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RESEARCH ARTICLE

Adenine type and diphenyl urea derived cytokinins improve the postharvest performance of *Iris germanica* L. cut scapes

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Abstract An experiment was designed to evaluate the effect of various adenine derived cytokinins (kinetin and 6-benzylaminopurine) and diphenyl urea cytokinin (thidiazuron) on the postharvest performance of cut scapes of *Iris germanica*. Flower scapes were harvested with the oldest bud at '1 day before anthesis stage', brought to laboratory under water, cut to a uniform length of 35 cm, divided into three sets viz., kinetin (KIN), 6-benzyl aminopurine (BAP) and thidiazuron (TDZ). Each set of scapes was treated with a particular cytokinin alone or in combination with 0.1 M sucrose. TDZ was effective than KIN and BAP in improving the postharvest life of the *I. germanica* scapes by 5.4 days as compared to the control (untreated scapes held in distilled water). This was because of the minimum percentage of bud abortion by TDZ application. Cytokinin application resulted in increased antioxidant activity, higher protein and phenolic content, besides a decrease in specific protease activity and α-amino acids in the tepal tissues. Application of TDZ resulted in the maximum increase in the superoxide dismutase, catalase and ascorbate peroxidase activity in the tepal tissues. The scapes treated with BAP and KIN maintained higher carbohydrate content in the tissue samples as compared to control and TDZ treated scapes. TDZ and BAP application resulted in increased membrane stability because of the decreased lipoxygenase activity which prevented membrane lipid peroxidation. Among the cytokinins tested, TDZ proved to be the promising cytokinin in improving the postharvest performance of beautiful flowers of *I. germanica* scapes.

Keywords Antioxidant enzymes · Benzylaminopurine · Kinetin · Senescence · Thidiazuron

Introduction

Studies on flower senescence have always fascinated plant biologists because of its complexity in terms of involvement of various biomolecules. Flower senescence is characterized by the sequential changes that start at the molecular level, involve physiological and biochemical changes which are ultimately visible at the organism level (Ichimura et al. 2009; Trivellini et al. 2014; Saeed et al. 2014). Flower senescence is influenced by a turnover in the synthesis and expression of various endogenous phytohormones like ethylene, abscisic acid, cytokinins, auxins and gibberellins. In some flowers, ethylene plays the main role in flower senescence (ethylene sensitive flower senescence) while as in others, ethylene has a little or no role to play (ethylene insensitive flower senescence) (van Doorn and Woltering 2008; Shahri and Tahir 2014; Ahmad and Tahir 2016a; Iqbal et al. 2017). Although ethylene and abscisic acid promote flower senescence, yet cytokinins are known to delay senescence (Hunter et al. 2004; Arrom and Munne-Bosch 2012). The inhibitory effect of cytokinins on flower senescence makes it a hormone of choice in extending the postharvest performance of various cut flowers. It has been shown that young buds contain high cytokinin levels which decline sharply towards senescence (Xu et al. 2007; van Doorn et al. 2013; Rogers 2013; Dar et al. 2014a). During the recent advances about the application of cytokinins in postharvest technology, researchers

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have tried both adenine type cytokinins such as benzyl adenine, zeatin, and kinetin or diphenyl urea derived non-metabolizable cytokinins such as thidiazuron (*N*-phenyl-*N*-1,2,3-thiadiazol-5-ylurea) (van Doorn 2004; Sankhla et al. 2005; Mortazavi et al. 2011). These studies have revealed that exogenous application of cytokinins improve the postharvest life of various cut flowers like *Lilium*, *Dianthus*, *Nelumbo*, *Gerbera*, *Iris*, *Hemerocallis* (Macnish et al. 2010a; Imsabai and van Doorn 2013; Reid and Wu 2018). The role of endogenous and exogenously applied cytokinins has been studied at transgenic level in some flowers like *Petunia, Nicotiana*, *Chimonanthus*, *Rosa* and *Dianthus* (Mor et al. 1983; Trivellini et al. 2014; Sui et al. 2015). Although both classes of cytokinins (adenine type and phenyl urea derivatives) proved effective in improving postharvest performance of cut flowers, yet the effect of thidiazuron was more promising. Cytokinins delay flower senescence by promoting transport, accumulation and retention of metabolites in flower petals, besides preventing membrane degradation (Imsabai and van Doorn 2013; Shahri and Tahir 2014; Radio et al. 2017; Iqbal et al. 2017). Alternatively, cytokinins may result in delay of senescence by influencing cytokinin/auxin activity or by causing a decrease in sensitivity of flowers to ethylene in ethylene dependent flowers (Macnish et al. 2010a; Ferrante et al. 2009; Liu et al. 2016a).

During our earlier studies, a simulated transportation protocol was proposed for long term transportation of the cut scapes of *Iris germanica,* an ethylene insensitive flower system (Ahmad et al. 2013). The present investigation was carried out to increase the vase life and improve the postharvest quality of cut scapes of *I. germanica* by the application of various cytokinins and thidiazuron. The effect of these chemicals on the vase life and postharvest performance was analyzed at biochemical level to assess the various physiological and biochemical changes during senescence.

