



Trends in **Horticultural Research**

ISSN 1996-0735



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Efficacy of STS Pulsing and Floral Preservative Solutions on Senescence and Post Harvest Performance of *Narcissus pseudonarcissus* Cv. Emperor

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ABSTRACT

Ornamental Daffodils form a prized cut flower class. Silver Thiosulfate Solution (STS) besides some floral preservative solutions were examined for their efficacy to enhance the longevity of daffodil (*Narcissus pseudonarcissus* cv. Emperor). The spikes were divided into two sets. One set of spikes was pulsed with Silver thiosulfate solution (STS 0.5 mM) for 1 h. Both the pulsed and unpulsed sets of spikes were separately transferred to Distilled Water (DW), Sucrose (SUC 0.15 M), Sucrose (SUC, 0.15 M)+8-Hydroxy Quinoline Sulphate (HQS, 50 mg L⁻¹), Sucrose (SUC, 0.15 M)+8-Hydroxy Quinoline Sulphate (HQS, 50 mg L⁻¹)+Kinetin (Kin, 25 mg L⁻¹). Improved vase life and postharvest performance was observed in all treatment as compared to the control (DW). Pulse treatment of spikes STS (0.5 mM, 1 h) increased solution uptake, maintained high fresh and dry mass of flowers and lowered electrical conductivity of leachates particularly in spikes transferred to SUC+8-HQS. The content of sugars decreased in all treatments. Proteins and phenol content increased, in the pulsed set of spikes; whereas that of α -amino acids followed a contrary pattern. STS (0.5 mM, 1 h) pulse treatment of spikes with transfer to SUC+8-HQS enhanced vase life and improved postharvest performance in this flower system.

Key words: Silver thiosulfate solution, senescence, 8-hydroxyquinone sulphate (8-HQS), sucrose solution, vase life, postharvest performance, sugars, proteins

INTRODUCTION

Flowering represents the heart of plant biology being the principal organs of reproduction for sexual reproduction flowers represent the ultimate site for genetic recombination. Since ages flowers have attracted the interest of mankind for being a symbol of beauty and peace. Daffodils are one of the prized ornamental flowers across the world. *Narcissus pseudonarcissus* is a perennial bulbous species of the genus *Narcissus*. It has pale yellow flowers with a darker central trumpet and is therefore also called as "Trumpet Daffodil". The species is native to Western Europe ranging from Spain and Portugal to Germany, England and Wales. Senescence is a dynamic, well regulated developmental event in plant life cycle; comprising of active gene expression (Yamada *et al.*, 2003; Hoerberichts *et al.*, 2005; Chapin and Jones, 2007; Shahri and Tahir, 2011). Rapid senescence of ornamental flowers is highly undesirable from a postharvest perspective. Strategies for senescence delay involve differential hormonal status; particularly ethylene and cytokinins; application of floral preservatives or respiratory source, temperature control and protease inhibition (Mayak and

Halevy, 1972; Mayak and Kofranek, 1976; Taverner *et al.*, 1999; Lara *et al.*, 2004; Woltering and Van Doorn, 1988; Nowak and Rudnicki, 1990; Ketsa *et al.*, 1995; Gul *et al.*, 2007; Gul and Tahir, 2009, 2012a,b; Reddy *et al.*, 1996; Pun and Ichimura, 2003; Shahri and Tahir, 2011; Gul *et al.*, 2012). Ethylene has been found to play a pivotal role in senescence of ethylene sensitive flowers (Woltering and Van Doorn, 1988; Trobacher, 2009; Shahri and Tahir, 2011). Endogenously produced ethylene in such flowers initiates senescence and coordinates the expression of genes required for the process (Jones *et al.*, 1994, 2005; Narumi *et al.*, 2006; Ichimura *et al.*, 1999; Lerslerwong *et al.*, 2009; Shahri and Tahir, 2011). After harvest the longevity of flowers is limited by rapid petal wilting. Floral preservatives have been found to delay senescence by suppressing the microbial growth and promoting solution uptake due to reduction in vascular blockades (Halevy and Mayak, 1979, 1981; Reid *et al.*, 1980; Taverner *et al.*, 1999; Elhindi, 2012; Asrar, 2012). *N. pseudonarcissus* has been found to be responsive to ethylene (Hunter *et al.*, 2002; Van Doorn, 2004). The use of ethylene antagonist Silver Thiosulfate Solution (STS) and floral preservative (8-Hydroxy quinoline sulphate) will therefore allow for a better adjustment of flowers supply to the requirements of the market and to a greater extent eliminate the postharvest losses. It is in this perspective that the present study was conducted on cut spikes of *N. pseudonarcissus* cv. Emperor with the ultimate aim to delay senescence and improve their postharvest performance.

