Physiol Mol Biol Plants (April 2021) 27(4):835–845
https://doi.org/10.1007/s12298-021-00969-5

RESEARCH ARTICLE

Nitric oxide effectively curtails neck bending and mitigates senescence in isolated flowers of *Calendula officinalis* L.

Mohammad Lateef Lone[1] · Aehsan ul Haq[1] · Sumira Farooq[1] · Foziya Altaf[1] · Inayatullah Tahir[1]

Received: 23 September 2020 / Revised: 2 March 2021 / Accepted: 7 March 2021 / Published online: 23 March 2021

Abstract In recent years, there has been a considerable and renewed upsurge in research to ascertain the physiological and biochemical role of Nitric oxide (NO) in plants. The present investigation is focused to study the role of NO on neck bending associated with senescence and postharvest performance in isolated flowers of *Calendula officinalis*. The flower buds harvested at one day before anthesis stage were supplied with sodium nitroprusside (SNP) as a source of NO at different concentrations viz., 50, 100, 150 and 200 µM. A distinct set of flowers held in distilled water designated the control. The investigation revealed that SNP delayed the senescence in flowers of *C. officinalis* significantly manifested by prolonged longevity. The maximum longevity of 12 days was recorded in flowers supplemented with 100 µM SNP. The flowers held in distilled water (control) displayed early senescence symptoms and lasted for 6 days only. Our research suggested that improved flower longevity by SNP was commensurate with delayed neck bending, inhibition of bacterial growth in the vase, increased solution uptake, high membrane stability, besides an up-regulated activities of antioxidant enzymes in the tissue samples. In addition, the treated flowers exhibited increased content of sugar fractions, total phenols and soluble proteins in the petal tissues compared to control. Further, 100 µM SNP was observed as most effective treatment and increased the longevity of flowers by 6 days. The concentration above 150 µM provoked early senescence compared to control, whereas concentration lower than 100 µM was less efficacious in improving the postharvest life and longevity of cut *Calendula* flowers.

Keywords Sodium nitroprusside · Neck bending · Vase life · Flower senescence · Petal tissues

✉ Inayatullah Tahir
tahir.inayatullah@gmail.com

[1] Plant Physiology and Biochemistry Research Laboratory, Department of Botany, University of Kashmir, Srinagar 190006, India

Introduction

Senescence, a genetically programmed event represents the final phase in ontogeny of flowers in which a sequence of irreversible events are initiated, inevitably leading to cellular breakdown and organ death. The process is orchestrated by changes in the levels of growth regulators and their crosstalk acts as regulatory signals for the commencement or cessation of specific reactions. The biochemical and physiological indices that affect the postharvest flower senescence include; water uptake, lipid peroxidation rates, membrane stability, respiratory activity, protein and sugar content, besides activities of antioxidant enzymes in the petal tissues (Reid and Jiang 2012; Rani and Singh 2014; Hemati et al. 2019). Neck (stem) bending has been reported as a primary reason for limited life of many cut flowers which precedes well before the wilting of the petals (Naing et al. 2017; Gómez-Merino et al. 2020). It is caused by insufficient mechanical support in the xylem, inadequate lignin rich sclerenchyma cells in the flower stems and microbial contamination (van Doorn and de Witte 1994; Williamson et al. 2002; Perik et al. 2012; Naing et al. 2017; Shabanian et al. 2018).

From villain to hero to biochemistry's new superstar, NO has emerged as a novel molecule regulating diverse set of biological processes in both animal and plant kingdom (Moroz et al. 2020; Verma et al. 2020). This enigmatic,

though unique transportable multidimensional signaling molecule due to its lipophilic nature plays pivotal role in regulating various processes in plants such as resistance to untoward environmental stresses, defense responses, hormonal modulation and programmed cell death (Shi et al. 2016; Ahmad et al. 2018; Kumar Rai et al. 2018). NO is involved in the broad spectrum of physiological and developmental processes and in multiple modes of action associated with flower senescence (Lamattina et al. 2003; Hasanuzzaman et al. 2016). It has been well-documented that NO functions as a negative regulator during flower senescence hence acts as a novel substitute for some other toxic chemicals like silver thiosulphate (STS) in postharvest technology (Procházková and Wilhelmová 2011; Rabiei et al. 2019; Deng et al. 2019). Exogenously applied NO has significantly increased the vase life of various cut flowers such as gerbera, iris, tulip, snapdragon, delphinium, oriental lily, rose, gladiolus, chrysanthemum and carnations (Badiyan et al. 2004; Naing et al. 2017; Kazemzadeh-Beneh et al. 2018; Zhang et al. 2018; Deng et al. 2019). Indeed application of NO has been shown to extend not only the longevity of cut flowers but also the shelf life of various fruits, e.g. peach, cherry (Saba and Moradi 2017; Rabiei et al. 2019) and vegetables, e.g. broccoli (Shi et al. 2016). NO has been found to delay senescence at lower optimal concentration, while its higher concentration (super-optimal) reportedly causes nitrosative damage to plants and provokes early senescence (Naing et al. 2017; Sami et al. 2018).

Calendula (Marigold) of *Asteraceae* family is one of the leading commercial cut flowers in the world securing top ranks in global floricultural industry. Being prolific and floriferous, besides having medicinal and culinary importance, their pungent blooms bring bursts of sunshine into the room and smiles to the face. However, short flower longevity due to early neck (stem) bending and associated physiological and biochemical instability reduces its potential export and trade value. Despite the application of various anti-senescence agents to combat such problems, the role of NO remains unexplored till date for this flower. *Calendula* is insensitive to ethylene (Kondo et al. 2017). Pertinently, NO is highly efficacious in flowers in which senescence progresses independently of ethylene (Woltering and Van Doorn 1988; Dwivedi et al. 2016). It is in this perspective that the study was undertaken to ascertain the implication of SNP as a source of NO on neck bending associated with senescence and postharvest performance in flowers of *C. officinalis* by investigating various physiological and biochemical parameters with the particular aim to ameliorate the longevity of these joyful and bright flowers.