Materials and methods

Plant material

Uniform and healthy scapes of *I. germanica* grown in the Kashmir University Botanic Garden (KUBG) were utilized in this study. The scapes were collected at 8:00 h with their oldest bud at pencil stage (1 day before anthesis) (Fig. 1), brought to laboratory, cut to identical size of 35 cm. The scapes were then held in 170 ml of 75 µM KIN, 75 µM BAP and 50 µM TDZ separately, alone or in combination with 0.1 M sucrose in 250 ml flasks. Each treatment had 10 replicates (flasks) and each flask contained two scapes. A separate set of scapes were held either in distilled water or sucrose without the cytokinin treatment, designated the control. In all the flasks, 0.1 mM 8-HQS (8-hydroxy quinoline sulfate) was added to prevent microbial growth in the vase solutions. All the biochemical parameters were analyzed from the tepal tissues on day 4 of transfer to the respective vase solutions. The oldest bud present on the scape was used for the biochemical analysis. The experiment was conducted under controlled conditions with relative humidity (RH) of 60 ± 10% and 12 h light period a day.

Assessment of vase life and floral diameter

The average vase life of the cut scapes was counted from the day of transfer of scapes to holding solutions and assessed to be terminated when the last flower lost its ornamental/display value. The floral diameter was measured on day 2 and 6 as the mean of two perpendicular measurements across the flower.

Membrane stability index (MSI)

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

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

Lipid peroxidation (LPO)

Lipid peroxidation was determined by the method of Heath and Packer (1968). 0.5 g of tepal tissue was macerated in 15 ml of 0.1% trichloroacetic acid (TCA) and centrifuged at 15,000×*g* for 10 min under refrigeration. 1 ml of supernatant was taken and mixed with 4 ml of 0.5% TBA diluted in TCA (20%). The reaction was started by incubating the mixture at 95 °C in water bath for 25 min and reaction was ended by placing it in ice. Absorbance was taken at 532 and 600 nm. Non-specific absorbance at 600 nm was subtracted from the value at 532 nm.

Estimation of sugar fractions, amino acids and phenols

1 g chopped tepal tissue from each treatment was fixed in hot 70% ethanol, macerated and centrifuged thrice. Total phenols, α-amino acids, reducing, non-reducing and total

Fig. 1 Stages of flower development and senescence in *Iris germanica*. Scapes with the oldest buds at stage III (1 day before anthesis) were used for the present study

sugars were estimated from a suitable aliquot taken from the supernatant. Rosen's method (1957) was employed for α-amino acid quantification with glycine as standard. Total phenolics were quantified by Swain and Hillis method (1959) using gallic acid as standard. Nelson's method (1944) was used for determining reducing sugars with glucose acting as standard. Non-reducing sugars were converted to reducing sugars by invertase for the estimation of total sugars. Difference between total and reducing sugars revealed the amount of non-reducing sugars.

Protein estimation and specific protease activity

For protein estimation, 1 g of tepal tissue was macerated in 100 mM (pH 7.2) phosphate buffer containing 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 10% glycerol, 10% polyvinyl pyrrolidone (PVP) and 1 mM Dithiothreitol (DTT). The mixture was centrifuged at 12,000×*g* at 5 °C in a refrigerated centrifuge for 15 min. The supernatant was collected and used for protein estimation. Proteins were estimated by Lowry et al. method (1951) from a suitable volume of aliquot taken from the supernatant. Specific protease activity was determined from 1 g of tepal tissue by the modified method as described by Tayyab and Qamar (1992).

Enzyme extraction and assays

Superoxide dismutase (SOD)

1 g of tepal tissue was macerated in a mortar and homogenized with 0.1 mM potassium phosphate buffer (pH = 7.8) containing 0.1 mM EDTA, 1% PVP and 0.5% (*v*/*v*) Triton X-100. The homogenate was centrifuged at 15000xg for 10 min. The supernatant was filtered through Mira cloth and used for the enzyme assay.

SOD activity was measured by the method of Dhindsa et al. (1981) by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture contained 50 mM sodium carbonate, 75 µM nitroblue tetrazolium (NBT), 0.1 mM EDTA, 13 mM methionine in 50 mM phosphate buffer (pH = 7.8) and 0.1 ml of the enzyme extract in a final volume of 3 ml. The reaction was started by adding 2 µM riboflavin and placing the test tubes in water bath at 25 °C and illuminated with a 30 W fluorescent lamp. The reaction was stopped by switching off the light and keeping the test tubes in darkness. Identical test tubes which were not illuminated served as blanks. Absorbance was measured at 560 nm and one unit of SOD activity was defined as the quantity of the enzyme which inhibits the photoreduction of NBT to blue formazan by 50% as compared to the reaction mixture kept in dark without the enzyme extract. The SOD activity was expressed as units min^{-1} mg^{-1} protein.

