Effect of Cytokinins on the Senescence and Longevity of Isolated Flowers of Day Lily (*Hemerocallis fulva*) cv. Royal Crown Sprayed with Cycloheximide

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Keywords: Flower senescence, Membrane permeability, Sugars, Proteins, α-Amino acids, Phenols

Abstract

We studied the effects of cycloheximide (CHI) and cytokinins on the senescence and longevity of isolated flowers of *Hemerocallis fulva*. Mature buds were detached from the scapes in the field at 1700h one day before anthesis and transferred to 15 ml glass vials containing distilled water. On the subsequent day at 800 h one set of partially open flowers was sprayed with CHI (0.5mM 25° C) and the other set with plain water. The flowers were transferred to 15ml glass vials containing 0.5mM of each of the Kinetin, BAP and DPU besides distilled water which served as control. Flowers sprayed with CHI significantly enhanced longevity to 3.3 days in distilled water. Kinetin and BAP markedly delayed senescence and prolonged longevity in CHI sprayed flowers. The present study reveals that spray treatment of partially open flowers with CHI followed by transfer to BAP or Kinetin were the effective treatments in delaying senescence, maintaining flower quality and thereby prolonging longevity of isolated flowers of *Hemerocallis fulva*. In these treatments lesser ion leakage, besides higher fresh mass and water content was recorded suggesting maintenance of membrane integrity. These treatments also maintain the respiratory pool of sugars in the perianth tissues, besides being effective in slowing down protein degradation with flower opening and senescence.

INTRODUCTION

Ephemeral flowers, *Ipomoea* and *Hemerocallis* have been used as effective model systems to study the process of senescence because the events happen quickly. *Hemerocallis fulva* L. (Family: Liliaceae) with its showy flowers has considerable potential as a cut flower and for developing effective handling techniques to improve the keeping quality of this cut flower, an understanding of the pattern and mechanism of senescence is necessary. Flower senescence in *Hemerocallis* has been reported to be ethylene insensitive (Lukaszewski and Reid, 1989; Lay-Yee et al., 1992) and is accompanied by a decline in protein content as well as breakdown of specific proteins in petals; cycloheximide treatment maintains the protein content and effectively delays senescence of its flowers (Sultan and Farooq, 1996). Cycloheximide spray treatment soon after opening has also been shown to prolong the longevity of detached morning glory (*Ipomoea tricolor*) flowers (Sultan et al., 2002). Cytokinins slow down the process of senescence possibly by their ability to promote the transport, accumulation and retention of metabolites in tissues and organs; besides protecting membranes against degradation (Lesham, 1988; Beckman and Ingram, 1994). Applied cytokinins have been shown to slow down the aging process in rose and carnation petals (Borochov and Woodson, 1989; Van Staden et al., 1990).

The present study is aimed to study the effect of cycloheximide spray followed by transfer to cytokinins on the senescence and longevity of isolated flowers of *Hemerocalis fulva* with the aim of extending its vase life.

MATERIALS AND METHODS

Flowers of *Hemerocallis fulva* cv. Royal crown growing in the Kashmir University Botanic garden were used in the study. Mature buds were harvested from the scapes in the field at 1700h, one day before anthesis. The buds were immediately transferred to 15ml glass vials containing distilled water. On the subsequent day at 800h one set of partially open flowers was sprayed with CHI (0.5mM. 25^{o}C) and the other set with plain water. The flowers were transferred to 15ml glass vials containing 0.5mM each of the Kinetin (from CDH), Benzylaminopurine (BAP from HiMedia) and Diphenyl urea (DPU grom Fluka) besides distilled water which served as control. Each treatment had ten replicates with one flower in each vial. All vials were totally random. Test solutions were renewed everyday to minimise the microbial growth. Treatment effects were evaluated by keeping the flowers in the laboratory at a temperature of 30 ± 3°C under cool white fluorescent light with a mix of diffused natural light (10Wm^{-2})12h a day and RH of 60 ± 10%. The flower fresh and dry mass was determined at 0, 1, 2 and 3 days after harvesting the buds. Dry mass was determined after drying the flowers in a drying oven at 70°C for 48 h. Water content was determined as the difference between fresh mass and dry mass. Changes in membrane permeability were estimated by measuring ion leakage from 20 discs each of 7mm diameter punched from the flag region of the perianth lobes of five different flowers incubated in dark in 15ml glass distilled water for 12h at a temperature of 20°C.

