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Evaluation of sodium nitroprusside (NO donor) as pulsing solution in improving post harvest quality of gladiolus spikes

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Gladiolus is an important commercial cut flower of emerging floriculture industry. The prime concern in commercialization is postharvest management of flowers as flowers are biological systems that perish after harvest. Among different chemicals used to improve vase life of gladiolus, nitric oxide has emerged as potent signaling molecule that can delay senescence and improve post harvest life of flowers. Here, we studied the potential of sodium nitroprusside (SNP) as pulsing solution on post harvest quality of gladiolus. Spikes of gladiolus were harvested at tight bud stage and pulsed for 20 h with SNP solutions of different concentrations and various physiological parameters were recorded to evaluate the keeping quality. The pulsing solution of SNP @200 mg L^{-1} with sucrose and aluminium sulphate significantly improved the vase life whereas pulsing with SNP @400 mg L^{-1} adversely affected the quality parameters. Spikes pulsed with SNP @200 mg L^{-1} supplemented with sucrose and aluminium sulphate had higher membrane stability index, relative water content, sugar content, protein content antioxidant enzymatic activity and lower TBARS content as compared to control supporting improved postharvest quality. Thus, sodium nitroprusside @200 mg L^{-1} with sucrose and aluminium sulphate could be used as pulsing solution to improve the postharvest quality of spike.

Keywords: Floriculture, Physiological weight loss, Protein content, Senescence, Vase life, Water absorption

Post harvest quality is the major concern in floricultural crops as after harvest, the undesirable changes due to flower senescence begins and ultimately marks the end of its vase life. Thus, maintaining the keeping quality of cut flowers and enhancement of their vase life are important thrust areas of floricultural research. *Gladiolus grandiflorus* (Hort.), gladiolus is commercially cut, stored, packed and transported to distant markets to regulate the supply of flowers for better remunerative prices. Gladiolus is being grown in an area of 11660 ha in India with an estimated production of 106 crore cut flowers. Amongst the cut flowers, in terms of area and production, it occupies third position. There is considerable demand for gladiolus cut flowers in both domestic and export markets due their attractive flowers. The major setback to the quality of spikes or post harvest losses take place during its supply chain. The poor-quality leads to difficulties in long-distance transportation and decreased market value[1]. The typical vase life of individual florets is just 6 to 8 days and senescent florets remain at bottom of the spikes after opening of the upper florets. The post harvest quality of flowers is dependent upon stored metabolic reserves in the form of carbohydrates, fats and proteins, cellular turgidity and responsiveness to ethylene. So, the quality of cut spikes can be prolonged either by pulsing them with high concentration of carbohydrates along with biocide or holding them in floral preservative comprising of carbohydrates and inhibitors of microorganism's synthesis and action. This will simultaneously promote the growth and retard the senescence of cut flowers. Pulsing is a pre-shipment short term treatment by the growers, the effect of which should last throughout the shelf life of flower[2].

Application of sucrose in vase or pulsing solutions improves the keeping quality of several flowers by delaying protein degradation, increasing water uptake and inhibiting ethylene production[3]. The senescence of flower petals is followed by a series of physiological and biochemical mechanisms including production of reactive oxygen species (ROS), activation of hydrolytic enzymes, changes in ethylene production and respiration, increase in malondialdehyde/ thiobarbituric acid reactive substances (TBARS) and reduction of plasma membrane integrity[4]. Thus, pulsing flowers with an appropriate solution in combination with sucrose,

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biocide and growth promoter could help in maintaining the keeping quality of spikes during supply chain from fields to market.

Recently, an impressive upsurge has revealed the pivotal role of nitric oxide (NO) in plants in elucidating the various physiological and biochemical functions[5]. It acts as a natural senescence delaying plant growth regulator primarily by down regulating ethylene production[6,7]. This led to the use of NO in extending the post harvest life of horticultural commodities[8,9]. The gaseous nature of this compound offers hindrance to its commercial usage, so exogenous application of NO using solid NO donors is done. Among different solid NO donors like SNAP (S-Nitros-N-acetylpenicilamine), DETA/NO (2,2-(Hydroxynitrosohydrazino)-bisethanamine), Sin-1 (3-mopholinosil-nonomine),PBN (N-Butil-α-phenil nitrone) and SNP (Sodium nitroprusside), SNP is the most important compound that could be used for elongation of post harvest life of ethylene insensitive flowers as compared to other compounds which are used relatively on ethylene responsive flowers[10-13]. Based on response to exogenous application of ethylene, gladiolus do not show accelerated senescence so hence regarded as ethylene insensitive flower[14]. The likely differential action of SNP and sucrose alone raise the possibility that SNP maybe useful as a synergistic co-treatment with sucrose. Thus, keeping in view commercial significance of gladiolus[15], its ethylene insensitivity, the potential role of sucrose, aluminium sulphate and nitric oxide in delaying senescence and upregulating antioxidant enzymes[16], the studies were conducted to investigate the role of SNP as pulsing solution in improving post harvest quality of gladiolus spikes through its influence on physiological and biochemical attributes.

