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Thidiazuron outpaces 6-benzylamino purine and kinetin in delaying flower senescence in *Gladiolus grandiflora* by alleviating physiological and biochemical responses

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ABSTRACT

An experiment was carried out to elucidate the role of thidiazuron (TDZ), 6-benzylamino purine (BAP), and kinetin on the vase life of *Gladiolus grandiflora* cut flower. Harvested flowers were treated with BAP, kinetin, and TDZ of various concentrations, viz., 100 µM, 0.5 mM, and 1 mM. Our findings revealed that there was a significant increase in flower longevity in treatment with BAP, kinetin, and TDZ as compared to untreated flowers. Vase solutions which contain BAP (0.5 mM), kinetin (1 mM), and TDZ (100 µM) were most effective in improving vase life (14, 13, and 15 days, respectively) of cut *Gladiolus* floral spike. Flower vase life was extended with increase in fresh weight, vase solution uptake, high membrane integrity, besides reduced pH of vase solution, malondialdehyde content, and lipoxygenase activity in the flowers. However, TDZ outpaced BAP and kinetin in improving the vase life of *G. grandiflora*.

1. INTRODUCTION

Flowers are the excellent model system to unveil the physiological and biochemical processes during senescence as the senescence process in flowers is rapid and foreseeable. Senescence is defined as an age-dependent degenerative process in plants leading to death [1]. The senescence process involves halt of various biochemical pathways and up-regulation of many degradative pathways, finally leading to the cell, tissue, or whole plant death [2]. Genetically, senescence is determined and it is governed by the various plant hormones and environmental factors during plant development process. During senescence process, a number of changes such as reduced fresh weight, vase solution uptake, loss of membrane integrity, increase in lipid peroxidation, production of reactive oxygen species (ROS), increase in membrane lipid peroxidation, degradation of fatty acids, proteins, DNA, RNA, and sugars are induced [3–5]. Morphological changes which are visible during petal senescence are change of petal color and rolling of petals [6], and anatomical changes include degradation of mesophyll cells before morphological changes are apparent [7,8].

It is the life span and quality of the cut flower that determine the commercial value of flowers [9]. To increase flower life span, it is important to understand physiological and biochemical processes associated with petal senescence [10]. Cytokinins act as senescence retarding hormones that increase the life span in both the ethene-sensitive and ethene-insensitive flowers [1,11]. Because of cytokinin delays senescence process, it is used in postharvest technology to enhance the postharvest life of flowers [12]. Cytokinin delays flower senescence by increasing uptake of water, fresh weight sink activity, metabolites contents in flower petals, besides preventing membrane lipid peroxidation [11,13]. Cytokinin both adenine type 6-benzylamino purine (BAP, kinetin) and diphenyl urea derived thidiazuron (TDZ) [14] retard senescence process by increasing the content of various metabolites, vase solution uptake, besides preventing peroxidation of membrane lipids [12]. Exogenous application of cytokinin has important

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role in enhancing vase life of flowers like lotus, Gerbera, Iris and daylily [15–17]. Although both classes of cytokinin are effective in enhancing vase life, however, the role of TDZ is more promising in cut flowers.*Gladiolus grandiflora* "*White Prosperity"* cultivar is a mid-season bloomer which is grown in India as an ornamental plant and has a great role in floriculture market [18]. However, short span of vase life of flower reduces its potential commercial value. *Gladiolus* is ethylene insensitive; therefore, the reduced vase life is because of oxidative stress [19] and role of cytokinin's in ameliorating oxidative stress during flower senescence in *G. grandiflora* "*White Prosperity" cultivar* has not been explored yet. In this respect, an experiment was depicted to study the effect of BAP, kinetin, and TDZ in alleviating flower senescence in *G. grandiflora* by assessing physiological and biochemical parameters to improve its postharvest life span.

2. MATERIALS AND METHODS

All the experiments were performed in the Department of Botany, SSN College, University of Delhi.

2.1. Plant Material

Gladiolus grandiflora, i.e., "*White Prosperity." Gladiolus* spikes used in the experiment were collected from Indian Agricultural Research Institute, New Delhi.