Catalase (CAT)

Catalase activity was estimated by the method of Aebi (1984). 1 g of tepal tissue was macerated in motor and homogenized in 100 mM potassium phosphate buffer (pH = 7.0) containing 1 mM EDTA. The reaction mixture contained 50 mM potassium phosphate buffer (pH = 7.0), 12.5 mM H_2O_2, 50 µl enzyme extract and distilled water to make the volume to 3 ml. Reaction was started by adding H_2O_2 and the catalase activity was determined by the consumption of H_2O_2 for 3 min at 240 nm and was expressed as µmol H_2O_2 red. min^{-1} mg^{-1}.

Ascorbate peroxidase (APX)

For the determination of APX activity, flower petals were macerated in 100 mM sodium phosphate buffer containing 5 mM Ascorbate, 10% glycerol and 1 mM EDTA. The APX activity was determined in 1 ml reaction mixture containing 50 mM potassium phosphate buffer (pH = 7.0), 0.1 mM Ascorbate, 0.3 mM H_2O_2. The decrease in the absorbance was recorded for 3 min at 290 nm (Chen and Asada 1989).

Lipoxygenase (LOX)

LOX activity was determined by the method of Axerold et al. (1981). 1 g tepal tissue was macerated in 1 ml extraction buffer containing 50 mM potassium phosphate buffer (pH = 6.5), 10% polyvinyl pyrrolidone (PVP), 0.25% Triton X-100 and 1 mM phenyl methyl sulfonyl fluoride (PMSF). The reaction mixture (1 ml) contained 50 mM Tris–HCl buffer (pH = 6.5) and 0.4 mM linoleic acid. The reaction was started by adding 10 µl crude tepal extract to the reaction mixture and absorbance was recorded at 234 nm for 5 min.

All the biochemical parameters studied were analyzed from the tepal tissues which were last to open on the scape.

Statistical analysis

Completely randomized experimental design was followed during the experiment. Each treatment was represented by ten replicates (flasks) and each flask contained two scapes. Each value represents the mean of ten replicates. Treatment means were compared by analysis of variance using SPSS® (SPSS version 16; Chicago, USA). Least significant difference (LSD) was calculated at the 5% level of probability. Standard error between the replicates was also calculated. The Duncan's multiple range test (DMRT) has been applied to the data to separate the means.

Results

Just prior to this experiment, normalization/standardization of the concentrations of 6-benzyl aminopurine, kinetin and thidiazuron on the postharvest performance of cut scapes of *I. germanica* was carried out. Concentrations ranging from 25 to 150 µM through 50, 75, 100, 125 µM were used. Application of BAP (75 µM) and KIN (75 µM) individually resulted in the maximum enhancement of vase life of cut scapes held in various test concentrations (25, 50, 75, 100, 125, and 150 µM). The increase in the vase life was commensurate with the increase in various biochemical parameters like membrane stability index, soluble proteins, total phenols, sugar fractions and antioxidant enzymes; besides a decrease in lipid peroxidation, specific protease activity, α-amino acids and lipoxygenase activity was recorded in the tissue samples from tepals. The concentrations of BAP and Kin higher than 75 µM showed a significant decrease in all the biochemical parameters studied except lipid peroxidation, specific protease activity, α-amino acids and lipoxygenase activity. Among the various concentration of TDZ (25, 50, 75, 100, 125, and 150 µM) tested, maximum vase life was recorded in the scapes treated with 50 µM concentration. Moreover, these scapes showed the highest values for membrane stability index, soluble proteins, total phenols, sugars fractions and antioxidant enzymes; besides maintaining the lower values for lipid peroxidation, specific protease activity, α-amino acids and lipoxygenase activity. The concentrations (75, 100, 125, and 150 µM) above 50 µM showed a significant decrease in membrane stability index, soluble proteins, total phenols, sugars fractions and antioxidant enzymes; besides an increase in lipid peroxidation, specific protease activity, α-amino acids and lipoxygenase activity. Among all the cytokinins (BAP, KIN and TDZ) tested, TDZ proved to be the most effective in increasing vase life and postharvest performance of the cut scapes of *I. germanica*.

Vase life, % blooms per scape and floral diameter

The scapes of *I. germanica* bear 4–5 buds with the oldest bud at the top. The individual flowers remain open for 2 days in the field as also under laboratory conditions. Flower senescence is marked by turgor loss in the tepals followed by inrolling of the distal ends of the tepals and the blue color of the tepals intensifies and ultimately the flowers turn pale towards the peak of senescence. Among the cytokinins tested, TDZ was the most effective in increasing the vase life of cut scapes of *I. germanica* by 5.4 days followed by 3.7 days in KIN and 2.8 days in BAP (Figs. 2, 3) as compared to control. This profound increase in the vase life by TDZ treatment is due to the normal opening of all the buds on the scapes (i.e., 100% blooming) in the treated scapes in comparison to control where only 37% flowers opened. BAP and KIN treatment resulted in 66 and 77% blooms (Fig. 4). Addition of sucrose along with the cytokinins in the vase showed improved vase life than the DW. Application of cytokinins resulted in the increase in floral diameter than the control. TDZ treatment resulted in an increase in the floral diameter by 11.6% followed by KIN (6.6%) and BAP (5%). Application of sucrose further augmented this increase in floral diameter in all the cytokinins used by 2.8%. Flower diameter decreased with the progression in time from day 2 to day 6.