MATERIALS AND METHODS

Experimental plant material: Spikes of *N. pseudonarcissus* growing in the Kashmir University Botanical Garden (KUBG) were used for the present study. The spikes were harvested at 08.00 h when the spikes were at goose neck stage. The spikes were brought to the laboratory and cut to a uniform spike length of 25 cm, held in 1000 mL borosil beakers containing distilled water for 1 h for the slime to drain off. The spikes were divided into two sets. One set of spikes was pulsed with STS (0.5 mM) for 1 h. Both the pulsed and unpulsed sets of spikes were separately transferred to 250 mL conical flasks containing 200 mL of DW, SUC (0.15 M), SUC (0.15 M)+8-HQS (50 mg L⁻¹), SUC (0.15 M)+8-HQS (50 mg L⁻¹)+Kin (25 mg L⁻¹) in triplicate. Treatment effects were evaluated by keeping the flowers in the laboratory at a temperature of 12±2°C under cool white fluorescent light with a mix of diffused natural light (10 W m⁻²) 12 h a day and Relative Humidity (RH) of 60±10%. Treatment was given on the day of harvest which was designated as day zero.

Assessment of vase life, blooming and solution uptake: The average vase life of the spikes was counted from the day of transfer of spikes to the holding solution and was assessed to be terminated when the flowers reached senescent stage (Table 1). The volume of holding solution absorbed by the spikes was calculated by measuring the volume of solution on a particular day and subtracting it from the initial quantity of the vase solution kept in the flasks. To account for the volume of solution evaporated blank flasks (containing particular vase solutions without spikes) were used in triplicates alongside the flasks with spikes. Volume of holding solution absorbed per spike was recorded on every second day of the experiment till the controls senesced.

Conductivity of leachates, fresh and dry mass: Conductivity of leachates, fresh and dry mass of the flowers was determined on 4th and 8th day of harvest. Dry mass was determined by drying the material in an oven for 48 h at 70°C. Membrane permeability was studied by measuring ion leakage from the petal discs (5 mm in diameter) incubated in the dark in 15 mL glass double

distilled water for 15 h at 20°C. The discs were punched from the flag region of petals of 5 flowers. The discs were floated with their abaxial surface downwards and were removed after 15 h of incubation. Conductivity of leachates was measured by CM-180 ELICO Conductivity meter and was expressed in μS .

Tissue constituents: At each stage 0.5 g chopped material of petal tissue was fixed in triplicate in hot 80% ethanol. The material was macerated and centrifuged three times. The supernatants were pooled and used for the estimation of sugars and total phenols. Reducing sugars were estimated by the method of Nelson (1944) using glucose as the standard. Total sugars were estimated after enzymatic conversion of non-reducing sugars into reducing sugars with invertase. Non-reducing sugars were calculated as the difference between total and reducing sugars. Amino acids were estimated by the method of Rosen (1957) using Glycine as standard. Total phenols were estimated by the method of Swain and Hillis (1959) using Gallic acid as standard. Tissue constituents were estimated on day 4 and 8 of the treatment taking into consideration the already senescent status of flowers from controls i.e., DW or SUC (0.15 M).

Soluble proteins: Proteins were extracted from 0.5 g perianth tissue drawn separately from different flowers. The tissue was homogenized in 5 mL of 5% sodium sulphite (w/v) adding 0.1 g of polyvinylpyrrolidone and centrifuged. Proteins were precipitated from a suitable volume of cleared supernatant with equal volume of 20% trichloroacetic acid, centrifuged at 1000 \times g for 15 min and the pellet redissolved in 0.1 N NaOH. Proteins were estimated from a suitable aliquot by the method of Lowry *et al.* (1951) using Bovine Serum Albumin (BSA) as the standard. Soluble proteins were estimated on day 4 and 8 of the treatment.

Statistical analysis: Each value represents the mean of five independent replicates. Differences between the treatments have been calculated by simple analysis of variance and least significant difference (LSD) at 5% computed at $p = 0.05$ using Minitab version 11.

RESULTS

Vase life and solution uptake: The average life of an individual flower under field conditions after it had opened fully was about 3 days. Senescence is characterized by loss of turgor petals initiating at margins; petals turn paper like, translucent with flaccid corona followed by complete wilting (Table 1). The spike finally dislodges in the surrounding bunch of leaves. The average vase life of spikes harvested at goose neck stage was about 6-8 days in DW or SUC. STS pulsing increased longevity in all holding solutions (Fig. 1). The average vase life of flowers from STS

Table 1: Assessment of stages of flower development and senescence based on development of morphological features in *Narcissus pseudonarcissus* cv. Emperor

Stage	Description	Display
I	Tight bud stage	Buds tightly closed, tepals yellow in colour, spathe attached.
II	Loose bud stage	Buds loosely closed, tepals yellow in colour.
III	Half open stage	Flowers half open, corona visible, tepals yellow in colour.
IV	Fully open stage	Flowers fully open, tepals and corona turgid, stamens and pistils visible.
V	Partially senescent stage	Corona dilated, tepals flaccid.
VI	Senescent stage	Tepals papery, translucent and senescent with flaccid corona.