Materials and methods

Plant material and SNP treatment

Fresh uniform isolated flower buds of *C. officinalis* grown in Kashmir University Botanic Garden (KUBG) were utilized for this study. The flowers at one day before anthesis stage were harvested at 9:00 h and quickly transported to laboratory in distilled water. The flower stems were re-cut to an approximate 8 cm length in accordance with marketable size. Two flowers of approximately same size were placed in 100 ml Ehrylenmeyer flasks (vases) filled with distilled water containing different concentrations of SNP viz., 50, 100, 150 and 200 µM. Each treatment had ten replicates (flasks) wrapped with aluminum foil in order to avoid photo-degradation of SNP (release of a nitrosyl ligand and cyanide ion). A distinct set of flowers held in distilled water served as control. The mouth of each flask was sealed with parafilm to keep the flowers upright and to avoid evaporation. The day of application of SNP treatments to isolated flowers was designated as day zero. The laboratory conditions were adjusted with relative humidity of 55 ± 10% and 12 h light period a day and mean temperature of 22 ± 2 ^{O}C. Visual assessment of flowers was performed from day zero till last day of experiment and changes in various parameters were recorded on day 2 and 5 of application of different treatments.

Measurements

Flower longevity and floral diameter

The longevity of cut *Calendula* flowers was calculated from the second day of experiment (day 1) when almost all the flowers of the treatments were opened and assessed to be expired when last flower displayed signs of senescence and lost its decorative/market value. The floral diameter was evaluated as the mean of two perpendicular measurements of flower heads.

Bacterial density, solution uptake and neck bending

The bacterial density was determined by recording the optical density (OD) of 1 ml of vase solution taken from each treatment including control at 600 nm using PC-based UV–VIS spectrophotometer (Systronics) by the method of Naing et al. (2017) taking *E. coli* as standard (OD 1 = 8 × 10^8 cfu ml^{-1}). The bacterial density was expressed as cfu ml^{-1}. Solution uptake (ml) was evaluated as the difference between unutilized volume of solution remained at the completion of the experiment and total volume of

solution in the vase. The timing of neck bending was determined by assessing the change in the position of the flower heads according to the method of Perik et al. (2012).

Membrane stability index

Electrolyte seepage provides a measure of membrane integrity of plant cells, which was calculated by incubating 500 mg petal tissue in 25 ml deionized water at 25 °C for 30 min and 95 °C for 15 min employing Sairam (1994) method. The conductivities of the samples incubated at 25 °C (C1) and those incubated at 95 °C (C2) were recorded by using Elico CM180 conductivity meter. MSI was evaluated by computing the formulae:

$$\mathrm{MSI} = [1 - \mathrm{C1}/\mathrm{C2}] \times 100$$

Estimation of total phenols and sugar fractions

Quantification of phenols was performed by the method as described by Swain and Hillis (1959) using gallic acid as standard. A suitable volume of aliquot from the alcohol-soluble fraction of the tissue extract was diluted to 7 ml with distilled water, followed by the addition of 0.5 ml of Folin–Dennis reagent. After 3 min, 1 ml of saturated solution of sodium carbonate was added and the total volume was made to 10 ml with distilled water. Absorbance was measured after 30 min at 725 nm. Nelson's (1944) protocol was employed for quantification of reducing sugars using glucose as standard. A suitable volume of aliquot from the alcohol-soluble fraction of the tissue extract was made up to 5 ml with distilled water, followed by the addition of 1 ml of copper reagent prepared by mixing copper reagent A and B in the ratio of 50:1. The mixture was heated at 100 °C for 20 min in water bath. After cooling at room temperature, 1 ml of arsenomolybdate reagent was added and the volume was made to 25 ml with distilled water. Absorbance was measured at 520 nm. Total sugars were estimated after enzyme mediated conversion of non-reducing sugars into reducing sugars by invertase. The volume of a suitable aliquot from the alcohol-soluble fraction of the fixed material was made to 4 ml with distilled water followed by the addition of 1 ml of 0.3% invertase. A drop of toluene was layered on the top and the solution was incubated overnight at 25 °C. Total sugars were then estimated by employing Nelson's (1944) protocol. Non-reducing sugars were determined by evaluating the difference between total and reducing sugars. The parameters were expressed as mg g^{-1} fm.

Quantification of soluble proteins and α-amino acids

For quantification of soluble proteins, 1 g of petal tissue was macerated in 100 mM phosphate buffer (pH 7.2) comprising NaCl (150 mM), ethylenediamine tetraaceticacid (1 mM), Triton X-100 (1%), glycerol (10%), polyvinyl pyrrolidone (10%) and Dithiothreitol (1 mM). The mixture was subjected to centrifugation at 12,000 g at 4 °C in a refrigerated centrifuge for 15 min. Employing Lowry et al. (1951) protocol, a suitable aliquot taken from the supernatant was used for quantifications of proteins. Estimation of α-amino acids was performed by following Rosen's (1957) protocol using glycine as standard. The soluble protein and α-amino acid content was expressed as mg g^{-1} fm.

Determination of enzyme activities

Superoxide dismutase (SOD)

SOD activity was determined according to Dhindsa et al. (1981). One unit of SOD activity was defined as the quantity of enzyme reducing 50% absorbance of reaction mixture with enzyme in comparison to reaction mixture lacking enzyme. The reaction mixture contained sodium carbonate (50 mM), nitroblue tetrazolium (75 µM), ethylenediamine tetraacetic acid (0.1 M), methionine (13 mM) in 50 mM phosphate buffer (pH 7.8). The absorbance was monitored at 560 nm on spectrophotometer. The SOD activity was expressed as units min^{-1} mg^{-1} protein.

Catalase (CAT)

CAT activity was determined by employing Aebi (1984) protocol based on the consumption of hydrogen peroxide (H_2O_2) in the reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), enzyme extract (50 µl) and distilled water making a final volume of 3 ml. Absorbance of reaction mixture was recorded at 240 nm for three minutes using spectrophotometer. The activity was expressed as µM H_2O_2 red. min^{-1} mg^{-1} protein.

Ascorbate peroxidase (APX)

APX activity was assayed according to the protocol as described by Chen and Asada (1989) which is based on decrease in absorbance at 290 nm due to the oxidation of ascorbate (0.1 mM) in the reaction mixture. The reaction mixture in addition to ascorbate contained potassium phosphate buffer (50 mM) at neutral pH and H_2O_2 (0.3 mM). The absorbance was recorded at 290 nm for

3 min. The activity was expressed as μmol min^{-1} mg^{-1} protein.

Lipoxygenase activity (LOX)

LOX activity was determined by Axerold et al. (1981) method. To begin the reaction, 10 μl of petal extract was added to the mixture containing Tris–Hydrochloric acid buffer (50 mM) at pH 6.5 and linoleic acid (0.4 mM). The absorbance was recorded at 234 nm for 5 min and activity was expressed as μmol. min^{-1} mg^{-1} protein.

Statistical analysis

For determination of various parameters 3 replicates (each comprising two flowers) from each treatment were used except for 5 biological replicates used for assessment of vase life and floral diameter. Completely randomized experimental design was performed during the present study. Treatment means were compared by analysis of variance using SPSS (SPSS version 16; Chicago, USA). The significant difference among various treatments was analyzed by comparing treatments applying Duncan's multiple range test (DMRT $P < 0.05$).

