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Efficiency of Benzyladenine reduced ethylene production and extended vase life of cut *Eustoma* flowers

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Abstract

In this research, Benzyladenine (BA) was used for improving quality and vase life of cut *Eustoma* flowers. BA (0, 25, 50 or 75 mg L^{-1}) was sprayed on the flowers with a fine mist to cover all surfaces of the flowers and foliage. BA extended the vase life at all concentrations, but at 25, 50 mg L^{-1} were the more effective treatments. Ethylene production of cut flowers increased flower senescence. BA delayed ethylene production compared with the control. The weight loss, chlorophyll and anthocyanin degradation were significantly reduced by the application of 25 and 50 mg L^{-1} BA. Water uptake was higher in all treatments. This results show that ethylene production is an important factor in determining the vase life of cut *Eustoma* flowers.

Keywords: anthocyanin; chlorophyll; lisianthus; vase life; weight loss
Abbreviations: BA_Benzyladenine

Introduction

Postharvest life is influenced by many factors such as genetic (cultivar difference), season; stage of development when harvested; cultivated condition, ethylene effects (Doel and Wilkins, 1999) and etc. The postharvest quality of many flowers is reduced by ethylene. Ethylene causes premature wilting, color fading, abscission of flower petals and leaf yellowing (Joyce and Poole, 1993; Cameron and Reid, 2001; Celikel et al., 2002). Clearly, senescence of many flowers is coordinated by a rise in ethylene biosynthesis. The life of such flowers can be improved through manipulation of their production and sensitivity to ethylene (Amarjit, 2000). A rise in ethylene production that accelerates senescence has been found in cut carnations, roses and lisianthuses (Mayak and Halevy, 1980; Halevy and Mayak, 1981; Farokhzad et al., 2005; Hojjati et al., 2007). Cytokinins have been particularly effective in delaying senescence of cut carnation by inhibiting ethylene biosynthesis (Cook et al., 1985). The cytokine, benzyladenine increase the vase life of Eustoma (Huang and Chen, 2002). Other flowers, Such as carnations (Mac Clean and Dodelf, 1962), irises, roses, tulips and daffodils, show a slight positive effect or no increase in vase life in response to BA application (Halevy and Mayak, 1979). The effect of growth regulators, such as BA and GA_{4+7} on delaying chlorophyll degradation are well documented (Han, 1995, 1997). Spraying leaves of Oriental and Asiatic lily with 25 mg L^{-1} each of BA and GA_{4+7} completely prevented postharvest leaf yellowing (Han, 2001). The purpose of this study was to evaluate the response of flowers and foliage to BA application and roll of BA in ethylene production inhibition.

Materials and methods

Plant materials and treatments

Cut Eustoma (*Eustoma grandiflorum* cv. Azuma-no-kasumi) flowers were purchased when at least one bud was fully open from a commercial grower at karaj, Iran. Flower stems were trimmed to 40 cm. BA (0, 25, 50 or 75 mg L^{-1}) was sprayed on the flowers with a fine mist to cover all surfaces of the flowers and foliage. Then cut flowers were placed in a 500 ml flask with 400 ml of distilled water. Cut flowers were kept at 22˚C, 70% relative humidity, and 12h photoperiod with 15µmol$m^{-2}s^{-1}$ irradiance from cool – white fluorescent lamps throughout the experiment period.

Evaluation of vase life, Fresh weight loss and Water uptake

The vase life of cut *Eustoma* flowers were completed when the petals or stem below the flower head lost turgidity. Fresh weight loss and water uptake were determined 4, 8, 12 and 16 days after treatment. The fresh weight of each flower was expressed relative to the initial weight to represent the % of weight losses cut flowers. The rate of water uptake of cut flowers was measured by subtracting the volume of water

Table 1. Effect of BA on water uptake, vase life, chlorophyll content and total anthocyanin.

BA (mg L^{-1})	Water uptake(ml g^{-1} FW)				Vase life (day)	Chlorophyll (µg ml^{-1})	Anthocyanin (mg L^{-1})
	4 days	8 days	12 days	16 days			
0	1.04	1.70	1.12	0.35	12.25	8.53	86.42
25	0.97	2.11	2.02	2.04	16.26	11.06	99.50
50	0.89	2.15	2.06	2.05	16	11.17	105.77
75	0.99	1.79	1.42	0.57	12.75	9.17	89.69
LSD(0.05)	0.18	0.18	1.42	0.50	0.91	0.81	7.79

Table 2. Correlation coefficients between studied traits in cut Eustoma flowers.

