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Research Paper

Screening Eight Cultivars of *Alstroemeria* Cut Flower for Vase Life and Biochemical Traits

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Abstract

Alstroemeria is one of the most popular cut flowers in European Union owing to its postharvest longevity and broad color spectra. Nevertheless, premature wilting decreases the esthetic value of flowering branches even before the opening of the secondary florets in the fluorescence. The present study evaluated different biochemical and morphological traits of *Alstroemeria* cut flowers in order to screen the longevity of eight cultivars of *Alstroemeria* cut flowers including (Topaz, Chicago, Mayfair, Onyx, Frosty, Bellevue, Samantha, and Dimension). Traits such as longevity, wilting, water uptake, chlorophyll content, total soluble solids, relative water content, catalase activity, peroxidase activity, and protein content were evaluated. Data were subjected to the statistical and cluster analysis. The results showed that Mayfair and Frosty cultivars have the greatest distance from another. Mayfair had the lowest longevity. Frosty exhibited the most significant difference and was the most different cultivar from other cultivars in terms of biochemical traits, so that it was clustered in a separate group. As a result, two cultivars with the longest and the shortest longevity was determined. Maximum peroxidase activity was related to Frosty, differing significantly from that of Mayfair.

Keywords: *Alstroemeria*, Catalase, Peroxidase, Screening, Vase life.

INTRODUCTION

Alstroemeria, commonly known as Peruvian lily, belongs to the family *Alstroemeriaceae*. It is an important cut flower in Northern Europe (Breeze *et al*., 2004) and is popularly used in bouquets and flower arrangements. Because of its good postharvest longevity and broad color spectra, *Alstroemeria* has become one of the most adorable cut flowers. The flowering shoots of *Alstroemeria* bear numerous leaves and end with cymose inflorescences with three or four florets percyme (Hicklenton, 1991). The vase life can be long, up to 14 days, and it is usually ended by petal abscission of the flower (Chanasut *et al*., 2003; Wagstaff *et al*., 2005).

Postharvest senescence is a major limitation to the marketing of many cut flower species so that substantial efforts have been devoted to improving postharvest treatments to expand the marketing period (Nichols, 1977; Rattanawisalanona *et al*., 2003). Ethylene is a major regulator of senescence in many flowers (Nichols, 1977). It is a gaseous plant hormone synthesized by oxidation of 1-aminocyclopropane-1-carboxylicacid (ACC). In case of flowers in which ethylene has been implicated in the control of floral senescence, the use of ethylene inhibitors such as aminooxyacetic acid (AOA), norbornadiene, or 1-methylcyclopropene (1-MCP) has usually prolonged the vase life of the cut flowers (Serek *et al*., 1995). Reactive oxygen species (ROS) participate in plant senescence (Dhindsa *et al*., 1981). Cells are equipped with defense response against aggregation of free radicals by increasing catalase (CAT) and superoxide dismutase (SOD) activity (Khan, 2006). Ethylene causes a decline in peroxidation and prolongs the life of cut carnations. This phenomenon suggests a relationship between ROS generation and ethylene production (Mayak *et al*., 1983). In carnation petals, SOD activity decreases, but CAT activity increases during senescence (Droillard *et al*., 1989). The purpose of this study was to screen eight cultivars of *Alstroemeria* cut flowers based on morphological and biochemical traits in the postharvest period.

MARERIALS AND METHODS

Cut flowers of eight *Alstroemeria* cultivars (Topaz, Chicago, Mayfair, Onyx**,** Frosty, Bellevue, Samantha, and Dimension) were purchased from a commercial grower in Mahalat in Markazi Province, Iran, and were immediately transferred to the lab. The research was conducted in 2014 in the lab of the Department of Horticulture of Abhar Branch, Islamic Azad University, Abhar, Iran and the Biotechnology Research Center of Zanjan University, Zanjan, Iran. The flower stalks were cut at the time when all buds were closed, but the primary florets were already colored. The stalks were shortened to 40 cm. The flowers were kept at 20°C ±2 °C, 70-80% relative humidity, and 12 h photoperiod with 15 µmol m^{-2} s^{-1} irradiance of cool white fluorescent lamps throughout the experiment. Vase life was measured as the time passed to wilting of more than one-third of the flowers in the inflorescences.

