International Journal of Botany Studies
ISSN: 2455-541X
Impact Factor: RJIF 5.12
www.botanyjournals.com
Volume 3; Issue 3; May 2018; Page No. 71-77

Salicylic acid extends flower longevity in isolated flowers of *Petunia hybrida* Vilm

Shaziya Nisar, Riyaz Ahmad Dar, Inayatullah Tahir*
Department of Botany, University of Kashmir, Srinagar, Jammu & Kashmir, India

Abstract
An experiment was conducted to study the effect of different grades of salicylic acid (SA) on flower longevity of *Petunia hybrida* Vilm. The buds were subjected to different grades (0.10, 0.15, 0.20 and 0.25 mM respectively) of SA. A separate set of flowers kept in distilled water designated the control. As compared to distilled water (DW) as the control, the greatest delay in petals senescence was obtained in cut flowers treated with 0.25 mM SA. During the present study flower longevity increased by 3.7 days in the above mentioned treatment. The other parameters such as flower diameter, membrane stability index (MSI), soluble proteins, total phenols, α-amino acids and sugar fractions (total, reducing and non-reducing) were also greater in the floral buds treated with 0.25 mM SA. However, lipid peroxidase activity was suppressed in the floral buds held in 0.25 mM SA. Overall, the results suggest that SA can increase the vase life of *P. hybrida* isolated flowers by maintaining the MSI and soluble protein content during senescence.

Keywords: flower longevity, soluble protein, senescence

Introduction
Flower senescence is accompanied by many physiological, biochemical and molecular changes. It is a major issue regarding the postharvest handling of cut flowers. Petal senescence is the final stage of display life that follows the physiological maturity, ultimately leading to the death of cell, organ or the whole plant. Flower longevity and quality are requisites for the cut flower marketing which ensure that the consumers will be satisfied and will return back to procure more flowers. Commercially, the customers need a guarantee for potential decorative life of cut flowers (Stead, 2004; Bhattacharjee and De, 2005; Gebremedhin *et al*., 2013; Saeed *et al*., 2016) [20, 3, 8, 18]. The onset of petal senescence is triggered by an array of events orchestrated by plant growth regulators including ethylene, ABA, auxins, cytokinins, polyamines, jasmonic acid, salicylic acid and brassinosteriods (Rubinstein, 2000; Rogers, 2006; van Doorn and Woltering, 2008; Ichimura *et al*., 2009; Schaller, 2012; Saeed *et al*., 2016) [17, 16, 21 18].
Salicylic acid (SA) has been extensively studied both as a plant growth regulator and for its potential role in the maintenance of postharvest quality of vegetable, fruits and cut flowers (Ezhilmathiet *et al*., 2007) [6, 7]. It belongs to a plant phenolics group, widely distributed within plants and plays a vital role in plant growth metabolism and developmental phenomena. It has also been given more attention for the last two decades due its capability to persuade systemic acquired resistance (SAR) both for biotic and abiotic stress and signaling mechanism in plants (Rejeb *et al*., 2014) [15]. Salicylic acid influences cut flower quality and longevity and involved in the regulation of several physiological processes. Its exogenous application may influence ion uptake, stomatal conductance and transpiration (Eraslan *et al*., 2007) [5], and reduce the postharvest water loss (Hassan *et al*., 2007) [11]. It also has stimulatory effect on flowering and acts as endogenous regulator for flower induction (Pacheco *et al*., 2013) [14].
During the present study, we investigated the effect of SA treatment on the flower longevity of isolated flowers of *Petunia hybrida*, as well as physiological and biochemical changes during its petal senescence.

Materials and Methods
Isolated floral buds of *Petunia hybrida* were collected at 800 h when the buds were at stage III (one day before anthesis) from Kashmir University Botanic Garden (KUBG). These harvested floral buds were brought to the laboratory, cut to a uniform pedicel length of 3 cm and divided into five sets each set comprising of 25 vials with 5 ml of respective test solutions. Each set was supplied with various grades of SA viz. 0.10 mM, 0.15 mM, 0.20 mM and 0.25 mM. A separate set of 25 vials containing buds held in distilled water (DW) designated the control. The experiment was conducted under laboratory conditions with relative humidity (RH) of 60±10%, 12 h light period a day and average temperature of 20±2 ºC. The day of transfer of isolated flowers to test solutions was designated as day zero.

Flower longevity
Longevity of isolated flowers was measured as the time taken in days by an open flower to show visible signs of senescence like inrolling of petals and loss of turgidity.

Floral diameter
Diameter of 10 flowers from each treatment was recorded on day 2 and 5 of transfer to different concentrations of salicylic acid and was taken as mean of two perpendicular measurements of the flower.