Materials and Methods

Plant material

The plants of gladiolus variety Punjab Glad-1 (orange in color with a tint of yellow at the center of florets) were raised in the Research Farms of the Department of Floriculture and Landscaping, Punjab Agricultural University, Ludhiana from the uniform sized corms (3.5-4 cm diameter). The experimental field was thoroughly ploughed and levelled, and the plots of 3×3 m size were prepared. Farmyard manure @5 Kg/m^2 was uniformly mixed in the soil one month before the planting of corms. Before planting, the corms were treated with bavistin (0.2%) for 30 min as a protective measure against fungal diseases and were planted 30 × 20 cm apart at a depth of 7 cm by keeping the terminal buds upward. All the recommended agronomical practices were followed to raise the healthy crop.

Treatment with SNP

The plants came to flowering after 90-95 days of sowing. The spikes were harvested early in the morning at tight bud stage (when 1-2 basal florets showed colour) and all the leaves were removed below the floret before subjecting to preliminary experiment of 10 pulsing treatments (100 mg L^{-1} SNP, 100 mg L^{-1} SNP + 20% Sucrose + 400 mg L^{-1} Aluminium sulphate, 200 mg L^{-1} SNP, 200 mg L^{-1} SNP + 20% Sucrose + 400 mg L^{-1} Aluminium sulphate, 300 mg L^{-1} SNP, 300 mg L^{-1} SNP + 20% Sucrose + 400 mg L^{-1} Aluminium sulphate, 400 mg L^{-1} SNP, 400 mg L^{-1} SNP + 20% Sucrose + 400 mg L^{-1} Aluminium sulphate, Water control, 20% Sucrose + 400 mg L^{-1} Aluminium sulphate). The treatments were done by dipping the basal 5-7 cm portion of bundle of five spikes in three replications each in the respective solution for 20 h at 23±2°C. The treated spikes were transferred to vase containing distilled water and were evaluated on basis of physiological parameters for post harvest quality under ambient conditions. Based on results of preliminary experiment, two best treatments (100 mg L^{-1} SNP and 200 mg L^{-1} SNP + 20% Sucrose + 400 mg L^{-1} Aluminium sulphate) and two treatments showing least performance (300 mg L^{-1} SNP and 400 mg L^{-1} SNP) along with water control and 20% Sucrose + 400 mg L^{-1} Aluminium sulphate were selected for further physiological and biochemical evaluation.

Vase life, days to opening of basal floret, total volume of water absorbed per spike and change in pH of pulsing solutions

Vase life was measured in number of days from the opening of one basal floret till the wilting of 50% of total number of opened florets occurred. Days taken for complete opening of the basal floret were recorded from the day of placing the spikes in water following treatment. Total volume of water absorbed by the spike till the end of vase life was measured in ml and expressed as water/solution absorbed per spike. The pH of the pulsing solutions was measured using pH meter before (initial) and after (final) treating spikes.

$$\text{Change in pH} = \text{Final pH} - \text{Initial pH}$$

Maximum number of florets opens at one time, size of fully expanded floret, per cent opening of florets and per cent loss in physiological weight at end of vase life

The number of florets open on each spike were counted daily and the maximum number of florets opened at one time on the spike was recorded till the

wilting of first floret started. The maximum diameter of the 2nd fully opened floret from the base was measured in cm and expressed as floret size. The number of fully opened florets were counted daily throughout the duration of the experiment and expressed as per cent opening of florets on the spike.

$$\text{Per cent opening of florets} = \frac{\text{No. of opened florets}}{\text{Total no. of florets}} \times 100$$

The per cent loss in physiological weight was calculated based on consecutive difference between initial weight of spike after treatment and at the end of vase life.

$$\text{Per cent loss in physiological weight} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Membrane stability index (MSI) and relative water content (RWC)