2.2. Experimental Treatments

Uniform length (15 cm) of the floral spikes was cut and it is divided into four sets. Three sets of spikes were subjected to different concentrations of BAP, kinetin, and TDZ, viz., 100 µM, 0.5 mM, 1 mM and another set of floral spikes were kept in Milli Q and referred as control. Each treatment was represented by three replicates and the experiment was performed at temperature of 22°C ± 3°C and relative humidity 67% ±_3% and light intensity 1000 lux.

2.3. Estimation of Vase Life

The flower spikes fresh weight was determined on a daily basis till the end of the vase life of flower. The vase life of floral spikes was defined as when the fresh weight of floral spikes was less than its initials fresh weight.

2.4. Vase Solution Uptake Estimation

The floral spikes were kept undisturbed in the test tubes containing 25 ml of solution. The uptake of vase solution by spikes was measured and expressed as cumulative uptake of vase solution in ml/spike.

2.5. pH of Vase Solution

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

2.6. Membrane Stability Index (MSI)

The MSI of *Gladiolus* flower petals was measured using the experimental protocol of Bailly *et al.* [20]. Petal tissue (200 mg) was immersed in the tube containing 10 ml Milli Q water. After 5 hours of incubation, the electrical conductivity (C_1) of the solution was determined using conductometer (HACH analyzer, HQ440d multi). The petal tissue was then heated for 30 minutes and the conductivity (C_2) was measured.

$$MSI = [1-(C_1/C_{-2})] \times 100$$

2.7. Malondialdehyde (MDA) Content

The lipid peroxidation was measured in terms of MDA content by following the experimental protocol of Heath and Packer [21]. Petal (500 mg) was homogenized in 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged for 5 minutes at 15,000 rpm (Beckman refrigerated centrifuge, Model J2-21). To the supernatant, 4 ml of 0.5% thiobarbituric acid in 20% TCA was added [21]. The content was boiled for 30 minutes and then quickly cooled. Then centrifuged for 10 minutes at 10,000 rpm (Beckman refrigerated centrifuge, Model J2-21). The absorbance data of the supernatant was recorded at 532 nm using Eppendorf UV-VIS Spectrophotometer, 5810 R. The MDA content of the sample was calculated using its absorption coefficient of 155 m mol^{-1} cm^{-1}.

2.8. Lipoxygenase (LOX) Activity

The LOX enzyme activity was measured using the experimental protocol given by Doderer *et al.* [22]. Flower petals (500 mg) were homogenized in phosphate buffer solution (0.1 M) containing ethylenediaminetetraacetic acid (EDTA) (0.5 mM). The homogenate was centrifuged (Beckman refrigerated centrifuge, Model J2-21) for 15 minutes at 15,000 rpm. The supernatant was referred as enzyme extract. LOX activity was measured by adding enzyme (50 µl) to substrate solution made by adding 70 µl linoleic acid to 10 ml Milli Q having 100 µl tween 20, final volume adjusted to 200 ml using 0.1 M phosphate buffer. Absorbance data was recorded at 234 nm and the enzyme activity was measured as change in ΔA_{234} $minutes^{-1}$ g^{-1} protein [22].

2.9. Statistical Analysis

We used a completely randomized design with three replicates. The experimental data was examined using Statistical Package for the Social Sciences (SPSS) 21 Statistical program (IBM SPSS Statistics 21) by one way analysis analysis of variance. Comparison of the means was determined by post hoc Duncan's test ($p < 0.05$).

3. RESULTS

The standardization of BAP, kinetin, and TDZ concentration in extending postharvest life of cut flowers of *G. grandiflora* was carried out. Various concentrations of BAP, kinetin, and TDZ, viz. 100 µM, 0.5 mM, and 1 mM were used. Application of 0.5 mM BAP and 1 mM kinetin and 100 µM TDZ exhibited maximum enhancement of vase life of *Gladiolus* (Fig. 1) due to increase in various physiological parameters (fresh weight of flowers, uptake of vase solution, membrane integrity) and decrease in biochemical parameters (lipid peroxidation and LOX activity). However, among all the cytokinin treatments (BAP, kinetin, and TDZ), TDZ was found as most effective in enhancing the vase life of *Gladiolus*.