Membrane stability index (MSI)

Solute leakage of the petal tissues was calculated by incubating 250 mg of petal tissue in 5 mL of deionized water at 25 °C for 30 minutes and 100 °C for 15 minutes (Sairam, 1994). The conductivity of the samples incubated at 25 °C was designated as C1 and those incubated at 100 °C was designated as C2 after recording the values on Elico CM180 Conductivity meter. MSI was computed as:

$$MSI = \left[1 - \frac{C1}{C2}\right] \times 100$$

Lipid peroxidation

LPO activity, expressed as TBARS content, was determined by the method of Heath and Packer (1968). 0.5 g of petal tissue was macerated in 15 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 15,000xg for 10 min under refrigeration. 1 mL of supernatant was taken and mixed with 4 mL of 0.5% TBA diluted in TCA (20%). The reaction was started by incubating the mixture at 95 °C in water bath for 25 min and ended by placing the reaction mixture in ice. Absorbance was taken at 532 and 600 nm. Non-specific absorbance was subtracted from the value obtained at 532 nm.

Estimation of tissue constituents

Soluble proteins were estimated by the method of Lowry *et al*. (1951) using BSA as standard.

α-amino acids were estimated by the method of Rosen (1957) using glycine as the standard.

Total phenols were estimated by the method of Swain and Hillis (1969) using gallic acid as standard. Reducing sugars were estimated by the method of Nelson (1944) using D-Glucose as the standard. Total sugars were estimated after the enzymatic conversion of non-reducing sugars into reducing sugars by invertase. Non-reducing sugars were calculated as the difference between total sugars and reducing sugars.

The data were analyzed statistically and LSD computed at P0.05 using SPSS software.

Results

Flower senescence in *Petunia hybrida* was characterised by the loss of petal turgidity followed by inrolling of petals (Fig 1). Treatment of isolated buds of *P. hybrida* with different grades of salicylic acid (SA) resulted in the improvement of its flower longevity and various biochemical attributes viz. soluble proteins, α-amino acids, total phenols and sugar fractions (total, reducing and non-reducing sugars) (Table 1). Floral buds treated with 0.25 mM SA showed improved flower longevity (3.7 d) as compared to the other concentrations as well as control where it was 4 days. Isolated flower buds of *P. hybrida* treated with different grades of SA showed improved flower diameter as compared to the control. Maximum flower diameter was recorded in the floral buds on day 2 as well as on 5 which were treated with 0.25 mM SA. Membrane stability (MSI) was found to increase with the increase in the SA concentration and was recorded to be maximum in the tissue samples from floral buds held in 0.25 mM SA. Floral buds of *P. hybrida* held in 0.25 mM SA showed minimum lipid peroxidase activity (LPO) as compared to the floral buds held in other concentrations as well as control. Tissue samples from floral buds treated with 0.25 mM SA showed maximum soluble protein content on day 2 as compared to the other concentrations including control. The concentration of soluble proteins was found to decrease with the progression in time from day 2 to 5. Maximum α-amino acid content was registered in the tissue samples from floral buds which were held in 0.25 mM SA and was found to increase with the progression in time from day 2 to 5. A higher content of total phenols was maintained by the tissue samples from floral buds which were treated with 0.25 mM SA as compared to the tissue samples from floral buds held in control. Treatment of isolated floral buds of *P. hybrida* with SA resulted in an increased sugar content and maximum total sugar content was registered in the tissue samples from floral buds held in 0.25 mM SA as compared to the other concentrations as well as control. The concentration of sugar fractions was found to decrease with the progression in time from day 3 to 5.

Table 1: Effect of different grades of salicylic acid (SA) on the flower longevity, floral diameter, membrane stability index, lipid peroxidase activity, soluble proteins, α-amino acids, total phenols and sugar fractions (total, reducing and non-reducing) in isolated flowers/petal tissues of *Petunia hybrid**a***.

Days after transfer	Control (DW)	0.10 mM SA	0.15 mM SA	0.20 mM SA	0.25 mM SA	LSD P=0.05
Flower longevity (days)						
-	4	5.7	5.0	4.5	7.7	0.17
Floral diameter(cm)						
2	2.99	3.85	3.78	3.60	4.15	0.12
5	0	2.75	2.95	0	2.97	0.09
Membrane stability index (%)						
2	48.12	41.84	57.30	64.22	66.18	1.25
5	0	23.15	34.72	0	39.20	0.95
Lipid peroxidase activity (µM TBARS g^{-1} fm)						
2	28.22	32.10	25.54	38.33	22.14	0.88
5	0	22.04	18.34	0	12.22	0.75
Soluble proteins (mg g^{-1}fm)						
2	4.22	5.88	9.21	14.60	19.74	0.35
5	0	2.18	5.66	0	10.10	0.30
α-amino acids (mg g^{-1} fm)						
2	3.55	4.14	6.20	9.32	11.04	0.32

