POLISH SOCIETY FOR HORTICULTURAL SCIENCE

Folia Hort. 27/2 (2015): 115-121

DOI: 10.1515/fhort-2015-0021

FOLIA HORTICULTURAE

Published by the Polish Society for Horticultural Science since 1989

ORIGINAL ARTICLE | Open access | http://www.foliahort.ogr.ur.krakow.pl

Flower development and senescence in *Narcissus tazetta* 'Kashmir Local'

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ABSTRACT

Flower development and senescence was studied in *Narcissus tazetta* 'Kashmir Local'. Flower development was divided into six stages (I-VI), from the tight bud stage to the senescent stage. Flower fresh and dry weight increased as the flowers progressed from bud to bloom and then declined during senescence. Membrane permeability of tepal tissues increased as the flower progressed through various stages. The content of sugars (reducing and total) increased during flower development and declined thereafter during senescence. The α-amino acid content registered an increase during flower development with a concomitant decrease in soluble protein content. The SDS-PAGE of protein extracts from tepal tissues revealed a general decrease in the expression of some high molecular weight proteins and an increase in low molecular weight proteins during flower development and senescence. It may be suggested that the flower senescence of *N. tazetta* may be linked to the protein turnover and sugar status of the perianth tissues and that these newly synthesized proteins may be involved in proteolysis. At this stage, it is not known whether these polypeptides play an important role in senescence but revealing the nature of these proteins can provide new insights into the pathways executing senescence in this flower system.

Key words: α-amino acids, flower, membrane permeability, SDS-PAGE, senescence, soluble proteins

Abbreviations:

PCD – Programmed cell death; SDS – Sodium dodecylsulphate; PAGE – Polyacrylamide gel electrophoresis; TEMED – Tetramethyl ethylenediamine; BSA – Bovine serum albumin

INTRODUCTION

Senescence or biological aging is the change in the biology of an organism as it ages after its maturity. Such changes range from those affecting its cells and their function to that of the whole organism. Senescence in flower petals, regarded as a form of programmed cell death; PCD (Woltering and van Doorn 2009, Shahri and Tahir 2011a), involves the organized break down of cells or tissues in a predictable manner and the reutilization of nutrients by other cells, tissues or plant parts (carpels) (Shahri and Tahir 2011b, Shahri et al. 2011). The process of tepal or petal senescence has been found to be accompanied by a decline in petal or tepal fresh/dry weight, soluble proteins (Lay-Yee et al. 1992), soluble carbohydrates and, nucleic acids and an increase in the activity of catabolic enzymes, amino acid content, ion leakage (van Doorn and Woltering 2008), DNA degradation and nuclear fragmentation (Yamada et al. 2006a, b, Shahri and Tahir 2014).

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Rapid senescence of flowers is highly undesirable from the postharvest perspective. The present investigation has been undertaken to study the changes occurring during flower development and senescence in *Narcissus tazetta* cv. Kashmir Local, with the ultimate aim to improve its postharvest performance.

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 regions with the Mediterranean climate extending from Spain, Iran, and Kashmir to China and Japan (Coats 1971). Beauty, delicate fragrance and the presence of a multi floret head makes *N. tazetta* superior to other Narcissi species. The bunch flowered 'Tazetta' bears an average of 2 to 7 flowers per scape, entitling them to also be pronounced as 'Polyanthus Narcissus'. In Kashmir *Narcissus tazetta* is one of the earliest spring blooming species marking it as an obvious choice for cut flower study. The present study was conducted to study some physiological and biochemical changes associated with flower development and senescence in *Narcissus tazetta* in order to understand the basic senescence mechanism in this flower system so that future strategies can be made to improve its postharvest performance.

