



RESEARCH ARTICLE

# Role of homobrassinolide, abscisic acid, and 6-benzylamino purine on delaying flower senescence in *Gladiolus grandiflora*

Madhulika Singh<sup>1</sup> & Sunil K. Singh<sup>2\*</sup>

<sup>1</sup>Department of Botany, Swami Shradhanand College, University of Delhi, Delhi-110 036, India

<sup>2</sup>Department of Chemistry, Kirori Mal College, University of Delhi, Delhi-110 007, India

\*Email: chem.sunil@kmc.du.ac.in



## ARTICLE HISTORY

Received: 19 November 2022

Accepted: 15 March 2023

Available online

Version 1.0 : 27 July 2023



## Additional information

**Peer review:** Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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## CITE THIS ARTICLE

Singh M, Singh S K. Role of homobrassinolide, abscisic acid, and 6-benzylamino purine on delaying flower senescence in *Gladiolus grandiflora*. Plant Science Today (Early Access). <https://doi.org/10.14719/pst.2245>

## Abstract

A study was conducted to investigate the effects of homobrassinolide (HBL), abscisic acid (ABA) and 6-benzylamino purine (BAP) on the post-harvest life of two *Gladiolus grandiflora* cultivars, snow princess and nova lux. Different HBL, ABA and BAP concentrations were applied to cut flowers. The experimental results showed that ABA and BAP treatments significantly increased post-harvest life in snow princess and nova lux cultivars compared to untreated flowers. However, HBL treatment was unsuccessful in delaying senescence in gladiolus. Furthermore, ABA and BAP treatments were more effective in delaying senescence in the nova lux cultivar than in the snow princess cultivar. Vase solutions containing BAP (500 µM) and ABA (10 µM) were the most efficient in extending the life of the cut floral spike of the nova lux variety (10 and 9 days, respectively) followed by the snow princess variety (8 and 7 days respectively). Increased fresh weight of flowers, vase solution uptake and membrane integrity along with decreased pH, malondialdehyde content, and lipoxygenase (LOX) activity prolonged flowers' post-harvest life. In terms of the post-harvest life of *G. grandiflora*, BAP outperformed ABA in improving the flower longevity of *Gladiolus* by maintaining higher physiological and biochemical stability in petals.

## Keywords

Membrane integrity, nova lux, snow princess, lipoxygenase, malondialdehyde, phytohormone

## Introduction

The rapid and predictable nature of the senescence process makes flowers an excellent model for revealing physiological and biochemical processes. The deterioration of plants with age, which leads to death, is called senescence (1). A plant's tissues, cells, or whole body die as a result of the down-regulation of several physiological and biochemical processes during senescence (2). During plant development, senescence is genetically determined as well as controlled by various plant hormones and environmental factors. Senescence causes numerous changes, including decreased fresh weight, reduced vase solution uptake, membrane degradation and an increase in lipid peroxidation (3-5).

Flowers' commercial value is determined by the life expectancy and quality of the cut flower (6). Understanding physiological and biochemical processes associated with petal senescence is essential to increasing the life span of flowers (7). Both ethene-sensitive and ethene-insensitive flowers extend their lives by responding to phytohormones (1, 8). Phytohormones

delay the senescence process, making them suitable for postharvest technology.

In cut roses, ABA treatment enhanced vase life, flower diameter, superoxide dismutase, peroxidase and ascorbate peroxidase activity (9). ABA is a natural floral senescence promoter (10). In some flowers, such as daffodils (11) exogenous administration of ABA has been demonstrated to accelerate the senescence process. A crucial role is played by abscisic acid in inducing floral senescence, while cytokinins inhibit it (12).

Several researchers have found that various compounds are involved in flower opening, including ethylene, gibberellin, abscisic acid and brassinolide (13). Flowers such as lotus, Gerbera, *Iris* and daylilies retain longer vase lives when applied exogenously with phytohormones (14-16). The overall vase life of cut wintersweet flowers was reduced by ABA treatment to 7.8 days, although this was not substantially different from the control (17). In wheat, brassinolide administration increased relative water content, membrane stability, nitrate reductase activity, chlorophyll content, and photosynthesis. BAP, an adenine cytokinin (20), inhibited the senescence process by increasing the content of various metabolites, preventing lipid peroxidation in membranes, as well as regulating vase solution uptake (8). These favorable effects resulted in an increased area of leaf, biomass, grain production and yield-related metrics (18). In *Vigna radiata*, HBL improved physiological and biochemical processes (19).

*G. grandiflora* cultivars (snow princess and nova lux) are mid-season bloomers that are popular in India as ornamentals and play a key role in the floriculture industry. Gladiolus is used to cure diarrhea, constipation and colds. However, the short vase life of the flower reduces its market value. As Gladiolus is not sensitive to ethene, the shorter vase life is caused by oxidative stress (21) and the impact of phytohormones in reducing oxidative stress during floral senescence in *G. grandiflora* "snow princess" and "nova lux" cultivars have yet to be investigated. In this regard, an experiment was planned to investigate the effects of HBL, ABA and BAP on flower senescence in *G. grandiflora* by analyzing physiological and biochemical parameters with the ultimate aim to extend its post-harvest life span.

## Materials and Methods

All the experiments were performed in the Department of Botany, SSN College and Department of Chemistry, Kirori Mal College, University of Delhi.

### Plant Material

*Gladiolus grandiflora* cultivars snow princess and nova lux floral spikes used in the experimental setup were collected from the Indian Agricultural Research Institute (IARI), New Delhi.

### Experimental Treatments

Eighteen floral spikes of uniform length (120 cm) were cut and divided into 4 sets. Three sets of floral spikes were treated with 70  $\mu$ M HBL, 300  $\mu$ M HBL, 10  $\mu$ M ABA, 50  $\mu$ M ABA,

500  $\mu$ M BAP and 1000  $\mu$ M BAP and another set of floral spikes were held in distilled water and considered as control. Each experimental treatment was in replicates of three and the experiment was performed at a temperature of  $21 \pm 2$  °C and relative humidity of  $65 \pm 2\%$  and light intensity of 950 lux.