5	0	7.33	9.86	0	16.50	0.28
Total phenols (mg g^{-1} fm)						
2	5.15	6.42	8.15	10.30	15.44	0.36
5	0	3.22	4.09	0	9.60	0.30
Total sugars (mg g^{-1} fm)						
2	14.22	16.28	16.50	19.45	21.36	0.40
5	0	10.11	9.65	0	15.23	0.38
Reducing sugars (mg g^{-1} fm)						
2	7.12	9.13	9.20	9.90	11.23	0.36
5	0	6.22	5.40	0	9.13	0.32
Non-reducing sugars (mg g^{-1} fm)						
2	7.10	7.15	7.30	9.55	10.13	0.28
5	0	3.89	4.25	0	6.10	0.25

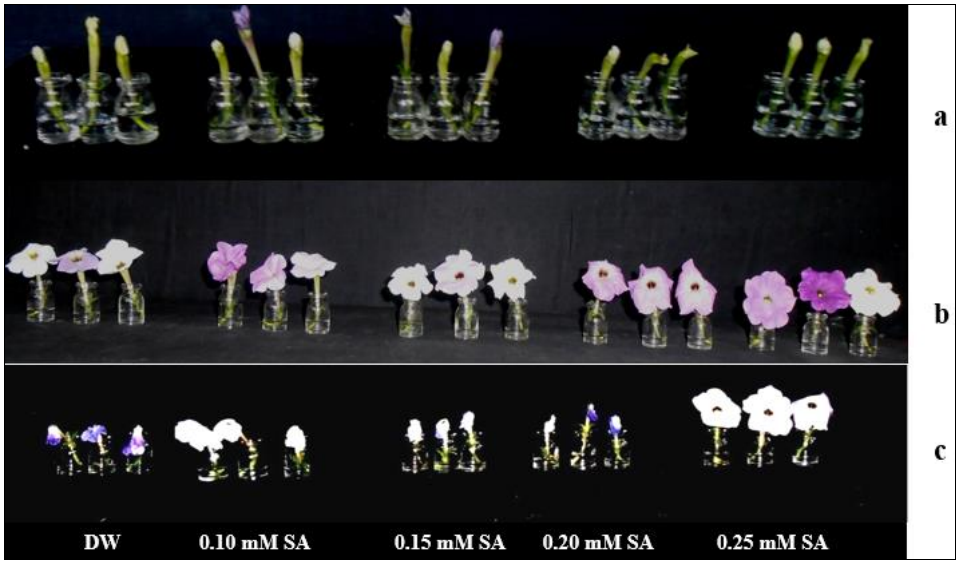

Fig 1: Effect of various grades of salicylic acid (SA) on flower senescence in isolated flowers of *Petunia hybrida* on day 0 (Fig a), day 3 (Fig b) and day 6 (Fig c) of transfer to the test solutions.