Lipid peroxidation and membrane stability index

Lipid peroxidation showed a decrease by 57.9% in the samples from the scapes treated with TDZ along with sucrose followed by BAP and KIN which showed a decrease of 49.1 and 39.6% respectively as compared to control where lipid peroxidation peaked (Fig. 5). Because of decreased lipid peroxidation in samples from TDZ treated scapes, MSI index was found to be highest i.e. 79%. MSI values of the tissue samples from BAP (76%) and KIN (63%) treated scapes were marginally lower than TDZ treated scapes but significantly higher than the control.

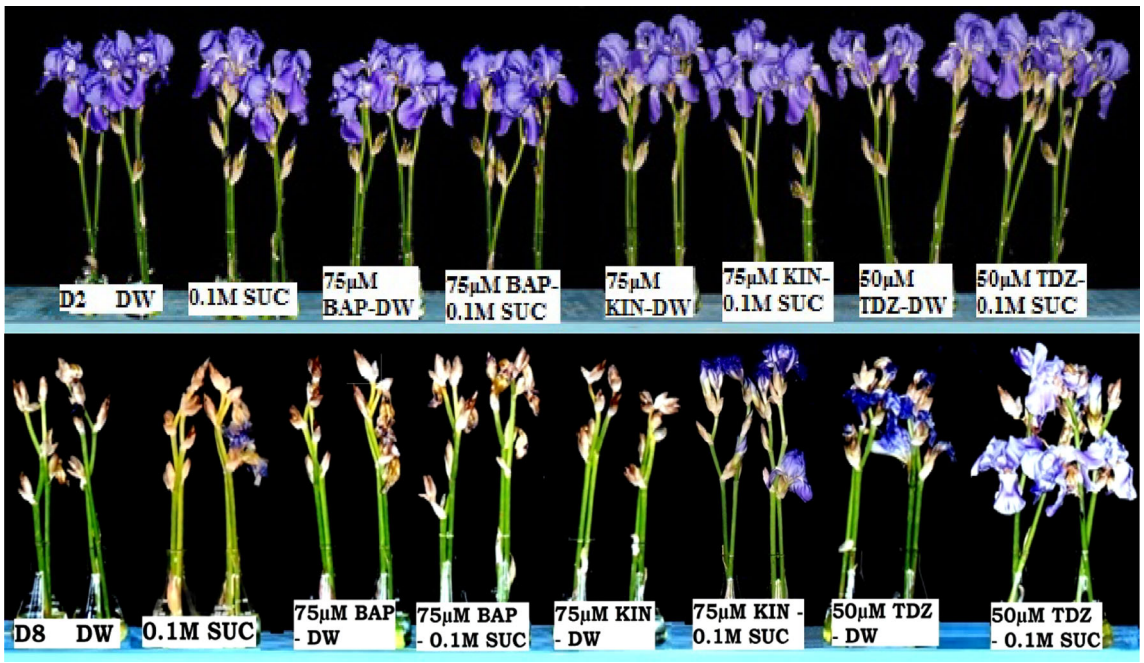

Fig. 2 Effect of benzyl amino purine (BAP), kinetin (KIN) and thidiazuron (TDZ) on the postharvest performance and vase life of cut scapes of *Iris germanica* on day 2 and day 8 of transfer of the scapes to the respective vase solutions

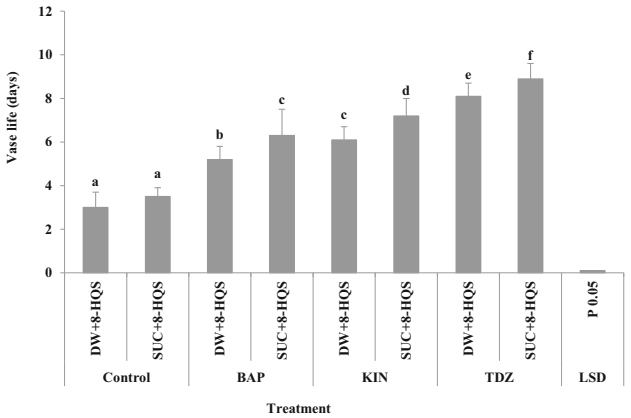

Fig. 3 Effect of benzyl amino purine (BAP), kinetin (KIN) and thidiazuron (TDZ) on the vase life of cut scapes of *Iris germanica*. The letters a–f above the bars denote the statistical significance (Duncan's multiple range test) of the differences between individual treatments (only those marked with different letter differ significantly at $P < 0.05$)

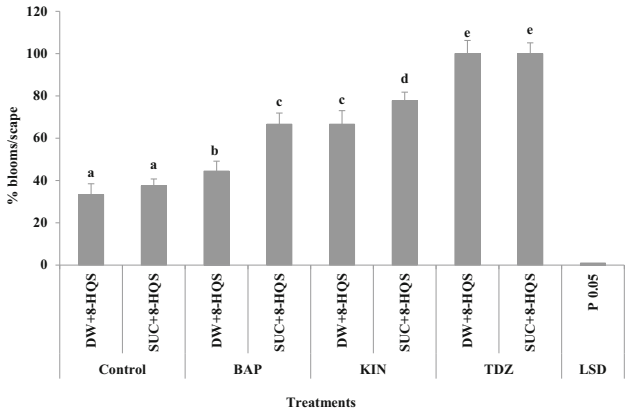

Fig. 4 Effect of benzyl amino purine (BAP), kinetin (KIN) and thidiazuron (TDZ) on the percent blooms per scape in cut scapes of *Iris germanica*. The letters a–e above the bars denote the statistical significance (Duncan's multiple range test) of the differences between individual treatments (only those marked with different letter differ significantly at $P < 0.05$)

