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Effect of plant bioregulators on the vase life of snapdragon (*Antirrhinum majus*) cut flowers

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ABSTRACT

The present study on effect of plant bioregulators on the vase life of snapdragon *(Antirrhinum majus* L*)* cut flowers was conducted at Directorate of Floricultural Research, Pusa Campus, New Delhi during 2013-14 to evaluate snapdragon flower spikes as cut flower based on vase life and the effect of plant bioregulators on the vase life of snapdragon flower. Plant bioregulators namely aminooxyacetic acid (AOA) 0.5mM, salicylic acid (SA) 1mM and benzyl adenine (BA) 0.2mM along with 2% sucrose was evaluated. Vase life of snapdragon flower spikes were significantly increased in treatments with plant bio regulators BA+ sucrose, SA + sucrose and AOA + sucrose (9.3, 9 and 8.3 days) in comparison with control (5.6 days) and sucrose (2%) alone (7.3days). Treatments with plant bioregulators delayed the flower opening and flower senescence. Change in fresh weight was less in treatments with plant bioregulators and vase solution uptake rate was also more in the same treatments when compared to Control. Membrane stability index of spikes treated with BA + sucrose was better (64.99%) after 9 days of experiment. Bract chlorophyll content and petal carotenoid content after 9 days of vase life were found better in BA+ sucrose treatment (3.2 mg/g and 0.896 mg/g/respectively). BA performed better than other bio regulators in terms of vase life, colour retention and membrane stability. Sucrose (2%) alone improved the vase life but not significant when compared with solutions containing plant growth regulators along with it.

Key words: Amino-oxy acetic acid, Benzyl adenine, Chlorophyll, Membrane stability index, Salicylic acid, Snapdragon, Sucrose, Vase life

Snapdragon (*Antirrhinum majus* L.) is an herbaceous winter annual plant and it is widely planted as land cover during winter season in India. It belongs to *Scrophulariaceae* family and the genus *Antirrhinum* comprises more than 20 species of attractive colors and mild fragrance. The stem length varies from 3 ft in tall varieties to 15 inches in dwarf bedding varieties (Gilman 2011).

Vase life is an important criterion which determines the potentiality of any flower as cut flower and successive marketability. Earlier studies show that adding chemicals to the vase solution will increase the vase life of cut flowers. Any vase solution should contain an energy source and an antimicrobial component. Recently growth regulators are being used to improve the flower turgidity and to delay the senescence during vase life. Sugar is most commonly used energy source in vase solutions. After harvest the availability of sugar is limited in flowers. Adding sugars mostly in the form of sucrose will delay the senescence and improve the vase life of the flowers. However, Han (2003) reported that sugar improved the intensity of petal color but had no effect on longevity, bud size or bud opening of Oriental lily cv. Stargazer.

Snapdragon is highly sensitive to ethylene and susceptible for gravitropic bending it was reported that ethylene causes flower abscission and gravitropic bending and treating the flowers with ethylene inhibitors delays abscission but it does not make significant change in gravitropic bending (Fisun *et al.* 2010 and Serek *et al*. 1995). Ethylene produced during senescence of flowers further accelerates senescence. This makes petal wilting, permeability of petal cells and degrades the membrane lipids. The senescence effects can be reduced by inhibitors of ethylene biosynthesis (Kazemi *et al.* 2012). AOA has been reported as an inhibitor of ethylene synthesis in flowers during vase life (Zuliana *et al.* 2008, Chaturaphat *et al*. 2003, Sodi and Ferrante 2005). AOA along with sugars improved the vase life of *Dendrobium* flowers but had no significant effect on vase life when used alone. Further, AOA reduced the bud drop and improved the bud opening in *Dendrobium* flowers (Chaturaphat *et al*. 2003). Vase life and membrane stability of the cut spike of gladiolus was increased by using benzyl adenine (Singh *et al*. 2008). Danaee *et al*. (2011) reported that, BA along with ethanol

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and sucrose increase the vase life of gerbera by increasing solution uptake, fresh weight, flower diameter, and anthocyanin content, therefore enhancing flower quality and delaying senescence. Salicylic acid (SA) is a well-known phenol that can extend the vase life of cut flowers by decreasing ethylene (Srivastava and Dwivedi 2000). SA reduced the anthocyanin leakage and increased the chlorophyll content in highly ethylene sensitive carnation flowers (Kazemi *et al*. 2012). Increase in vase life by SA in vase solution is also reported in gladiolus and cut rose flowers (Ezhilmathi *et al*. 2007 and Alaey *et al*. 2011).

