Effect of 5-Aminolevulinic Acid (ALA) on the Biochemical and Physiological Parameters of Postharvest Quality of Polygonatum multiflorum L. All. ‘Variegatum’ Cut Foliage

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Abstract: Due to the dynamic development of the floriculture market, new species and varieties of plants that can potentially be used as a source of cut greenery are constantly being sought. The experiment was conducted to analyze the effect of the cultivation site (unheated foil tunnel and open field) and treatments with the Pentakeep V formulation (0.00%, 0.02%, 0.04%, and 0.06%) on the vase life and postharvest quality of Polygonatum multiflorum ‘Variegatum’ cut foliage. There was a positive effect of the cultivation in the unheated foil tunnel on the vase life and biochemical parameters, namely the electrolyte leakage (E_L), thiobarbituric acid reactive substances (TBARS), chlorophyll a + b (Chl. a + b), proline, peroxidase (POD), and ascorbate peroxidase (APX), of P. multiflorum cut foliage. Foliar application of Pentakeep V formulation at concentrations of 0.04% and 0.06% contributed to a decrease in the TBARS content in the P. multiflorum cut leaves, regardless of the plant cultivation site. Additionally, it reduced proline production in cut leaves of P. multiflorum cultivated in the unheated foil tunnel, which was confirmed by the analyses carried out after the 30 days of the experiment. Furthermore, the exogenous application of ALA during P. multiflorum cultivation inhibited POD activity, irrespective of its concentration and the site of plant cultivation.

Keywords: chlorophyll; cut greenery; electrolyte leakage; peroxidase; proline; TBARS; vase life

1. Introduction

In the last decade, there has been a continuous increase in the importance of cut greenery as a floristic material worldwide [1]. Cut foliage is gaining popularity, as it complements floral arrangements and often improves their shape [1]. Senescence of leaves that have been cut off from the parent plant is an inevitable rapid process, evident especially in the final phase of ontogenesis, in which irreversible changes are initiated, leading to gradual cell degradation and death of the organism [2]. Excision of an organ from the mother plant results in water and oxidative stress, the adverse effect of which is intensified at the successive stages of trading and accelerates the process of aging of cut foliage. Oxidative stress, which is defined as imbalance between reactions generating reactive oxygen species (ROS) and neutralizing reactions [3], may also appear in plant cells as secondary stress, caused by, e.g., water stress [4]. To assure the best quality of plant material, several studies have been undertaken to understand and control physiological and biochemical changes during the postharvest and postproduction life [5]. One of the phenomena occurring during senescence of cut foliage and inflorescence is accumulation of ROS and activation of the enzymatic antioxidant defense...
mechanism [6]. In plant cells, overproduced hydrogen peroxide (H$_2$O$_2$) can rapidly diffuse across the membrane and is toxic [7]. Plant defense mechanisms against oxidative stress are based on enzymatic reactions involving catalase and peroxidases and nonenzymatic reactions with the participation of glutathione, flavonoids, or carotenoids [3]. An important role in neutralization of a common ROS, H$_2$O$_2$, is played by peroxidases, which reduce the reactive species to water, thus oxidizing the organic substrate, i.e., the electron donor. Flavonoids, ascorbic acid, or substances containing thiol groups can be substrates of reactions catalyzed by peroxidases [8–10].

The physiological and anatomical characteristics that ultimately determine the vase life potential of cut inflorescence and foliage are formed during the preharvest period [11–13]. It is estimated that the contribution of cultivation conditions to the quality and vase life of plant material is 30–70% [14,15]. The highest quality of ornamental material is obtained from cultivation in fully automated greenhouses. However, the costs of construction and maintenance of such a greenhouse are very high. A much cheaper option is cultivation in an unheated foil tunnel, which allows plants to start vegetation earlier, protects plants against spring frosts, and reduces damage caused by atmospheric precipitation and pest attacks, thereby ensuring substantially higher quality of the ornamental material [16].