The biochemical studies were done from tepals at following stages: S_1 - Tepals just emerging from the bract, S_2 - Fully open floret, S_3 - Floret after two days of opening, S_4 - Florets showing complete senescence. For the membrane stability index (MSI)[17], the conductivity was measured before (C1) and after (C2) the leakage of electrolytes using standard method and following formula was used to calculate MSI:

$$\text{MSI} = (1 - \text{C1/C2}) \times 100$$

Relative water content (RWC) was determined using florets from different stages. After detachment, florets were kept in distilled water in a pre-weighed sealed test tube. The tubes were again weighed, and the increased weight of the tubes was used to calculate fresh weight (FW). After 28 h, the saturated florets were again weighed to determine turgid weight (TW)[18]. Dry weight (DW) was then obtained after oven-drying at 70°C for 48 h and RWC was calculated using following formula:

$$\frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Total soluble sugars and total protein content

For estimation of total soluble sugars, the concentration of total soluble sugars was calculated against standard curve prepared by using glucose standards (10-100 µg/mL) and was expressed as µg/gFW[19]. The total protein content was also expressed as µg/g FW[20]. The following formula was used to calculate total soluble sugars and total protein content:

$$\frac{\text{Conc. of standard} \times \text{OD of test sample} \times \text{Total vol. of extract}}{\text{OD of standard} \times \text{Vol. of sample taken from extract} \times \text{amount of tissue taken for extraction}}$$

Catalase (CAT), peroxidase (POD) and lipid peroxidation assay

For the catalase enzyme assay, the tissue weighing 500 mg was crushed in pestle and mortar containing 5 mL of 0.1 M phosphate buffer (pH 7) at 0°C. The homogenate was centrifuged at 10,000 ×g for 15 min in refrigerated centrifuge at 0°C. The supernatant was collected and made to final volume of 10 mL for estimation of catalase activity[21]. CAT was expressed as in mM H_2O_2 hydrolyzed min^{-1} g^{-1} FW.

For the peroxidase activity, the tissue weighing 500 mg was crushed in pestle and mortar containing 5 mL of 0.1 M phosphate buffer (pH 6) at 0°C. The homogenate was centrifuged at 10,000 g for 15 min in refrigerated centrifuge at 0°C. The supernatant was collected and made to final volume to 10 mL for estimation of peroxidase activity[22]. POD was expressed as in ΔA min^{-1} g^{-1} FW. Five samples per treatment were used and the analysis was repeated thrice.

The level of lipid peroxidation was measured in terms of a product of lipid peroxidation, TBARS content by following the method of Heath and Packer[23]. Absorbance of the supernatant was recorded at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The TBARS content was calculated using absorption coefficient of 155 mM^{-1} cm^{-1}.

Statistical analysis

The experiment was carried out in factorial completely randomized design (CRD). Data was subjected to statistical analysis of variance (ANOVA) using SAS software (version 9.2, SAS Institute Inc., Cary, NC, USA). Mean comparisons to calculate significant differences between treatments were performed using Least Significant Difference (LSD) test at 0.05 level of probability.

Results

Vase life, days to opening of basal floret, total volume of water absorbed per spike and change in pH of pulsing solutions

Vase life is the prime concern of cut flowers and it was recorded to be 7.67 days in control and when pulsed with sucrose and aluminium sulphate, it increased significantly to 8.67 days (Table 1). The pulsing of spikes with different concentrations of SNP further significantly increased the vase life to 12.67 in SNP @200 mg L^{-1} supplemented with sucrose and aluminium sulphate, 11.00 days in SNP @100 mg L^{-1}, 9.67 days in SNP@300 mg L^{-1} whereas the vase life was at par with control i.e., 8.67 days in SNP @400 mg L^{-1}.

Table 1 — Effect of SNP as pulsing solution on vase life, days to opening of basal floret, total water absorbed per spike, change in pH of pulsing solutions

Treatment	Vase life (days)	Days to opening of basal floret in vase	Total water absorbed per spike (ml)	Change in pH of the pulsing solutions
SNP, 100 mg L^{-1}	11.00[b]	3.00[d]	52.91[b]	1.35[b]
SNP, 200 mg L^{-1} + Sucrose, 20% + Aluminium sulphate, 400 mg L^{-1}	12.67[a]	2.66[d]	69.84[a]	2.33[a]
SNP, 300 mg L^{-1}	9.67[c]	5.00[bc]	29.64[d]	0.91[bc]
SNP, 400 mg L^{-1}	8.67[d]	7.00[a]	22.99[e]	0.73[c]
Sucrose, 20% + Aluminium sulphate, 400 mg L^{-1}	8.67[d]	4.66[c]	55.98[b]	1.35[b]
Water (control)	7.67[e]	5.33[b]	38.79[c]	0.63[c]
LSD	0.52	0.52	3.21	0.47