Results

Effect of SNP on flower longevity and floral diameter

Senescence commenced with the loss of turgidity in petals followed by upward erection and complete wilting of flowers (Fig. 1a–c). Flowers treated with SNP showed significant improvement in flower longevity. The recorded longevity of flowers treated with different concentrations of SNP viz., 50, 100, 150 and 200 μM was 8, 12, 10 and 4 days respectively. The flowers held in distilled water lasted for 6 days confirming that higher SNP concentration hastened the process of senescence. Moreover, the longer flower longevity was associated with larger floral heads compared to control which however showed a decreasing trend with the advancement of flower development from day 2 to 5 (Fig. 2a–b).

Effect of SNP on bacterial density, solution uptake and neck bending

SNP was highly effective in inhibiting the microbial growth and neck bending in cut *Calendula* flowers. Least bacterial growth was recorded in vase solutions containing 100 μM SNP. The flowers held in this solution resulted in maximum solution uptake besides minimum neck bending in flower stems. Maximum bacterial density was recorded in the control which showed reduced solution uptake and faster rate of neck bending. Neck bending in untreated flowers was initiated on day 5, while as in flowers treated with various concentration of SNP viz., 50, 100, 150 and 200 μM, it was observed on day 7, 11, 9 and 3 respectively. With the advancement of flower development, a spike in bacterial density and rapid bent in flower stems was observed in flowers from day 2 to 5 of experiment. Since flowers treated with 200 μM SNP registered early wilting, so the bacterial density was determined on day 5 for the sake of convenience (Fig. 3a–c).

Effect of SNP on membrane stability index (MSI)

As flower development progresses, membranes lose their integrity, leading to electrolyte leakage and loss of intracellular compartmentalization. However, SNP at 100 μM was highly effective in protecting the membrane deterioration by maintaining high membrane stability in petal tissues as compared to control. However, MSI values were observed as decreased as the flowers approached senescence from day 2 to 5 of the experiment. The observations pertaining to MSI are pictured in Fig. 4.

Effect of SNP on total phenols and sugar fractions

Flowers held in SNP showed higher content of sugar fractions and total phenols in comparison to untreated samples. 100 μM SNP was most effective in maintaining high content of these parameters followed by 150 μM SNP. The results also indicate the effectiveness of SNP in maintaining marginally higher content of reducing sugars than non-reducing sugars in the petal tissues. In addition, untreated floral tissues registered a sharp decrease in these parameters with the advancement of flower development compared to flowers treated with SNP at optimal concentration (Fig. 5a–d).

Effect of SNP on soluble proteins and α-amino acids

Flowers treated with SNP at 50, 100 and 150 μM recorded a significant increase in the amount of soluble proteins. Maximum protein enrichment was recorded in the petal tissues treated with 100 μM SNP. Pertinently, the samples which showed higher protein content recorded lower α-amino acids. Maximum α-amino acid content was recorded in the samples treated with 200 μM SNP followed by control. However, protein content decreased with the concomitant increase in α-amino acids as flower development progressed from day 2 to 5 as depicted in the Fig. 6a–b.

Fig. 1 Effect of different concentrations of SNP on postharvest performance of isolated flowers of *Calendula officinalis* on day 0 (**a**), day 2 (**b**) and day 11 (**c**) of transfer of flower buds to the test solutions

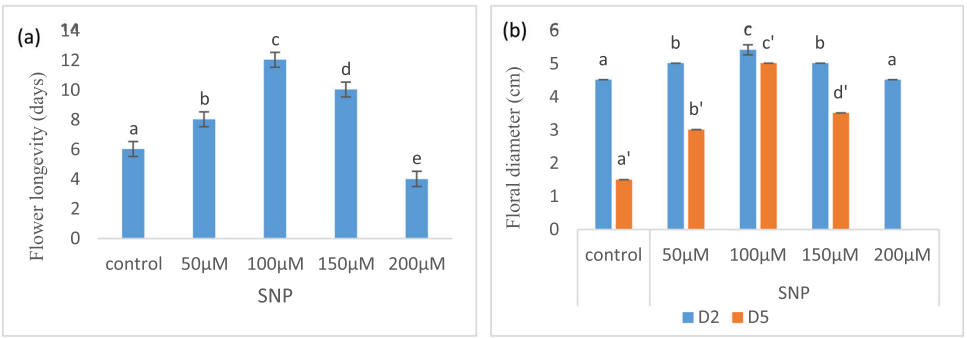

Fig. 2 Effect of different concentrations of SNP on flower longevity (**a**) and floral diameter (**b**) of *Calendula officinalis*. Each value represents the mean of 5 replicates. Letters above the bars denote the statistical significance between individual treatments. Bars with different letters indicate significant differences by Duncan's multiple range test ($P < 0.05$)

Effect of SNP on activities of antioxidant and lipoxygenase enzymes

Application of SNP resulted in substantial augmentation in the activities of SOD, CAT and APX, besides maintaining an attenuated LOX activity in petal tissues. Maximum SOD activity was registered in flower petals treated with 150 µM SNP, while as 100 µM SNP was highly effective in maintaining elevated activities of APX and CAT enzymes. In addition, high LOX activity besides low SOD, CAT and APX activity was recorded in 200 µM SNP treated samples followed by petal tissues of untreated flowers. However, SOD, CAT and APX activities were observed as decreased at the later stages of experiment as the flowers approached senescence, besides a significant increase in LOX activity as depicted in Fig. 7a–d.