Adding new speciality flowers as cut flower will improve the floriculture trade and widen the scope of flower cultivation. However, available studies show that snapdragon is sensitive to ethylene and has shorter vase life, (Ichimura *et al.* 2008). Hence this work was carried out with the objective of evaluating the effect of plant bio-regulators on the post harvest vase life of snapdragon flowers.

MATERIAL AND METHODS

Snapdragon cv. Triumph Scarlet was cultivated in the research field of Directorate of Floricultural Research, IARI, New Delhi. Spikes were harvested when 2-3 flowers were open and the spikes were defoliated and cut to 40 cm length. Individual spikes were held in centrifuge tubes filled with 50 ml distilled water (control) or other vase solutions. All the chemicals, viz. sucrose, salicylic acid (SA), benzyladenine (BA) (Hi Media, Mumbai) and aminooxyacetic acid (AOA) (Sigma, USA) in distilled water were used as vase solution in different combinations [T_0 Control (Distilled water), T_1 : sucrose 2%, T_2 : AOA (0.5mM) + sucrose 2%, T_3 : SA (1mM) + sucrose 2%, T_4 : BA (0.22mM) + sucrose 2%]. The tubes were covered with cotton plug and aluminium foil to avoid evaporative losses and kept in a room with natural light (12 hr with 1600 lux light intensity). The average room temperature and humidity during the study was recorded as 25±2ºC and 80±5%, respectively. Each experiment was replicated four times with 3 spikes per replication.

Weight of each spick was measured at 2 days interval for 10 consequent days. The relative fresh weight of spikes was calculated as:

$$\text{Relative fresh weight} = \frac{\text{weight of flower spike at day t}}{\text{initial flower spike weight}}$$

where t = 0,2,4,6,8,10 (He *et al*. 2006)

Difference in the stem/spike height was recorded at 2 days interval. Change in volume of solution and weight of tubes without spikes was recorded at 2 days interval. Following formulae were used to find the water relations and per cent flower opening:

$$\text{VSUR (ml/g Initial fresh weight (IFW)/day)} = \frac{(St-2)-(St)}{(IFW\times 2)}$$

where t = 0,2,4,6,8,10 [He *et al*. 2006]

$$\text{Flower opening(\%)} = \frac{\text{No. of flowers at day t}}{\text{Maximum No. of flowers}} \times 100$$

Membrane stability index of petals was analysed at 3 days interval. It was calculated on the basis of the electrolyte leakage of petals. The electrolyte was measured by taking 1 g of petals. The petal discs were rinsed well in deionised water prior to incubation in 10 ml of deionised water for 3 hr at room temperature. After incubation, the conductivity (C_1) of the bathing solution was measured with the conductivity meter. Petals discs were boiled with bathing solution for 15 min to kill tissue. After cooling to room temperature, the conductivity (C_2) of the bathing solution was again measured. The membrane stability index was expressed as per cent value according to the formula given below

$$\text{Membrane Stability Index (\%)} = \left[1-\left(\frac{C_1}{C_2}\right)\right]\times 100$$

(Danaee *et al*. 2011)

Chlorophyll from the bracts and petals were estimated by DMSO (Dimethtyl Sulphoxide) method (Hiscox and Israelstam 1979). Chlorophyll solution was prepared by incubating the 50 mg sample tissues in 10 ml DMSO at 65ºC for 4 hr. After 4 hr the absorbance of the chlorophyll solution is read at 663 and 645 nm using DMSO as blank in UV-Vis spectrophotometer. Chlorophyll content (Chl a, Chl b and total chlorophyll) was calculated using the following formulae:

$$\text{Chlorophyll a (mg/g fw)} = \frac{[12.7(0\,D_{663})-2.69(0\,D_{645})]\times \text{Volume}\times \text{dilution}}{100\times \text{wt. of sample}}$$

$$\text{Chlorophyll a (mg/g fw)} = \frac{[22.9(0\,D_{645})-2.69(0\,D_{663})]\times \text{Volume}\times \text{dilution}}{100\times \text{wt. of sample}}$$

$$\text{Total chlorophyll (mg/g fw)} = \frac{[20.2(0\,D_{645})-8.02(0\,D_{663})]\times \text{Volume}\times \text{dilution}}{100\times \text{wt. of sample}}$$

$$\text{Total carotenoid} = \frac{[1000(0\,D470)-(3.27\,\text{Chl a}+104\,\text{Chl b})]/229\times \text{Volume}\times \text{dilution}}{1000\times \text{wt. of sample}}$$

(Lichtenthaler and Wellburn 1983)

Vase life of the snapdragon spikes was characterised based on petal wilting, flower drop, bud drop, stem bending and stem rotting. The average vase life of spikes was assessed as terminated when 50% of the flowers in a spike were senesced/dropped. Experiment was carried out in completely randomized block design (CRBD) with 4 treatments and 4 replications. Data were analyzed by Generalized Linear Model (GLM) procedure and means were compared using Duncan's multiple range tests at P=0.05 in SAS version 9.3.

