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MODULATION OF LEAF SENESCENCE IN *BERGENIA CILIATA* (HAW.) STERNB. THROUGH THE SUPPLEMENTATION OF KINETIN AND METHYL JASMONATE

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Abstract

Optimizing senescence in specific plant species is a significant objective in crop breeding programs. Senescence in plants serves to redistribute nutrients from older tissues to new organs at the end of the growing season, contributing to parental investment for improved growth fitness. This study aimed to investigate the potential role of exogenous kinetin (KIN) and methyl jasmonate (MeJ) in regulating postharvest leaf senescence in *Bergenia ciliata* at two stages of leaf development (referred to as S1 and S2). Various physiological and biochemical parameters were evaluated to understand hormonal control and crosstalk during senescence, considering the integration of developmental signals. Leaf discs were prepared from excised leaves of *B. ciliata* and treated with different concentrations of KIN and MeJ (0.01, 0.1, 1 and 10 µM), alongside a control set. Compared to the control, KIN application extended the green color of leaf discs by approximately 4 days, delaying leaf senescence. This delayed senescence was accompanied by higher levels of chlorophyll and soluble proteins, along with a noticeable decrease in sugar levels. Moreover, leaf discs treated with various KIN concentrations exhibited a significant increase in antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), while lipoxygenase (LOX) activity decreased significantly. On the other hand, leaf discs supplemented with different concentrations of MeJ showed discoloration, lower chlorophyll content, reduced soluble proteins and increased sugar levels compared to the control. However, MeJ application enhanced the activity of antioxidant enzymes in the leaf discs, demonstrating a synergistic effect with KIN in augmenting antioxidant enzyme activity. By investigating these parameters, the researchers aim to elucidate how KIN and MeJ modulate postharvest leaf senescence in *B. ciliata* by analyzing underlying physiological and biochemical mechanisms involved.

Key words: Chlorophylls, Crosstalk, Developmental signals, Hormonal control, Soluble proteins.

Introduction

Leaf senescence is a well-coordinated process involving sequential changes in cell physiology, biochemistry, gene expression and ultimately cell death (Lim *et al*., 2003; Jan *et al*., 2018). This process is regulated by a tightly controlled genetic switch, ensuring the plant's survival and the accumulation of nutrients for future generations. When leaves detach, early senescence is triggered, leading to the rapid breakdown of macromolecules such as proteins, nucleic acids and membrane lipids. Consequently, the products of these degraded molecules are released through leaky membranes (Subhashini *et al*., 2012; Qi *et al*., 2015).

The initial visible sign of leaf senescence is the yellowing of leaves, primarily due to the decrease in chlorophyll content (Skutnik *et al*., 2004). Additionally, protein degradation (Breeze *et al*., 2011) and an increase in reactive oxygen species (ROS) levels are characteristic of leaf senescence (Breusegem & Dat, 2006). These processes collectively contribute to the overall deterioration of leaf structure and function during senescence.

Various growth regulators, such as abscisic acid, salicylic acid, jasmonates, gibberellins, auxins, ethylene and cytokinins, can interact either harmoniously or antagonistically to initiate or halt leaf senescence (Lim *et al*., 2007; Gan, 2014; Qiu *et al*., 2015; Schippers, 2015; Zhao *et al*., 2016). Kinetin, a synthetic cytokinin, has been identified as an effective growth regulator that delays senescence by scavenging radicals, promoting the synthesis of chloroplast proteins and chlorophyll pigments (Pandey *et al*., 2017; Abeed *et al*., 2020). Decreased levels of cytokinins have been associated with impaired nutrient translocation to leaves, which promotes leaf senescence (Paparozzi *et al*., 2016; Guo *et al*., 2017).

Cytokinins play a crucial role in preventing leaf senescence by maintaining the integrity of the tonoplast membrane, which acts as a protective barrier against protease leakage. This preservation of the tonoplast membrane inhibits the activity of proteases that can hydrolyze soluble proteins in chloroplast and mitochondrial membranes. Preserving the integrity of the tonoplast membrane is essential for the stability and preservation of essential soluble proteins within chloroplast and mitochondrial membranes, thereby sustaining leaf functionality and overall health (Subhashini *et al*., 2012; Moura *et al*., 2018). Additionally, cytokinins delay senescence by enhancing sink activity, facilitating transport, maintaining increased metabolite levels in plant tissues and preventing lipid peroxidation of membranes (Iqbal *et al*., 2017). They also regulate genes associated with oxidative stress, inhibiting chlorophyll degradation and preserving chloroplast structure (Lu *et al*., 2017).

