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

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## 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 µM HBL, 300 µM HBL, 10 µM ABA, 50 µM ABA, 500 µM BAP and 1000 µM 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 ± 2 $^0$C and relative humidity of 65 ± 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 m mol$^{-1}$ 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 µl) 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 µl) to the substrate solution. The absorbance was recorded at 234 nm and LOX activity was determined as a change in $\Delta A_{234}$ min$^{-1}$ g$^{-1}$ 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 µM ABA and 500 µM 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 µM HBL-treated floral spikes and control in both cultivars of Gladiolus. Moreover, 300 µM 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).

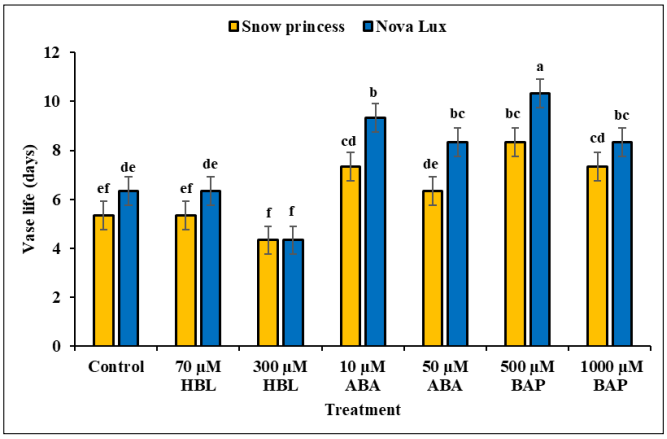


**Fig. 1.** Influence of different concentrations of HBL, ABA and BAP on vase life of Gladiolus. Data represent mean values ± SD; n=3. Bars bearing different letters indicate significance difference means (Duncan's test; P≤0.05).

### *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 µM HBL exhibited not much significant difference in cumulative uptake of vase solution in both cultivars as compared to control. Moreover, 300 µM 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 µM BAP concentration by floral spikes of nova lux and snow princess (64.33 ml/spike and 47.33 ml/spike, respectively) followed by 10 µM 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).

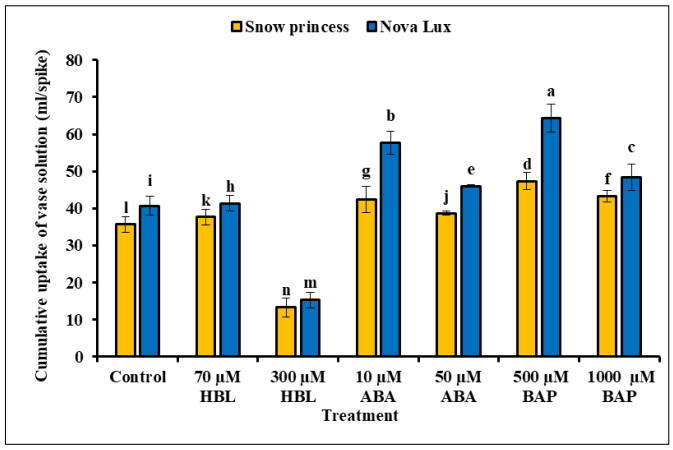


**Fig. 2.** Influence of different concentrations of HBL, ABA and BAP on cumulative uptake of vase solution in Gladiolus. Data represent mean values ± SD; n=3. Bars bearing different letters indicate significance difference means (Duncan's test; P≤0.05).

### *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 µM 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 µM 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 µM BAP and 10 µM 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 µM HBL, 10 µM ABA and 500 µM BAP concentrations on membrane integrity, lipid peroxidation and lipoxygenase activity in floral spikes of nova lux and snow princess cultivars of Gladiolus during senes-

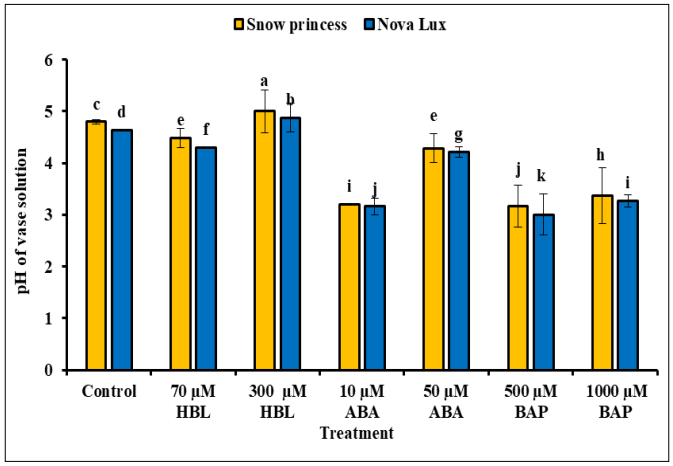


**Fig. 3.** Influence of different concentrations of HBL, ABA and BAP on the pH of vase solution of Gladiolus. Data represent mean values ± SD; n=3. Bars bearing different letters indicate significance difference means (Duncan's test; P≤0.05).

