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EFFECT OF AMINOOXYACETIC ACID (*AOA*) ON *ACS* AND *ACO* GENES EXPRESSION AND THE VASE LIFE OF ALSTROEMERIA CUT FLOWER

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Aging in the limbs is divided into two categories: ethylene-sensitive limbs, and ethylene-insensitive limbs. Extracellular synthesis of ethylene in the first group is carried out through a specific biochemical pathway that results from flower development and pollination. The present study aims to explore the effects of aminooxyacetic acid (AOA) on the expression of ethylene biosynthesis pathway genes (1-aminocyclopropane-1-carboxylate synthase (*ACS)* and 1-aminocyclopropane-1-carboxylate oxidase (*ACO*)) and the vase life of the Alstroemeria cut flowers 'Mayfair' and 'Frosty' varieties. Also, the effect of AOA on at the rates of 0, 50, 100 and 150 mg/l was studied on the morphological and physiological traits. The comparison of the vase life of Mayfair and Frosty showed that Frosty had a longer vase life than Mayfair. Frosty exhibited lower levels of cell degradation, higher relative water content, more chlorophyll content, more

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total soluble solids, and higher peroxidase level than Mayfair. Higher AOA levels were significantly associated with higher activity of catalase and peroxidase, so that the 150 mg/l AOA had the greatest effect on the activity of these two enzymes. Also, the increase in AOArate to 150 mg/l resulted in the retention of leaf chlorophyll and the reduction of leaf chlorosis percentage in both studied varieties.The results indicated that the highest *ACO* and *ACS* gene transcripts were related to the 7th flower opening stage. The use of AOA was effective in reducing the gene expression in Frosty. Also, the application of 150 mg/l AOA had a significant reducing effect on the expression of the *ACS* gene in Frosty.

Keywords: *AC*S, Alstroemeria, peroxidase, leaf chlorosis, chlorophyll

INTRODUCTION

Alstroemeria is one of the most important cut flowers from the family Alstroemeriaceae. This flower is very much appreciated for its variety of colors, beautiful flowers, and high yield. The main problem with alstroemeria is the development of early chlorosis in the leaves and the subsequent abscission of the petals (HALEVY and MAYAK, 1981). This problem is a complex genetically programmed cell death, which is associated with a decrease in the concentration of macromolecules (proteins, ribonucleic acids, membrane lipids) and chloroplast destruction (WEAVER *et al.,* 1998). This is induced by the imbalance of hormones after harvest, and the use of hormones delays chlorophyll depletion and postpones flower senescence (VAN DOORN and STEAD, 1997). Ethylene plays a significant role in regulating the aging process of most plant organs such as flowers. In most flowers, wilting is associated with self-regulation of ethylene production (YANG and HOFFMAN, 1984). Ethylene-stimulated aging can be exacerbated by changes in cell structure and the increase in the concentration of reactive oxygen species such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide (ABELES *et al*., 1992). In ethylene synthesis pathway, first, conversion of S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylate (ACC) and methylthioadenosine, is performed by the enzyme ACC synthase (ACS). In the second step, ACC is converted to ethylene, CO_2, and cyanide by 1-aminocyclopropane-1-carboxylate oxidase (ACO). Finally, oxygen-dependent conversion of ACC to ethylene, is catalyzed by ACC oxidase (ACO) (BOOKER and DELONG, 2015; RUDUŚ, 2013).

Ethylene stimulates the aging of leaves and petals, resulting in the drying of sepals, the shedding of florets, the rolling of petals, wilting, or even the change in the color (DE LA TORRE *et al*., 2006). In fact, ethylene as a gas can quickly penetrate into the tissue of flowers and affect the quality of their maintenance. In fact, ethylene gas binds to very specific sites called ethylene-receptor molecule in plant tissues and alters the biochemistry of plant cells (NICHOLS, 1980). Oxidative stress has been long known as an effective factor in the onset of aging. The most important ethylene-induced aging inhibitors are the ACC synthase inhibitors, such as AVG and AOA, and ethylene-inhibitors, such as cyclopropane and silver ions in the form of silver thiosulfate complex. Aminooxyacetic acid (AOA) is an ethylene-inhibitor that inhibits the activity of the ACC synthase and maintains the ACC at a low level. AOA prevents the synthesis of endogenous ethylene, prevents water stress, and postpones flower wilting (PALIYATH *et al*., 2008). It has been documented that 1mM AOA treatment had no effect on the change in the activity of antioxidant enzymes (superoxide dismutase and catalase) in cut carnation flowers (BARTOLI *et al*., 1996).

