

Effect of Benzyladenine on the Vase Life and Keeping Quality of *Alstroemeria* Cut Flowers

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ABSTRACT

Two experiments were conducted to investigate the response of Benzyladenine (BA) on the vase life and the physiological changes in the leaves of *Alstroemeria* cut flowers. The treatments were combined in a factorial manner and laid down in a completely randomised design with 3 replicates. Treatment of *Alstroemeria* cut flowers with 25 or 50 mg/litre BA consistently increased the number of days to full opening of primary florets and delayed the onset of flower senescence as measured by days to 50 % petal fall and 50 % leaf yellowing. BA at 25, 50 or 75 mg/litre increased both the leaf nitrogen and chlorophyll content of the *Alstroemeria* cut flowers compared to the control. However, 75 and 100 mg/litre BA gave the highest values of leaf dry weight. BA decreased the leaf water content of *Alstroemeria* cut flowers. These results suggests that 25 mg/litre BA has the potential to be used as a commercial cut flower preservative to prevent leaf yellowing and prolonging the vase life of *Alstroemeria* cut flowers.

KEY WORDS: *Alstroemeria*, benzyladenine, post harvest quality, vase life

1.0 INTRODUCTION

Cytokinins are known to delay leaf senescence (Richmond and Lang, 1957) and improve the keeping qualities of cut flowers (Halevy and Mayak, 1981). Exogenous application of cytokinins have been shown to reduce water stress damage in carnations (Paulin and Muloway, 1979), improve water uptake and maintain petal turgidity in roses (Mayak and Halevy, 1974), reduce respiration rate (MacLean and Dedolph, 1962; Heide and Oydvin, 1969), inhibit ethylene production and reduce sensitivity to ethylene (Eisinger, 1977; 1982; Mor *et al.*, 1983).

A flower dip in Benzyladenine (BA), a synthetic cytokinin, increases flower life of field grown Narcissus cut flowers by one day (Ballantyne, 1963). BA is effective when flowers

are dipped before they are given a dry storage. However, BA alone has no effect on fresh weight, dry weight and moisture content of freshly cut 'King Alfred' daffodils (*Narcissus pseudonarcissus*) (Ballantyne, 1965). Supplements of BA in the holding solutions of cut carnations extends their post harvest life (Heide and Oydvin, 1969). Applications of BA offer a very effective treatment to reduce leaf chlorosis in Easter lilies, whether the chemical is sprayed at harvest (Han, 1997) or during production (Heins *et al.*, 1996). BA treated flowers did not show a decline in quality and there was no significant change in the fresh weight, throughout the experiment (Cook *et al.*, 1985). Pulsing carnation buds with BA before cold storage causes a slight acceleration of bud opening and increases the vase life of fully open flowers (Goszczyńska and Nowak, 1979).

Cytokinins are thought to regulate carnation senescence through interaction with ethylene (Eisinger, 1977; 1982; Mor *et al.*, 1983). Cytokinin treatment appears to reduce ethylene synthase activity, since pre-treatment with BA results in a 90 % reduction in the capacity of carnation flowers to convert exogenously applied 1 - aminocyclopropane - 1 - carboxylic acid (ACC) to ethylene (Eisinger, 1982). Mor *et al.* (1983) reported that BA pretreatment prevents the normal rise in endogenous ACC levels, in detached carnation petals, associated with the onset of senescence. However, BA does not inhibit ethylene production in green leaves but rather slightly but significantly increases it (Mor *et al.*, 1983). Hence, cytokinin action on ethylene synthesis in green foliage leaves differs from that in petals. Due to the contradictory effects which cytokinins have on flower senescence, the present study was initiated to investigate the effect of BA on the vase life and post harvest quality of *Alstroemeria* cut flowers.

2.0 MATERIALS AND METHODS

Plant material

Alstroemeria flowers cvs 'Yellow King' and 'Marina' were harvested, just as the primary florets opened on 2nd April and 23rd May 1997 from a 24 month old plants, grown in the open, on a commercial farm in South Kinangop (2,558m) for the first and second experiments, respectively. Shoots between 70 cm and 90 cm long were pulled from the rhizomes in the morning, sorted, graded to 62 cm, packed and received the same day in our laboratory at the Crop Science Department, University of Nairobi.

The flowers were immediately unpacked, the lower 10 cm of the stems were defoliated and 2 cm was cut off under water to avoid air embolism. Eight stems were used for each treatment. The stems of the flowers were then placed in glass jars that contained deionized water, Accel or GA₄₊₇ solutions. The glass jars contained 5 levels of BA and were applied to 2 cultivars of *Alstroemeria* cut flowers, combined in a 5x2 factorial experiments and laid down in a completely randomised design with 3 replicates. The experiments were carried out in a laboratory at room temperature ($23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$), 74-81 % RH and continuous lighting with cool white Sylvania fluorescent lamps (65W, 240V) at an intensity of 4160 J/S.