Growth stimulants applied during cultivation also exert a positive effect on the postharvest quality of cut foliage. Pentakeep V is a multicomponent fertilizer with 5-aminolevulinic acid (ALA) as an active substance [17]. ALA is naturally found in plants, animals, algae, and photosynthetic bacteria, and it is known as the first common precursor of all tetrapyrroles such as vitamin B12, chlorophyll, heme, and phytochrome [18,19]. Foliar application of ALA to plants can increase chlorophyll content in leaves and increases plants' ability to absorb light, which is especially important in cover cultivations and in the spring and autumn-winter periods when light deficiency is the basic factor inhibiting plant growth and development [20,21]. Additionally, as a biological metabolic intermediate, ALA can not only improve the quality of plant [20], but also has a similar physiological function of plant hormones and can regulate physiological activity of protective enzyme system and osmotic regulation substance content to improve the performance of plants from adversity [21]. The excision of the foliage from the mother plant results in water stress, which is known to induce substantial accumulation of proline, an important organic osmolyte for improving plant stress tolerance. The biosynthesis of ALA and proline share the same precursor, glutamic acid [22]. The exogenous application of ALA may increase cellular levels of ALA, and the precursor is then consumed for proline biosynthesis [23]. Proline buffers cytosolic pH, protects subcellular compartments, and scavenges ROS [24]. ALA supplementation has a significant potential in ornamental plants sector and a positive effect on growth parameters and chlorophyll content was observed, i.e., for Begonia x tuberhybrida ‘Nonstop’ [25], Saintpaulia ionantha, Tillandsia usneoides [26], and Phoenix dactylifera ‘Kalas’ [27].

P. multiflorum can grow to a height of 80 cm in deciduous European and Asian forests (beech, oak, and hornbeam) [28]. In recent years, this plant has become more and more popular in European gardens. P. multiflorum is a perennial that produces long, profuse leafy stems, which, in floral arrangements, might successfully substitute fern or palm leaves [29]. An especially decorative variety of the species is ‘Variegatum,’ with white edges of the leaves [29].

The aim of the study was to determine the effect of application of ALA and the impact of the site of cultivation on the postharvest quality of P. multiflorum cut foliage. The determination of E$_L$ and the level of peroxidation of membrane lipids were assessed. The content of assimilation pigments (chl. a + b) and proline was analyzed, and the activity of antioxidative system enzymes (POD and APX) and postharvest foliage vase life were assessed.

2. Materials and Methods

2.1. Plant Material and Treatments

The foliage of Polygonatum multiflorum L. All. ‘Variegatum’ plants used in the experiment originated from plants grown in an unheated foil tunnel and in an open field. During cultivation,
the plants growing in the tunnel and in the field were divided into four plots and sprayed six times with aqueous solutions of Pentakeep V (Pentakeep®, Cosmo Seiwa Agriculture Co., Ltd., Osaka City, China), which contains 0.3% ALA, 9.5% N, 5.7% Mg, 0.3% Mn, and 0.45% B, as well as DTPA-Fe, ZnSO₄, CuSO₄, and dinatiummolibdenat. Pentakeep V were used in concentrations of 0.02%, 0.04%, and 0.06%. The plants were always sprayed in the morning every week, starting from 2 May to 6 June in the tunnel and 9 May to 16 June in the field. Plants sprayed with distilled water constituted the control group. At the stage of plant morphological maturity (2 weeks after the last treatment), foliage were excised in the morning, transferred to containers with distilled water, and transported to the phytotron with controlled conditions (light and temperature) for the next stage of the experiment. The experiment was carried out in controlled thermal and light conditions: Temperature 21/18 °C (day/night), relative air humidity 60%, irradiation intensity 35 µmol m⁻² s⁻¹, and photoperiod 12 h light/12 h dark. The experiment consisted of eight combinations: Four from the open field and four from the unheated foil tunnel (Pentakeep V at concentrations 0.00%, 0.02%, 0.04%, 0.06%). Each combination included 15 equal-length leafy stems (50–60 cm), marked individually and regarded as replicates. The distilled water in which the foliage were kept was replaced every day until the end of the experiment. At each change of water, the foliage were trimmed by approximately 0.5 cm.

2.2. Vase Life

To assay the postharvest vase life of *P. multiflorum* foliage, 15 cut steams of every 4 combination were kept in distilled water for 30 days. The postharvest vase life of the *P. multiflorum* leafy steams was determined by recording the number of days between excision of the foliage and appearance of signs of loss of decorativeness (30% of leaves on the foliage having turned yellow or brown and withered).

