DOI: 10.15740/HAS/AU/12.TECHSEAR(1)2017/218-223
Agriculture Update
Volume **12** | TECHSEAR-1 | 2017 | 218-223
Visit us : *www.researchjournal.co.in*
e ISSN-0976-6847

RESEARCH ARTICLE :

Allevation of oxidative stress and increase of vase life by exogenous proline in rose (*Rosa hybrida* L. cv. 'MINUPARLE')

PRAGNYASHREE MISHRA

ARTICLE CHRONICLE :
Received :
11.07.2017;
Accepted :
26.07.2017

SUMMARY : The effect of exogenous proline on vase-life of cv. 'MINUPARLE' rose (*Rosa hybrida* L.) was studied. Application of 5mM proline enhanced the vase life of 'Minuparle' roses by 3.5 days by suppressing the oxidative stress. The increase in vase life was associated with higher concentration of endogenous proline and lower levels of superoxide radicals (O_2^-). Proline treated flowers showed was lowest production of O_2^- 1.2-fold (Stage-2), 1.6-fold (Stage-3), and 1.7-fold decline at Stage-4 of flower senescence in comparison to control. Various iso-forms of superoxide dismutase (SOD) were found in senescing rose petals in all the treatments. Proline dehydrogenase (PDH) activity was high in proline treated flowers upto Stage-6 of flower senescence. Higher energy production from proline catabolism helped in delaying the ageing process of flower petals. Reciprocal relationship was observed between GSSG and GSH/GSSG Ratio and higher GSH/GSSG ratios were observed upto Stage-6 in petal of treated flowers in comparisons to control.

KEY WORDS :
Oxidative stress, Rose, Exogenous proline

How to cite this article : Mishra, Pragnyashree (2017). Allevation of oxidative stress and increase of vase life by exogenous proline in rose (*Rosa hybrida* L. cv. 'MINUPARLE'). *Agric. Update*, **12**(TECHSEAR-1) **: 218-223; DOI: 10.15740/HAS/AU/12.TECHSEAR(1)2017/218-223.**

Author for correspondence :
PRAGNYASHREE MISHRA
Department of Floriculture and Landscaping, College of Horticulture, Orissa University of Agriculture and Technology, CHIPLIMA (ODISHA) INDIA
Email:pragnyashree.mishra@gmail.com

BACKGROUND AND OBJECTIVES

Minuparle cultivar of Rose (*Rosa hybrida* L.) is one of the most important cultivar used as cut flower in India. Vase life of a flower is the most important factor while choosing for the cut flower. Effective pre-harvest and postharvest treatments can delay flower senescence and control quality maintenance which are very important for the development of the rose industry. The loss of membrane permeability, increase inoxidative stress and decreased level of antioxidant enzymes leads to the death of petals during flower senescence (Tripathi and Tuteja; 2007). It is important to study the mechanisms of oxidative stress management to understand petal senescence (Gerailoo and Ghasemnezhad, 2011).superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and highly toxic hydroxyl radicals (OH) are the main components of ROS (Danon *et al.*, 2005). ROS are the byproduct of normal metabolic process and generated by membrane bound oxidases, peroxisomes, chloroplast, and

mitochondria (Apel and Hirt, 2004). At early stages of development Flower bud is well protected from the deleterious effectof O_2^- (Kumar *et al.*, 2008a) but at later stages limited antioxidant defense capacity made the flower more susceptibleto ROS (Kumar *et al.*, 2008b). In order to modulate the levels of ROS and associated cellular redox in senescing rose in this investigation exogenous proline is used to avoid cellular ROS toxicity. The α-imino acid proline functions as a potent antioxidant by scavenging intracellular ROS (Chen and Dickman, 2005).

RESOURCES AND METHODS

Collection of flowers :

Flowers were harvested from rosary of College of Horticulture, OUAT ,Chiplima at the commercial stage, *i.e.*, flowers with their outer petal whorl just unfurled, and were re-cut under water to a stem-length of 60 cm, with one pair of leaves. Flowers were kept in distilled water and proline solutions for 1–12 d at 20±2^0 C at a relative humidity of 65±5%. Water and proline solution (1, 2, 4, 5, 8, and 10mM) were refreshed every third day. Analysis has been done during different developmental stages: Stage- 1, commercial stage; Stage-2, flowers 3 d after harvest; Stage-3, flowers 6 d after harvest; Stage-4, flowers 9 d after harvest; Stage- 5, flowers 10 d after harvest; Stage-6, flowers 12 d after harvest. The end of their vase-life was calculated on bluing of petals (Pompodakis and Joyce, 2003). In initial experiment with various proline concentrations (1, 2, 4, 5, 8, and 10mM) determined that 5mM proline enhanced the maximum vase life.