Jasmonates, a class of plant growth regulators derived from enzymatic oxygenation of triunsaturated fatty acids, have regulatory roles in various developmental processes in plants (Ahmad *et al*., 2016; Sirhindi *et al*., 2016; Ahmad *et al*., 2018). Methyl jasmonate (MeJ), among jasmonates,

plays a crucial role in leaf senescence regulation (He *et al*., 2002; Lim *et al*., 2007; Saini *et al*., 2015; Hu *et al*., 2017; Pandey *et al*., 2017; Zhang *et al*., 2018). MeJ mediates leaf senescence by modulating cytosolic calcium levels (Huang *et al*., 1990) and accelerates leaf senescence in both attached and detached *Arabidopsis* leaves, leading to increased chlorophyll degradation, protein turnover and sugar accumulation (Hu *et al*., 2017; Jan *et al*., 2018). However, MeJ also enhances the elimination of reactive oxygen species (ROS) by increasing antioxidant activity (Ahmad *et al*., 2016; Sirhindi *et al*., 2016; Ahmad *et al*., 2018; Abeed *et al*., 2020). Furthermore, MeJ levels increase in senescing leaves compared to non-senescing leaves in various plants, including *Arabidopsis thaliana* and *Oryza sativa* (Hung *et al*., 2006; Hu *et al*., 2017; Zhang *et al*., 2018; Wang *et al*., 2019). MeJ treatment has been shown to increase the expression of *SAG12* (*SENESCENCE ASSOCIATED GENE12*), a molecular marker of senescing leaves, in leaves (He *et al*., 2002).

B. ciliata, a perennial herbaceous plant belonging to the Saxifragaceae family, has a long history of use in Ayurvedic and traditional medicine for treating various diseases. It is known for its effectiveness against bacterial infections and possesses notable anti-inflammatory properties (Pokhrel *et al*., 2014; Singh *et al*., 2017). In our laboratory, we have chosen this plant as a model organism to study leaf senescence due to its persistent leaf nature and the interconnected signaling pathways involving jasmonates and cytokinins during this process.

The present research aims to investigate the role of two specific compounds, KIN and MeJ, in modulating leaf senescence in *B. ciliata*. By studying these compounds, we hope to gain a deeper understanding of the temporal changes associated with leaf senescence in this plant, as this area of study remains largely unexplored but has garnered significant attention in recent years. Revealing the regulatory roles of these phytohormones in senescence could have broad applications, not only in controlling leaf senescence in *B. ciliata* but also in other economically important crops, thereby enhancing their growth and productivity.

Additionally, it is worth noting that there is a limited body of research focused on postharvest senescence of cut foliage, especially when compared to studies on cut flowers (Qu *et al*., 2020). Therefore, investigating the senescence process in cut foliage is an important aspect that warrants further exploration.

Material and Methods

Plant material: This study made use of healthy *B. ciliata* plants cultivated at the Kashmir University Botanic Garden (KUBG). During the month of October 2018, plant rhizomes were sown in 4x2m experimental beds. Plants were irrigated twice a week and analyzed at two developmental stages, S1 and S2. At 8:00 a.m., healthy leaf samples of the same age were collected with a sharp scalpel, transported to the laboratory, rinsed and dried in the pleats of absorbent paper. Leaf discs were punched from leaf samples with the help of a hole punch. Punched-out leaf discs of 2 cm diameter with their abaxial surface downward were floated in 50 ml solutions of 0.01, 0.1, 1 and 10 µM KIN and MeJ in separate borosilicate petriplates of 15cm diameter. In an incubator, petriplates were maintained at 18 photon m^{-2} s^{-1} of photon flux density (PFD). Each treatment comprised 10 replicates (petriplates), with 25 leaf discs per petriplate. The control group consisted of 25 leaf discs floating in distilled water. Samples were collected on the first, fourth- and eighth days following transfer. The research was carried out under precise conditions of relative humidity (RH) of 60% and a temperature of 20±2°C.

Estimation of chlorophyll: The Liao *et al*., (2012) method was used to determine the chlorophyll content of leaves. 0.25 g of leaves were crushed to a powder and extracted in 3 mL of 80% (v/v) acetone after being frozen in liquid nitrogen. Chlorophyll content was determined by measuring absorption at 510, 649 and 665 nm.

Methods for enzyme assays and extraction: 0.25 g of leaf tissue was crushed to a fine powder with liquid nitrogen and extracted by homogenization in 5 mL of phosphate buffer for enzyme liquid extraction (0.05 M, pH 7.8). The homogenate was centrifuged at 12,000g for 20 minutes at 4°C and the supernatant was collected for enzyme tests**.**

Estimation of superoxide dismutase (SOD): The suppression of photoreduction of nitroblue tetrazolium was used to test superoxide dismutase (SOD) activity following the Dhindsa *et al*., (1981) method. The enzyme extract (20 µL) was mixed with 3 mL of a substrate combination containing 0.05 M potassium phosphate buffer, 130 mM Met solution, 750 mM NBT solution, 1 mM EDTA-Na_2 and 20 µM riboflavin and incubated at 30°C for 30 minutes. At 560 nm, the optical density was found. The amount of enzyme required to inhibit nitroblue tetrazolium photoreduction by 50% was determined as one unit of SOD activity and calculated as unit min^{-1} mg^{-1} protein.

Catalase (CAT): The method of Aebi (1984) was used to estimate catalase activity. Each reaction was started by adding 100 µL of supernatant to 2.9 mL of reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0) and 20 mM H_2O_2 and the absorbance was measured at 240 nm. The decrease in absorbance at 240 nm for 1 minute was used to calculate CAT activity and was represented as µmol H_2O_2 red. min^{-1} mg^{-1} protein.

Ascorbate peroxidase (APX): APX activity was calculated using the method given by Chen & Asada (1989). Each reaction was started by adding 100 µL supernatant to a 2.9 mL reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate and 2 mM H_2O_2 for ascorbate peroxidase (APX) activity. The decrease in absorbance at 290 nm was used to assess APX activity and was expressed as µmol min^{-1} mg^{-1}protein.