2.3. Membrane Permeability

\( E_L \) was measured with the method described by Kościelnia [30] using an Elmetron CC-317 microcomputer conductometer. Ten rings (0.9-cm diameter) were cut with a cork borer from the leaves of each series, covered with 20 mL of redistilled water, and shaken at room temperature for 24 h. Next, the first electroconductivity measurement was made (\( K_1 \)). The plant material was then boiled at 100 °C (15 min). After another 24 h of shaking, electroconductivity was measured again to determine the total electrolyte content (\( K_2 \)). \( E_L \) was expressed as a percentage of its total content in the tissue, according to the formula:

\[
E_L = \left( \frac{K_1}{K_2} \right) 100\%.
\]

The level of membrane lipid peroxidation was assessed by determination of the content of thiobarbituric acid reactive substances (TBARS) according to Heath and Packer [31]. Crushed plant material (0.2 g) was homogenized in 0.1 M of potassium phosphate buffer, pH 7.0, and then centrifuged at 12,000×g for 20 min. Next, 0.5 mL of the homogenate was added to 2 mL of 20% trichloroacetic acid (TCA) containing 0.5% thiobarbituric acid (TBA) and incubated for 30 min in a water bath at 95 °C. After incubation, the samples were quickly cooled and centrifuged again at 10,000×g for 10 min. Absorbance was measured at 532 nm and 600 nm with a Cecil CE 9500 spectrophotometer. The TBARS concentration in the sample was calculated using the molar absorbance coefficient (155 mM⁻¹ cm⁻¹ for malondialdehyde (MDA)) and expressed as nanomoles per 1 g of fresh weight.

2.4. Chlorophyll Content

The content of the chl. \( a + b \) pigments in plant tissues was determined according to the method proposed by Lichtenthaler and Wellburn [32], i.e., extraction of 0.5 g of the leaf fresh weight in 80% acetone. The measurement of absorbance was performed at two wavelengths (\( \lambda \)), 646 nm (chl. \( b \)) and 663 nm (chl. \( a \)), using a Cecil spectrophotometer CE 9500. The concentration of each pigment was calculated according to the following formulas:

\[
C_{\text{chl.a}} = 12.21 \cdot A_{663} - 2.81 \cdot A_{646}
\]
\[ C_{\text{chl.b}} = 20.13 \cdot A_{646} - 5.03 \cdot A_{663} \]

where \( A_\lambda \) — the absorbance value for wavelength \( \lambda \).

Next, the concentrations of the pigments were converted into their content in the leaf fresh weight.

2.5. Free Proline Assay

To determine the free proline level, 0.5 g of leaf samples from each group were homogenized in 3% (w/v) sulphosalicylic acid and the homogenate was filtered through filter paper [33]. The mixture was heated at 100 °C for 1 h in a water bath after addition of acid ninhydrin and glacial acetic acid. The reaction was then stopped in an ice bath. The mixture was extracted with toluene and the absorbance of the fraction with toluene aspirated from the liquid phase was read at 520 nm. The proline concentration was determined using a calibration curve and expressed as \( \mu g \) proline \( g^{-1} FW \).

2.6. Enzyme Assay

For preparation of the enzymatic extract, the leaf samples (100 mg) were homogenized in a cooled mortar with 0.05 M of phosphate buffer (pH = 7.0), containing 0.2 M of EDTA and 2% PVP and centrifuged (10,000 rpm; 10 min; 4 °C). The obtained supernatant was used for POD and APX determination.

POD activity was measured following the method described by Małolepsza et al. [34]. The reaction mixture contained 0.5 mL of 0.05-M phosphate buffer, pH 5.6, 0.5 mL of 0.02-M guaiacol, 0.5 mL of 0.06-M \( H_2O_2 \), and 0.5 mL of enzymatic extract. Extinction was measured at 1 min intervals for 4 min with a Cecil CE 9500 spectrophotometer at 480 nm. POD activity was determined using the absorbance coefficient for this enzyme, which is 26.6 mM\(^{-1}\) cm\(^{-1}\). The result was converted to POD activity per fresh weight and expressed as U mg\(^{-1}\) FW.

