Increased oxidative stress, lipid peroxidation and protein degradation trigger senescence in *Iris versicolor* L. flowers

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Abstract Dynamics in various physiological and biochemical aspects were studied during various stages (I—tight bud stage to VI—senescent stage) of flower development in *Iris versicolor*. Floral diameter, fresh & dry mass and water content increased during flower opening and decreased towards senescence. Senescence was found to be related to the increased lipid peroxidation which was reflected in the decreased membrane stability index towards senescence. This increase in the lipid peroxidation was probably initiated by increased lipoxygenase activity which shot up just prior to the increase in lipid peroxidation. Soluble protein content showed a marginal decrease towards senescence with a corresponding increase in specific protease activity. Sugar fractions and α -amino acids showed a significant decrease towards senescence. Superoxide dismutase and ascorbate peroxidase activity increased as the flowers opened and thereafter a significant decrease was registered towards senescence. Catalase activity improved as the flower matures, but decreased prior to flower opening through senescence. The protein patterns from the tepal tissues resolved through electrophoresis showed a consistency in proteins upto the flower opening but a marginal decrease was registered in both high and low molecular weight proteins towards senescence. However, a protein of molecular weight 76.5 kDa showed up during senescent stages which may have a role in flower senescence.

Keywords Ascorbate peroxidase · Catalase · Lipid peroxidation · Lipoxygenase · Protease · Proteins

Introduction

Associated with a continuum of physiological, biochemical and hormonal changes, flower senescence is a genetically programmed process that results in the death of plant organs as the plant matures (Rogers 2012). Flower petal tissues are preferred for cell death studies because of homogeneity in the tissue and the ease with which chemical manipulation may be done to the petals (Hoeberichts et al. 2005). Flower senescence is often being regarded as the terminal stage of flower development, but in most plants it is not a final stage, but a part of the developmental cycle that helps to remobilize the nutrients from the flowers to the developing organs (Ahmad and Tahir 2015). A turnover in various biomolecules like proteins, carbohydrates, lipids and nucleic acids, besides an accumulation of reactive oxygen species (ROS) are evident during senescence that makes the process easy to study (Edwards et al. 2012).

Protein degradation by proteolytic cleavage has been found to be one of the earliest steps towards senescence. Proteomic studies have revealed the expression of cysteine proteases towards senescence and the application of inhibitors of these proteases have been found to delay senescence in flowers (van Doorn and Woltering 2008). Upregulated expression of proteases and a decrease in proteins has often been shown to be associated with senescence. The decline in protein content could be due to minimal biosynthesis and/or improved degradation (Celikel and van Doorn 1995). Maintenance of adequate carbohydrate levels in the petal tissues has been reported to

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suspend flower senescence in flowers like rose and carnation (Dar et al. 2014a). This property of sugars to suspend senescence is credited with the supply of substrates for respiration, structural materials and osmoticum (van Doorn and Woltering 2008). Flower senescence results in the loss of membrane integrity which is because of the changes in membrane proteins and lipid content. This dynamics results in increased ratio of sterol/phospholipid, a decline in the phospholipid and proteins, besides a decrease in protein thiols (van Doorn and Woltering 2008). The age-related decrease in phospholipid content is reportedly one of the prime reasons for loss of membrane integrity.

Oxidative stress has been found to initiate senescence in some cases and hasten it in others. Oxidative stress arises because of the accumulation of ROS such as O_2^- , H_2O_2 or OH which in turn react with and degrade nucleic acids, proteins and lipids (Kou et al. 2014). The degradation of proteins, nucleic acids and lipids results in the onset of senescence. To scavenge and detoxify these ROS plant cells activate certain enzymes like catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD). Role of these antioxidant scavenging enzymes in plant development and senescence has led to the postulation that the activity of these enzymes is a system's ability to delay senescence (Kou et al. 2014).

Keeping in view the role of above discussed biomolecules in flower senescence, the present study was undertaken in so far unexplored *Iris versicolor* to study the quantitative changes in proteins, protease activity, amino acids, phenols and carbohydrates besides; the role of antioxidant enzymes (CAT, APX, SOD) in flower senescence. Changes in lipoxygenase (LOX), membrane stability index (MSI) and lipid peroxidase activity (LPO) were also studied. The electrophoretic study was carried out to study the turnover in various high and low molecular weight proteins.