Lipoxygenase (LOX) The leaf extract was made by homogenizing leaf tissue in a microtissue homogenizer for 20 minutes at 0–4°C with 100 vol of phosphate buffer (0.2

M, pH 6.8). At 4°C, the homogenized suspension was centrifuged at 10000 rpm for 10 minutes. Following the conventional procedure of Axerold *et al.*, (1981), the supernatant was employed as the crude extract for assaying the lipoxygenase enzyme. Crude extract as an enzyme source, phosphate buffer (0.2 M, pH 6.8) and 10 mM sodium linoleate as a substrate make up the reaction mixture. Because the enzyme assay was based on the appearance of conjugated diene in fatty acid hydroperoxides, the change in absorbance at 234 nm was recorded in a UV-visible spectrophotometer at 234 nm. One unit of enzyme was defined as the amount of enzyme that caused a 1.0 per minute increase in absorbance at 234 nm and the activity was expressed as μmol $min^{-1}mg^{-1}$ protein.

Protein estimation: In a 100 mM phosphate buffer with a pH of 7.2, 0.25 g of leaf tissue was homogenized. In a precooled centrifuge, the solution was centrifuged at 12,000xg for 15 minutes at 5°C. The supernatant was filtered and the protein concentrations were calculated in mg/gfm. Bradford (1976) method was used to determine protein concentrations using brilliant blue G-250 dye. To make 1 mL final volume, 20 μL supernatant was added to 800 μL coomassie Bradford reagent. Absorbance was read at 595 nm using a UV-Visible spectrophotometer.

Sugar fractions and α-amino acids: Each treatment's leaf tissue was fixed in hot 70% ethanol, macerated and centrifuged three times. Sugars and α-amino acids were calculated from a sufficient quantity of supernatant. Nelson (1944) approach was used to determine reducing sugars, with glucose serving as the reference. Rosen (1957) approach was used to quantify α-amino acids using glycine as the standard. To quantify total sugars, invertase was utilized to convert non-reducing sugars to reducing sugars. The proportion of non-reducing sugars was shown by the difference between total and reducing sugars.

Statistical Analysis

For each treatment, three duplicates were carried out. Leaf discs were selected in a completely randomized design in all of the studies. SPSS was used for all statistical analyses (version 17.0; SPSS Inc., Chicago, IL, USA). All data was presented as a mean value with a standard error (SE). For statistical analysis, the analysis of variance (ANOVA) at P 0.05 was employed.

Results

The present work revealed a visual change in the color of leaf discs from green to yellow and finally to black. Blackening started from the edges towards the center. At day 0 leaf discs in all the treatments were green and fresh. KIN treated leaf discs remained green up to day 8. The discs turned black after day 8. MeJ treated leaf discs displayed early discoloration and blackening from day 4 onwards.

Chlorophylls: Application of KIN increased the total chlorophyll content in the leaf tissues with an average increase of 78.5% at stage S1 and 81.5% at stage S2. Highest chlorophyll "a" content was verified from samples given 10 μM KIN with an average increase of 56.3% at stage S1and 42.1% at stage S2. Moreover, KIN at 0.1 μM was effective in maintaining higher chlorophyll "b" by 11.3% at stage S1 and 0.01 μM KIN was effective by 8.7% at stage S2. Chlorophyll a/b ratio decreased after the application of KIN with the average decrease of 37.2% at stage S1 and 32.6% at stage S2. In contrast to this, application of MeJ decreased the total chlorophyll content with an average decrease of 60.4% and 63.4% at stages S1 and S2 respectively. The use of MeJ resulted in a significant reduction in the amount of chlorophyll "a" with an average decrease of 45.2% and 34.6% at stages S1 and S2 respectively. MeJ treatments decreased the contents of chlorophyll "b" with an average decrease of 43.7% at stage S1 and 52.1% at stage S2. Chlorophyll a/b ratio increased after the application of MeJ with an average increase of 82.3% at stage S1 and 79.3% at stage S2 (Fig. 1).

Sugar fractions: In leaf samples treated with KIN, sugar fractions (reducing, non-reducing and total sugars) remained moderately low in comparison to control at both the stages S1 and S2. 10 μM concentration of KIN was most effective in maintaining sugar fractions. Furthermore, the sugar levels in the KIN-treated leaf samples decreased over time from D4 to D8. In contrast, when the leaf samples were treated with MeJ, there was an increase in the content of sugar fractions. This implies that MeJ treatment had a contrasting effect on sugar levels compared to the KIN treatment. The MeJ-treated leaf samples showed higher levels of reducing sugars, non-reducing sugars and total sugars compared to the control samples with 10 μM being most effective (Fig. 2).

Contents of α-Amino acids and soluble proteins: KIN treatment resulted in an increase in soluble proteins in leaf samples, with the highest content observed at a concentration of 10 μM at both stages S1 and S2. However, the application of KIN led to a decrease in α-amino acids in the leaf tissue samples, with the lowest content observed at a concentration of 10 μM in both stages S1 and S2. On the contrary, MeJ treatment decreased the soluble protein content in the leaf samples, with the minimum content observed at a concentration of 10 μM at both stages S1 and S2. Additionally, the reduced protein content in the MeJ-treated samples was accompanied by a simultaneous increase in α-amino acids, with the highest content observed at a concentration of 10 μM at both stages S1 and S2 (Fig. 3).