For the assessment of APX activity [35], the reaction mixture contained 0.5 mM of ascorbic acid in 0.1 M of phosphate buffer (pH 7.0), 100 mL of crude extract, and a final concentration of 0.1 mM of \( H_2O_2 \). The activity was determined by monitoring ascorbate oxidation at 290 nm. The APX activity was determined using the absorbance coefficient for this enzyme, which is 2.8 mM\(^{-1}\) cm\(^{-1}\). The result was converted to APX activity per fresh weight and expressed as U mg\(^{-1}\) FW.

2.7. Statistical Analysis

For the chemical analyses, five plant foliage (each year) were selected randomly from each combination and three leaves were sampled from the middle part of the foliage. All analyses and measurements were performed in five replications, with determination of the \( E_1 \) value and the content of TBARS, chlorophylls \( a + b \), and proline after 1, 17, and 30 days of storage of the \( P. multiflorum \) foliage in distilled water. In turn, the activity of antioxidant enzymes was determined after 17 and 30 days of the experiment. The entire experiment was repeated twice during two growing seasons and the two-year results were treated as replicates. All results were statistically analyzed using SAS version 9.1.3. All the data were subjected to three-way analysis of variance (ANOVA) with Tukey’s post-hoc test at \( p < 0.05 \). The first factor was the site of cultivation, the second factor was the concentration of ALA, and the third factor was the day of storage of plant material.

3. Results

3.1. Vase Life

The analysis of the length of the postharvest vase life of the \( P. multiflorum \) cut foliage revealed a favorable effect of the tunnel cultivation on the value of this parameter (Table 1). The leaves of the studied species retained their decorativeness in average more than 5 days longer than the leaves of plants cultivated in the field, irrespective of Pentakeep V treatment.
Table 1. Effect of growth conditions (unheated foil tunnel and open field) and foliar treatment (six times during vegetative period) with different concentrations of Pentakeep V (0.00%, 0.02%, 0.04%, 0.06%) on the vase life of P. multiflorum cut foliage.

| Cultivation Site | Pentakeep V Concentration (%) | Vase Life (Days) | Mean for the Cultivation Site |
|------------------|--------------------------------|-----------------|------------------------------|
| Tunnel           | 0.00                           | 28.00 bd        |                              |
|                  | 0.02                           | 30.00 d         | 28.75 b                      |
|                  | 0.04                           | 29.00 cd        |                              |
|                  | 0.06                           | 28.00 bd        |                              |
|                  | 0.00                           | 23.00 a         |                              |
| Field            | 0.02                           | 24.00 ab        | 23.25 a                      |
|                  | 0.04                           | 25.00 ac        |                              |
|                  | 0.06                           | 21.00 a         |                              |

1 Values in columns marked with the same letter do not differ statistically at \( p < 0.05, n = 15. \)

3.2. Membrane Permeability

The analysis of the status of cytoplasmic membranes by the determination of \( \varepsilon_L \) from leaf cells and the content of TBARS demonstrated that the cell membranes were degraded during the time of the experiment (Figures 1 and 2). An increase in the \( \varepsilon_L \) and TBARS values was noted during the postharvest senescence of P. multiflorum leaves. The cultivation site exerted an effect on the permeability of cytoplasmic membranes as well. Leaves of plants growing in the field conditions exhibited higher values of the \( \varepsilon_L \) and TBARS parameters (Table 2). Pentakeep V treatment reduced the \( \varepsilon_L \) parameter regardless of the concentration used, while only the two highest concentrations of Pentakeep V reduced the TBARS value (Table 3). The analysis carried out after 17 days of the experiment showed an inhibitory effect of the Pentakeep V treatment at a concentration of 0.04% on the degradation of cytoplasmic membranes expressed by the TBARS index both in the tunnel and field conditions (Figure 2). Whereas Pentakeep V 0.02% has no significant effect on TBARS value compared to control treatment, the variants treated with the two highest Pentakeep V concentrations showed significant reduced TBARS values (Figure 2).

