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RESEARCH ARTICLE

Putrescine and Jasmonates Outplay Conventional Growth Regulators in Improving Postharvest Performance of *Iris germanica* L. Cut Scapes

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Abstract Efficiency of various growth regulators viz. gibberellic acid, salicylic acid, jasmonic acid, methyl jasmonate, putrescine, spermine and spermidine in improving the postharvest performance of cut scapes of *Iris germanica* L. was examined. Flower scapes harvested with the oldest bud at pencil stage (1 day before anthesis) were cut to a uniform length of 35 cm under water and divided into eight sets. Each set was provided with a different vase solution viz. gibberellic acid, salicylic acid, jasmonic acid, methyl jasmonate, putrescine, spermine and spermidine alone or in combination with 0.1 M sucrose. A separate set of scapes held in distilled water was designated as control. Application of putrescine in the vase resulted in the maximum enhancement of vase life by 6.4 days as compared to the control. All the growth regulators applied resulted in the significant increment of vase life against the control except for gibberellic acid which showed comparable vase life to control. Full blooming (100 %) was achieved in the scapes treated with putrescine and spermine alone or in combination with sucrose. Spermine treatment resulted in the burst of flowering on day 4 against the normal sequential blooming behavior of *Iris* species. Improved postharvest performance by the application of various growth regulators was associated with decrease in lipid peroxidase and lipoxygenase activity and an increased activity of various antioxidant enzymes like catalase, superoxide dismutase and ascorbate peroxidase besides maintaining higher values of soluble proteins, sugar fractions, total phenols and membrane stability index.

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Introduction

Flower senescence although a crucial and important event in the life cycle of the angiosperms, is considered as a major problem regarding the postharvest management of cut flowers. The onset of flower senescence is triggered by a wide array of internal and external factors which initiate a series of physiological events orchestrated by plant growth regulators [1, 2]. Ethylene is the main candidate for senescence regulation in ethylene sensitive flowers while it has little or no role in ethylene insensitive flower systems. The role of various other growth regulators such as auxins, gibberellic acid, cytokinins, salicylic acid, jasmonic acid, methyl jasmonate and polyamines has also an important part to play in the regulation of flower senescence [1, 3]. Studies on various flowers have confirmed that ethylene and abscisic acid trigger senescence, whereas cytokinins and polyamines have been found to delay it. The role of auxins, gibberellins, jasmonates and salicylic acid in flower senescence has been shown to vary from one species to another [4].

Auxins have been found to trigger flower senescence in some ethylene sensitive flowers like carnation by stimulating ethylene production [5], while as in ethylene insensitive *Lilium longiflorum*, flower senescence was shown to be triggered by an increase in the endogenous level of auxins without the involvement of ethylene [6]. In *Lilium longiflorum* Asiatic hybrid (L.A), *Hemerocallis*, *Ipomoea* and *Ranunculus* flower senescence has been shown to be initiated and executed without changes in the level of endogenous auxins [6]. This dynamic role of auxins in

flower senescence has opened new windows for understanding the precise role of auxins in flower development and senescence. Gibberellins have been found to delay senescence in *Dianthus* sp., *Lupinous desiflorus* and *Gerbera jamesonii* by acting as ethylene antagonists but in *Grevillea* sp., increase in the gibberellins resulted in the flower abscission but not senescence [7, 8]. Gibberellins have been shown to have no role in the regulation of flower senescence in *Hemerocallis* sp. and tulips. Gibberellins show their involvement in the regulation of flower development and senescence in ethylene sensitive flower systems by acting as ethylene antagonists, but their role in the regulation of ethylene independent flower senescence has not been detailed so far. Cytokinins delay flower senescence by promoting transport, accumulation and retention of metabolites in flower petals, besides preventing membrane degradation [2, 4]. Alternatively, cytokinins may delay senescence by regulating cytokinin/auxin activity or by decreasing flower's sensitivity to ethylene in ethylene dependent flowers [4]. Applied jasmonates (jasmonic acid and methyl jasmonate) have been shown to trigger flower senescence in *Dendrobium* sp., *Phalaenopsis* sp. and *Petunia hybrida* accompanied by an increase in the ethylene production [9]. Contrary to this, flower senescence in *Nicotiana attennuta* and *Iris hollandica* has been shown to be delayed by the application of jasmonic acid [4]. Thus the precise role of jasmonates in flower development and senescence is unclear and needs to be studied individually at species level. Salicylic acid has recently been found to play an important role in the regulation of flower development in some ethylene sensitive flowers. Salicylic acid has been found to delay senescence in *Eustoma grandiflorum* and *Dianthus* sp. by delaying lipid peroxidation of membrane lipids [10]. Senescence in many other flowers has been shown to be dependent on salicylic acid but its role has not been studied in detail. Polyamines have generally been found to delay flower senescence in ethylene sensitive flowers like *Nicotiana* sp. and *Dianthus* sp. as polyamines inhibit the synthesis of ethylene [11]. Polyamines and ethylene share a common precursor, S-adenosyl methionine (SAM) for their synthesis and thus increased polyamine synthesis declines the biosynthesis of ethylene thus preventing ethylene sensitive flower senescence [12]. Application of polyamines has also been found to delay senescence by preventing chlorophyll loss, membrane deterioration and protease activity. Although the role of polyamines in ethylene sensitive flower senescence is clear, but their role in the regulation of ethylene insensitive flower senescence is still unclear.