Chemicals

Accel^R [a liquid concentrate containing 20 g a.i/litre (w/w) 6 -benzyladenine and 2 g a.i/litre (w/w) gibberellins (GA₄₊₇), Abbott, Illinois] was used to prepare 0, 25, 50, 75 and 100 mg/litre BA equivalent solutions. To assess if the gibberellins found in Accel affected BA efficacy, Provide^R [a liquid concentrate containing 21 g a.i/litre (w/w) gibberellins (GA₄₊₇) Abbott, Illinois] at 0, 2.5, 5.0, 7.5 and 10.0 mg/litre was applied alone to *Alstroemeria* cut flowers in amounts equivalent to GA₄₊₇ found when Accel was applied at 0, 25, 50, 75 or 100 mg/litre BA equivalent. Therefore, the effects of BA reported in this paper was obtained by subtracting the values of GA₄₊₇ from the values of Accel (Accel-GA₄₊₇), for all dependent variables determined.

Vase life determination

This was determined by counting the number of days from harvest to full opening of the primary florets, number of days from harvest to 50 % petal fall and 50 % leaf yellowing from daily observations.

Chlorophyll content

This was determined after 7, 14 and 21 days after harvest from 2 discs per leaf (9 mm diameter) cut using a cork borer from 5 leaves per replicate. The 10 discs were extracted in 4 ml of 0.1N HCl in methanol at 21°C in a dark room for 24 hours. Absorbance of extracts was measured using a WPA S105 Spectrophotometer. The leaf chlorophyll content was measured as absorbance of these extracts at 653 nm (Holden, 1965; Douglas, 1983).

The following equation was used to calculate the relative total chlorophyll content (Douglas, 1983).

$$\text{Chlorophyll (mg/cm}^2 \text{ of } Alstroemeria \text{ leaf)} = 24.88 \times A_{653}.$$

Dry weight and moisture content

These were determined 7, 14, and 21 days after harvest. Eight grams of lower fresh leaves were weighed immediately after removal from the shoots, using Sartorius digital balance ELE. The leaf samples were put in brown paper bags and oven dried at 66°C to constant weight (72 hours) using Memmert, UL80 780218 incubator, then re-weighed for dry weight. Moisture content of the leaves was determined by subtracting dry weights from their corresponding fresh weights (8 grams) and transformed into percentage.

Total leaf nitrogen content

This was determined after 7, 14 and 21 days after harvest. The dried leaves used in dry weight determination for each treatment were ground separately, using a Coffee mill (Moulinex, Superior 'S'). Total nitrogen content was analysed using Microkjeldahl method according to the Association of Official Analytical Chemists (A.O.A.C., 1984) methods. Total nitrogen content in the samples was calculated using the equation:

$$\% \text{ N} = \frac{\text{Titre (ml)} \times \text{Normality of acid} \times 14.007 \times 100}{\text{Oven dried weight of sample (0.5 g)} \times 1000}$$

Where: 14.007 is the equivalent weight of nitrogen according to A.O.A.C. (1984).

Statistical analysis

The % N was transformed to mg n/g dry weight of leaf tissue. Analysis of variance was performed on the data using the general linear models (GLM) procedure of Statistical Analysis System (SAS, 1990) program package. Proc univariate procedure was carried out on residuals to support the assumptions of normality made by the researchers. Multiple comparisons among treatment means was done using the Protected Least Significant Difference (LSD) at P=0.05.

3.0 RESULTS

There were no interactions between benzyladenine and *Alstroemeria* cultivars, therefore only the main effects are reported.

3.1 Vase life of *Alstroemeria* cut flowers

(a) Days from harvest to full opening of the primary florets

The lower BA levels, 25 or 50 mg/litre delayed the full opening of the primary florets while 75 or 100 mg/litre BA had no effect, in both experiments (Table 1). In the first experiment, there were no BA differences between 25, 50, or 75 mg/litre with respect to days to full opening of the primary florets. However, in the second experiment, 25 mg/litre BA significantly delayed the opening of the primary florets compared to other BA concentrations and the control.