![Figure 1](image-url)
Figure 2. Effect of growth conditions (unheated foil tunnel and open field) and foliar treatment (six times during vegetative period) with different concentrations of Pentakeep V (0.00%, 0.02%, 0.04%, 0.06%) on the content of thiobarbituric acid reactive substances (TBARS) in leaves measured after 1, 17, and 30 days of storage of the *P. multiflorum* cut foliage in water. Values are mean ± SE, n = 5. The bars marked with the same letters are not significantly different at p < 0.05.

Table 2. Effect of growth conditions (unheated foil tunnel and open field) on the mean values (comparative from days 1, 17, and 30) of parameters (electrolyte leakage, thiobarbituric acid reactive substances, chlorophyll *a* + *b*, proline, ascorbate peroxidase, guaiacol peroxidase) in *P. multiflorum* leaves. The averages in the table result from a three-factor statistical analysis.

| Cultivation Site | E<sub>L</sub> | TBARS | Chl. *a* + *b* | Proline | APX | POD |
|------------------|--------------|--------|----------------|---------|-----|-----|
| Tunnel           | 15.00 a<sup>1</sup> | 36.32 a | 1.50 b         | 109.17 a | 0.26 a | 15.20 a |
| Field            | 17.08 b | 39.03 b | 1.40 a         | 138.22 b | 0.65 b | 19.07 b |

<sup>1</sup> Values in columns marked with the same letter do not differ statistically at p < 0.05.

Table 3. Effect of Pentakeep V concentration (0.00%, 0.02%, 0.04%, 0.06%) on the mean values (comparative from days 1, 17, and 30) of parameters (electrolyte leakage, thiobarbituric acid reactive substances, chlorophyll *a* + *b*, proline, ascorbate peroxidase, guaiacol peroxidase) in *P. multiflorum* leaves. The averages in the table result from a three-factor statistical analysis.

| Pentakeep V Concentration (%) | E<sub>L</sub> | TBARS | Chl. *a* + *b* | Proline | APX | POD |
|-------------------------------|--------------|--------|----------------|---------|-----|-----|
| 0.00                          | 18.01 b<sup>1</sup> | 43.74 b | 1.40 ab | 178.89 c | 0.47 a | 22.07 b |
| 0.02                          | 15.87 a | 43.29 b | 1.51 bc | 120.42 b | 0.48 a | 16.05 a |
| 0.04                          | 15.09 a | 31.00 a | 1.57 c  | 99.03 ab | 0.44 a | 15.61 a |
| 0.06                          | 15.20 a | 32.68 a | 1.32 a  | 96.27 a  | 0.40 a | 14.87 a |

<sup>1</sup> Values in columns marked with the same letter do not differ statistically at p < 0.05.

3.3. Chlorophyll *a* + *b* Content

The senescence of the *P. multiflorum* cut foliage was associated with gradual degradation of chl. *a* + *b*, as evidenced by the reduced content of the analyzed pigments after 17 and 30 days of the experiment (Figure 3). The site of cultivation had a significant effect on the content of the pigment as well (Table 2). A higher chl. *a* + *b* concentration was detected in the leaves of *P. multiflorum* grown in the tunnel compared to the level of the pigment in the leaves of plants grown in the field.
Figure 3. Effect of growth conditions (unheated foil tunnel and open field) and foliar treatment (six times during vegetative period) with different concentrations of Pentakeep V (0.00%, 0.02%, 0.04%, 0.06%) on the content of chlorophyll $a + b$ in leaves measured after 1, 17, and 30 days of storage of the $P. multiflorum$ cut foliage in water. Values are mean ± SE, $n = 5$. The bars marked with the same letters are not significantly different at $p < 0.05$.

The Pentakeep V applied at the concentration of 0.04% significantly inhibited the degradation of chlorophyll compared to the control (Table 3). According to Figure 3, there were no statistically significant differences in the sum of chl. $a + b$ between the treatments with the different Pentakeep V concentrations and control. After 1 and 17 days of storage of $P. multiflorum$ foliage, an inhibitory effect of Pentakeep V at the concentration of 0.06% on the chl. $a + b$ concentration was observed compared to lower concentrations of Pentakeep V.