MATERIAL AND METHODS

Flowers of *Narcissus tazetta* growing in the University Botanic Garden (KUBG) were used. The plants were grown from bulbs in experimental fields under optimal environmental (Feb-Mar) conditions without any special pre-harvest treatments. Flower development and senescence was divided into six stages (Fig. 1). These stages were designated as the tight bud stage (I), loose bud stage (II), half open stage (III), fully open stage (IV), partially senescent stage (V) and senescent stage (VI). The morphological details of the different stages are listed in Table 1. Visible changes were recorded throughout flower development and senescence. Flower diameter, fresh and dry weight were determined at each stage (15-20 replicates). Changes in membrane permeability were estimated by measuring the electrical conductivity of ion leachates (μS cm^{-1}) from the tepal discs (5 mm in diameter) of five different flowers (10 replicates) incubated in 15 mL distilled water for 20 h at 20°C. For the estimation of tissue constituents, 1 g chopped material of tepal tissues was fixed in hot 80% ethanol at each stage of flower development and senescence. The material was macerated and centrifuged three times at 1000 rpm. The supernatants were pooled, made to volume and suitable aliquots (0.2 mL) were used for the estimation of reducing sugars, non-reducing sugars, total sugars, α-amino acids and total phenolic content (10 replicates). Reducing sugars were determined by the method of Nelson (1944) using glucose as the standard. Total soluble sugars were estimated after the enzymatic conversion of non-reducing sugars into reducing sugars with invertase. Non-reducing sugars were calculated as the difference between total and reducing sugars while α-amino acids were estimated by the method of Rosen (1957) using glycine as the standard. Total phenolic contents were estimated by the method of Swain and Hillis (1959) using gallic acid as the standard. Soluble proteins were extracted from 1 g of the tepal tissue at each of the six stages and estimated by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as the standard with the necessary precautions as highlighted by Mattoo et al. (1987). Electrophoretic profiles were studied at various stages of flower development and senescence. 80 μL of the SDS-denatured protein

Table 1. Flower development and senescence stages in *Narcissus tazetta* 'Kashmir Local'

No.	Name	Description
I	Tight bud stage	Buds tightly closed, tepals greenish in colour
II	Loose bud stage	Buds loosely closed, tepals white in colour.
III	Half open stage	Flowers half open, cup shaped yellow corona visible, tepals white in colour.
IV	Fully open stage	Flowers fully open, tepals and corona turgid, stamens and pistils visible.
V	Partially senescent stage	Corona dilated, pushed outward, tepals flaccid.
VI	Senescent stage	Tepals papery, sticky margins transparent corona flaccid.

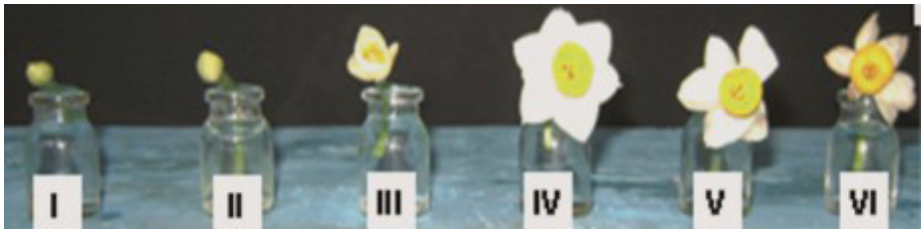

Figure 1. Stages of flower development and senescence in *Narcissus tazetta* 'Kashmir Local'

extract was loaded into each lane and was carried out according to the method described by Ausubel et al. (1989). Following electrophoresis the gels were stained overnight in 0.25% Coomassie brilliant blue in 45% methanol: 10% acetic acid. Gels were destained in 45% methanol: 10% acetic acid, then in 7% methanol: 5% acetic acid. Each value represented in the tables corresponds to the mean of 10 independent replicates. The data have been analyzed statistically by computing standard deviation.

