Indian Journal of Experimental Biology
Vol. 61, June 2023, pp. 450-462
DOI: 10.56042/ijeb.v61i06.1942

Plant growth regulators improved cutting induced physio-biochemical responses to postponement senescence in chrysanthemum

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Received 27 January 2023; *revised 13 April 2023*

The marketability of cut flowers relies on its postharvest longevity, making it crucial to develop technologies that can extend its vase life. The vase life in chrysanthemum is dependent upon floret senescence and leaf yellowing. In view of extending the vase life, the present study elucidates the implication of plant growth regulators viz. benzyl adenine (BA), thidiazuron (TDZ) and salicylic acid (SA) on postharvest performance and flower longevity of chrysanthemum stems. The harvested stems were treated with various concentrations viz. BA and SA at 50,100,150 and 200 µM and TDZ at 5,10,15 and 20 µM. Treatments containing 10 µM TDZ and 100 µM BA were most effective in improving the longevity (23.35 and 20.32 days respectively) of cut chrysanthemum stems. However, TDZ outplayed BA followed by SA extends the flower longevity through the maintenance of higher physiological and biochemical characteristics. Cluster analysis inferred that the treatments of 50 and100 µM BA and 5 and10 µM TDZ were found to be efficient in delaying floret senescence (24.01 days) and leaf yellowing (20.54 days) in cut chrysanthemum stems. Postharvest longevity exhibited a positive correlation with antioxidant activities, total soluble sugars and proteins, while it showed a negative correlation with anthocyanin and carotenoid contents. The results have demonstrated that use of growth regulators such as benzyl adenine, thidiazuron and salicylic acid may preserve the quality of cut chrysanthemum stems and delay their senescence.

Keywords: Benzyl adenine, Flower longevity, Flower senescence, Post-harvest quality, Salicylic acid, Thidiazuron, Vase life

The natural developmental stage of flowers is flower senescence, which involves various biochemical and physiological changes. These changes include loss of water from the senescing tissue, leakage of ions, increased membrane fluidity, generation of reactive oxygen species (ROS) and hydrolysis of proteins, carbohydrates, and nucleic acids[1]. The loss of cut flower quality and shorter vase life is attributed to premature senescence, which occurs soon after harvesting, negatively affecting market demand and industry profitability[2]. The postharvest lifespan of flowers plays a crucial role in determining the commercial worth and consumer satisfaction of decorative flowers[3]. A major challenge for postharvest researchers is to reduce the damage to flowers caused by cutting in order to alleviate petal senescence symptoms[4]. Farmer and wholesalers would be able to transport cut flowers with desirable quality further away from their farms, and sellers and consumers would be able to store them for longer time by delaying the senescence process[5].

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Chrysanthemum is one of the most valuable decorative flower in the global flower industry, appreciated for its broad range of colors and shapes, which makes it popular among consumers[6]. In India, total chrysanthemum production during 2021-22 has been estimated to be 470,150 metric tons led by Andhra Pradesh with 144.14 MT followed by Karnataka (133.66 MT) and Tamil Nadu (128.86MT). Loose flowers alone contribute to a remarkable amount of 454,200 MT, while 15,950 MT come from cut flowers[7]. Yellowing of leaves prior to floret senescence is the major issue with cut chrysanthemum stems, resulting in reduced vase life. Therefore, there is a significant demand for a preservative solution to address physiological and biochemical attributes that can effectively prolong the marketing period for cut chrysanthemum stems.

To increase the longevity of flowers, it is crucial to comprehend the biological processes that regulate postharvest physiology, hormonal imbalances, transpiration and the mechanisms linked with petal discoloration[8]. Cytokinins serve as primary anti-senescence hormones that enhance the longevity of

both ethylene-sensitive and insensitive cut flowers[9]. Because of their anti-senescence properties, cytokinins like benzyl adenine (BA) and thidiazuron (TDZ) are in high demand in postharvest technology to enhance the vase life of cut flowers[10].

Salicylic acid (SA), a plant hormone, plays a crucial part in regulating several biological processes and acclimation reactions to different environmental stresses[11]. Moreover, SA has gained more recognition because of its potential role in maintaining the postharvest quality of flowers, such as gladiolus[12].

In this study, we examined the effects of plant growth regulators viz. benzyl adenine, thidiazuron and salicylic acid on morpho-physiological and biochemical parameters to find differentials associated with prolonged postharvest longevity of cut chrysanthemum stems.

Material and Methods

Plant materials and treatments

This study was carried out on cut chrysanthemum stems var. White Star (spray type). The plants of chrysanthemum were raised through rooting of terminal cuttings at the Research Farm of the Department of Floriculture and Landscaping, PAU, Ludhiana. For post-harvest studies, flowers were chosen based on their comparable maturity stage and consistent size and quality. The leaves from lower one-third portion of cut stem were removed and the cut ends were recut under PGRs solutions in which these stems were to be pulsed for 24 h. The solutions were of BA (50,100, 150 and 200 µM), TDZ (5, 10, 15 and 20 µM) and SA (50, 100, 150 and 200 µM). After the treatment, the stems were transferred to flasks with 500 ml of distilled water for evaluating their quality parameters. Until the florets were fully senesced, all of the stems were stored at room temperature, specifically at 15 ± 2°C. Morphological observations viz. the number of days to initiation and complete floret senescence, number of days to initiation and 50% yellowing of leaves were recorded on the basis of visual analysis. The amount of water uptake by stems was calculated by measuring the volume of water at the termination of vase life and subtracting it from initial quantity of distilled water in containers. The biochemical analysis was conducted on florets (those from the outer, middle, and inner whorls were combined) while for the leaves (the middle part of the stems).

Membrane stability index (MSI) and Relative water content (RWC)

Premchandra *et al*.[13] method was used to assess the MSI by measuring the amount of electrolyte leakage. Samples were washed with deionised water and incubated for 30 min at 25°C, measure the electrical conductivity of the solution (EC_1) with a conductometer. After boiling the samples at 100°C for 30 min, the conductivity of the solution was measured again (EC2). The MSI was then computed using the following formula:

$$\text{MSI} = \frac{EC1}{EC2} \times 100$$

The method given by Weatherly[14] was used for determination of relative water content. The samples were placed in pre-weighed test tubes containing distilled water. The tubes were then re-weighed, and the increased weight was used to determine the fresh weight (FW). After 28 h, the saturated samples were weighed again to obtain the turgid weight (TW). The dry weight (DW) was obtained through oven-drying and RWC was calculated as following:

$$\text{RWC} = \frac{FW-DW}{TW-DW} \times 100$$

Total chlorophyll, anthocyanin and carotenoid content

The leaf samples were weighed, finely chopped, and homogenized in 5 mL of acetone[15] using a pestle and mortar. After homogenizing the mixture, it was centrifuged at 10,000 rpm for 15 min. The supernatant was diluted with 5 mL of acetone. The absorbance was measured using a spectrophotometer at 645 and 663 nm. Total chlorophyll content was determined using following formulas:

$$\text{Total chlorophyll content} = \frac{20.2\,(\text{A645}) + 8.02\,(\text{A663})\text{volume of extract}}{(100 \times \text{weight of sample})}$$