Antioxidant Enzymes

Superoxide dismutase (SOD) activity: The activity of superoxide dismutase (SOD) in the leaf discs decreased with progression in time from D0 to D4 both at stages S1 and S2, but increased as the senescence progressed from D4 to D8 at stage S1 with the application of KIN, however the SOD activity was generally maintained at both D4 and D8 of the stage S2. Similarly, the application of MeJ increased and maintained higher activity of SOD from D0 to D4 at both the stages S1 and S2 (Fig. 4).

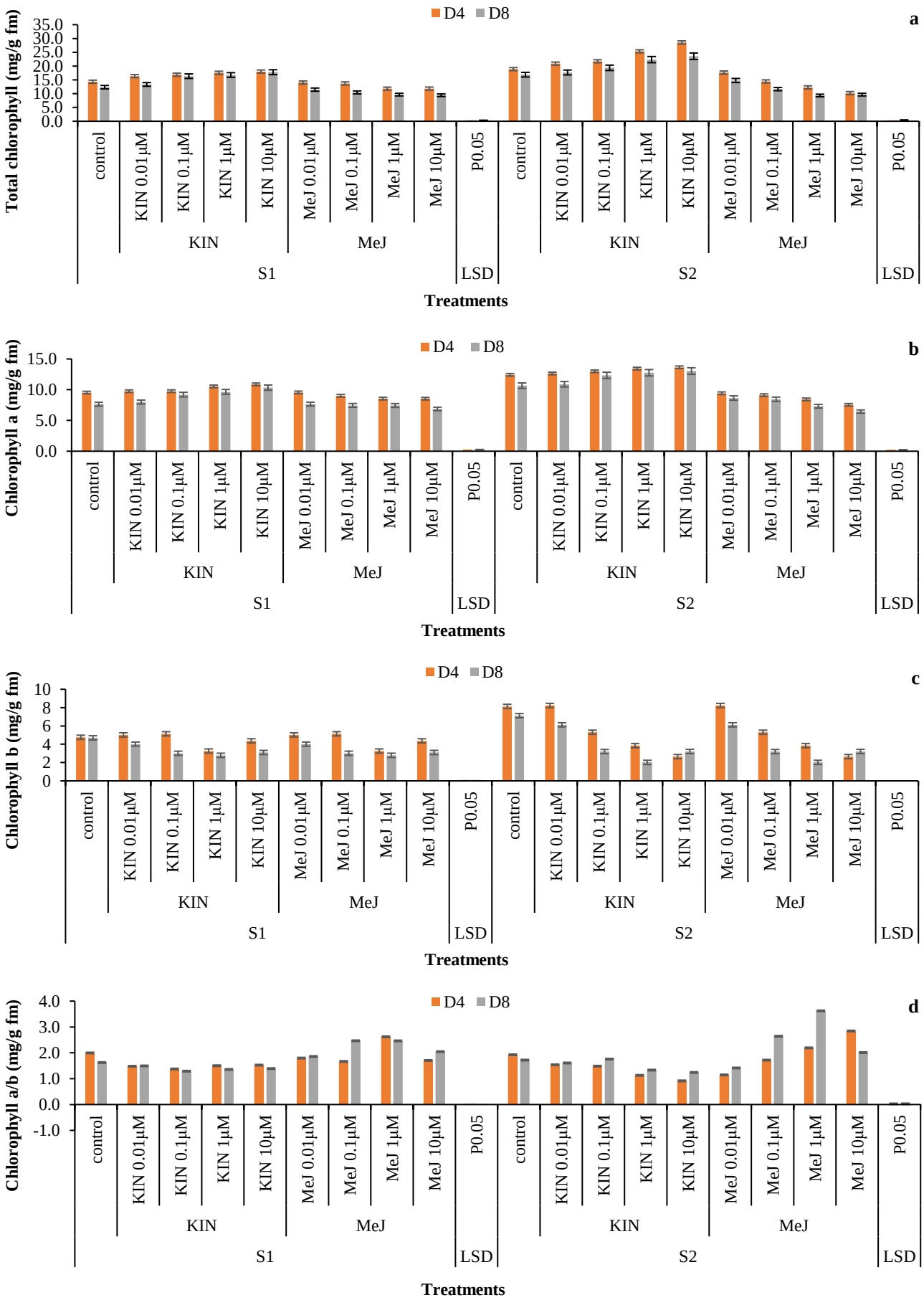

Fig. 1. Kinetin and Methyl jasmonate effects assessed at D4 and D8 in leaf tissues of *Bergenia ciliata* on total chlorophyll, chlorophyll a, chlorophyll b and chlorophyll a/b ratio at S1 and S2 with error bars symbolizing ± standard error. Three replicates are represented by each value.