RESULTS AND DISCUSSION

Flowering buds of *Narcissus tazetta* open into beautiful flowers, with white perianth surrounding a bright yellow corona, providing the flowers with a delinquent appearance. The flowers lasted for five days in full bloom and exhibited the wilting pattern of senescence. The tepals became papery and developed water soaked areas that progressed from the margins towards the base (Fig. 1). Flower senescence, in general, is accompanied by some distinct morphological and biochemical changes which allow the process to be easily documented. Flowers generally have ethylene-sensitive, ethylene-insensitive or intermediate pattern of senescence characterized by petal/sepal abscission (A), wilting (W) or wilting followed by abscission (W/A) (van Doorn 2001, Shahri and Tahir 2011a). In *Narcissus tazetta*, flower senescence was marked first by slight and then considerable loss of turgor, and the perianth lobes finally became flaccid and wilted. Flower senescence was found to be accompanied by a decrease in fresh or dry weight of flowers, increase in the electrical conductivity of ion leachates, protein turn over (synthesis and degradation), reduction in the pool of respiratory sugars along with an increase in the content of α-amino acids and a gradual decrease in the tissue content of total phenols. Flower diameter registered a gradual increase upto stage III, then a sharp increase as the flowers opened (stages IV and V) and a decrease thereafter as the flowers wilted (Fig. 2). Fresh weight, dry weight and water content of the flowers registered a gradual increase as the development progressed from stages I to V and a decrease thereafter (Fig. 2). The greenish buds opened into beautiful white flowers. Flower bud opening involves the expansion of existing cells by promoting the water influx, which inturn leads to an increase in fresh and dry weight (Evans and Reid 1988). Sugar accumulation in cells has been suggested to be a possible mechanism for reducing water potential and promoting water influx (Yamada et al. 2007). It may therefore be suggested that decrease in the fresh and dry weight of the flowers during senescence (stage VI) could be due to the decrease in sugar status of perianth, which leads to decreased water uptake, hence a reduction in the fresh and dry weight of flowers. The observation is supported by the estimation of the sugar status (reducing, non-reducing and total) of the perianth tissues at various stages of flower development and senescence. The tissue content of total and reducing sugars registered an increase as the development progressed from bud to full bloom and a gradual decrease as the flowers senesced (stages V and VI). However, the content of non-reducing sugars registered a gradual decrease throughout flower development and senescence (Fig. 2). Membrane permeability of tepal tissues estimated as electrical conductivity of ion leachates increased as the flowers progressed through various developmental stages (Fig. 2). Throughout various stages of flower development and senescence, membrane permeability (as indicated by the estimation of electrical conductivity of ion leachates) registered a gradual increase first (stages I to IV) and then a sharp increase during senescence (stages IV to VI). Membrane permeability has been shown to increase with age in various flower systems such as *Consolida* (Shahri and Tahir 2011b), *Hemerocallis* (Gulzar et al. 2005), *Helleborus* (Shahri et al. 2011) and *Iris* (Celikel and van Doorn 1995). The increase in ion leakage preceded the visible senescence symptoms and is suggestive of the fact that membrane disintegration occurs before the visible signs of senescence becomes apparent, possibly involving PCD. The increased ion leakage