The content of total anthocyanins from florets was determined by Harborne's method[16]. The florets were homogenised in ethanolic HCl and absorbance was measured at 535 nm against blank in spectrophotometer. The total anthocyanin content was calculated using following formula:

$$\text{Total absorbance/100 mL} = \frac{(\text{e} \times \text{b} \times \text{c})}{(\text{d} \times \text{a})} \times 100$$

$$\text{Total anthocyanin (mg/100 mL)} = \frac{\text{Total absorbance for the sample}}{98.2}$$

The variables are defined as follows: 'a' represents the sample volume, 'b' represents the volume of extract utilized for colour measurement, 'c' represents the total volume, 'd' represents the volume of extract used, and 'e' represents the absorbance measured at a wavelength of 535 nm

The carotenoids from florets were determined using DMSO reagent[17] and absorbance was read at 470, 645 and 663 nm. The total carotenoid content was calculated using formula:

$$\text{Chl a} = 12.25\,(A663) + 2.79\,(A645) \times \frac{\text{volume of extract}}{(100 \times \text{weight of sample})}$$

$$\text{Chl b} = 21.5\,(A645) - 5.10\,(A663) \times \frac{\text{volume of extract}}{(100 \times \text{weight of sample})}$$

$$\text{Total carotenoids} = 1000\,(A470) - 1.82\,(\text{Chl a}) - 85.02\,(\text{Chl b}) \times \frac{\text{volume of extract}}{(100 \times \text{weight of sample})}$$

Total soluble sugars (TSS) and Total soluble protein (TSP) contents

Total soluble sugars extraction and determination was performed according to Dubois *et al.*[18] procedure. Florets and leaves were homogenised in ethanol and centrifuged at 5000 rpm for 10 min. The supernatant was collected, mixed with phenol and sulphuric acid and incubated for 20 min, the absorbance of orange brown colour was measured at 490 nm against blank in spectrophotometer. After homogenizing 100 mg of florets and leaves in a 0.1 M sodium phosphate buffer with a pH of 7.5 for protein extraction, the mixture was centrifuged at 5000 rpm for 10 min. The soluble protein determination was performed by Lowry *et al.*[19]. The results were expressed in mg/g FW. Total soluble sugars and total soluble proteins were determined using the formula below:

$$\frac{\text{Conc. of standard} \times \text{Absorbance of test sample} \times \text{Total volume of extract}}{\text{Absorbance of standard} \times \text{Volume of sample taken from extract} \times \text{Amount of tissue taken for extraction}}$$

Antioxidant enzyme activities

The peroxidase (POX) (EC 1.11.1.7)[20] and catalase (CAT) (EC 1.11.1.6)[21] enzymes were evaluated using established standard protocols for measuring enzyme activity.

A 0.1 M phosphate buffer with a pH of 7 was added to 500 mg of tissue and crushed, after which the homogenate was centrifuged in a chilled centrifuge at 0°C for 15 min at 10,000 rpm. The resulting supernatant was collected and diluted to a final volume of 10 mL for estimation. Catalase activity was measured in mmol H_2O_2 hydrolysed min^{-1} g^{-1} FW.

To determine the peroxidase activity, a mixture of 500 mg of tissue, 5 mL of 0.1 M phosphate buffer (pH 6) was crushed using a pestle and mortar and then centrifuged at 0°C. The resulting supernatant was collected and diluted to a final volume of 10 mL. For the enzyme assay, 0.1 mL of enzyme extract was mixed with 2.4 mL of 0.1 M phosphate buffer and 0.1 mL of 6 mM o-dianisidine in a test tube. The mixture was then subjected to spectrophotometric analysis at 430 nm, with absorbance readings taken every 30 s over a 3-min period. The POX activity was expressed as the change in absorbance per minute per gram of fresh weight (Δ in absorbance $min^{-1}g^{-1}$ FW).

Stastical analysis

The experiment followed a completely randomized design (CRD), with three replications per treatment. The data obtained underwent statistical analysis of variance (ANOVA) through SPSS software (version 24.0.0). Significant differences between treatments were calculated by conducting mean comparisons using the least significant differences (LSD) test with a 0.05% probability. Additionally, the SPSS software was used to perform hierarchical cluster analysis and correlation analysis.

Results

Floret senescence

The cut chrysanthemum stems treated with different growth regulators showed significant variations (Fig. 1) in the number of days until the initiation of florets, as well as complete floret senescence. The florets treated with distilled water (control) and 50 µM SA exhibited an inferior commercial aspect as they initiated floret senescence after 11.15 and 11.20 days, respectively. However, 10 µM TDZ treatment had the most influential effect in delaying the initiation of floret senescence among all the treatments, as they initiated senescence after 17.25 days. Cut stems of chrysanthemums held in distilled water lasted for only 17.30 days. However, the use of plant growth regulators (PGRs) extended the lifespan of the stems in nearly all treatments. Furthermore, the use of 10 µM TDZ and 100 µM BA resulted in the greatest improvement in the longevity of the flowers, with the blooms lasting for 25.40 and 24.20 days, respectively. Among all the treatments, the least days for floret senescence were recorded in control (17.30 days) followed by 50 µM SA (18.20

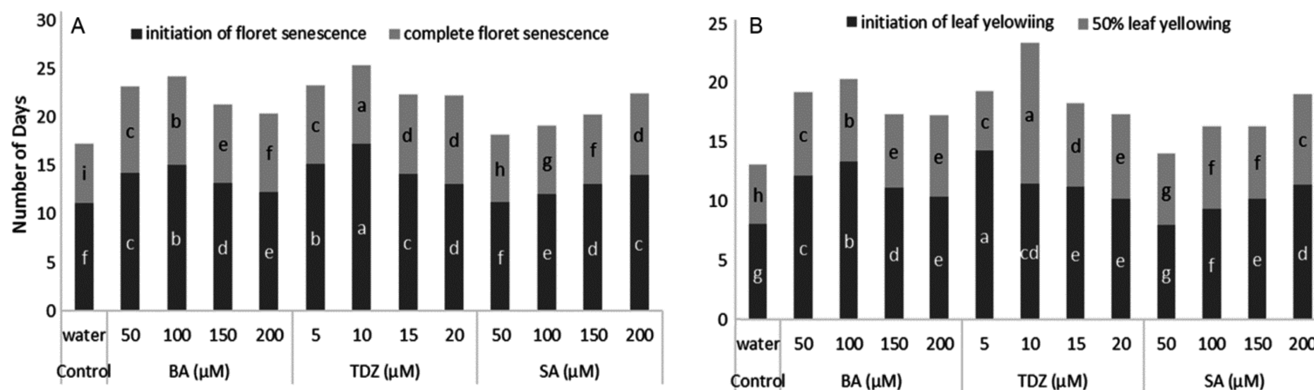


Fig. 1 — Effect of different concentrations of plant growth regulators on floret senescence and leaf yellowing of cut chrysanthemum stems

days). Among different concentrations of SA, 200 µM SA was the most effective in delaying senescence, with 22.40 days, followed by 150 µM SA which resulted in 20.25 days which was statistically at par with the 200 µM BA (20.25 days).