During the experiment, traits such as longevity, willting, water uptake, chlorophyll content, total soluble solids, relative water content, catalase activity, peroxidase activity, and protein content were investigated. Coloration of the buds and the flowers to the wilting and the jaundice of the leaves expressed in days this was calculated (Ezhilmathi *et al*., 2007). The traits considered as symptoms of senescence wilting, flower color change, and flower fall. The willting was estimated by the method of Bahremand *et al.* (2014), and the number of chlorotic leaves in each experiment was counted to measure this trait. It was expressed in percent. During the experiment, traits such as chlorophyll content, total soluble solids (TSS), relative water content (RWC) percentage, cell corruption, catalase activity, peroxidase activity, and protein content were evaluated. Chlorophyll index was measured with a chlorophyll-meter (model SPAD-502) according to Jordi *et al.* (1994) method. TSS content was estimated with a handheld refractometer (model ATAGO) according to Chang (2003) method. In order to define the impact of different treatments on water content in *Alstroemeria* cut flowers, changes during storage were monitored. Relative water content (RWC)

was measured after turgor and drying. Beltrano *et al.* (2006) method was applied using the following formula:

$$RWC = \frac{\text{Fresh weig ht - dry weight}}{\text{Saturation weight - dry weight}} \times 100$$

Electrolyte leakage was measured using an EC meter according to Rabiei and Jozghasemi (2013) and Lim *et al*. (1998) procedure and EL was calculated by the following formula:

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

Potassium phosphate buffer was used to measure catalase activity according to Pereira *et al* (2002) method. Catalase activity and the decrease in H_2O_2 content were investigated by delving the alternations in light absorbance at 240 nm for 1 minute. First, 3.5 ml of H_2O_2 was mixed well with 50 ml of distilled water, then 70 ml of that mixture was taken to which 2.83 ml of potassium phosphate buffer without polyvinylpyrrolidone (PVP) and ethylene diamine tetra acetic acid (EDTA) was added. Finally, 100 ml from the extract supplied in protein assay was added to the prepared solution, and the absorbance was observed and recorded at 240 nm after 1 min. The control solution for this assay was obtained by adding 2.83 ml of potassium phosphate buffer without PVP and EDTA to 70 µl of H_2O_2 2% and then adding 100 µl of potassium phosphate including PVP and EDTA; in the end, relevant digits of absorbance were plugged in the formula so that the concentration of H_2O_2 which corresponded to the concentration of catalase enzyme was achieved.

In order to measure peroxidase activity, the method used by Biles and Abeles (1991) was followed. First, some lab tubes were put in ice, then 4 ml of sodium acetate buffer 0.4 M, 5 and 0.4 ml of H_2O_2 3%, and 0.2 ml of benzidine 2-methanol 50% were added to each of them. Finally, 0.2 ml of the extract was added to each tube, and absorbance was read at 530 nm, instantaneously. In the control sample, 0.2 ml of tris-HCl was added instead of 0.2 ml of extract. To measure protein content, the method of Bradford (1976) was applied. So, 100 µl of the already extracted solution was poured into a lab tube, then 5 ml of Bradford reagent was added (for each group of alga, one separate lab tube was considered). All of these stages were done while the tubes were located in ice. After 20 minutes, the absorbance of each tube was measured at 595 nm in comparison with the control sample. The control sample was achieved by adding 100 µl of buffer tris-HCl to 5 ml of Bradford reagent. The concentration of extracted protein was determined using the standard curve. The results were statistically analyzed using the MSTATC software package, and the means were compared by Duncan's multiple range test. Cluster analysis was based on the traits that were performed using the SYSTAT software package.

RESULTS AND DISCUSSION

According to the results in table 1, no significant difference was observed among cultivars in protein content and catalase enzyme activity. Topaz and Frosty did not differ significantly, but they had the highest peroxidase enzyme activity among all cultivars (Table 1). The highest electrolyte leakage was seen in Mayfair. Frosty and Dimension exhibited the longest longevity. Mayfair had the lowest longevity, RWC, water uptake , chlorophyll content, and peroxidase activity and the highest EL, TSS, and willting. There was no significant difference between 'Mayfair' and other cultivars except Frosty in longevity. The lowest RWC was of Samantha, Topaz, Chicago, and Mayfair. The highest TSS was observed in Mayfair, but there was no significant difference between this variety and Dimension and Bellevue in this respect. The lowest peroxidase activity was reported for Frosty showing significant differences with Mayfair. The lowest flower diameter was observed in Chicago and Topaz cultivars (Table 1) significantly difference from that of other cultivars.

