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Effect of cycloheximide on postharvest performance of cut spikes of *Consolida ajacis* cv. Violet Blue

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Abstract

A study was conducted to examine the effects of pulse treatment with different concentrations of cycloheximide (CHI) on postharvest performance of cut spikes of *Consolida ajacis* cv. Violet Blue in distilled water and sucrose 0.2 M+HQS 100 mg/L. The present investigation revealed that at a particular threshold concentration (0.01 mM), it delays senescence and above that it prevents flower opening and promotes senescence. Cycloheximide at 0.01 mM concentration enhanced vase life, besides maintaining higher fresh and dry mass of flowers and soluble protein content in the sepal tissue. The fact that cycloheximide delays petal/sepal senescence demonstrates that the synthesis of particular suicide proteins, probably enzymes responsible for degradation of cellular constituents, orchestrates the cell death programme. Postharvest performance of spikes was much better in spikes pretreated with 0.01 mM CHI and transferred to sucrose+HQS and this can be used as an effective treatment to improve postharvest longevity in this flower system.

Key words: *Consolida ajacis*, vase life, postharvest performance, soluble proteins, sucrose, senescence.

Introduction

Flower senescence has been shown to be a genetically programmed event which is under tight developmental control and involves degradation of proteins, lipids, carbohydrates and nucleic acids (Rubinstein, 2000; Eason *et al.,* 2002; Wagstaff *et al.,* 2002; van Doorn, 2004; Hoeberichts *et al.,* 2005; Zhou *et al.,* 2005; Eason, 2006; Price *et al.,* 2008; Shibuya *et al.,* 2008; van Doorn and Woltering, 2008). Keeping in view the diversity of floral behaviour it is realized that detailed studies are required to fully understand the process in different genera, species and perhaps even cultivars (Reid, 2005). The interaction between proteases and their inhibitor proteins have been linked to modulation of cell death processes in plants and in certain cut flowers, chemical inhibition of protease action delays the onset of senescence (Eason *et al.,* 2002; Sin and Chy, 2004; Pak and van Doorn, 2005).

Cycloheximide (a protein synthesis inhibitor at the translational level) has been implicated to effectively delay senescence in flowers such as *Dianthus*, *Gladiolus*, *Hemerocallis*, *Ipomoea*, *Iris* and *Narcissus* (Wulster *et al.,* 1982; Lukaszewski and Reid, 1989; Courtney *et al.,* 1994; Jones *et al.,* 1994; Celikel and van Doorn, 1995; van Doorn *et al.,* 1995; Sugawara *et al.,* 2002; Gulzar *et al.,* 2005). During postharvest life, the preservative solutions comprising carbohydrates and biocides have been found effective in preventing many disorders apart from providing nutrients necessary for flower opening, sustaining normal development and preventing microbial growth within the vase (Ichimura *et al.,* 1999; Eason *et al.,* 2002; Redman *et al.,* 2002; Janowska and Jerzy, 2004).

Consolida ajacis (Ranunculaceae) commonly called as "Rocket Larkspur" blooms from May to July in Kashmir. The plants possess blue to violet flowers borne on long erect spikes (40 - 50 cm) in racemes. The present study was undertaken to determine the postharvest performance in holding solutions; distilled water (DW) and sucrose (SUC+HQS) after pulse treatment for 1 h with different concentrations of cycloheximide in cut spikes of *C. ajacis* cv. Violet Blue with the ultimate aim to improve the postharvest life of this beautiful flower.

Materials and methods

Plant materials: Spikes of *C. ajacis* Nieuwl. cv. Violet Blue growing in the University Botanic Garden were used for the present study. The spikes were harvested at 0800 h at 1-2 floret open stage. The harvested spikes were brought to the laboratory, defoliated, cut to a uniform size of 35 cm and pulse treated for 1 h separately in different concentrations of cycloheximide (0.01, 0.05, 0.1 and 0.25 mM CHI). After pulse treatment the spike ends were washed with distilled water thrice. In each case two spikes were transferred to 250 mL Ehrlenmeyer flasks containing 200 mL of distilled water (DW) or sucrose 0.2 M+ 8-HQS 100 mg/L (SUC+HQS). A separate set of five flasks each containing untreated spikes represented control (DW) and (SUC+HQS). Overall there were ten treatments including controls. Treatment effects were evaluated by keeping the flowers in the laboratory at a temperature of 22 ± 2 ºC under cool white fluorescent light with a mix of diffused natural light (10 W m^{-2}) 12 h a day and RH of 60 ± 10 %. The day of harvest was designated as day zero.