### Vase Life estimation

The fresh-weight floral spikes were measured daily with the help of a weighing balance. The floral spikes vase life was defined as when the floral spike's final fresh weight was less than its initial fresh weight.

### Vase Solution uptake

The Gladiolus spikes were held in test tubes containing 30 ml of vase solution. The cumulative uptake of vase solution by floral spikes was determined and expressed in ml/spike.

### pH of Vase Solution

The pH vase solution was determined using the pH meter (HACH analyzer, HQ440d multi).

### Membrane Stability Index (MSI)

The membrane stability index of petals was determined using the standard protocol (22). Flower petals (200 mg) were kept in the test tubes containing 10 ml Milli Q water. After incubating for 5 hrs, the conductivity ( $C_1$ ) of the vase solution was measured using a conductivity meter (HACH analyzer, HQ440d multi). The vase solution was then heated for half an hr and the conductivity ( $C_2$ ) was determined again.

$$\text{Membrane Stability Index} = [1 - (C_1/C_2)] \times 100$$

### Lipid peroxidation

The lipid peroxidation was measured in terms of malondialdehyde content using the standard protocol (23). Flower petals (500 mg) were homogenized in 0.1% trichloroacetic acid (TCA). Centrifuged the homogenate for 5 min. Four milliliters thiobarbituric acid (0.5%) in TCA (20%) was added to the supernatant. The content was heated for half an hr and then quickly cooled. The content was centrifuged for 10 min. The absorbance was measured at 532 nm. The sample malondialdehyde content was determined using an absorption coefficient  $155 \text{ m mol}^{-1} \text{ cm}^{-1}$ .

### Estimation of Lipoxygenase Activity

The lipoxygenase activity was determined following the standard method (24). Flower petal tissue (500 mg) was homogenized in 0.1 M phosphate buffer comprising EDTA (0.5 mM). The content was centrifuged for 15 min at 15,000 rpm. The supernatant was denoted as an enzyme extract. The substrate was made by adding linoleic acid (70  $\mu$ l) to Milli Q having tween 20 and the final volume was made to 200 ml using phosphate buffer. Lipoxygenase enzyme activity was measured by adding an enzyme (50  $\mu$ l) to the substrate solution. The absorbance was recorded at 234 nm and LOX activity was determined as a change in  $\Delta A_{234} \text{ min}^{-1} \text{ g}^{-1} \text{ protein}$ .

### Statistical Analysis

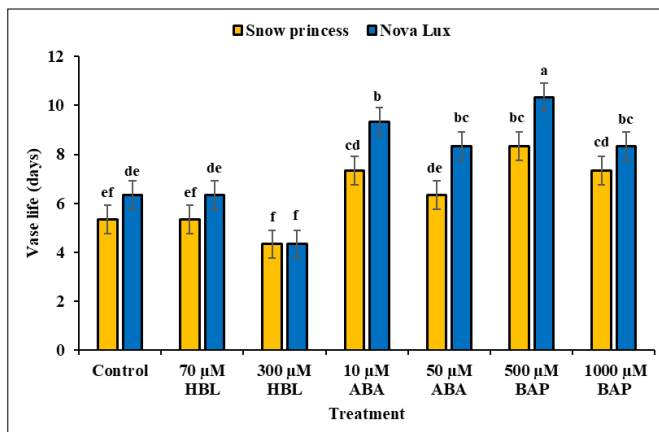
A completely randomized design with replicates of three

was used in this experiment. The data were analyzed using the IBM SPSS Statistics 21 statistical program. A comparison of the means was done by post hoc Duncan's test ( $p < 0.05$ ).

## Results

### Vase Life

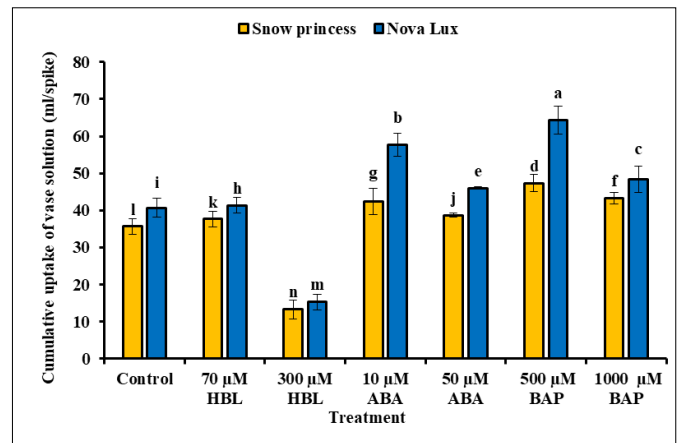
The ABA and BAP-treated floral spikes of snow princess and nova lux cultivars of *Gladiolus* showed a significant increase in vase life as compared to the control. Moreover, the nova lux cultivar exhibited more vase life than snow princess in ABA and BAP treatments. The vase life of floral spikes kept in 10  $\mu\text{M}$  ABA and 500  $\mu\text{M}$  BAP solutions increased to 7 and 8 days respectively, as compared to the control (5 days). There was not much significant difference in the vase life of 70  $\mu\text{M}$  HBL-treated floral spikes and control in both cultivars of *Gladiolus*. Moreover, 300  $\mu\text{M}$  HBL caused a significant reduction in the vase life of nova lux and snow as compared to the control. The most effective phytohormone for enhancing *Gladiolus* vase life was BAP (Fig. 1).



**Fig. 1.** Influence of different concentrations of HBL, ABA and BAP on vase life of *Gladiolus*. Data represent mean values  $\pm$  SD;  $n=3$ . Bars bearing different letters indicate significance difference means (Duncan's test;  $P \leq 0.05$ ).