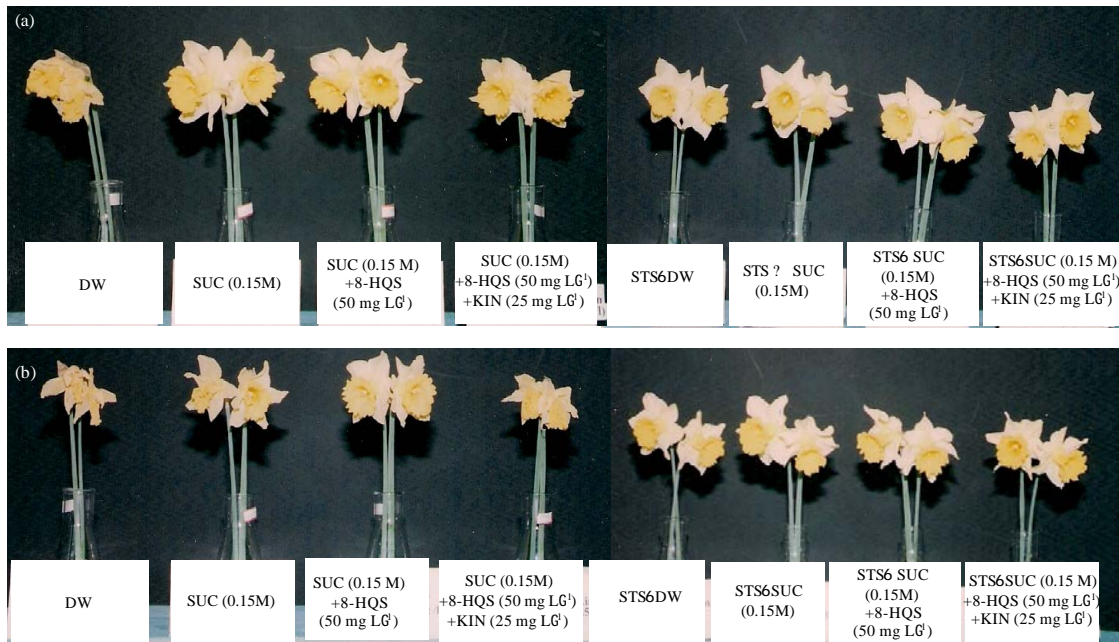


Fig. 1(a-b): Effect of pulse treatment with STS (0.5 mM, 1 h) before transfer to DW, SUC (0.15 M), SUC (0.15 M)+8-HQS (50 mg L⁻¹), SUC(0.15 M)+8-HQS (50 mg L⁻¹)+Kin (25 mg L⁻¹) on vase life and senescence on day 4 and 8 of transfer of scapes to holding solutions in *Narcissus pseudonarcissus* cv. Emperor. From left to right are arranged scapes held in DW, SUC (0.15 M), SUC (0.15 M)+8-HQS (50 mg L⁻¹), SUC (0.15 M)+8-HQS (50 mg L⁻¹)+Kin (25 mg L⁻¹), STS (0.5 mM, 1 h)-DW, STS (0.5 mM, 1 h)-SUC (0.15 M), STS (0.5 mM, 1 h)-SUC (0.15 M)+8-HQS (50 mg L⁻¹), STS (0.5 mM, 1 h)-SUC(0.15 M)+8-HQS (50 mg L⁻¹)+Kin (25 mg L⁻¹)

pulsed spikes and transferred to SUC+8-HQS and SUC+8-HQS+Kin was enhanced to 14-12 days (Fig. 2a). Solution uptake increased with progression in time (days after harvest) in all treatments. Marginal difference was observed in solution uptake among the STS pulsed and unpulsed spikes. SUC+HQS was absorbed more against the other holding solutions (Fig. 2b).

Membrane permeability, fresh and dry mass: The ion leakage of the petal discs was lowered in the STS pulsed spikes particularly in SUC+HQS. Conductivity of leachates increased with progression in time from day 4 to day 8 of the treatment; suggesting increased membrane permeability (Fig. 3a). Generally pulse treatment of spikes with STS resulted in an increase in fresh and dry mass of flowers up to day 8 of the treatment. However, decrease in fresh and dry mass of flowers was generally recorded in samples from unpulsed set of spikes; with progression in time from 4 to 8 days after harvest. A higher fresh and dry mass was, however maintained with progression in time, in samples from spikes pulsed with STS and transferred to SUC, SUC+8-HQS or SUC+8-HQS+Kin (Fig. 3d, 4a).