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 µM BAP (73.85% and 64.56%, respectively) followed by 10 µM ABA (45.86% and 36.35%, respectively) and 70 µM HBL (5.36%, 5.13%, respectively) in comparison to control

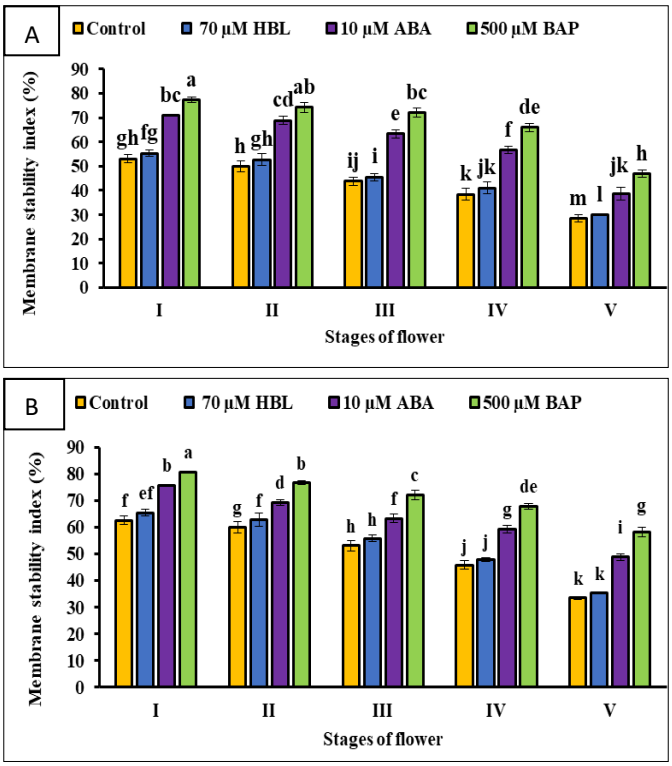


**Fig. 4.** Effect of 70 µM HBL, 10 µM ABA and 500 µM BAP on membrane stability index (%) in (**A**) Snow princess and (**B**) Nova lux cultivars of Gladiolus during flower senescence. Different stages of flower development of Gladiolus are: I stage -Slightly opened / point brush stage; II stage- Half-opened stage; III stage - Fully opened stage; IV stage - Incipient senescence; stage; V stage – Senescent stage. Data represent mean values ± SD; n=3. Bars bearing different letters indicate significance difference means (Duncan's test; P≤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 µM 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 µM ABA (43.71% and 38.18%, respectively) and 70 µM HBL