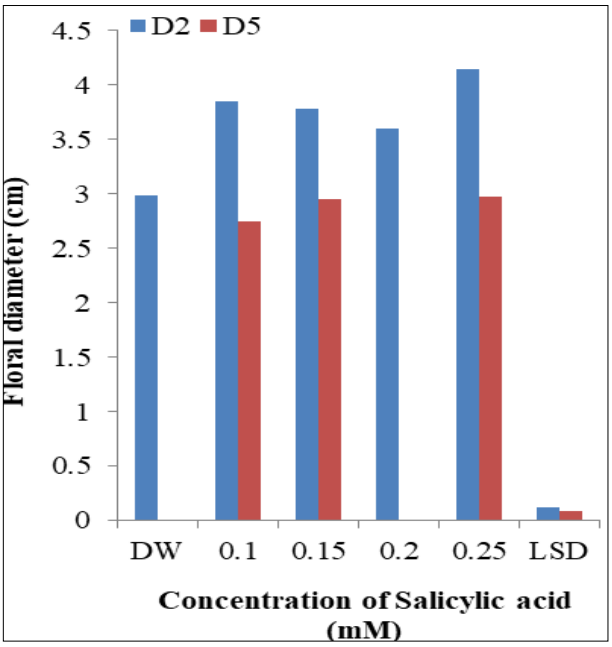

Fig 1

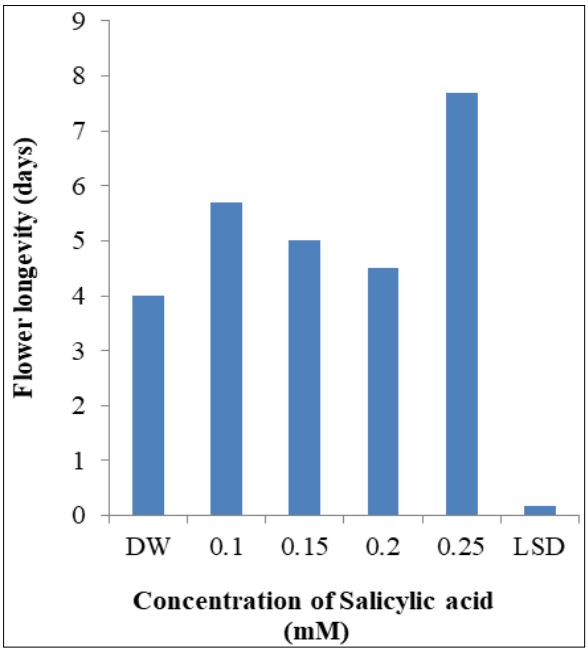

Fig 2

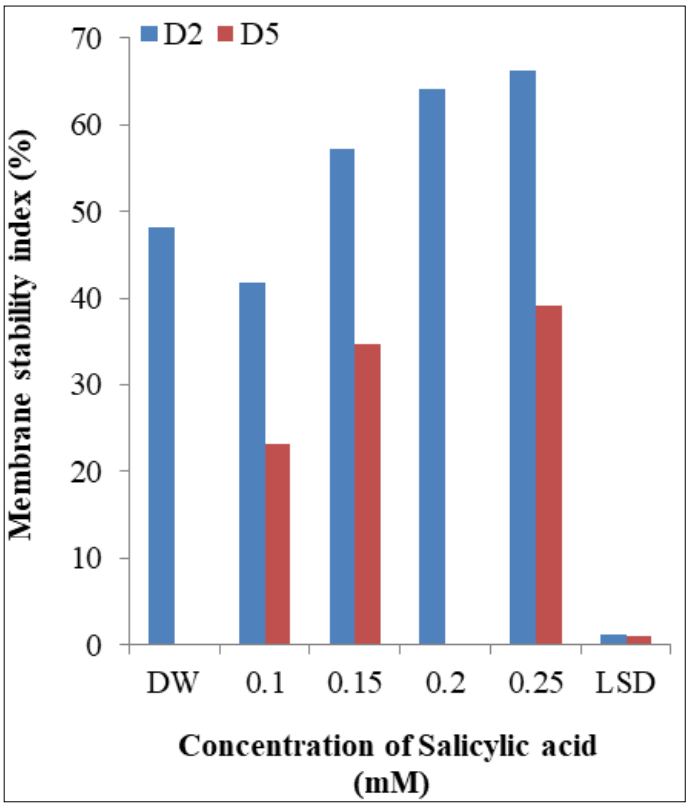

Fig 3

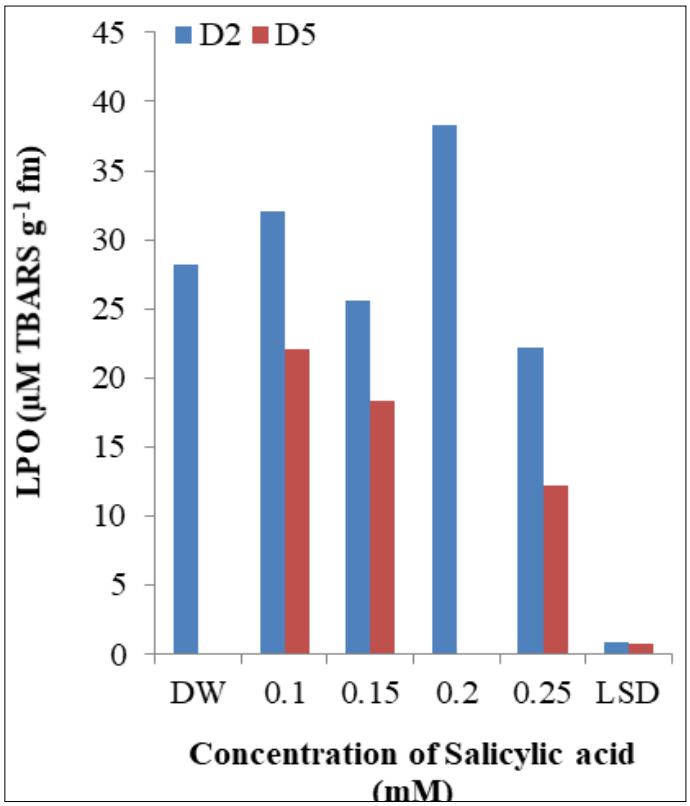

Fig 4

Fig 1-4: Effect of different grades of salicylic acid (SA) on the flower longevity, floral diameter, membrane stability index and lipid peroxidase activity (LPO) in isolated flowers/petal tissues of *Petunia hybrida***.**