Yellowing of leaves

Stems treated with 50 µM SA showed the earliest onset of leaf yellowing, occurring at 8.05 days, which was comparable to stems treated with water at 8.11 days. (Fig. 1). Data depicted that the maximum delay in initiation of leaf yellowing was observed in stems treated with 5 µM TDZ (14.27 days). The stems treated with 100 µM BA prolonged the longevity via a delay in the onset of leaf yellowing (13.37 days), compared to other concentrations of BA (12.10 days with 50 µM BA, 11.20 days with 150 µM BA and 10.36 days with 200 µM BA). Among all the concentrations of SA, maximum delay was observed in stems treated with 200 µM SA (11.40 days). Stems treated with 10 µM TDZ exhibited the longest delay in 50% yellowing (23.35 days), whereas in control, the cut stems showed the symptoms of leaf yellowing after 13.07 days only. Salicylic acid also showed a significant delay in leaf senescence, as 50% yellowing of leaves was observed after 14.06, 16.32, 16.34 and 19.10 days at 50, 100, 150 and 200 µM SA, respectively.

Vase life

The vase life of cut chrysanthemum stems was significantly affected by the different treatments applied. The vase life was notably longer when growth regulators were applied compared to the control treatment (water). The application of 10 µM TDZ resulted in maximum enhancement in flower longevity, with a lifespan of 25.40 days, meaning the florets can be acceptable for that long. While the maximum delay in 50% yellowing with 10 µM TDZ was 23.35 days. If the leaves on cut stems displayed 50% senescence or yellowing, even if the florets remained fresh, it is worth noting that the stems lost their vase life or became unacceptable. Therefore, 10 µM TDZ was the most effective treatment for chrysanthemums with a vase life of 23.35 days before 50% leaf yellowing was reached. In comparison, the control and 50 µM SA treatments reached this stage several days earlier, at 13.07 and 14.06 days, respectively. Thus, 10 µM TDZ extended the storage time of the flowers by almost 10 days compared to the control, leading to the preservation of the flowers' aesthetic appeal, which is closely linked to their economic worth. The maximum vase life was observed with 10µM TDZ (23.35 days) followed by 100 µM BA (20.32 days). Among SA, 200 µM SA maintained maximum vase life for 19.10 days as compared to other concentrations (14.06 with 50 µM, 16.32 with 100 µM and 16.34 with 150 µM, respectively).

Water absorption

The results depicted that the maximum water absorption was seen in the treatment 10 µM TDZ, which was 68.52 mL as compared to 40.35 mL in control (Fig. 2). Among all the concentrations of TDZ, 10 µM TDZ (68.52 mL) showed best results, which was significantly higher followed by 5 µM TDZ (60.28 mL), whereas minimum amount of water was absorbed by stems treated with 20 µM TDZ (53.57 mL). The stems treated with 100 µM BA showed the highest water uptake among the various concentrations of BA tested, with a value of 67.07 mL. On the other hand, the highest water uptake for SA was observed at 200 µM concentration, with a value of 58.55 mL.

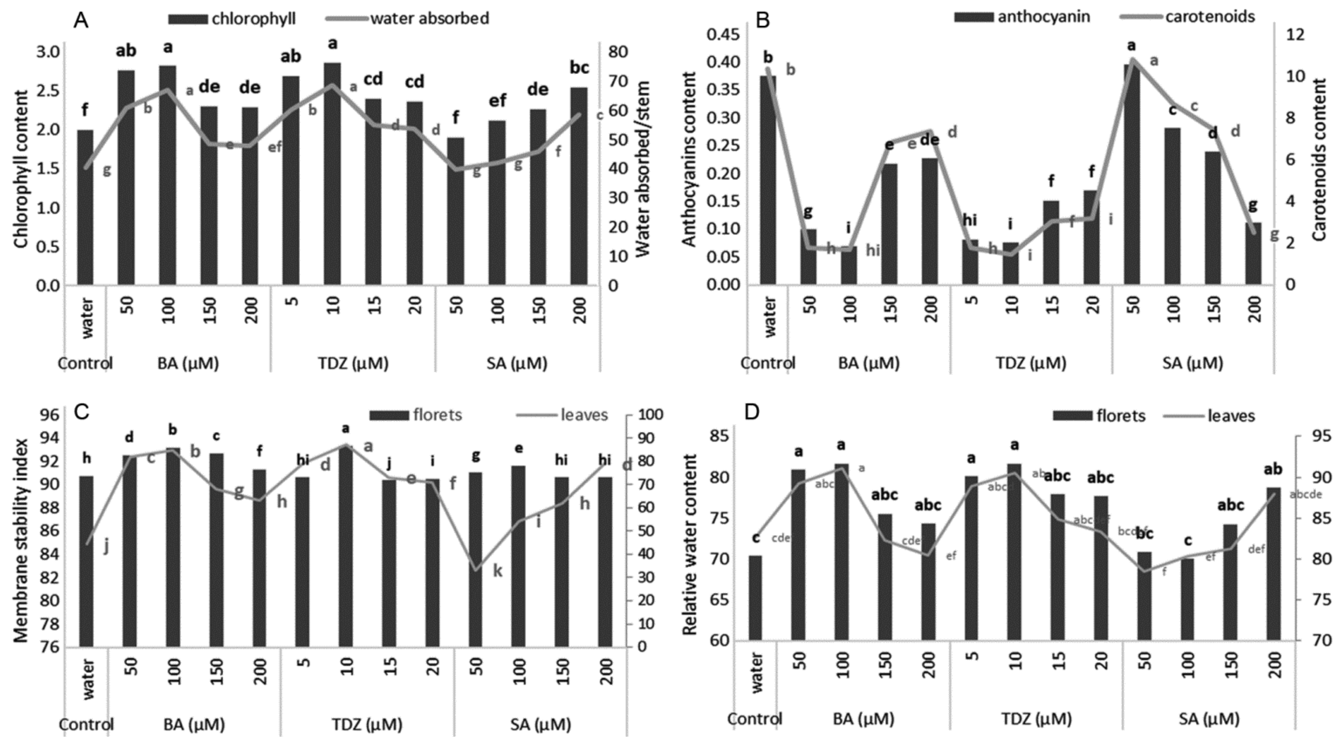


Fig. 2 — Effect of different concentrations of plant growth regulators on water absorbed/stem and total chlorophyll, anthocyanins and carotenoids, relative water content and membrane stability index in cut chrysanthemum stems

