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FULL LENGTH ARTICLE

An effective protocol for improving vaselife and postharvest performance of cut *Narcissus tazetta* flowers

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Abstract A study was made to investigate differential responses of petal senescence and postharvest performance at varying concentrations of Cycloheximide (CHI) in cut spikes of *Narcissus tazetta* cv. Kashmir Local. Cycloheximide at 0.01 and 0.05 mM concentrations delayed senescence. Above 0.05 mM concentrations CHI prevents flower opening and promotes senescence. Senescence delay by CHI points to the synthesis of some specific proteins (enzymes) responsible for execution of cell death programme in flower petals. Cycloheximide at lower concentrations (0.01 and 0.05 mM) enhanced longevity, maintained a sustained rate of flower blooms, delayed senescence and optimized postharvest performance. Pulse treatment of spikes with CHI concentrations at 0.01 and 0.05 mM concentrations maintained high fresh and dry mass of flowers and lowered electrical conductivity of leachates. The content of sugars and proteins decreased, whereas that of α-amino acids and total phenolics increased in the petal tissues with CHI treatment; besides improving postharvest performance. Pretreatment of flowers with 0.01 or 0.05 mM CHI concentrations for 1 h enhanced vase life and improved postharvest performance in this flower system.

1. Introduction

Petal senescence is a part of a developmental continuum in the flower and preceded by tissue differentiation, growth and development of seeds and coordinated by plant hormones. Senescence can be studied at cellular, tissue, organ or organization level as a genetically programmed event (Rubinstein, 2000; Eason et al., 2002; Wagstaff et al., 2002; van Doorn, 2004; Hoeberichts et al., 2005; Zhou et al., 2005; Price et al., 2008; van Doorn and Woltering, 2008; Shibuya et al., 2009; Woltering and van Doorn, 2009; Shahri and Tahir, 2010; Shahri, 2011). Enhanced expression of protease genes is one of the earliest senescence related gene changes to be identified in various ornamental cut flowers pointing to the fact that protein cleavage is a prime step during petal senescence (Eason et al., 2002; Wagstaff et al., 2002; DaSilva, 2003; Jones et al., 2005; van Doorn and Woltering, 2008). The interactions between proteases and their inhibitors have been linked to modulation of cell death processes in plants and in certain cut flowers. Inhibition of protease action chemically modulates the onset of cellular senescence thereby developing a new strategy of delaying flower senescence and improving the postharvest performance in ornamental flowers (Eason et al., 2002; Da Silva, 2003; Sin and Chy, 2004; Pak

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and van Doorn, 2005; Shahri and Tahir, 2010; Gul et al., 2012). Longevity of flowers can therefore be enhanced with application of specific protein synthesis inhibitors.

Actinomycin D and Cycloheximide have been identified as protein synthesis inhibitors at transcriptional and translational levels. Cycloheximide ($C_{15}H_{23}NO_4$), is a protein synthesis inhibitor at translational level (Tobita and Shono, 2001). It has been found to delay senescence in *Dianthus*, *Hemerocallis*, *Gladiolus*, *Iris*, *Ranunculus* and *Nerine* (Wulster et al., 1982; Lukaszewski and Reid, 1989; Jones et al., 1994; van Doorn et al., 1995; Gulzar et al., 2005; Shahri and Tahir, 2010; Gul et al., 2012). Cycloheximide has been shown to inhibit the flower opening and also delay senescence depending upon the stages at which it is included in the experiment (Celikel and van Doorn, 1995; Gulzar et al., 2005; Zhou et al., 2005).

Narcissus is a genus of hardy, spring-blooming, bulbous plants in the family Amaryllidaceae. Earlier reports suggested that the genus *Narcissus* contained around 26 wild species (Third, 1976). The number has been reported to be between 50 and 100 including species variants and wild hybrids (Brent and Becky, 2001). The species *Narcissus tazetta* derives its name from the word ''Tazetta'' which in Italian means ''little cups'' with reference to the centrally placed little yellow corona cups. It is the most widespread species of the genus *Narcissus* found in region with Mediterranean type of climate extending from Spain, Iran, Kashmir to China and Japan (Coats, 1971). Beauty, delicate fragrance and presence of a multi floret head places ''Tazetta'' superior to other Narcissi species. The bunch flowered ''Tazetta'' bears an average of 2–7 flowers per spike, entitling them to be pronounced as ''Polyanthus Narcissus'' also. In Kashmir *N. tazetta* grows wild and is one of the earliest spring blooming species marking it as an obvious choice for cut flower study. The present study was undertaken to investigate the effects of Cycloheximide at different concentrations on regulation of senescence and a means for improvement of postharvest performance in *N. tazetta* cv. Kashmir Local.