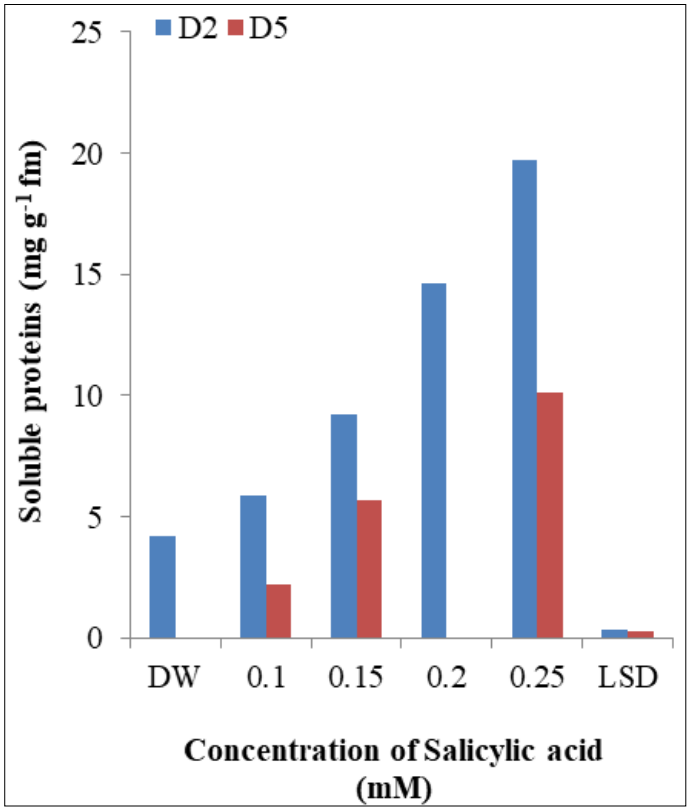

Fig 5

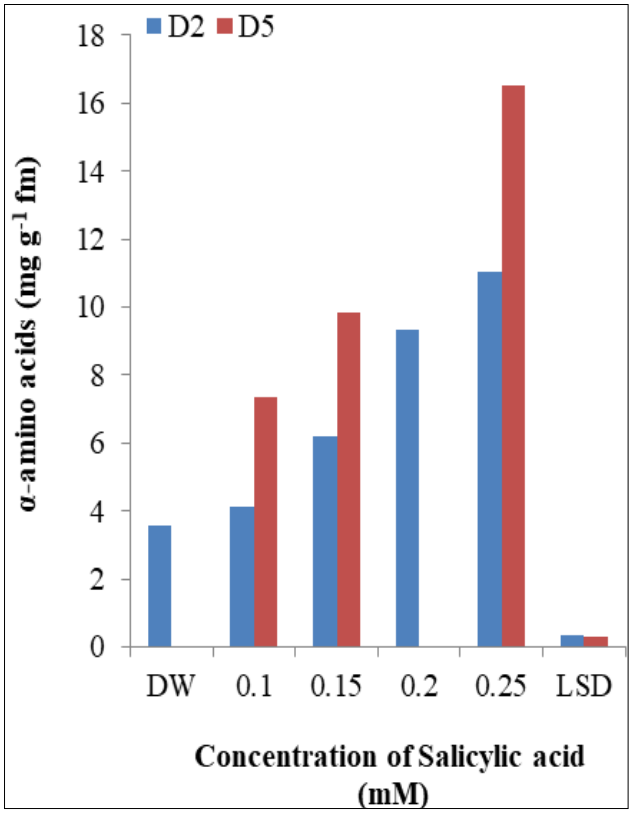

Fig 6

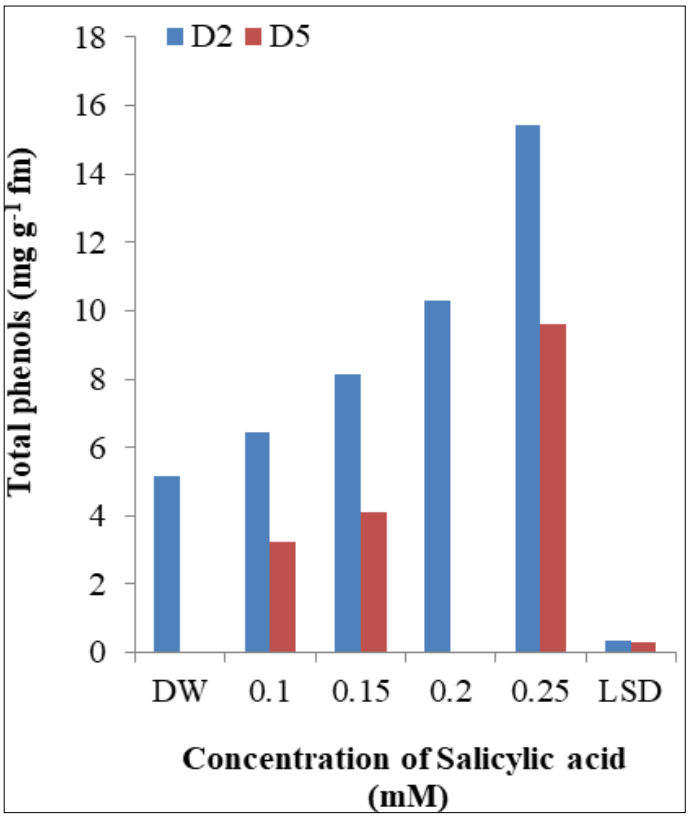

Fig 7

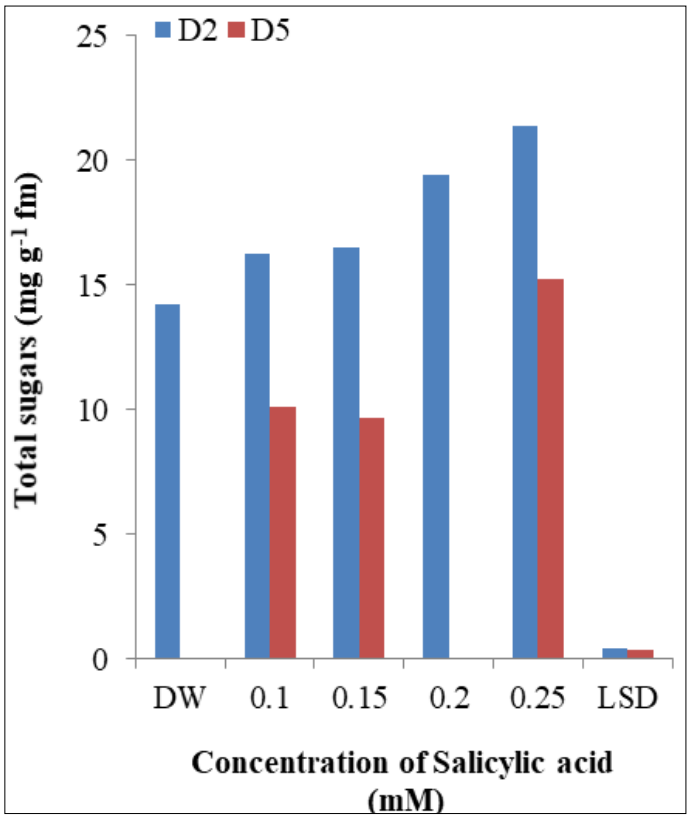

Fig 8

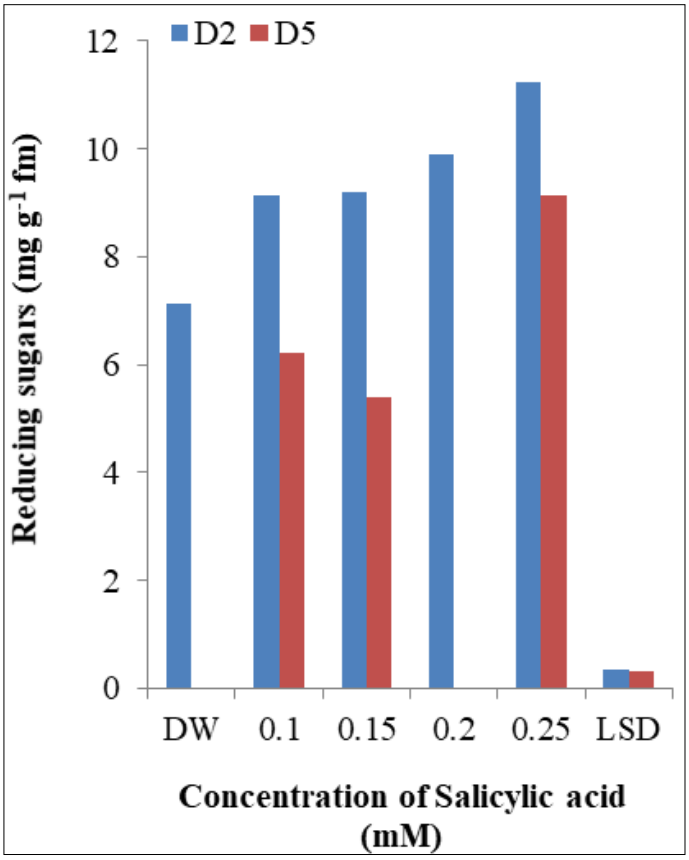

Fig 9

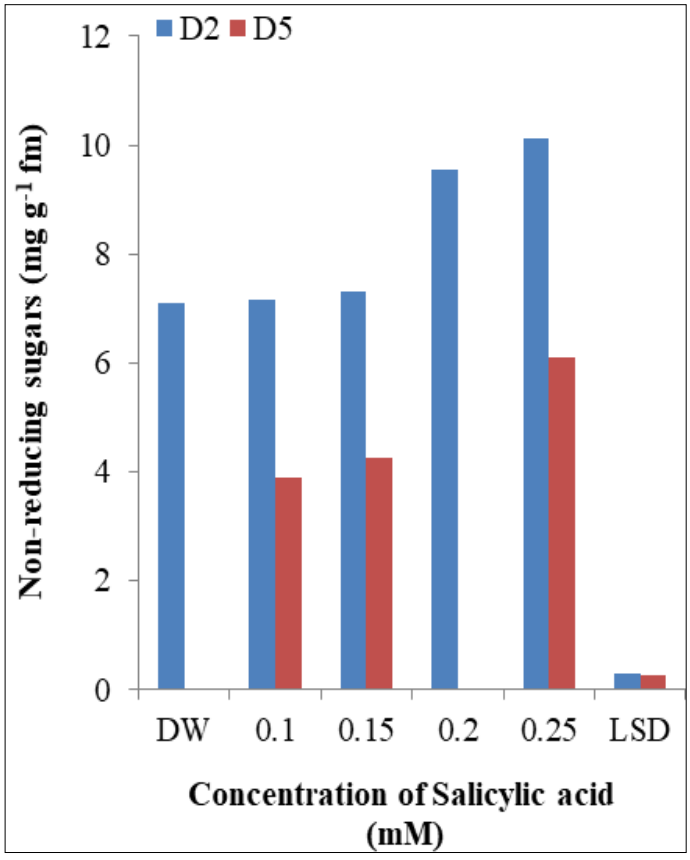

Fig 10

Fig. 9-10: Effect of different grades of salicylic acid (SA) on reducing and non-reducing sugars in petal tissues of *Petunia hybrida*.

Discussion

The present study revealed that flower senescence in *P.*

hybrida is characterised by the loss of petal turgidity followed by inrolling of petals. SA was found to improve the flower longevity of isolated floral buds of *P. hybrida* and maximum flower longevity of 3.7 days was recorded in the floral buds which were held in 0.25 mM SA as compared to the other concentrations as well as control, where it was 4 days. SA improves flower longevity as a consequence of reduced rates of ethylene biosynthesis because it is known to block the conversion of ACC to ethylene (Bueno and Del Rio, 1992; Gerailoo and Ghasemnezhad, 2011; Hassan and Ali., 2014) [4, 9, 10]. SA also