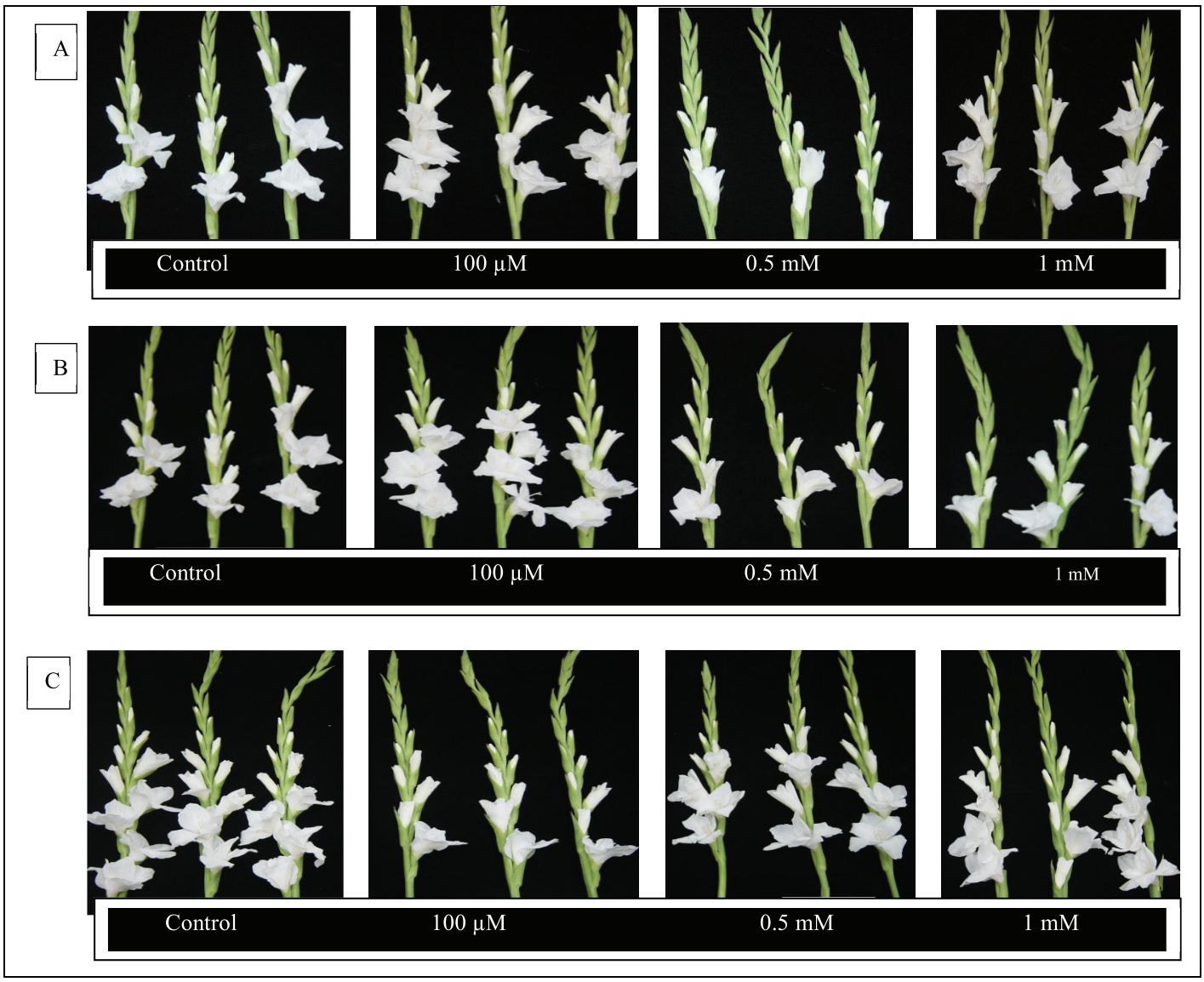

Figure 1: Influence of different concentrations of (A) BAP (B) kinetin, and (C) TDZ during flower senescence in comparison to control.

3.1. Vase Life

The cut flower kept in Milli Q water (control) had a vase life of 11 days only; however, treatment with cytokinin increased the vase life of cut *Gladiolus* flowers evidently. The vase life of flowers kept in 0.5 mM BAP, 1 mM kinetin, and 100 µM TDZ solutions increased to14, 13, and 15 days, respectively, indicating that TDZ was the most efficient cytokinin in enhancing the vase life of *Gladiolus* (Fig. 2).