At each stage, 2g chopped material of perianth tissue was fixed in tripicate in hot 80% ethanol. The material was macerated and centrifuged three times. The supernatants were pooled and used for the estimation of sugars, free amino acids and total phenols. Reducing sugars were estimated by the method of Nelson (1944) using glucose as standard. Total soluble sugars were estimated after enzymatic conversion of non reducing sugars into reducing sugars with invertase (BDH). Non-reducing sugars were calculated as the difference between total and reducing sugars. α- amino acids were estimated by the method of Rosen (1957) using glycine as standard. Total phenols were estimated by the method of Swain and Hillis (1959) using gallic acid as standard. Proteins were extracted from 1g perianth tissue drawn separately from three different flowers. The tissue was homogenized in 10ml of 5% sodium sulphite (w/v) adding 0.1g polyvinylpyrrolidone and centrifuged. Proteins were precipitated from a suitable volume of cleared supernatant with equal volume of 20% w/v trichloracetic acid, centrifuged at 1000x g for 15 minutes and the pellet redissolved in 0.5 N NaOH. Proteins were estimated form a suitable aliquot by the method of Lowry et al. (1951) using BSA as the standard.

The data has been analysed statistically and LSD between the treatments computed at P = 0.05.

RESULTS AND DISCUSSION

The individual flowers of *Hemerocallis* (daylily) have a life span of one day, opening in the morning and wilting in the evening of the same day. This short life span of individual flowers is compensated by the profusion and continuity with which buds bloom into flowers. Scape in the field produces about 16 flowers during its life span of three weeks. Mature *Hemerocallis* buds held in distilled water opened and wilted in the same pattern as that of the attached buds in the field. The first visible signs of senescence were the appearance of translucent areas on perianth margins which began to appear just in the afternoon followed by a gradual closing of petals with complete collapse from 2000 - 2100h, thus the longevity per flower was approximately 14 h (Fig. 1 a-f). In CHI sprayed flowers water soaked areas did not show up during senescence besides the petals did not collapse (Fig. 2 a -c).

Compared to distilled water controls, Kinetin, BAP and DPU marginally prolonged longevity of unsprayed flowers but markedly delayed senescence and prolonged longevity in CHI sprayed flowers. Fresh mass, dry mass and water content declined with opening and senescence in flowers held in distilled water, but increased with opening and then declined in flowers held in various cytokinins with or without CHI

spray treatment (Table 1). Compared to unsprayed flowers, higher freshmass, drymass and water content was maintained in the flowers sprayed with CHI (Table 1).

Compared to distilled water controls and other treatments, higher values for longevity, dry mass and water content were recorded in flowers sprayed with CHI and then transferred to BAP. Maintenance of higher dry mass could be due to lower respiratory losses in CHI sprayed flowers as CHI has been found to suppress respiration in certain plant tissues (Ellis and MacDonald, 1970); besides in *Hemerocallis* it has been shown to abolish the peak in respiration at the start of senescence (Bieleski and Reid, 1991).

Membrane permeability increased with time irrespective of the treatment. The increase was however, much less marked in CHI sprayed flowers and more pronounced in flowers held in distilled water without or with CHI spray (Table 1). The increase was least marked in CHI sprayed flowers held in BAP or Kinetin. Cytokinins have been suggested to maintain the integrity of tonoplast membranes preventing the leakage of proteases from the vacuoles into the cytoplasm which could otherwise hydrolyze soluble proteins (Thimann, 1987). A highly significant negative correlation was obtained between conductivity and moisture content (Fig. 3). The loss of membrane integrity causes an increase in permeability and leakage during senescence in flowers such as *Arum, Ipomoea, Dianthus, Iris, Hemerocallis, Rosa* and *Gerbera* (van Meeteren, 1979; Halevy and Mayak, 1979; Lay-Yee et al., 1992; Celikel and van Doorn, 1995).

Total soluble sugars and reducing sugars in the perianth tissues declined as the flowers opened but the decline was marked during senescence (Table 2). Flower maturation and senescence is accompanied by decline in total soluble carbohydrate content as in flowers such as carnations (Nichols, 1973; Paulin and Jamain, 1982). It has been reported that there is a small but consistent respiratory climacteric coinciding with the early senescence in *Hemerocllis* flowers (Lukaszewski and Reid, 1989; Lay-Yee et al., 1992). In CHI sprayed flowers the concentration of these tissue constituents decreased as the flowers opened and then remained more of less constant as the flower senescence progressed (Table 2). Non reducing sugars decreased as the flowers opened and senesced, the decrease was much less marked in CHI sprayed flowers held in various cytokinins especially BAP (Table 2). The decline in sugars suggests that an increased rate of respiration in fully open flowers is responsible for their rapid utilization as also suggested by (Mwangi et al., 2003) in cut rose petals.