improves sugar translocation in plants which could accumulate more resources and exert turgor pressure for cell division and elongation. Increased turgidity and resource accumulation in turn improve the flower longevity (Ezhilmathi *et al*., 2007; Saeed *et al*., 2016) [6, 7, 18]. Our results corroborate with those of Gladiolus cut flowers wherein SA application was found to improve its flower longevity (Anjum *et al*., 2001; Saeed *et al*., 2016) [1, 18]. Treatment of isolated floral buds of *P. hybrida* with SA also resulted in improved flower diameter as compared to the control. Similar results have been obtained in case of Gladiolus flowers wherein SA treatment showed delayed dehydration of flowers and improved fresh weight and floral diameter (Saeed *et al*., 2016) [18]. The reason for this increased floral diameter could be due to the fact that SA might delay the dehydration either due to increase in solution uptake or lowering the water loss (Saeed *et al*., 2016) [18]. Lipid peroxidation and membrane stability are inversely proportional and closely associated with flower senescence. During the present study MSI and LPO activity showed opposite effects upon treatment with SA. Similar profiles had been observed in chrysanthemum petal senescence by Bartoli *et al*. (1995) [2]. Treated flowers with SA maintained a significantly lower level of lipid peroxidation (Hatamzadeh *et al*., 2012) [12]. Treatment of isolated floral buds of *P. hybrida* with SA showed maximum soluble protein content as compared to the tissue samples from floral buds held in control. The soluble proteins play an important role until final stages of vase life. Therefore, high protein content of SA treated flowers may help flowers to extend postharvest longevity (Hassan and Ali., 2014) [10]. SA treatment was also found to increase the total sugar content of isolated floral buds of *P. hybrida*. The reason for this increase could be due to the fact that SA improves the water uptake and sugar translocation in plants which could accumulate more resources and exert turgor pressure and in turn delay flower senescence as seen in case of Gladiolus flowers (Ezhilmathi *et al*., 2007; Saeed *et al*., 2016) [6, 7,18].