	Variables											
Characters	Vase life	Chlo	Anth	Wu8	Wu12	Wu16	Fw8	Fw12	Fw16	Ep8	Ep12	Ep16
Vase life	1											
Chlo	0.99**	1										
Anth	0.94	0.96*	1									
Wu8	0.99**	0.99**	0.99*	1								
Wu12	0.98*	0.99**	0.96*	0.99**	1							
Wu16	0.99**	0.99**	0.95*	0.99**	0.98*	1						
Fw8	-0.98*	-0.99**	-0.98*	-0.99**	-0.98*	-0.99**	1					
Fw12	-0.98*	-0.99**	-0.94	-0.99*	-0.99**	-0.98*	0.97*	1				
Fw16	-0.98*	-0.99**	-0.97*	-0.99**	-0.99**	-0.98**	0.99**	0.99**	1			
Ep8	-0.99**	-1.00**	-0.96*	-0.99**	-0.99**	-0.99**	-0.99**	-0.99**	-0.99**	1		
Ep12	-1.00**	-0.99**	-0.94	-0.99**	-0.98*	-1.00**	0.98*	0.98*	0.98*	0.99**	1	
Ep16	0.95	0.92	0.89	0.93	0.893	0.95*	-0.94	-0.89	-0.90	-0.93	-0.96	1

*correlation is significant at the 0.05 level, **correlation is significant at the 0.01 level Chlo(Chlorophyll), Anth(Anthocyanin), Wu8,12,16(water uptake after 8, 12 and 16 day), Fw8, 12, 16(fresh weight loss after 8, 12 and 16 day), Ep8, 12, 16(Ethylene production after 8, 12 and 16 day)

evaporated from a flask of the same volume without cut flowers.

Measurement of ethylene production

One flower was sealed in a 230 ml glass vessel, for the measurement of ethylene production. Various organs, combined organs (pistil, stamen, and calyx) from three flowers were placed in a test-tube (15 ml), except for petals which were placed in a 50-ml erlenmeyer flask (66 ml). All vessels were sealed and kept at 22°C (Ichimura et al., 1998). One milliliter of the extracted gases was injected in a gas chromatograph equipped with an activated alumina column fitted with a flame ionization detector. Nitrogen was used as a carrier gas.

Measurement of Anthocyanin concentration

To determine the effect of BA concentrations on Anthocyanin, petal slices was extracted with methanol containing 1% HCL overnight at 4°C. The absorbance of the extracts was measured at 530–700 nm with a spectrophotometer. (Murr et al., 2008).

Measurement of Chlorophyll content

For testing chlorophyll content six circular disks, each 6.25 mm in diameter, were punched from the same general area of the leaf. The disks were placed immediately into 8 ml of 100% methanol, and pigments were allowed to extract in the dark at 30˚C for 24 h. Absorbance of the extracts was measured using spectrophotometer at 652 and 665 nm (Porra, 1989).

Statistical analysis

This experiment was conducted in completely randomized design with four replications. Three stems were used for each replication. Results were analyzed by using SAS software. Mean comparisons to identify significant difference between treatments were performed using least significant difference (LSD).

Results

Effect of BA on the vase life

The results showed that in comparison to the control, all concentrations of BA prolonged the vase life cut *Eustoma* flowers.

The use of 25 and 50 mg L^{-1} BA resulted in a greater extension in vase life. There were no significant ($p<0.01$) differences between 75 mg L^{-1} BA and control. Vase life of cut flowers held in distilled water received to 12.25 days (Table 1).