Assessment of vase life and blooming: The average vase life of spikes was counted from the day of harvest and was assessed to be terminated when approximately 70% florets senesced on each spike. The experiment was maintained till the vase life in the last set of spikes was regarded to be terminated. Number of blooms per spike was recorded at regular intervals till maximum number of buds bloomed in a particular treatment including control. Total number of buds on each spike was also counted to express the data on percentage basis.

Diameter, fresh and dry mass of flowers: Diameter, fresh and dry mass of the flowers was determined on 2nd, 4th and 6th day of harvest (transfer of spikes to the test solutions). Dry mass was determined by drying the material in an oven for 48 h at 70 ºC.

Determination of soluble protein content: Proteins were extracted from 0.5 g sepal tissue drawn separately from 3-5 different flowers. The tissue was homogenized in 5 mL of 5% sodium sulphite (w/v) adding 0.1 g of polyvinylpyrrolidone and centrifuged. Proteins were precipitated from a suitable volume of cleared supernatant with equal volume of 20 % trichloroacetic acid, centrifuged at 1000 × g for 15 minutes and the pellet redissolved in 0.1 N NaOH. Proteins were estimated from a suitable aliquot by the method of Lowry *et al.* (1951) using BSA as the standard.

Statistical analysis: Each treatment was represented by five replicates (flasks) and each flask contained two spikes. The data was analyzed statistically and LSD computed at P=0.05 using MINITAB (v 15. 1.2- EQUINOX_Softddl.net) software.

Results

Vase life and blooming: The average life of an individual floret on the spike after it had opened fully was about 4-5 days. The flowers opened in an acropetal order. Almost all the florets in a particular spike opened in 6 days (Fig. 2). Flower senescence is characterized by loss of turgor in sepals and finally abscission of sepals and petals leaving behind the carpel on the spike which finally develops into fruit (follicle). Pretreatment of spikes with higher concentrations of CHI resulted in spike bending and the extent of bending increased with the increase in CHI concentration (Fig. 2). The average life of untreated spikes was about 7 days in (DW) and 10 days in (SUC+HQS). The spikes pretreated with 0.01 mM CHI before transfer to (DW) showed a marked increase in longevity by an increment of about 6 days in (DW) and 9 days in (SUC+HQS). The spikes pretreated with 0.05, 0.1 and 0.25 mM CHI registered a decrease in longevity after transferring them to (SUC+HQS) as compared to corresponding spikes transferred to (DW). Pretreatment with higher concentrations of CHI (0.1 and 0.25 mM) registered a decrease in vase life as compared to distilled water (control) or sucrose (Fig. 1). The rate of blooming and number of blooms per spike in spikes pretreated with 0.01 mM before transfer to (DW) or (SUC+HQS) as also spikes pretreated with 0.05 mM CHI before transfer to (DW) was comparable to the corresponding untreated spikes. Pulse treatment with higher concentrations of cycloheximide (0.1 and 0.25 mM) prevented flower opening and promoted premature senescence (Table 1).

Diameter, fresh and dry mass of flowers: Pretreatment of spikes with different concentrations of CHI resulted in a general reduction in floral diameter as compared to corresponding untreated spikes (controls). The floral diameter registered a gradual decrease with the progression in time from 2nd to 6th day of transfer. An increase in floral diameter was recorded in samples from unpulsed spikes as also spikes held in (SUC+HQS) compared to corresponding spikes held in (DW) (Table 1). In some cases floral diameter could not be recorded due to the abscission of florets. Generally, pretreatment of spikes with 0.01 and 0.05 mM CHI before transfer to (DW) or

Table 1. Effect of pretreatment with different concentrations of cycloheximide (CHI, 1h pulse) and subsequent transfer to (DW) and (SUC+HQS) on number of blooms per spike and floral diameter at day 2, 4 and 6 (D2, D4 & D6) of transfer in *C. ajacis* cv. Violet Blue spikes. Each value is a mean of 6 independent replicates. Room temperature = 22±2ºC. Figures in parentheses represent percent blooms on a particular day