Materials and methods

Plant material

Iris versicolor L. belonging to the family Iridaceae, flowers from May to July in Kashmir. The plant bears hard textured scapes (55–65 cm) on which are borne blue colored flowers (Fig. 1). Flowers of *Iris versicolor* were harvested from Kashmir University Botanic Garden (KUBG) for this study. Flower development and senescence was divided into 6 stages viz., tight bud (I), mature bud (II), pencil (III), open flower (IV) partially senescent (V) and senescent (VI) stage, depending on their development (Fig. 2). Visible, physiological and biochemical changes were monitored during flower development and senescence.

Fig. 1 A field view of the flowers of *Iris versicolor* L. in full bloom in Kashmir University Botanic Garden (KUBG)

Floral diameter, water content, fresh and dry mass

Floral diameter, water content, fresh and dry mass was recorded at each stage of flower development. Fresh material was oven dried at 70 °C for 48 h for the calculation of dry mass and the difference between fresh and dry mass gave the value for the water content.

Membrane stability index (MSI)

Solute leakage of the tepal tissues was calculated by incubating 100 mg of tepal tissue in 5 ml of 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:

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

Lipid peroxidase activity (LPO)

LPO activity, expressed as TBARS content, 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 the reaction mixture in ice. Absorbance was taken at 532 and 600 nm. Non-specific absorbance at 600 nm was subtracted from the value obtained at 532 nm.

Fig. 2 Stages of flower development and senescence in *Iris versicolor* L. The different stages correspond to tight bud stage (I), mature bud stage (II), pencil stage (III), open flower stage (IV) partially senescent stage (V) and senescent stage (VI)

Estimation of sugar fractions, amino acids, phenols and specific protease activity

At each developmental stage, 1 g chopped tepal tissue 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 volume taken from the supernatant. Rosen's method (1957) was employed for α -amino acid quantification with glycine acting as standard. Total phenolics were quantified by Swain and Hillis (1959) method 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. 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 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 $15,000 \times g$ 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 μ M 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 μ M 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 $\text{min}^{-1} \text{mg}^{-1}$ protein.

Catalase activity (CAT)

CAT activity was estimated by the method of Aebi (1984). 1 g of tepal tissue 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 μ l enzyme extract and distilled water to make the volume to 3 ml. Reaction was started by adding H_2O_2 and the CAT activity was determined by the consumption of H_2O_2 for 3 min at 240 nm and was expressed as $\mu\text{mol H}_2\text{O}_2$ reduced $\text{min}^{-1} \text{mg}^{-1}$ 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 and 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 Axelrod et al. (1981). 1 g tepal tissue was macerated in 1 ml extraction buffer containing 50 mM potassium phosphate buffer (pH = 6.5), 10% polyvinylpyrrolidone (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.

Protein estimation and SDS-PAGE analysis

For the estimation of proteins and for running SDS-PAGE, proteins were extracted from 1 g of tepal tissue which was macerated in 100 mM phosphate buffer (pH 7.2) 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 and SDS-PAGE analysis. Proteins were estimated by Lowry et al. (1951) method from a suitable volume of aliquot taken from the supernatant. For SDS-PAGE, one-dimensional vertical gel electrophoresis was carried out according to the method described by Ausubel et al. (1989).

Statistical analysis of the data

The values depicted in the figures represent the mean of 10 independent replicates. LSD was computed at $P_{0.05}$ using SPSS 16 software for analyzing the data statistically. The data was also statistically analyzed by computing standard deviation as:

$$S = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}.$$

Table 1 Changes in the floral diameter, fresh & dry mass and water content, during flower development and senescence in *Iris versicolor* L

Parameters	Floral diameter (cm)	Fresh mass (g)	Dry mass (g)	Water content (g)
Stage I	0.63 ± 0.05	1.13 ± 0.12	0.222 ± 0.03	0.91 ± 0.04
Stage II	0.83 ± 0.07	1.64 ± 0.20	0.309 ± 0.03	1.33 ± 0.07
Stage III	1.15 ± 0.10	2.33 ± 0.10	0.432 ± 0.02	1.90 ± 0.02
Stage IV	12.25 ± 0.80	3.17 ± 0.03	0.488 ± 0.04	2.68 ± 0.04
Stage V	5.92 ± 0.20	1.66 ± 0.20	0.402 ± 0.05	1.26 ± 0.04
Stage VI	3.45 ± 0.10	1.01 ± 0.10	0.366 ± 0.02	0.64 ± 0.02
LSD $P = 0.05$	0.07	0.09	0.05	0.08

Results

The green buds of *Iris versicolor* open into bluish flowers with six tepals (3 standards and 3 falls). The young buds remain enclosed in the green spathe (stage I) and as the development proceeds, the spathe opens and the bluish tepals are exposed (stage III). The falls are hard in texture with a prominent midrib while as the standards are soft textured with no midrib. In the field and under the laboratory conditions, the flower remains open for 2.5 days. Flower senescence is marked by the loss of tepal turgidity followed by inrolling of tepal margins. The color of the tepals fades away as the senescence peaks. Floral diameter, fresh and dry mass and water content increased from stage I to IV and decreased thereafter during senescent stages V and VI (Table 1).