Tissue constituents (sugars): The content of reducing and total sugars decreased with the progression in age from 4 to 8 days after harvest. However a higher content of both reducing and

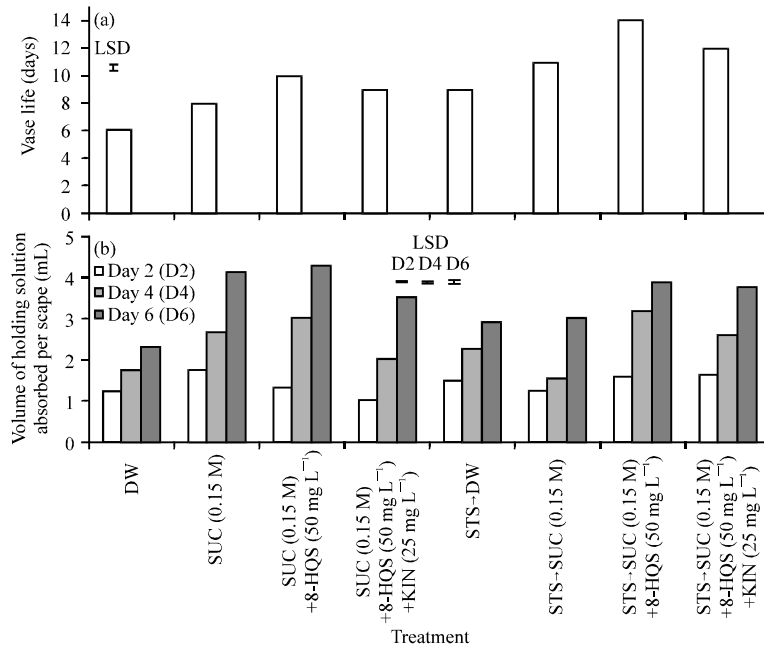


Fig. 2(a-b): Histograms showing effect of pulse treatment with STS (0.5 mM, 1 h) before transfer to DW, SUC (0.15 M), SUC (0.15 M)+8-HQS (50 mg L⁻¹), SUC (0.15 M)+8-HQS (50 mg L⁻¹)+Kin (25 mg L⁻¹) on (a) Vase life and (b) Solution s absorbed per scape mL, on day 2, 4 and 6 of transfer of scapes to holding solutions in *Narcissus pseudonarcissus* cv. Emperor. Vertical bars represent LSD at p = 0.05

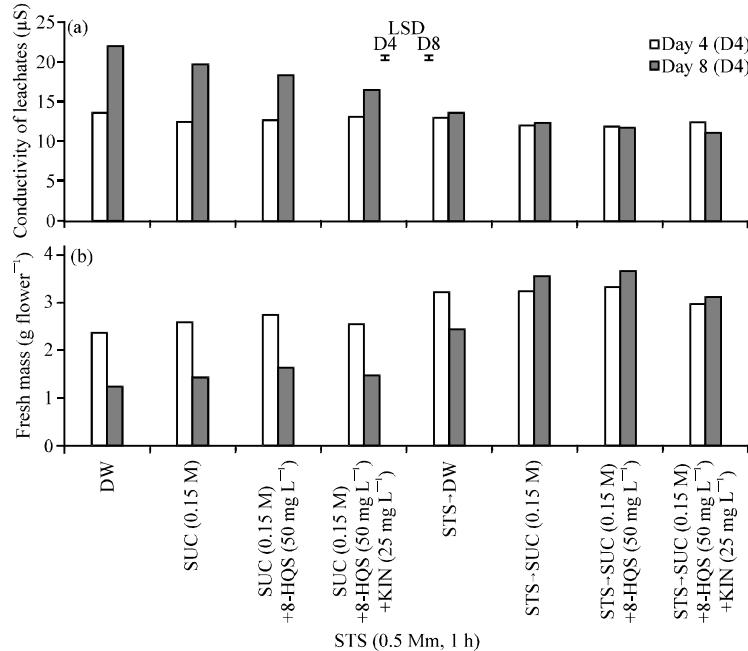


Fig. 3(a-b): Histograms showing effect of pulse treatment with STS (0.5 mM, 1 h) before transfer to DW, SUC (0.15 M), SUC (0.15 M)+8-HQS (50 mg L⁻¹), SUC(0.15 M)+8-HQS (50 mg L⁻¹)+Kin (25 mg L⁻¹) on conductivity of leachates (a) in tepal tissues and fresh mass of flowers (b) on day 4 and 8 of transfer of scapes to holding solutions in *Narcissus pseudonarcissus* cv. Emperor. Vertical bars represent LSD at p = 0.05