[*Different lowercase letters in the vertical column represent significant differences (P >0.05) between treatments]

Table 2 — Effect of SNP as pulsing solution on maximum no. of florets open at one time, size of fully expanded floret, per cent opening of florets and per cent loss in physiological weight at the end of vase life

Treatment	Maximum number of florets open at one time	Size of fully expanded floret (cm)	Per cent opening of florets	Per cent loss in physiological weight at end of vase life
SNP, 100 mg L^{-1}	3.00[b]	10.03[b]	55.88[a] (48.36)	16.26[c] (23.77)
SNP, 200 mg L^{-1} + Sucrose, 20% + Aluminium sulphate, 400 mg L^{-1}	4.34[a]	10.90[a]	56.60[a] (48.77)	3.68[d] (11.00)
SNP, 300 mg L^{-1}	1.67[d]	9.40[bc]	32.63[d] (34.82)	23.51[b] (28.99)
SNP, 400 mg L^{-1}	1.34[d]	7.30[d]	31.28[d] (33.99)	25.83[a] (30.53)
Sucrose, 20% + Aluminium sulphate, 400 mg L^{-1}	3.00[b]	9.70[bc]	52.34[b] (46.32)	3.71[d] (11.05)
Water (control)	2.34[c]	9.00[c]	42.64[c] (40.75)	26.31[a] (30.84)
LSD	0.52	0.77	0.91	1.65

[*Different lowercase letters in the vertical column represent significant differences (P >0.05) between treatments. *Figures in parentheses are arc sine transformed values]

Among the different post harvest quality parameters influencing the vase life, days to opening of basal floret is one of the most important quality parameters. Pulsing gladiolus spikes with SNP significantly influenced the time taken to opening of basal florets of gladiolus spikes (Table 1). The basal floret took 5.33 days to open in control whereas the number of days significantly increased to 7.00 with SNP treatment @400 mg L^{-1} and decreased to least 2.66 days with SNP @200 mg L^{-1} supplemented with sucrose and aluminium sulphate. The water absorbed by the spikes is the key determinant of the vase life. Among different treatments, the spikes pulsed in SNP @200 mg L^{-1} supplemented with sucrose and aluminium sulphate absorbed 69.84 mL of water which was significantly highest followed by 55.98 mL in sucrose and aluminium sulphate and 52.91 mL in SNP @100 mg L^{-1} (Table 1). The water absorbed by spikes pulsed with 300 mg L^{-1} SNP was 29.64 mL and 400 mg L^{-1} SNP was 22.99 mL which was less than control (38.79 mL). The decrease in pH was more in solutions supplemented with sucrose and aluminium sulphate than those in solutions containing SNP alone (Table 1). Maximum fall (2.33) in pH was noticed in solution of 200 mg L^{-1} SNP supplemented with sucrose and aluminium sulphate and minimum change of 0.73 in pH was observed in 400 mg L^{-1} SNP pulsed spikes and control (water).

Maximum number of florets open at one time, size of fully expanded floret, per cent opening of florets and per cent loss in physiological weight at end of vase life

The pulsing treatments significantly influenced the number of florets open at one time (Table 2). Maximum number of florets opened at one time were 4.34 in spikes pulsed with SNP@ 200 mg L^{-1} supplemented with sucrose and aluminium sulphate as compared to 2.34 in control. The number of florets which are indicative of flower quality declined to 1.67 and 1.34 when spikes were pulsed, respectively @300 and 400 mg L^{-1} SNP.

The size of floret is the genetic character but internal turgor pressure of petals affects the opening of florets. SNP @200 mg L^{-1} in combination with sucrose and aluminium sulphate resulted in significant increase in floret diameter to 10.90 cm in comparison to 9.00 in spikes pulsed in control (water) and 9.70 in spikes pulsed with sucrose and aluminium sulphate (Table 2). The floret size decreased to 7.30 cm by pulsing spikes with SNP @400 mg L^{-1} indicating differential behaviour of SNP at high concentration.

The opening of florets was significantly enhanced with pulsing treatment containing SNP (Table 2). Among the different concentrations of SNP, highest percentage of floret opening (56.60%) was recorded with SNP @200 mg L^{-1} supplemented with sucrose and aluminium sulphate whereas minimum per cent opening of florets (31.28%) was exhibited by spikes pulsed with SNP @400 mg L^{-1} which was at par with SNP @300 mg L^{-1}. The loss in physiological weight of flowers is an important parameter affecting vase life. Higher the loss in weight, lower is the vase life. The loss in weight was 26.31% in control that declined to 3.71% when supplemented with sucrose and aluminium sulphate and 3.68% with SNP @200 mg L^{-1} supplemented with sucrose and aluminium sulphate. The spikes pulsed with SNP alone @100 mg L^{-1} lost 16.26%, @300 mg L^{-1} lost 23.51% and @400 mg L^{-1} lost 25.83% of weight.