3.2. Vase Solution Uptake

Various concentrations of BAP, kinetin, and TDZ increased the cumulative uptake of vase solution as compared to untreated flowers. It was found that the 100 µM TDZ concentration was most effective in increasing uptake of vase solution (118 ml/spike) by flower spike followed by 0.5 mM BAP (98.66 ml/spike) and 1 mM kinetin (95.00 ml/spike) as compared to control (53.99 ml/spike) (Fig. 3).

3.3. pH of Vase Solution

Different concentrations of BAP, kinetin, and TDZ decreased the pH of vase solution as compared to control. It is revealed from Figure 4 that 100 µM TDZ concentration was most effective in reducing pH of vase solution (pH 3.00) followed by 0.5 mM BAP (pH 3.17) and 1 mM kinetin (pH 3.45) as compared to control (pH 4.95).From the aforesaid results, it is clear that the 0.5 mM BAP, 1mM kinetin, and 100 µM TDZ treatments had maximum effect on improving vase life of *Gladiolus*. Further, studies were made to understand the effect of 0.5 mM BAP, 1mM kinetin, and 100 µM TDZ on membrane stability index, MDA content, and LOX activity in *Gladiolus* during senescence.

3.4. Membrane Stability Index (MSI)

A linear decline in MSI was observed in both cytokinin treated and untreated flowers during the flower development process (Fig. 5). There was a significant difference in MSI cytokinin treated flowers

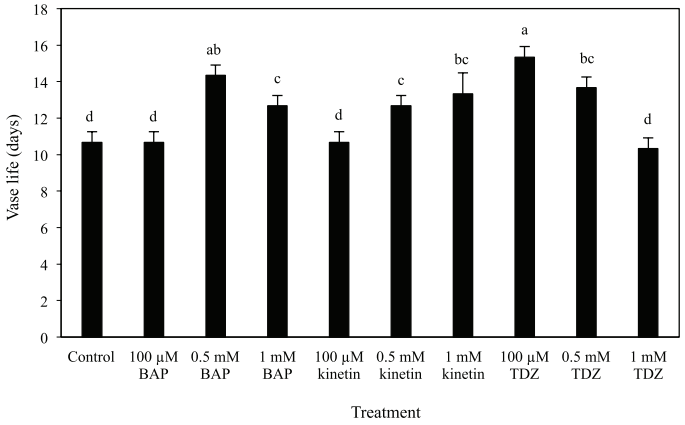

Figure 2: Influence of different concentrations of BAP, kinetin, and TDZ on vase life of *Gladiolus.*

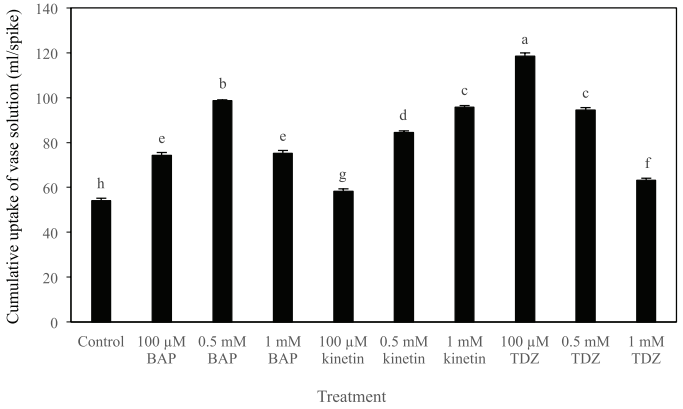

Figure 3: Influence of different concentrations of BAP, kinetin, and TDZ on vase solution uptake of *Gladiolus.*

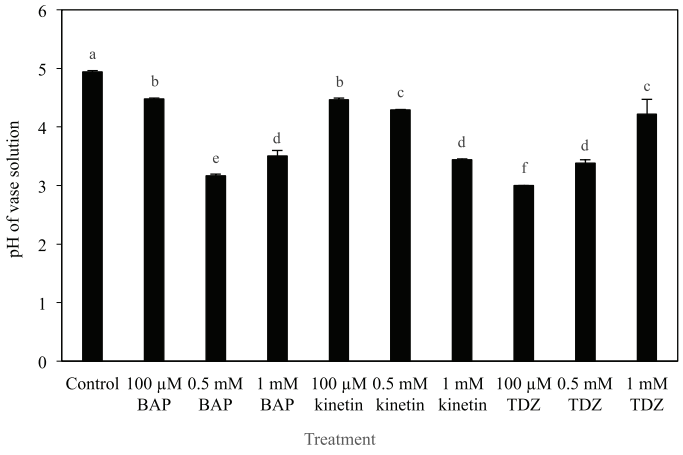

Figure 4: Influence of different concentrations of BAP, kinetin, and TDZ on the pH of vase solution of *Gladiolus.*

and control. The decrease in MSI was significantly alleviated at stage V by treatment with 100 µM TDZ (62.07%) followed by 0.5 mM BAP (36.76%) and 1 mM kinetin (25.75%) in comparison to control (Fig. 6).

