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Effect of polyamine and ethylene inhibitors on post harvest physiology of cut stems in chrysanthemum cv. Reagan White

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ABSTRACT

Experiments were carried out to investigate the effect of different concentration of polyamine (putrescine) and ethylene inhibitors (AOA and 1-MCP) on post-harvest physiology of chrysanthemum cv. Reagan White. The results obtained showed that the most effective treatments in prolonging the vase-life were AOA (5 and 10 mg/l). Both treatments showed vase-life upto 25.67 and 21.33 days, about double than that of control and decreased floret and leaf senescence but also improve the water uptake and membrane stability index. Percentages of leaf senescence were earlier in all treatments as compared to floret senescence. However, both the concentrations of AOA effectively retarded the yellowing of leaf up to 18 days. Percentage decrease in fresh weight was also minimum in AOA treated flower stem as compared to other treatments. There was less reduction in total soluble solids and reducing sugar in AOA treated flower stem than control. Amino-oxyacetic acid and low concentration of 1-MCP lowered the ethylene production, but later was not effective in prolonging the vase-life.

Key words: Amino-oxyacetic acid, chrysanthemum, senescence, vase-life.

INTRODUCTION

Cut flowers are highly perishable as respire very actively. Postharvest physiology of flowers is jointly dependent on its sugar reservoirs, chlorophyll contents, antioxidant enzymes and amount of ethylene evolved. Prolonging of vase-life of cut flowers is one of the important desires of consumer as well as farmer for preference of a genotype. Senescence of flower petals is a complex process involving an increase of cell membrane permeability that results in wilting, pigment degradation and petal collapse (Jones and McConchie, 8). The biochemical changes associated with petal senescence include increase in hydrolytic enzymes, degradation of macro-molecules and an increase in respiratory activity (Ezhilmathi *et al*., 3). The point of termination of vase-life starts from the first sign of wilting to the complete death of flowers, involves physiological changes ongoing in cells of petal. But this termination of vase-life is largely dependent on pre-harvest, harvest and post-harvest factors. The use of polyamines and ethylene inhibitor delay termination of vase-life of flowers and has been demonstrated in different flowers (Razali *et al*.,11; Chandran *et al*., 2; Jámbor-Benczúr *et al*., 7; Mahgoub *et al*., 9). Hence, an experiment was conducted on chrysanthemum by addition of polyamine and two ethylene inhibitors on vase-life and physiological and biochemical changes in holding solutions.

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MATERIALS AND METHODS

Experiments were carried out in the Research Farm of the Directorate of Floricultural Research, IARI, New Delhi, during 2013-2014. Chrysanthemum cv. Reagan White flowers of similar maturity were harvested with stem length of 50 cm for the study. The treatments used for experiment were T_1 = Putrescine (25 mg/l), T_2 = Putrescine (50 mg/l), T_3 = Amino-oxyaceticacid (AOA) (5 mg/l), T_4 = amino-oxyaceticacid (10 mg/l), T_5 = 1-methylcyclo propene (1-MCP) (50 ml/l), T_6 = 1-MCP (100 ml/l), T_7 = 1-MCP (200 ml/l). Double-distilled water was used as control (T_0). Single flowers stems were transferred in the treatment solutions separately in (100 ml) graduated test-tube and plugged the open mouth with non-absorbent cotton plug. The day of transferring flower stem in vase solution was considered as zero day. Five stems in three replications for eight different treatments were conducted. The experiment was conducted at ambient conditions, *i.e.* temp 16°-18°C and relative humidity of 65%, PPF 16/8 h of 350-400 µmol $m^{-2}s^{-1}$. A separate set of treatment combinations were kept for destructive sample analysis. The day of transfer of flower stems to the vase solution was considered as zero day.

Observations were collected at 6-day interval on 6, 12, 18, and 24 days after treatment. Flower stem weight was calculated on percentage basis by taking initial minus fresh weight. The water uptake by cut stems was recorded. Membrane stability

index was estimated according to Bailey *et al.* (1). The chlorophyll content of leaves were measured by DMSO method (Hiscox and Israelstam, 5), tissue total soluble solids (TSS) and reducing sugar of floret and leaf tissues were estimated by Nelson's arsenomolybdate method. Ethylene evolution from ray florets was measured by incubating three flowers for 6 h in airtight container provided with a sub-seal septum for gas sampling. Aliquots of 1 ml of head space were injected in gas chromatograph. The termination of vase-life was determined as that time pollens burst from outer part of disc florets or flower showing symptoms of floret wilting. The experiment was conducted in factorial randomized block design having eight treatments including control with single flower stem as one unit and three replications using statistical analysis system software (SAS version 2).