It becomes evident that the role of various growth regulators like gibberellic acid, salicylic acid, jasmonates and polyamines is understood to some extent in ethylene sensitive flowers while their role in ethylene insensitive flowers is still elusive. The present study was undertaken on the role of various growth regulators on flower development and senescence in ethylene insensitive *Iris germanica*. The effect of the exogenous application of these growth regulators on vase life and postharvest performance of cut scapes of *Iris germanica* was monitored at physiological and biochemical level. Changes in soluble proteins, specific protease activity, sugar fractions, total phenols, α-amino acids and membrane stability index besides changes in the activity of various enzymes like catalase, superoxide dismutase, lipid peroxidase, lipoxygenase and ascorbate peroxidase were studied.

Material and Methods

Plant Material

Healthy scapes of *Iris germanica* were collected at 8:00 a.m with the oldest bud at pencil stage i.e. stage IV (1 day before anthesis) from Kashmir University Botanic Garden (KUBG), brought to laboratory under water, cut to a uniform length of 35 cm and divided into eight sets. The scapes were subjected to treatment of seven different growth regulators viz. gibberellic acid (100 μM), salicylic acid (400 μM), jasmonic acid (20 μM), methyl jasmonate (0.5 μM), putrescine (500 μM), spermine (500 μM) and spermidine (500 μM) alone or in combination with sucrose (0.1 M). A separate set of scapes held in distilled water was designated as control. The scapes were held in 200 ml of the respective vase solutions. Each treatment had 10 replicates (flasks) and each flask contained two scapes. In all the flasks, 0.1 mM 8-hydroxy quinoline sulphate (8-HQS) was added to prevent microbial growth in the vase solutions. The experiment was conducted under controlled conditions with relative humidity (RH) of 60 ± 10 %, 12 h light period a day and average temperature of 23 ± 2 °C.

Just prior to this experiment, the optimal concentration was standardization for sucrose and all the growth regulators (gibberellic acid, salicylic acid, jasmonic acid, methyl jasmonate, putrescine, spermine and spermidine) used in the present experiment. For that purpose, grades of all the growth regulators were prepared and the response of scapes was tested for various concentrations of the growth regulators. The experiment was monitored keenly and the concentrations which gave best results either in the improvement of vase life and postharvest performance or least deleterious effects on the cut scapes of the *Iris germanica* were selected for each growth regulator for this experiment.

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. Floral diameter was recorded at periodic intervals during the course of the experiment.

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 [13]. 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 under.

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

Lipid Peroxidase Activity (LPO)

Lipid peroxidation was determined by the method of Heath and Packer [14]. Tepal tissue (0.5 g) was macerated in 15 ml of 0.1 % trichloroacetic acid (TCA) and centrifuged at 15,000×*g* for 10 min under refrigeration. One 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 for 95 °C in water bath for 25 min. Reaction was ended by placing it in ice. Absorbance was recorded 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

Chopped tepal tissue (1 g) from each treatment was fixed in hot 70 % ethanol, macerated and centrifuged thrice. Total phenols, α-amino acids, reducing, non-reducing and total sugars were estimated from a suitable aliquot taken from the supernatant. Rosen's method [15] was employed for α-amino acid quantification with glycine acting as standard. Total phenolics were quantified by Swain and Hillis method [16] using gallic acid as standard. Nelson's method [17] 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 4 °C in a refrigerated centrifuge for 15 min. The supernatant was collected and used for protein estimation. Proteins were estimated by the method of Lowry et al. [18] from a suitable volume of aliquot taken from the supernatant. Specific protease activity was determined from 1 g of tepal tissue by the modification of the method as described by Tayyab and Qamar [19].

Enzyme Extraction and Assays

Superoxide Dismutase Activity (SOD)

Tepal tissue (1 g) 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 15,000×*g* for 10 min. The supernatant was filtered through Mira cloth and used for the enzyme assay.

SOD activity was measured using the method of Dhindsa et al. [20] 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 illuminating 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 Activity (CAT)

Catalase activity was estimated by the method of Aebi [21]. Tepal tissue (1 g) was macerated in mortar 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} protein.

Ascorbate Peroxidase Activity (APX)

For the determination of APX activity, flower tepals 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 [22].

Lipoxygenase Activity (LOX)

LOX activity was determined by the method of Axerold et al. [23]. Tepal tissue (1 g) 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 phenylmethanesulfonyl fluoride (PMSF). The 1 ml reaction mixture 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.

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 in figures and table represents the mean of ten replicates. LSD was computed at $P_{0.05}$ using SPSS 16 software for analyzing the data statistically. Standard deviation has been computed as under.

$$S = \sqrt{\frac{\sum x^2}{N-1}}$$

Results and Discussion

Cut flowers face the problem of short vase life and poor postharvest performance, once they are detached from the parent plant. This postharvest deterioration of the quality comes in the way of their efficient marketability and as such techniques and methods are to be devised for improving the vase life and postharvest quality of the various important cut flowers. The present experiment was aimed to improve the postharvest performance of the cut scapes of *Iris germanica,* an important ethylene insensitive flower by the exogenous application of various growth regulators viz. gibberellic acid, salicylic acid, jasmonic acid, methyl jasmonate, putrescine, spermine and spermidine alone or in combination with sucrose. The scapes of *Iris germanica* bear 4–7 buds which are enclosed within two green leaves up to maturity. The flower opening is initiated by the elongation of pedicel and ovary so that the flower is relieved of the green leaves which mechanically prevent the opening of the floral bud. The life of an individual flower after it is fully open is about 2 days in the field as well as under laboratory conditions. Flower senescence is marked by loss of turgor followed by inrolling of the distal ends of the tepals and ultimately the flowers turn pale towards the peak of senescence.