LPO activity, expressed as TBARS content, remained almost constant from the bud to open flower stage. However, significant increase in LPO was registered during the process of senescence in V and VI stages which have a close proximity with the MSI values (Fig. 3). Higher values of LPO in senescent stages V and VI was reflected in higher electrolyte leakage indicating higher degree of membrane deterioration and thereby least MSI values, whereas membrane integrity was maintained from stage I to stage IV as the flowers opened (Fig. 3). Lowest MSI values and highest values for LPO recorded in the complete senescent stage (stage VI) reflected total loss of membrane integrity and severe membrane deterioration.

The soluble proteins remained almost constant up to stage IV and showed a sharp decrease thereafter during senescence with an increase in specific protease activity (Fig. 4). The specific protease activity remained more or less constant from bud to bloom, but increased marginally thereafter as the flower senesced through V and VI stage. The α -amino acids increased up to stage V and decreased abruptly as the senescence peaked (Table 2). The concentration of total phenols remained more or less constant up to stage IV with an increase towards senescence through stages V and VI (Table 2). The content of reducing, non-reducing and total sugars showed a continuous increase from stages I to IV as the flower opened up and registered a

Fig. 3 Changes in membrane stability index (% MSI) and lipid peroxidase activity (TBARS content g^{-1} fm) during various stages of flower development and senescence in *Iris versicolor* L

Fig. 4 Changes in the content of soluble proteins (mg g^{-1} fm), specific protease activity (μg tyrosine equivalents liberated per mg protein per minute) during various stages of flower development and senescence in *Iris versicolor*

decrease through stages V and VI during senescence (Table 2). The SDS-PAGE analysis of tepal proteins revealed that high and low molecular weight proteins show consistency from stage I to IV but decreased significantly through stages V and VI. However, a protein with molecular mass of approximately 76.5 kDa showed up during senescence (Fig. 5).

The behavior of antioxidant enzymes through different stages of flower development in *Iris versicolor* reflected their active role in governing this developmental process. SOD and APX activity registered a continuous increase from the bud to the open flower stage but a significant decrease was registered through senescent stages V and VI (Table 3). CAT activity showed an increase from the stage I to Stage III, but a significant decrease in CAT activity was registered from the open flower stage (stage IV) till the

senescence progressed via stages V and VI (Table 3). LOX activity remained more or less constant up to stage III and increased marginally thereafter through stages IV, V and VI. Maximum LOX activity was registered in the fully senescent stage (Table 3).

Discussion

The present study on the flower development and senescence in *Iris versicolor* provided insights into analyzing the dynamics in various physiological and biochemical parameters that leads to senescence of this beautiful ornamental flower. Senescence in *Iris versicolor* flowers is marked by the loss of tepal turgidity followed by inrolling of tepal margins. Increase in the floral diameter, water

Table 2 Changes in the α -amino acids, total phenols and sugar fractions (total, reducing and non-reducing) during flower development and senescence in *Iris versicolor* L

Parameters	α -amino acids (mg g ⁻¹ fm)	Total phenols (mg g ⁻¹ fm)	Total sugars (mg g ⁻¹ fm)	Reducing sugars (mg g ⁻¹ fm)	Non-reducing sugars (mg g ⁻¹ fm)
Stage I	3.05 ± 0.21	2.87 ± 0.00	19.64 ± 0.20	7.143 ± 0.90	12.497 ± 0.05
Stage II	2.11 ± 0.06	2.94 ± 0.03	31.25 ± 0.40	7.143 ± 0.23	24.107 ± 0.20
Stage III	4.22 ± 0.02	2.50 ± 0.02	58.99 ± 0.32	29.64 ± 0.40	29.35 ± 0.50
Stage IV	4.93 ± 0.00	2.37 ± 0.07	62.50 ± 0.20	32.29 ± 0.70	30.21 ± 0.00
Stage V	7.04 ± 0.04	5.25 ± 0.03	49.50 ± 0.43	30.71 ± 0.50	18.79 ± 0.30
Stage VI	2.82 ± 0.03	2.37 ± 0.02	33.04 ± 0.17	14.28 ± 0.30	18.76 ± 0.40
LSD	0.09	0.09	0.58	0.43	0.38
<i>P</i> = 0.05

