



Research Journal of
Botany

ISSN 1816-4919



Academic
Journals Inc.

www.academicjournals.com

Effect of Ethylene Antagonists (STS and AOA) on Postharvest Senescence of *Ranunculus asiaticus* L. Flowers

Waseem Shahri, Inayatullah Tahir, Sheikh Tajamul Islam and Mushtaq Ahmad Bhat
Plant Physiology and Biochemistry Research Laboratory, Department of Botany, University of Kashmir,
Srinagar, 190006, India

Corresponding Author: Waseem Shahri, Plant Physiology and Biochemistry Research Laboratory, Department of Botany, University of Kashmir, Srinagar, 190006, India

ABSTRACT

The present study was conducted on isolated flowers of *Ranunculus asiaticus* L. to assess the effect of different ethylene antagonists: Silver Thiosulphate (STS) and Aminooxyacetic Acid (AOA) on the postharvest performance. Uniform floral buds (at half open stage) of *R. asiaticus* were pulse treated for 1 h with different concentrations of STS and AOA (0.25 and 0.5 mM) and transferred to Distilled Water (DW) to compare the treatment effects. One set of buds was kept unpulsed which represented control. Pretreatment of flowers with either STS or AOA at any concentration was not found to be effective in delaying senescence and enhancing the postharvest life. It is therefore, suggested that ethylene plays little or no role in senescence in isolated flowers of *R. asiaticus*. The overall strategy of petal senescence in *R. asiaticus* is initial wilting followed by ethylene-independent abscission at later stages. The study also recommends that ethylene sensitivity and patterns of flower senescence should not be assigned to plants at the family level on the basis of response of a few representative members.

Key words: Abscission, aminooxyacetic acid, fresh and dry mass, *Ranunculus asiaticus*, silver thiosulphate, soluble proteins

INTRODUCTION

Senescence is an integral part of the normal developmental cycle of plants and can be viewed on a cell, tissue, organ or organization level. It is the final event in the life of many plant tissues and is highly regulated process that involves structural, biochemical and molecular changes that in many cases bear the hallmarks of programmed cell death, PCD (Da Silva, 2003; Shahri, 2011; Islam *et al.*, 2011). Ethylene is a plant hormone that accelerates the aging process leading to a shortened vase life in several flowers (Woltering and van Doorn, 1988; Chutichudet *et al.*, 2011). Flower senescence varies widely between plant genera; therefore a number of senescence parameters have been used to group plants into some arbitrary categories. One distinction is made on the basis of response of flowers to ethylene resulting in the recognition of ethylene-sensitive (*Consolida*, *Dianthus*, *Ipomoea*, *Petunia*) and ethylene-insensitive (*Hemerocallis*, *Iris*, *Narcissus*) flower systems (Lukaszewski and Reid, 1989; Lay-Yee *et al.*, 1992; Van Staden, 1995; Voleti *et al.*, 2000; Finger *et al.*, 2004; Tassoni *et al.*, 2006; Zuliana *et al.*, 2008). The other distinction is made on the basis of whether perianth (sepals/petals) wilts or undergoes abscission or even wilting followed by abscission (Stead and van Doorn, 1994; Van Doorn and Stead, 1994). On the basis of how flowers respond to ethylene (Shahri and Tahir, 2011) has categorized

sepal/petal senescence into five different patterns. The flowers belonging to family Ranunculaceae have been defined to be highly sensitive to ethylene (Woltering and van Doorn, 1988). In order to check the ethylene-sensitivity of a flower system, two general strategies are employed; one involves the application of exogenous ethylene while the other involves the use of ethylene antagonists (STS, AOA and MCP) either as pulse treatment or continuous supply.

Ranunculus asiaticus L. commonly known as 'butter cup' blooms from April to June in Kashmir. It possesses dark red terminal flowers with a cluster of brownish anthers at centre surrounding the carpel. It is widely grown as a garden plant, cut flower and flowering potted plants. Only scanty information is available on the studies related to senescence and improvement of *Ranunculus asiaticus* as a cut flower crop (Kenza *et al.*, 2000; Dole *et al.*, 2005). The present study was undertaken to investigate the effect of pretreatment with different concentrations of STS and AOA before transfer to Distilled Water (DW) on senescence with the ultimate aim to develop strategies to improve its postharvest performance.