Application of all the growth regulators except gibberellic acid resulted in significant increment in the vase life against control. Putrescine and jasmonic acid were most effective in delaying flower senescence in the scapes of *Iris germanica* by 6.4 and 4.8 days respectively against the control (Figs. 1, 2). This increased flower longevity was commensurate with the increased percent blooms per scape. All the growth regulators resulted in the improved rate of blooming of the floral buds as compared to the control with 100 % blooming in putrescine and spermine and 93.7 % in jasmonic acid (Table 1). Polyamines like putrescine, spermine and spermidine have been found to improve the postharvest performance of various ethylene sensitive flowers like *Dianthus* sp., *Nicotiana* sp. and *Narcissus* sp. but their role in the regulation of flower senescence in ethylene insensitive flowers has not been studied [1, 3, 11]. Delay of flower senescence in ethylene sensitive flowers can be ascribed to its anti-ethylene property but the precise mechanism employed in delaying the flower senescence in *Iris germanica* (an ethylene insensitive flower) is yet to be studied in detail. A possible explanation can be that putrescine maintains higher activity of antioxidant enzymes and a low lipid peroxidase activity. This may in turn lead to delay in programmed cell death and thereby improve the postharvest performance of *Iris germanica* cut scapes. The effect of jasmonic acid in improving flower longevity was unexpected as jasmonates have been found to accelerate senescence in leaves, fruits, cotyledons and various ethylene sensitive flowers like *Petunia* sp., *Dendrobium* sp. and *Phalaenopsis* sp. by causing an increase in ethylene production. Moreover, the leaf senescence in *Iris* was also hastened by the application of Jasmonic acid [4]. The delay in flower senescence by jasmonic acid in the present study may be because jasmonates prevent protein synthesis as senescence in *Iris* flowers is thought to get induced by specific proteins that are formed towards latter stages of flower development [24]. Gibberellic acid has been shown to be ineffective in improving the postharvest performance of *Iris germanica* during the present investigation. Earlier studies have revealed that

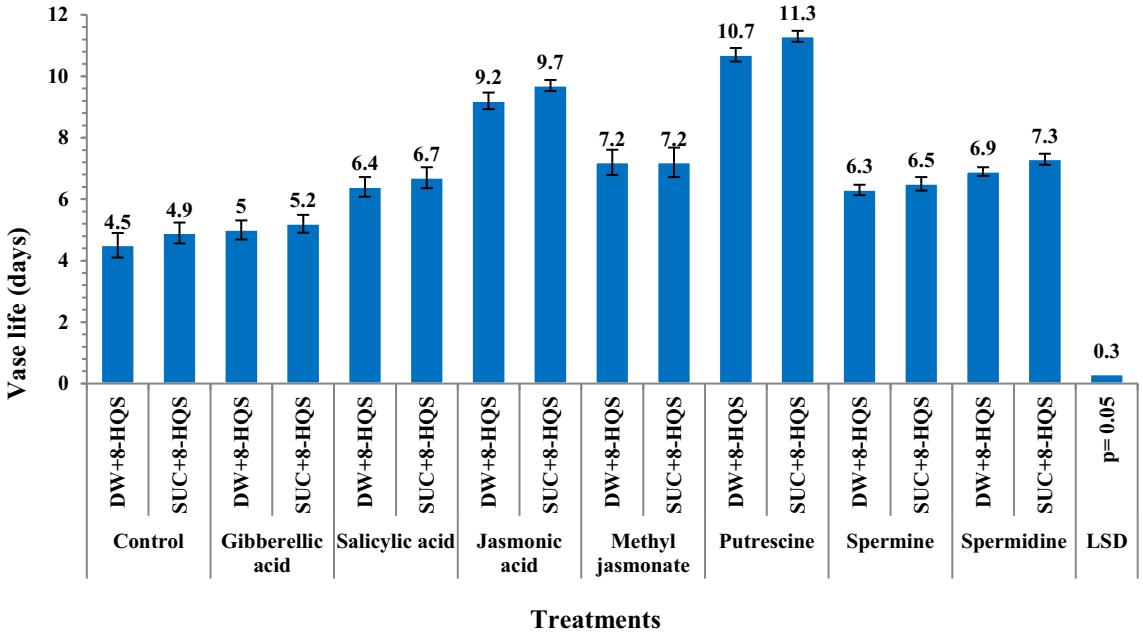

Fig. 1 Effect of various growth regulators viz. gibberellic acid, salicylic acid, jasmonic acid, methyl jasmonate, putrescine, spermine and spermidine on the vase life of cut scapes of *Iris germanica*