Treatment	Days after transfer					
	Number of blooms spike^{-1}			Floral diameter (cm)		
	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6
Set A (DW)						
Control	10.50	13.00	16.33	3.67	3.52	-
	(64.3)	(79.6)	(100)			
0.01 mM CHI	11.00	14.50	17.50	3.48	3.33	3.09
	(62.8)	(82.8)	(100)			
0.05 mM CHI	11.50	13.17	15.67	3.48	3.15	2.73
	(64.2)	(73.5)	(87.5)			
0.1 mM CHI	8.67	8.67	8.67	2.73	-	-
	(49.6)	(49.6)	(49.6)			
0.25 mM CHI	-	-	-	1.20	-	-
	(0.0)	(0.0)	(0.0)			
Set B (SUC+HQS)						
Control	12.60	14.67	17.50	4.24	3.83	3.31
	(72.0)	(83.8)	(100)			
0.01 mM CHI	11.00	13.33	16.83	3.68	3.40	3.38
	(65.3)	(79.2)	(100)			
0.05 mM CHI	7.20	7.33	8.50	3.23	3.15	-
	(43.2)	(43.9)	(51.0)			
0.1 mM CHI	6.00	6.00	6.00	2.45	-	-
	(38.0)	(38.0)	(38.0)			
0.25 mM CHI	-	-	-	1.21	-	-
	(0.0)	(0.0)	(0.0)			
LSD (P=0.05)	2.23	3.15	2.29	0.23	-	-

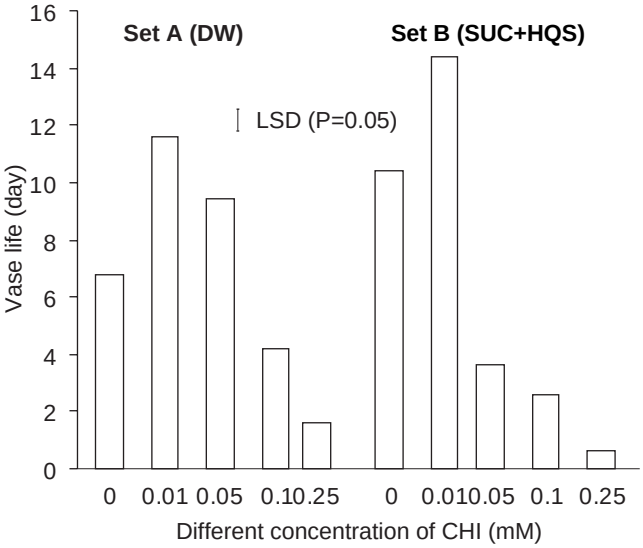

Fig. 1. Effect of pretreatment with varying grades of cycloheximide (CHI, 1h pulse) and subsequent transfer to (DW) and (SUC+HQS) on vase life in *C. ajacis* cv. Violet Blue spikes.

Table 2. Effect of pretreatment with different concentrations of cycloheximide (CHI, 1h pulse) and subsequent transfer to (DW) and (SUC+HQS) on fresh mass, dry mass and soluble proteins from sepals of flowers at day 2, 4 and 6 (D2, D4 & D6) of transfer in *C. ajacis* cv. Violet Blue spikes at room temperature (22±2°C). Each value is a mean of 6 independent replicates

Treatment	Days after transfer								
	Fresh mass (g flower^{-1})			Dry mass (g flower^{-1})			Soluble proteins (mg g^{-1}fw)		
	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6
Set A (DW)									
Control	0.115	0.095	-	0.023	0.014	-	4.54	3.41	-
0.01 mM CHI	0.130	0.123	0.079	0.023	0.022	0.017	4.31	5.00	7.73
0.05 mM CHI	0.122	0.098	0.075	0.019	0.018	0.017	3.64	4.54	5.68
0.10 mM CHI	0.116	-	-	0.018	-	-	2.72	-	-
0.25 mM CHI	0.118	-	-	0.018	-	-	1.36	-	-
Set B (SUC+HQS)									
Control	0.126	0.159	0.098	0.026	0.033	0.020	6.59	5.91	4.99
0.01 mM CHI	0.139	0.164	0.124	0.029	0.032	0.038	5.00	6.59	8.64
0.05 mM CHI	0.132	0.108	-	0.026	0.020	-	5.45	5.45	-
0.10 mM CHI	0.110	-	-	0.022	-	-	3.86	-	-
0.25 mM CHI	0.112	-	-	0.022	-	-	2.95	-	-
LSD (P=0.05)	0.003	-	-	0.001	-	-	0.17	-	-

(SUC+HQS) resulted in an increase in fresh and dry mass of flowers as compared to corresponding untreated spikes (Table 2). A decrease in fresh and dry mass of flowers was generally recorded in samples from spikes pretreated with CHI at 0.1 and 0.25 mM concentrations, particularly when transferred to (SUC+HQS).

Soluble protein content: A higher content of soluble proteins was initially maintained (at day 2 of transfer) in samples from untreated spikes as compared to corresponding spikes pretreated with different concentrations of CHI before transfer to holding solutions. With the progression in time from 2nd to 6th day of transfer, the soluble protein content registered an increase in samples from spikes pretreated with 0.01 and 0.05mM CHI as compared to samples from untreated spikes and spikes pretreated with higher concentrations of CHI, which registered a decrease in the protein content with the progression in time (Table 2). A higher content of soluble protein content was maintained in samples from spikes transferred to SUC+HQS as compared to corresponding spikes transferred to DW.