Figure 5: Different stages of flower development of *Gladiolus*. 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.

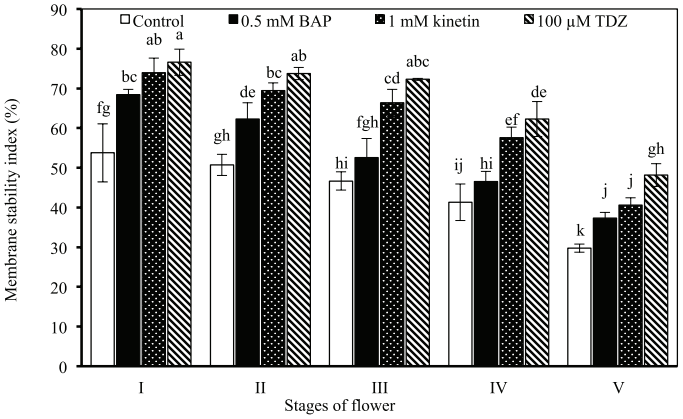

Figure 6: Effect of 1 mM kinetin, 0.5 mM BAP, and 100 µ M TDZ on membrane stability index (%) as compared to control in *Gladiolus* flower during senescence.

3.5. MDA Content

Lipid peroxidation level was studied in terms of MDA content and decrease in the MDA content was observed till stage II, then gradual increase was observed till stage V in both cytokinin treated flowers and in control. Cytokinin-treated flowers had decreased amount of MDA content throughout flower development as compared to the untreated flowers. MDA content displayed a decrease by 63.70% in flowers treated with TDZ that is followed by BAP and kinetin where the MDA content was reduced by 36.72% and 22.53%, respectively, as compared to untreated flowers where MDA level was maximum (Fig. 7).

3.6. LOX Activity

The LOX activity decreased gradually till stage II and then activity increased till stage V in both control and cytokinin treated flowers (Fig. 8). At any given stage, the LOX activity was remarkably less in cytokinin-treated flowers as compared to controlled one. LOX activity significantly decreased by 44.20%, 36.10%, and 12.15% in the flowers treated with 100 µM TDZ, 0.5 mM BAP, and 1 mM

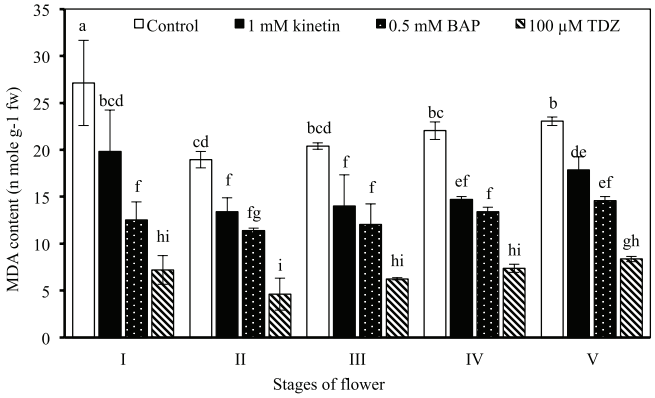

Figure 7: Effect of 1 mM kinetin, 0.5 mM BAP, and 100 µM TDZ on MDA content (n mole g^{-1} fw) as compared to control in *Gladiolus* flower during senescence.