3.4. Free Proline Content

Proline accumulation in leaf cells was found to increase with the progressing senescence of the $P. multiflorum$ foliage (Figure 4). Similarly, the site of cultivation exerted a significant impact on the value of this parameter (Table 2). According to Table 3, Pentakeep V decreased the proline level in $P. multiflorum$ leaves compared to the control, regardless of the concentration used. The level of this amino acid in the leaves of plants growing in the unheated tunnel was 21.0% lower than that determined for the open-field-cultivated plants (Table 2). The analysis of the proline content in the $P. multiflorum$ leaves after 30 days of the experiment revealed a beneficial effect to plants supplemented with ALA via Pentakeep V spraying (Figure 4). In the tunnel cultivation variant, the lowest content of proline in the $P. multiflorum$ leaves was a result of the application of Pentakeep V at the concentration of 0.04% and 0.06%, which corresponded to a 78.9% and 87.0% decrease, respectively, compared to the control. In turn, the most effective inhibition of proline synthesis in plants growing in the open field was noted upon application of 0.02% and 0.04% of the formulation. The amount of the amino acid was reduced by 51.2% and 35.9%, respectively, compared to the control.
3.5. Enzyme Activity

The aging of the *P. multiflorum* leaves was accompanied by enhanced APX and POD activities (Figure 5). The site of cultivation exerted an effect on the activity of the analyzed enzymes as well (Table 2). The leaves of the field-grown plants exhibited significantly higher activity of these enzymes. The inhibitory effect of ALA on POD activity was shown by the analysis (Table 3), but there were no significant differences in the mean values of the examined parameter between the treatments with the different concentrations of the formulation used (Figure 5). After 30 days of storage, a decrease in POD activity was observed due to ALA application as compared to controls, irrespective of the place of cultivation (Figure 5).

4. Discussion

Ornamental plants grown as floristic material are most often produced in specialized greenhouses, whose construction and maintenance costs are quite high. A much cheaper alternative is the cultivation of plants in an unheated foil tunnel or in field conditions [36]. Compared to field-grown crops, plants
cultivated in a tunnel are characterized by higher yield, quality, and marketability [37]. The present analysis of the postharvest vase life of *P. multiflorum* leaves showed a positive effect of the tunnel cultivation on the assessed parameters. Similarly, Oh et al. [37] reported a positive impact of this type of cultivation on the quality parameters in lettuce leaves. Additionally, *Antirrhinum majus*, *Celosia argentea*, *Dahlia x hybrida*, *Dianthus barbatus*, *Eustoma russellianum*, *Helianthus annuus*, *Mathiola incana*, and *Zinnia elegans* cultivated in tunnels to be used as cut flowers were characterized by higher values of quality parameters (i.e. stem length and caliper, inflorescence length, flower width) compared to plants grown in field conditions [38]. On the other hand, Salachna and Byczyńska [39] reported that production location (foil tunnel and field) had no effect on vase life of cut *Eucomis ‘Sparkling Burgundy’* flowers.

Exogenous application of ALA regulates antioxidant enzyme activity, thereby enhancing plant resistance to different stresses [40–42]. At the time of excision of a foliage from the mother plant and during gradual aging of cut greenery, changes in the content of various chemical substances indicating progressing water and oxidative stress are observed in the plant cells. The exogenous application of ALA during plant growth can inhibit subsequent destructive consequences of stress and thus extend the postharvest vase life of cut greenery [23]. In the present experiment, no significant visual differences were found in the *P. multiflorum* vase life between the ALA concentration used, indicating that ALA impacts postharvest by decreasing TBARS value, proline content, and POD activity.

The loss of green color due to chlorophyll degradation in leaves is usually the first visible symptom of plant senescence [43]. Simultaneously, it is the most commonly used measure of the decorative value of cut greenery [44]. During the experiment, a reduced level of chl. *a* + *b* was observed in the leaves of the open-field-cultivated *P. multiflorum* plants. This confirms the results obtained by Rubinowska et al. [45,46], who studied the postharvest vase life of *Rosa* sp. and *Waigela florinda*. The site of cultivation was found to exert an impact on the chlorophyll content in the *P. multiflorum* leaves. A higher level of the pigment was detected in the leaves of plants grown in the unheated foil tunnel, which confirms the results obtained in previous studies of the same species [47].