Chlorophyll content

The results from the analysis of chlorophyll content in leaves showed that the application of PGRs significantly increased the chlorophyll levels, except for the 50 µM SA treatment (Fig. 2). Among the selected growth regulators, it was observed that 10 µM TDZ had the highest chlorophyll content (2.85 mg/g FW) followed by 100 µM BA (2.81 mg/g FW) but the least in 50 µM SA (1.89 mg/g FW) which was statistically comparable with water (1.99 mg/g FW). Among varied doses of SA, 200 µM SA had significantly higher chlorophyll content (2.54 mg/g FW). When different concentrations of BA were tested, it was observed that 100 µM BA had the highest chlorophyll content (2.91 mg/g FW) followed closely by 50 µM BA (2.76 mg/g FW). These findings suggest that the use of PGRs can effectively maintain higher chlorophyll content and delay leaf yellowing during vase life.

Anthocyanin and carotenoid Content

The application of plant growth regulators had a significant effect on the petal anthocyanin content of white-coloured chrysanthemums. Compared to the control, petals treated with PGRs displayed a reduced level of pigment content. The lowest anthocyanin content was observed in cut stems treated with 100 µM BA and 10 µM TDZ, with a value of 0.07 mg/g FW (Fig. 2). Conversely, the highest anthocyanin content was observed with 50 µM SA (0.39 mg/g FW) followed by the control (0.37 mg/g FW). Additionally, the results indicated that 200 µM SA had the least anthocyanin content (0.11 mg/g FW) among different concentrations of SA.

Similarly, cut chrysanthemum stems treated with 10 µM TDZ exhibited the lowest carotenoid content (1.44 mg/g FW), while the highest carotenoid content (10.78 mg/g FW) was observed in florets treated with 50 µM SA. Interestingly, stems treated with 100 µM BA had relatively lower carotenoid content (1.68 mg/g FW) compared to other concentrations of BA (1.77 mg/g FW with 50 µM BA, 6.80 mg/g FW with 150 µM BA, and 7.36 mg/g FW with 200 µM BA). Furthermore, 200 µM SA had the least carotenoid content (2.49 mg/g FW) compared to other doses (10.78 mg/g FW with 50 µM SA, 8.64 mg/g FW with 100 µM SA, and 7.42 mg/g FW with 150 µM SA).

Membrane stability index (MSI) and Relative water content (RWC)

The use of growth regulators proved to be effective in protecting cell membranes, as demonstrated by the results of the study. Among the selected growth regulators, TDZ showed the highest MSI values in

both florets and leaves (86.84 and 93.43%, respectively, with 10µM TDZ), while those treated with BA (85.93 and 92.94% with 100 µM BA) and salicylic acid (77.30 and 86.80% with 200 µM SA) registered marginally lower values but significantly higher than the control (73.09 and 84.86%, respectively) (Fig. 2). The results demonstrated that growth regulators revealed their effectiveness in protecting cell membranes and maintaining membrane stability in florets and leaves almost at all concentrations. Among different doses of BA, 100 µM BA (92.94%) considerably improved membrane stability of leaves as compared to other concentrations of BA (92.40% with 50 µM BA, 89.64% with 150 µM BA and 88.65% with 200 µM BA). Further, data revealed that florets and leaves treated with distilled water, i.e. control (73.69 and 84.86%, respectively), showed the greatest destruction of cell membranes.

Nearly all the treatments maintained higher values RWC in florets and leaves compared to the control, indicating better visual quality for a longer period. The florets treated with 10 µM TDZ (91.67%) and 100 µM BA (91.67%) showed significantly higher RWC content compared to other treatments. Whereas, florets treated with distilled water i.e. control (80.36%) under had lower RWC. Comparable trends were seen in leaves treated with various treatments. As the higher RWC content in leaves was maintained by 100 µM BA (81.15%) followed by 10 µM TDZ (80.54%).

Total soluble sugars (TSS) and Total soluble proteins (TSP)

The sugar content is an important factor controlling vase life. When compared to the control group (157.03 in florets and 126.24 in leaves), a significantly elevated amount of total soluble sugars was observed in stems treated with 10 µM TDZ (188.24 mg/g FW in florets and 157.56 mg/g FW in leaves) and 100 µM BA (187.26 mg/g FW in florets and 152.67 mg/g FW in leaves) (Table 1). Leaves recorded significantly lower total soluble sugar content than florets. Leaves treated with 200 µM SA maintained the highest TSS content (137.09 mg/g FW) compared to other doses (122.32 mg/g FW with 50 µM SA, 130.15 mg/g FW with 100 µM SA and 133.04 mg/g FW with 150 µM SA). The PGR's treatments maintained higher total soluble sugars and slowed down their reduction.

Statistically significant variations were observed in total soluble proteins in response to various PGRs (Table 2). The use of various growth regulators on stems resulted in higher levels of total soluble proteins. Among the treatments, florets treated with 10 µM TDZ (198.36 mg/g FW) showed highest maintenance of total soluble proteins followed by 100 µM BA (196.31 mg/g FW) which was statistically at par with and 50 µM BA (194.26 mg/g FW). The lowest content was recorded in samples treated with 50 µM SA, with values of 161.54 mg/g FW in florets and 123.52 mg/g FW in leaves, followed by the control treatment (165.63 and 126.61 mg/g FW in florets and

Table 1 — Effect of different concentrations of plant growth regulators (BA, TDZ and SA) on TSS, TSP, CAT and POX activities in florets and leaves of cut chrysanthemum stems