The improvement of post harvest quality of gladiolus spikes and vase life with SNP at low concentration could be supported through the action of NO on different physiological and biochemical attributes *viz*. membrane stability index, relative water content, total soluble sugars, proteins content, TBARS content, catalase and peroxidase activity.

Membrane stability index (MSI) and relative water content (RWC)

The membrane stability index which is related to membrane integrity was high in immature florets i.e. at S_1 and decreased with the advancement in the stage of floret development from 76.47 to 29.98 indicating membrane deterioration during senescence in all the treatments (Fig. 1A). Maximum MSI was observed in florets of spikes treated with SNP @200 mg L^{-1} supplemented with sucrose and aluminium sulphate and minimum in water. Among different concentrations of SNP, spikes pulsed @200 mg L^{-1} SNP with sucrose and aluminium sulphate had significantly highest MSI (65.11) and lowest was recorded in control (40.46). The relative water content (RWC) assesses the degree of water deficit stress. Like MSI, the relative water content was high in tepals of immature florets at S_1 (66.37) and gradually increased with opening of florets and was maximum in fully opened florets at S_2 (79.21). The RWC reached the lowest level in the tepals of florets showing complete senescence at S_4 (39.48) (Fig. 1B). However, pulsing treatment with SNP @200 mg L^{-1} supplemented with sucrose and aluminium sulphate had significantly higher RWC (44.39) at S_4 stage followed by treatment with SNP @100 mg L^{-1} (42.60) indicating the ameliorative role of SNP under water stress.

Total soluble sugars and total protein content

The sugar content was highest in florets of spikes pulsed with SNP @200 mg L^{-1} in combination with sucrose and aluminium sulphate at all the stages (Fig. 1C). Among different stages, the TSS content was maximum in fully opened florets in all the treatments and showed a decline with progressing stages (762.44, 917.26, 563.32 and 373.13 µg/g FW at S_1, S_2, S_3 and S_4 respectively). The sugar content in florets of spikes pulsed @300 mg L^{-1} SNP and 400 mg L^{-1} SNP were at par with control (water) at S_4.

During the development of florets, the level of proteins increased initially but later it declined. The florets pulsed with SNP had higher protein level in the tepals in comparison to the control (Fig. 1D). The protein concentration decreased significantly with the onset of senescence in spikes kept in distilled water. Maximum protein content was found in tepals of florets pulsed in SNP @200 mg L^{-1} supplemented with sucrose and aluminium sulphate (195.47 µg/g FW) at S_4 stage.

Catalase (CAT), peroxidase (POD) and lipid peroxidation assay

The senescence is always accompanied by change in activity of antioxidant enzymes. Catalase, a key antioxidant enzyme, showed a steady decrease in its activity from flower opening to the senescence stage (Table 3). In our studies, gladiolus spikes treated with SNP @200 mg L^{-1} supplemented with sucrose and aluminium sulphate showed highest levels of CAT and minimum CAT activity was observed in florets of spikes in SNP @400 mg L^{-1}. Another antioxidant enzyme, peroxidase (POD) activity was low initially

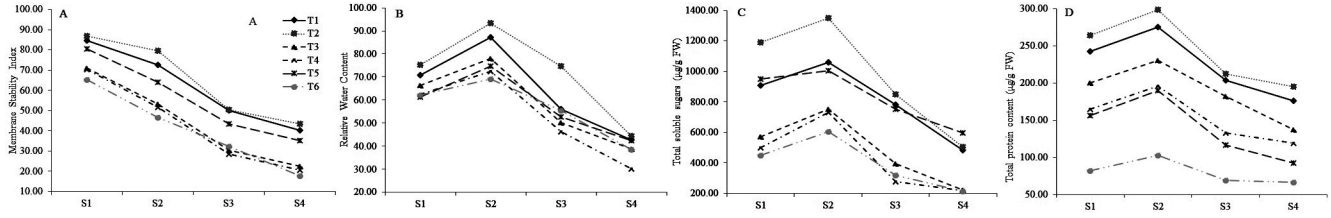

Fig. 1 — Effect of SNP as pulsing solution on (A) Membrane stability index; (B) Relative water content; (C) Total soluble sugars; and (D) Total protein content in tepals at different developmental stages gladiolus spikes