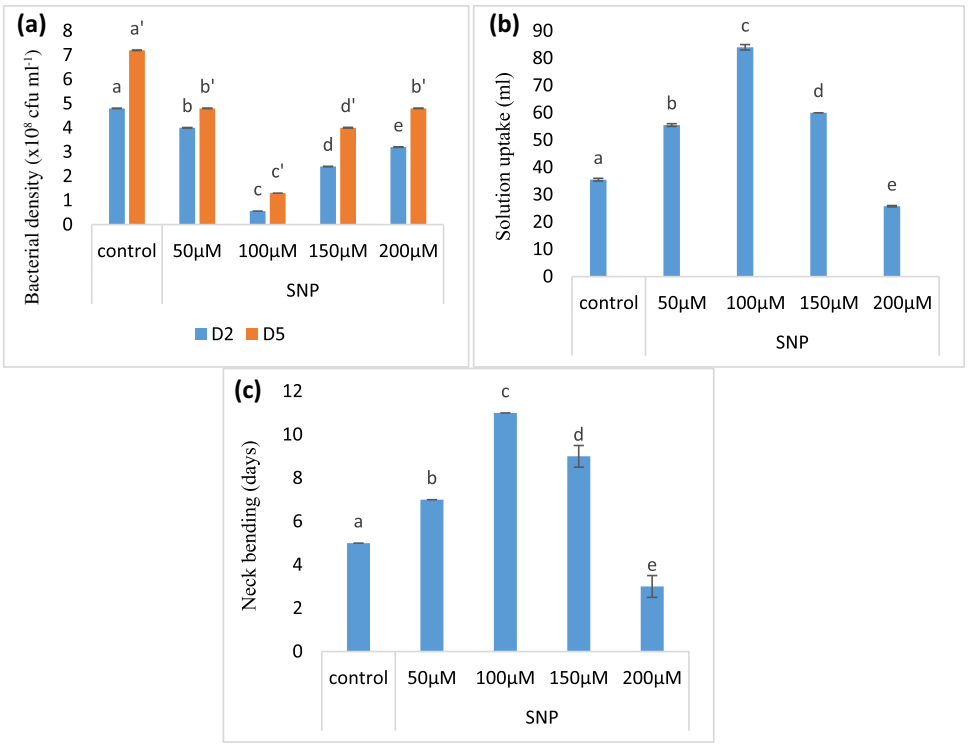

Fig. 3 Effect of different concentrations of SNP on bacterial density (**a**), solution uptake (**b**) and neck bending (**c**) in flowers of *Calendula officinalis*. Each value represents the mean of 3 replicates. Letters above the bars denote the statistical significance between individual treatments. Bars with different letters indicate significant differences by Duncan's multiple range test ($P < 0.05$)

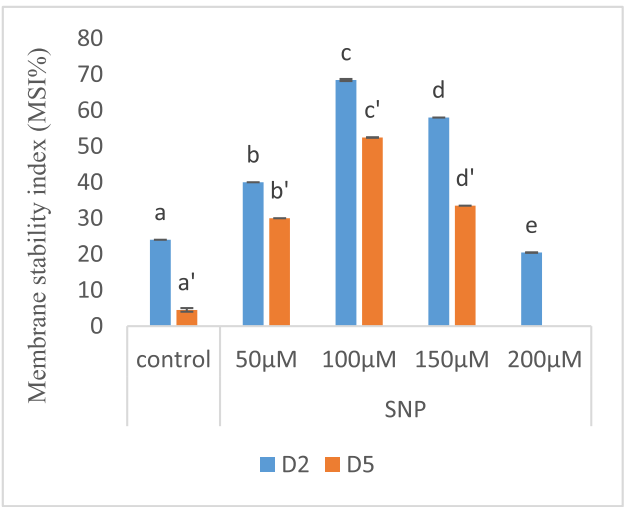

Fig. 4 Effect of different concentrations of SNP on MSI in flowers of *Calendula officinalis*. Each value represents the mean of 3 replicates. Letters above the bars denote the statistical significance between the individual treatments. Bars with different letters indicate significant differences by Duncan's multiple range test ($P < 0.05$)

Discussion

Since the realization that NO plays a key role in the regulation of cellular functions in plants, it has garnered a profound attention amongst researchers carrying investigations on postharvest physiology of cut flowers keeping in view its non-hazardous and eco-friendly nature. NO has been revealed as an exceptional signaling molecule due to its versatile functions in physiology and biochemistry of living organisms (Lamattina et al. 2003; Astier et al. 2018). Improved longevity of *Calendula* flowers could be attributed to the involvement of NO in maitaining proper water conduction in the flowers by inhibiting the bacterial growth in vase solutions that otherwise lead to neck bending (Perik et al. 2014). Maintenance of proper turgor pressure, achieved by sufficient water uptake and/or reduced rates of water loss in flower heads is a prerequisite to prevent head drop (Hemati et al. 2019). Additionally, NO could prevent neck bending by inducing lignification that has considerable scope in strengthening the flower pedicels (Böhm et al. 2010; Naing et al. 2017; Hemati et al. 2019). It has been observed that NO up-regulates the phenylalanine ammonia lyase (PAL) activity which is involved in synthesis of many phenolics and lignin like compounds associated with plant metabolism (Liu et al. 2005; Naing et al. 2017; Rezayian et al. 2020). So our analysis suggest that NO could prevent neck bending by inhibiting bacterial growth and xylem blockage, besides up-regulating the expression levels of lignin biosynthesis, gene and antioxidant activities. Furthermore, maintenance of proper water balance in petals resulted substantial improvement in head diameter of *Calendula* flowers consistent with the earlier findings by Zhang et al. (2018) and Deng et al. (2019) in cut lily and roses respectively.

Membrane stability plays a key role in preventing excessive leakage of electrolytes, sugars and pigments in order to delay senescence (Ezhilmathi et al. 2007;