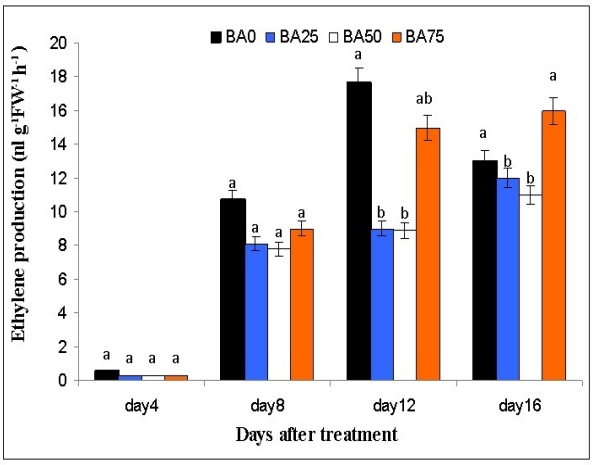

Fig 1. Effect of BA application on ethylene production of cut *Eustoma* flowers

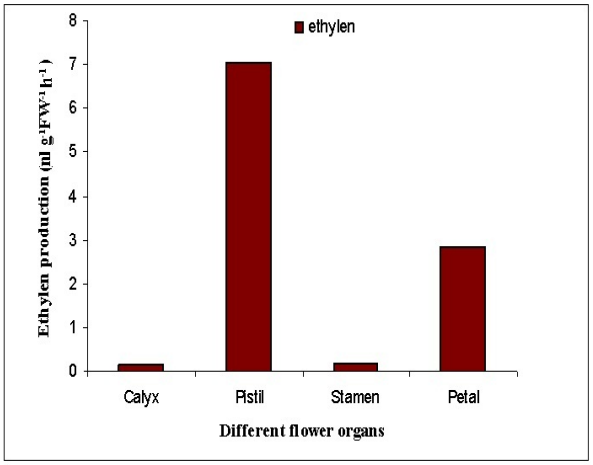

Fig 2. Ethylene production in various organs of cut Eustoma flower

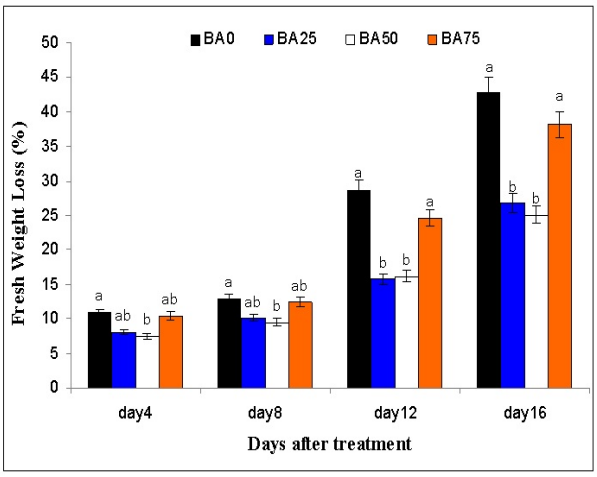

Fig 3. Effect of BA application on fresh weight loss of cut *Eustoma* flowers

Ethylene production

The results showed that the ethylene production by untreated flowers rapidly increased. The ethylene production increased as the floral senescence advance and then reduced (Fig. 1). Cut *Eustoma* flowers showed typical climacteric patterns of ethylene production during senescence. To spray with BA decreased ethylene production in all concentration. Significant differences ($p < 0.01$) were obtained for ethylene production, after 8, 12 and 16 Days. Highest means of ethylene production inhibition was found with 25 and 50 mg L^{-1} BA. There was negative correlation and significant difference between increasing ethylene production and decreasing vase life (table 2). The amount of ethylene production from the stamen and calyx was very low but the petal and pistil showed climacteric ethylene production, the pistil produced a larger rate of ethylene than the petal (Fig. 2).

Water uptake and Fresh weight loss

Water uptake rate increased at the first days of experiment in all treatments tested and then decreased. Uptake rate decreased rapidly in control and 75 mg L^{-1} BA, while flowers that were sprayed by 25 or 50 mg L^{-1} showed the minimum decrease to day 8 (Table 1). All the treatments significantly minimized the % of weight loss in comparison with the control. The data showed no significant difference between 75 mg L^{-1} BA and control (Fig. 3). Correlation of vase life with water uptake and fresh weight loss was significant (Table 2).

Chlorophyll and Anthocyanin content

The application of different BA concentrations delayed the Chlorophyll and anthocyanin degradation in comparison to untreated control. The best treatment in this regards was BA 25 and 50 mg L^{-1}. There were no significant ($p<0.01$) difference between BA 75 mg L^{-1} and control. Also correlation between vase life and chlorophyll was significant (Table 2).