Hotta et al. [48] reported that ALA can stimulate chlorophyll synthesis. However, the effect of the analyzed substance on pigment production depends on its concentration, mode of application (foliar, soil), plant species, and light intensity. In their analysis of the effect of ALA on the growth and senescence of pak choi leaves, Wei et al. [49] evidenced that exogenously applied ALA both stimulates the production of chlorophyll and delays its degradation, thus inhibiting leaf aging. Similar conclusions were also formulated by Kisvarga et al. [25], who found that ALA stimulates chlorophyll synthesis in the *Begonia x tuberhybrida* ‘Nonstop’ line. In the present experiment, there were statistically significant differences in the inhibition of chlorophyll degradation upon application of Pentakeep V at the concentration 0.04% during *P. multiflorum* growth. The absence of an effect of ALA on the value of the measured parameter in other variants may be associated with the mode of application or insufficient concentrations of the active substance. Ördögh et al. [50] observed a negative effect on chlorophyll content in micropropagated *Hosta ‘Gold Drop’* with addition of 0.1–0.8 mL L⁻¹ Pentakeep V to the growth medium. Liu et al. [20] reported ineffectiveness of sprayed ALA in the enhancement of chl. *b* synthesis during the cultivation of Swiss chard exposed to salinity-induced osmotic stress. Also, Kisvarga et al. [25] reported no differences in chlorophyll synthesis between Pentakeep V concentrations and ways of treatment in *Begonia x tuberhybrida* ‘Nonstop’ leaves.

Increased proline synthesis is observed in plant response to environmental stress, especially osmotic stress associated with aging of cut leaves. Free proline protects cellular structures, especially proteins and cytoplasmic membranes, against damage caused by various environmental stresses [51] such as those associated with aging and excision of cut foliage from the mother plant. A decline in the level of free proline after exogenous application of ALA in plants subjected to stress was reported by Zhang et al. [52]. The authors showed that ALA inhibited proline accumulation in *Solanum tuberosum* micro-seedlings exposed to salinity stress. In the present experiment, the lowest proline content was detected in the leaves of tunnel-cultivated plants sprayed with the two highest concentrations of
Pentakeep V during growth. These results may indicate that ALA effectively alleviates the effects of water stress accompanying senescence of cut plant material. In contrast, Liu et al. [21] observed enhancement of proline synthesis in Leymus chinensis seedlings, which were exogenously treated with ALA at a concentration of 50 mg L$^{-1}$ during drought stress. The analysis of the proline content in the P. multiflorum leaves depending on the site of cultivation demonstrated a higher level of the analyzed amino acid in the foliage of the field-grown plants. These results may indicate higher levels of stress to which plants are exposed in these growing conditions. As suggested by Zhang et al. [42], foliar treatment of plants with an ALA solution before the occurrence of stress may indirectly increase the activity of antioxidant enzymes via stimulation of proline production in plant cells. Additionally, in the case of cut plant material, the ability to retain water in tissues is highly important. Like soluble proteins and sugars, proline is classified as an osmoregulatory substance contributing to proper hydration of cells and maintenance of cell membrane stability in water stress conditions [21,53].

Leaf senescence is accompanied by accumulation of the ROS, especially H$_2$O$_2$ [54], which is directly associated with damage to cell membranes and an increase in their permeability [55]. This results in an increase in the E$_L$ and TBARS parameters in leaf tissues. The treatment of plants with ALA during growth can effectively inhibit the process of stress-induced disintegration of cell membranes, as demonstrated by Zhang et al. [42]. The authors showed that foliar application of ALA to cucumber plants during growth inhibited cell membrane damage caused by high temperatures, as evidenced by lower MDA values than those in water-sprayed plants. Similarly, Liu et al. [20] confirmed the beneficial effect of foliar application of low ALA concentrations on the maintenance of the functionality and integrity of cell membranes in Swiss chard leaves exposed to salinity stress. In contrast, Liu et al. [56] reported an increase in the MDA content in Brassica napus leaves as an effect of application of 10 mg L$^{-1}$ of ALA in water stress conditions. In the present experiment, a decrease in the level of TBARS in P. multiflorum leaves was observed in the variants treated with the highest ALA concentrations, regardless of the site of cultivation, which was confirmed by the analyses carried out after 30 days of the experiment. The foliar ALA treatment during plant growth may also influence the activity of antioxidant enzymes, which, in turn, alleviates the destructive effect of free oxygen radicals on the stability of cell membranes [42]. The analysis of the impact of the site of P. multiflorum cultivation on the APX and POD activity revealed a significant increase in the activity of these antioxidant enzymes during the senescence of the leaves of the open-field-grown plants. Furthermore, it was found during the experiment that the progression of aging of the P. multiflorum foliage was accompanied by an increase in the activity of APX and POD. Water stress progressing along the duration of the experiment increased the activity of antioxidant enzymes, which confirms the results presented by Li et al. [57].