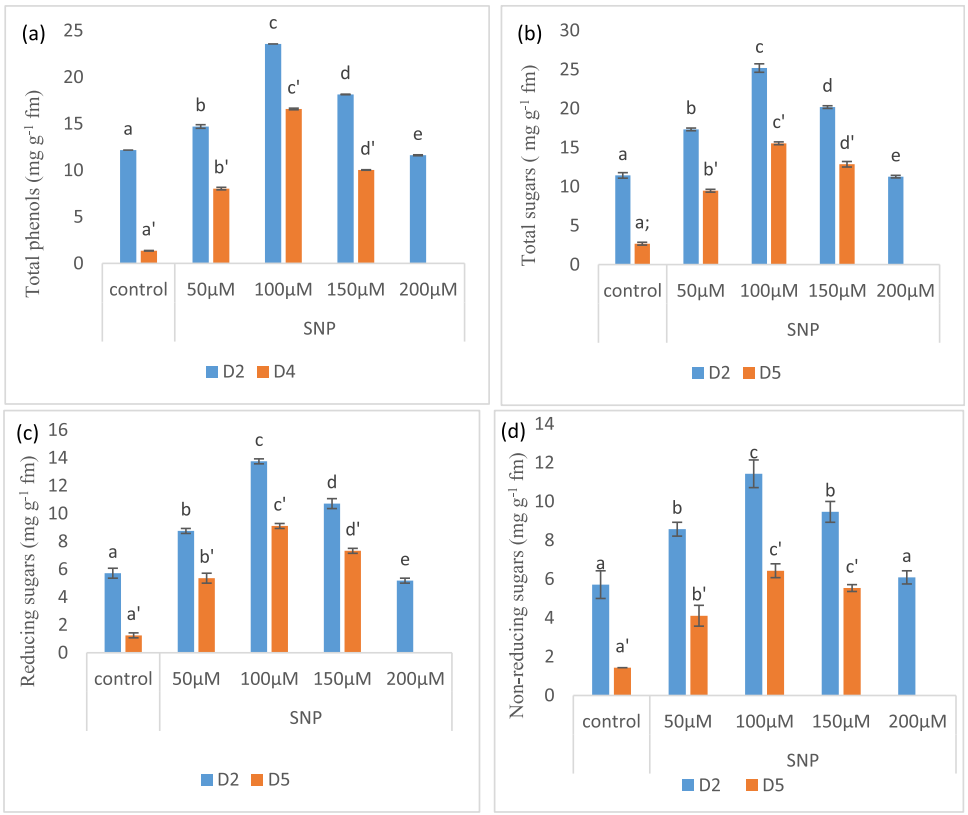

Fig. 5 Effect of different concentrations of SNP on total phenols (**a**), total sugars (**b**), reducing sugars (**c**) and non-reducing sugars (**d**) in flowers of *Calendula officinalis*. Each value represents the mean of 3 replicates. Letters above the bars denote the statistical significance between the individual treatments. Bars with different letters indicate significant differences by Duncan's multiple range test ($P < 0.05$)

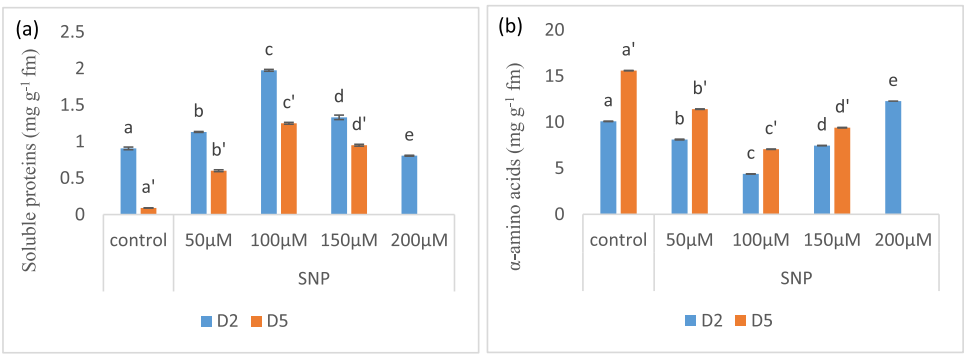

Fig. 6 Effect of different concentrations of SNP on soluble proteins (**a**) and α-amino acids (**b**) in flowers of *Calendula officinalis*. Each value represents the mean of 3 replicates. Letters above the bars denote the statistical significance between individual treatments. Bars with different letters indicate significant differences by Duncan's multiple range test ($P < 0.05$)

Ghadakchiasl et al. 2017). As the senescence advances, oxidative stress due to free radical production leads to deterioration of membranes hence loss of membrane integrity. NO could maintain high membrane stability in *Calendula* by inhibiting lipid peroxidation either directly by interacting with lipid peroxyl radicals or indirecty by maintaining a decreased LOX activity in the petal tissues (Shabanian et al. 2018; Singh et al. 2020). NO application has been reported to improve the vase life of various cut flowers such as carnations, gladiolus, chrysanthemums and lily by maintaining high membrane stability in the petal tissues. (Mansouri 2012; Naing et al. 2017; Kazemzadeh-Beneh et al. 2018; Zhang et al. 2018).

The role of NO to confront senescence in *Calendula* flowers might be related with its regulatory role in maintaining higher sugar levels in the petal tissues. Limited information is available about the mechanism involved in NO induced carbohydrate metabolism in cut flowers. Our observations suggest that NO maintained high sugar content in petals possibly by modulating the enzyme activities of carbohydrate metabolism and/or by acting as a potent inhibitor of the mitochondrial respiratory chain which prevented rapid degradation of sugars (Mason et al. 2006;

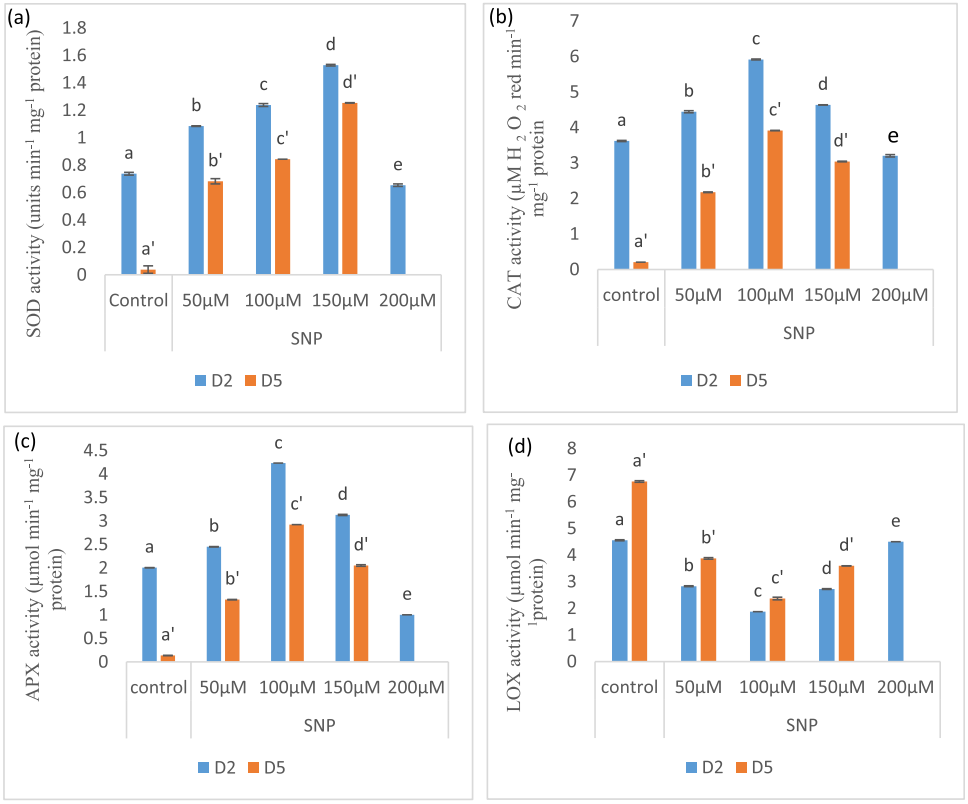

Fig. 7 Effect of different concentrations of SNP on activities of SOD (**a**), CAT (**b**), APX (**c**) and LOX (**d**) in flowers of *Calendula officinalis*. Each value represents the mean of 3 replicates. Letters above the bars denote the statistical significance between individual treatments. Bars with different letters indicate significant differences by Duncan's multiple range test ($P < 0.05$)