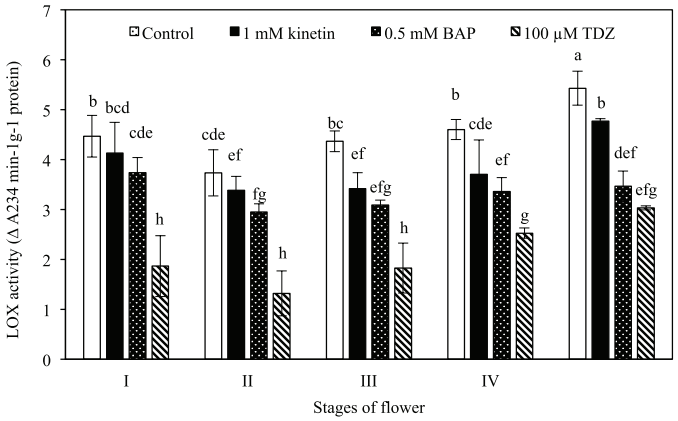

Figure 8: Effect of 1 mM kinetin, 0.5 mM BAP, and 100 µ M TDZ on LOX activity (ΔA_{234} $minute^{-1}g^{-1}$ protein) as compared to control in *Gladiolus* flower during senescence.

kinetin as compared to control. Minimum LOX activity was seen in the flowers subjected to 100 µM TDZ treatment.

4. DISCUSSION

Senescence is defined as age-dependent degenerative process leading to plant death [1]. Indeed, flowers have the showiest petals but have the shortest life span too. The senescence of flowers is fast and foreseeable; therefore, flowers are the perfect model for the study of the senescence [23]. In the market, the life of petals determines the life span of the cut flower [9]. The life span of cut flowers is influenced by number of factors which vary in different ornamental plants [24]. Therefore, the determination of petal senescence helps us to enhance the life span of flowers, as well as in understanding the mechanisms underlying petal senescence [25].

The difference between vase life of cytokinin treated flowers was remarkable than the control. The vase life was increased by treatment with kinetin (1 mM), TDZ (100 µM), and BAP (0.5 mM) (Fig. 2). However, the vase life of TDZ (100 µM) treated flowers was more pronounced than BAP (0.5 mM) followed by kinetin (1 mM). The vase life was enhanced due to increased fresh weight, vase solution uptake, and decreased ion leakage. The vase life of cut rose flowers was increased due to kinetin treatment [26]. Chamani *et al.* [27] reported that TDZ significantly enhanced the fresh biomass and life span of cut carnation flowers. Gulzar *et al*. [28] reported that kinetin and cycloheximide increased the vase life of daylily flowers by maintaining higher fresh mass. Marandi *et al*. [29] showed that essential oils, salicylic acid increased the vase life of cut Gladiolus flowers by increasing fresh weight and vase solution uptake. BAP, kinetin, and TDZ increased the life span of cut *Gladiolus* flowers by influencing membrane stability and water balance [30,31]. Cytokinin application delays senescence in cut flowers such as *Chrysanthemum, Hemerocallis, Eustoma, Anthurium,* and *Nicotiana* [1,17,31–33]. Application of cytokinins might improve flower vase life by providing more photosynthates to the developing flower bud which creates a water potential gradient, leading to more uptake of water making petal tissue more turgid [17]. This reduction in vase life of control may be due to decreased water uptake [34] and increased permeability of cell membrane.

There was a significant difference between vase solution uptake by cytokinin treated flowers and control. The vase solution uptake was increased by treatment with kinetin (1 mM), TDZ (100 µM), and BAP (0.5 mM) (Fig. 3). However, the vase uptake by TDZ (100 µM) treated flowers was more pronounced than BAP (0.5 mM) followed by kinetin (1 mM). This may be due to decreased pH of vase solution. When the pH of vase solution was lowered, the vase solution traveled faster in the water conducting system. Similar work was reported [35], where salicylic acid enhanced the uptake of vase solution of cut flowers by reducing the pH of vase solution.

There was a remarkable difference in pH value of cytokinin treated flowers and control. The BAP (0.5 mM), kinetin (1 mM), and TDZ (100 µM) acidified (pH 3.17, 3.45, 3.00, respectively) (Fig. 4) the vase solution. When the pH of vase solution was lowered the vase solution travelled faster in the water conducting system.At this pH, the bacterial growth might be reduced, thus improving water uptake. Gowda and Gowda [36] also showed that aluminum sulfate treated flowers lowered the pH of vase solution and reduced the growth of microbes in vase solution during senescence.