Proline content :

Proline content was determined by the method of Bates (1973) with some modifications using l-proline a standard.

Superoxide radical generation :

The rate of superoxide anion production was measured following the method of Chaitanya and Naithani (1994).

SOD assay :

Superoxide dismutase activity was determined by the method of Dhindsa *et al.* (1981).

PDH assay :

The fresh petals (0.5 g) were homogenized in 50mM Tris–HCl buffer (having 7mM $MgCl_2$, 0.6M KCl, 3mM EDTA, 1mM dithiothreitol, and 5% (w/v) and polyvinylpolypyrrolidone. pH was adjusted to 7.4. The homogenate was filtered and centrifuged at 39,000×g for 20 min. at 4^{0}C (Rosales *et al.*, 2007). The supernatant was used to determine PDH activity. PDH activity was assayed by reduction of NAD^+ (or $NADP^+$) at 340 nm. The reaction mixture contained 0.15mM. Na_2CO_3–HCl buffer, pH 10.3 with 15mM l-proline and 1.5mM NAD^+ or $NADP^+$ (Miller and Stewart, 1976).

Glutathione assays :

Glutathione in its reduced (GSH) and oxidized (GSSG) form were determined according to Smith (1985).

Statistical analysis :

The experiment was designed in Randomized Complete Block Design. All experiments were repeated three times with 15 flowers and three replicates were used for all biochemical estimations. The means per plant were determined and subjected to analysis of variance (SAS Institute, Cary, NC) and separated using a least significant difference (LSD) at $P<0.05$.

OBSERVATIONS AND ANALYSIS

In this experiment continuous pulse of 5mM proline enhanced the vase life of 'Minuparle' rose by 3.4 days. However this dose is taken into the experiment as higher doses such as 8mM and 10mM of proline induced the early senescence in petals and lower doses had non-significant on vase life.

Application of exogenous 5mM proline gradually increased the endogenous levels of proline level in the petal. The proline concentration in the petal increased1.4-fold, 1.2-fold and 2.8-fold at Stages 2, 3, and 4, respectively. Highest proline concentration was observed at Stage-5 in petals of treated flowers (Table 1).

Levels of O_2^- production were steady in bothtreated and control flowers (Table 2). The maximum generation of O_2^- was recorded at Stage-3 in petals of both flowers. Low O_2^- production was observed in proline treated flowersand showed 1.2-fold (Stage-2), 1.6-fold (Stage-3), and 1.7-fold decline at Stage-4 of flower senescence in comparison to control flowers.

Table 1 : Effect of exogenous proline on endogenous proline concentration (µmol g^{-1} Fw)

Treatments	Stages						Mean
	S_1	S_2	S_3	S_4	S_5	S_6	
Control	0.24	0.29	0.78	0. 39	-	-	0.43
5mM proline	0. 26	0.39	0.93	1.06	1. 27	1.23	0.68

Table 2 : Effects of exogenous proline on superoxide production (ΔA540 min^{-1} mg^{-1} protein)

Treatments	Stages						Mean
	S_1	S_2	S_3	S_4	S_5	S_6	
Control	0.44	1.2	3.18	1.84	-	-	1.67
5mM proline	0.57 NS	0.99 NS	2.03	1.06	1.27	0.22 NS	1.02

NS=Non-significant

Total SOD activity declined at successive stages of petal senescence in both treatments (Table 3). The decline of total SOD was more pronounced (6.9-fold) in the petals of control flowers and appeared to be moderate (1.4-fold) in treated flowers during different stages. Consistently higher SOD activity was maintained in petals of treated flowers than control during the vase life.

Higher PDH activity was observed in proline treated flowers in comparison to control (Table 4). 1.2-fold at Stage-3 and 5.0-fold at Stage-4 rise in PDH activity has been seen. PDH activity was observed upto Stage-6 in treated flowers.

Reduced glutathione content increased upto Stage-3 then declined in both treatments (Table 5). However, the decline was more pronounced in control. Three fold decline was observed in between Stage-3 and Stage-4. GSH content remained higher in petals of treated flowers in comparison to control.GSH content was 1.1-fold, 1.1-fold and 1.3-fold higher in Stage-2, Stage-3 and Stage-4, respectively in proline treated flowers than control flowers of same age. After wards GSH content declined in treated flowers drastically. Oxidized glutathione content was higher in control flowers at all the stages during senescence. The GSSG content was highest at Stage-3 in control treatment.