RESULTS AND DISCUSSION

Flowers were fresh at harvest, thereafter they start wilting as day proceeds. Ray florets are aesthetic part of flower. Hence, turgidity of floret is utmost for keeping quality that depends on relative water content and membrane stability index. The different vase solutions important had significantly effect on MSI of ray florets and leaves. Among the treatment T_3 recorded 59.63 followed non-significantly by 57.42 (T_4), but significantly differed from other treatments and control (Table 1). Among the different sampling durations in vase, maximum MSI was observed at 6 days (67.95), which declined thereafter. In case of leaf MSI, T_2 recorded the maximum MSI with 63.66 followed significantly by T_5 (60.54) and T_3 (59.89). Among the duration in vase, maximum MSI of leaf was at par with ray florets. The senescence process is accompanied by a dramatic increase in the ion leakage. Earlier, Mayak and Halevy (4) showed that the ion leakage rate in ethylene induced senescence of carnation was usually 2-5 times higher than in non-senescing petals.

There was a significant reduction in physiological fresh weight of flower stems in vase solutions. This reduction in weight could be due to decreased water uptake or increase in transpiration loss due to high metabolism in the tissue. Similar findings on chrysanthemum have also been reported earlier (Mansouri, 10). Our results showed that there was less decrease in fresh weight in T_3 (24.31%) followed non-significantly by T_2 (25.49%), though they differed significantly with other treatments. Maximum reduction in stem fresh weight was 54.94% in control (T_0). Amongst the duration intervals in vase, the maximum fresh weight was recorded at zero day, which thereafter decreased continuously in all the treatments (Table 2). The extended vase-life in AOA treated stem is accompanied by less reduction in loss of fresh weight, improved membrane stability of flowers and leaves, stabilized leaf chlorophyll contents and less reduction in reducing sugar and total soluble solids.

The findings showed that there was continuous decline of water uptake in all treatments, but maximum uptake was observed during initial days, which was non-significantly different with other treatments.

Table 1. Effect of different vase solutions on membrane stability index of stem and leaf tissue of chrysanthemum stem.

Treatment	Ray floret					Ray floret leaf				
	6 D	12 D	18 D	24 D	Mean	6 D	12 D	18 D	24 D	Mean
T_0	53.67	34.98	19.69	10.35	29.67	66.25	40.49	18.00	11.46	34.05
T_1	77.24	64.33	45.72	38.18	56.36	77.21	57.42	37.27	30.08	50.49
T_2	66.99	58.08	40.61	36.93	50.65	82.31	67.83	57.74	46.75	63.66
T_3	76.08	65.06	51.72	45.67	59.63	80.45	66.36	51.25	41.53	59.89
T_4	67.06	64.79	51.61	46.25	57.42	78.49	61.86	51.2	41.18	58.18
T_5	65.37	61.35	34.87	29.03	47.65	78.23	70.82	55.82	37.30	60.54
T_6	66.63	52.34	30.78	23.61	43.34	75.38	60.11	45.65	39.08	55.05
T_7	70.62	47.08	32.57	24.52	43.69	77.33	57.51	42.77	32.47	52.52
Mean	67.95	56.00	38.44	31.82		76.95	60.3	44.96	34.98	
CD at 5%										
Treat. (T)					6.65					6.84
Dur.					3.87					1.18
T × Dur.					10.30					10.90

D= Days after treatment

Ray floret MSI at 0 day = 84.28; leaf MSI at 0 day = 85.62

Table 2. Effect of different vase solutions on stem fresh weight and water uptake in chrysanthemum cv. Reagen White.

Treatment	Stem FW (g)					Water uptake (ml)				
	6 D	12 D	18 D	24 D	Mean	6 D	12 D	18 D	24 D	Mean
T_0	23.68	16.81	14.04	10.67	16.3	27.38	16.14	9.01	4.58	14.28
T_1	25.50	21.94	18.20	16.14	20.44	27.36	21.8	13.21	7.4	17.44
T_2	22.01	19.51	17.42	15.51	18.61	27.6	22.11	14.76	9.03	18.37
T_3	25.66	24.55	21.7	19.42	22.83	28.61	24.33	17.28	10.77	20.25
T_4	24.32	22.18	19.26	16.75	20.63	26.22	21.74	14.09	8.04	17.52
T_5	25.05	22.16	17.42	16.16	20.19	27.15	21.56	11.84	7.67	17.05
T_6	25.5	23.90	20.01	16.84	21.56	26.12	20.49	10.06	6.67	15.84
T_7	21.7	19.00	16.62	15.47	18.19	28.44	23.49	10.44	6.15	17.13
Mean	24.18	21.26	18.08	15.87		27.36	21.46	12.58	7.54	
CD at 5%										
Treat. (T)					3.20					3.07
Dur.					2.94					2.08
T × Dur.					4.86					6.16

D= Days after treatment; FW at 0 day = 26.57 g

There was significant difference in water uptake in all treatments at 6, 12 and 24 days (Table 2).