Table 3 — Effect of SNP as pulsing solution on CAT activity and POD activity at different stages of floret development in gladiolus spikes

Treatment	CAT activity (mM H_2O_2 hydrolyzed min^{-1} g^{-1} FW)					POD activity (ΔA min^{-1} g^{-1} FW)				
	Stage of floret development				Mean	Stage of floret development				Mean
	S_1	S_2	S_3	S_4		S_1	S_2	S_3	S_4	
SNP, 100 mg L^{-1}	2.59^{cd}	3.22^{ab}	2.06^{e}	1.58^{fg}	2.36^{B}	7.18^{de}	8.69^{b}	6.58^{efg}	5.82^{ghi}	7.07^{B}
SNP, 200 mg L^{-1} + Sucrose, 20% + Aluminium sulphate, 400 mg L^{-1}	3.01^{bc}	3.56^{a}	2.67^{c}	2.13^{de}	2.84^{A}	8.36^{bc}	10.65^{a}	7.62^{cd}	7.13^{def}	8.44^{A}
SNP, 300 mg L^{-1}	1.68^{efg}	1.89^{ef}	1.10^{hijk}	0.74^{k}	1.35^{C}	6.09^{gh}	6.54^{efg}	4.53^{klmn}	4.17^{lmno}	5.33^{C}
SNP, 400 mg L^{-1}	1.06^{ijk}	1.43^{fghi}	0.82^{k}	0.67^{k}	1.00^{D}	4.92^{jkl}	5.61^{hij}	3.76^{no}	3.49^{o}	4.45^{D}
Sucrose, 20% + Aluminium sulphate, 400 mg L^{-1}	1.84^{efg}	2.11^{e}	1.37^{ghij}	0.81^{k}	1.53^{C}	7.10^{def}	7.68^{cd}	6.38^{fgh}	6.04^{gh}	6.80^{B}
Water (control)	1.02^{ijk}	1.56^{fgh}	0.94^{jk}	0.67^{k}	1.05^{D}	4.83^{jklm}	5.16^{ijk}	4.06^{mno}	3.58^{o}	4.41^{D}
Mean	1.87^{B}	2.30^{A}	1.49^{C}	1.10^{D}		6.41^{B}	7.39^{A}	5.49^{C}	5.04^{D}	
LSD	Treatment (A)= 0.24; Stage (B)= 0.19; A×B= 0.45					Treatment (A)= 0.39; Stage (B)= 0.32; A×B= 0.78				

[*S_1-Tepals just emerging from the bract, S_2-Fully open floret, S_3-Floret after two days of opening, S_4-Florets showing complete senescence. *Different uppercase letters in the vertical column represent significant differences (P >0.05) between treatments and in the horizontal row represent significant differences (P >0.05) between stages. Different lowercase letters represent significant differences (P >0.05) between interaction of treatments and stages]

Table 4 — Effect of SNP as pulsing solution on TBARS content at different stages of floret development in gladiolus spikes

Treatment	TBARS content (nM g^{-1} FW)				
	Stage of floret development				Mean
	S_1	S_2	S_3	S_4	
SNP, 100 mg L^{-1}	22.83^{o}	24.98^{k}	25.07^{k}	27.22^{d}	25.03^{E}
SNP, 200 mg L^{-1} + Sucrose, 20% + Aluminium sulphate, 400 mg L^{-1}	22.17^{p}	24.15^{m}	24.23^{m}	26.54^{fg}	24.27^{F}
SNP, 300 mg L^{-1}	23.29^{n}	25.47^{j}	25.83^{i}	27.83^{c}	25.61^{D}
SNP, 400 mg L^{-1}	24.56^{l}	25.85^{i}	26.45^{g}	28.07^{b}	26.23^{C}
Sucrose, 20% + Aluminium sulphate, 400 mg L^{-1}	25.04^{k}	26.17^{h}	26.66^{f}	27.92^{bc}	26.45^{B}
Water (control)	25.87^{i}	26.65^{f}	26.94^{e}	28.47^{a}	26.98^{A}
Mean	23.96^{D}	25.55^{C}	25.87^{B}	27.68^{A}	
LSD	Treatment (A)= 0.05; Stage (B)= 0.04; A×B= 0.002				

[*S_1-Tepals just emerging from the bract, S_2-Fully open floret, S_3-Floret after two days of opening, S_4-Florets showing complete senescence. *Different uppercase letters in the vertical column represent significant differences (P >0.05) between treatments and in the horizontal row represent significant differences (P >0.05) between stages. Different lowercase letters represent significant differences (P >0.05) between interaction of treatments and stages]

in the florets of SNP treated spikes (Table 3). The POD activity showed considerable increase in florets of spikes treated with SNP @200 mg L^{-1} supplemented with sucrose and aluminium sulphate as compared to the control. TBARS content gradually increased with the advancement of senescence from stage 1 until stage 4 (Table 4). Among all the treatments, spikes pulsed with SNP @200 mg L^{-1} in combination with sucrose and aluminium sulphate maintained lower level of lipid peroxidation throughout the stages of floret development (24.27 nM g^{-1} FW) as compared to highest in water control (26.98 nM g^{-1} FW).