Discussion

The results of the present study suggests that pretreatment of spikes at 0.01 and 0.05 mM CHI before transfer to holding solutions enhanced vase life by an increment of about 2-5 days in DW and about 8 days in SUC+HQS. Pretreatment of spikes with 0.05, 0.1and 0.25 mM CHI followed by transfer to SUC+HQS was found to reduce the postharvest life of spikes. It appears that sucrose in combination with higher concentrations of CHI may exercise its effect on the osmotic potential of cut spikes, which in turn negate their longevity. Spikes pretreated with different concentrations of cycloheximide showed spike bending and delayed abscission of florets. It may be due to the inhibitory action of cycloheximide on the synthesis of proteins responsible for maintaining spike health (shape and condition) and promoting floral abscission. The ultrastructural data have indicated that floral abscission requires high protein synthesis and secretory activity of material towards cell walls of the abscission zone cells (van Doorn and Stead, 1994). Pretreatment with lower concentrations (0.01 and 0.05 mM) of CHI did not have any significant effect on rate of blooming, which was comparable to that of corresponding unpulsed spikes. However, at high concentrations (0.1 and 0.25 mM), the opening of flowers was prevented. Cycloheximide has been shown to inhibit the flower opening and also delay senescence depending on the stage at which it is included in the experiment (Celikel and van Doorn, 1995; Gulzar *et al.,* 2005; Zhou *et al.,* 2005).

In the present study, a general decrease in floral diameter was observed in samples from spikes pretreated with different concentrations of CHI as compared to untreated spikes. Floral diameter decreased with the increase in CHI concentration. Maintenance of higher fresh and dry mass of flowers particularly at low concentration of CHI (0.01 mM) could be due to lower respiratory losses as CHI has been found to suppress respiration in certain plant tissues. Besides, in *Hemerocallis,* it has been shown to abolish the peak in respiration at the start of senescence (Ellis and Macdonald, 1970; Beileski and Reid, 1991). During the current investigation, higher content of soluble proteins was found in samples from spikes pretreated with lower concentrations (0.01 and 0.05 mM) of CHI, however a reduction in the protein content was observed when the spikes were pretreated with CHI at 0.1 and 0.25 mM concentrations. Treatment of flowers with compounds that inhibit protein synthesis, have been found to delay the decrease in protein levels and increased the time to visible symptoms of petal senescence, revealing that active protein synthesis is required for the execution of cell death in petals (Lay-yee *et al.,* 1992; Courtney *et al.,* 1994; Celikel and van Doorn, 1995; Sultan and Farooq, 1997; Wagstaff *et al.,* 2002; Xu *et al.,* 2007). It may be suggested that CHI maintained a high protein content in the sepal tissue by inhibiting the synthesis of specific proteases responsible for protein degradation.