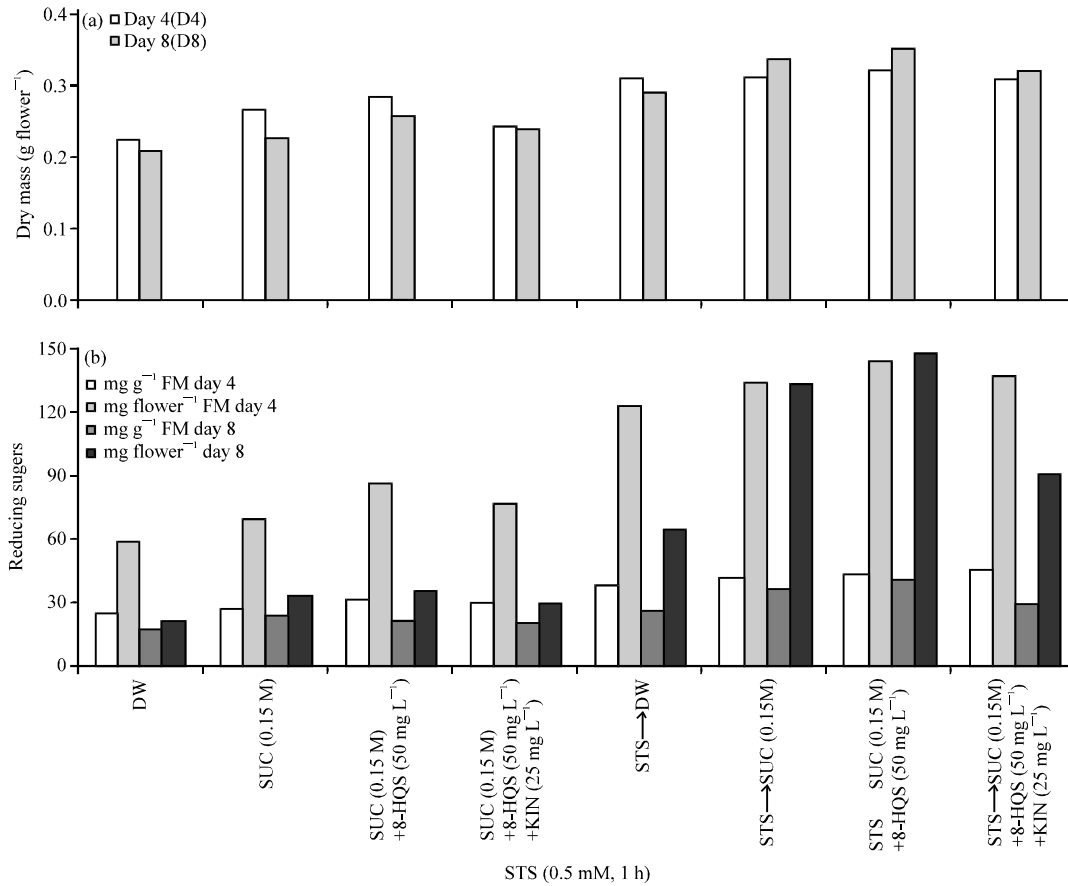


Fig. 4(a-b): Histograms showing effect of pulse treatment with STS (0.5 mM, 1 h) before transfer to DW, SUC (0.15 M), SUC (0.15 M)+8-HQS (50 mg L⁻¹), SUC (0.15 M)+8-HQS (50 mg L⁻¹)+Kin (25 mg L⁻¹) on (a) Dry mass of flowers and (b) Reducing sugars in tepal tissues on day 4 and 8 of transfer of scapes to holding solutions in *Narcissus pseudonarcissus* cv. Emperor. Vertical bars represent LSD at p = 0.05

total soluble sugars was recorded in samples from pulsed spikes transferred particularly to SUC or SUC+8-HQS. The non-reducing sugar content generally registered an increase with progression in time from day 4 to day 8 of transfer of spikes to various holding solutions particularly in samples from spikes held in SUC+8-HQS or SUC+HQS+KIN (Fig. 4b, 5a-b).

Soluble proteins, α -amino acids and phenols: The soluble protein content decreased with the progression in age from 4 to 8 days after harvest in the unpulsed set of spikes. However, the protein content showed an increase in samples from pulsed spikes transferred particularly to SUC or SUC+8-HQS over the period of time. A higher protein content was maintained in the samples from the pulsed spikes as compared to the samples from corresponding unpulsed spikes (Fig. 6a). Amino acids followed a reverse trend as that of soluble proteins. The α -amino acid content registered an increase with the progression in time from day 4 to day 8 of the transfer of unpulsed spikes to various holding solutions. However the α -amino acid content decreased with the progression in time in samples from pulsed spikes particularly transferred SUC+8-HQS. A lower α -amino acid content