Han et al., 2018). Sugars play an indispensable role in preventing senescence not only by acting as typical osmoprotectants and membrane stabilizers (Couée et al. 2006) but also by eliciting signals during sugar sensing and signaling systems (Chen et al. 2009). Sugar molecules have been shown to stabilize the membrane lipids via hydrogen-bonding interactions between sugar OH and lipid headgroups. Such interactions are beneficial in ROS balancing and preventing the phase transitions that otherwise elicit extravasation of solutes (Vital et al. 2019). Furthermore, petal senescence occurs concomitant with rise in ratio of non-reducing to reducing sugars (Nichols 1973). However, NO maintained higher ratio of reducing to non-reducing sugars in petal tissues of *Calendula* flowers. NO has reportedly increased sugar content in petal tissues of many cut flowers such as chrysanthemums and gerbera due to which the longevity in these flowers was significantly enhanced (Mansouri 2012; Hemati et al. 2019).

Petal browning and discoloration are important parameters which determine display quality and market value of cut flowers and in many cases are responsible for the termination of vase life (Khalaj et al. 2017; Salehi Salmi et al. 2018). Flowers treated with NO recorded high phenolic content in petal tissues due to increased PAL activity as discussed above, which is responsible for the synthesis of several defense-related secondary compounds that prevent cell membranes from lipid peroxidation by scavenging free superoxide radicals (Soleimani Aghdam et al. 2015; Naing et al. 2017; Shabanian et al. 2018) consistent with the earlier observations by Naing et al. (2017) in carnations.

Higher levels of soluble proteins in SNP treated petal tissues could be owed to the involvement of NO in up-regulation of m-RNA transcription and protein synthesis (Beligni and Lamattina 2001; Zhang 2007). Moreover, NO might have inhibited the activity of proteases by down-regulating their gene expression, thus reducing the α-amino acid content in the petal tissues (Pak and Doorn 2005; Dwivedi et al. 2016). Furthermore, the accumulation of proteins may be involved in enhancing the activities of antioxidant enzymes and synthesis of specific stress-related proteins as defense mechanism (Doganlar et al. 2010; Promyou et al. 2012). Noticeable accumulation of total soluble proteins by NO treatment might alternatively amplify tolerance of cells through osmotic regulation synergistically with sugars. Studies also suggest that protein enrichment minimizes the starving effect of carbohydrates/sugars by serving as alternative respiratory substrates (Rezvanypour and Osfoori 2011). Pertinently, NO has been shown to maintain an elevated protein levels in lilium, gladiolus, gerbera and white prosperity flowers improving their longevity (Kaviani et al. 2013; Dwivedi

et al. 2016; Shabanian et al. 2018; Kazemzadeh-Beneh et al. 2018).

Antioxidant system is regarded as one of the most efficient systems to counter deleterious effects of ROS associated oxidative stress (Soares et al. 2019). It comprises of both enzyme and non-enzyme players which function synergistically to protect cells from oxidative imbalances and stress (Hossain et al. 2015; Hoque et al. 2016; Soares et al. 2019). NO has been found to act as an antioxidant molecule either directly by scavenging the ROS or indirectly by modulating other antioxidant systems such as antioxidant enzymes to diminish the obnoxious effects of both biotic and abiotic stresses in plants (Zeng et al. 2011; Ahmad et al. 2016; Dwivedi et al. 2016; AbuQamar et al. 2017; Siddiqui et al. 2017; Deng et al. 2019; Hemati et al. 2019; Rabiei et al., 2019; Sharma et al. 2020; Souri et al. 2020). In the present investigation, NO positively amplified the activities of antioxidant enzymes possibly by up-regulating the gene expression and protein functions (Nabi et al. 2019; Sharma et al. 2020). Validating our results, NO has improved the vase life of gladiolus, gerbera, carnations and rose by modulating the antioxidant enzymes (Dwivedi et al. 2016; Naing et al. 2017; Kazemzadeh-Beneh et al. 2018; Deng et al. 2019). Furthermore, our result has been consistent with the observations of Dwivedi et al. (2016) which stated that in addition to increased activities of SOD, CAT and APX, an attenuated LOX activity was recorded in SNP treated gladiolus florets/spikes.

Conclusion and future outlook

The present investigation reveals promising effect of NO on maintaining the postharvest quality of *Calendula* flowers. Among the range of treatments, 100 µM SNP was observed as most effective in improving the postharvest performance and longevity in this flower followed by 150 µM SNP. The important finding of this investigation was that NO through SNP, delayed neck bending in *Calendula* by inhibiting bacterial growth and xylem blockage, besides promoting accumulation of phenolic compounds. The delayed neck bending was corroborated with the up-regulated activities of antioxidant enzymes, high membrane stability, in addition to an elevated sugar content and protein levels in petals of *Calendula officinalis*. However, critical revision needs to be undertaken at molecular level to explain how exactly NO affects various parameters during senescence in cut *Calendula* flowers. The authors realize that *Calendula officinalis* could act as a model system for studies related to postharvest physiology and petal cell death.

References

AbuQamar S, Moustafa K, Tran LS (2017) Mechanisms and strategies of plant defense against *Botrytis cinerea*. Crit Rev Biotechnol 37:262–274

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Ahmad P, Abdel Latef AA, Hashem A, Abd Allah EF, Gucel S, Tran LS (2016) Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea. Front Plant Sci 7:347

Ahmad P, Ahanger MA, Alyemeni MN, Wijaya L, Alam P (2018) Exogenous application of nitric oxide modulates osmolyte metabolism, antioxidants, enzymes of ascorbate-glutathione cycle and promotes growth under cadmium stress in tomato. Protoplasma 255(1):79–93

Astier J, Gross I, Durner J (2018) Nitric oxide production in plants: an update. J Exp Bot 69(14):3401–3411

Axerold B, Chesbrough TM, Laakso S (1981) Lipoxygenase from soybean. In: Lowenstein JM (ed) Methods enzymology. Academic Press, New York, pp 441–451

Badiyan D, Wills RBH, Bowyer MC (2004) Use of a nitric oxide donor compound to extend the vase life of cut flowers. Hortic Sci 39:1371–1372

Beligni MV, Lamattina L (2001) Nitric oxide in plants: the history is just beginning. Plant Cell Environ 24:267–278

Böhm FMLZ, Ferrarese MDLL, ZanardoDIL MJR, Ferrarese-Filho O (2010) Nitric oxide affecting root growth, lignification and related enzymes in soybean seedlings. Acta Physiol Plant 32(6):1039–1046