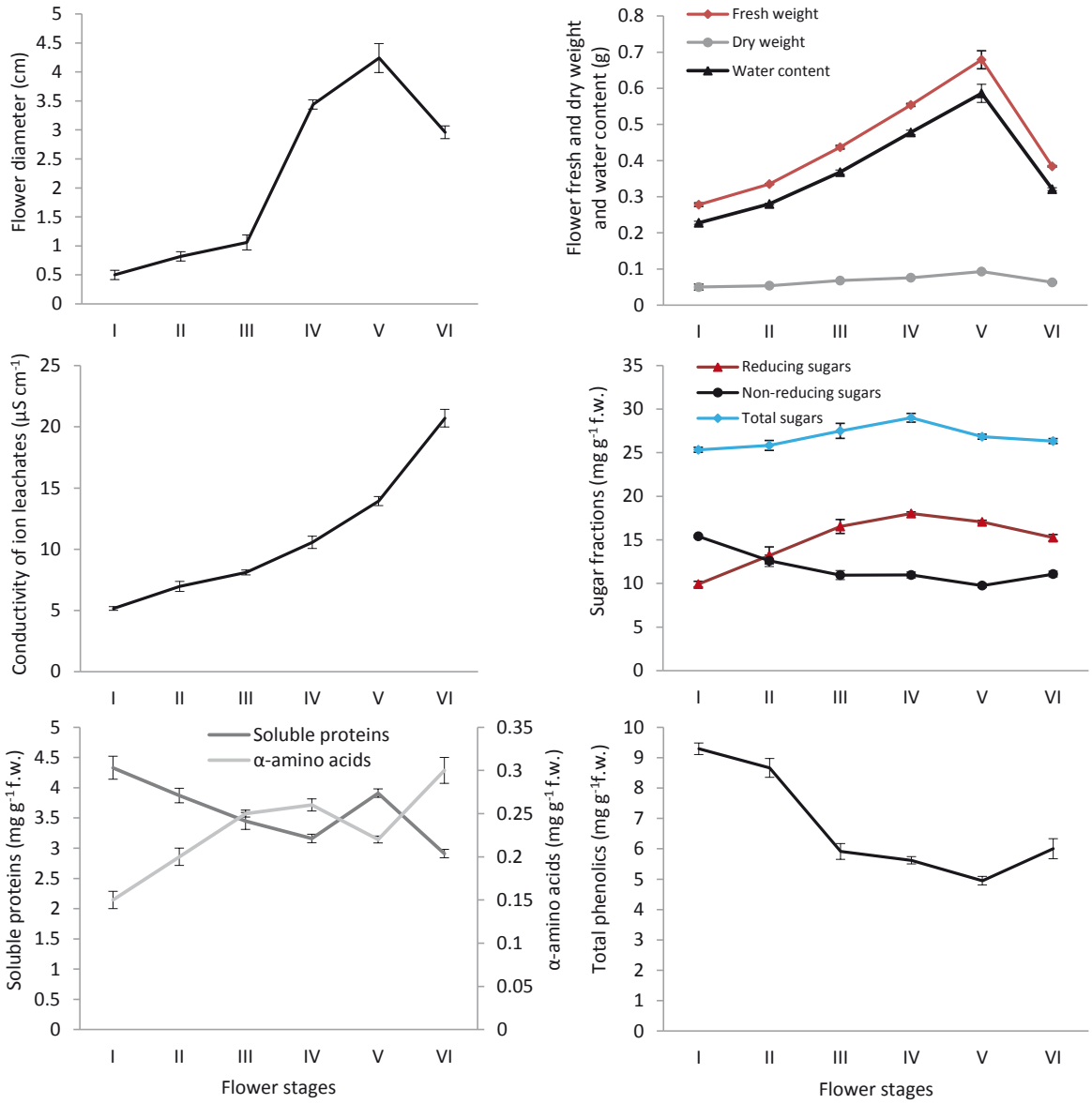

Figure 2. Changes in morphological and biochemical parameters observed at consecutive flower development and senescence stages. See Table 1 for stage description

from perianth tissue has been suggested to be one of the factors responsible for reduction in flower fresh weight and as an indicator of cell death correlated to the measure of dead cells (Celikel and van Doorn 1995). The tissue content of soluble proteins registered a gradual decrease with the concomitant increase in α-amino acids as the flower development progressed through various stages (Fig. 2). It is a well known fact that there is a progressive loss of proteins during flower senescence. The decrease in protein levels has been reported in various flowers systems irrespective of ethylene sensitivity or insensitivity (Williams et al. 1995, Lay-Yee et al. 2002, Shahri and Tahir 2011a, b, c, Shahri et al. 2011). The decrease may be due to increased proteolytic activity or decreased synthesis. A marked increase in proteolytic activity during senescence has been reported in flowers such as *Consolida* (Shahri and Tahir 2011b), *Helleborus* (Shahri et al. 2011), *Iris* (Pak and van Doorn 2005), *Ranunculus* (Shahri and Tahir 2011c) and *Petunia* (Jones et al. 2005). Moreover, the treatment of flowers with compounds that inhibit protein synthesis (cycloheximide) has been found to delay the symptoms of petal senescence, indicating that protein synthesis is required (Gulzar et al. 2005, Shahri and Tahir 2010, Islam et al. 2011). Although the quantitative estimation of soluble proteins from perianth tissue at various stages of flower development and senescence suggested an overall decrease in the protein content, the SDS-PAGE of the protein extracts showed a differential display of protein bands. Some polypeptides were