### Vase Solution Uptake

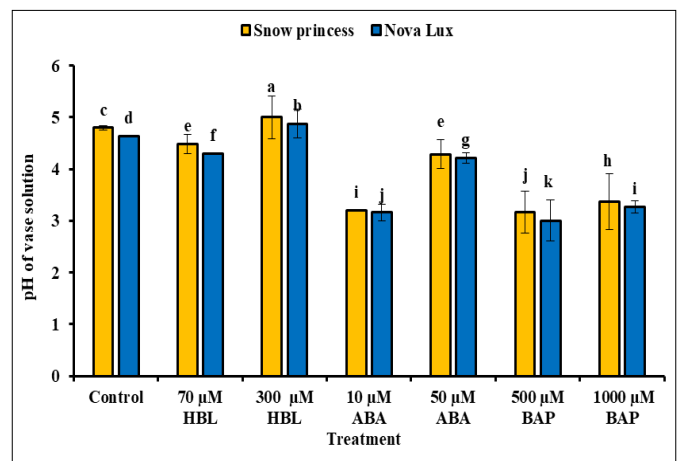
Various concentrations of ABA and BAP increased the vase solution uptake in both cultivars of *Gladiolus* as compared to the control. However, vase solution uptake was significantly higher in the nova lux cultivar as compared to snow princess. Floral spikes treated with 70  $\mu\text{M}$  HBL exhibited not much significant difference in cumulative uptake of vase solution in both cultivars as compared to control. Moreover, 300  $\mu\text{M}$  HBL caused a significant reduction in the uptake of vase solution in nova lux and snow princess (15.33 ml/spike and 13.33 ml/spike, respectively) as compared to the control. Vase solution uptake was increased most effectively with a 500  $\mu\text{M}$  BAP concentration by floral spikes of nova lux and snow princess (64.33 ml/spike and 47.33 ml/spike, respectively) followed by 10  $\mu\text{M}$  ABA treated nova lux and snow princess cultivars (57.67 ml/spike and 42.33 ml/spike, respectively) as compared to control floral spikes of nova lux and snow princess (40.67 ml/spike and 35.67, respectively) (Fig. 2).



**Fig. 2.** Influence of different concentrations of HBL, ABA and BAP on cumulative uptake of vase solution in *Gladiolus*. Data represent mean values  $\pm$  SD;  $n=3$ . Bars bearing different letters indicate significance difference means (Duncan's test;  $P \leq 0.05$ ).

### pH of Vase Solution

Vase solution pH was significantly decreased by different concentrations of ABA and BAP of nova lux and snow princess cultivar as compared to the control. However, the pH value of the vase solution was much reduced in the nova lux cultivar compared to the snow princess cultivar. There was not much significant difference in the pH value of HBL-treated floral spikes and control in both cultivars of *Gladiolus*. It is revealed from (Fig. 3), that 500  $\mu\text{M}$  BAP treatment was most effective in reducing the pH of the vase solution of nova lux and snow princess (pH 3.00 and 3.17, respectively) followed by 10  $\mu\text{M}$  ABA treated floral spikes of nova lux and snow princess (pH 3.16 and 3.20, respectively) compared to the control of nova lux and snow princess cultivar (pH 4.64 and 4.80, respectively). From the aforesaid results, it is evident that the 500  $\mu\text{M}$  BAP and 10  $\mu\text{M}$  ABA treatments had maximum effect on improving the vase life of both cultivars *Gladiolus*. However, HBL treatments were ineffective in improving the vase life of both cultivars of *Gladiolus*. Further, studies were performed to evaluate the effect of 70  $\mu\text{M}$  HBL, 10  $\mu\text{M}$  ABA and 500  $\mu\text{M}$  BAP concentrations on membrane integrity, lipid peroxidation and lipoxygenase activity in floral spikes of nova lux and snow princess cultivars of *Gladiolus* during senes-

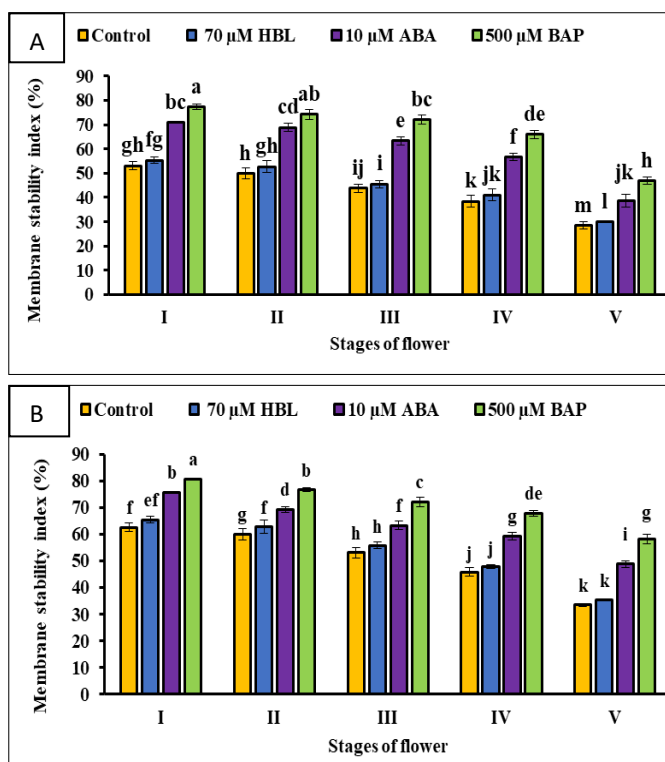


**Fig. 3.** Influence of different concentrations of HBL, ABA and BAP on the pH of vase solution of *Gladiolus*. Data represent mean values  $\pm$  SD;  $n=3$ . Bars bearing different letters indicate significance difference means (Duncan's test;  $P \leq 0.05$ ).

cence.