It has been reported that AOA reduced the synthesis of ethylene in Dendrobium flowers treated with or without external ethylene. The levels of DEN-ETR, DEN-ACO and DEN-ACS genes transcription were measured by RT-PCR in the flowers treated with ethylene, and the results showed their significant increase. Pre-treatment of flowers with or without ethylene reduced the expression of DEN-ETR1 in perianth and stem. The amount of DEN-ACS transcription in the perianth was inhibited and decreased in the stems (KETSA and NARKBUA, 2001).

Given the increasing rate of the production and export of ornamental plants, the high costs of their production and their sensitivity to the storage conditions, it is becoming more important to consider post-harvest problems. The loss of post-harvest quality in most ornamental plants may be caused by one or more factors, including wilting or abscission of leaves and petals. The limiting factor of post-harvest life in some cut flowers is the disturbance of the hormonal balance in that ethylene may be involved. Regarding the fact that leaf and petal abscission in Alstroemeria cut flowers may be related to the presence and effect of ethylene, it is important to study the ethylene biosynthesis pathway and the use of its inhibitors, as well as the vase life of different varieties. Therefore, the purpose of this study was to investigate the effect of AOA on reducing ethylene biosynthesis pathway genes expression (ACS and ACO) and comparing the vase life of Alstroemeria cut flowers 'Mayfair' and 'Frosty' varieties.

MATERIALS AND METHODS

This research was conducted as two separate experiments in the laboratory of the Department of Horticulture, Islamic Azad University, Abhar Branch and in the laboratory of Biotechnology Research Center of Zanjan University, Zanjan, Iran in 2014. The first experiment was to investigate the effect of aminooxyacetic acid (AOA) on the vase life of Alstroemeria 'Mayfair' and 'Frosty' cut flowers, and the second experiment aimed to investigate the expression of ethylene biosynthesis pathway genes in Alstroemeria flowers. Each experimental unit consisted of a 0.5-L glass and three stalk of Alstroemeria with three replications. Samples were taken at four different stages of flower opening. The temperature of the laboratory was about 16-21°C and relative humidity was higher than 50 percent. Sunlight was the only source of light. The ventilation device also applied the laboratory air. In the first experiment, 200 mg/l aluminum sulfate was prepared using distilled water and 3% sucrose solution. Morphological and physiological traits of Mayfair and Frosty plants were measured. In this experiment, 3% sucrose and 200 mg/l aluminum sulfate were also applied uniformly to all experimental units.

Plant materials

Alstroemeria 'Mayfair' and 'Frosty' cut flowers were procured from commercial greenhouses. The irrigation system of the greenhouse was drip where the flowers were fertigated twice a week with the water containing fertilizers such as calcium nitrate, ammonium nitrate, potassium nitrate, iron chelate, molybdate, potassium sulfate, zinc sulfate, and copper sulfate. The flowers were picked from the lowest possible place (the white part) in the morning. The flowers, which were opened from 1 to 2 buds, were picked and were immediately placed to the refrigerator before packing. Then, they were immediately transferred to the laboratory. In the laboratory, the lower leaves of the stalks were removed and the stem end was cut under a water flow of 50 cm, they were placed inside glasses containing the experiment solution.

Evaluated Traits

During the experiment, the cut flowers were subjected to the measurement of vase life, percentage of flower opening, chlorophyll content, relative water content, carbohydrate content, leaf chlorosis percentage and cell degradation, total protein content, catalase and peroxidase activities.

Chlorophyll measurement

Chlorophyll content was measured using a CCM-200 chlorophyll meter.

Total soluble solids (TSS)

This measurement was performed with a manual refractometer (model ATAGD) by the CHANG (2003) method.

Relative water content

It was calculated according to the method used by BELTRANO *et al.* (2006) using the following formula:

$$\text{relative water content}(\%) = \frac{\text{Dry weight} - \text{fresh weight}}{\text{Dry weight} - \text{saturation weight}} \times 100$$

The fresh weight of the tissues was about 0.5 g. The saturation weight was obtained of weighing leaves saturated in water after 24 h. Dry weight was obtained from weighing tissue after drying at 80°C for 48 h.

Vase life

In order to measure the lifespan of the flowers, REID (1996)'s method was applied to estimate such traits as wilting, flower color changes and their abscission, indicating senescence. Then, the vase life was expressed in percentage.