Reciprocal relationship was observed between GSSG and GSH/GSSG in the flowers in all the treatments.GSH/GSSG ratio decreased in flowers of both treatments from Stage-2 onward. The decline in GSH/GSSG ratio was more in control. Upto Stage-6 GSH/GSSG ratios was at its higher levelin petals of treated flowers than control.

Proline is a compatible osmolyte with respect to its ability to scavenge free radicals (Rontein *et al.*, 2002 and Matysik *et al.*, 2002). Flower senescence in 'Minuparle' rose is associated with huge amount production of free radical (Kumar *et al.*, 2007). Exogenous 5mM proline application enhanced the level of endogenousproline gradually in petals and simultaneously slowed the senescence. Therefore it enhanced the longevity of this cultivar by 3.4 days. This increment in vase life was mainly governed by reduced O_2^- generation (1.2-fold, Stage-2; 1.6-fold, Stage-3; and 1.7-fold, Stage-4).

Physical quenching of singlet oxygen and chemical reactions with hydroxyl radicals are the main mechanism for which proline reduces free radicall damage (Mohanty

Table 3 : Effects of exogenous proline on total SOD (units min^{-1} mg^{-1} protein)

Treatments	Stages						Mean
	S_1	S_2	S_3	S_4	S_5	S_6	
Control	5.08	3.62	2.17	0.735	-	-	2.90
5mM proline	4.51 NS	3.8 NS	3.48	2.68	2.55	0.32 NS	2.85

NS=Non-significant

Table 4 : Effects of exogenous proline on Proline dehydrogenase (µmol NAD(P)+ reduced mg^{-1} protein min^{-1})

Treatments	Stages						Mean
	S_1	S_2	S_3	S_4	S_5	S_6	
Control	0.19	0.23	0.38	0.06	-	-	0.21
5mM proline	0.16 NS	0.3	0.44	0.35	0.3	0.13	0.26

NS=Non-significant

Table 5 : Effects of exogenous proline on GSH (μmol g^{-1}Fw), GSSG (μmol g^{-1}Fw) and GSH/GSSG

Treatments	Stages						Mean
	S_1	S_2	S_3	S_4	S_5	S_6	
GSH							
Control	0.58	0.64	0.78	0.28	-	-	0.57
5mM proline	0.55 NS	0.73	0.84	0.36	0.28	0.23	0.49
GSSG							
Control	0.27	0.24	0.57	0.28	-	-	0.34
5mM proline	0.26NS	0.21	0.44	0.2	0.22 NS	0.25	0.26
GSH/GSSG							
Control	2.07	2.74	1.35	1.01	-	-	1.79
5mM proline	2.1 NS	3.61	1.94	1.43	1.47	0.88	1.91

LSD at P = 0.05 for total SOD (S: 1.12; T: 0.91; S×T: 0.82)
Stage-1:commercial stage; Stage-2: flowers 3 d after harvest; Stage-3: flowers 6 d after harvest; Stage-4: flowers 9 d after harvest; Stage-5:flowers 10 d after harvest; Stage-6: flowers 12 d after harvest and X vase life terminated.
ns: values that are significantly different from the controls (means separated using LSD at P = 0.05).

and Matysik, 2001). Due to low ionization potential of proline, itis capable to forma reversible charge-transfer complex with singlet oxygen and effectivelyquenches this ROS (Mohanty *et al.*, 2002). According to Chen and Dickman (2005) an exogenous application of 1.6mM proline inhibit the ROS induced programmed cell death in dominant active Ras mutant of phytopathogenic fungus *Colletotricuhm trifolii*. It can be concluded from these that proline acts as a potent antioxidant against ROS.

Total SOD activity was higher in treated flower which might suppressed the rate of O_2^- generation. SOD constitutesthe first line of defense against ROS and changes in its activity and amount have been identified as indicator of oxidative stress (Bowler *et al.*, 1992). Total SOD activity decline in bothtreated and control flowers with ageing. Decline in total SOD activity may be regulated by developmental physiology. SOD genes are regulated by developmental physiology (Kurepa *et al.*, 1997). Total SOD activity was higher and sustained for a longer duration (3.4 more days) in treated flowers in comparison to the short lived (8.5 days) control. This observation can be justified by prolinemediated increase in total SOD activity. Similar observations were also recorded by Hua and Guo (2002) and Yan *et al.* (2000) in stress condition.

Proline induced higher PDH activity was observed in treated flowers and that continued upto Stage-6 of flower senescence. According to Verbruggen *et al.* (1996) exogenous proline induced the expression of At-PDH gene in Arabidopsis thaliana and it is correlated with higher levels of free proline. Low availability of energy in Flower petals of tulip during flower bud opening leads to petalsenescence (Azad *et al.*, 2008). Under these conditions higher PDH activity seems to be beneficial in terms of energy output and thus contributes to vase life of flowers (Hare and Cress, 1997).