### Membrane Stability Index

Phytohormones treated and untreated floral spikes of nova lux and snow princess cultivar of *Gladiolus* showed a linear decline in membrane integrity during the flower development process (Fig. 4). However, there was a significant difference in membrane integrity of phytohormones treated and untreated floral spikes of both cultivars. The membrane stability index of phytohormones treated flowers was much higher than those of untreated flowers in both cultivars of *Gladiolus* however, membrane integrity was higher in the nova lux cultivar than snow princess. The membrane stability index was alleviated at stage V by treatment of nova lux and snow princess floral spikes with 500  $\mu\text{M}$  BAP (73.85% and 64.56%, respectively) followed by 10  $\mu\text{M}$  ABA (45.86% and 36.35%, respectively) and 70  $\mu\text{M}$  HBL (5.36%, 5.13%, respectively) in comparison to control



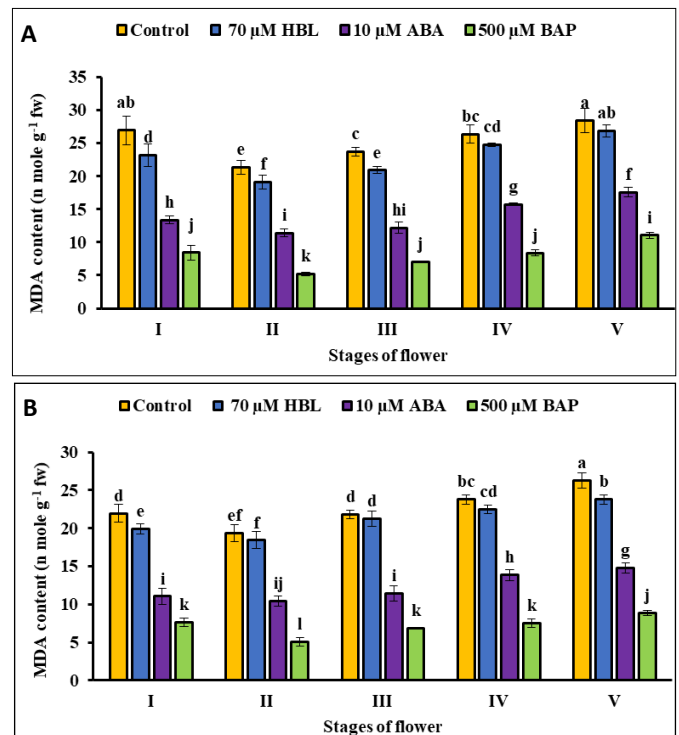
**Fig. 4.** Effect of 70  $\mu\text{M}$  HBL, 10  $\mu\text{M}$  ABA and 500  $\mu\text{M}$  BAP on membrane stability index (%) in (A) Snow princess and (B) Nova lux cultivars of *Gladiolus* during flower senescence. Different stages of flower development of *Gladiolus* are: I stage - Slightly opened / point brush stage; II stage - Half-opened stage; III stage - Fully opened stage; IV stage - Incipient senescence; stage; V stage - Senescent stage. Data represent mean values  $\pm$  SD;  $n=3$ . Bars bearing different letters indicate significance difference means (Duncan's test;  $P\leq 0.05$ ).

(Fig. 4). There was not much significant difference between HBL treated and untreated floral spikes of both cultivars.

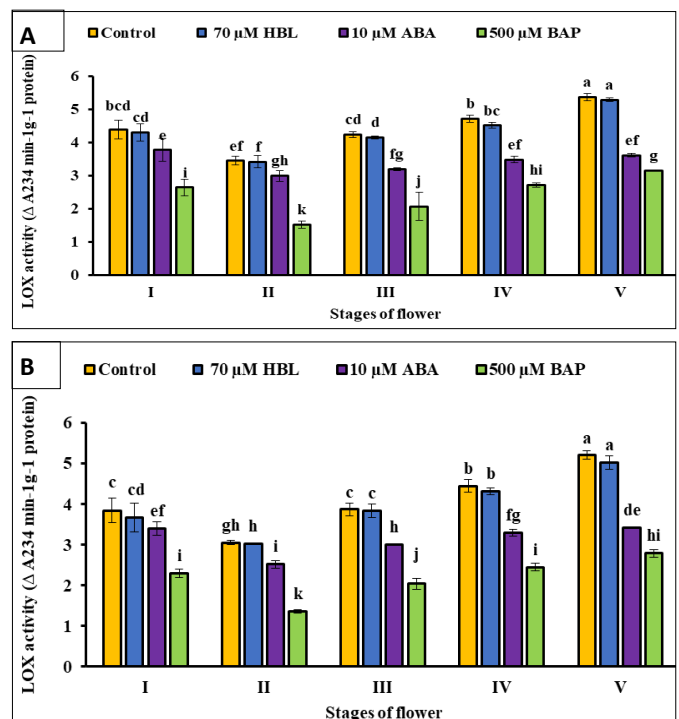
### Lipid peroxidation

Malondialdehyde content decreased till stage II (Half-opened stage) and then there was a gradual increase till stage V (Senescent stage) in phytohormones treated and untreated floral spikes of both cultivars of *Gladiolus*. Phytohormones-treated floral spikes had decreased malondialdehyde content throughout the flower developmental process as compared to untreated floral spikes. 500  $\mu\text{M}$  BAP-treated floral spikes of nova lux and snow

princess exhibited a decrease in malondialdehyde by 66.22% and 61.22%, respectively that is followed by 10  $\mu\text{M}$  ABA (43.71% and 38.18%, respectively) and 70  $\mu\text{M}$  HBL



**Fig. 5.** Effect of 70  $\mu\text{M}$  HBL, 10  $\mu\text{M}$  ABA and 500  $\mu\text{M}$  BAP on MDA content (n mole  $\text{g}^{-1}$  fw) in (A) Snow princess and (B) Nova lux cultivars of *Gladiolus* during flower senescence. Different stages of flower development of *Gladiolus* are: I stage - Slightly opened / point brush stage; II stage - Half-opened stage; III stage - Fully opened stage; IV stage - Incipient senescence; stage; V stage - Senescent stage. Data represent mean values  $\pm$  SD;  $n=3$ . Bars bearing different letters indicate significance difference means (Duncan's test;  $P\leq 0.05$ ).