Leaf yellowing

This was calculated by REID (1996)'s method after obtaining the number of yellow leaves per experimental unit. The results were expressed in percentage.

Cell degradation measurement

Cell destruction was evaluated based on electrolyte leakage. EC_1 was obtained at 30°C after 1 h. EC_2 was calculated after autoclaving at 120°C at 1 atmosphere for 20 min. Electrolyte leakage (EL) was calculated by the following formula:

$$EL = \frac{EC2 - EC1}{EC2} \times 100$$

Protein content measurement

The Bradford method (BRADFORD, 1976) was used to measure protein content.

Catalase activity

The catalase enzyme activity was measured by PEREIRA *et al*. (2002) method in which 0.01 g of frozen algae was ground in 5 ml of 5 mM potassium phosphate buffer with pH 7.5,

containing polyvinylpyrrolidone (PVP) 1% and emulsifolium EDTA for 10 min. It should be noted that all the extraction steps were carried out in ice. Then, the extracts were centrifuged at 18000 rpm for 15 min at 4°C and the supernatant was used to determine the activity of catalase.

Peroxidase activity

The ABELES and BILES (1991) method was employed to measure the peroxidase enzyme activity in which 0.1 g of dissolved algae was ground with 5 ml of Tris-Glycine buffer in a wooden mortar in ice for 10 min. The resulting solution was centrifuged for at 18000 rpm at 4°C for 1 min. Then, the supernatant was used to measure the enzymatic activity. If the enzyme could not be measured immediately after extraction, the supernatant was stored in a freezer.

Second experiment

Sampling

The two varieties of Alstroemeria were sampled at different stages of flower opening (4 stages) based on the BREEZE *et al*. (2004) method. The samples were frozen immediately and stored in a freezer at -80°C.Flower samples taken at different stages of opening were first well-powdered in liquid nitrogen and then, they were extracted by Extract Kit Protocol in 20 stages of RNA.

Determining the quality and quantity of RNA extracted

The RNA extracted quality assay was performed using a Thermo Scientific N200 Nanotrope device, which could determine the quality of RNA from a wavelength of 260 to 280 nm received by the device. If this ratio was between 1.8 and 2, it was a sign of good RNA quality.

TBE buffer preparation

107.8 g of Tris-HCl, 7.44 g of EDTA, and 77 g of boric acid with pH8.3 were mixed and adjusted to 1000 mL using distilled water. Then, this ×1 buffer solution was prepared and used in the preparation of gel.

Gel preparation and loading of Real Time-PCR product

To prepare the gel, Agarose and buffer were mixed together and were heated for 40 seconds for Agarose to completely dissolve in the buffer. Then, it was poured into a mold that was already prepared and the shoulders to create well were placed in it. After cooling the gel, the Real Time-PCR products were mixed with color and loaded into the well. Leader was also used in the first well.

Synthesis of cDNA

For the synthesis of cDNA, a pioneering company kit containing the materials needed for cDNA synthesis was used. To this end, the amount of micro-l/ml was mixed with each other from the RNA of the sample and then were added to the MIX and placed under reaction conditions according to the instructions given in the kit (Pishgam Kit). Microtibum containing reaction mixture was performed at 95°C for 5 min, 30 cycles for 45" seconds at 95°C, 1 min at 52-62°C, and 45 seconds at 72°C with a final set at a temperature of 72°C for 7 min.

Primer design

The primer design in this study was performed manually for three GAPDH, *ACS* and *ACO* genes, using different software. First, different sequences were taken from the NCBI site. Then, CLC Main Workbench and Primer3 software were used to design the primers individually. Forward and reverse primers were designed from variable portions of the sequence. Then, they were investigated in Fast software of PCR (for a Daimer survey), Mfold (for T folding test), Folding and Blast (for examination of similar sequences to other organisms).

Duplication of the cDNA and the real-time PCR conditions

Real-Time PCR reactions were performed using a Rotor Gene (model 3000). To duplicate by each pair of primers, a major site (including all experiment materials except cDNA) was prepared. The names of primers used along with the connection temperature are given in Table 1.