Soluble proteins showed a decreasing trend with time during flower opening and senescence, whereas α-amino acids showed a concomitant increase irrespective of the treatment (Table 3). In CHI sprayed flowers soluble proteins decreased with opening and then remained more or less constant. The present study corroborates the finding of (Lay-Yee et al., 1992) who found that flower maturation and senescence is accompanied by a decline in protein content, whereas CHI has been shown to delay the flower senescence in *Hemerocallis*. CHI has also been found to inhibit senescence in carnation, gladiolus, iris and narcissus flowers (Dilley and carpenter 1975; Wulster et al., 1982; Jones et al., 1994; Sultan et al., 2002). In *Iris germanica* the detachment of flowers has been suggested to cause a water stress which in turn causes a decline in protein content and synthesis (Paulin, 1972; 1975). The decline in protein content with the opening and senescence of flowers in *Hemerocallis fulva* suggests protein degradation to be an important factor contributing to senescence. CHI maintained a high protein content in the perianth tissue probably by inhibiting the synthesis of specific proteases responsible for protein degradation. Total phenols generally increased with flower opening and senescence irrespective of the treatment but the increase was much less marked in CHI sprayed flowers transferred to various cytokinins (Table 3). Higher content of total phenols has been associated with longer vase life in cut rose petals (Mwangi et al., 2003).

The study suggests that changes in membrane integrity and protein degradation by specific proteases are the main causes of flower senescence in *Hemerocallis fulva.*

ACKNOWLEDGEMENTS

The authors are grateful to Prof. B.A. Wafai, Head, Department of Botany for providing encouragement and facilities. One of us Miss Shabana Gulzar is indebted to CSIR (New Delhi) for a research fellowship.

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Tables

Table 1. Effect of CHI (0.5mM, 25°C) spray treatment and subsequent transfer to Kinetin (0.5 mM), BAP (0.5 mM) and DPU (0.5mM) on the fresh mass, dry mass, moisture content and conductivity (perianth discs) of isolated flowers of *Hemerocallis fulva*.

Days after harvest	Treatments								
	UN SPRAYED				CHI SPRAYED				
	D.W (Control)	Kinetin (0.5mM)	BAP (0.5mM)	DPU (0.5mM)	D.W	Kinetin (0.5mM)	BAP (0.5mM)	DPU (0.5mM)	LSD (P = 0.05)
	Fresh Mass Flower $^{-1}$(g)								
0	**2.100**	-	-	-	-	-	-	-	-
1	2.000	2.183	2.200	2.116	2.216	2.358	2.466	2.250	0.041
2	0.883	1.066	1.233	1.566	1.725	2.092	1.961	1.866	0.069
3	0.750	0.864	0.863	0.783	1.485	1.383	1.400	1.225	0.035
	Dry Mass Flower^{-1} (g)								
0	**0.206**	-	-	-	-	-	-	-	-
1	0.200	0.205	0.209	0.206	0.211	0.225	0.230	0.213	0.008
2	0.144	0.168	0.190	0.167	0.191	0.204	0.210	0.192	0.006
3	0.145	0.161	0.164	0.158	0.172	0.181	0.195	0.183	0.007
	Moisture Content Flower^{-1} (g)								
0	**1.893**	-	-	-	-	-	-	-	-
1	1.800	1.978	2.032	1.895	1.990	2.080	2.170	1.990	0.032
2	0.739	1.231	1.985	1.940	1.990	2.170	2.270	2.085	0.200
3	0.604	0.685	1.955	1.895	2.035	2.150	2.270	2.038	0.008
	Conductivity (µS)								
0	**31.7**	-	-	-	-	-	-	-	-
1	120.5	88.3	80.0	92.3	70.4	61.2	53.1	63.1	3.4
2	174.9	144.6	130.2	162.7	106.6	67.6	64.3	98.1	5.2
3	215.2	173.0	167.9	188.5	145.1	95.8	92.8	112.7	4.7

Each value represents the mean of 5 readings

Table 2. Effect of CHI (0.5mM, 25°C) spray treatment and subsequent transfer to Kinetin (0.5 mM), BAP (0.5 mM) and DPU (0.5mM) on total soluble sugars, reducing sugars and non-reducing sugars in the perianth tissue of isolated flowers of *Hemerocallis fulva*.