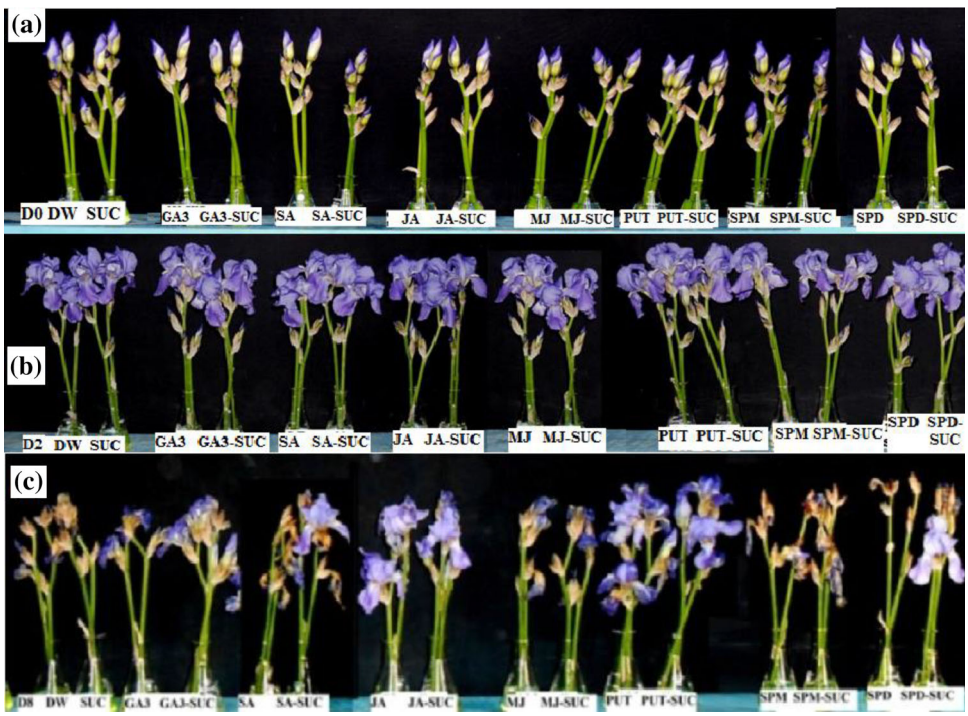

Fig. 2 Effect of various growth regulators viz. gibberellic acid (*GA3*), salicylic acid (*SA*), jasmonic acid (*JA*), methyl jasmonate (*MJ*), putrescine (*PUT*), spermine (*SPM*) and spermidine (*SPD*) on day 0 (**a**), day 2 (**b**) and day 8 (**c**) on the postharvest performance and vase life of cut scapes of *Iris germanica*

gibberellins delay senescence in some ethylene sensitive flowers like *Dianthus* sp. but had no effect on ethylene insensitive flower senescence [25]. Application of salicylic acid to the cut scapes of *Iris germanica* slightly extended the vase life of the cut scapes. These scapes also showed lower lipid peroxidase activity which may be the possible reason for the role of salicylic acid in improving the postharvest performance of cut scapes of *Iris germanica*. Lipid peroxidation of membrane lipids have been shown to be the basic event in the initiation of flower senescence in various flowers like Carnation, *Gladiolus* sp. and *Gerbera* sp. [10]. Spermine treatment although resulted in 100 %

Table 1 Effect of various growth regulators viz. gibberellic acid, salicylic acid, jasmonic acid, methyl jasmonate, putrescine, spermine and spermidine on floral diameter, %blooms/scape, membrane stability index (MSI), α-amino acids, total phenols and sugar fractions (total, reducing and non-reducing) in the tepal samples of the flowers from cut scapes of *Iris germanica*

Days after transfer	Control		Gibberellic acid (GA_3)		Salicylic acid		Jasmonic acid		Methyl jasmonate		Putrescine		Spermine		Spermidine		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	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)																	
3	9.1	9.3	9.3	9.3	9.1	9.5	9.2	9.5	8.9	9.6	9.3	9.7	10.6	11.2	9.2	9.8	0.2
6	–	–	–	–	8.6	8.7	9.1	9.3	8.3	8.5	8.7	9.3	9.5	9.9	8.6	8.9	0.1
% Blooms $scape^{-1}$																	
–	33.30	37.50	40.50	40.50	58.35	65.70	89.35	93.70	85.00	93.75	100	100	100	100	67.35	70.80	0.9
Membrane stability index (%)																	
3	57	60	67	68	68	73	81	79	66	69	80	80	65	65	70	77	1.3
7	–	–	35	43	29	53	62	68	49	49	69	72	25	25	39	44	1.1
α-amino acids (mg g^{-1} fm)																	
3	11.32	10.17	4.68	4.70	4.69	4.73	4.68	4.54	4.73	4.74	4.52	4.26	4.66	4.65	4.75	4.71	0.11
7	–	–	9.89	8.99	10.15	9.06	8.33	8.15	9.29	9.37	6.57	6.34	11.12	10.97	9.33	8.98	0.13
Total phenols (mg g^{-1} fm)																	
3	3.11	3.29	3.37	3.36	3.12	3.29	3.11	3.16	3.07	3.15	3.39	3.31	3.21	3.17	3.10	3.11	0.09
7	–	–	6.58	6.61	6.53	6.57	4.77	4.19	5.52	5.57	4.89	4.91	6.15	6.31	6.47	5.68	0.14
Total sugars (mg g^{-1} fm)																	
3	8.34	8.54	16.19	18.78	16.11	17.76	19.16	22.14	16.12	16.23	22.79	24.73	16.13	16.16	16.26	18.85	0.79
7	–	–	4.86	5.54	4.69	6.33	9.18	10.84	5.97	6.03	10.93	11.15	4.18	4.29	5.98	6.73	0.28
Reducing sugars (mg g^{-1} fm)																	
3	5.12	5.23	8.01	11.79	8.04	9.17	13.35	15.01	8.19	8.27	14.19	15.92	7.96	8.03	8.21	12.11	0.52
7	–	–	3.78	4.01	3.03	4.79	6.33	7.54	3.91	3.87	6.98	7.54	2.95	3.00	3.95	5.13	0.27
Non-reducing sugars (mg g^{-1} fm)																	
3	3.22	3.31	8.18	6.99	8.07	8.59	5.81	7.13	7.93	7.96	8.60	8.81	8.17	8.13	8.05	6.74	0.15
7	–	–	1.08	1.53	1.66	1.54	2.85	3.30	2.06	2.16	3.95	3.61	1.23	1.29	2.03	1.6	0.13