Table 1. Primers used along with the connection temperatures

Primer name	Sequence	TM(°C)
Leader GAPDH	5'-AGGAAYCCTGAGGAGAT-3'	50
Returned GAPDH	5'-ACCTTCTTRGCACCACC-3'	52.4
Leader *ACO*	5'-MAGCCGGABCTGATCAA-3'	50
Returned *ACO*	5'-CCGCTCACCTTGTCGTCCTG-3'	46.6
Leader *ACS*	5'-GGCSRTSGCBRRHTTCATG-3'	55.2
Returned *ACS*	5'-GGRTARTAHGGNGWWGG-3'	47.6

Analysis of the Ct data of the Real Time PCR reaction

The gene expression of the Ct size, as well as the absolute size of the number of copies of DNA, was studied obtained in comparison with the standard curve were obtained. Comparison of Means of treatment samples were compared with the control group was performed. The control was used to normalize the data and eliminate the possible errors.

Statistical analysis

A factorial experiment was conducted in a completely randomized design with three replications in the laboratory. Data were subjected to statistical analysis by MSTATC software package and means were compared by LSD test at the $p< 0.01$ and $p< 0.05$ levels. Graphswere drawn using MS-Excel software package. REST software was used to obtain primer efficiency.

RESULTS

Vase life related traits

The comparison of the vase life traits of two varieties of Mayfair and Frosty showed a significant difference between the two varieties so that Frosty had a longer vase life than Mayfair. Frosty had less cell degradation than Mayfair. Frosty had significantly higher relative water content and chlorophyll content than Mayfair. The two varieties had a significant difference in total soluble solids so that the percentage of total soluble solids in Frosty was greater than that in Mayfair (Fig. 1). Peroxidase enzyme activity of Frosty was significantly higher than that of Mayfair.

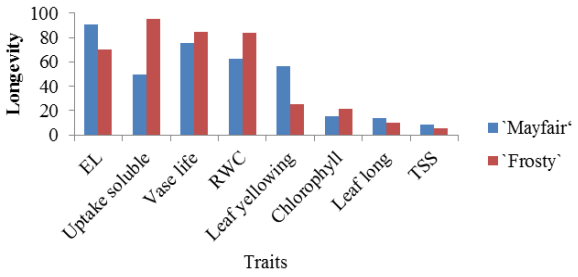

Fig 1. Measurement of traits related to the vase life of two varieties of Mayfair and Frosty

Effect of AOA on the studied traits

The increase in AOA rate significantly increased the catalase enzyme activity so that the application of AOA at 150 mg/l had the greatest effect on the catalase activity compared to control and other AOA levels. Mayfair had the highest catalase enzyme activity at 150 mg/L. However, no significant difference was found in Mayfair plants among other levels of AOA. Catalase activity in Frosty was increased with the increase in AOA concentration, but this increase was not significant versus other levels (Table 2).

Table 2. Results of comparison of mean of studied traits in variety and AOA interactions

Variety	*AOA(mg/l)*	Catalase (nmolmin^{-1}mg Pro^{-1})	Peroxidase (nmolmin^{-1}mg Pro^{-1})	Chlorophyll	Solution uptake (ml)	Leaf yellowing (%)
Mayfair	Control	0.016 b	0.127 a	15.07 f	50.00 d	58.33 a
	50	0.032 b	0.016 b	16.9 e	53.33 d	48.33 b
	100	0.08 b	0.032 b	20.8 d	61.67 c	23.33 c
	150	0.201 a	0.071 ab	22.57 c	63.33 c	19.33 c
Frosty	Control	0.032 b	0.016 b	17.93 e	88.33 a	25.00 c
	50	0.048 b	0.024 b	21.00 d	91.67 a	12.33 d
	100	0.048 b	0.016 b	24.4 b	71.67 b	5.333 e
	150	0.064 b	0.024 b	27.83 a	73.33 b	3.333 e

In each column, the means of the common letters do not differ significantly.

The peroxidase activity was significantly increased in Mayfair with the increase in AOA rate so that Mayfair exhibited the highest peroxidase activity at AOA level of 150 mg/L. The effect of different levels of AOA was not significant on peroxidase activity of Frosty plants (Table 2). AOA application had a significant increase in chlorophyll content compared to control. The increase in AOA concentration resulted in the preservation of leaf chlorophyll content of Mayfair and Frosty plants. Frosty plants treated with 150 mg/l AOA had the highest chlorophyll content. Mayfair variety had the highest chlorophyll content at 150 mg/l AOA (Table 2). The yellow leaf percentage was significantly decreased with AOA application. The highest chlorosis was measured in control treatment and the lowest was observed in plants treated with 150 mg/l AOA. In Frosty, the lowest percentage of chlorosis was observed at AOA

concentrations of 150 and 100 mg/l, respectively. In Mayfair, the lowest percentage of chlorosis was observed in the plants exposed to 150 mg/l AOA (Table 2).