Soluble proteins, specific protease activity and α-amino acids

Treatment of scapes with various cytokinins resulted in the maintenance of increased soluble protein content in tepal tissues with a concomitant decrease in the specific protease activity (Fig. 6). The tepal samples from the scapes treated with TDZ along with sucrose showed maximum increase in the protein content by 264.3% and a decrease in the specific protease activity by 55.6%. Scapes treated with TDZ showed a decrease in the α-amino acid content by 63% followed by KIN and BAP with a decrease of 49.5 and 47.6% respectively. Maximum α-amino acid content was registered in the samples taken from the control (Table 1).

Phenols and sugar fractions

The total phenolic content of the tepal tissues showed an increase with the application of BAP, KIN and TDZ. The

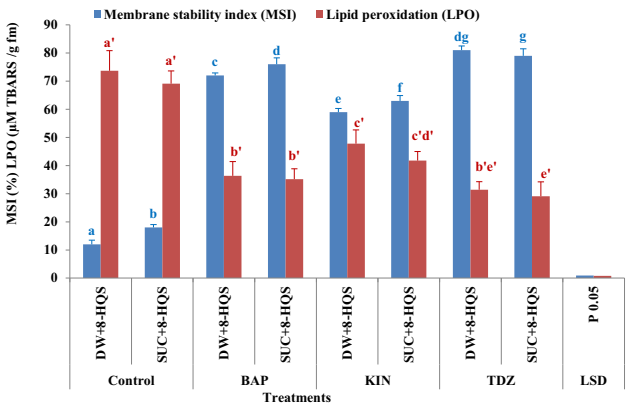

Fig. 5 Effect of benzyl amino purine (BAP), kinetin (KIN) and thidiazuron (TDZ) on the membrane stability index (MSI) and lipid peroxidation (LPO) of the tepal tissue in cut scapes of *Iris germanica*. The letters a–g and a′–e′ above the bars denote the statistical significance (Duncan's multiple range test) of the differences between individual treatments (only those marked with different letter differ significantly at $P < 0.05$)

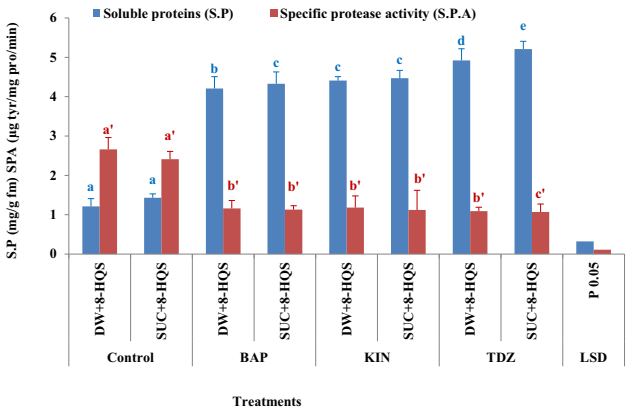

Fig. 6 Effect of benzyl amino purine (BAP), kinetin (KIN) and thidiazuron (TDZ) on the soluble proteins and specific protease activity of the tepal tissue in cut scapes of *Iris germanica*. The letters a–e and a′–c′ above the bars denote the statistical significance (Duncan's multiple range test) of the differences between individual treatments (only those marked with different letter differ significantly at $P < 0.05$)

tissue samples from the scapes treated with TDZ showed an increase in the phenolic content by 88.9% followed by KIN (71.2%) and BAP (58.9%) (Table 1). Minimum sugar fractions (reducing, non-reducing and total) were found in the control and maximum in tissue samples from BAP treated scapes with an increase by 125% (Fig. 7). Application of cytokinins resulted in the maintenance of higher sugar levels in the tepal tissues irrespective of nature of the vase solution.

Activities of antioxidant and lipoxygenase enzymes

Highest SOD, CAT and APX activity was found in the scapes treated with TDZ followed by scapes treated with KIN and BAP (Figs. 8, 9). TDZ treatment resulted in an increase in the SOD, CAT and APX activity by 170, 295 and 247.8% respectively. Lipoxygenase (LOX) activity was significantly decreased by 67, 59 and 54.6% in the samples from the scapes treated with TDZ, BAP and KIN. Minimum LOX activity was found in the samples from TDZ treated scapes (Fig. 9).