Discussion

Sodium nitroprusside, NO donor, has attained considerable attention in floricultural research particularly in post harvest management of cut flowers, due to the evidence for its role in delaying senescence and extending the vase life of various cut flowers such as rose, gladiolus, and carnations[24]. In above studies, the role of SNP in improving the vase life was found to be by inhibiting the ethylene production or promoting antioxidant activities[25]. In the present investigation, the effect of different concentration of SNP application alone or in combination with sucrose and aluminium sulphate was done to understand the crosstalk between NO and various physiological parameters determining the post harvest quality of gladiolus spikes. The treatments for present studies were selected from preliminary studies in which different concentrations of SNP viz. 100, 200, 300 and 400 mg L^{-1} alone and with 20% sucrose and 400 mg L^{-1} aluminium sulphate were used as pulsing solutions and its effect on post harvest quality of gladiolus spikes were studied. The results revealed that based on quality parameters especially days to opening of basal floret, vase life, total water/solution absorbed per spike and per cent physiological loss in weight, pulsing the gladiolus spikes with 100 mg L^{-1} SNP alone and 200 mg L^{-1} SNP with sucrose and aluminium sulphate were found to give

better results than control whereas spikes pulsed with 300 mg L^{-1} SNP and 400 mg L^{-1} SNP were found to perform at par or below the control in terms of quality parameters. The lower concentration of SNP was less effective in enhancing the vase life but higher concentrations of SNP (200 mg L^{-1}) significantly promoted the vase life but further increase in concentration of SNP reduced the vase life, indicating negative role of SNP[8,16]. Zheng *et al.*[24] also reported similar findings in gladiolus and carnation supporting dose dependent role of SNP. Keeping in view the above results, two pulsing treatments of 100 mg L^{-1} SNP alone and 200 mg L^{-1} SNP with 20% sucrose and 400 mg L^{-1} aluminium sulphate that were at par in improving vase life along with 300 mg L^{-1} S.

NP and 400 mg L^{-1} SNP that reduced the vase life than control were selected for physiological studies. The pulsing with sucrose and aluminium sulphate @200 mg L^{-1} significantly improved the vase life in gladiolus in comparison to control (water) as they respectively provided respiratory substrate and inhibited microbial contamination of vase solution[26]. High concentration of SNP *viz*. 300 and 400 mg L^{-1} reduced the vase life of gladiolus below the level of controls. High concentrations of SNP were found to reduce vase life in other cut flowers. This increase in vase life with SNP could be accounted for the role of NO in delaying senescence. The other physiological parameters *viz*. days to opening of basal floret, amount of solution absorbed, change in pH of solution, maximum number of florets opened, size of floret, per cent opening of florets and per cent loss in weight were found to be concomitant with vase life. Thus, the beneficial or detrimental effect of SNP, NO donor, might depend upon concentration, genotype, and presence of sucrose.

Sucrose induced acceleration of floret opening in gladiolus has also been reported earlier[3]. The results suggested that application of high concentrations of SNP alone reduces the water absorbed by interfering with the translocation channel. The effect of pulsing with SNP might be due to decrease in xylem blockage[27]. It was reported that increase in water uptake by pulsing of gladiolus might be due to improvement in translocation of water and sugars accumulated in flowers that increase the osmotic potential thereby improving the ability of spikes to absorb water[28,29]. The source of energy associated with low pH might be useful for enhancing the vase life of gladiolus flowers and one of the main advantages of low pH is decrease in pathogen development. The effect of SNP on size of floret is concentration dependent and might be due to regulatory role of NO released from SNP on several metabolic processes[30]. This increase in size might be since SNP and sucrose acts as food material by providing respiratory substrate, which provides energy for all metabolic activities, thus helps in increasing flower diameter and maintaining turgidity for expansion. Hence, use of vase preservatives or pulsing of spikes with sucrose increased the opening of florets as well as vase life[31]. Moreover, weight loss is one of the most important physiological disorders of cut flowers[4] and fruits[32]. The effect of SNP in decreasing the loss in weight could be attributed to increased water uptake thus, preventing weight loss[33].