Effect of AOA on ACS and ACO (AL-ACS gene expression)

The application of AOA did not affect Mayfair, but *ACS* gene expression was increased at higher concentrations. However, in the Frosty variety, the increase in AOA level entailed the significant reduction of *ACC* synthase gene expression compared to control.

Effect of time on AL-ACS gene expression

The Alstroemeria flowers were sampled in four stages (0, 2, 5 and 7 days). It was found that the highest expression of *ACC* synthase gene was in stage 7, which is significantly different from the control and the rest of the stages.

Effect of variety on AL-ACS gene expression

The level of *AL-ACS* expression in Mayfair with the lowest survival rate was significantly higher than Frosty with the highest survival rate.

Effect of time and variety on AL-ACS gene expression

In cv. Mayfair, the effect of sampling time and variety showed that the transcription rates of *ACS* was increased gradually while in cv. Frosty, it was decreased in stages 5 and 7.

Effect of time and AOA on AL-ACS gene expression

The effect of AOA and harvesting stages showed that the application of 150 mg/l AOA in stage 5 had a significant reduction in *ACS* gene expression compared to the control.

Effect of variety, time and AOA on AL-ACS gene expression

In cv. Mayfair in Stage 7, the application of *AOA* has no effect on reducing gene expression and, it even increased the expression of this gene. But in cv. Frosty in stages 5 and 7, there was a decrease in *ACS* gene expression, which indicates that AOA has been effective in reducing the expression of this gene in cv. Frosty (Table 3).

Effect of time on AL-ACO gene expression

ACO gene expression was the highest in stage 7 showing a significant difference with the control.

Effect of variety on AL-ACO gene expression

ACO gene expression in cv. Mayfair is more than in cv. Frosty, and it shows a significant difference.

Effect of AOA on AL-ACO gene expression

The application of AOA has no effect on the reduction of *ACO* gene expression.

Effect of AOA and variety on AL-ACO gene expression

Application of 150 mg/l AOA increased *AL-ACO* gene expression in a way that is significantly different with others.

Table 3. Interaction of variety, time and AOA on ACS gene expression

Variety name	*AOA (mg/l)*	Opening stage	*ACS* gene expression
Mayfair	Control	0	11.40 i
		2	15.85 h
		5	21.31 cde
		7	23.77 b
	150	0	18.23 fg
		2	20.62 e
		5	22.18 cd
		7	25.12 a
Frosty	Control	0	21.26 de
		2	22.63 bc
		5	19.02 f
		7	20.65 e
	150	0	17.29 g
		2	21.92 cde
		5	11.80 i
		7	15.47 h

In each column, the means followed with the same of the common letter(s) do not differ significantly.

Effect of time and variety on AL-ACO gene expression

Cv. Mayfair showed the highest *ACO* expression in stage 5, and the lowest was related to stage 0. But, cv. Frosty showed a decrease in *ACO* gene expression in comparison to cv. Mayfair, while Frosty also had the highest level of gene expression in stage 5 and the lowest in stage 7 (Table 4).

Table 4. Effect of time and variety on ACO gene expression

Variety name	Opening stage	*ACO* gene expression
Mayfair	0	4.692 e
	2	6.715 b
	5	8.73 a
	7	6.482 b
Frosty	0	5.343 d
	2	5.81 c
	5	6.562 b
	7	4.542 e

Interaction of time and AOA for AL-ACO gene expression

The use of 150 mg/l AOA increased *ACO* gene expression so that the highest expression was related to stage 5.

Effect of variety, time and AOA on AL-ACO gene expression

In Mayfair, the highest gene expression was observed in plants treated with 150 mg/l AOA in stage 7, and the lowest was related to plants not treated with AOA in stage 0 mg/l, but Frosty had the lowest *ACO* gene expression at AOA rate of 150 mg/l in stage 7 which didn't show a significant difference with AOA rate of 0 mg/l in stage 7 (Table 5).