Chen GX, Asada K (1989) Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant Cell Physiol 30:987–998

Chen S, Gollop N, Heuer B (2009) Proteomic analysis of salt-stressed tomato (*Solanum lycopersicum*) seedlings: effect of genotype and exogenous application of glycinebetaine. J Exp Bot 60:2005–2019

Couée I, Sulmon C, Gouesbet G, El Amrani A (2006) Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. J Exp Bot 57:449–459

Deng Y, Wang C, Huo J, Hu W, Liao W (2019) The involvement of NO in ABA-delayed the senescence of cut roses by maintaining water content and antioxidant enzymes activity. Sci Hortic 247:35–41

Dhindsa RS, Plumb-Dhindsa D, Thorpe TA (1981) Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot 32:93–101

Doganlar ZB, Demir K, Basak H, Gul I (2010) Effects of salt stress on pigment and total soluble protein contents of three different tomato cultivars. Afr J Agric Res 5:2056–2065

Dwivedi SK, Arora A, Singh VP, Sairama R, Bhattachary RC (2016) Effect of sodium nitroprusside on differential activity of antioxidants and expression of SAGs in relation to vase life of gladiolus cut flowers. Sci Hortic 210:158–165

Ezhilmathi K, Singh VP, Arora A, Sairam RK (2007) Effect of 5-sulfosalicylic acid on antioxidant activity in relation to vase life of Gladiolus cut flowers. Plant Growth Regul 51(2):99

Ghadakchiasl A, Mozafari AA, Ghaderi N (2017) Mitigation by sodium nitroprusside of the effects of salinity on the morpho-physiological and biochemical characteristics of *Rubus idaeus* under in vitro conditions. Physiol Mol Biol Plants 23(1):73–83

Gómez-Merino FC, Castillo-González AM, Ramírez-Martínez M, Trejo-Téllez LI (2020) Lanthanum delays senescence and

improves postharvest quality in cut tulip (*Tulipa gesneriana* L.) flowers. Sci Rep 10(1):1–12

Han S, Cai H, An X, Huan C, Wu X, Jiang L, Yu M, Ma R, Yu Z (2018) Effect of nitric oxide on sugar metabolism in peach fruit (cv. Xiahui 6) during cold storage. Postharvest Biol Technol 142:72–80

Hasanuzzaman M, Nahar K, Mahmud JA, Ahmad P, Fujita M (2016) Nitric oxide: a Jack of all trades for drought stress tolerance in plants. In: Ahmad P (ed) Water stress and crop plants: a sustainable approach, 1st edn. Wiley, West Sussex, pp 628–648

Hemati E, Salehi Salmi MR, Daneshvar MH, Heidari M (2019) The roles of sodium nitroprusside, salicylic acid, and methyl jasmonate as hold solutions on vase life of *Gerbera jamesonii* 'Sun Spot.' Adv Hort Sci 33(2):187–195

Hoque TS, Hossain MA, Mostofa MG, Burritt DJ, Fujita M, Tran LSP (2016) Methylglyoxal: an emerging signaling molecule in plant abiotic stress responses and tolerance. Front Plant Sci 7:1341

Hossain MA, Bhattacharjee S, Armin SM, Qian P, Xin W, Li HY, Burritt DJ, Fujita M, Tran LSP (2015) Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. Front Plant Sci 6:420

Kaviani M, Mortazavi SN, Talebi SF, Sharafi Y (2013) Investigating enzyme activity and protein changes in postharvest (*Lilium candidum* cv. Navona) cut flower. Intl J Agron Plant Prod 4(9):2354–2360

Kazemzadeh-Beneh H, Samsampour D, Zarbakhsh S (2018) Biochemical, physiological changes and antioxidant responses of cut gladiolus flower 'White Prosperity' induced by nitric oxide. Adv Hortic Sci 32(3):421–431

Khalaj M, Kiani S, Khoshgoftarmanesh AH, Amoaghaie R (2017) Growth, quality and physiological characteristics of gerbera (*Gerbera jamesonii* L.) cut flowers in response to different NO_3:NH_4 ratios. Hortic Environ Biotechnol 58:313–323

Kondo M, Shibuya K, Ichimura K (2017) Sensitivity to ethylene and availability of preservative on the vase life of 15 important cut flowers. Hortic Res 16(1):438

Kumar Rai K, Rai N, Pandey Rai S (2018) Salicylic acid and nitric oxide alleviate high temperature induced oxidative damage in *Lablab purpureus* L. plants by regulating bio-physical processes and DNA methylation. Plant Physiol Biochem 128:72–88

Lamattina L, Garcia-Mata C, Graziano M, Pagnussat G (2003) Nitric oxide: the versatility of an extensive signal molecule. Ann Rev Plant Biol 54:109–136

Liu H, Jiang W, Bi Y, Luo Y (2005) Postharvest BTH treatment induces resistance of peach (*Prunus persica* L. cv. Jiubao) fruit to infection by *Penicillium expansum* and enhances activity of fruit defense mechanisms. Postharvest Biol Technol 35(3):263–269

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193(1):265–275

Mansouri H (2012) Salicylic acid and sodium nitroprusside improve postharvest life of chrysanthemums. Sci Hortic 145:29–33

Mason MG, Nicholls P, Wilson MT, Cooper CE (2006) Nitric oxide inhibition of respiration involves both competitive (heme) and noncompetitive (copper) binding to cytochrome c oxidase. Proc Natl Acad Sci USA 103:708–713

Moroz LL, Romanova DY, Nikitin MA, Sohn D, Kohn AB, Neveu E, Varoqueaux F, Fasshauer D (2020) Nitric oxide (NO) signaling in Trichoplax and related species: microchemical characterization and the lineage-specific diversification. bioRxiv

Nabi RBS, Tayade R, Hussain A, Kulkarni KP, Imran QM, Mun B-G, Yun B-W (2019) Nitric oxide regulates plant responses to drought, salinity, and heavy metal stress. Environ Exp Bot 161:120–133

Naing AH, Lee K, Kim KO, Ai TN, Kim CK (2017) Involvement of sodium nitroprusside (SNP)in the mechanism that delays stem bending of different gerbera cultivars. Front Plant Sci 8:2045

Nelson N (1944) A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem 153:375–380

Nichols R (1973) Senescence of the cut carnation flower: respiration and sugar status. J Hortic Sci 48(1):111–121

Pak C, Van Doorn WG (2005) Delay of iris flower senescence by protease inhibitors. New Phytol 165:473–480