Discussion

Deterioration of quality and short vase life of various beautiful ornamental flowers comes in the way of their marketability in floriculture trade (Rogers 2013; Ahmad and Tahir 2015). Thus, the techniques to maintain the quality; besides the means for the extension of vase life are of immense importance. Several chemical treatments like silver thiosulphate, aminooxyacetic acid, 1-methylcyclopropene, auxins, gibberellins, sugars, sugar alcohols, jasmonates and polyamines have been tried in various flower systems like *Dianthus*, *Lilium*, *Rosa*, *Hemerocallis*, *Petunia*, *Consolida* and *Narcissus* which have proved successful in improving their postharvest performance (Have and Woltering 1997; van Doorn et al. 2013; Dar et al. 2014a; Mortazavi et al. 2011; Ahmad et al. 2013; Saeed et al. 2014). The present study was carried on *I. germanica* which has a lot of potential in the cut flower business. Postharvest and senescence modulation studies on this species are limited but studies have been carried out on *Iris hollandica* for the extension of vase life and improving the postharvest quality (Celikel and van Doorn 1995; van Doorn et al. 1995, 2003; Lee et al. 2005; Pak and van Doorn 2005; Macnish et al. 2010a; Celikel and van Doorn 2012; van Doorn et al. 2013). A simulated transportation protocol was earlier proposed by us for long term transport of this ornamental flower (Ahmad et al. 2013). In the present experiment, we have proposed the use of various cytokinins (as continuous vase treatments) for their ability to improve vase life and postharvest performance of *I. germanica*. Although Macnish et al. (2010a) have recommended 24 h pulse of 1 mM TDZ for improving the vase life of *Iris hollandica*, but the effect of continuous TDZ supply in the vase solution has not been assessed so far. Moreover, the underlying biochemical changes that result in the increase of vase life by cytokinin treatment are yet to be studied. This experiment was aimed to enhance our knowledge about the role of various cytokinins on vase life of *I. germanica* at biochemical level.

Table 1 Effect of benzyl amino purine (BAP), kinetin (KIN) and thidiazuron (TDZ) on the floral diameter, α-amino acids and total phenols on the postharvest performance of cut scapes of *Iris germanica*

Days after transfer	Control		6-Benzyl amino purine (BAP)		Kinetin (KIN)		Thidiazuron (TDZ)		LSD $P = 0.05$
	DW + 8-HQS	SUC + 8-HQS	DW + 8-HQS	SUC + 8-HQS	DW + 8-HQS	SUC + 8-HQS	DW + 8-HQS	SUC + 8-HQS	
Floral diameter (cm)									
2	8.13^{a}	8.36^{a}	8.34^{a}	8.76^{b}	8.54^{b}	8.91^{c}	8.97^{c}	9.33^{d}	0.09
6	–	–	8.12$^{a'}$	8.15$^{a'}$	8.31$^{b'}$	8.27$^{b'}$	8.88$^{c'}$	8.97$^{c'}$	0.04
α-amino acids (mg g^{-1} fm)									
4	11.32^{a}	10.17^{b}	5.71^{c}	5.33^{d}	5.37^{d}	5.13^{e}	4.11^{f}	3.76 ^{g}	0.11
Total phenols (mg g^{-1} fm)									
4	3.81^{a}	3.99^{a}	6.61^{b}	6.34^{b}	6.97^{c}	6.83^{c}	7.33^{d}	7.54^{d}	0.08

The letters a–d and a'–c' (floral diameter); a–g (α-amino acids) and a–d (total phenols) denote the statistical significance (Duncan's multiple range test) of the differences between individual treatments (only those marked with different letters differ significantly at $P < 0.05$)

The values presented in the table are mean of 10 independent replicates (n = 10)

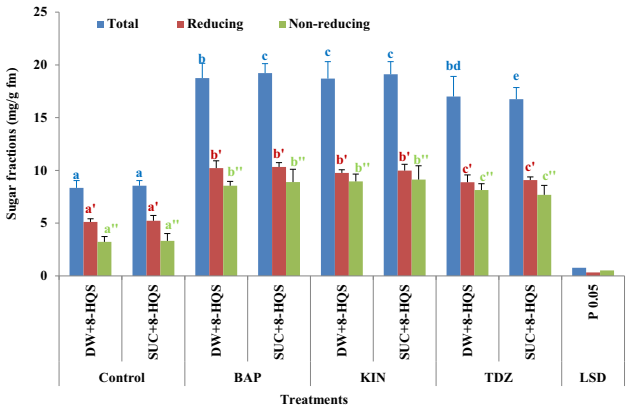

Fig. 7 Effect of benzyl amino purine (BAP), kinetin (KIN) and thidiazuron (TDZ) on the sugar fractions (reducing, non-reducing and total) of the tepal tissue in cut scapes of *Iris germanica*. The letters a–e, a'–c' and a''–c'' above the bars denote the statistical significance (Duncan's multiple range test) of the differences between individual treatments (only those marked with different letter differ significantly at $P < 0.05$)