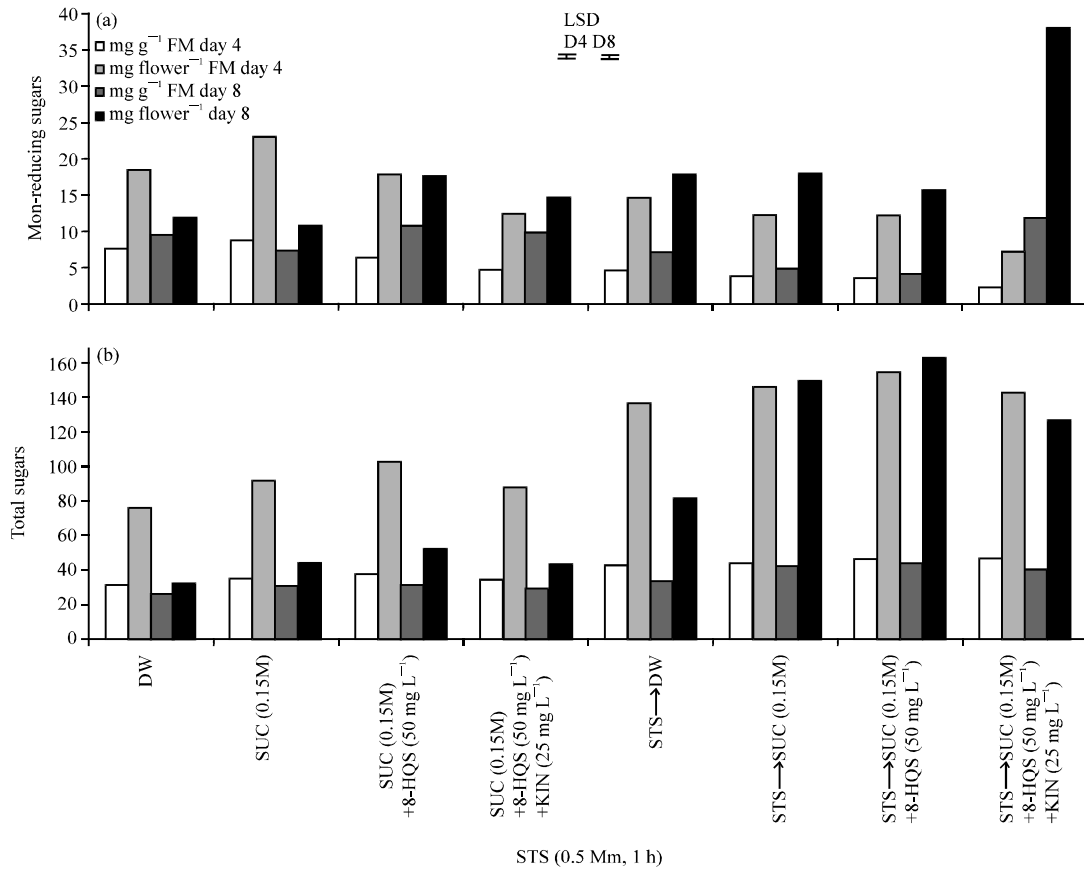


Fig. 5(a-b): Histograms showing effect of pulse treatment with STS (0.5 mM, 1 h) before transfer to DW, SUC (0.15 M), SUC (0.15 M)+8-HQS (50 mg L⁻¹), SUC (0.15 M)+8-HQS (50 mg L⁻¹)+Kin (25 mg L⁻¹) on (a) Non-reducing sugars and (b) Total sugars in tepal tissues on day 4 and 8 of transfer of scapes to holding solutions in *Narcissus pseudonarcissus* cv. Emperor. Vertical bars represent LSD at p = 0.05

was maintained in the samples from the pulsed spikes as compared to the samples from unpulsed spikes irrespective of the transfer to various holding solutions (Fig. 6a). The content of total phenols registered an increase with the progression in age from day 4 to day 8 of harvest. The increase of phenols was more in the samples from the pulsed set of spikes particularly held in SUC+8-HQS or SUC+HQS+KIN (Fig. 7).

DISCUSSION

The current study suggests that pulse treatment with STS followed by transfer to SUC+HQS or SUC+HQS+Kin enhanced vase life by an increment of eight or six days in *N. pseudonarcissus*. Silver ions present in STS have been found to be effective ethylene blockers. STS has been shown to inhibit the increase in the climacteric respiration and ethylene production of flowers (Finger *et al.*, 2004). Inhibition of ethylene action by STS, has been found to prolong the vase life of many cut flowers as *Petunia*, *Lathyrus*, *Thalictrum*, *Consolida* and *Antirrhinum* (Hansen *et al.*, 1996; Uda *et al.*, 1997; Ichimura and Hiraya, 1999; Shahri and Tahir, 2010; Asrar, 2012). Application of Cytokinins has been found to delay senescence in *Petunia* and roses (Mac Lean and