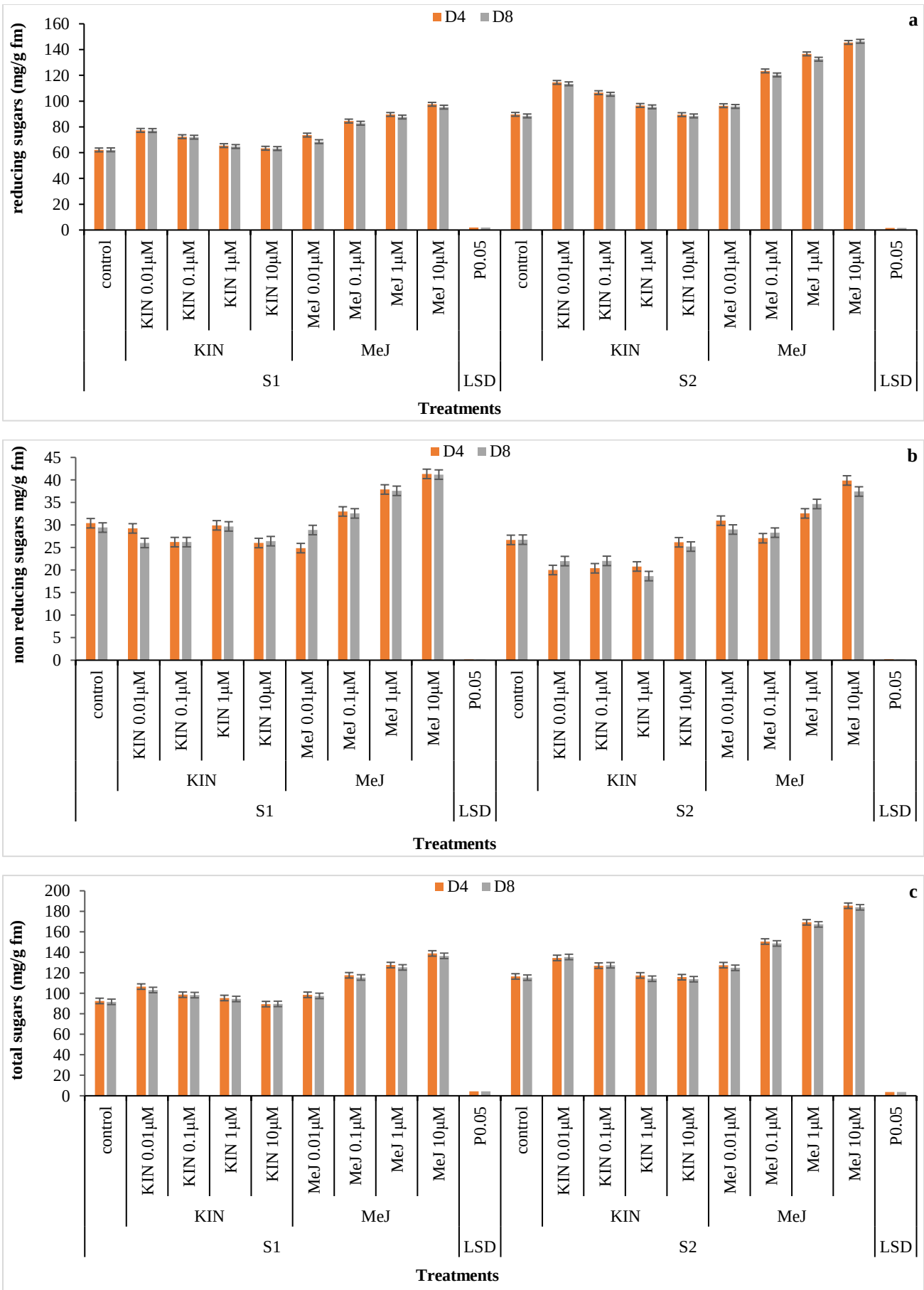

Fig. 2. Kinetin and Methyl jasmonate effects assessed at D4 and D8 in leaf tissues of *Bergenia ciliata* on reducing sugars, non-reducing sugars and total sugars at S1 and S2 with error bars symbolizing ± standard error. Three replicates are represented by each value.