RESULTS AND DISCUSSION

Flower weight

Flower weight was increasing gradually in all treatments up to 6 days except control. There was a sudden decrease in flower weight in control after 2 days. Maximum relative flower weight was achieved in flowers in T_4 (BA+Sucrose) (160.62%) on day 6. Increase in relative fresh weight in sucrose, T_2 and T_3 solution was on par on 6th day (144.30%, 142.53% and 141.52% respectively). T_3 showed a continuous increase in weight up to 8 days (Fig 1a). Plant bioregulators increased the relative fresh weight of flower spikes due to delayed senescence, increasing the solution uptake and reduced tissue transpiration and respiration (Keramat *et al.* 2012). This may also be due to the negative correlation between plant bioregulators and microbial population as reported by Kazemi *et al.* (2012) in carnation flowers.

Water relation

Maximum vase solution uptake was observed in both T_1 and T_2 on day 4 (8.33 ml/spike/day) followed by T_4 (7.17 ml/spike/day) on day 6. All treatments showed a decreasing trend in volume of vase solution uptake after 4 days except T_4. Maximum vase solution uptake rate was observed in sucrose treatment on day 4 followed by control (0.70 ml/g IFW/day) on day 2 and T_4 (0.61 ml/g IFW/day) on day 6. The data on vase solution uptake rate (Table 1) clearly shows that all the treatments with plant regulators were steadily increasing up to 4 days and then it reduced.

Percentage open flower and stem elongation

Maximum number of open flowers (16.33) was recorded in T_3 on day 8 followed by T_4 (14.33) on day 10 and T_2 (14.33) on day 6 (Table 1). In case of control after 4 days 100% flowering was observed whereas in treatments with plant bioregulators flowering continued up to 8 days. At the end of 10 days T_4 has 98.15% open flowers when compared to 23.48% of control and 60.48% of T_1. It was reported that sucrose increases the number of fully open flowers in cut spray carnation (Shigeru *et al*. 2005). From the data (Table 1) it is evident that flower opening percentage was gradually increasing in T_4 and it reached the maximum flower opening on day 10. BA is known to delay the flower opening as cytokinin reported for its negative effect on flower senescence (Mutui *et al.* 2001). The same effect was reported in roses (Sakine *et al*. 2011). Maximum stem elongation of 24.17cm was achieved in T_4 followed by T_3 (22.50cm) and T_2 (20.83 cm). Stem elongation of samples with T_1 was reached the maximum of 11.67cm on day 6 and then it started reducing.

Membrane stability index (MSI)

Initial Membrane stability index of the fresh snapdragon flower petal was recorded as 70.84%. Membrane stability of petals in control was reduced drastically during vase life and after 9 days it was recorded

Table 1 Effect of different treatments on vase solution uptake rate, flower opening and stem elongation of snapdragon flower spikes during vase life

Treatment	Vase solution uptake rate (ml/g IFW/day/spike)					Flower opening (%)					Stem elongation (%)				
	0 days	2 days	4 days	6 days	8 days	10 days	0 days	2 days	4 days	6 days	8 days	10 days	0 days	2 days	4 days	6 days	8 days	10 days
T_0 (Control)	0a	0.71a	0.51b	0.38d	0.19c	0.047e	29.29a	82.32a	100.0a	85.35b	47.22c	23.48d	0a	5.83c	10.83c	10.83b	10.05b	6.66c
T_1 (Sucrose 2%)	0a	0.67a	0.88a	0.48c	0.23bc	0.11d	18.57b	60.63b	93.17a	100.0a	86.03b	60.47c	0a	7.91bc	10.83c	11.66b	10.83b	8.33c
T_2 (AOA 0.5mM + sucrose 2%)	0a	0.48b	0.58b	0.36d	0.29b	0.195	15.72b	43.12c	76.53b	94.22ab	100.0a	80.46b	0a	11.25a	17.08a	18.33a	20.83a	20.83b
T_3 (SA (1mM) + sucrose 2%)	0a	0.54b	0.59b	0.55b	0.41a	0.24b	14.15b	45.88c	73.92b	96.76a	100.0a	94.82a	0a	11.66a	15.83ab	19.00a	22.083a	22.51ab
T_4 (BA (0.2mM) + sucrose 2%)	0a	0.46b	0.58b	0.61a	0.45a	0.29a	16.88b	56.64b	79.30b	92.48ab	96.29a	98.14a	0a	9.58ab	13.75bc	17.51a	20.42a	24.16a

Means with the same letter are not significantly different at P = 0.05.