2. Materials and methods

2.1. Experimental site and plant material

Uniform sized bulbs *N. tazetta* were planted in Kashmir University Botanical Garden (KUBG) in first week of November 2009. The bulbs were allowed to grow under natural environmental conditions. The emergent spikes form the bulbs were used for the present study in March 2010. The spikes were harvested at 08.00 h at mature stage characterized by the presence of a mature bud about to open the next day.

2.2. Procedure and experimental design

The spikes were brought to the laboratory and cut to a uniform spike length of 20 cm and pulse treated for 1 h separately with varying concentrations of Cycloheximide (0.01, 0.05, 0.5 and 1.0 mM). After pulse treatment the spike ends were washed thrice with distilled water. Each treatment consisted of five replicates. Overall there were 10 treatments including control and sucrose. In each case two spikes were transferred to 250 ml Borosil conical flasks containing 200 ml of distilled water (Set A) or sucrose (Set B). A separate set of five flasks each containing unpulsed spikes represented controls DW and sucrose SUC (0.15 M). The experiments were carried out in Plant Physiology and Biochemistry Laboratory, Department of Botany, Kashmir University, J&K, India. The laboratory maintained a temperature of 12 ± 2 °C, in cool white fluorescent light with a mix of diffused natural light (10 W m^{-2}) 12 h a day and RH of 60 ± 10%. Treatment was given on the day of harvest which was designated as day zero. Data were recorded on vase life, blooming, solution uptake, conductivity of leachates, fresh and dry mass of flowers, petal tissue constituents and proteins.

2.3. Assessment of vase life, blooming and solution uptake

The average vase life of the spikes was counted from the day of transfer of spikes to the holding solution and was assessed to be terminated when 50% flowers had senesced, which was characterized by loss of turgor followed by petal wilting. Petal senescence was marked by the loss of turgor in the petal tissue followed by complete wilting. Number of blooms per spike was recorded till maximum numbers of buds bloomed in a particular treatment including the controls. The volume of holding solution absorbed by the spikes was calculated by measuring the volume of solution on a particular day and subtracting it from the initial quantity of the vase solution kept in the flasks. To account for the volume of solution evaporated blank flasks (containing particular vase solutions without spikes) were used in triplicates alongside the flasks with spikes. Volume of holding solution absorbed per spike was recorded on every second day of the experiment till the controls senesced.

2.4. Conductivity of leachates, fresh and dry mass

Conductivity of leachates, fresh and dry mass of the flowers was determined on 4th and 8th days of harvest. Dry mass was determined by drying the material in an oven for 48 h at 70 °C. Membrane permeability was studied by measuring ion leakage from the petal discs (5 mm in diameter) incubated in the dark in 15 ml glass double distilled water for 15 h at 20 °C. The discs were punched from the flag region of petals of 5 flowers. The discs were floated with their abaxial surface downwards and were removed after 15 h of incubation. Conductivity of leachates was measured by CM-180 ELICO Conductivity metre and was expressed in μS.

2.5. Tissue constituents

At each stage 0.5 g chopped material of petal tissue was fixed in triplicate in hot 80% ethanol. The material was macerated and centrifuged three times at 3000*g* for 20 min. The supernatants were pooled and used for the estimation of sugars and total phenols. Reducing sugars were estimated by the method of Nelson (1944) using glucose as the standard. Total sugars were estimated after enzymatic conversion of non-reducing sugars into reducing sugars with invertase. 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. Tissue constituents were estimated on day 4 and 8 in all the pulse treatments of CHI at 0.01, 0.05, 0.5 and 1.0 mM concentrations including the controls i.e. DW or SUC (0.15 M).