Perik RR, Razé D, Harkema H, Zhong Y, Van Doorn WG (2012) Bending in cut *Gerbera jamesonii*flowers relates to adverse water relations and lack of stem sclerenchyma development, not to expansion of the stem central cavity or stem elongation. Postharvest Biol Technol 74:11–18

Perik RR, Razé D, Ferrante A, Van Doorn WG (2014) Stem bending in cut *Gerbera jamesonii* flowers: effects of a pulse treatment with sucrose and calcium ions. Postharvest Biol Technol 98:7–13

Procházková D, Wilhelmová NA (2011) Nitric oxide, reactive nitrogen species and associated enzymes during plant senescence. Nitric Oxide 24(2):61–65

Promyou S, Ketsa S, van Doorn WG (2012) Salicylic acid alleviates chilling injury in anthurium (*Anthurium andraeanum*L.) flowers. Postharvest Biol Technol 64:104–110

Rabiei V, Kakavand F, Zaare-Nahandi F, Razavi F, Aghdam MS (2019) Nitric oxide and γ-aminobutyric acid treatments delay senescence of cornelian cherry fruits during postharvest cold storage by enhancing antioxidant system activity. Sci Hortic 243:268–273

Rani P, Singh N (2014) Senescence and postharvest studies of cut flowers: a critical review. Pertanika J Trop Agric Sci 32(2):159–201

Reid MS, Jiang CZ (2012) Postharvest biology and technology of cut flowers and potted plants. In: Janick J (ed) Horticultural reviews. Wiley, Hoboken, pp 1–54

Rezayian M, Ebrahimzadeh H, Niknam V (2020) Nitric oxide stimulates antioxidant system and osmotic adjustment in soybean under drought stress. J Soil Sci Plant Nutr 1–11

Rezvanypour S, Osfoori M (2011) Effect of chemical treatments and sucrose on vase life of three cut rose cultivars. J Res Agric Sci 7:133–139

Rosen H (1957) A modified ninhydrin colorimetric analysis for amino acids. Arch Biochem Biophys 67(1):10–15

Saba MK, Moradi S (2017) Sodium nitroprusside (SNP) spray to maintain fruit quality and alleviate postharvest chilling injury of peach fruit. Sci Hortic 216:193–199

Sairam RK (1994) Effect of moisture stress on physiological activities of two contrasting wheat genotypes. Indian J Exp Biol 32:584–593

Salehi Salmi MR, Falehi Hoseini M, Heidari M, Daneshvar MH (2018) Extending vase life of cut rose (*Rosa hybrid* L.) cv. Bacara by essential oils. Adv Hortic Sci 32(1):61–69

Sami F, Faizan M, Faraz A, Siddiqui H, Yusuf M, Hayat S (2018) Nitric oxide-mediated integrative alterations in plant metabolism to confer abiotic stress tolerance, NO crosstalk with phytohormones and NO-mediated post translational modifications in modulating diverse plant stress. Nitric Oxide 73:22–38

Shabanian S, Esfahani MN, Karamian R, Tran LSP (2018) Physiological and biochemical modifications by postharvest treatment with sodium nitroprusside extend vase life of cut flowers of two gerbera cultivars. Postharvest Biol Technol 137:1–8

Sharma A, Soares C, Sousa B, Martins M, Kumar V, Shahzad B, Sidhu GP, Bali AS, Asgher M, Bhardwaj R, Thukral AK (2020) Nitric oxide-mediated regulation of oxidative stress in plants under metal stress: a review on molecular and biochemical aspects. Physiol Plant 68(2):318–344

Shi J, Gao L, Zuo J, Wang Q, Wang Q, Fan L (2016) Exogenous sodium nitroprusside treatment of broccoli florets extends shelf life, enhances antioxidant enzyme activity, and inhibits chlorophyll-degradation. Postharvest Biol Technol 116:98–104

Siddiqui MH, Alamri SA, Al-Khaishany MY, Al-Qutami MA, Ali HM, Hala AR, Kalaji HM (2017) Exogenous application of nitric oxide and spermidine reduces the negative effects of salt stress on tomato. Hortic Environ Biotechnol 58(6):537–547

Singh P, Singh I, Shah K (2020) Alterations in antioxidative machinery and growth parameters upon application of nitric oxide donor that reduces detrimental effects of cadmium in rice seedlings with increasing days of growth. S Afr J Bot 131:283–294

Soares C, Carvalho M, Azevedo R, Fidalgo F (2019) Plants facing oxidative challenges: a little help from the antioxidant networks. Environ Exp Bot 161:4–25

Soleimani Aghdam M, Naderi R, Sarcheshmeh MAA, Babalar M (2015) Amelioration of postharvest chilling injury in anthurium cut flowers by γ-aminobutyric acid (GABA) treatments. Postharvest Biol Technol 110:70–76

Souri Z, Karimi N, Farooq MA, Sandalio LM (2020) Nitric oxide improves tolerance to arsenic stress in *Isatis cappadocica* desv. Shoots by enhancing antioxidant defenses. Chemosphere 239:124523

Swain T, Hillis WE (1959) The phenolic constituents of *Prunus domestica* I. The quantitative analysis of phenolic constituents. J Sci Food Agric 10(1):63–68

van Doorn WG, de Witte Y (1994) Effect of bacteria on scape bending in cut *Gerbera jamesonii*flowers. J Am Soc Hortic Sci 119(3):568–571

Verma N, Tiwari S, Singh VP, Prasad SM (2020) Nitric oxide in plants: an ancient molecule with new tasks. Plant Growth Regul 90(1):1–13

Vital RG, Müller C, da Silva FB, Batista PF, Merchant A, Fuentes D, Rodrigues AA, Costa AC (2019) Nitric oxide increases the physiological and biochemical stability of soybean plants under high temperature. Agronomy 9(8):412

Williamson VG, Faragher J, Parsons S, Franz P (2002) Inhibiting the postharvest wounding response in wildflowers. Rural Industries Research and Development Corporation, Barton

Woltering EJ, Van Doorn WG (1988) Role of ethylene in senescence of petals: morphological and taxonomical relationships. J Exp Bot 39(11):1605–1616

Zeng CL, Liu L, Guo X (2011) The physiological responses of carnation cut flower to exogenous nitric oxide. Sci Hortic 127:424–430

Zhang A (2007) Nitric oxide induced by hydrogen peroxide mediates abscisic acid-induced activation of the mitogen-activated protein kinase cascade involved in antioxidant defense in maize leaves. New Phytol 175:36–50

Zhang J, Fang H, Huo J, Huang D, Wang B, Liao W (2018) Involvement of calcium and calmodulin in nitric oxide-regulated senescence of cut lily flowers. Front Plant Sci 9:1284