MATERIALS AND METHODS

Isolated flowers of *Ranunculus asiaticus* L. growing in open in the Kashmir University Botanic Garden (KUBG) were used for the present study (2010). The flowers were harvested at 0800 h at half-open stage. The harvested flowers were immediately brought to the laboratory, cut to a uniform size of 15 cm and pulse treated for 1 h separately in different concentrations of STS (0.25 and 0.5 mM) and AOA (0.25 and 0.5 mM). After pulse treatment the pedicle ends were washed with distilled water thrice. In each case two flowers were transferred to 100 mL Ehrlenmeyer flasks containing 75 mL of Distilled Water (DW). A separate set of five flasks each containing untreated flowers represented control. Overall there were 5 treatments including control. Treatment effects were evaluated by keeping the flowers in the laboratory at a temperature of $25\pm 2^{\circ}\text{C}$ under cool white fluorescent light with a mix of diffused natural light (10 W m^{-2}) 12 h a day and RH of $60\pm 10\%$. The day of harvest was designated as day zero. The average vase life of the flowers was counted from the day of transfer of flowers to holding solutions and was assessed to be terminated when flowers lost their ornamental/display value (underwent colour change; wilt and loose turgidity). The volume of holding solution absorbed by the buds 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; taking into account the volume of solutions evaporated by using blank flasks in triplicate (containing particular vase solutions without buds) alongside the flasks with buds. Conductivity of leachates from petal samples, diameter, fresh and dry mass of the flowers was determined on 3rd and 6th day of harvest (transfer of buds to distilled water). Dry mass was determined by drying the material in an oven for 48 h at 70°C . The changes in membrane permeability were estimated by measuring the electrical conductivity of leachates (μS) of petal discs (5 mm in diameter) incubated in dark in 15 mL glass distilled water for 15 h at 20°C . Proteins were extracted from 1g petal 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 (PVP) and centrifuged. Proteins were precipitated from a suitable volume of the cleared supernatant with equal volume of 20% Trichloroacetic Acid (TCA), 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. 0.5 mM STS was prepared by mixing equal volumes of 1mM silver nitrate (AgNO_3) and 4 mM sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$).

Statistical analysis: Each value represents the mean of six independent replicates. The data has been analyzed statistically and LSD computed at $p = 0.05$ using Minitab Software.

RESULTS AND DISCUSSION

All the mature buds opened on the subsequent day of transfer to DW irrespective of the treatment. The flower senescence under laboratory conditions was comparable to that under field conditions. The average life of an individual flower after it opens fully was about 5 days. Flower senescence was characterized by loss of turgor in petals and change in petal colour from dark red to brick red. Finally the petals wilted and drop when slightly touched. It is therefore, evident that the initial symptom of senescence was wilting of petals and abscission occurred much later. Pretreatment of flowers with either STS or AOA before transfer to DW did not seem to have any effect on overall postharvest performance. Vase life of pretreated flowers was comparable to that of untreated flowers and no significant difference was observed (Table 1). Inefficiency of the ethylene antagonists to increase vase life of cut flowers of *R. asiaticus* strongly points out that ethylene play little or no role in senescence. Pretreatment of flowers with 0.5 mM STS or AOA resulted in a slight increase in fresh and dry mass as compared to control. Fresh and dry mass of flowers registered a decrease with the progression in time from day 3 to 6 of transfer to DW. However, the decrease was least in flowers pretreated with 0.5 mM AOA (Table 1). Maintenance of high fresh and dry mass by AOA has been attributed to the fact that it is effective in reducing respiration in cut flowers (Bichara and Staden, 1993; Uda *et al.*, 1997). Pretreatment of flowers with different concentrations of AOA resulted in a decrease in floral diameter while as STS treated flowers registered an increase in floral diameter as compared to control. Floral diameter registered a decrease with the progression in time from 3rd to 6th day of transfer, in case of control but an increase was registered in flowers pretreated with STS or AOA. The increase was higher in flowers pretreated with STS (Table 2). Silver in flower preservative solutions has been shown to be effective in preventing the bacterial growth, maintaining low water potential in cells and promoting solution uptake thereby leading to opening of flowers (Kofranek and Paul, 1975). The electrical conductivity of ion leachates from petal discs did not show any significant difference between treatments (Table 2). Volume of holding solution absorbed was more in flowers pretreated with STS as

Table 1: Effect of pretreatment with different concentrations of silver thiosulphate (STS) and aminoxyacetic acid (AOA) for 1 h and subsequent transfer to distilled water (DW) on longevity, fresh mass and dry mass at day 3 and 6 (D3 and D6) of transfer in isolated flowers of *Ranunculus asiaticus* L.