Table 5. Effect of variety, time and AOA on ACO gene expression

Variety name	AOA(mg/l)	Opening stage	*ACO* gene expression
Mayfair	Control	0	2.527 k
		2	4.883 hi
		5	8.437 c
		7	3.237 j
	150	0	6.857 d
		2	8.547 bc
		5	9.023 b
		7	9.727 a
Frosty	Control	0	5.297 gh
		2	5.86 ef
		5	6.243 e
		7	4.43 i
	150	0	5.39 fgh
		2	5.76 efg
		5	6.88 d
		7	4.653 i

DISCUSSION

Vase life

The decrease in water absorption by cut flowers results in the loss of their relative water content. Also, less water absorption entails the decline of turgor and thus, chlorophyll degradation (HUNT *et al*., 2002). By decreasing chlorophyll content and relative water content, we observed a decrease in the vase life and increased leaf chlorosis in cv. Mayfair. The differences in vase life traits between two cultivars may be attributed to the differences in water absorption or their ability of osmoregulation to maintain tissue turgor and increase physiological activity. As it was observed, there was a significant difference between the two cultivars in terms of solution uptake and relative water content. Water absorption was the lowest in Mayfair and the highest in Frosty. The highest relative water content was observed in Frosty and the lowest in Mayfair. The inability to absorb water and wilting are the main symptoms of senescence so that the absorption of water and transpiration of cut flowers during the senescence period becomes unbalanced, cellular turgor disappears, and flowers suffer from early wilting (VAN MEETEREN *et al*., 2000).

The rate of lipid peroxidation increases with the senescence of flowers, but in some varieties, after the complete senescence of petals, a decrease in lipid peroxidation is also observed. Studies have shown that membrane damage and lipid peroxidation are increased during senescence (EZHILMATHI *et al.,* 2007). The increase in the permeability of the membrane at the senescence stage aggravates the loss of petal water content. Therefore, preserving petal water with different treatments plays an important role in preventing senescence. Free radicals (ROS) are naturally occurring plant metabolic substances that occur in different intracellular locations (HOSSAIN *et al.,* 2005).

Peroxidase enzyme activity in cv. Frosty was significantly higher than in Mayfair (GASPAR *et al.,* 1985) stated that peroxidase is an important antioxidant enzyme that neutralizes the toxic effect of free oxygen from H_2O_2 and thus, it prevents the senescence of petals. Free oxygen resulting from the decomposition of H_2O_2 is one of the important factors in the early premature senescence of petals. In general, it can be argued that the differences in the studied traits may be attributed to the differences in the two cultivars in terms of water absorption or the differences in their ability for osmoregulation to maintain tissue turgor and enhance physiological activity. In addition, the difference in the vase life of Alstroemeria cut flowers is brought about by the differences in their physiological and biochemical changes. Differences in carbohydrate stored in different varieties may also be due to this difference (VAN MEETEREN *et al.,* 2000).The difference in the vase life of the varieties may be caused by the greater sensitivity of these varieties to ethylene. Different sensitivity to ethylene has also been reported in different varieties of roses (REID, 1996).

Different varieties of Alstroemeria cut flowers can vary in their response to post-harvest treatments. Petal abscission and leaf chlorosis signal sensitivity to ethylene in this flower. There is a close relationship between ethylene biosynthesis and membrane degradation. Investigations show that lipid deformation leads to membrane degradation, and then peroxidation produces free radicals which augment ethylene synthesis (KIM *et al*., 2014). Flower senescence is associated with morphological, physiological, and biochemical deterioration. Ethylene plays a vital role in the regulation of flower senescence, and its production increases with the senescence of flowers (KETSA and RUGKONG, 2000). Ethylene causes early wilting, the loss of flower color, petal abscission, and leaf chlorosis (CAMERON and REID, 2001). Among the ethylene biosynthesis inhibitors, AOA hinders the synthesis of ethylene and prevents *ACC* synthase activity (KHAN, 2006). AOA prevents the production of endogenous ethylene and postpones the wilting of flowers (PALIYATH *et al.,* 2008). Alstroemeria is one of the flowers that are sensitive to ethylene. Probably, an intrinsic ethylene-inhibitor in the faded petals is synthesized in some flowers such as cloves, Alstroemeria, gladiolus, and rose, which affects the senescence of petals by blocking the hydrolysis of sucrose into glucose and fructose in old and worn out tissue. The reason is to control the transfer of sucrose from the wilted petals to other flower organs (WAGSTAFF *et al*., 2003).