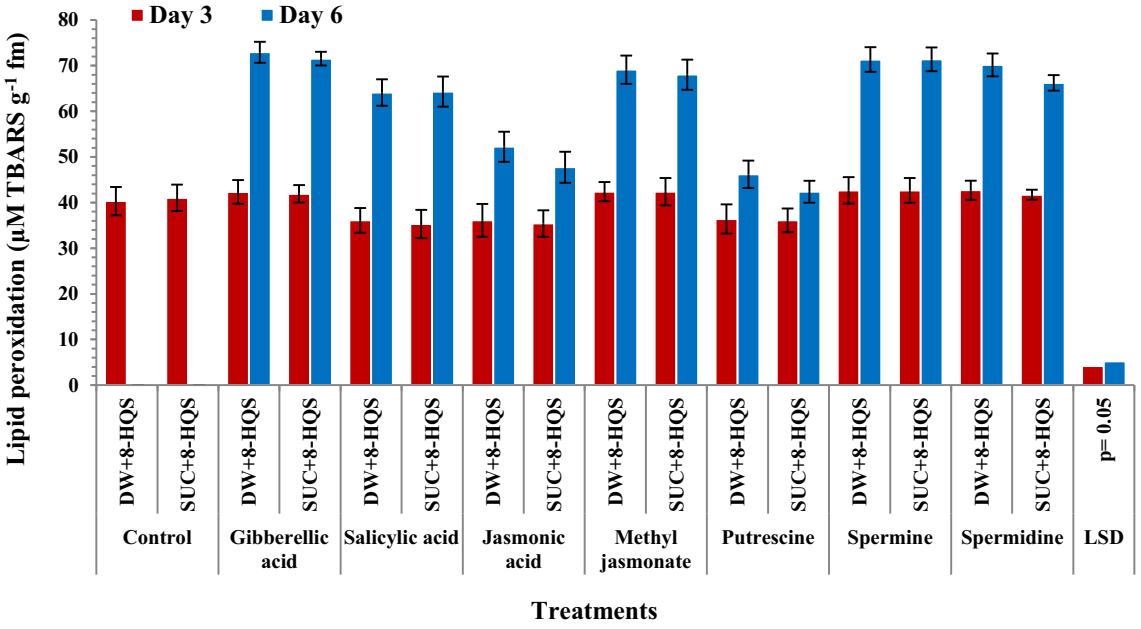

Fig. 3 Effect of various growth regulators viz. gibberellic acid, salicylic acid, jasmonic acid, methyl jasmonate, putrescine, spermine and spermidine on lipid peroxidation (LPO) in the tepal samples of flowers from cut scapes of *Iris germanica*

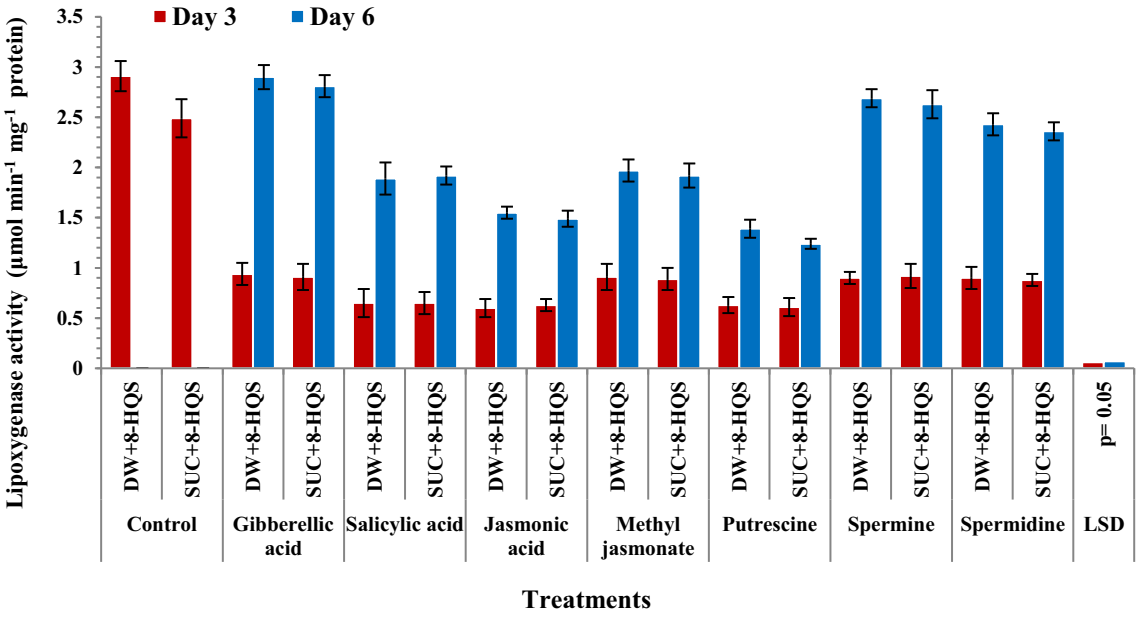

Fig. 4 Effect of various growth regulators viz. gibberellic acid, salicylic acid, jasmonic acid, methyl jasmonate, putrescine, spermine and spermidine on lipoxygenase (LOX) in the tepal samples of flowers from cut scapes of *Iris germanica*

blooming, but the vase life was not extended significantly as its treatment resulted in the burst of flowering on day 4 against the normal sequential blooming behavior of *Iris* species (Fig. 1; Table 1). This may be because spermine helps in maintaining higher water status of the cells which ultimately make the cells turgid resulting in flower opening. This can be substantiated by the significantly increased flower diameter in the scapes treated with spermine than the control and other treatments which showed more or less same flower diameter (Table 1). The increased turgidity of the cells is the basic criteria for opening of various flowers like *Iris* sp., *Dianthus* sp. and *Hemerocallis* sp. [3, 8]. Addition of sucrose along with different growth regulators augments the effect of growth regulators on vase life and

postharvest performance. This may be because sucrose acts as a respiratory substrate for the better functioning of the plant tissue besides helping in maintaining higher water balance of the cells and acting as a signal molecule.