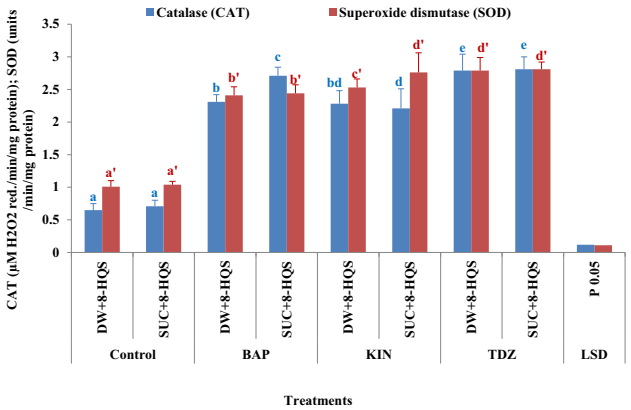

Fig. 8 Effect of benzyl amino purine (BAP), kinetin (KIN) and thidiazuron (TDZ) on the catalase (CAT) and superoxide dismutase (SOD) activity of the tepal tissue in cut scapes of *Iris germanica*. The letters a–e and a′–d′ above the bars denote the statistical significance (Duncan's multiple range test) of the differences between individual treatments (only those marked with different letter differ significantly at $P < 0.05$)

Effect on vase life, percent blooms and floral diameter

During the present study, TDZ was found to be most effective in improving the quality as well as vase life of *I. germanica* by 5.4 days followed by KIN and BAP. This increase in the vase life was because of the 100% blooming that took place in the TDZ treated scapes. Macnish et al. (2010a) have earlier reported that application of TDZ resulted in the increase in the number of blooms in *I. hollandica*. The effectiveness of TDZ than BAP and KIN can be attributed to its non-metabolizable nature (Macnish et al. 2010a). Our results about increase in vase life by cytokinin treatment on *Iris* is contrary to the studies on *Hemerocallis* where the cytokinins did not manifest the effect, but are in conformity with the results on carnation where vase life was considerably increased by cytokinin application (Lukaszewski and Reid 1989; Ferrante et al. 2009). Treatment of scapes with BAP, KIN and TDZ resulted in increased floral diameter than the control. This increased floral diameter by cytokinins is because of the maintenance of increased carbohydrate levels in the tepal tissues as is evident from the current investigation. Presence of more solutes will develop water potential gradient helping the water to move inside the tepal tissues making them turgid resulting in the increased floral diameter (van Doorn 2004; Dar et al. 2014a; Dar et al. 2014b; Iqbal et al. 2017; Reid and Wu 2018).

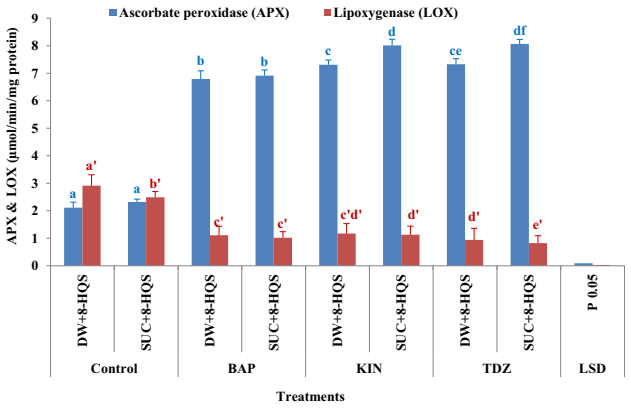

Fig. 9 Effect of benzyl amino purine (BAP), kinetin (KIN) and thidiazuron (TDZ) on the ascorbate peroxidase (APX) and lipoxygenase (LOX) activity of the tepal tissue in cut scapes of *Iris germanica*. The letters a–f and a′–e′ above the bars denote the statistical significance (Duncan's multiple range test) of the differences between individual treatments (only those marked with different letter differ significantly at $P < 0.05$)

Changes in lipid peroxidation, lipoxygenase activity and membrane stability index

Treatment with cytokinins resulted in the decreased lipid peroxidase activity (LPO) with least in TDZ treated scapes. This decrease in LPO can be attributed to the decreased lipoxygenase activity (LOX) of the tepal samples in the cytokinin treated scapes. Decreased LOX activity resulted in the decreased lipid peroxidation which can be visualized by the increased membrane stability index in the tissue samples from cytokinin treated scapes. This decrease in the LOX activity and LPO by cytokinin treatment can be attributed to maintenance of adequate phospholipids, proteins and thiols by preventing the leakage of proteases from vacuoles into the cytoplasm (Fukuchi-Mizutani et al. 2000; Pak and van Doorn 2005; Liu et al. 2016b; Dek et al. 2017).

Changes in soluble proteins, specific protease activity and α-amino acids

Protein levels were maintained with the treatment of scapes by the cytokinins, with the maximum protein levels in the tissue samples from TDZ treated scapes. The maintenance of increased proteins levels was concomitant with the decreased specific protease activity in the tepal tissues. Cytokinins have been found to delay senescence in *Andrepogon gerardi* and *Nicotiana* by preventing protein degradation and inhibiting the activity of proteases (Towne and Owensby 1983; Macnish et al. 2010b; Nisar et al. 2015). This suggests that cytokinins delay senescence by limiting programmed cell death due to prevention of an upsurge in the proteasomes (Pak and van Doorn 2005; van Doorn et al. 2013). Cytokinin treatment resulted in the maintenance of lower α-amino acid content as compared to the control. This decreased quantity of α-amino acids can be attributed to the lower protein breakdown in the tepal tissues from cytokinin treated scapes. During senescence process, proteins are transported as α- amino acid from the flowers back to the developing organs (Tripathi and Tuteja 2007; Ahmad et al. 2013; Ahmad and Tahir 2016a; Williamson and Hepworth 2018). Thus, the treatment of cytokinins during the present study showed curtailed protein degradation resulting in the lower α- amino acid content in the tepal tissues.