Table 1. Results of comparison of mean of studied traits in the eight cultivars in *Alstroemeria* cut flower.

Cultivars	Peroxidase (nmol $min^{-1}mg\ Pro^{-1}$)	Electrolyte leakage (%)	Chlorophyll index (SPAD)	Water uptake (ml)	RWC (%)	TSS (%)	Flower diameter (cm)	Wilting (%)	Longevity (day)
'Chicago'	0.07367bc	80.95bc	18.50ab	56.67de	67.40c	7.167de	4.833de	43.33ab	8.35b
'Mayfair'	0.03700c	91.01a	15.23b	50e	62.62c	8.6a	5d	56.67a	8.01b
'Topaz'	0.1613ab	80.63bc	21.57a	80b	66.01c	7.467cd	4.76e	33.33bc	8.40b
'Onyx'	0.1257bc	85.58b	17.30b	56.67de	73.96b	6.8e	5.033d	53.33a	8.62b
'Frosty'	0.2420a	70.0d	21.40a	95a	84.11a	5.733f	6.567a	25c	10.86a
'Bellevue'	0.05233bc	73.65d	15.90b	50e	74.67b	8.433a	5.467c	53.33a	8.73b
'Samantha'	0.04967bc	79.24c	15.53b	70bc	64.90c	7.8bc	5.933b	51.67a	8.59b
'Dimension'	0.1210bc	73.16d	16.37b	66.67cd	74.94b	8.3ab	5.467c	50a	9.92ab

Means with different letters on the same column are significantly different ($P<0.05$) based on LSD contrast test. (A1:Chicago , A2:Mayfair, A3:Topaz , A4:Onyx, A5: Frosty, A6:Bellevue , A7:Samantha , A8: Dimension).

Cluster analysis

Morphological traits

Fig. 1. shows the dendrogram derived from cluster analysis of the eight cultivars of *Alstroemeria* cut flower based on the morphological traits (flower diameter, longevity, and wilting). The dendrogram cutting divided the studied genotypes based on the nearest neighbor distance into two large groups and each group has two subgroups:

Group 1: Chicago, Topaz, Frosty.

Group 1 has two subgroups: Subgroup 1 includes Frosty and subgroup 2 includes Chicago and Topaz.

Group 2: Mayfair, Onyx, Bellevue, Samantha and Dimension.

Group 2 has two subgroups including Mayfair constituting subgroup 1 and Onyx, Bellevue, Samantha and Dimension forming subgroup 2.

Mayfair and Frosty were the most different from one another so that Onyx and Bellevue showed no significant differences in terms of the morphological traits and they were placed in the same sub-cluster with no gap.

Biochemical traits

Fig. 2. shows the dendrogram derived from cluster analysis of the eight cultivars of *Alstroemeria* cut flower based on the biochemical traits (peroxidase enzyme activity, chlorophyll, TSS, water uptake and EL). The dendrogram cutting divided by the studied genotypes based on the nearest neighbor distance into three large groups and some subgroups as below:

Group 1: Mayfair, Onyx, Chicago and Bellevue.

Group 1 has three subgroups: Subgroup1 composed of Mayfair, subgroup 2 containing Onyx and Chicago, and subgroup 3 includes Bellevue.

Group 2: Topaz, Samantha, Dimension.

Group 2 has two subgroups: Subgroup 1 containing Dimension and subgroup 2 containing Samantha and Topaz.

Group 3: Frosty.