Fig. 5 Electrophoretogram of the different stages (I–VI) of flower development and senescence in *Iris versicolor* L

content, fresh mass and dry mass was associated with flower opening and a decrease in these attributes was closely related with the flower senescence. Similar pattern of changes in these parameters towards senescence was recorded in *Iris germanica*, *Dianthus chinensis* and *Consolida ajacis* (Ahmad et al. 2013; Dar et al. 2014a; Ahmad and Tahir 2015). Decreased floral diameter towards

senescence is attributed to the loss of tepal turgidity which makes the cells flaccid. Water is required for cell expansion which is an important criterion for flower opening and maintaining the shape of flowers (Ahmad et al. 2013; Kou et al. 2014). Flowers maintained decreased fresh and dry mass towards senescence to reallocate the nutrients back to the plant for economic benefits (Dar et al. 2014b, 2015). Recycling of the nutrients towards the developing organs is the underlying mechanism for shedding off flowers once they are pollinated (Dar et al. 2014b).

In the present study, extent of LPO activity, represented by the thiobarbaturic acid reactive substance (TBARS) content was almost constant up to flower opening and thereafter a marginal increase was shown with senescence. This increase in the LPO with senescence can be attributed to the damage of the plasma membrane by ROS which is also depicted by increased ion leakage through the membrane towards senescence. Rise in the LPO and thereby a decrease in the MSI, an index of membrane leakiness with senescence has been recorded in *Hemerocallis* flowers (Panavas and Rubinstein 1998), however Celikel and van Doorn (1995) reported that LPO activity remains unaltered in *Iris hollandica* tepals which are contrary to our results on *Iris versicolor*. During senescence, lipid biosynthesis shuts down and an increase in the lipolytic enzyme activity

Table 3 Changes in the activities of superoxide dismutase, catalase, ascorbate peroxidase and lipoxygenase during flower development and senescence in *Iris versicolor* L

Parameters	Superoxide dismutase (SOD) (units min ⁻¹ mg ⁻¹ protein)	Catalase activity (CAT) (μmol H ₂ O ₂ red. min ⁻¹ mg ⁻¹ protein)	Ascorbate peroxidase (APX) (μmol min ⁻¹ mg ⁻¹ protein)	Lipoxygenase (LOX) (μmol min ⁻¹ mg ⁻¹ protein)
Stage I	2.76 ± 0.21	2.14 ± 0.15	6.34 ± 0.21	0.71 ± 0.04
Stage II	2.98 ± 0.17	2.31 ± 0.17	6.89 ± 0.17	0.69 ± 0.07
Stage III	3.20 ± 0.11	2.63 ± 0.19	7.54 ± 0.11	0.71 ± 0.03
Stage IV	3.35 ± 0.18	2.32 ± 0.12	8.76 ± 0.22	1.60 ± 0.04
Stage V	2.51 ± 0.19	2.01 ± 0.18	5.37 ± 0.16	1.90 ± 0.05
Stage VI	2.23 ± 0.14	1.73 ± 0.16	3.11 ± 0.11	2.30 ± 0.02
LSD <i>P</i> = 0.05	0.06	0.08	0.11	0.06

especially LOX occurs (van Doorn and Woltering 2008; Rogers 2012). In the current study, LOX activity increased sharply towards senescence just prior to the increase in LPO activity which suggests that lipid peroxidation in this flower system is chiefly because of the activity of LOX. LOX causes peroxidative damage to membrane lipids which results in a decrease in lipid unsaturation and membrane fluidity (Fukuchi-Mizutani et al. 2000).

During the present study it was observed that total, reducing and non-reducing sugars registered an increase from bud to bloom and then showed a gradual decline in V and VI stage. Senescence in flowers is often associated with a decrease in the sugar fractions in flowers like *Dianthus* and rose (Dar et al. 2014b; Mwangi et al. 2003). Till late in the flower development, sugar metabolism remains active in the tepal tissues to remobilize the macromolecules back to the developing parts of the plant in the form of sucrose (van Doorn and Woltering 2008). Metabolism or abundance/lack of sugars in the cells may form an endogenous signal that initiates senescence (Dar et al. 2014b). Phenols have been shown to increase significantly towards senescence and attained a peak at the fully senescent stage. This increased phenolic content can be attributed to the loss of membrane permeability towards senescence because of the decreased membrane proteins and phospholipids (Celikel and van Doorn 1995).