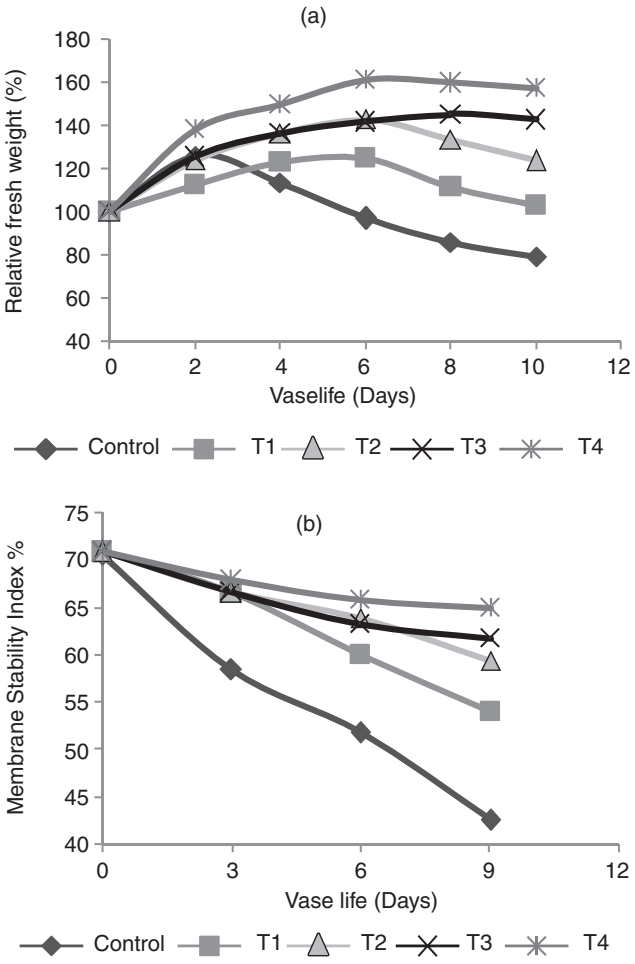

Fig 1 Effect of treatments on vase life of snapdragon flowers with respect to (a) relative fresh weight and (b) Membrane stability index.

as 42.56% (Fig 1b). Reduction of MSI in T_1, T_2 and T_3 treatments was similar on day 3 but after that it was reduced to 53.97% in T_1. Maximum membrane stability index after 9 days was recorded in T_4 (64.99%) followed by T_3 (61.72%) and T_2 (59.37%). Membrane stability was more in T_4 when compared to other treatments till the completion of 9 days. From our study it is evident that membrane stability and vase life are positively correlated. It was observed that the degradation of membrane was slower in T2, T3 and T4 when compared to T1 and control. It is also noted that the water balance and vase solution uptake was more in these treatments and these parameters are associated with the stomatal conductance and membrane integrity. Membrane stability expressed as petal leakage of electrolytes of petal discs (Memon *et al.* 2012). It was reported by Bartoli *et al.* (1996), in carnation that petal electrolytic leakage by membrane disruption is due to ethylene and by controlling ethylene production membrane stability of the petals can be improved. It was also reported by same that adding PGR like AOA reduces the electrolytic leakage in carnation flowers. It was reported that BA improves the membrane stability index in gerbera flowers (Danaee *et al*. 2011). Adding exogenous SA to vase solution to decrease the

Table 2 Effect of different treatments on Membrane stability index, bract chlorophyll content, petal carotenoid content and vase life of snapdragon flower spikes