**Fig. 6.** Effect of 70  $\mu\text{M}$  HBL, 10  $\mu\text{M}$  ABA and 500  $\mu\text{M}$  BAP on LOX activity ( $\Delta A_{234} \text{ min}^{-1} \text{ Ig-1 protein}$ ) in (A) Snow princess and (B) Nova lux cultivars of *Gladiolus* during flower senescence. Different stages of flower development of *Gladiolus* are: I stage - Slightly opened / point brush stage; II stage - Half-opened stage; III stage - Fully opened stage; IV stage - Incipient senescence; stage; V stage - Senescent stage. Data represent mean values  $\pm$  SD;  $n=3$ . Bars bearing different letters indicate significance difference means (Duncan's test;  $P\leq 0.05$ ).

(9.30% and 5.60% respectively), as compared to untreated floral spikes where malondialdehyde level was highest (Fig. 5).

### Lipoxygenase Activity

The lipoxygenase activity decreased gradually till stage II and then the enzyme activity showed a linear increase till stage V in both phytohormones treated and untreated floral spikes (Fig. 6). At any given stage the lipoxygenase activity was significantly less in phytohormone-treated floral spikes as compared to untreated floral spikes in both cultivars of *Gladiolus*. However, the lipoxygenase was very much reduced in phytohormones treated nova lux cultivar than in the snow princess cultivar. Lipoxygenase activity significantly decreased in nova lux and snow princess cultivars by 44.64% and 41.35%, respectively with 500  $\mu\text{M}$  BAP treatment that is followed by 10  $\mu\text{M}$  ABA (34.10% and 32.71%, respectively) and 70  $\mu\text{M}$  HBL (3.41% and 1.50%, respectively), as compared to untreated floral spikes where lipoxygenase activity was highest. Minimum lipoxygenase activity was seen in the floral spikes subjected to 500  $\mu\text{M}$  BAP treatment.

### Discussion

Senescence is a gradual deterioration process leading to the death of living organisms (1). Flower petals are showy, but their lifespan is short. Floral senescence is fast and predictable; therefore, flowers make an ideal model for studying senescence (25). Petal life determines how long a cut flower will last in the market. It is therefore essential to determine petal senescence so that we can maximize the life span of flowers and better understand their mechanisms.

There was a remarkable difference between the vase life of phytohormone (ABA and BAP) treated and untreated floral spikes. *Gladiolus* fresh weight and uptake of vase solution extend the vase life. In control flowers, vase life decreased because water uptake was reduced and membrane permeability increased. In control flowers, vase life decreased because water uptake was reduced (30) and membrane permeability increased. This is in accordance with the work reported by Mayak and Halevy (26) that treatment with kinetin increased the vase life of cut rose flowers. Carnation flowers were reported to significantly improved in terms of biomass and vase life after treatment with thiadiazuron (27). Daylily flowers were reported to have a longer vase life by maintaining higher fresh mass when treated with kinetin and cycloheximide (28). According to one report, salicylic acid could extend the vase life of horticultural crops by enhancing vase solution uptake of vase solution, antioxidant enzyme activities and membrane integrity (29).

The uptake of vase solution by phytohormone-treated (ABA and BAP) floral spikes and control spikes differed significantly. By treating the vase solution with 500  $\mu\text{M}$  BAP and 10  $\mu\text{M}$  ABA, the uptake of the vase solution was enhanced. However, BAP (500  $\mu\text{M}$ ) treated flowers exhibited a greater vase uptake than ABA (10  $\mu\text{M}$ ) treated

flowers. A reduced pH in the vase solution could be responsible for this. The vase solution moved more quickly in the xylem when the pH was lowered. In similar work (31), salicylic acid reduced the pH of the vase solution to enhance the uptake of cut flowers. HBL (70  $\mu\text{M}$ ) treatment had no significant effect on vase solution uptake of either cultivar of *Gladiolus*.

Compared to control flowers, phytohormone-treated (ABA and BAP) flowers showed a remarkable pH value difference. BAP (500  $\mu\text{M}$ ) treatment reduced the pH of the vase solution of nova lux and snow princess most effectively followed by 10  $\mu\text{M}$  ABA-treated floral spikes of nova lux and snow princess compared to the control of nova lux and snow princess cultivar. The vase solution was able to travel faster in the water-conducting system when the pH was lowered. A lower pH might improve water absorption by reducing bacterial growth. HBL (70  $\mu\text{M}$ ) treatment had no significant effect on the pH of the vase solution of either cultivar of *Gladiolus*. It was reported that salicylic acid treatment of cut rose flowers increased the vase solution uptake by reducing microbial growth which otherwise would obstruct the vascular system (32).

Treatment with 500  $\mu\text{M}$  BAP and 10  $\mu\text{M}$  ABA significantly increased the membrane stability index in comparison with control in both cultivars of *Gladiolus*. The membrane stability index changed significantly after 500  $\mu\text{M}$  BAP treatment compared to 10  $\mu\text{M}$  ABA in both cultivars of *Gladiolus*. The change in the integrity of the membrane indicates the amount of leakage of ions from flower petals. Similar work was reported that salicylic acid maintained the integrity of the membrane by lowering lipid peroxidation and lipoxygenase activity (33). There is evidence that free radical scavengers are involved in flower senescence by modulating the life span of flowers (34). It was reported in *Dendrobium nobile* thiadiazuron treatment preserved the integrity of the cell membrane, preventing senescence (26). Similar work was reported where cycloheximide- and kinetin-treated daylily flowers have retained the integrity of the membrane (20, 28). It was found that thiadiazuron maintained the integrity of the membrane of the cut rose flower, which delayed senescence (20). It is now reasonable to postulate that plant hormones can act as free radical scavengers, thereby maintaining membrane integrity for longer life spans. Phytohormones maintain the integrity of the cell membrane through improved water absorption and reduction of lipid peroxidation in plants during senescence which was also reported (35). It was also suggested that BAP may improve the membrane integrity of *Calendula officinalis* during senescence (36). HBL (70  $\mu\text{M}$ ) treatment had no significant effect on the membrane stability index of either cultivar of *Gladiolus*.