The leaf total soluble solids (TSS) were maximum in leaf tissue in T_2, which was significantly different from T_6, T_7 and control but was non-significantly different with other treatments. While reducing sugars in leaf was maximum in T_3 that was highly significantly compared to control, but was non-significantly different with remaining treatments (Table 3). It was evident that the TSS and reducing sugar contents in leaf declined with increment of duration. In ray florets, the maximum TSS was estimated in T_3 (51.42 mg/g FW) followed by T_4 (50.17 mg/g FW), which was significantly different with other treatments. Among the sampling interval in vase, the maximum TSS was observed at 6 day (58.77 mg/g FW) that was significantly higher compared to 12, 18 and 24 days (Table 4). Sugars has a role as source of energy and

Table 3. Effect of different vase solutions on leaf total soluble solids and reducing sugar contents in chrysanthemum.

Treatment	TSS mg/g FW					RS mg/g FW				
	6 D	12 D	18 D	24 D	Mean	6 D	12 D	18 D	24 D	Mean
T_0	45.31	36.40	28.09	19.10	32.23	22.86	14.62	9.3	8.61	13.85
T_1	45.19	37.88	33.86	26.69	35.91	26.85	22.06	17.97	16.27	20.78
T_2	47.04	41.18	36.67	33.65	39.63	26.76	22.37	19.43	16.31	21.22
T_3	45.25	37.85	33.27	31.16	36.88	26.79	24.64	22.26	17.82	22.87
T_4	44.44	39.14	36.08	27.84	36.87	24.97	22.07	19.23	16.96	20.81
T_5	44.75	37.81	33.87	27.91	36.08	24.71	21.48	18.56	16.39	20.28
T_6	44.6	37.25	31.34	25.89	34.77	26.54	21.83	16.35	14.78	19.87
T_7	44.03	36.89	30.7	26.31	34.48	25.78	20.62	18.46	15.81	20.17
Mean	45.08	38.05	32.98	27.32		25.65	21.21	17.69	15.37	
CD at 5%										
Treat. (T)					3.42					3.77
Dur.					4.57					3.36
T × Dur.					6.64					5.37

D= Days after treatment

TSS at 0 day = 48.38 mg/g FW; RS at 0 day = 26.65 mg/g FW

Table 4. Effect of different vase solutions on ray floret total soluble solids and reducing sugar contents in chrysanthemum stems.

Treatment	TSS (%)					Reducing sugars (%)				
	6 D	12 D	18 D	24 D	Mean	6 D	12 D	18 D	24 D	Mean
T_0	57.4	45.84	28.35	21.17	38.19	27.97	16.12	9.56	7.35	15.25
T_1	60.45	51.58	41.98	38.01	48.01	29.72	22.29	14.12	10.64	19.19
T_2	59.37	48.75	42.63	39.00	47.44	30.09	24.59	17.76	12.44	21.22
T_3	58.87	54.19	48.02	44.61	51.42	31.92	28.84	22.10	15.28	24.54
T_4	61.12	52.56	46.23	40.79	50.17	30.44	27.08	24.18	15.8	24.38
T_5	58.18	50.48	45.43	38.21	48.07	28.72	24.92	20.80	15.18	22.41
T_6	58.00	51.28	42.84	42.72	48.71	29.45	27.17	18.97	13.92	22.37
T_7	56.75	49.19	42.72	36.03	46.17	32.53	24.36	18.46	13.32	22.16
Mean	58.77	50.48	42.27	37.57		30.105	24.42	18.24	12.99	
CD at 5%										
Treat. (T)					4.57					3.83
Dur.					0.98					1.14
T × Dur.					7.94					6.30

D= Days after treatment
TSS at 0 day = 63.14 mg/g FW; RS at 0 day = 26.65 mg/g FW

also regulate gene expression. It has been reported that there is complex interaction between ethylene signaling mechanisms that are tissue dependent. This indicates sugar may be early indicators of senescence (Zhuo *et al*., 12). Lower reduction in reducing sugar may be due to lower metabolism in flower stem, accompanied by least respiration in vase.