GSH is present in plant cells in millimolar concentration and regarded as a major determinant of cellularredox (Mullineaux and Rausch, 2005). In this case accumulation of higher endogenous proline in petals appeared to be an effective strategy topreserve the glutathione mediated redox of the cell by direct scavenging of the ROS (Krishnan *et al.*, 2008). Higher levels ofAccording to Ghezzi *et al.* (2005) GSH might sustain the activity of GSH dependent enzymesof antioxidant defense system such as glutathione peroxidase, *Glutathion ereductase*, and glutathione-s-tranferase to scavenge ROS and therefore, primed a more reducing environment which was evident from the lower pool of GSSG in treated flowers.

Many reports showed external application of proline is toxic to plants (Deuschle *et al.*, 2001). Proline degradation is mediated by PDH.Excess proline supply induced the activity of PDH (Miller *et al.*, 2009) but the activity of P5CDH remains unchanged (Forlani *et al.*, 2000) during proline degradation. SoP5C get accumulated in mitochondria due to variability in enzymes activities (Miller *et al.*, 2009). Under suchconditions excess of P5C is transported to the cytoplasm andre-converted to proline by the action of P5CR (α1-pyrroline-5-carboxylate reductase) and transported back to mitochondria (Miller *et al.*, 2009). Enhanced P5C-proline

cycling increases thelevel of electron flow through PDH to mitochondria and molecularoxygen and caused the generation of ROS (Miller *et al.*, 2009) which seems to be a possible cause of early petal senescence inflowers treated with higher level (8mM and 10mM) of prolinepulse.

Conclusion :

Exogenous proline (5mM) is capable of suppressing the oxidative stress and enhanced the vase life of 'Minuparle' roses. Lower level of superoxide radicals were maintained throughout the senescence process by higher activity of SOD and indirectly byhigher levels of endogenous proline. Larger GSH pool and lower GSSG content further ameliorate the oxidative stress in roses. From this investigation I can conclude that for longer vase life of roses use of proline can be commercialized.

REFERENCES

Apel, K. and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress and signal transduction. *Annu. Rev. Plant Biol.*, **55** : 373–399.

Azad, A., Ishikawa, T., Sawa, Y. and Shibata, H. (2008). Intracellular energy depletion triggers programmed cell death during petal senescence in tulip. *J. Exp. Bot.*, **59** : 2085–2095.

Bates, L.S. (1973). Rapid determination of free proline for water stress studies. *Plant Soil.*, **39** : 205–207.

Bowler, C.H., Van Montagu, M. and Inze, D. (1992). Superoxide dismutase and stress tolerance. Annu. Rev. *Plant Physiol. Plant Mol. Biol.*, **43** : 83–116.

Chaitanya, K.S.K. and Naithani, S.C. (1994). Role of superoxide lipid peroxidation and superoxide dismutase in membrane perturbation during loss of viability in seedsof Shorearobusta Farten. *F. New Phytol.*, **126** : 623–627.

Chen, C. and Dickman, M.B. (2005). Proline suppresses apoptosis in the fungal pathogen of Colletotrichumtrifolii. *Proc. Natl. Acad. Sci. U.S.A.*,**102** : 3459–3464.

Danon, A., Miersch, O., Felix, G., Camp, R.G.L. and Apel, K. (2005). Concurrent activation of cell death regulating signaling pathways by singlet oxygen in *Arabidopsisthaliana*. *Plant J.*, **41** : 68–80.

Deuschle, K., Funck, D., Hellman, H., Daschner, K., Binder, S. and Frommer, W.B. (2001). A nuclear gene encoding mitochondrial _1-pyrroline-5-carboxylate dehydrogenase and its potential role in protection from proline toxicity. *Plant J.*, **27** : 345–355.

Dhindsa, R.A.P., Plumb-Dhindsa, P. and Thorpe, T.A. (1981). Leaf senescence: correlated with increased permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.*, **126** : 93–101.

Forlani, G., Mangiagalli, A., Pinter, C. and Nielsen, E. (2000). Expression of _1-pyrroline- 5-carboxylate dehydrogenase and proline/arginine homeostasis in Solanumtuberosum. *Physiol. Plant.*, **110** : 22–27.

Gerailoo, S. and Ghasemnezhad, M. (2011). Effect of salicylic acid on antioxidant enzyme activity and petal senescence in 'Yellow Island' cut rose flowers. *J. Fruit & Ornamental Plant Res.*, **19** : 183-193.