The malondialdehyde level showed a linear increase from stage III (Fully opened stage) to stage (V) in the treated and untreated floral spikes of both cultivars of *Gladiolus*. However, floral spikes treated with 500  $\mu\text{M}$  BAP and 10  $\mu\text{M}$  ABA showed a reduction in malondialdehyde content during flower development compared to untreated floral spikes in both cultivars of *Gladiolus*. In addition, flowers treated with 500  $\mu\text{M}$  BAP exhibited a reduced rate

of lipid peroxidation compared to 10  $\mu\text{M}$  ABA in both cultivars. However, 70  $\mu\text{M}$  HBL treatment had no significant role in reducing MDA content in both cultivars. The Nova lux cultivar responded better to phytohormone treatments in extending post-harvest life than the snow princess cultivar by the reduced level of lipid peroxidation. The malondialdehyde content by lipid peroxidation is increased by ROS which is in accordance with the work reported earlier (37), therefore, it can be concluded that 500  $\mu\text{M}$  BAP and 10  $\mu\text{M}$  ABA could play a critical role in decreasing lipoxygenase activity thereby reducing the levels of free radicals. Phytohormone treatment has enhanced the integrity of the cellular membrane due to the decrease in lipoxygenase activity which has reduced lipid peroxidation which is in accordance with one earlier work (38). In addition, BAP-treated flowers show a marked reduction in malondialdehyde levels and an increase in membrane integrity in cauliflower and carnations during senescence, as reported earlier (39, 40). This treatment reduced lipid peroxidation, thus confirming the role of plant hormones as free radical scavengers.

Gladiolus is insensitive to ethene, hence oxidative stress is the primary cause of Gladiolus senescence. There is a remarkable difference in lipoxygenase activity in phytohormone-treated and untreated floral spikes. The lipoxygenase activity was reduced in 500  $\mu\text{M}$  BAP and 10  $\mu\text{M}$  ABA-treated floral spikes compared to untreated floral spikes. Additionally, BAP was highly efficient in maintaining lower lipoxygenase activity than ABA, as indicated by a lower level of MDA and increased integrity of the membrane. The 70  $\mu\text{M}$  HBL treatment had no significant role in reducing lipoxygenase activity in both the cultivars of Gladiolus. It was reported that the reduction in lipoxygenase activity in phytohormone-treated plants leads to the formation of phospholipids and proteins by lowering protease secretion into the cellular cytoplasm (41, 42). Scientists have reported that several chemicals viz. boric acid and BAP have proven beneficial in enhancing post-harvest attributes of various flowers such as *Calendula*, and *Digitalis* (43, 44) A similar study was reported where phytohormone treatment increased the post-harvest life of *Iris germanica* by lowering lipoxygenase activity and avoiding membrane lipid peroxidation (38).

## Conclusion

The standardization of HBL, ABA and BAP concentrations in increasing the post-harvest life of Gladiolus was performed. Various concentrations of phytohormones viz., 70  $\mu\text{M}$  HBL, 300  $\mu\text{M}$  HBL, 10  $\mu\text{M}$  ABA, 50  $\mu\text{M}$  ABA, 500  $\mu\text{M}$  BAP and 1000  $\mu\text{M}$  BAP were used. It is concluded from the present study that 500  $\mu\text{M}$  BAP and 10  $\mu\text{M}$  ABA treatment reduced senescence-induced oxidative damage in Gladiolus cultivars nova lux and snow princess, as indicated by increased flower life span and post-harvest characteristics. 500  $\mu\text{M}$  BAP was shown to be the most effective and outperformed 10  $\mu\text{M}$  ABA in extending the post-harvest life of both cultivars of Gladiolus. Furthermore, the nova lux cultivar responded to phytohormone treatment better than the

snow princess cultivar in terms of extending post-harvest life. The 70  $\mu\text{M}$  HBL treatment had no effect on the post-harvest life of both Gladiolus cultivars. Treatment with phytohormones resulted in higher fresh weight, vase life and cellular membrane integrity, as well as decreased pH, malondialdehyde content and lipoxygenase activity. The involvement of phytohormones (BAP, ABA and HBL) in senescence regulation in *G. grandiflora* nova lux and snow princess cultivars has yet to be investigated. Among all the phytohormone treatments (HBL, ABA and BAP), BAP was most effective in enhancing the post-harvest life of the snow princess and nova lux cultivar of Gladiolus. However, BAP treatment was most effective in enhancing the post-harvest life of the nova lux cultivar than the snow princess cultivar.

At the physiological and biochemical level, a detailed investigation of the critical role of BAP, ABA and HBL in delaying senescence and enhancing the postharvest life of flowers is required. Furthermore, the mode of action of BAP should be investigated further, as it has been shown to be the most effective at delaying senescence at lower doses.

The study offers opportunities for mitigating flower senescence and postharvest losses at the molecular level, in addition to studying complex interactions of phytohormones and related compounds in this fascinating flower. The mechanism of flower senescence in this flower will unveil approaches to modulate its efficiency and productivity through various chemical formulations and/or molecular interventions.

## Acknowledgements

MS is appreciative for the financial support received from Swami Shraddhanand College, University of Delhi, Delhi, India. Author is also grateful to the Department of Floriculture, IARI, New Delhi for providing gladiolus floral spikes.

## Authors contributions

MS and SKS both carried out the experiments and participated in the design of the study and performed the statistical analysis. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

## References

1. Ahmad SS, Tahir I. Increased oxidative stress, lipid peroxidation and protein degradation trigger senescence in *Iris versicolor* L. flowers. *Physiol Mol Biol Plants*. 2016;22(4):507-14. <https://doi.org/10.1007/s12298-016-0392-9>
2. Sarwat M, Tuteja N. Chapter 13 – Flower senescence: present status and future aspects. In: Sarwat M, Tuteja N (ed.). *Senes-*