Treatment of scapes with salicylic acid, putrescine and jasmonic acid resulted in significantly lower lipid peroxidase activity than the other treatments and control which is reflected by their increased membrane stability index values (Table 1; Fig. 3). Lipid peroxidation increases with the progression in time from day 3 to 6 with a concomitant decrease in the membrane stability index (Fig. 3). The decreased values for lipid peroxidation in salicylic acid, putrescine and jasmonic acid treated scapes is mainly due to lower lipoxygenase activity. This decreased lipoxygenase activity helps to maintain adequate phospholipids, proteins and thiols by preventing the leakage of proteases from vacuoles into the cytoplasm [26].

The lipoxygenase (LOX) showed a minimal activity in the tissue samples from scapes treated with various growth regulators as compared to the control where it was very high (Fig. 4). The LOX activity was slightly lower in the samples from scapes treated with various growth regulators along with sucrose than the corresponding scapes held in various growth regulators without sucrose. LOX activity showed a sharp increase from day 3 to 6 in the samples from scapes held in gibberellic acid, spermine and spermidine but a marginal increase was recorded in the samples from scapes held in salicylic acid, putrescine, methyl jasmonate and jasmonic acid (Fig. 4). Increased lipoxygenase activity has been shown to be the basic biochemical change that takes place during the onset of senescence in various flowers like *Gladiolus* sp., *Dianthus* sp. and *Gerbera* sp. [27].

The total phenolic content has been shown to remain more or less constant in all the treatments including control on day 3 but a sharp increase has been registered towards day 7 with maximum in the tissue samples from scapes treated with gibberellic acid and salicylic acid (which showed least improvement in vase life and postharvest performance against the other treatments) (Table 1). The finding that increased phenolic content is associated with shorter vase life does not corroborate with the earlier studies on *Nerine sarniensis*, *Rosa hybrida* and *Tithonia rotundifolia* wherein the improvement in postharvest life is associated with increased content of total phenols as the phenolics have been suggested to play an important role in antioxidant defense by scavenging the free radicals and preventing the flower from oxidative stress [28, 29]. The increased concentration of phenols in the present study towards senescence can be due to failure of reallocation of the phenols towards the developing parts or by the increased synthesis of phenols towards senescence as part of the defense mechanism employed by flowers to delay senescence [29, 30].

Increased vase life and postharvest performance was found to be associated with increased levels of proteins with maximum in the scapes held in putrescine and jasmonic acid. Sucrose treated scapes showed higher values for soluble proteins in the tepal tissues as compared to the corresponding scapes transferred to DW irrespective of the growth regulator tested (Figs. 5, 6). The increased levels of the proteins in the treated scapes were manifested because of the decreased specific protease activity of the tepal tissues. This decreased specific protease activity in the samples from the treated scapes is reflected in the lower content of α-amino acids which are the break down products of the proteins. The tissue content of α-amino acids in all the treatments was almost comparable but significantly lower than the control (Table 1). Application of putrescine, spermine and spermidine has been found to delay senescence in *Nicotiana* sp. by maintaining an increased level of proteins which suggests that polyamines delay

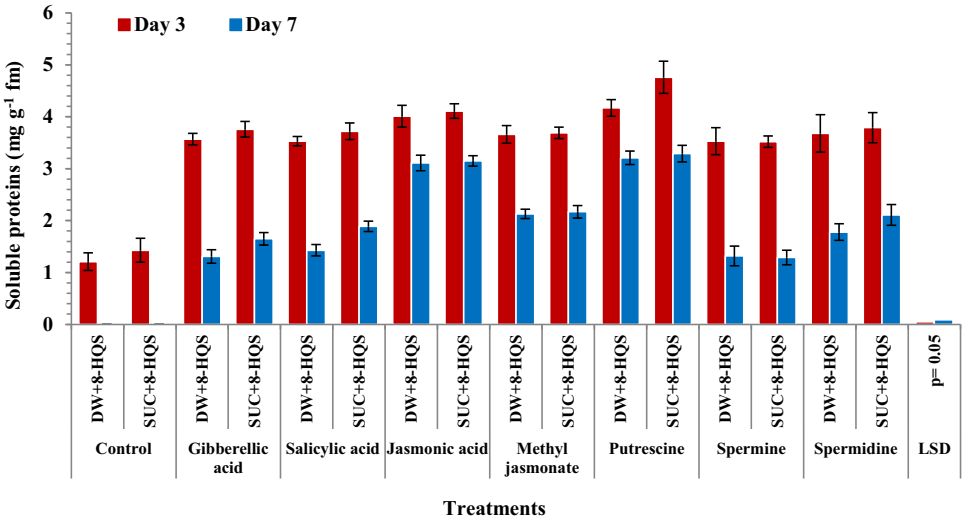

Fig. 5 Effect of various growth regulators viz. gibberellic acid, salicylic acid, jasmonic acid, methyl jasmonate, putrescine, spermine and spermidine on soluble proteins in the tepal samples of flowers from cut scapes of *Iris germanica*