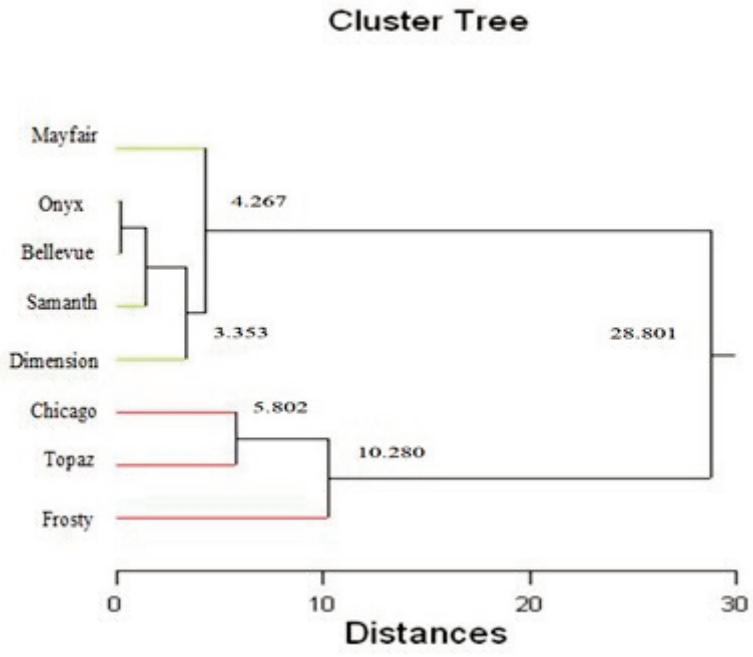

Fig. 1. The cluster analysis, the eight cultivars were evaluated based on morphological traits and the nearest neighbor distance.

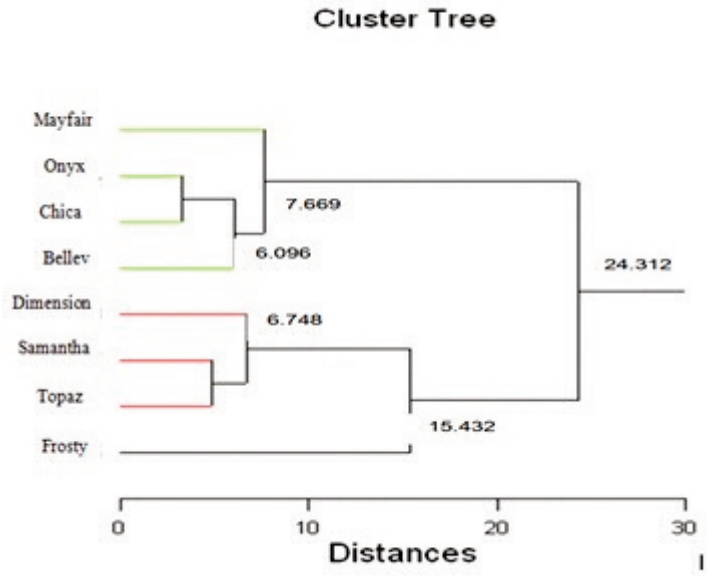

Fig. 2. The cluster analysis, the eight cultivars were evaluated based on biochemical traits and the nearest neighbor distance.

Frosty had the most significant difference and have the greatest distance from the other cultivars based on biochemical traits so that it was placed in a separate group. Onyx and Chicago showed no significant difference in terms of the biochemical traits and they were placed in the same sub-cluster with no gap. Likewise, Samantha and Topaz had no significant difference in terms of the biochemical traits and they were placed in the same sub-cluster with no gap.

The results showed a significant difference among cultivars in water uptake, RWC, flower diameter, and chlorophyll content. Also, longevity was significantly different among the cultivars. The short postharvest longevity of some cultivars can be attributed to their higher susceptibility (Solomos and Gross,1997). Different sensitivities to ethylene were reported in various rose flowers, too (Reid, 2002). This difference may come from the difference in the amount of carbohydrate storage in cultivars (van Meeteren *et al*., 2000). The findings of van Meeteren *et al.,* (2001) showed that the difference in the longevity of *Alstroemeria* cut flowers was due to the difference in physiological and biochemical perturbations. In this flower, petal abscission and wilting are symptoms of sensitivity to ethylene.

The increase in membrane permeability at senescence leads to more water loss from petals. So, the retention of water in petals plays a vital role in the prevention of senescence. In addition, the decrease in water absorbance causes the decline of RWC, turgor, and flower diameter, so it instigates chlorophyll degradation and destruction. The decrease in fresh weight at the end of the storage period is one of the senescence symptoms in flowers. This stage is seen in *Alstroemeria* flowers, too. The limitation of water uptake may be caused by various factors such as vascular obstruction in shoots (van Meeteren *et al*., 2001).