Treatment	Vase life (days)	Days after transfer			
		Fresh mass (g flower ⁻¹)		Dry mass (g flower ⁻¹)	
		D3	D6	D3	D6
DW (control)	5.30	1.48	1.10	0.17	0.14
0.25 mM STS	5.00	1.38	1.10	0.15	0.14
0.5 mM STS	5.40	1.56	1.18	0.17	0.15
0.25 mM AOA	5.00	1.45	1.14	0.17	0.14
0.5 mM AOA	5.60	1.58	1.25	0.18	0.15
LSD at p0.05	0.23	0.01	0.02	0.001	0.003

Each value is a mean of 6 independent replicates. Room temperature = 15±2°C. Fresh and dry mass of flowers registered a decrease with the progression in time from day 3 to 6 of transfer to DW

Table 2: Effect of pretreatment with different concentrations of silver thiosulphate (STS) and aminoxyacetic acid (AOA) for 1 h and subsequent transfer to distilled water (DW) on floral diameter, conductivity of leachates, volume of holding solution absorbed and soluble proteins from petal tissues at day 3 and day 6 (D3 and D6) of transfer in isolated flowers of *Ranunculus asiaticus* L.

Treatment	Days after transfer							
	Floral diameter (cm)		Conductivity of leachates (μ S)		Volume of holding solution absorbed (mL)		Soluble proteins (mg g^{-1} fm)	
	D3	D6	D3	D6	D3	D6	D3	D6
DW (control)	5.36	4.98	4.30	7.00	5.50	8.50	2.87	1.25
0.25 mM STS	5.43	5.85	4.50	7.00	6.50	8.00	2.16	1.06
0.5 mM STS	5.50	6.05	4.50	7.00	6.50	8.00	2.46	1.19
0.25 mM AOA	5.20	5.55	5.00	6.00	5.00	7.50	2.70	1.08
0.5 mM AOA	5.22	5.82	4.00	6.00	5.20	7.50	2.70	1.19
LSD at $p_{0.05}$	0.08	0.08	0.19	0.13	0.16	0.27	0.02	0.08

Each value is a mean of 6 independent replicates. Room temperature = $15\pm 2^\circ\text{C}$

compared to that of AOA and control. There was no significant difference in volume of holding solution absorbed between control and flowers treated with AOA. It increased with the progression in time from day 3 to 6 of transfer irrespective of the treatment (Table 2). A higher content of soluble proteins was recorded in samples from flowers kept untreated (control) as compared to that of samples from flowers pretreated with either STS or AOA. The soluble protein content registered a general decrease with the progression in time from day 3 to 6 of transfer irrespective of the treatment (Table 2). Protein turnover has been found to regulate both ethylene biosynthesis and ethylene response. Recently it has been found that ethylene receptors are controlled by protein turnover as well (McClellan and Chang, 2008). In the present study, no such effects were observed suggesting that flower senescence in *R. asiaticus* flowers is independent of ethylene as ethylene antagonists were found to have no effect on the overall postharvest performance.

CONCLUSIONS

The present results suggest that the family Ranunculaceae, even though considered to be an ethylene-sensitive family, consists of an ethylene-insensitive member (*R. asiaticus* L.) in which the initial symptom of senescence is petal wilting rather than abscission. The overall strategy of petal senescence in *R. asiaticus* is initial wilting followed by ethylene-independent abscission at later stages.

ACKNOWLEDGMENTS

The authors thank Head Department of Botany for providing facilities. Waseem Shahri thanks UGC (University Grants Commission, India) for providing Junior Research fellowship. We also acknowledge Prof. A.Q. John (Professor Emeritus, SKUAST) for cultivar identification.