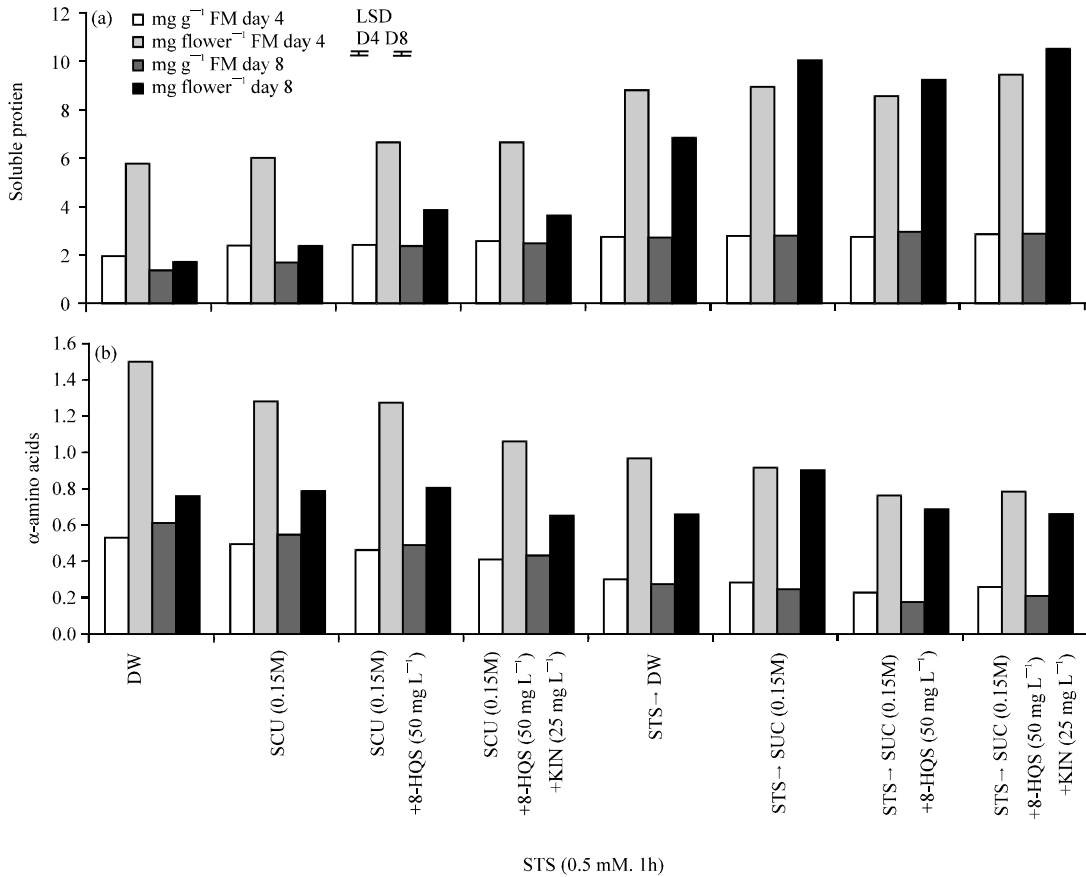


Fig. 6(a-b): Histograms showing effect of pulse treatment with STS (0.5 mM, 1 h) before transfer to DW, SUC (0.15 M), SUC (0.15 M)+8-HQS (50 mg L⁻¹), SUC (0.15 M)+8-HQS (50 mg L⁻¹)+Kin (25 mg L⁻¹) on (a) Soluble proteins and (b) α -amino acids in tepal tissues on day 4 and 8 of transfer of scapes to holding solutions in *Narcissus pseudonarcissus* cv. Emperor. Vertical bars represent LSD at p = 0.05

Dedolph, 1962; Mayak and Halevy, 1972; Mayak and Kofranek, 1976; Taverner *et al.*, 1999; Lara *et al.*, 2004). Cytokinins are natural anti-senescence factors their declining levels serve as trigger for increased ethylene production (Eisinger, 1977; Shahri and Tahir, 2011). Recent studies by Chang *et al.* (2003) confirmed interdependent role of Cytokinins and ethylene for regulating senescence in transgenic plants.

Pulse treatment with STS had negligible effect on solution uptake. Results indicate enhancement of vase life in spikes transferred to 8-HQS+SUC or 8-HQS+SUC+KIN. Higher preferential absorption of HQS+SUC against DW or SUC; suggests the synergistic effect of preservative and respiratory. Sucrose or its combinations with biocides or antioxidants, improved postharvest performance in *Tuberosa*, *Phalaenopsis*, *Leptospermum*, *Amaryllis nerine*, *Lathyrus*, *Antirrhinum* (Huang *et al.*, 1995; Burge *et al.*, 1996; Gul *et al.*, 2007; Gul and Tahir, 2009; Elhindi, 2012; Asrar, 2012). Part of the beneficial effects of 8-HQS have been attributed due to its effect on stomatal closure and in part due to its antibacterial or antifungal activity; resulting in reduced vascular blockage (Halevy *et al.*, 1978; Halevy and Mayak, 1981; Taverner *et al.*, 1999; Elhindi, 2012; Asrar, 2012). STS pulsing and 8-HQS+SUC vase solution significantly maintained