Our results showed that leaf is senescent more advance than ray floret of chrysanthemum. The maximum leaf senescence was retarded by T_3 and T_4 upto 18 days, which was highly significant with other treatments, even up to 24 day only 44% leaves are yellow. While, in T_3 and T_4 treatments recorded the minimum retardation in senescence of ray florets (Table 5).

Leaf total chlorophyll content showed less reduction during the vase-life (Fig. 1a). This indicates that polyamines and ethylene inhibitors prevent the degradation of chlorophyll pigment, thereby

Table 5. Effect of different vase solutions on ray floret and leaf senescence after different days in chrysanthemum stems.

Treatment	Floret senescence (%)					Leaf senescence (%)				
	6 D	12 D	18 D	24 D	Mean	6 D	12 D	18 D	24 D	Mean
T_0	10.47	57.37	0.00	0.00	16.96	17.22	98	0.00	0.00	28.81
T_1	0.00	16.52	34.67	62.41	28.4	8.74	32	89.66	0.00	32.6
T_2	0.00	23.06	42.92	74.08	35.02	4.40	20.13	72.66	0.00	24.29
T_3	0.00	0.00	17.19	43.30	15.12	0.00	0.00	1.95	43.66	11.40
T_4	0.00	2.69	25.50	46.56	18.68	0.00	0.00	7.53	50.00	14.38
T_5	0.00	7.04	28.75	73.21	27.25	0.00	1.53	24.66	69.00	23.79
T_6	0.00	14.82	44.15	86.19	36.29	0.00	10.00	36.66	82.66	32.33
T_7	0.00	17.51	54.07	94.52	41.52	0.00	16.00	49.00	87.66	38.16
Mean	1.31	17.37	30.91	60.034		3.79	22.21	35.26	41.62	
Treat. (T)					5.78					6.50
Dur.					3.98					3.16
T × Dur.					12.22					11.77