Treatments	Total soluble proteins (TSP)		Total soluble sugars (TSS)		Catalase activity (CAT)		Peroxidase activity (POX)	
	Florets	Leaves	Florets	Leaves	Florets	Leaves	Florets	Leaves
50µM BA	194.26^{a}±4.7	156.46^{a}±3.6	186.29^{ab}±4.7	149.73^{ab}±3.5	0.57^{abc}±0.02	0.99^{a}±0.03	8.36±0.25	10.57^{a}±0.32
100µM BA	196.31^{a}±4.7	153.38^{a}±3.6	187.26^{ab}±5.0	152.67^{ab}±3.5	0.58^{ab}±0.02	0.98^{ab}±0.03	8.40±0.25	10.60^{a}±0.32
150µM BA	180.97^{bcd}±4.4	142.05^{bcd}±3.3	174.58^{bcde}±4.6	138.96^{cd}±3.2	0.53^{bcde}±0.02	0.92^{abcde}±0.03	7.96±0.24	10.05^{abcd}±0.30
200µM BA	178.92^{bcd}±4.3	141.02^{bcd}±3.3	170.68^{cde}±4.5	136.03^{d}±3.1	0.52^{cde}±0.02	0.91^{abcde}±0.03	7.95±0.24	10.02^{abcd}±0.30
5µM TDZ	191.19^{ab}±4.6	154.40^{a}±3.6	184.34^{ab}±4.9	150.70^{ab}±3.5	0.58^{a}±0.02	0.98^{ab}±0.03	8.25±0.25	10.51^{a}±0.32
10µM TDZ	198.35^{a}±4.8	157.49^{a}±3.7	188.24^{a}±5.0	157.56^{a}±3.7	0.59^{a}±0.02	0.99^{a}±0.03	8.48±0.25	10.73^{a}±0.32
15µM TDZ	189.15^{abc}±4.6	150.29^{ab}±3.5	182.39^{abc}±4.8	148.75^{ab}±3.4	0.55^{abcd}±0.02	0.96^{abc}±0.03	8.11±0.24	10.28^{ab}±0.31
20µM TDZ	186.08^{abc}±4.5	148.23^{abc}±3.4	179.46^{abcd}±4.7	145.81^{bc}±3.4	0.55^{abcd}±0.02	0.94^{abcd}±0.03	8.05±0.24	10.24^{ab}±0.31
50µM SA	161.54^{f}±3.9	123.52e±2.9	153.13^{g}±4.0	122.32^{f}±2.8	0.45^{g}±0.02	0.84^{e}±0.03	7.47±0.22	9.17^{d}±0.28
100µM SA	172.79^{def}±4.2	132.79^{de}±3.1	161.90^{efg}±4.3	130.15^{def}±3.0	0.49^{efg}±0.02	0.86^{cde}±0.03	7.71±0.23	9.52^{bcd}±0.29
150µM SA	176.88^{cde}±4.3	138.96^{cd}±3.2	168.73^{def}±4.5	133.04^{de}±3.1	0.51^{def}±0.02	0.89^{bcde}±0.03	7.78±0.23	9.90^{abcd}±0.30
200µM SA	189.15^{abc}±4.6	155.43^{a}±3.6	185.31^{ab}±4.9	137.09^{cd}±3.2	0.56^{abcd}±0.02	0.98^{ab}±0.03	8.17±0.25	10.48^{a}±0.32
WATER	165.63^{ef}±4.0	126.61^{e}±2.9	157.03f^{g}±4.1	126.24^{ef}±2.9	0.46^{fg}±0.02	0.85^{de}±0.03	7.55±0.23	9.25^{cd}±0.28
CD (5%)	13.06	9.96	13.72	9.68	0.05	0.09	NS	0.89
SE(m)	4.41	3.40	4.69	3.31	0.02	0.03	0.24	0.30
SE(d)	6.33	4.82	6.64	4.68	0.03	0.05	0.34	0.43
C.V.	4.24	4.08	4.63	4.08	6.02	6.01	5.21	5.21

[*a±b, a represents the mean value and b represents the standard error of mean; and **Different lowercase letters represent significant differences between different treatments]

Table 2 — Total soluble proteins (TSP) and Total soluble sugars (TSS): Analysis of Variance

Source of Variation (TSP Florets)	DF (TSP florets)	Sum of squares (TSP florets)	Mean squares (TSP florets)	F-calculated (TSP florets)	Significance (TSP florets)
Treatment	12	4847.671	403.973	6.737	0.00003
Error	26	1559.074	59.964		
Total	38	6406.746			
Source of Variation (TSP Leaves)	DF (TSP leaves)	Sum of squares (TSP leaves)	Mean squares (TSP leaves)	F-calculated (TSP leaves)	Significance (TSP leaves)
Treatment	12	4803.862	400.322	11.484	0.00000
Error	26	906.366	34.860		
Total	38	5710.228			
Source of Variation (TSS Florets)	DF (TSS florets)	Sum of squares (TSS florets)	Mean squares (TSS florets)	F-calculated (TSS Florets)	Significance
Treatment	12	5251.999	437.667	6.615	0.00003
Error	26	1720.171	66.160		
Total	38	6972.170			
Source of Variation (TSS Leaves)	DF (TSS leaves)	Sum of squares (TSS leaves)	Mean squares (TSS leaves)	F-calculated (TSS leaves)	Significance (TSS leaves)
Treatment	12	4362.248	363.521	11.031	0.00000
Error	26	856.797	32.954		
Total	38	5219.045			

Table 3 — Catalase and Peroxidase: Analysis of Variance

Source of variation (catalase florets)	DF (catalase florets)	Sum of squares (catalase florets)	Mean squares (catalase florets)	F-calculated (catalase florets)	Significance (catalase florets)
Treatment	12	0.078	0.007	6.331	0.00004
Error	26	0.027	0.001		
Total	38	0.105			
Source of variation (catalase leaves)	DF (catalase leaves)	Sum of squares (catalase leaves)	Mean squares (catalase leaves)	F-calculated (catalase leaves)	Significance (catalase leaves)
Treatment	12	0.110	0.009	2.938	0.01039
Error	26	0.081	0.003		
Total	38	0.191			
Source of variation (peroxidase florets)	DF (peroxidase florets)	Sum of squares (peroxidase florets)	Mean squares (peroxidase florets)	F-calculated (peroxidase florets)	Significance (peroxidase florets)
Treatment	12	3.718	0.310	1.779	0.10624
Error	26	4.527	0.174		
Total	38	8.245			
Source of variation (peroxidase leaves)	DF (peroxidase leaves)	Sum of squares (peroxidase leaves)	Mean squares (peroxidase leaves)	F-calculated (peroxidase leaves)	Significance (peroxidase leaves)
Treatment	12	9.621	0.802	2.899	0.01120
Error	26	7.191	0.277		
Total	38	16.813			

leaves respectively). In leaves, higher total soluble protein contents were observed in those treated with 5 and 10 μM TDZ, with values of 154.40 mg/g FW and 157.49 mg/g FW, respectively. However, the content decreased when higher concentrations of TDZ were used, with 15 and 20 μM TDZ resulting in values of 150.29 and 148.23 mg/g FW, respectively.

Antioxidant enzyme activities

The overall trend of peroxidase activity showed that florets and leaves treated with 10 μM TDZ (8.48 and 10.73 Δ in absorbance min^{-1} g^{-1} FW, respectively) had significantly higher POX activity, and the rate of downregulation was slower than stems treated with 100 μM BA (8.40 and 10.60 Δ in absorbance min^{-1} g^{-1} FW, respectively) (Table 1). Water had the lowest peroxidase activity (7.55 and 9.25 Δ in absorbance min^{-1} g^{-1} FW) followed by 50 μM SA (8.05 and 10.24 Δ in absorbance $min^{-1}g^{-1}$ FW) in florets and leaves, respectively.