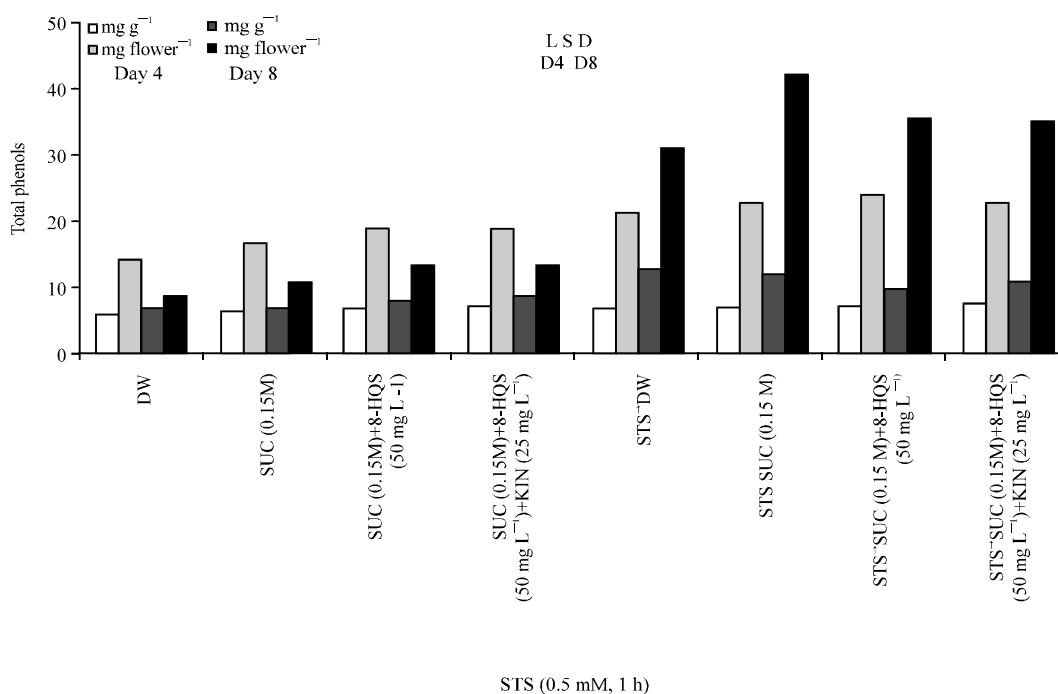


Fig. 7: Histograms showing effect of pulse treatment with STS (0.5 mM, 1 h) before transfer to DW, SUC (0.15 M), SUC (0.15 M)+8-HQS (50 mg L⁻¹), SUC (0.15 M)+8-HQS (50 mg L⁻¹)+Kin (25 mg L⁻¹) total phenols in tepal tissues on day 4 and 8 of transfer of scapes to holding solutions in *Narcissus pseudonarcissus* cv. Emperor. Vertical bars represent LSD at p = 0.05

the membrane integrity in *N. pseudonarcissus*. Supply of exogenous sucrose has been shown to increase water absorption and maintain turgidity of cut flowers by increasing the osmotic concentration of flowers after translocation to petals and subsequent hydrolysis to reducing sugars (Halevy and Mayak, 1979; Gul *et al.*, 2007). Similar findings were obtained by Ichimura (1998), Beura *et al.* (2001), Dineshbabu *et al.* (2002) and Moneruzzaman *et al.* (2010).

Enhancement of vase life of flowers bears a positive relation with the delay in loss of fresh weight (Zuliana *et al.*, 2008; Chutichudet *et al.*, 2011). Maintenance of higher fresh and dry mass of flowers pulse treated with STS and transferred to SUC+HQS could be due to lower respiratory losses. Silver thiosulfate with Sucrose has been found to suppress respiration in cut inflorescences of Esporinha (Finger *et al.*, 2004). Postharvest life of cut flowers has been shown to be dependent on the carbohydrate status (Emongor, 2004; Kazemi *et al.*, 2010; Kazemi *et al.*, 2011). Higher sugar content in STS pulsed spikes particularly in 8-HQS +SUC or 8-HQS+SUC+Kin vase solution. The maintenance of sugars in the STS pulsed set of spikes suggests that senescence is delayed by curtailment in respiratory climacteric otherwise in normal senescence sugars are rapidly respired and utilized (Mwangi *et al.*, 2003; Gulzar *et al.*, 2005). Sugars have been found to the suppression of ethylene biosynthesis or sensitivity to ethylene (Ichimura and Hiraya, 1999; Ichimura *et al.*, 1999; Pun and Ichimura, 2003). These findings suggests an existence of a pathway linking the production of ethylene vis a vis sugar status of the plant. Maintenance of higher phenolic content

in the pulsed set of spikes extended the vase life particularly in 8-HQS+SUC vase solution. The higher content of total phenols has been shown to be associated with longer vase life in cut rose petals and *Hemerocallis* (Mwangi *et al.*, 2003; Gulzar *et al.*, 2005).

The soluble protein content increased whereas α -amino acid content decreased with progression in age from 4-8 days of harvest in STS pulsed set of spikes. Recently it has been found that ethylene receptors are controlled by protein turnover as well (McClellan and Chang, 2008). Protein turnover has been found to regulate both ethylene biosynthesis and ethylene response (Shahri and Tahir, 2010). Maintenance of protein levels in STS pulsed spikes suggest protein status to be an the important factor in regulating the ethylene production and enhancing vase life of the cut spikes.

CONCLUSION

The present study suggests that STS pulse treatment (0.5 mM, 1 h) delayed senescence and improved postharvest performance in *N. pseudonarcissus* spikes. The vase solution 8-HQS+SUC is effective in enhancing the longevity in this flower system. Combination of STS pulse treatment and 8-HQS+SUC improved vase life, maintained membrane integrity, fresh and drymass of flowers; besides a higher sugar and protein content in *N. pseudonarcissus*. Therefore STS (0.5 mM, 1 h) pulse treatment followed by transfer to 8-HQS+SUC can be used for prolonging the vase life and improving the postharvest performance in *N. pseudonarcissus*.

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