Treatment	Bract chlorophyll a/b ratio				Bract total chlorophyll (mg/g)				Petal total carotenoid (mg/g)				Vase life (Days)
	0 day	3 day	6 day	9 day	0 day	3 day	6 day	9 day	0 day	3 day	6 day	9 day	
T_0 (Control)	1.57a	1.29b	1.13b	1.08b	3.756a	2.804c	2.061c	1.863c	1.023a	0.84b	0.639c	0.541d	5.66d
T_1 (Sucrose 2%)	1.57a	1.45ab	1.39a	1.27ab	3.756a	2.895bc	2.561b	2.390b	1.023a	0.948a	0.860b	0.713c	7.33c
T_2 (AOA 0.5mM + sucrose 2%)	1.57a	1.67a	1.62a	1.49a	3.756a	2.697c	2.581b	2.362bc	1.023a	0.977a	0.913ab	0.889a	8.33b
T_3 (SA + sucrose 2%)	1.57a	1.60a	1.62a	1.58a	3.756a	3.177b	2.856b	2.738ab	1.023a	0.979a	0.917ab	0.803b	9.00ab
T_4 (BA + sucrose 2%)	1.57a	1.61a	1.64a	1.6a	3.756a	3.809a	3.517a	3.201a	1.023a	1.017a	0.941a	0.896a	9.33a

Means with the same letter are not significantly different at P= 0.05.

permeability of the plasma membrane and membrane lipid per oxidation and to maintain the membrane integrity was reported by (Kazemi and Shokri 2011).

Total chlorophyll (Bracts) and total carotenoid (Petals) content

Initial total chlorophyll in bracts of fresh snapdragon flower was estimated as 3.756 mg/g. Total chlorophyll content in bract was showing reducing trend in all treatments (Table 2). Minimum total chlorophyll content was recorded in control (1.86 mg/g) and maximum of (3.20 mg/g) was recorded in T_4 after 9 days of experiment. Chl a/b ratio increase in T_4. This may be due to decrease in Chl b as Chl b degrades faster than Chl a and conversion of Chl b into Chl a during vase life. It was reported by Mutui *et al*. (2001) and Sakine *et al. (*2011)*,* that cytokinins prevent the leaf senescence by arresting degradation of protein and chlorophyll in alestromeria and gladiolus, respectively. It is also reported that cytokinins activate the NADH protochlorophyllide reductase, an enzyme involved in chlorophyll biosysnthesis and reduces chlorophyll loss (Asil and Karimi 2010).

Similar trend was found in T_3 and T_4 with respect to petal total carotenoid content during vaselife. T_2 was showing a decreasing trend and minimum caretonoid content in petal was found in control (0.54 mg/g) after 9 days. Caroteniods are one of the major components contributing flower color. Ethylene synthesis during senescence leads to fading of petal color. It was reported that AOA and BA reduces the petal discoloration in potted carnation flowers (Karimi *et al.* 2012). SA pre-treatment reduces the rate of chlorophyll and caroteniod degradation in *Nigell sativa* plant (Kabiri *et al.* 2014). It was reported by (Moharekar *et al*. 2003) that SA in higher concentration reduces the chlorophyll content in plant leaves and in contrast it increases the caroteniod content. An increase in oxidative stress may induce reduction in total chlorophyll content or reduction in chlorophyll content might have caused the oxidative stress with an increase in SA concentration. Fard *et al*. (2013) reported better chlorophyll content in SA treatment in alesteromeria flower when compared to the control.

Vase life

There was a significant difference in the vase life of snapdragon flowers with respect to treatments (Table 2). Maximum vase life of 9.33 days was recorded in T_4 treatment followed by 9 days in T_3. Minimum of 5.6 days vase life was recorded in control. T_1 increased the vase life of flowers to 7.33 days. Sucrose solution without biocide or lower pH will promote the microbial growth and subsequent stem plugging (Asrar 2012). This could be the reason behind lower vase life of flowers in T_1 despite better performance of flower in the initial stage of vase life.

Chaturaphat *et al*. (2003) reported that using AOA alone did not improve the vase life of *Dendrobium* flowers AOA along with sucrose delayed the longevity of flowers. Further increase in vase life and other parameters was found better in AOA+ sucrose solution than in treatment with sucrose alone. This clearly indicated the ethylene sensitivity of flower and the effect of AOA on senescence (Chaturaphat *et al.* 2003 and Zuliana *et al.* 2008). The results of the present study are in line with the above findings. It was reported by Danaee *et al*. (2013) in gerbera flowers that SA also delays the senescence process in flowers due to its ethylene inhibition property. SA suppresses the conversion of ACC into ethylene by inhibiting ACC oxidanse activity and it is involved in local and systemic resistance to pathogens (Danaee *et al.* 2003). A significant increase in vase life of gladiolus cut flowers variety White Prosperity by adding BA in the vase solution was reported by Sakine *et al*. (2011).

In conclusion, snapdragon can be used as a potential cut flower with the minimum vase life of H" 6 days without any preservatives in the vase solution. Snapdragon is an ethylene sensitive cut flower and vase life of the flower can be improved by adding plant bio regulators in the vase solution.

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