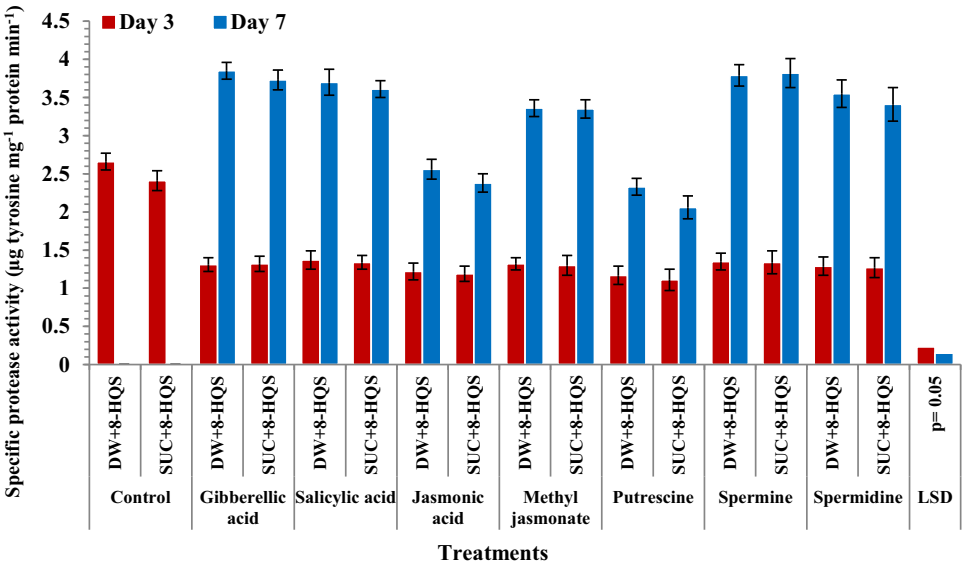

Fig. 6 Effect of various growth regulators viz. gibberellic acid, salicylic acid, jasmonic acid, methyl jasmonate, putrescine, spermine and spermidine on specific protease activity in the tepal samples of flowers from cut scapes of *Iris germanica*

programmed cell death by preventing the proteins from proteolysis. Jasmonates have been shown to have inhibitory effect on the activity of the proteolytic enzymes in flowers of *Petunia* sp. which prevents protein degradation thereby delay flower senescence. The specific protease activity has been shown to increase with the progression in time from day 3 to 7 resulting in a sharp decline in the soluble protein content and an increase in its breakdown product i.e. α-amino acids. Protein degradation and increased protease activity is the most important step in the initiation of senescence in various flowers like *Dianthus* sp., *Nicotiana* sp. and *Petunia* sp. [1, 2, 11, 31].

The tissue content of sugar fractions (total,reducing and non-reducing) showed that increased vase life was associated with higher content of sugar fractions. The scapes treated with various growth regulators maintained significantly higher content of sugar fraction (total, reducing, non-reducing) as compared to the control with maximum in the tissue samples from scapes treated with putrescine in combination with sucrose (Table 1). Sugar fractions have been shown to decrease sharply with the progression in time from day 3 to 7 with least decrease in the samples from scapes held in putrescine. Maintenance of higher levels of sugar fractions by the application of putrescine can be ascribed to its property of delaying cellular respiration and thereby maintaining higher levels of internal sugars for longer duration [11, 32]. Maintenance of higher sugar fractions in various other flowers like *Dianthus* sp., *Iris* sp., *Nicotiana* sp., *Petunia* sp., *Narcissus* sp., *Nerine* sp. and *Hemerocallis* sp. has been shown to lead to improved vase life and postharvest performance by serving

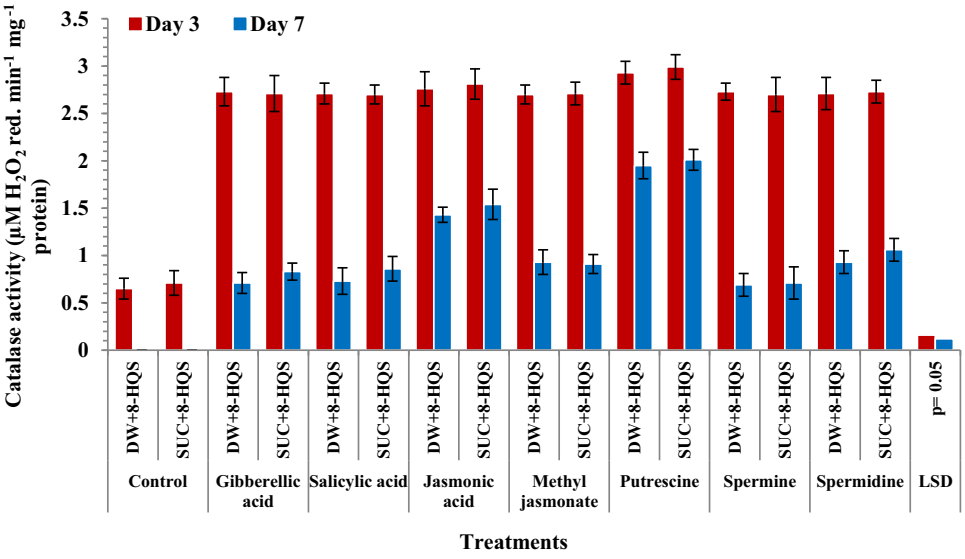

Fig. 7 Effect of various growth regulators viz. gibberellic acid, salicylic acid, jasmonic acid, methyl jasmonate, putrescine, spermine and spermidine on catalase activity (CAT) in the tepal samples of flowers from cut scapes of *Iris germanica*