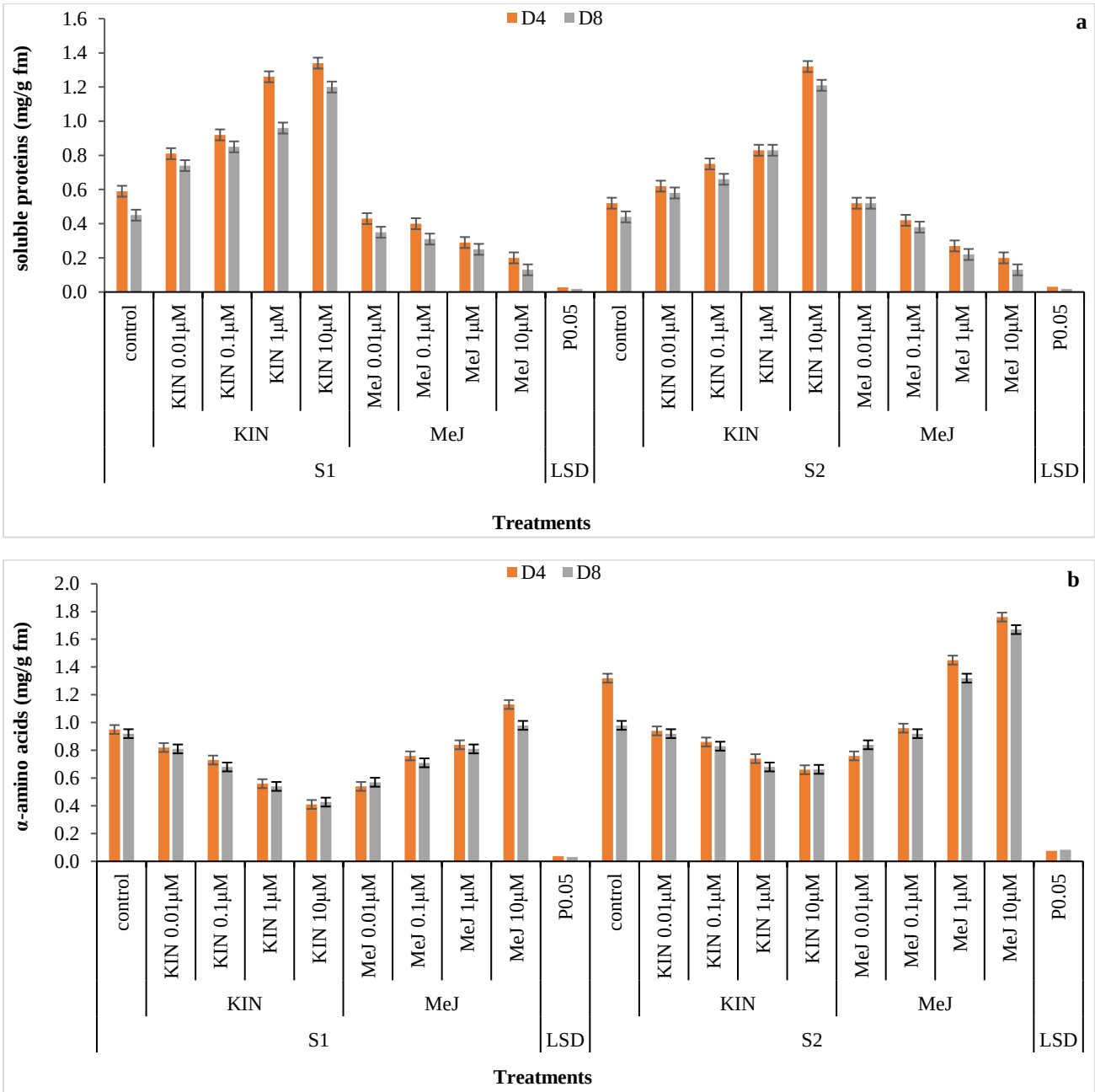

Fig. 3. Kinetin and Methyl jasmonate effects assessed at D4 and D8 in leaf tissues of *Bergenia ciliata* on soluble proteins and α-amino acids at S1 and S2 with error bars symbolizing ± standard error. Three replicates are represented by each value

Catalase (CAT) activity: In the tissue samples from leaf discs, activity of CAT decreased with advancement in time from D0 to D4 at both the stages S1 and S2, however the CAT activity was generally maintained as the senescence progressed from D4 to D8 at stage S1 with the application of either KIN or MeJ. With the treatment of either KIN or MeJ at stage S2, there was a significant decrease in CAT activity from D4 to D8. The CAT activity decreased both at D4 and D8 of the stages S1 and S2. 10 µM of both KIN and MeJ were highly effective in increasing the activity of CAT (Fig. 4).

Ascorbate peroxidase (APX) activity: The activity of APX in the tissue samples from leaf discs decreased with progression in time from D0 to D4 at both the stages S1 and S2, however the APX activity was maintained as the leaf senescence progressed from D4 to D8 at the stage S1 with the application of either KIN or MeJ. The APX activity in the tissue samples of the leaf discs increased with the increasing concentration of KIN and MeJ both at the stages S1 and S2 (Fig. 4).

Lipoxygenase (LOX) Activity: The activity of LOX in tissue samples rose as the time passed from D0 to D4 and then decreased from D4 to D8 at stage S1 with the application of either KIN or MeJ, however at S2 the LOX activity was more or less maintained with the application of KIN. The LOX activity increased sharply from D0 to D4 with the application of MeJ. Further, the LOX activity decreased with increasing concentration of KIN, however the concentration of LOX increased with increasing concentration of MeJ (Fig. 4d).