In conclusion, SA enhanced the flower longevity of isolated flowers of *P. hybrida* by maintaining a higher protein, sugar content and reducing lipid peroxidase activity during the process of flower senescence in this beautiful ornamental flower of Solanaceae.

References

1. Anjum MA, Naveed F, Shakeel F, Amin S. Effect of some chemicals onkeeping quality and vase life of tuberose (Polianthes tuberosa L.) cut flowers. J.Res. (Sci.). 2001; 12(1):1-7.
2. Bartoli CG, Simomtacchi M, Guiamet JJ, Montaldi E, Puntarulo S. Antioxidant enzymes and lipid peroxidation during aging of *Chrysanthemum morifolium* RAM petals. Plant Sci. 1995; 104:161-168.
3. Bhattacharjee S, De LC. Post-harvest technology of flowers and ornamental plants. Aavishkar Publishers. Jaipur, India, 2005, 11-19.
4. Bueno P, Del Rio LA. Purification and properties of lyoxysomal cuperozinc superoxide dismutase from watermelon (*Citrullus vulgaris* Scrad.). Plant Physiol. 1992; 8:331-336.
5. Eraslan F, Inal A, Gunes A, Alpaslan M. Impact of exogenous salicylic acid on the growth, antioxidant activity and physiology of carrot plants subjected to combined salinity and boron toxicity. Sci. Hortic. 2007; 113:120-138.
6. Ezhilmathi K, Singh VP, Arora A, Sairam RK. Effect of 5-sulfosalicylic acid on antioxidant activity in relation to vase life of Gladiolus cut flowers. Plant Growth Regulation. 2007; 51:99-108.
7. Ezhilmathi K, Singh VP, Arora A, Sairam RK. Effect of 5-sulfosalicylic acid on antioxidant activity in relation to vase life of Gladiolus cut flowers. Plant Growth Regulation, 2007; 51:99-108.
8. Gebremedhin H, Tesfaye B, Mohammed A, Tsegay D. Influence of preservative solutions on vase life and postharvest characteristics of rose (Rosahybrid) cut flowers. Int. J. Biotechnol. Mol. Biol. Res. 2013; 4(8): 111–118.
9. Gerailoo S, Ghasemnezhad M. Effect of salicylic acid on antioxidant enzyme activity and petal senescence in 'yellow island' cut rose flowers. Journal of Fruit and Ornamental Plant Research. 2011; 19(1):183-193.
10. Hassan FAS, Ali EF. Protective effects of 1-methylcyclopropene and salicylic acid on senescence regulation of gladiolus cut spikes. Sci. Hortic. 2014; 179:146-152.
11. Hassan I, Zhang Y, Du G, Wang G, Zhang J. Effect of salicylic acid (SA) ondelaying fruit senescence of Huang Kum Pear. Front. Agric. China. 2007; 1(4):456-459.
12. Hatamzadeh A, Hatami M, Ghasemnezhad M. Efficiency of salicylic acid delay petal senescence and extended quality of cut spikes of *Gladiolus grandiflora* cv 'Wing's Sensation'. African Journal of Agricultural Research. 2012; 7:540-545.
13. Ichimura K, Yamada T, Yoshioka S, Pun UK, Tanase K, Shimizu-Yumoto H. Ethylene regulates programmed cell death (PCD) associated with petal senescence incarnation flowers. Acta Horticulturae. 2009; 847:185-190.
14. Pacheco AC, da Silva Cabral C. da Silva Fermino, É.S and Aleman, C.C. Salicylic acid-induced changes to growth, flowering and flavonoids production in marigold plants. J Med. Plant Res. 2013; 7(42):3158-3163.
15. Rejeb IB, Pastor V, Mauch-Mani B. Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. Plants. 2014; 3:458-475.
16. Rogers HJ. Programmed cell death in floral organs: how and why do flowers die? Ann. Bot. 2006; 97(3):309-315.
17. Rubinstein B. Regulation of cell death in flower petals. J. Plant Mol. Biol. 2000; 44(3):303-318.
18. Saeed T, Hassan I, Abbasi NA, Jilani G. Antioxidative activities and qualitative changes in gladiolus cut flowers

in response to salicylic acid application. Scientia Horticulturae. 2016; 210:236-241.
19. Schaller GE. Ethylene and the regulation of plant development. BMC Biology, 2012; 10:9.
20. Stead AD. Cut flowers in the US—can Europe teach us anything? FOA Bull. 885, July/August, 2004.
21. van Doorn WG, Woltering EJ. Physiology and molecular biology of petal senescence. J Exp. Bot. 2008; 59:453-480.