Table 1: Effect of Benzyladenine on vase life of *Alstroemeria* cut flowers

BA (mg/litre)	First Experiment			Second Experiment		
	Days to full opening of primary florets	Days to 50% petal fall	Days to 50% leaf yellowing	Days to full opening of primary florets	Days to 50% petal fall	Days to 50% leaf yellowing
0 (Control)	4.5bc	14.2a	18.5a	4.2c	14.3ab	14.5ab
25	5.8a	15.2a	18.3a	6.3a	15.2a	15.7a
50	5.3ab	14.0a	18.3a	5.2b	14.2b	13.5b
75	5.3ab	12.3b	15.5b	4.8bc	13.7b	11.0c
100	4.2c	10.3c	13.5c	4.0c	12.5c	9.5c
Significance	**	****	****	****	****	****
LSD	0.86	1.58	1.47	0.88	0.84	1.89

Means separated by the protected LSD ($P=0.05$); means with the same letter(s) within columns are not significantly different.

(b) Days from harvest to 50 % petal fall

In experiment one, 75 and 100 mg/litre BA significantly accelerated the onset of days to 50 % petal fall (Table 1). While in the second experiment only 100 mg/litre BA accelerated the onset of days to 50 % petal fall (Table 1). In both experiments, 25 and 50 mg/litre BA had no effect on days to 50 % petal fall of *Alstroemeria* cut flowers (Table 1). However, in the second experiment, 50 mg/litre BA accelerated the onset of 50 % petal fall compared to 25 mg/litre BA, but it was not different from 75 mg/litre BA.

(c) Days from harvest to 50 % leaf yellowing

Holding *Alstroemeria* cut flowers in 75 or 100 mg/litre BA promoted senescence as measured by days to 50 % leaf yellowing in both experiments (Table 1). In the first experiment, there were significant differences between 75 and 100 mg/litre BA, with 100 mg/litre BA significantly accelerating leaf yellowing. However, 25 or 50 mg/litre BA had no effect on leaf yellowing of *Alstroemeria* cut flowers, in the two experiments (Table 1).

3.2 Leaf chlorophyll content

Treating *Alstroemeria* cut flowers with 25 or 50 mg/litre BA significantly retarded chlorophyll degradation as evidenced by high chlorophyll content in the leaves, but 100 mg/litre BA had no effect, in both experiments (Table 2). BA at 75 mg/litre had significantly high leaf chlorophyll content, 14 days after treatment, in the first experiment. However, it had no effect on the leaf chlorophyll content, 7 and 21 days after treatment (Table 2). In the second experiment, 75 mg/litre BA at 7 and 21 days after harvest delayed the degradation of leaf chlorophyll compared to the control (Table 2).

Table 2: Effect of Benzyladenine on the chlorophyll content of the leaves (mg/cm²) of *Alstroemeria* cut flowers

BA (mg/litre)	First Experiment			Second Experiment		
	Leaf chlorophyll content (mg/cm ²)			Leaf chlorophyll content (mg/cm ²)		
	7 days	14 days	21 days	7 days	14 days	21 days
0 (control)	1.52b	1.34c	0.81b	0.74c	0.55c	0.38d
25	1.92a	1.78ab	1.35a	1.84a	1.65a	1.19a
50	1.97a	2.04a	1.42a	1.50a	1.18b	0.83b
75	1.79ab	1.78ab	1.08ab	1.34ab	0.83c	0.71bc
100	1.50b	1.51bc	0.68b	0.83bc	0.64c	0.49cd
Significance	*	**	**	***	****	****
LSD	0.39	0.35	0.47	0.53	0.31	0.23

Means separated by the protected LSD ($P=0.05$); means with the same letter(s) within columns are not significantly different.

3.3 Leaf dry weight

The cut flowers were randomly selected and their initial (day zero) leaf dry weight were: 8 g for both 'Yellow King' and 'Marina', in the first and second experiments, respectively. Generally, the leaf dry weight decreased, however, holding *Alstroemeria* cut flowers in 25 mg/litre BA had no effect, in respect to the leaf dry weight and it was also not significantly different from 50 mg/litre BA (Table 3). Higher levels of BA (75 or 100 mg/litre) maintained high leaf dry weight, with 100 mg/litre giving the highest values, since the two levels were significantly different in the first experiment (Table 3).

Table 3: Effect of Benzyladenine on the dry weight of the leaves of *Alstroemeria* cut flowers

BA (mg/litre)	First Experiment		Second Experiment		
	Dry weight (g) of the leaves		Dry weight (g) of the leaves		
	7 days	21 days	7 days	14 days	21 days
0 (control)	1.26c	1.31d	1.10c	3.28c	3.87d
25	1.28c	1.34cd	1.39bc	5.09b	6.34c
50	1.43bc	1.47c	1.24bc	4.61b	6.63c
75	1.58b	1.66b	1.52b	4.93b	7.10b
100	1.96a	1.81a	2.22a	5.83a	7.50a
Significance	****	****	****	****	****
LSD	0.25	0.14	0.41	0.68	1.30

Means separated by the protected LSD (P=0.05); means with the same letter(s) within columns are not significantly different.