- cence signalling and control in plants. Cambridge: Academic Press, MA; 2019. p. 211-25. <https://doi.org/10.1016/B978-0-12-813187-9.00013-5>
3. Salleh FM, Mariotti L, Spadafora ND, Price AM, Picciarelli P, Wagstaff C *et al.* Interaction of plant growth regulators and reactive oxygen species to regulate petal senescence in wallflowers (*Erysimum linifolium*). *BMC Plant Biol.* 2016;16:77. <https://doi.org/10.1186/s12870-016-0766-8>
  4. Chen C, Zeng L, Ye Q. Proteomic and biochemical changes during senescence of phalaenopsis 'Red Dragon' petals. *Int J Mol Sci.* 2018;19:1317. <https://doi.org/10.3390/ijms19051317>
  5. Hemati E, Daneshvar MH, Heidari M. The roles of sodium nitroprusside, salicylic acid and methyl jasmonate as hold solutions on vase life of *Gerbera jamesonii* 'Sun Spot.' *Adv Hortic Sci.* 2019;33:187-95.
  6. Kumari P and Bhargava B. Phytochemicals from edible flowers: Opening a new arena for healthy lifestyle. *Journal of Functional Foods.* 2021;78:104375. <https://doi.org/10.1016/j.jff.2021.104375>
  7. Wojciechowska N, Sobieszczuk-Nowicka E, Bagniewska-Zadworna A. Plant organ senescence—regulation by manifold pathways. *Plant Biol.* 2018;20:167-81. <https://doi.org/10.1111/plb.12672>
  8. Iqbal N, Khan NA, Ferrante A, Trivellini A, Francini A, Khan MIR. Ethylene role in plant growth, development and senescence: interaction with other phytohormones. *Front Plant Sci.* 2017;8:475. <https://doi.org/10.3389/fpls.2017.00475>
  9. Deng Y, Wang C, Huo J, Hu W, Liao W. The involvement of NO in ABA-delayed the senescence of cut roses by maintaining water content and antioxidant enzymes activity. *Scientia Horticulturae.* 2019;247:35-41. <https://doi.org/10.1016/j.scienta.2018.12.006>
  10. Reid MS, Chen JC. Flower senescence. In: S. Gan editor. *Senescence processes in plants.* Oxford: Blackwell Publishing; 2007. p. 256-77. <https://doi.org/10.1002/9780470988855.ch11>
  11. Hunter DA, Ferrante A, Vernieri P, Reid MS. Role of abscisic acid in perianth senescence of daffodil (*Narcissus pseudonarcissus* 'Dutch Master'). *Physiol Plant.* 2004A;121:313-21. <https://doi.org/10.1111/j.0031-9317.2004.0311.x>
  12. Chen HW, Jiang YZ, Hsu FH, Yang HC. Silencing of Forever Young Flower-Like Genes from Phalaenopsis orchids Promotes Flower Senescence and Abscission. *Plant cell Physiol.* 2021;62(1):111-124. <https://doi.org/10.1093/pcp/pcaa145>
  13. Sun X, Qin M, Yu Q, Huang Z, Xiao Y, Li Y, Ma N, Gao J. Molecular understanding of postharvest flower opening and senescence. *Molecular Horticulture.* 2021;1(1):7. <https://doi.org/10.1186/s43897-021-00015-8>
  14. Macnish AJ, Jiang CZ, Negre-Zakharov F, Reid MS. Physiological and molecular changes during opening and senescence of *Nicotiana glauca* flowers. *Plant Sci.* 2010;179:267-72. <https://doi.org/10.1016/j.plantsci.2010.05.011>
  15. Imsabai W, van Doorn WG. Effects of auxin, gibberellin and cytokinin on petal blackening and flower opening in cut lotus flowers (*Nelumbo nucifera*). *Postharvest Biol Technol.* 2013;75:54-57. <https://doi.org/10.1016/j.postharvbio.2012.05.015>
  16. Reid MS, Wu MJ. Ethylene in flower development and senescence. In: Mattoo AK, Suttle JC, editors. *The plant hormone ethylene.* Boca Raton: CRC Press, Taylor and Francis Group, FL; 2018. P. 4-32. <https://doi.org/10.1201/9781351075763-12>
  17. Sui S, Luo J, Liu D, Ma J, Men W, Fan L, Bai Y, Li M. Effects of hormone treatments on cut flower opening and senescence in wintersweet (*Chimonanthus praecox*). *Hort Science horts.* 2015;50(9):1365-69. <https://doi.org/10.21273/HORTSCI.50.9.1365>
  18. Sairam R K. Effects of homobrassinolide application on plant metabolism and grain yield under irrigated and moisture-stress conditions of two wheat varieties. *Plant Growth Regulation.* 1994;14(2):173-81. <https://doi.org/10.1007/BF00025220>
  19. Alyemeni MN, Al-Quwaiz SM. Effect of 28-homobrassinolide on the performance of sensitive and resistant varieties of *Vigna radiata*. *Saudi Journal of Biological Sciences.* 2016;23(6):698-705. <https://doi.org/10.1016/j.sjbs.2016.01.002>
  20. Mortazavi SN, Talebi SF, Naderi RA, Sharafi Y. Regulation of ethylene biosynthesis by nitric acid and thiazurion during post-harvest of rose. *J Med Plant Res.* 2011;5(20):5177-83.
  21. Rahmani I, Ahmadi N, Ghanati F, Sadeghi M. Effects of salicylic acid applied pre- or post-transport on post-harvest characteristics and antioxidant enzyme activity of Gladiolus cut flower spikes. *N Z J Crop Hortic Sci.* 2015;43:294-305. <https://doi.org/10.1080/01140671.2015.1096799>
  22. Bailly C, Benamar A, Corbineau F, Come D. Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. *Physiol Plant.* 1996;97:104-10. <https://doi.org/10.1111/j.1399-3054.1996.tb00485.x>
  23. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys.* 1968;125:189-98. [https://doi.org/10.1016/0003-9861\(68\)90654-1](https://doi.org/10.1016/0003-9861(68)90654-1)
  24. Doderer A, Kokkelink I, van der Veen S, Valk BE, Schram André W, Douma AC. Purification and characterization of two lipoxygenase isoenzymes from germinating barley. *Biochim Biophys Acta.* 1992;1120:97-104. [https://doi.org/10.1016/0167-4838\(92\)90429-H](https://doi.org/10.1016/0167-4838(92)90429-H)
  25. Tripathi SK, Tuteja N. Integrated signaling in flower senescence. *Plant Signal Behav.* 2007;2:437-45. <https://doi.org/10.4161/psb.2.6.4991>
  26. Ren S, Hu M, Wu Q, Wang L, Gu H, Chen Z, Ming Z, Li Z. Flowering Time and Physiological Reaction of *Dendrobium nobile* Lindl in Response to TDZ Application. *Horticulturae.* 2023;9:129. <https://doi.org/10.3390/horticulturae9020129>
  27. Chamani E, Irving DE, Joyce DC, Arshad M. Studies with thiazurion on the vase life of cut rose flowers. *J Appl Hortic.* 2006;8:42-44. <https://doi.org/10.37855/jah.2006.v08i01.10>
  28. Gulzar S, Tahir I, Amin I, Farooq S, Sultan SM. Effect of cytokinins on the senescence and longevity of isolated flowers of day lily (*Heemerocallis fulva*) cv. royal crown sprayed with cycloheximide. *Acta Hort.* 2005;669:395-404. <https://doi.org/10.17660/ActaHortic.2005.669.52>
  29. Abdelaal KA, Attia KA, Alamery SF, ElAfry MM, Ghazy AI, Tantawy DS, Aldoss AA, El-Shawy ESE, M Abu-Elsaoud A and Hafez YM. Exogenous application of proline and salicylic acid can mitigate the injurious impacts of drought stress on barley plants associated with physiological and histological characters. *Sustainability.* 2020 12(5): 1736. <https://doi.org/10.3390/su12051736>
  30. Gul F, Tahir I, Shahri W. Flower senescence and some postharvest considerations of *Amaryllis belladonna* cut scapes. *Plant Physiol Rep.* 2020;25:315-24. <https://doi.org/10.1007/s40502-020-00506-8>
  31. Bayat H, Aminifard MH. Salicylic acid treatment extends the vase life of five commercial cut flowers. *Electron J.* 2017;13:1
  32. Ghadimian SV., Orojloo M. 2020. Evaluation of the physical characteristics and nutritional value of five varieties of dates (*Phoenix dactylifera* L.) in two years of storage. *ALKHAS; The Journal of Environment, Agriculture and Biological Sciences.* 2020,2(1):1-7. <http://dx.doi.org/10.29252/alkhass.2.1.1>
  33. Farooq S, Haq A, Lone ML, Parveen S, Altaf F, and Tahir I. Salicylic acid enhances the vase life and improves the postharvest attributes — A case study of *Antirrhinum majus* L. *East African Scholars J Agri Life Sci.* 2022;5(6):87-96. <https://doi.org/10.3390/easjals506087>