Days after harvest	Treatments								
	UN SPRAYED				CHI SPRAYED				
	D.W (Control)	Kinetin (0.5mM)	BAP (0.5mM)	DPU (0.5mM)	D.W	Kinetin (0.5mM)	BAP (0.5mM)	DPU (0.5mM)	LSD (P = 0.05)
	Total Soluble Sugars (mg g^{-1} F.M)								
0	**29.584**	-	-	-	-	-	-	-	-
1	23.048	26.488	27.176	24.080	27.176	27.864	28.208	27.520	4.724
2	7.568	10.320	10.320	8.256	14.104	18.920	20.640	14.448	1.966
3	3.096	7.568	8.256	5.160	15.136	19.264	21.328	16.856	1.768
	Reducing Sugars (mg g^{-1} F.M)								
0	**18.576**	-	-	-	-	-	-	-	-
1	13.416	14.448	14.448	13.760	15.480	16.512	16.856	15.824	0.468
2	6.192	8.256	8.600	6.192	9.288	10.320	10.320	9.632	0.581
3	2.408	6.192	7.568	4.472	14.104	18.232	19.264	15.824	2.164
	Non Reducing Sugars (mg g^{-1} F.M)								
0	**11.008**	-	-	-	-	-	-	-	-
1	9.632	12.040	12.728	10.320	11.696	11.352	11.352	11.696	4.764
2	1.376	2.064	1.720	2.064	4.816	8.600	10.320	4.816	1.461
3	0.688	1.376	0.688	0.688	1.032	1.032	2.064	1.032	1.056

Each value represents mean of three replicates

Table 3. Effect of CHI (0.5mM, 25°C) spray treatment and subsequent transfer to Kinetin (0.5 mM), BAP (0.5 mM) and DPU (0.5mM) on soluble proteins, α-amino acids and total phenols in the perianth tissues of isolated flowers of *Hemerocallis fulva*.

Days after harvest	Treatments								
	UN SPRAYED				CHI SPRAYED				
	D.W (Control)	Kinetin (0.5mM)	BAP (0.5mM)	DPU (0.5mM)	D.W	Kinetin (0.5mM)	BAP (0.5mM)	DPU (0.5mM)	LSD (P=0.05)
	Soluble Proteins (mg g^{-1} F.M)								
0	**3.067**	-	-	-	-	-	-	-	-
1	1.431	1.840	1.840	1.636	2.045	2.454	2.863	2.249	0.415
2	0.818	1.227	1.227	1.022	1.431	1.840	2.046	1.636	2.691
3	0.409	1.022	1.022	0.613	1.840	2.045	2.045	1.840	2.876
	α -Amino acids (mg g^{-1} F.M)								
0	**0.675**	-	-	-	-	-	-	-	-
1	1.416	1.268	1.268	1.357	1.062	0.737	0.708	0.973	0.217
2	2.006	1.711	1.622	1.888	1.416	1.298	1.209	1.386	0.203
3	2.625	2.419	2.330	2.478	1.357	1.180	1.121	1.209	0.218
	Total Phenols (mg g^{-1} F.M)								
0	**2.500**	-	-	-	-	-	-	-	-
1	3.862	2.953	2.612	3.237	2.783	2.612	2.556	2.669	3.671
2	5.850	4.998	4.600	5.452	3.407	3.180	2.953	3.237	0.850
3	8.804	8.520	6.748	8.520	4.771	3.976	3.805	4.146	0.462