The present study further revealed that postharvest performance

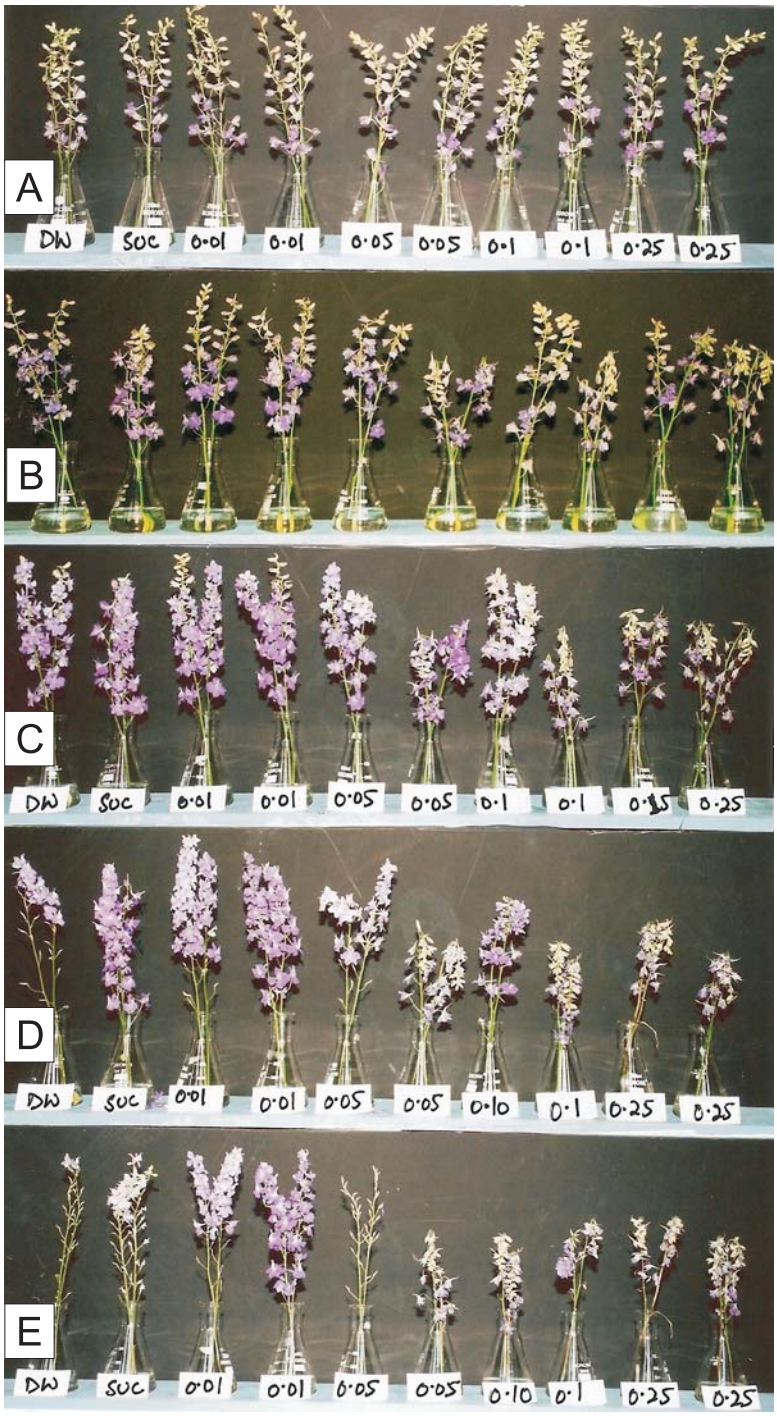

Fig. 2. Spikes of *C. ajacis* cv. Violet Blue held in distilled water (DW) or sucrose 0.2 M+ 8-HQS 100 mg/L (SUC+ HQS) after 1h pulse treatment with different concentrations of cycloheximide (CHI) at day zero of transfer (A), day 1 of transfer (B), day 4 of transfer (C), day 8 of transfer (D) and day 12 of transfer (E). From left to right are flasks containing spikes arranged as DW (control), SUC+HQS, 0.01mM CHI–DW, 0.01mM CHI–SUC+HQS, 0.05mM CHI–DW, 0.05mM CHI–SUC+HQS, 0.1mM CHI–DW, 0.1mM CHI–SUC+HQS, 0.25mM CHI–DW and 0.25mM CHI– SUC+HQS.

of untreated spikes as also spikes pretreated with 0.01 mM CHI was better in SUC+HQS as compared to corresponding spikes transferred to DW. Sugars besides supplying respiratory substrates maintain adequate water balance, decrease sensitivity to ethylene and delay the climacteric ethylene biosynthesis (Ichimura *et al.,* 2000; Pun and Ichimura, 2003). Sucrose alone, or in combination with the antioxidant, 8-hydroxyquinoline sulphate (8-HQS) has been found to improve the postharvest performance of many cut flowers such as tuberose, *Phalaenopsis*, *Leptospermum*, *Amaryllis* and *Delphinium* (Huang *et al.,* 1995; Reddy *et al.,* 1995; Burge *et al.,* 1996; Ichimura *et al.,* 2000; Gul *et al.,* 2007). Part of the beneficial effect of 8-HQS has been attributed to its effect on stomatal closure (Halevy and Mayak, 1979; Ichimura *et al.,* 1999) and in part due to its antibacterial and antifungal activity (Reid and Kofranek, 1980). Sugars have also been found to delay the increase in mRNA abundance of a number of senescence-associated genes (Eason *et al.,* 2002; Hoeberichts *et al.,* 2007).

The present investigation suggest that the effects of cycloheximide are the result of modulation at the cellular level. The fact that cycloheximide delays petal/sepal senescence demonstrates that the synthesis of particular suicide proteins orchestrates the cell death programme, however it is necessary to show that these proteins and their products actually play a causal role. Pretreatment of spikes (harvested at 1-2 floret open stage) with CHI (0.01 mM) before transfer to (DW) or (SUC 0.2M+HQS 100 mg/L) resulted in marked improvement in vase life and can be used as effective postharvest treatment for this cut flower.

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