REFERENCES

- Bichara, A.E. and J. Staden, 1993. The effect of aminoxyacetic acid and cytokinin combinations on carnation flower longevity. *Plant Growth Regul.*, 13: 161-167.
- Chutichudet, P., B. Chutichudet and K. Boontiang, 2011. Influence of 1-MCP fumigation on flowering weight loss, water uptake, longevity, anthocyanin content and colour of patumma (*Curcuma alismatifolia*) cv. chiang mai pink. *Int. J. Agric. Res.*, 6: 29-39.

- Da Silva, J.A.T., 2003. The cut flower: Postharvest considerations. *J. Biol. Sci.*, 3: 406-442.
- Dole, J.M., W.C. Fonteno and S.L. Blankenship, 2005. Comparison of silver thiosulphate with 1-Methyl cyclopropene on 19 cut flower taxa. *Acta Hort.*, 682: 249-256.
- Finger, F.L., T.F. Carneiro and J.G. Barbosa, 2004. Post-harvest senescence of inflorescencias of esporinha (*Consolida ajacis*). *Brasilia*, 39: 533-537.
- Islam, S.T., I. Tahir, W. Shahri and M.A. Bhat, 2011. Effect of Cycloheximide on Senescence and Postharvest Performance in *Hemerocallis fulva* cv. Royal Crown. *J. Plant Sci.*, 6: 14-25.
- Kenza, M., N. Umeil and A. Borochoy, 2000. The involvement of ethylene in the senescence of ranunculus cut flowers. *Postharvest Biol. Technol.*, 19: 287-290.
- Kofranek, A.M. and J.L. Paul, 1975. The value of impregnating cut stems with high concentrations of silver nitrate. *Acta Hort.*, 41: 199-206.
- Lay-Yee, M., A.D. Stead and M.S. Reid, 1992. Flower senescence in daylily (*Hemerocallis*). *Physiol. Plant.*, 86: 308-314.
- Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
- Lukaszewski, T.A. and M.S. Reid, 1989. Bulb type flower senescence. *Acta. Horticult.*, 261: 59-62.
- McClellan, C.M. and C. Chang, 2008. The role of protein turnover in ethylene biosynthesis and response. *Plant Sci.*, 175: 24-31.
- Shahri, W. and I. Tahir, 2011. Flower senescence: Strategies and some associated events. *Bot. Rev.*,
- Shahri, W., 2011. Senescence: Concepts and synonyms. *Asian J. Plant Sci.*, 10: 24-28.
- Stead, A.D. and W.G. van Doorn, 1994. Strategies of Flower Senescence-A Review. In: *Society for Experimental Biology Seminar Series 55: Molecular and Cellular Aspects of Plant Reproduction*, Scott, R.J. and A.D. Stead (Eds.). Cambridge University Press, Cambridge, pp: 215-237.
- Tassoni, A., P. Accettulli and N. Bagni, 2006. Exogenous spermidine delays senescence of *Dianthus caryophyllus* flowers. *Plant Biosyst.*, 140: 107-114.
- Uda, A., M. Yamanaka, K. Fukushima and Y. Koyama, 1997. Effects of various concentrations and duration of treatment with silver thiosulphate (STS) on Ag absorption, distribution and the vase life of cut carnations. *J. Jpn. Soc. Hortic. Sci.*, 64: 927-933.
- Van Doorn, W.G. and A.D. Stead, 1994. The Physiology of Petal Senescence which is not Initiated by Ethylene. In: *Molecular and Cellular Aspects of Plant Reproduction*, Scott, R.J. and A.D. Stead (Eds.). Cambridge University Press, Cambridge, pp: 239-254.
- Van Staden, J., 1995. Hormonal control of carnation flower senescence. *Acta Hort.*, 405: 232-239.
- Voleti, S.R., V.P. Singh, A. Arora, N. Singh and S.R. Kushwaha, 2000. Physiology of Flower Senescence in Floriculture Crops. In: *Advances in Plant Physiology*, Hemantaranjan, A. (Ed.). Scientific Publishers, India, Jodhpur, pp: 423-439.
- Woltering, E.J. and W.G. van Doorn, 1988. Role of ethylene in senescence of petals-morphological and taxonomic relationships. *J. Exp. Bot.*, 39: 1605-1616.
- Zuliana, R., A.N. Boyce, H. Nair and S. Chandran, 2008. Effects of aminooxyacetic acid and sugar on the longevity of pollinated *Dendrobium pompadour*. *Asian J. Plant Sci.*, 7: 654-659.