Ghezzi, P., Bonetto, V. and Fratelli, M. (2005). Thiol-disulfide: balance from the concept of oxidative stress to that of redox regulation. *Antioxid. Redox Signal*, **7** : 964–972.

Hare, P.D. and Cress, W.A. (1997). Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regul.*, **21** : 79–102.

Hua, B. and Guo, W.Y. (2002). Effect of exogenous proline on SOD and POD activity of soybean callus under salt stress. *Acta Agric. Boreali-Sinica*, **17** : 37–40.

Kumar, N., Srivastava, G.C. and Kiran, D. (2008a). Senescence in rose (Rosa hybrida L.): role of endogenous antioxidant system. *J. Hort. Sci. Biotechnol.*, **83** : 125–131.

Kumar, N., Srivastava, G.C. and Kiran, D. (2008b). Antioxidant metabolism during rose (Rosa hybrida L.) petal senescence. *J. Hort. Sci. Biotechnol.*, **83** : 755–759.

Kumar, Naveen, Srivastava, Girish Chand and Dixit, Kiran (2007). Flower bud opening and senescence in roses (*Rosa hybrida* L.). *Plant Growth Regulation*, **55** : 81.

Krishnan, N., Dickman, M.B. and Becker, D.F. (2008). Proline modulates the intracellular redox environment and protects mammalian cells against oxidative stress. *Free Radic. Bio. Med.*, **15** : 671–681.

Kurepa, J.D., Herourepa, D., Van Montagu, M. and Inze, D. (1997). Differential expressionof Cu–Zn and Fe-superoxide dismutase genes of tobacco during development, oxidative stress, and hormonal treatments. *Plant Cell Physiol.*, **38** : 463–470.

Matysik, J., Bhalu, Alia.,Mohanty, B., Atysik, P., Alia, J., Bhalu, B. and Mohanty, P. (2002). Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr. Sci.*, **82** : 525–532.

Miller, G., Honig, A., Stein, H., Suxuki, N., Mittler, R., Zilberstein, A. (2009). Unraveling _1-pyrroline-5-carboxylate-proline cycle in plants by uncoupled expression of proline oxidation enzymes.

J. Biol. Chem., **284** : 26482–26492.

Miller, P.M. and Stewart, C.R. (1976). Pyrroline-5-carboxylic acid reductase from soybean leaves. *Phytochem.*, **15** : 1855–1857.

Mohanty, Alia and Matysik, J. (2001). Effect of proline on the production of singlet oxygen. *Amino Acids*, **21**(2):195-200.

Mohanty, C.R., Mishra, N.K. and Patnaik, P. (2002). Effect of nitrogen and phosphorus on flower production in marigold. *J. Ornamental Hort.*, **5** (1) : 67-68

Mullineaux, P.M. and Rausch, T. (2005). Glutathione, photosynthesis and the redox regulation of stress-responsive gene expression. *Photosynth. Res.*, **86** : 459–474.

Pompodakis, N.E. and Joyce, D.C. (2003).ABA analogue effects on vase life and leaf crisping of cut 'Baccara' roses. *Australia J. Agric. Sci.*, **43** : 425–428.

Rontein, D., Basset, G. and Hanson, A.D. (2002). Metabolic engineering of osmo-protectant accumulation in plants. *Metab. Eng.*, **4** : 49–56.

Rosales, M.A., Rios, J.J., Castellano, R., Lopez-Carrion, A.I. and Ruiz, I.M. (2007). Proline metabolism in cherry tomato exocarp in relation to temperature and solar radiation. *J. Hort. Sci. Biotechnol*., **82** : 739–744.

Smith, I.K. (1985). Stimulation of glutathione synthesis in photorespiring plants by catalase inhibitors. *Plant Physiol*., **79** : 1044–1047.

Tripathi, S.K. and Tuteja, N. (2007). Integrated signaling in flower senescence: An overview. *Plant Signal Behavior*., **2** : 437-445.

Verbruggen, N., Hua, X.-J., May, M. and Van Montagu, M. (1996). Environmental and developmental signals modulate proline homeostasis: evidence for a negative transcriptional regulator. *Proc. Natl. Acad. Sci. U.S.A.*, **93** : 8787–8791.

Yan, H., gang, L.Z., Zhao, C.Y. and Guo, W.Y. (2000). Effects of exogenous proline on the physiology of soybean plantlets regenerated from embryos in vitro and on the ultrastructure of their mitochondria under NaCl stress. *Soybean Sci*., **19** : 314–319.