Discussion

The obtained results indicate the effective role of BA to extend the postharvest quality of cut Eustoma flowers. The vase life of Eustoma was extended slightly by 75 mg L^{-1} and significantly by 25 and 50 mg L^{-1} BA that suggests benzyladenine at a lower concentration is required to delay flower senescence. Autocatalytic ethylene production was inhibited by BA (Huang and Chen, 2002; Han and Miller, 2003). Cut flowers showed typical climacteric patterns of ethylene production during senescence. This present studies have reconfirmed the role of ethylene on the vase life of cut Eustoma flowers (Farokhzad et al., 2005; Hojjati et al., 2007). These results suggest that *Eustoma grandiflorum* cv. Azuma-no-kasumi is very sensitive to ethylene. Ethylene causes premature wilting; color fading; abscission of flower petals; leaf yellowing (Joyce and Poole, 1993; Camerone and Reid, 2001; Celikel et al., 2002). In our study correlation of ethylene production with vase life and chlorophyll was significant. Ethylene production on various organs was different among plant species. In carnation, the petal produces the most ethylene (Nichols, 1977). In our study the pistil produced a larger rate of ethylene than the petal

(Ichimura et al, 1998). These differences may be partially due to gene expression and activity of various enzymes involved in ethylene biosynthesis. For example in *Phalaenopsis*, gene expression of ACC synthase is absent in the petal and 1-aminocyclopropane-1-carboxylic acid (ACC) transported from other organs to the petals (Oneill et al., 1993). Leaf yellowing is characterized by breakdown of chlorophylls, proteins, and nucleic acid in the detached leaves. The role of BA in delaying senescence of leafy vegetable is well established and it has been used to prevent leaf chlorosis in chrysanthemum during storage (Kofranek and Halevy, 1981). The data on chlorophyll content showed the positive role of BA (25 and 50 mg L^{-1}) on preserving the leaves in a good condition (state) by lowering the percent of weight loss and inhibiting the chlorophyll degradation. As a result the vase life could be increased. Similar results were obtained by Emongor et al. (2000) and Mutui et al. (2001) who reported cytokinins treatment delaying leaf senescence and improve the keeping quality of many cut flowers. Cytokinins have also been reported to promote chloroplast development and chlorophyll synthesis (Sallsbury and Ross, 1996). Color fading and discoloration is an important factor in determining display quality of cut flowers and in many cases is the main reason for determination of vase life. The major types of pigments contributing to the color of the flowers are cartenoids and anthocyanins (Amarjitt, 2000). Ethylene causes petal color fading, in our study BA treatments reduced ethylene production in cut Eustoma flowers. Thus anthocyanin was preserve in comparison to untreated control. In the present study BA at 25, 50 or 75 mg L^{-1} reduced petal color fading. Petridou et al. (2001) reported that treatment of cut chrysanthemum flowers by BA inhibit completely anthocyanin (cv. Reagan White) formation in the petals, along with its beneficial effect on chlorophyll content. Extending vase life for cut flowers depends absolutely on a continuing and adequate supply of water. Failure of water supply, results in rapid wilting of stem, petal, and leaves. At the higher BA concentration (75 mg L^{-1}) and control treatment water uptake rapidly reduced and the rate of senescence increased. Beginning of the senescence phase in cut flowers is characterized by a decrease in fresh weight (Adachi et al., 2000; Ichimura and Goto, 2002) and water uptake (Burge et al., 1996). BA treatment increased water uptake therefore retarding weight loss in comparison with control treatment.

Conclusion

According to these results it was possible to conclude that, spraying with BA at 25 and 50 mg L^{-1} concentrations extend the vase life of cut Eustoma flowers by retarding fresh weight loss, ethylene production, leaves chlorophyll, and anthocyanin degradation. Our results showed that the minimum treatment extended the vase life of cut flowers of *Eustoma grandiflorum* cv. Azuma-no-kasumi was 75 mg L^{-1} BA. Our findings also indicated the significant relationship between ethylene production and vase life cut flowers. However, we believed that more investigation is necessary to detect of BA treatment on the quality parameters of *Eustoma grandiflorum* cv. Azuma-no-kasumi.

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