The current investigation on *Iris versicolor* revealed a decrease in the soluble protein content towards senescence which was commensurate with an upsurge in specific protease activity. Specific protease activity remained almost constant up to flower opening and thereafter showed a marginal increase with senescence. However, the α -amino acid content (a breakdown product of proteins) showed an abrupt decrease as the senescence peaked. Decreased content of amino acids can be ascribed to the hypothesis that amino acids are pooled back from the senescing flowers to the developing organs as a part of nutrient remobilization. Protein degradation as a result of proteolytic cleavage and the transfer of the degradation product (amino acids) to the other parts of the plant is a part of the resource pooling process back to the developing organs of the plant. This possibility is strengthened by the reports that an early expression of protease genes occurs before the initiation of senescence in flowers like *Alstroemeria*, *Petunia*, roses and *Hemerocallis* (Tripathi et al. 2009). As a prominent decrease in the total soluble proteins content was recorded, so was the result obtained from SDS-PAGE of the tepal tissue. However, SDS-PAGE showed that a new protein with molecular weight of about 76.5 kDa showed up during senescence. This protein might have a role in the initiation of senescence in this flower system. Studies on various flowers like carnation

and daylily have shown that senescence in these flowers was triggered by specific proteins referred to as ‘death proteins’ which were expressed during senescence (Hoeberichts et al. 2005; Dar et al. 2014b).

Senescence in plants or plant parts is often marked by the abundant generation of ROS like superoxides (O_2^-) and hydrogen peroxides (H_2O_2) by cells (Dhindsa et al. 1981; Ezhilmathi et al. 2007; Saeed et al. 2014). To prevent plants from this damage, plant cells employ various scavenging enzymes like SOD, CAT and APX by activating specific signaling pathways (Kou et al. 2014). In our study, SOD activity has been shown to increase as the flower opens and thereafter a significant decrease was registered towards senescence with the least activity in the complete senescent stage. Our results are although partly in conformity with the earlier results on carnation and daylily, but the decrease in the SOD activity was found to occur much later in the progression of flower senescence than found in this study (Panavas and Rubinstein 1998; Ezhilmathi et al. 2007). CAT activity showed an increase as the flower matures and showed a marginal decrease in the open flower stage which continued through senescent stages. A decrease in CAT activity is initiated 6 h before flower opening till the last stage of flower development in daylily flowers (Bielecki 1993). However, Ezhilmathi et al. (2007) reported a continuous decrease in the CAT activity right from the bud to senescent stage in the flowers of gladiolus. Activities of SOD and CAT exhibit the stabilizing effects on lipid bilayer and membranous system and their higher ROS scavenging activity eliminates oxidative stress from flowers and prevents the oxidation of unsaturated fatty acids (Saeed et al. 2014). Our results revealed a steady increase in the APX activity towards flower opening and a significant decrease towards senescence. Down regulation of APX activity was also found to initiate senescence in various flowers such as daylily, gladiolus and carnation (Panavas and Rubinstein 1998). The primary function of APX is to reduce H_2O_2 with the simultaneous oxidation of ascorbate to dehydroascorbate. APX is the key enzyme in detoxifying H_2O_2 in plants through ascorbate glutathione cycle (Mittler et al. 2004). Promotion of senescence by decreased APX activity is due to the endogenous accumulation of H_2O_2 that in turn inhibits SOD gene activity (Mittler et al. 2004).

We conclude that flower senescence in *Iris versicolor* is associated with a decrease in the activity of various antioxidant enzymes (CAT, SOD and APX), sugar fractions, α -amino acids and proteins besides a significant increase in the lipid peroxidation and specific protease activity. Investigation on the protein patterns revealed an upregulation of a protein with molecular weight of 76.5 kDa which might have a role in flower senescence of *Iris versicolor*. Detailed proteomic studies are required to

know the precise role of this protein during senescence in this flower.

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Author contribution Syed Sabhi Ahmad carried out the experiments, obtained results, analyzed and compiled the data. Prof. Inayatullah Tahir helped in designing the experiment, supervised the laboratory work, took the photographs and edited the manuscript.

Compliance with ethical standards

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

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