D = Days after treatment

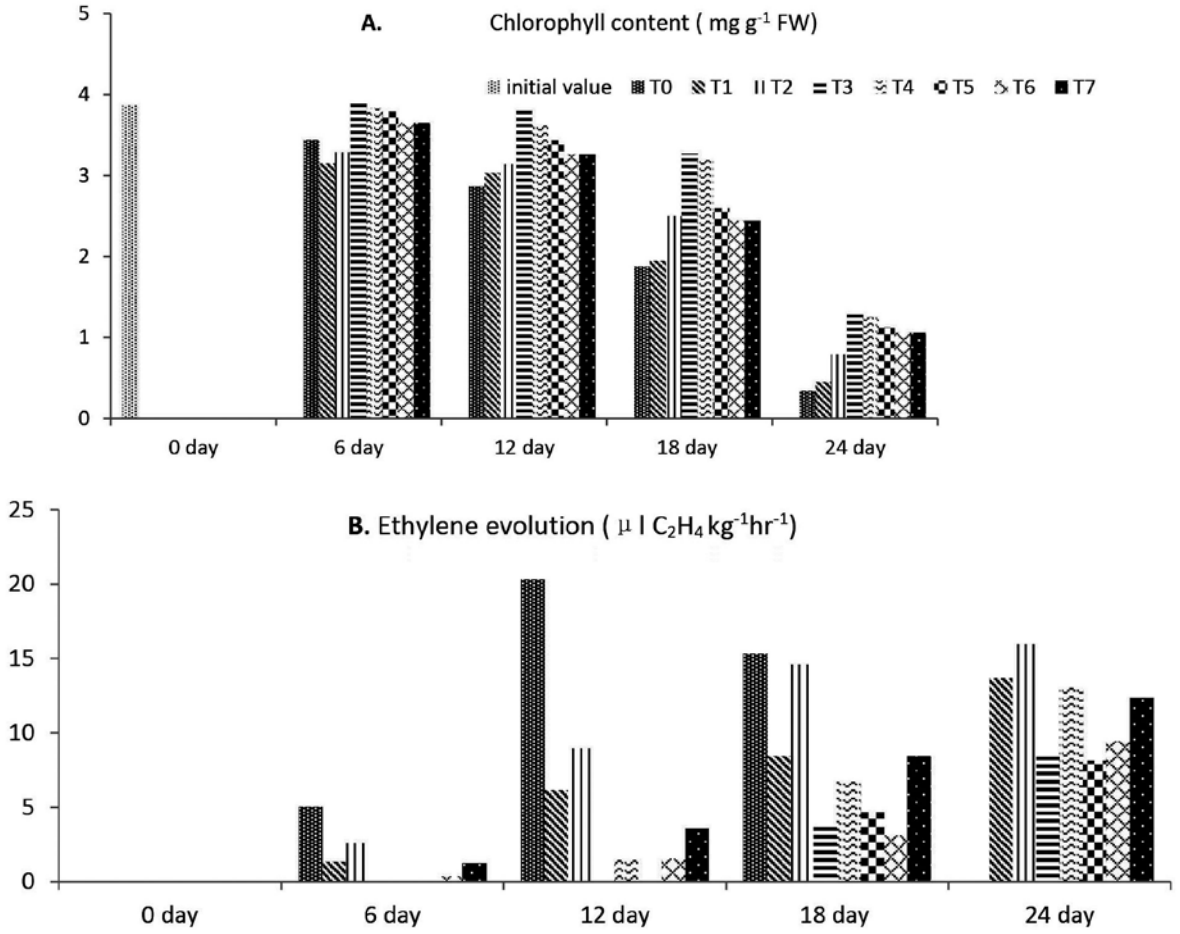

Fig. 1. (a) Total leaf chlorophyll content and (b) ethylene evolution from ray florets in chrysanthemum cut stems held in different vase solutions.

preventing leaf yellowing. Our results showed that the chlorophyll content at zero day (3.87 mg/ g FW), was maximum. Treatment T_3 was the best and was non-significantly followed by T_4 and T_5 but significantly differed from other treatments. Among the different intervals in vase, the maximum chlorophyll content was observed in T_3 at 6, 12, 18 and 24 days. The percentage decrease in chlorophyll content was minimum in T_3 (1.29%) followed by T_4 (6.21%) as compared to control (25.84%) at 12 days. The data presented in Fig. 1b revealed that there was less ethylene evolution in treated stem as compared to control. There was remarkably higher production of ethylene in T_0 (5.02 µl C_2H_4 $kg^{-1}hr^{-1}$) at 6 days, while it was not detectable in T_3, T_4 and T_5 treatments. However, ethylene evolution was minimum upto 24 day in T_3, T_4, T_5 and T_6 treatments.

Vase-life of chrysanthemum flower stems treated with polyamine and ethylene inhibitors was enhanced significantly compared control. The stem treated with amino-oxyacetic acid (T_3, T_4) had more than twice (25.67 and 21.33 days, respectively) longer life as compared to control (9.33). The minimum vase-life was recorded in T_1 (13.67 days), T_2 (12 days) and T_7 (13 days) compared to control (Fig. 2). The pH of T3 and T4 was lower throughout the experiment as compared to other treatments, may be the factor for prolonging the vase-life of flower stems. The AOA 50 ppm was the effective treatment for increasing the vase-life and delay in senescence in bougainvillea (Hossain, 6). From the study it was concluded that supplementation of amino-oxyacetic acid (5 and 10 mg/l) in vase solution was most effective in prolonging the vase-life of chrysanthemum cut stem of cv. Reagan White.

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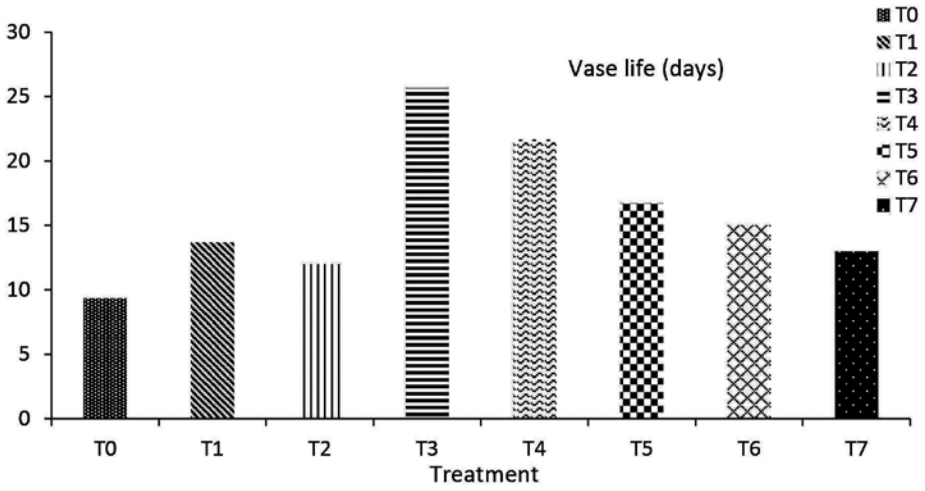

Fig. 2. Vase-life of cut stems of chrysanthemum cv. Reagan White as influenced by different vase solutions under ambient conditions.

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