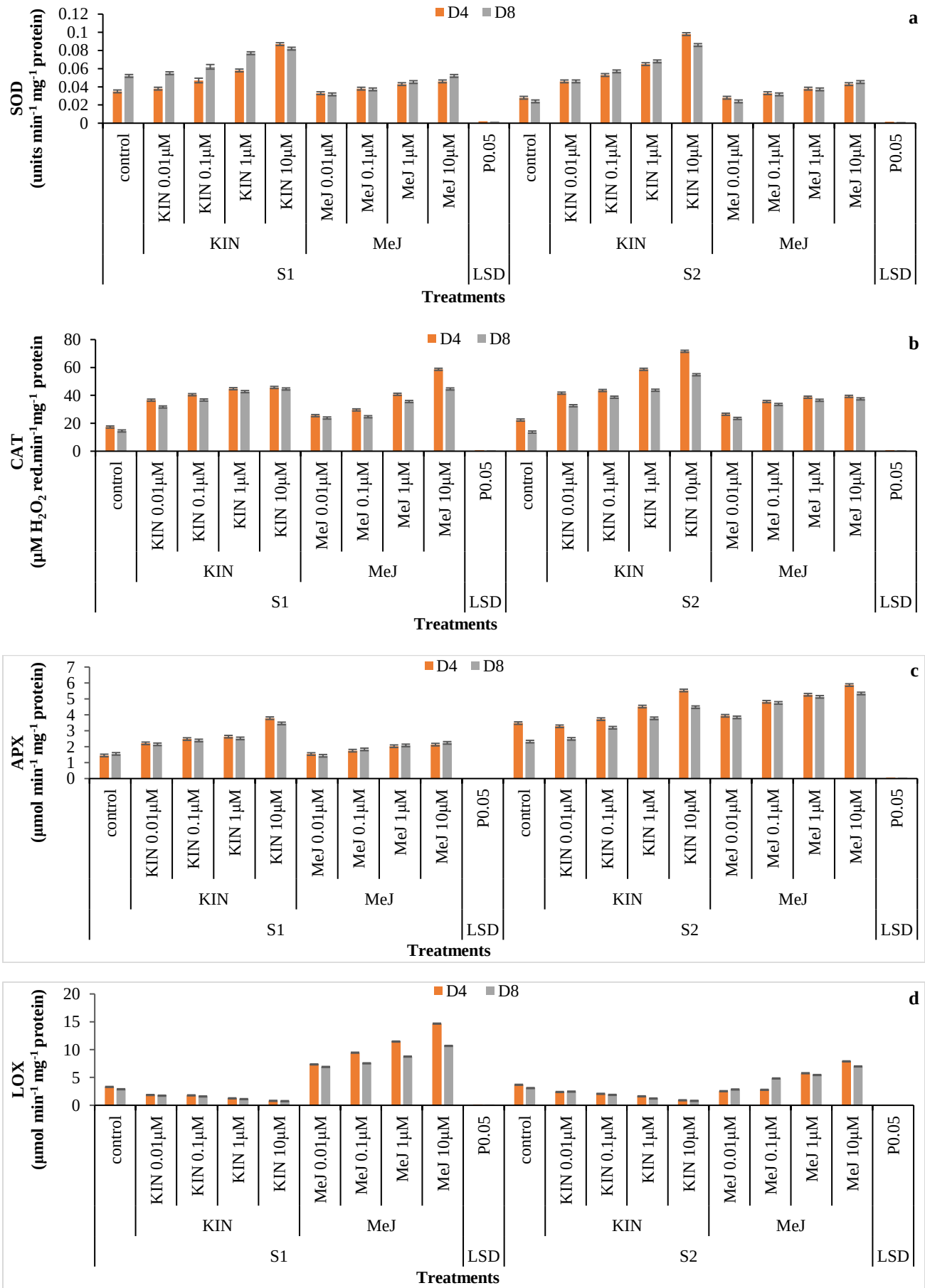

Fig. 4. Kinetin and Methyl jasmonate effects assessed at D4 and D8 in leaf tissues of *Bergenia ciliata* on SOD, CAT, APX and LOX activity at S1 and S2 with error bars symbolizing ± standard error. Three replicates are represented by each value.

Discussion

Leaf senescence is a complex process regulated by multiple genes and signaling pathways, integrating age-related information and various internal and external signals throughout the lifespan (Woo *et al*., 2013). One of the characteristic features of leaf senescence is the dismantling of chlorophyll. However, interestingly, the leaf samples treated with KIN exhibited higher levels of chlorophyll content. This increase in chlorophyll levels can be attributed to the potential influence of KIN in promoting chlorophyll production and inhibiting its breakdown, as reported by Moura *et al*., (2018). Previous studies by Richmond & Lang (1957) demonstrated that cytokinins, such as zeatin can delay leaf senescence by preventing chlorophyll breakdown. Similarly, KIN treatment has been documented to reduce chlorophyll degradation in maize plants under stress conditions (Kaya *et al*., 2010). Additional studies have shown that exogenous applications or endogenous enhancement of KIN can inhibit senescence in oat leaf segments (Tetley & Thimann, 1974) and Rumex leaf discs (Dhindsa *et al*., 1982) by preventing chlorophyll loss and maintaining chloroplast integrity through the upregulation of antioxidative responses. The concentration of endogenous cytokinins decreases rapidly during leaf senescence and exogenous application has been shown to prolong leaf lifespan (Oneto *et al*., 2016; Ma *et al*., 2018).

Sugars have been reported to actively promote leaf senescence by inducing the expression of senescence-specific genes, such as the cysteine protease gene *SAG12* (Wingler *et al*., 1998; Pourtau *et al*., 2006). Li *et al*., (2019) demonstrated that long-term high light-induced sugar enrichment is associated with leaf senescence in wheat. However, KIN has been found to inhibit the response of sugars, thereby delaying senescence in tomato plants (Niakan & Ahmadi, 2014).

Proteolysis, initiated by proteases, is a crucial phase in leaf senescence, leading to protein breakdown and subsequent mobilization of amino acids to developing organs (Liu *et al*., 2008). In contrast, cytokinins prevent protein degradation by inhibiting protease activity in senescing leaves, as demonstrated in *Hordeum vulgare* and *Arabidopsis thaliana* (Peterson & Huffaker, 1975; Buet *et al*., 2019). This suggests that cytokinins delay senescence by suppressing the increase in cell death associated with the proteasome (Ahmad *et al*., 2018). Furthermore, KIN treatment has been shown to increase the soluble protein content in *Sinapis alba* leaves (Zerbe & Wild, 1980).