The sharp decline in MSI with the progress of senescence is indicative of the loss of membrane integrity which is the final and irreversible phase of senescence associated with membrane lipid peroxidation. The eventual ion leakage due to loss of membrane permeability can be used to determine the extent of tissue damage[34]. The RWC of the tepals of developing florets is indicative of metabolic activity of the tepals during their expansion. The young and expanding tepals exhibit a greater capacity to retain water which accounts for their high relative water content. Sugars control the plant metabolism, growth and development and are correlated with light, stress, and hormone signaling[35]. The protein degradation and an increase of electrolyte leakage are the two major attributes leading to petal senescence. The decrease in protein content as the flowers approaches senescence is due to the expression of proteolytic enzymes or serine proteases[36]. Kumar & Gupta[37] studied the petal senescence and post harvest life of gladiolus and observed a significant decrease in the total protein content of petals at the time of senescence.

The activity of catalase enzyme is considered as an adaptive response to defend cells against oxidative stress[38]. The peroxidase enzyme is known to play a key role in scavenging of ROS by regulating the levels of the peroxide hydrogen (H_2O_2) produced in plant cells thereby, preventing its contact with superoxide and formation of hydroxyl radicals[39]. Induction in antioxidants was positively associated with alleviating harmful oxidation effects[40,41]. TBARS content was gradually increased with advancement of floret stage towards senescence. As the stage advances, free radical production increases the oxidative stress and hence leading to deterioration of membranes. But treatment with SNP decreased the TBARS content. Our

investigation is consistent with previous studies as progression in tepal senescence after full opening of tepal was associated with increase in lipid peroxidation and progressive decline in MSI, RWC, total soluble sugar and total protein content along with activity of antioxidants viz. catalase and peroxidase. The pulsing solution containing sucrose and aluminium sulphate improved all above parameters over control but exogenous application of NO, as SNP, affected the senescence in dose dependent manner. Among different treatments, the pulsing solution of SNP @200 mg L^{-1} supplemented with sucrose and aluminium sulphate performed better in terms of biochemical and physiological attributes affecting post harvest quality of gladiolus than SNP @100 mg L^{-1} due to synergistic effect of SNP with sucrose and aluminium sulphate. The key to improved vase life of cut flowers is membrane integrity of petals which further regulate relative water content and other metabolites. It has been shown that NO donors decrease lipid peroxidation and maintain membrane integrity. The SNP treatment ameliorates the oxidative stress induced in cut flowers after harvest as revealed by increased activity of CAT enzyme. The activity of this enzyme is considered as an adaptative response to defend cells against oxidative stress[38]. Further, the increased activity of antioxidant enzymes *viz*. catalase and peroxidase in tepals pulsed with SNP @200 mg L^{-1} supplemented with sucrose and aluminium sulphate in our studies could be accounted to the role of SNP in delaying senescence by maintaining the antioxidant activity, that scavenges the reactive oxygen species which damage the cell membrane. This could be supported by findings of workers[16,24] who found that SNP could significantly extend the vase life of carnation and gladiolus cut flowers by maintaining antioxidant activity.

The spikes pulsed with SNP @300 and 400 mg L^{-1} had lower vase life which could be related to their low MSI, RWC, higher TBARS content, lower total soluble sugar content and lower total protein content in tepals during floret development than control. Even the activities of antioxidant enzymes were found to be less than control or SNP at low concentrations. The dual function of NO as an effective antioxidant to ameliorate oxidative stress depends upon its concentration. The negative effect of SNP at higher concentrations could further be due to injury to membranes and nucleic acids, impairment of photosynthetic electron transport and DNA damage and cell death[8,16]. This might be due to perturbance caused to normal metabolism by high concentration of NO.

Conclusion

Results of the present study suggests that 200 mg L^{-1} SNP supplemented with 20% sucrose and 400 mg L^{-1} aluminium sulphate could be used as pulsing solution as it significantly improved the post harvest quality of gladiolus spikes. The various keeping quality parameters *viz*. vase life, days to opening of basal floret, maximum number of florets opens at one time, size of fully expanded floret, per cent opening of florets and per cent loss in physiological weight at end of vase life were found to be improved in spikes treated with this treatment as compared to the control. The biochemical attributes such as membrane stability index, relative water content, total soluble sugars, total protein content, catalase activity, peroxidase activity and lipid peroxidation supported the results of keeping quality parameters.

Conflict of Interest

Authors declare no competing interests.

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