In the second experiment, except at 7 days after harvest, all the levels of BA maintained high leaf dry weight, with higher concentrations (75 or 100 mg/litre) giving the highest values. Lower BA levels 25 or 50 mg/litre were not significantly different from the control, 7 days after treatment (Table 3). BA at 25, 50 or 75 mg/litre were not different from one another, except 21 days after treatment, when 75 mg/litre was different from the rest (Table 3).

3.4 Moisture content of the leaves

Generally, the BA levels decreased the moisture content of the *Alstroemeria* leaves, in both experiments (Table 4). At 7 days after treatment, lower BA levels 25 or 50 mg/litre were not different from each other and the control, in both experiments (Table 4). The higher BA levels, 75 or 100 mg/litre significantly decreased the moisture content, in both experiments, with 100 mg/litre BA giving the lowest values. Additionally, 75 and 100 mg/litre BA were significantly different (Table 4). In both experiments, at 7 and 14 days after harvest, BA at 50 and 75 mg/litre were not different in respect to lowering the leaf moisture content of *Alstroemeria* cut flowers (Table 4), except at 21 Days after harvest when they were different.

Table 4: Effect of Benzyladenine on moisture content of the leaves of *Alstroemeria* cut flowers

BA (mg/litre)	First Experiment		Second Experiment		
	Moisture content (%) of the leaves		Moisture content (%) of the leaves		
	7 days	21 days	7 days	14 days	21 days
0 (control)	84.38a	83.63a	86.25a	59.00a	51.63a
25	84.13a	83.25ab	82.63ab	36.38b	20.75b
50	82.25ab	81.63b	84.50ab	42.38b	17.13b
75	80.38b	79.25c	81.00b	38.38b	0.88c
100	75.63c	77.38d	72.25c	9.13c	0.13d
Significance	****	****	****	****	****
LSD	0.25	0.14	0.41	0.68	1.30

Means separated by the protected LSD (P=0.05); means with the same letter(s) within columns are not significantly different

Table 5: Effect of Benzyladenine on the total leaf nitrogen content of *Alstroemeria* cut flowers

BA (mg/litre)	First Experiment		Second Experiment		
	Total leaf nitrogen content (mg/g)		Total leaf nitrogen content (mg/g)		
	7 days	21 days	7 days	14 days	21 days
0 (control)	2.93c	2.26b	1.91c	1.70c	1.60c
25	3.49a	2.53ab	2.33a	1.99ab	2.12b
50	3.44a	2.76a	2.44a	2.12ab	2.19a
75	3.38a	2.72a	2.19ab	2.14a	2.16ab
100	3.20b	2.58ab	2.02bc	1.96b	2.14ab
Significance	****	*	**	****	****
LSD	0.15	0.34	0.26	0.17	0.06

Means separated by the protected LSD (P=0.05); means with the same letter(s) within columns are not significantly different

3.5 Total leaf nitrogen content

Treating *Alstroemeria* cut flowers with BA generally retarded the rate of nitrogen degradation as observed in the high retention of nitrogen in the leaves. In the first experiment, 21 days after treatment, 25 and 100 mg/litre BA had no effect on total leaf nitrogen content (Table 5). BA at, 100 mg/litre was not different from the control, 7 days after treatment, in the second experiment. BA at 25, 50 or 75 mg/litre significantly retarded nitrogen degradation on all days, in both experiments (Table 5), except at 21 days after harvest in the first experiment, when 25 mg/litre BA had no effect on total leaf nitrogen content (Table 5).

4.0 DISCUSSION

Benzyladenine extended the vase life of *Alstroemeria* flowers in this study, probably by replenishing the natural cytokinins that are normally supplied to the flower from the roots of parent plant. This indicates that the natural antisenesescence factor in *Alstroemeria* flowers may be endogenous cytokinins (Eisinger, 1977). In the present study, the lower BA levels (25 or 50 mg/litre) were the most effective in delaying the onset of senescence. Heide and Oydvin (1969) observed that too high cytokinin concentration might be detrimental to cut flowers. High concentration of cytokinin has been shown to promote the synthesis of ACC synthase, that lead to increased ethylene production (Yang, 1987). Ethylene has been shown to play an important role, in promoting cut flower senescence (Kader, 1985).