The application of KIN in the present study significantly enhanced the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). Previous research in Anthurium has also demonstrated the ability of KIN to enhance antioxidant enzyme activity (Moura *et al*., 2018). Antioxidant enzymes play a crucial role in combating the production of reactive oxygen species (ROS) and it has been observed that senescent leaves exhibit an inverse relationship between ROS levels and antioxidant enzyme activity (Prochazkova & Wilhelmova, 2007; Radhakrishnan & Lee, 2014). Cytokinins, including KIN, have been shown to delay the signs of aging in various studies. This can be attributed to their ability to either stimulate the production of antioxidant enzymes that scavenge ROS or directly scavenge ROS themselves (Ma *et al*., 2018; Lone *et al*., 2021).

Furthermore, the application of KIN in leaf tissues resulted in the downregulation of lipoxygenase (LOX) activity. This downregulation of LOX helps in preventing the leakage of phospholipids, proteins and thiols from the leaf tissues (Pak & Van-Doorn, 2005; Ahmad *et al*., 2018).

Recent studies have demonstrated the significant role of jasmonates in regulating leaf senescence (Goossens *et al*., 2016; Sharma & Laxmi, 2016). Methyl jasmonate (MeJ), known for its volatile and aromatic properties, has the potential to directly enter the internal atmosphere of leaf tissue, causing changes in gaseous components that trigger visible senescence (Takahashi, 1981; Yue *et al*., 2012; Qi *et al*., 2015). Furthermore, the analysis of gene expression and function has provided compelling evidence supporting the involvement of MeJ in the process of leaf senescence (Hu *et al*., 2017).

In the present study, the application of MeJ to *B. ciliata* resulted in noticeable changes in leaf color, including yellowing and browning. A similar response was observed in detached wild-type leaves of *Cucurbita pepo*, where exogenous MeJ treatment led to visible yellowing, indicating chlorophyll breakdown (Hu *et al*., 2017). Previous research has demonstrated that MeJ induces the activation of key enzymes involved in chlorophyll breakdown (Ullah *et al*., 2019). Additionally, the application of MeJ externally has been shown to induce leaf senescence symptoms (Jung, 2004; Hu *et al*., 2017).

The increase in sugars during the onset of senescence is a common phenomenon, attributed to the heightened respiration in aging organs (Chen *et al*., 2004; Kalra & Bhatla, 2018). In this study, the application of MeJ resulted in an increase in soluble sugar levels in leaf discs of *B. ciliata*, which is consistent with previous findings in detached leaves of *Oryza* and *Anchusa* (Chen *et al*., 2004; Taheri *et al*., 2020). Furthermore, jasmonates have been known to accelerate protein degradation, leading to elevated amino acid contents in leaf tissues (Takahashi, 1981; Chen & Kao, 2012). Additionally, the application of jasmonates has been shown to enhance cellular respiration and peroxidation kinetics, thereby promoting leaf senescence in *Arabidopsis* (Shan *et al*., 2011).

In the current investigation, MeJ exhibited a significant enhancement in the activities of antioxidant enzymes such as APX, CAT and SOD. Previous studies on *Matricaria chamomila* (Salimi *et al*., 2016) and *Zingiber officinale* (Li *et al*., 2019) have also reported that MeJ increases the activities of SOD, CAT and APX. The induction of leaf senescence by MeJ can be attributed to the heightened activity of lipoxygenase, as observed in *Arabidopsis* (Hu *et al*., 2017; Zhang *et al*., 2018). It has been demonstrated that the expression levels of *LOX* (*LIPOXYGENASE*) genes increase during leaf senescence (Takahashi, 1981; Ullah *et al*., 2019) and the application of exogenous MeJ leads to an upregulation of *LOX* expression (Schommer *et al*., 2008; Reinbothe *et al*., 2009; Repka *et al*., 2013).

Conclusion

The main discovery of this study highlights the contrasting effects of two phytohormones on the process of leaf senescence in *B. ciliata*. The hormone known as KIN was observed to have a delaying effect on leaf senescence. It achieved this by preserving elevated levels of chlorophylls, soluble proteins and reduced sugar fractions within the leaf tissues. Additionally, KIN also decreased the activity of an enzyme called LOX, which is associated with leaf senescence. On the contrary, the hormone MeJ was found to accelerate senescence by opposing the effects of KIN. Interestingly, both KIN and MeJ were found to increase the activities of antioxidant enzymes, suggesting their involvement in the cellular defense mechanisms during leaf senescence.

Future perspectives: Although this investigation provides valuable insights into the physiological mechanisms of KIN and MeJ regarding leaf senescence in *B. ciliata*, there remains a significant scope for further research to elucidate the molecular aspects of this process. An encouraging approach to prevent leaf senescence and boost plant productivity involves promoting cytokinin synthesis through innovative molecular interventions, such as the overexpression of the IPT (isopentenyl transferase) gene controlled by *SENESCENCE-ASSOCIATED GENES* (*SAGs*). Furthermore, a comprehensive understanding of leaf senescence can be attained by leveraging multi-omic technologies to analyze the dynamic changes in transcripts, proteins and other metabolites. Undertaking such investigations will bridge existing knowledge gaps and facilitate a thorough comprehension of the mechanisms governing leaf senescence. This, in turn, will pave the way for future agronomic advancements in diverse crop plants.

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