Statistically significant variations were observed in catalase activity in response to various PGRs (Table 3). The highest catalase activity was observed in 10 μM TDZ treatment, with 0.59 and 0.99 mmol H_2O_2 hydrolyzed $min^{-1}g^{-1}$ FW in florets and leaves, respectively, followed by 5 μM TDZ and 100 μM BA, which had equally effective results in enhancing catalase activity in both florets and leaves. TDZ was significantly more effective than other PGRs in

enhancing antioxidant enzyme activities. The lowest antioxidant enzyme activities were observed in florets and leaves treated with 50 µM SA (0.44 and 0.84 mM H_2O_2 hydrolyzed $min^{-1}g^{-1}$ FW), followed by untreated stems (control) (0.46 and 0.85 mmol H_2O_2 hydrolyzed $min^{-1}g^{-1}$ FW). Salicylic acid had a less pronounced effect on catalase activity, although both TDZ and BA had a slightly promoting effect.

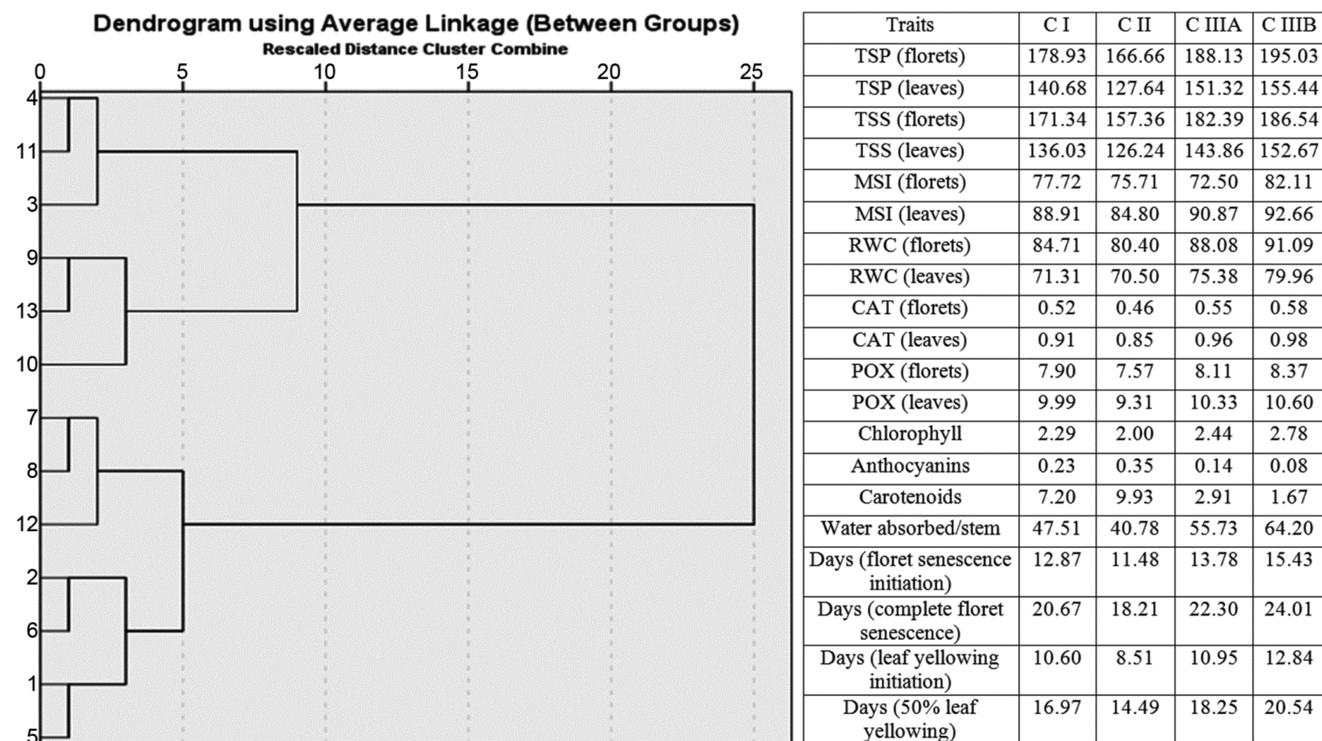
Hierarchical cluster analysis of morpho-physiological and biochemical traits

Using morpho-physiological and biochemical features, cluster analysis was performed, and the dendrogram (Fig. 3) indicates the effectiveness of this classification. The dendrogram grouped the thirteen treatments into three different clusters I, II, and III at 5 rescaled distance. Cluster I (CI) had three treatments (T-4, T-11and T-3), cluster II (CII) had three treatments (T-9, T-13 and T-10), and cluster III (CIII) had the remaining seven. Cluster III was further partitioned into III A and III B, with subcluster III A containing three treatments (T-7, T-8 and T-12) and subcluster III B containing four treatments (T-2, T-6, T-1 and T-5).

Cluster III B treatments, which included 10 µM TDZ, 5 µM TDZ, 50 µM BA, and 100 µM BA, had the highest relative water content (91.09 and 79.96%), membrane stability index ((91.09 and 79.96%), chlorophyll content (2.78 mg/g FW), catalase (0.58 and 0.98 mmol H_2O_2 hydrolyzed min^{-1} g^{-1} FW) and peroxidase activity (8.37 and 10.60 Δ in absorbance min^{-1} g^{-1} FW), total soluble sugar (186.54 and 152.67 mg/g FW) and protein content (195.03 and 155.44 mg/g FW) in florets and leaves, respectively. Treatments in this cluster had lowest carotenoid (1.67 mg/g FW) and anthocyanin content (0.08 mg/g FW) in florets while the highest content was observed in Cluster II (9.93 and 0.35 mg/g FW, respectively). In terms of all quality parameters, Cluster III B was followed by Cluster III A. Cluster II treatments had the least impact on post-harvest quality measures. Benzyl adenine (BA) @50 or 100 µM, 5 or 10 µM TDZ (cluster III B) could all be equally helpful in promoting physiological, biochemical and post-harvest quality attributes, suggests that utilizing these treatments could potentially extend the longevity of cut chrysanthemums

Correlation analysis of the morpho-physiological and biochemical traits

Correlation analysis was conducted to have an insight into the interdependence of floret senescence and morpho-physiological and biochemical traits. Significant and positive correlation was recorded between days to floret senescence and other morpho-