Benzyadenine at 100 mg/litre accelerated the opening of primary florets, though non significantly. Goszczyńska and Nowak (1979) reported similar results in carnations. In the present study, higher BA levels (75 or 100 mg/litre) hastened the onset of flower senescence, as measured by days to 50 % petal fall and 50 % leaf yellowing. Cytokinins have been shown to promote the synthesis of ACC synthase, an enzyme that catalyses the conversion of S-adenosylmethionine (SAM) to 1-aminocyclopropane - 1 - carboxylic acid (ACC) the immediate precursor of ethylene biogenesis (Yang, 1987), hence increased ethylene production. Therefore, high BA levels could have promoted *Alstroemeria* flower senescence indirectly via enhanced ethylene production. Ethylene has been shown to play an important role, in promoting cut flower senescence (Kader, 1985; Halevy and Mayak, 1981).

Benzyladenine at 25 or 50 mg/litre significantly retarded chlorophyll degradation as evidenced by high leaf chlorophyll content. This could be because BA is a synthetic cytokinin and cytokinins have been known to prevent leaf senescence by arresting degradation of protein and chlorophyll (Sacher, 1973). Cytokinins retarded the breakdown of chlorophyll and proteins in excised oat leaves (Thimann, 1987). Therefore, BA delayed the degradation of chlorophyll by probably delaying the rate of protein breakdown used in the synthesis of chlorophyll, rather than enhancing the rate of protein synthesis (Sacher, 1973; Thimann; 1987). This is evidenced by the high retention of nitrogen in the leaves of *Alstroemeria*, throughout the experimental period. Cytokinins have been shown to enhance development of etioplasts into chloroplasts, especially by promoting grana formation (Lew and Tsuji, 1982), thus increasing the rate of chlorophyll formation.

Though there was a decline in the leaf dry weight, BA treatment maintained high leaf dry weight in this study. This may be attributed to cytokinins' ability to promote carbohydrate metabolism and create new source - sink relationship (Mothes and Engelbretcht, 1961; Dyer *et al.*, 1990), thus leading to increased dry matter accumulation in the leaves. The high dry matter in the BA-treated cut stems of *Alstroemeria* could also be attributed to BA possibly reducing respiration rate. Respiration utilises carbohydrates (which are constituents of dry matter) as substrates for the production of carbon intermediates, usable energy (ATP), and reducing power [NAD(P)H] needed for maintenance processes in cut-flowers. Therefore, any treatment that reduces respiration rate in cut-flowers, leads to preservation of food reserves (dry matter) and prolonged postharvest vase-life (Hardenburg *et al.* 1986). Weaver and Johnson (1985) and Clifford *et al.* (1986) reported increased loading and unloading of assimilates across the membrane boundaries of the vascular tissues of plants sprayed with cytokinins, leading to enhanced crop growth and dry matter production.

One of the most important factors determining cut flower longevity, is the ability of the flower to maintain turgidity (Hardenburg *et al.*, 1986). A high level of turgidity is necessary for the development of lower buds to full bloom maturity. BA at 75 or 100 mg/litre, significantly decreased *Alstroemeria* leaf moisture content in this study. The decrease in leaf moisture content caused by BA treatment could be explained by BA inducing stomatal opening and increasing water loss. Arad *et al.* (1973) reported that Kinetin increased water

deficit in barley leaves, by inducing stomatal opening and increased water loss. Mayak and Halevy (1974) reported in cut rose flower shoots that the main effect of Kinetin on water balance was the enhancement of water uptake by enhancing the opening of stomata in the leaves. Such an effect increases transpiration leading to the development of water stress and enhancing wilting (Tal and Imber, 1971).

In our study, BA decreased the rate of nitrogen degradation in the leaves over the 3 weeks study period, compared to the control. Cytokinins are known to delay senescence by retarding the rate of protein breakdown rather than enhancing the rate of protein synthesis (Sacher, 1973). Salunke *et al.* (1962) explained that the primary step in the degradation of the soluble type ribonucleic acid is to invoke the loss of the end adenine. A treatment with BA therefore could provide the necessary adenine and restore the soluble ribonucleic acid molecule. Thus protein breakdown would be retarded and the treated produce can stay fresh for a longer time, as evidenced by high retention of total nitrogen in the leaves of *Alstroemeria* in this study. Mayak and Halevy (1974) and Ballantyne (1966) reported that cytokinins are involved in the regulation of flower senescence by maintaining high RNA and protein levels.

5.0 CONCLUSION

Our results indicate that BA at 25 and 50 mg/litre can be used to extend the vase life and improve the keeping quality of *Alstroemeria* cut flowers by preventing leaf yellowing. However, too high a BA concentration (75 or 100 mg/litre) is detrimental to *Alstroemeria*, since it hastens the onset of flower senescence.

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