As findings revealed, flowers of Frosty in storage had higher RWC than other cultivars (Table 1). Moreover, inability to intake water and wilting were of principal senescence symptoms. The loss of fresh weight at senescence has been reported in chrysanthemum cut flowers, too (van Meeteren *et al*., 2000). As such, water uptake and transpiration of cut flowers are in imbalance during senescence, and cells lose their turgor and premature wilting take place in flowers (van Meeteren *et al*., 2000).

As previously mentioned, peroxidase activity in Frosty was more than others, and this was a significant difference (Table 1). Free oxygen coming from H_2O_2 decomposition is one of the indispensable factors inducing premature senescence of petals, and on the other hand, peroxidase is an important antioxidant enzyme that neutralizes the toxic effect of free oxygen stemming out of H_2O_2, so it inhibits petal senescence (Gaspar *et al*., 1985).

The differences in the evaluated traits may come from variations among varieties in water uptake or different capacities for osmotic regulating for retaining turgor and augmenting physiological activities. Alterations in the membrane such as perturbation in viscosity, changes in the ratio of saturated to unsaturated fatty acids, and lipid peroxidation happen during petal senescence, and this is related to reactive oxygen species (ROS) that increases simultaneously at this period. Membrane destruction is usually related to the gradual decrease in membrane phospholipid content as the result of phospholipase activity. Augmentation of lipase content (lipolytic hydrolase acyl) and lipoxygenase activity are relevant to the commencement of membrane destruction in carnation (Hong *et al*., 2000) and rose (Fukuchi-Mizutani *et al*., 2000). In *Alstroemeria*, membrane performance loss occurs without lipoxygenase activity augmentation though (Leverentz *et al*., 2002).

Water balance is one of the major factors determining the quality and vase life of cut flowers. Water uptake potential and transpiration of cut flowers make a harmony between these two processes (da Silva, 2003). Once the transpiration surpasses water uptake, cut flowers confront water deficit, and flower wilting proceeds (Halevy and Mayak, 1981).Usually, in both water abundance and deficit, flowers will wilt after harvesting. In carnation cut-flowers, petal wilting was coupled with a decrease in water uptake without vascular bundle blockage, which implies the inability of petals to absorb water (Solomos and Gross, 1997). At senescence, there is a significant decrease in relative fresh weight of petals (Hossain *et al*., 2006). Commonly, the decrease in the weight of floral shoot is of the most important determinants in defining flower quality (Chutichuted *et al*., 2011).

There is an intimate relationship between ethylene biosynthesis and membrane destruction. Evaluations have shown that the transformation of lipids incurs membrane demolishment, then free radicals are released through peroxidation, and finally, these radicals cause the amplification of ethylene synthesis (Paulin *et al*., 1986). Kim *et al.* (2008) confirmed that catalase is one of the key antioxidant enzymes active in the defense system of cells. This enzyme prevents free radical formation by breaking H_2O_2 into water and oxygen. In another report, the activity of peroxidase was increased during gladiolus petal senescence (Yamane *et al*., 1999). Senescence of flowers is accompanied with morphological, physiological and biochemical decline. Ethylene has an important role in the regulation of flower senescence, and its quantity increases as flowers grow older (Ketsa and Rugkong, 2000). Ethylene causes precocious wilting, flower color vanishing, petal abscission, and wilting (Cameron and Reid, 2001).

CONCLUSIONS

Cultivars Topaz and Frosty did not differ significantly, but they had the highest peroxidase enzyme activity among all cultivars**.** Mayfair and Frosty exhibited the greatest distance from each other. Mayfair had the shortest longevity. Frosty had the most significant difference and indicated the greatest difference from others in biochemical traits and it was placed in a separate group in the cluster analysis. As a result, two cultivars with the shortest and longest longevity were determined. The lowest peroxidase activity was reported for Frosty that differed significantly from that of Mayfair. The shortest flower diameter was related to Chicago and Topaz.

Compliance with ethical standards

Disclosure of potential conflicts of interest. All authors have read and approved the final

manuscript. The authors have no conflict of interest.

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