The membrane stability index was significantly increased by treatment with 0.5 mM BAP, 1 mM kinetin, and 100 µM TDZ in comparison to control (Fig. 6). However, 100 µM TDZ treatment significantly increased the membrane stability index than 0.5 mM BAP treatment followed by 1 mM kinetin treatment. The change in membrane stability index demonstrates the rate of ion leakage from petals. Leakage of electrolyte has been observed in *Alstroemeria peruviana* [37] during flower senescence. The life span of flowers can be modulated by free radial scavengers [38] suggesting involvement of free radicals during senescence process. Kinetin delayed senescence in cut roses by maintaining cell membrane integrity [26]. The daylily flowers treated with cycloheximide and kinetin maintained the membrane integrity [14,28]. Mortazavi *et al*.[14] reported that TDZ and nitric oxide delayed senescence in cut rose flower by maintain membrane integrity. It is now reasonable to propose that cytokinin may act as free radical scavenger, thus maintaining membrane integrity for extended life span. Cytokinin maintains integrity of cellular membrane by

enhanced water uptake and reduced lipid peroxidation in plants during senescence [39] Kaur *et al.* [40] reported that BAP could enhance membrane stability index in *Calendula officinalis* during senescence. Cytokinin treatment enhanced the membrane integrity due to its participation in maintenance of highwater content, and decreased lipid peroxidation rates in petals, which are affected by senescence [32,39].

It was observed that the MDA content was increasing from the stage III to stage V in both treated and control flowers. However, 0.5 mM BAP, 1 mM kinetin, and 100 µM TDZ treated flowers showed reduced level of MDA during flower development as compared to untreated flowers (Fig. 7). Moreover, 100 µM TDZ treated flowers showed reduced level of lipid peroxidation as compared to 0.5 mM BAP followed by 1 mM kinetin. The MDA content via lipid peroxidation is enhanced by ROS [41]; therefore, 0.5 mM BAP, 1 mM kinetin, and 100 µM TDZ might play a pivotal role in abating the levels of free radicals by decreasing LOX activity. TDZ and BAP treatment increased the cell membrane integrity because of the decreased LOX activity which reduced peroxidation of membrane lipids [32]. Moreover, BAP treated flowers showed a remarkable decrease in MDA content and increase in MSI in cauliflower and carnations during senescence [42,43]. The treatment has reduced the lipid peroxidation; therefore, the role of cytokinin as free radical scavenger can be confirmed.

Gladiolus is ethylene insensitive; therefore, oxidative stress is the main reason of senescence in *Gladiolus*. There is a significant difference in LOX activity in cytokinin treated flowers and control. The LOX activity showed a significant decrease in 0.5 mM BAP, 1 mM kinetin, and 100 µM TDZ treatments as compared to control (Fig. 8). Moreover, TDZ was highly effective in maintaining reduced level LOX activity than 0.5 mM BAP followed by 1 mM kinetin, which is apparent from lower level of MDA and increased membrane stability index. The decrease in the LOX activity in cytokinin treated plants resulted in preventing the phospholipids and proteins structure by reducing the secretion of proteases into the cell cytoplasm [44,45]. Similar work was also reported by where TDZ, kinetin, and BAP application improved the postharvest life of *Iris germanica* by decreasing LOX activity and preventing membrane lipid peroxidation [32]. The decreased LOX activity maintained the membrane integrity by preventing the release of proteases into the cytoplasm from vacuoles thereby maintaining the structure of phospholipids, proteins, and thiols [44,45].

5. CONCLUSION

In conclusion, BAP, kinetin, and TDZ reduced the senescence induced oxidative damage in *G. grandiflora* which is evident from improved flower life span. TDZ was found most effective and outpaced BAP and kinetin in increasing vase life of cut flowers. Cytokinin treatment resulted in maintaining higher fresh weight, vase life membrane stability, besides maintaining lower pH, lipid peroxidation, and LOX activity. The possible role of cytokinins (BAP, kinetin, and TDZ) in controlling senescence process in *G. grandiflora* "White prosperity" cultivar is yet to be explored. Detailed study on a crucial role of cytokinin in retarding of senescence and increasing postharvest life of flowers need to be done at physiological and biochemical level. Furthermore, the mode of action of TDZ should be studied in detail, as it has been showing best results delaying senescence at comparatively lower concentrations. Understanding the mechanistic way of flower senescence in *Gladiolus* will unveil strategies to improve its post-harvest performance.

6. ACKNOWLEDGMENTS

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7. CONFLICT OF INTEREST

The authors do not have any conflict of interest in this research.

8. FUNDING

There is no funding to this research.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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