The results of this study showed that the use of 150 mg/l AOA prolonged the vase life of Alstroemeria. A similar result has been found in Orchid, according to which the short-term treatment of 5 mM AOA extended the vase life (LERSLERWONG and KETSA, 2008). In fact, AOA is a pyridoxal phosphate enzyme inhibitor that inhibits the production of *ACC* synthase in the ethylene biosynthesis pathway. Also, AOA is a synthesis *ACC* inhibitor. AOA and *AVG* are used for endogenous ethylene and do not affect exogenous ethylene. AOA is an agent for controlling ethylene biosynthesis and blocking *ACC* synthase activity (MENSUALI-SODI *et al*., 2005).

Effect of AOA on biosynthesis pathway genes expression (ACS and ACO)

Based on the results, the application of AOA did not affect *AL-ACS* gene expression in Mayfair. But in Frosty, the application of 150 mg/l AOAcaused significant reduction in *AL-ACS* gene expression, so that in stages 5 and 7 of sampling, a significant reduction was observed in *AL-ACS* expression. But, *AL-ACO* gene expression in Mayfair was greater than that of Frosty, and the highest level of gene expression was in stage 5, and the use of AOA did not have any effect on the expression of this gene. AOA inhibits the synthesis of ethylene and prevents *ACS* activity and maintains *ACC* at a low level. This agent is effective in preventing the production of endogenous ethylene. It also prevents water stress and delays flower wilting (PALIYATH *et al.,* 2008).

It has been documented that 1 mM AOA treatment had no effect on the activity of antioxidant enzymes (superoxide dismutase and catalase) in cut carnation (BARTOLI *et al.,* 1996). Also, the concentration of 250 mg/L AOA extended the vase life of cv. Regina from 4 days to 15 days. This treatment maintained α-amylase enzyme activity at a low level (YAKIMOVA *et al.*, 1997).

AOA is known as an inhibitor of ethylene biosynthesis, which prevents *ACS* activity (MENSUALI-SODI *et al.,* 2005). This material is used to preserve ethylene-sensitive cut flowers (RATTANAWISALANON *et al.,* 2003). Therefore, no ACC levels were observed after treatment with AOA, especially at the concentration of 150 mg/l, indicating that AOA is an *ACS* inhibitor. Ethylene is one of the most important causes of flower senescence. In Alstroemeria, petal abscission indicates flower decay. Although petal wilting is not always associated with increased ethylene production (SEXTON *et al.,* 2000), evidence from ethylene-insensitive transgenic varieties and natural varieties indicate that the senescence mechanism is accelerated in the presence of ethylene. For example, it was found that in transgenic Petunia, the physiological and morphological traits of senescence were similar to non-transgenic plants, with the difference being that the senescence process in transgenic plants was delayed (GUBRIUM *et al.,* 2000).

Research has shown that there was an increase in ethylene level during the separation of the Alstroemeria flowers. It was anticipated that the genes involved in the biosynthesis of ethylene would be involved in the senescence of the flowers. However, in the normal values of genes *ALSACS*1-1 and *ALSACS*2-1, variations of less than 2.5 were shown from stages 0 to 7, which suggests that there are a number of *ACS* genes that do not regulate the transcription of ethylene biosynthesis. This does not hold true for some flowers, such as Kardinal rose (WANG *et al.,* 2004). However, the regulation of ethylene biosynthesis transcription at the level of *ACOALSACO*1-1 showed very little change in the six stages of flower development. But after that, it was increased by 4.5 times in two stages before the abscission. Research has shown that Alstroemeria petals can convert less than 1 mM of *ACC* to ethylene (WAGSTAFF *et al.,* 2003). A similar pattern of gene expression in rose 'Bronze' did not show any change in *ACS* gene expression during senescence. However, the *ACO*gene transcription was increased before petal abscission (MULLER *et al.,* 2000).

In another study, regulatory genes have been identified at both higher and lower levels in stages 0 and 5 in which none of them was ethylene biosynthesis regulator. As a result, ethylene biosynthesis takes place only after this stage. Such a pattern of gene expression suggests that the biosynthesis genes of ethylene act very late – indeed, after the processes previously identified in stage 5, such as DNA laddering, electrolyte leakage, fresh weight loss and protease activity (WAGSTAFF *et al.,* 2003). Examination of sensitivity to ethylene and reaction of

Alstroemeria flowers using a wide range of techniques has shown that this type of senescence is independent of internal ethylene synthesis.