- [doi.org/10.36349/easjals.2022.v05i06.001](https://doi.org/10.36349/easjals.2022.v05i06.001)
34. Saeed T, Hassan I, Abbasi NA, Jilani G. Effect of gibberellic acid on the vase life and oxidative activities in senescing cut Gladiolus flowers. *Plant Growth Regul.* 2014;72:89-95. <https://doi.org/10.1007/s10725-013-9839-y>
  35. Honig M, Plíhalova L, Husickova A, Nisler J, Dolezal K. Role of cytokinins in senescence, antioxidant defence and photosynthesis. *Int J Mol Sci.* 2018;19(12):4045. <https://doi.org/10.3390/ijms19124045>
  36. Kaur P, Singh N, Mukherjee D. Regulation of membrane leakage and activities of some antioxidant enzymes in petals of cut flowers of *Calendula officinalis* and *Salvia splendens* with metabolites and plant growth regulators. *J Appl Hortic.* 2015;17:31-39. <https://doi.org/10.37855/jah.2015.v17i01.07>
  37. Kellogg DE. The role of phyletic change in the evolution of *Pseudocubus vema* (Radiolaria). *Paleobiology.* 1975;1:359-70. <https://doi.org/10.1017/S0094837300002669>
  38. Ahmad SS, Tahir I, Wani AS, Dar RA, Nisar S. Adenine type and diphenyl urea derived cytokinins improve the postharvest performance of *Iris germanica* L. cut scapes. *Physiol Mol Biol Plants.* 2018;24:1127-37. <https://doi.org/10.1007/s12298-018-0554-z>
  39. Siddiqui MW, Singh JP, Nayyar MdA, Barman K, Ahmad MS, Kumar V. 6-Benzylaminopurine affects lipid peroxidation and membrane permeability and thereby preserves curd quality and antioxidants during storage of cauliflower. *Acta Physiol Plant.* 2015;37:96. <https://doi.org/10.1007/s11738-015-1848-1>
  40. Ramtin A, Naderi R, Kalatejari S, Matinizadeh M. Comparison of plant growth regulators and exogenous ethylene effects on two types of cut carnation (*Dianthus caryophyllus* L.). *J Ornamental Plants.* 2019;9:55-64.
  41. Liu T, Longhurst AD, Talavera-Rauh F, Hokin SA, Barton MK. The Arabidopsis transcription factor ABIG1 relays ABA signaled growth inhibition and drought induced senescence. *Elife.* 2016;5:e13768. <https://doi.org/10.7554/eLife.13768>
  42. Dek MSP, Padmanabhan P, Sherif S, Subramanian J, Paliyath AG. Upregulation of phosphatidylinositol 3-Kinase (PI3K) enhances ethylene biosynthesis and accelerates flower senescence in transgenic *Nicotiana tabacum* L. *Int J Mol Sci.* 2017;18:1533. <https://doi.org/10.3390/ijms18071533>
  43. Farooq S, Lone M., Altaf F, Parveen S, Tahir I. Boric acid as a potential substitute for conventional ethylene antagonists in mitigating postharvest flower senescence of *Digitalis purpurea*. *Ornamental Horticulture.* 2021;27:516-25. <https://doi.org/10.1590/2447-536X.v27i4.2374>
  44. Lone ML, Farooq S, Parveen S, & Tahir I. 6-Benzylamino purine outperforms Kinetin and Thidiazuron in ameliorating flower longevity in *Calendula officinalis* L. by orchestrating physiological and biochemical responses. *Ornamental Horticulture.* 2021;27:183-95. <https://doi.org/10.1590/2447-536X.v27i2.2260>