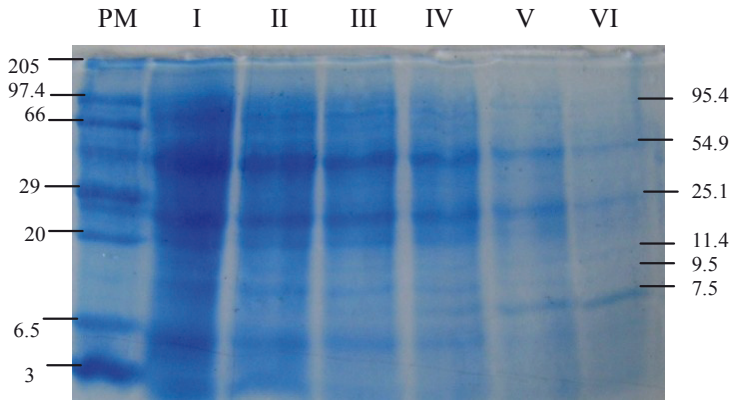

Figure 3. Electrophoretic profiles of crude protein extract from petal tissues at consecutive flower development and senescence stages. See Table 1 for stage description. PM – protein weight marker (GENEI, Bangalore)

found to be down-regulated while there was *de novo* synthesis of others. There was a general decrease in the expression of high molecular weight polypeptides. It corroborates with the earlier findings on other flower systems such as *Helleborus* (Shahri et al. 2011), *Consolida* (Shahri and Tahir 2011b), *Hibiscus* (Woodson and Handa 1987), *Hemerocallis* (Courtney et al. 1994) and *Petunia* (Bai et al. 2010). The decrease was particularly evident in polypeptides with the approximate molecular mass of 54.9, 25.1, 9.5 and 6.5 kDa. The polypeptide with ca. mol mass of 7.5 kDa showed increased expression during senescence (Fig. 3). The low molecular weight polypeptide may either result from the degradation of high molecular weight polypeptides or *de novo* synthesis of proteins which still needs to be ascertained. A gradual increase in α-amino acid content concomitant with the decrease in the soluble protein content may be due to increased protein degradation. The tissue content of total phenols registered a gradual decrease upto stage V and a slight increase during stage VI as the flowers senesced (Fig. 2). The increase of total phenols has been suggested to be due to their antioxidant properties and the scavenger's role that they may play during senescence (Trivellini et al. 2007). Further plant peroxidases are commonly known for their capability to reduce hydrogen peroxide to water at the expense of hydrogen donors like phenolics in most of the cases and recently the increment of antioxidants in broccoli florets has been related to the increment of phenols in the tissues (Hasperuéa et al. 2011). Our results also corroborate with those of Schmitzer et al. (2009), who reported the significantly higher content of phenolic compounds like gallic acid, Protocatechulic acid, chlorogenic acid, caffeic acid and *p*-coumaric acid in buds than the flowers of subsequent stages.

CONCLUSIONS

1. Flower senescence in *Narcissus tazetta* is accompanied by a general decrease in the fresh or dry weight of flowers and an increase in the electrical conductivity of ion leachates from tepals.
2. Tepal senescence also involves protein turn over (synthesis as well as degradation), a reduction in the pool of respiratory sugars, increase in the tissue content of α-amino acids and a gradual decrease in the tissue content of total phenolic content.
3. The present findings suggest that the regulation of flower senescence in Narcissus tazetta may be linked to protein turnover and the sugar status of the perianth tissue which inturn affects other important parameters such as fresh weight, dry weight and membrane permeability.

ACKNOWLEDGEMENTS

The authors thank Prof. Z.A. Reshi, Head of the Department of Botany for providing necessary laboratory facilities.

FUNDING

Fahima Gul received the research scholarship from University of Kashmir for carrying out the research work.

AUTHOR CONTRIBUTIONS

F.G. designed and conducted the experiments, W.S. performed the biochemical analyses, F.G. and W.S. equally contributed in writing the manuscript and I.T. supervised the research work and manuscript writing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Received June 7, 2015; accepted August 30, 2015