It seems that the decline of the gene expression is due to the reduction of ethylene production in the flower (SATOH and WAKI, 2006). *ACS* activity is limited to ethylene synthesis, which is significantly reduced by inhibiting the pyridoxal phosphate enzyme, for example by the application of AOA. AOA treatment on Dendrobium flowers prevented the appearance of senescence symptoms in Dendrobium treated with 60% ethylene for 18 hours prior to exposure to ethylene while AOA alone could not delay the senescence process compared to the control. (REID and WU, 1992). Also, AOA pretreatment failed to delay the senescence process of the Dendrobium. AOA did not affect the wilting time. It only delayed the senescence of flowers treated with external ethylene (RATTANAWISALANON *et al.,* 2003). These results are consistent with our findings.

ACS activity is limited to ethylene biosynthesis, which is significantly reduced by inhibiting the application of pyridoxal phosphate enzyme, such as AOA (REID and WU, 1992). It has been reported that internal ethylene induces senescence in the flowers by a signal (O'NEILL *et al.,* 1993). Typically, the accumulation of senescence mRNA, results in the senescence of flowers that are exposed to external ethylene (BUCHANAN-WOLLASTON, 1997).

The comparison of vase life traits of two cultivars of Mayfair and Frosty shows that Frosty had longer vase life than Mayfair. Frosty had lower levels of cell degradation and higher relative water content, soluble adsorption, chlorophyll content, total soluble solids, and peroxidase enzyme activity than Mayfair. Leaf length and chlorosis were higher in Mayfair than in Frosty. Increasing levels of AOA significantly increased the catalase activity and peroxidase enzymes so that the application of AOA at 150 mg/l had the greatest effect on the catalase and peroxidase enzymes activity compared to control and other levels. Also, higher AOA concentration (150 mg/l) resulted in an increase in the chlorophyll content of leaves and decreased yeast percentage of leaves in Mayfair and Frosty varieties.The application of AOA did not affect Mayfair, and the *ACC* synthase gene expression increased at the concentration of AOA. The highest expression of *ACS* and *ACO* genes was observed in stage 7 in comparison to control and the rest of the stages. In Mayfair in stage 7, the application of AOA did not have any effects on the reduction of *ACS* gene expression and even increased the expression of this gene. But in Frosty in stages 5 and 7, there was a reduction in *ACS* gene expression, which indicates that AOA has been effective in reducing the expression of this gene in Frosty.

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EFEKAT AMINOOKSIACETINSKE KISELINE (*AOA*) NA EKSPRESIJU *ACS* I *ACO* GENA I DUŽINU ŽIVOTA SEČENOG CVEĆA ALSTROMERIJE

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Izvod

Starenje grana deli se na dve kategorije: grane osetljive na etilen i grane koje nisu osetljivi na etilen. Vanćelijska sinteza etilena u prvoj grupi se vrši specifičnim biohemijskim putem koji je rezultat razvoja cveća i oprašivanja. Ovaj rad ima za cilj da istraži uticaje aminooksiacetne kiseline (AOA) na ekspresiju gena na putu biosinteze (1-aminociklopropan-1-karboksilat sintaza (ACS) i 1-aminociklopropan-1-karboksilatna oksidaza (ACO)) i dužinu života rezanog cveća Alstromerije vrsta „Maifair“ i „Frosti“.Takođe, proučavan je I uticaj sadržaja AOA od 0, 50, 100 i 150 mg/l na morfološka i fiziološka svojstva. Poređenje dužine života reyanog cveća u vazama, pokazalo je da je “Frosti” imao duži životni vek od “Maifair”-a. “Frosti” je pokazao niže nivoe razgradnje ćelija, veći relativni sadržaj vode, više sadržaja hlorofila, više ukupnih rastvorljivih čvrstih materija i veći nivo peroksidaze u odnosu na “Maifair”. Viši nivo AOA bio je značajno povezan sa većom aktivnošću katalaze i peroksidaze, tako da je 150 mg / l AOA imao najveći uticaj na aktivnost ova dva enzima. Takođe, povećanje AOA koncentracije na 150 mg / l rezultiralo je zadržavanjem hlorofila lista i smanjenjem procenta hloroze listova u obe ispitivane sorte. Rezultati su pokazali da su najviši transkripti gena ACO i ACS bili povezani sa 7. fazom otvaranja cveća. Upotreba AOA je bila efikasna u smanjenju ekspresije gena u sorti “Frosti”. Takođe, primena 150 mg / l AOA imala je značajan smanjujući efekat na ekspresiju ACS gena kod sorte “Frosti”.

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