In their study, Zhang et al. [42] showed that exogenous treatment of cucumber plants with ALA and subsequent exposure to high temperatures caused an increase in the activity of APX and POD. The authors indicated that the ALA pretreatment might modulate the activity of antioxidant enzymes indirectly via an increase in the proline and soluble sugar content. After 17 and 30 days of the present experiment, no significant effect of ALA on the APX activity was found in all experimental variants. Similarly, Liu et al. [20] did not detect an increase in APX activity in Swiss chard leaves subjected to salinity stress and treated with exogenous ALA. APX is a key enzyme in the ascorbate-glutathione pathway. It is commonly present in various cellular structures, e.g., chloroplasts, cytosol [58], mitochondria, peroxisomes [59], and apoplastic space [60]. Exogenous foliar application of ALA can stimulate APX activity, thereby protecting some of the organelles mentioned above without increasing the activity of total APX. This thesis was confirmed by Liu et al. [20], who observed a protective effect of chloroplast APX stimulated by exogenous ALA on chloroplasts in Swiss chard leaf cells but did not detect an increase in total APX activity. In turn, the activity of POD, i.e., another H$_2$O$_2$-scavenging peroxidase enzyme, declined upon the exogenous application of ALA. This relationship was confirmed by the results of analyses carried out after 30 days of storage of the P. multiflorum foliage. In contrast, Liu et al. [20] observed an increase in POD activity induced by exogenous ALA application to Swiss chard plants. Brassica napus seedlings were also found to have a higher ability to inactivate harmful
H2O2 after application of ALA [56]. This was confirmed by the results of analyses of APX and POD activity carried out as part of their experiment.

Summing up, the cultivation of P. multiflorum in the unheated foil tunnel contributed to the longer period of the postharvest decorativeness of the foliage, which was evidenced not only by the organoleptic measurements but also by the biochemical analyses. Plants grown in these conditions exhibited greater stability of cell membranes, reflected in the Ei indicator and TBARS content. The cultivation of plants in the unheated tunnel also resulted in higher content and slower degradation of chl. a + b during the storage of P. multiflorum leaves and in reduced content of proline and antioxidant enzyme activity. The present results allow a conclusion that the cultivation of plants in an unheated tunnel in the Central European climate ensures better quality of plant material with a longer postharvest vase life, compared to cut greenery from open-field-grown plants. The foliar ALA treatment of the P. multiflorum plants during cultivation did not extend the postharvest vase life of their leaves. ALA applied exogenously to plants as a Pentakeep V spray at a concentration of 0.04% and 0.06% resulted in a decrease in the TBARS content, regardless of the site of growth, and reduced the content of proline (in the tunnel cultivation), as evidenced by the analyses carried out after 30 days of storage of P. multiflorum foliage in water. Additionally, ALA inhibited POD activity, irrespective of its concentration and the site of plant cultivation. In summary, the treatment of the ALA-containing Pentakeep V formulation on growing plants exerted an impact on the physiological status of P. multiflorum foliage during storage. It influenced the state of cytoplasmic membranes, proline production, and activity of antioxidant enzymes. Therefore, the formulation can be recommended as an effective agent improving the quality of cut greenery, especially at insufficient precipitation during the growing season. However, these investigations should be continued in order to determine the optimal dose of ALA and an appropriate form of application of the formulation, especially in open-field cultivation, where plants are more frequently exposed to adverse weather conditions.

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