Each value represents mean of three replicates

Figures

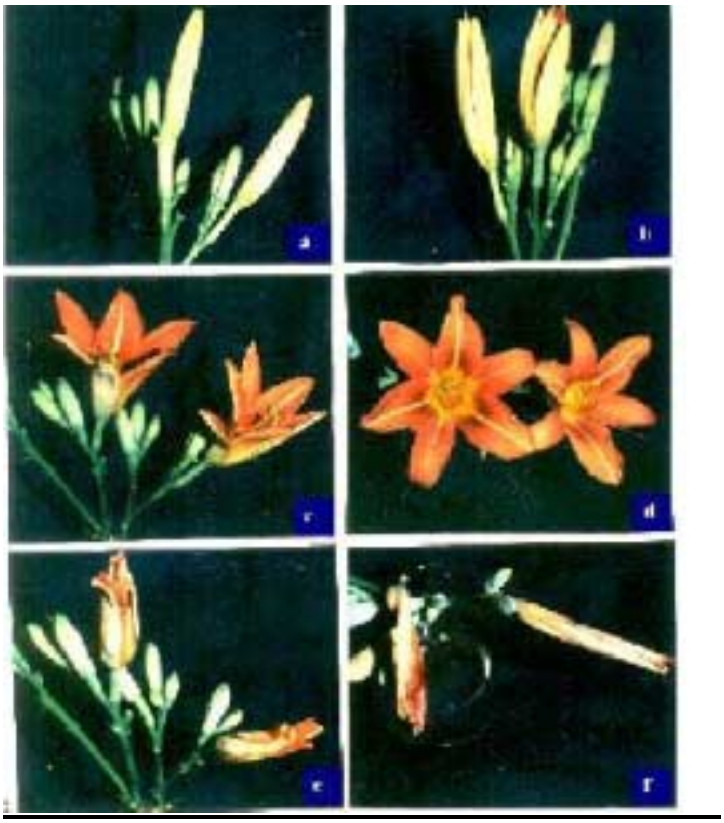

Fig. 1. Flower opening and wilting in *Hemerocallis fulva.* Photographs taken at 12:00h (a), 4:00h (b), 6:00h (c), 12:00h (d), 21:00h (e) and 12:00h (f).

Fig. 2. Stages of flower development and senescence in *Hemerocallis fulva.* Each stage was designated with reference to 12:00h with each stage spanning 24hours.

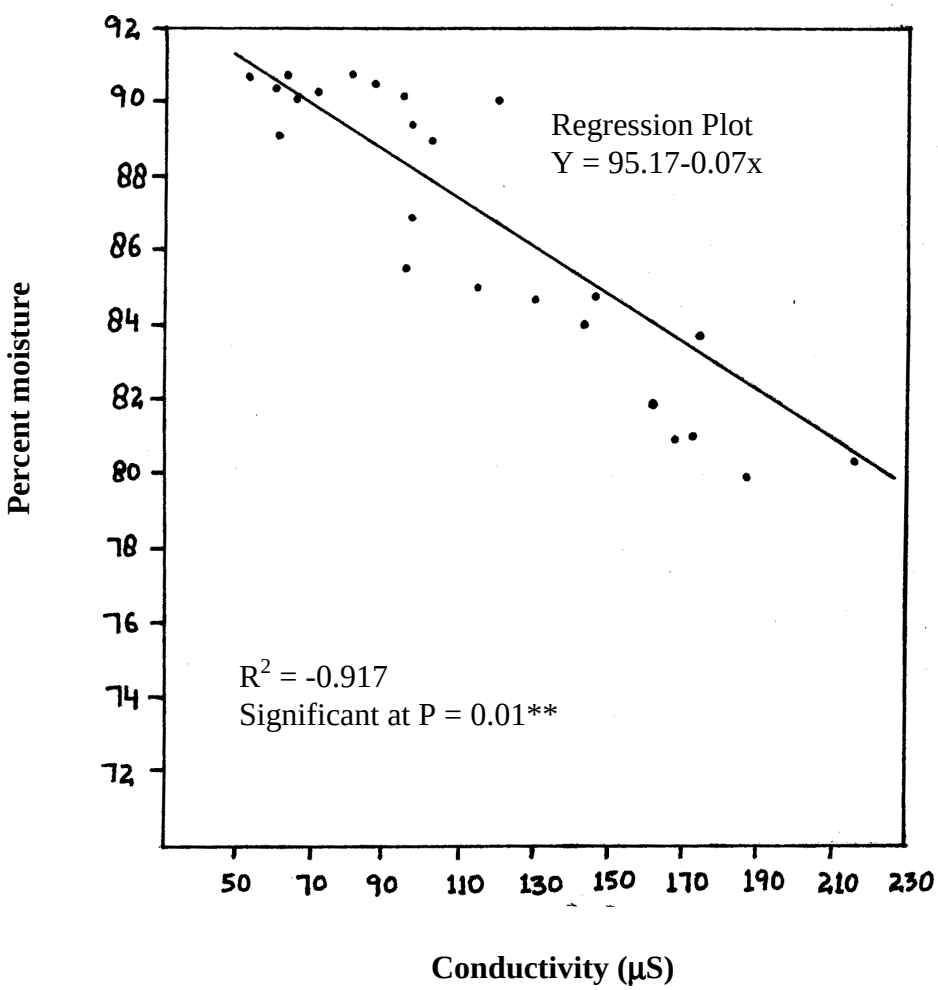

Fig. 3. Correlation between conductivity (µS) of leachates in perianth discs and percent moisture of isolated flowers of *Hemerocallis fulva.*