Traits	C I	C II	C IIIA	C IIIB
TSP (florets)	178.93	166.66	188.13	195.03
TSP (leaves)	140.68	127.64	151.32	155.44
TSS (florets)	171.34	157.36	182.39	186.54
TSS (leaves)	136.03	126.24	143.86	152.67
MSI (florets)	77.72	75.71	72.50	82.11
MSI (leaves)	88.91	84.80	90.87	92.66
RWC (florets)	84.71	80.40	88.08	91.09
RWC (leaves)	71.31	70.50	75.38	79.96
CAT (florets)	0.52	0.46	0.55	0.58
CAT (leaves)	0.91	0.85	0.96	0.98
POX (florets)	7.90	7.57	8.11	8.37
POX (leaves)	9.99	9.31	10.33	10.60
Chlorophyll	2.29	2.00	2.44	2.78
Anthocyanins	0.23	0.35	0.14	0.08
Carotenoids	7.20	9.93	2.91	1.67
Water absorbed/stem	47.51	40.78	55.73	64.20
Days (floret senescence initiation)	12.87	11.48	13.78	15.43
Days (complete floret senescence)	20.67	18.21	22.30	24.01
Days (leaf yellowing initiation)	10.60	8.51	10.95	12.84
Days (50% leaf yellowing)	16.97	14.49	18.25	20.54

Fig. 3 — Dendrogram representing clustering of different treatments of PGRs using squared Euclidean distance based on morpho-physiological and biochemical traits and their cluster centres

Table 4 — Correlation coefficients among various morpho-physiological and biochemical parameters

	Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	TSP (florets)	1																			
2	TSP (leaves)	.983**	1																		
3	TSS (florets)	.989**	.994**	1																	
4	TSS (leaves)	.957**	.909**	.926**	1																
5	MSI (florets)	0.429	0.31	0.345	0.448	1															
6	MSI (leaves)	.989**	.980**	.985**	.921**	0.419	1														
7	RWC (florets)	.967**	.960**	.969**	.933**	0.409	.942**	1													
8	RWC (leaves)	.888**	.865**	.872**	.843**	0.454	.867**	.908**	1												
9	CAT (florets)	.991**	.986**	.989**	.948**	0.382	.986**	.966**	.870**	1											
10	CAT (leaves)	.977**	.991**	.991**	.914**	0.336	.965**	.978**	.895**	.978**	1										
11	POX (florets)	.991**	.970**	.975**	.951**	0.499	.975**	.971**	.906**	.982**	.974**	1									
12	POX (leaves)	.989**	.992**	.992**	.926**	0.381	.988**	.971**	.860**	.993**	.984**	.981**	1								
13	Chlorophyll	.970**	.947**	.946**	.923**	0.516	.959**	.963**	.937**	.963**	.951**	.983**	.962**	1							
14	Anthocyanins	-.986**	-.986**	-.986**	-.920**	-0.349	-.985**	-.953**	-.872**	-.992**	-.975**	-.972**	-.988**	-.962**	1						
15	Carotenoids	-.977**	-.982**	-.983**	-.931**	-0.283	-.954**	-.968**	-.891**	-.977**	-.982**	-.961**	-.971**	-.940**	.977**	1					
16	Water absorbed/stem	.966**	.938**	.947**	.932**	0.49	.937**	.975**	.946**	.955**	.954**	.978**	.952**	.979**	-.946**	-.953**	1				
17	Days (floret senescence initiation)	.919**	.885**	.888**	.916**	0.494	.898**	.903**	.870**	.917**	.884**	.921**	.905**	.930**	-.897**	-.884**	.942**	1			
18	Days (complete floret senescence)	.976**	.952**	.961**	.955**	0.486	.953**	.968**	.864**	.977**	.952**	.976**	.971**	.960**	-.960**	-.954**	.973**	.957**	1		
19	Days (leaf yellowing initiation)	.865**	.859**	.867**	.828**	0.327	.871**	.866**	.809**	.897**	.871**	.864**	.875**	.884**	-.911**	-.857**	.839**	.794**	.846**	1	
20	Days (50% leaf yellowing)	.936**	.904**	.902**	.899**	.558*	.919**	.894**	.829**	.929**	.893**	.947**	.926**	.937**	-.915**	-.884**	.941**	.966**	.966**	.775**	1

physiological and biochemical traits. The highest positive and significant correlation (r = +0.986, 0.992, 0.989 and 0.991) was observed between antioxidant's activity (POX and CAT) and total soluble proteins in florets and leaves (Table 4). The correlation analysis suggested that using PGRs to boost antioxidant activity by scavenging reactive oxygen can help maintain protein levels in florets and leaves.

The total soluble proteins (r = +0.989) and peroxidase activity (r = +0.988) had strong relationships with membrane stability index. In this study, significant and direct correlation between membrane stability index and peroxidase activity suggested that increasing peroxidase activity reduced the destruction of membranes. Correlation coefficients revealed that chlorophyll content and total soluble sugars were significantly correlated (r = +0.946). The significant increase in the sugar content in florets and leaves might be correlated with the preserved photosynthetic ability in leaves[22]. Furthermore, days to floret senescence initiation and 50% leaf yellowing shows highly positive correlation with total soluble sugars (r = +0.916, 0.902) which are in accordance with the study, higher amount of sugars in petals is associated with a delay in the senescence of cut flowers[23]. There was significant correlation between days to floret senescence initiation and water absorbed (r = +0.942), supporting higher postharvest life as a result of increased solution uptake that maintained water balance. Correlation analysis deduced that an increasing the respiratory metabolites is accompanied by delaying floret and leaf yellowing. Moreover, anthocyanin and antioxidants activities had the strongest negative correlation (r = –0.922). It appears that these PGRs with effect on antioxidants activity eliminate free radicals and limits enzymatic browning, resulting in improved cut stems visual quality and longevity.

Discussion

Based on the current study, significant variations were revealed among the tested growth regulators (BA, TDZ, and SA) at different concentrations in prolonging the lifespan of cut chrysanthemum stems. The extended longevity corresponding with increased levels of various physiological and biochemical parameters, including relative water content, membrane stability index, total soluble sugars, total soluble proteins, and antioxidant activities in florets and leaves, besides decreased anthocyanin and carotenoids contents in florets was recorded. Application of BA at 50 and 100 µM, TDZ at 5 and 10 µM and SA at 200 µM resulted in substantial improvement in the longevity of flowers and postharvest performance of chrysanthemum stems. While the higher doses of BA and TDZ resulted in a significant decline in all the examined parameters, except for anthocyanin and carotenoids which exhibited a notable increase. TDZ was found to be the most effective among the selected growth regulators in enhancing the postharvest life of cut chrysanthemum stems.