Changes in total phenols and sugar fractions

Treatment of *I. germanica* scapes with cytokinins especially TDZ, resulted in the accumulation of larger quantities of phenols in the floral tissues. Cytokinins have the property to stimulate defense mechanism for prevention of senescence by enhancing the accumulation of phenols (Schnablova et al. 2006; Schmitzer et al. 2010; Ahmad and Tahir 2017). Phenol enrichment has been shown to occur in the petals of petunia by the application of cytokinins (Trivellini et al. 2014). Accumulation of phenols has been shown to help combat endogenous perturbations, biotic and abiotic stress in various flowers (Cvikrova et al. 1994; Siranidou et al. 2002; Lattanzio et al. 2006; Ahmad and Tahir 2017). Higher carbohydrate fractions were maintained in the tissue samples by the application of various cytokinins. Maintenance of higher carbohydrate content has been shown to be associated with longer vase life in various flower systems like *Dianthus* and rose (Hunter et al. 2002; Dar et al. 2014b; Jones et al. 2005). Cytokinins have long been implicated in maintaining the sink strength, thus preventing the movement of sugars from the floral parts back to ovary during nutrient remobilization (van Doorn and Woltering 2008; Javid et al. 2011; Ahmad et al. 2013). Moreover, it has been found that TDZ induced delay in flower senescence was associated with the movement of sugars from the leaves to the flowers for maintaining flower metabolism in *Iris hollandica* (van Doorn 2004; Tassoni et al. 2006; Macnish et al. 2010a; Shibuya and Ichimura 2016).

Changes in the activity of antioxidant enzymes

During the present investigation, antioxidant enzyme activities (CAT and SOD) of the tepal tissues were augmented by the application of cytokinins, thus helping to scavenge toxic and highly unstable reactive oxygen species (ROS) that otherwise trigger senescence. Maximum antioxidant activity was maintained in the samples from TDZ treated scapes. Application of cytokinins especially

TDZ resulted in increase in the ascorbate peroxidase (APX) activity. Decreased APX activity has been shown to trigger senescence by the accumulation of highly reactive H_2O_2 in the tepal tissues of various flowers like daylily, gladiolus and carnation (Panavas and Rubinstein 1998; Mittler et al. 2004; Saeed et al. 2014). Cytokinins have been found to delay senescence either by increasing the production of ROS scavenging enzymes (through its positive effect on protein synthesis) or directly by scavenging the free radicals (Synkova et al. 2006; Lattanzio et al. 2006; Liu et al. 2016a). Cytokinins have also been found to slow down the process of free radical formation during senescence in tobacco plant (Synkova et al. 2006; Ahmad and Tahir 2016b; Dek et al. 2017). POD, SOD and CAT activity was found to be modulated by the application of cytokinins for efficient scavenging of the reactive oxygen species (Petit-Paly et al. 1999, Mwangi et al. 2003; Mittler et al. 2004; Danilova et al. 2017). Application of the inhibitors of cytokinin oxidase on wall flowers has substantiated the role of cytokinins in maintaining the levels of antioxidant enzymes for longer vase life of the flowers (Price et al. 2008; Bartrina et al. 2017).

Conclusions and future prospects

The present investigation revealed that cytokinins, preferably TDZ, improve postharvest performance of *I. germanica* by maintaining a striking balance between various biochemical parameters. Cytokinins resulted in maintenance of higher proteins, carbohydrates, phenols and antioxidant enzymes; besides, maintaining lower specific protease activity, lipid peroxidation and α-amino acids. The possible mechanism of the involvement of cytokinins in regulating these processes is still unknown to a larger account. Detailed studies on the pathways of cytokinin action in delaying flower senescence and improving postharvest performance of cut scapes need to be taken at biochemical level. Understanding of the pathway by which cytokinins and thidiazuron regulate flower senescence at molecular level could help in gaining further insights. The understanding can greatly help us to delay senescence and devise more precise techniques for improving the postharvest performance of various cut flowers. Furthermore, the mode of action of the diphenyl urea derived cytokinin (TDZ) should be studied, as it has been showing promising results in the regulation of senescence in various flowers at relatively lower concentrations.

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Author's contribution Syed Sabhi Ahmad carried out the experiments, obtained results, analyzed, compiled the data and drafted the manuscript. Prof. Inayatullah Tahir helped in designing the experiment, supervised the laboratory work, took the photographs and edited the manuscript. Arif Shafi Wani, Riyaz Ahmad Dar and Shaziya Nisar helped in statistical analysis of the data and in the laboratory work.

Compliance with ethical standards

Conflict of interest The authors don't have any conflict of interest regarding this manuscript.

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