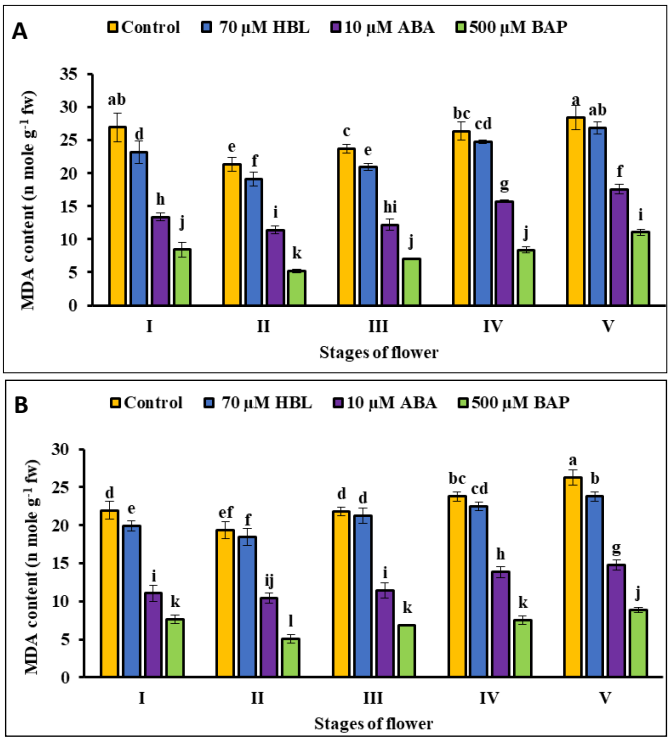


**Fig. 5.** Effect of 70 µM HBL, 10 µM ABA and 500 µM BAP on MDA content (n mole $g^{-1}$ fw) in (**A**) Snow princess and (**B**) Nova lux cultivars of Gladiolus during flower senescence. Different stages of flower development of Gladiolus are: I stage -Slightly opened / point brush stage; II stage-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

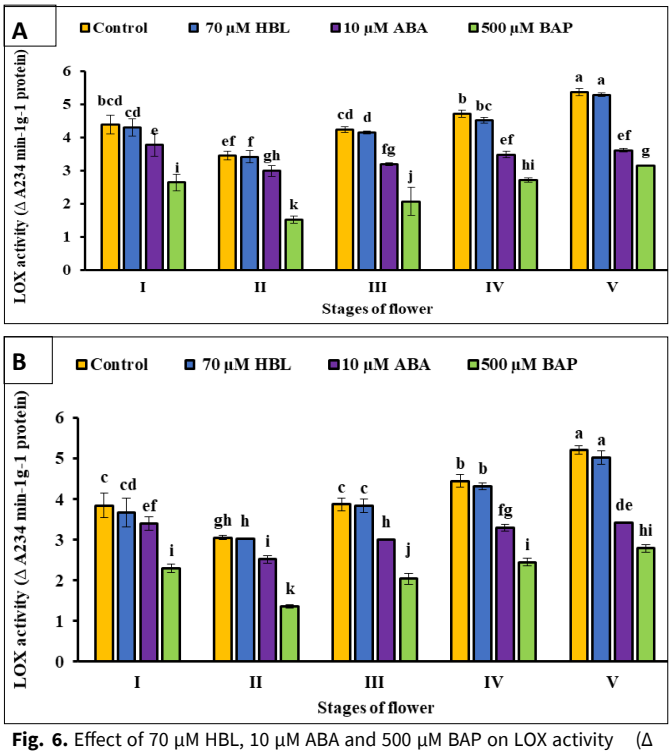


**Fig. 6.** Effect of 70 µM HBL, 10 µM ABA and 500 µM BAP on LOX activity (Δ $A_{234}$ $min^{-1}g^{-1}$ protein) in (**A**) Snow princess and (**B**) Nova lux cultivars of Gladiolus during flower senescence. Different stages of flower development of Gladiolus are: I stage -Slightly opened / point brush stage; II stage- Half-opened stage; III stage - Fully opened stage; IV stage - Incipient senescence; stage; V stage – Senescent stage. Data represent mean values ± SD; n=3. Bars bearing different letters indicate significance difference means (Duncan's test; P≤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 µM BAP treatment that is followed by 10 µM ABA (34.10% and 32.71%, respectively) and 70 µM 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 µM 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 thiadizuron (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 µM BAP and 10 µM ABA, the uptake of the vase solution was enhanced. However, BAP (500 µM) treated flowers exhibited a greater vase uptake than ABA (10 µM) 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 µM) 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 µM) treatment reduced the pH of the vase solution of nova lux and snow princess most effectively followed by 10 µM 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 µM) 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 µM BAP and 10 µM ABA significantly increased the membrane stability index in comparison with control in both cultivars of Gladiolus. The membrane stability index changed significantly after 500 µM BAP treatment compared to 10 µM 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* thiadizuron 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 thidiazuron 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 µM) 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 µM BAP and 10 µM 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 µM BAP exhibited a reduced rate

of lipid peroxidation compared to 10 µM ABA in both cultivars. However, 70 µM 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 µM BAP and 10 µM 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 µM BAP and 10 µM 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 µM 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 µM HBL, 300 µM HBL, 10 µM ABA, 50 µM ABA, 500 µM BAP and 1000 µM BAP were used. It is concluded from the present study that 500 µM BAP and 10 µM 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 µM BAP was shown to be the most effective and outperformed 10 µM 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 µM 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.

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## 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

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