PPO catalyzes the browning reaction and results in the formation of quinine, which is subsequently polymerized to varying degree leading to production of brown pigments (Dubravina *et al*., 2005). The PPO activity greatly was reduced by SNP, while the POD and CAT activity greatly promoted by SNP during progress senescence (Fig. 3). The results were in accordance with the findings of Ghadakchiasl *et al*. (2017) and Dwivedi *et al.* (2016). Indeed, the NO synthesized by SNP in tissue plant acts signaling

molecule to enhance the enzymatic antioxidant activity such as SOD and CAT and ultimately protects proteins degradation as well as lipid peroxidation against free radicals. So, POD and CAT high activity induced by SNP showed a negatively correlation with PPO activity and thus they blocked PPO activity during vase life (Table 2). Approximately, high and markedly negative between PPO and more traits examined in current study were also detected in Pearson correlation analysis. Furthermore, TMA degradation in flower petals protected by SNP, while TMA degradation increasable induced in flower petals without presence SNP during vase life period. However, positive effects induced by SNP in both POD activity and TMA were dose- and time-dependent, therefore, the 125 µM was selected as an optimal concentration for TMA and POD activity (Fig. 3B, D). Antioxidant compounds such as vitamin C, glutathione, and anthocyanin plays vital role, as non-enzymatic system, in protecting cell against destructive chemical compounds such as free radicals and reactive oxygen species (ROS) that are constantly produced by the cell metabolism and their concentration increases under stress conditions (Kazemzadeh *et al*., 2015). However, SNP increased TAM content at any time and level of SNP concentration in comparison to controls. The high and significantly positive correlation between TMA anthocyanin with CAT activity and total soluble protein was obtained by pearson analysis (Table 2). With progress senescence during vase life, TMA probably scavenged free radicals due to oxidative stress, consequently, inhibited more deterioration of membrane and protein degradation in flower petals.

In conclusion, vase life period in the *G. grandiflorus* cv. White Prosperity cut flower is likely to be associated with many parameters, particularly fresh weight content, water uptake, enzymatic or non-enzymatic antioxidant activities, membrane stability and lipid peroxidation. Furthermore, it was found that positive effects induced by SNP on vase life distinctly were dose- and time-dependent and were also genetic back ground cultivar-dependent in comparative responses between White Prosperity with Snow Princess, which has previously been reported by Dwivedi *et al*. (2016). Results showed that supplementing vase solution with SNP enhanced RFW and water uptake, maintained or increased antioxidant activity, leading to inhibit lipid peroxidation and protein degradation, scavenged free radical, and ultimately causing delay in the senescence of White Prosperity.

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