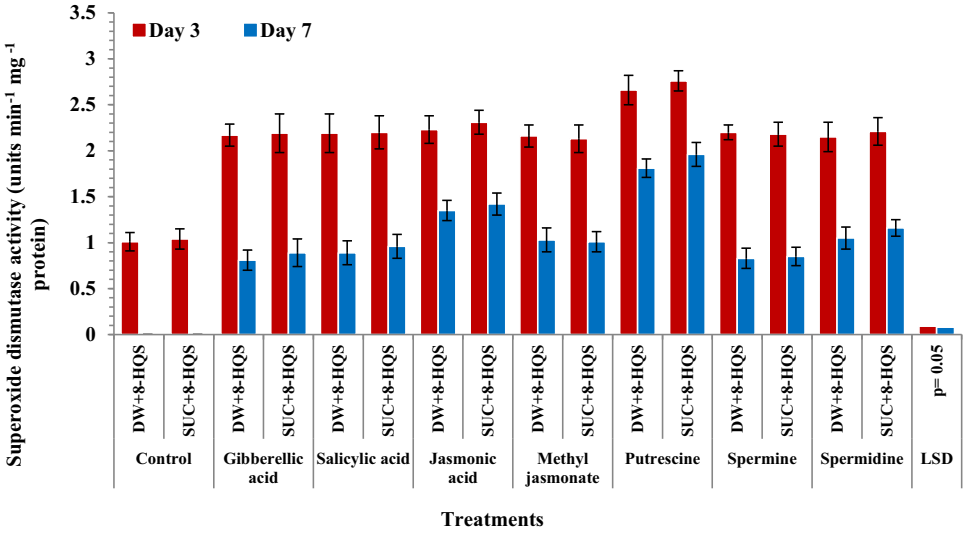

Fig. 8 Effect of various growth regulators viz. gibberellic acid, salicylic acid, jasmonic acid, methyl jasmonate, putrescine, spermine and spermidine on superoxide dismutase (SOD) activity in the tepal samples of flowers from cut scapes of *Iris germanica*

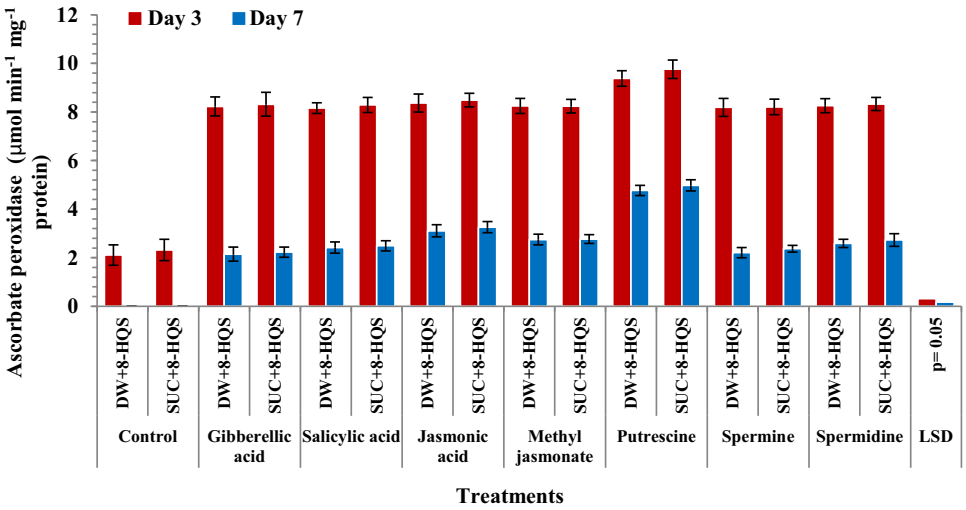

Fig. 9 Effect of various growth regulators viz. gibberellic acid, salicylic acid, jasmonic acid, methyl jasmonate, putrescine, spermine and spermidine on ascorbate peroxidase (APX) activity in the tepal samples of flowers from cut scapes of *Iris germanica*

to improve water relations and providing the energy for the cellular homeostasis [8, 31, 33, 34].

The increased vase life of the scapes of *Iris germanica* was associated with an increased activity of various oxidative enzymes like catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) (Figs. 7, 8, 9). The activity of these enzymes was found to be significantly higher in the scapes held in various growth regulators as compared to the control and the activity showed a pronounced decrease with the progression in time from day 3 onwards in all the treatments. Putrescine was found to be the most effective in increasing the antioxidant activity of CAT, SOD and APX. Polyamines like putrescine, spermine and spermidine have been shown to regulate the apoplastic antioxidant activity of various antioxidant enzymes. An endogenous or exogenous increase in the levels of the various polyamines results in the increased scavenging of oxygen and hydroxyl free radicals [35] which in turn helps the cells to maintain higher quantities of proteins and sugars to tackle the phenomenon of senescence. Increased activity of antioxidant enzymes by the application of jasmonates (methyl jasmonate and jasmonic acid) resulted in a decrease in protease activity which in turn significantly increased the vase life of the corresponding scapes. Gibberellins and salicylic acid have also been found to delay senescence in *Gladiolus* sp., *Dianthus* sp. and *Eustoma* sp. by the enhanced activity of CAT, SOD and APX which might be due to the catalyzation of peroxides [36]. The enhanced CAT and SOD activity has also been shown to improve vase life of various flowers by stabilizing the lipid bilayer and membranous system. The higher ROS scavenging activity of SOD, CAT and APX eliminates the oxidative stress from the cut flowers and reduces the ion

leakage by reducing membrane damage through the oxidation of polyunsaturated fatty acids (PUFA) [27].

Future Perspective

Although the biochemical mechanism employed in delaying senescence is known to some extent in ethylene sensitive flowers, but in ethylene insensitive flowers it is still elusive. Future studies are important to substantiate the role of polyamines and jasmonates in regulating programmed cell death of flowers. It could be very interesting to study the process at molecular level for understanding the pathway by which polyamines and jasmonates regulate flower senescence. The understanding can greatly help us to delay senescence and devise more precise techniques for improving the postharvest performance of various cut flowers.

Conclusion

The postharvest performance of the scapes of *Iris germanica* (an ethylene insensitive flower) was significantly improved by the application of various growth regulators like salicylic acid, methyl jasmonate, jasmonic acid, putrescine, spermine and spermidine. Gibberellic acid was ineffective in extending the vase life of cut scapes of *Iris germanica* despite being able to increase the antioxidant activity of SOD, CAT and APX. Putrescine and jasmonic acid were the most effective in extending the postharvest performance of cut *Iris germanica* scapes by maintaining an increased concentration of proteins and total sugars besides a decrease in the lipoxygenase activity which prevented lipid peroxidation and thus maintained membrane integrity.

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Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interest.

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