The use of plant growth regulators (PGRs) had a positive impact on the longevity of flowers by influencing several physiological processes, including membrane stability, water balance, free radical

formation, and sugar and protein metabolism. Benzyl adenine, for instance, delayed flower senescence by decreasing the production of abscisic acid (ABA), a known promoter of flower senescence in both ethylene-sensitive and insensitive flowers[24]. Adenine and phenyl urea derivatives have also been demonstrated to delay senescence in different cut flowers, including Iris[10]. Additionally, salicylic acid has been found to increase vase life and postpone floral senescence by preserving membrane integrity and elevating antioxidant activity[12].

The yellowing of leaves before petal wilting in cut chrysanthemum stems, is influenced by an internal hormonal imbalance, which can significantly impact their postharvest lifespan. To address this problem, plant growth regulators, such as cytokinins[25], can be used to delay the onset of leaf yellowing and preserve the leaves' quality. The effects of PGRs on days to leaf yellowing demonstrated that they positively impact the vase life of chrysanthemum stems by preventing chlorophyll degradation. Specifically, thidiazuron (TDZ) was found to be more effective than benzyl adenine (BA) and salicylic acid (SA) in inhibiting leaf yellowing, possibly due to its non-metabolizable nature. Concomitant to our findings, the use of exogenous melatonin in broccoli[26] has been shown to delay leaf yellowing.

Water status plays a crucial role in determining the vase life of flowers[27], as the turgidity of petals is essential for their acceptance. When a flower is harvested, uptake of water from parent plant ceases, leading to water deficiency and petal wilting[28] due to continued transpirational loss. Cut stems treated with various treatments absorbed more water and displayed superior performance compared to the control group. Early senescence of florets and leaves of control stems appeared to be the result of decreased water absorption and higher rate of transpiration that led to short vase life[29]. Lower rates of water absorption and transport in cut flower stems are attributed to blockage of xylem vessels by microbe agglomeration in the stem base[4]. Selected growth regulators significantly enhanced water absorption per stem, with TDZ-treated stems showing the most pronounced effects. Higher water uptake could be associated with greater sugar content, which establishes a water potential gradient and leads to water entry into tissues, resulting in increased turgidity and vase life.

Several studies have demonstrated that the use of floral preservatives can improve water absorption rates and extend the vase life of cut flowers[30,31]. However, the responses of TDZ and BA are dose-dependent, as low concentrations lead to better water uptake, while high concentrations interfere with the translocation channel. Antioxidants play a critical role in preventing the production of free radicals that cause the degradation of chlorophyll, and floral preservatives can prevent chlorophyll degradation by reducing the activity of hydrolytic enzymes in thylakoid membranes[32].

The browning of cut flowers occurs due to the oxidation of phenols, which produces quinones that eventually form brown pigments, resulting in changes in appearance and colour[33]. The stems treated with plant growth regulators had lower anthocyanin content as application of PGRs retarded enzymatic browning of cut flowers through completely inhibition of anthocyanin formation and provides the possibility to preserve natural white colour of florets[34].

Further, PGRs appear to have reduced browning in chrysanthemum florets by inhibiting the production of carotenoids in chrysanthemum petals, allowing flowers original colour to be preserved. The ABA biosynthesis may be derived by carotenoids through indirect biosynthesis pathway[35]. Our results showed that the highest carotenoid content was associated with shorter flower life, while the longest flower life had lower levels of pigments, suggesting their potential source for ABA biosynthesis in florets.

Maintaining adequate water content in cut flowers as long as possible is an important approach to extend their vase life. Our results showed that stems treated with growth regulators resulted in higher relative water content than untreated (control) stems, so sustained their better visual quality for longer periods. However, RWC were significantly higher in TDZ treated cut stems, providing an evidence to support the higher preservative effects of TDZ on postharvest performance. Lower relative water content, resulted in rapid wilting of florets and leaves. At higher concentration of TDZ and BA and control treatments relative water content in florets and leaves rapidly reduced and rate of senescence increased.

Early senescence in ethylene-insensitive flowers is thought to be caused by oxidative damage. As evidenced by higher values of the membrane stability

index, TDZ was the most effective in avoiding lipid peroxidation of membranes, followed by BA and SA. The role of cytokinin in maintaining high water content and antioxidant system, both of which are commonly affected by stresses such as senescence, could explain improved membrane integrity[36].

Application of PGRs leads to maintenance of total soluble sugars in the florets and leaves. Total soluble sugars maintained membrane stability and reduced floral withering. The results of our study also revealed that the flowers with greater survival rate had more total soluble sugars. These findings were further backed up with previous research that found a link between carbohydrate buildup and flower lifetime when cytokinin was applied[37]. Salicylic acid application also improved total soluble sugars as reported earlier in mungbean[38].

The content of total soluble proteins in stems treated with water was lesser which was consistent with a shorter vase life observed with control treatment. With supplementation of growth regulators, the levels of TSPs were maintained. These PGRs are believed to have a greater preserving effect due to their ability to strengthen the antioxidant system and reduce levels of reactive oxygen species (ROS) that cause protein oxidation, which occurs in specific amino acid sites within the proteins[39]. External application of cytokinins was found very effective in delaying senescence of *Phalaenopsis* by arresting degradation of proteins[40]. Hence, the use of postharvest preservatives that suppress protease activity could be considered as a strategy to extend vase life and minimize postharvest losses[41].

Cutting wounds and cellular dehydration trigger the generation and build-up of reactive oxygen species (ROS), resulting in oxidative stress, which is another key factor affecting flower quality and longevity after harvest[12]. High antioxidant enzyme activity is required for efficient scavenging of excessive ROS, which would otherwise damage cell membranes and promote flower degradation, in order to resist the negative consequences of oxidative stress. In various studies, different preservatives, such as SNP[37], SA[12] have been used to alleviating oxidative stress and extending vase life of cut flowers. Our results showed that these selected growth regulators were able to boost antioxidant enzyme activities (CAT and POX), suggesting their ability to diminish the damage of oxidative stress. These findings are in line with the role of PGRs in regulating antioxidant metabolism and ROS levels within cells[42.] Our present study showed that PGRs maintained higher catalase (CAT) activities. Increase in catalase activity may be due to an increase in transcription and translation of CAT-related genes[43]. The increased activity of these enzymes in our studies supports the improved postharvest quality attributes of treated stems.

Conclusion

The pulsing of cut chrysanthemum stems with various growth regulators improved the vase life of cut stems by maintenance of high membrane stability index, respirable substrates like total soluble sugars and proteins and improved water uptake by the stems. The increased catalase (CAT) and peeroxidase (POX) activity in florets and leaves scavenged reactive oxygen species (ROS) and retarded the senescence. Among all treatments, 5 μM thidiazuron (TDZ) was found to be best and could be employed as a pulsing solution to lengthen the vase life of cut chrysanthemums.

Conflict of interest

Authors declare no competing interests.

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