



Physiological and biochemical changes associated with flower development and senescence in *Dianthus chinensis* L

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Abstract Flowers of *Dianthus chinensis* growing in Kashmir University Botanic Garden (KUBG) were selected for the present study. Flower development and senescence was divided into six stages (I–VI), categorized as (I) tight bud stage, (II) mature bud stage, (III) paint brush stage, (IV) fully open/bloom stage, (V) partially senescent stage and (VI) senescent stage. Various physiological and biochemical changes associated with flower development and senescence were recorded. Fresh and dry mass, water content and flower diameter showed a continuous increase from bud to bloom, i.e., from stage I–IV and a significant decrease from stage V to VI. Scanning electron microscopic studies showed a clear degeneration of the cellular integrity and architecture with the onset of senescence in *Dianthus chinensis*. Soluble proteins, α -amino acids and sugar fractions increased with flower opening and showed a decrease as the senescence progressed. SDS-PAGE of the petal tissues revealed a decrease in both high and low molecular weight proteins. The present study suggests that the protein degradation is the key factor in regulating the process of flower senescence in this flower.

Keywords *Dianthus chinensis* · Proteins · α - amino acids · Senescence · SEM

Introduction

Dianthus chinensis is a prized ornamental and has a vast potential in floricultural industry. This beautiful

ornamental has a myriad of colors and hues. Flower senescence involves an ordered set of events coordinated at the plant, flower, organ and cellular level (Rogers 2012). The onset of flower senescence is triggered by an array of internal and external factors, which initiates a series of physiological events orchestrated by plant growth regulators (van Doorn and Woltering 2008). Ethylene has been reported to be the main regulator of flower senescence and distinctions have been made between ethylene sensitive and insensitive flower systems (Woltering and van Doorn 1988; Stead and van Doorn 1994; van Doorn and Woltering 2008). Flower senescence in *D. chinensis* has been shown to be dependent on ethylene accompanied by an upsurge in ethylene synthesis and a concomitant climacteric rise in respiration (Nichols 1966; Yang 1984; van Doorn and Reid 1992; van Doorn 2004; Arora et al. 2007; van Doorn and Woltering, 2008; Kazemi et al. 2011; Kazemi, 2012). Increase in membrane leakiness and phospholipid deterioration in petals of *Dianthus* has been found to be due to the increase in production of ethylene with senescence (van Doorn and Woltering 2008). Ethylene receptor genes and signal components have been characterized in several ethylene dependent flower systems such as *Dianthus*, *Als-troemeria*, *Hemerocallis* and *Petunia* (Satoh et al. 2005; Rogers 2012).

Petal senescence in *D. chinensis* has been found to be accompanied by increased protein degradation with a concomitant increase in the protease activity. The present study was undertaken to study the various physiological and biochemical changes associated with flower senescence in *D. chinensis* with the ultimate aim to modulate the senescence process in order to improve the life of this beautiful ornamental.

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Fig. 1 Stages of flower development and senescence in *Dianthus chinensis* L.

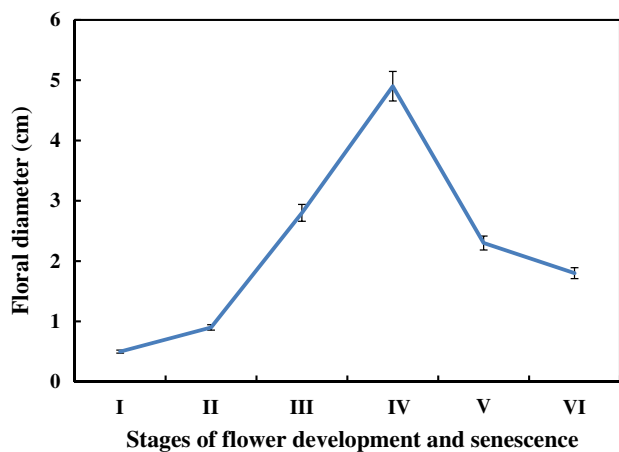


Fig. 2 Changes in floral diameter during various stages of flower development and senescence

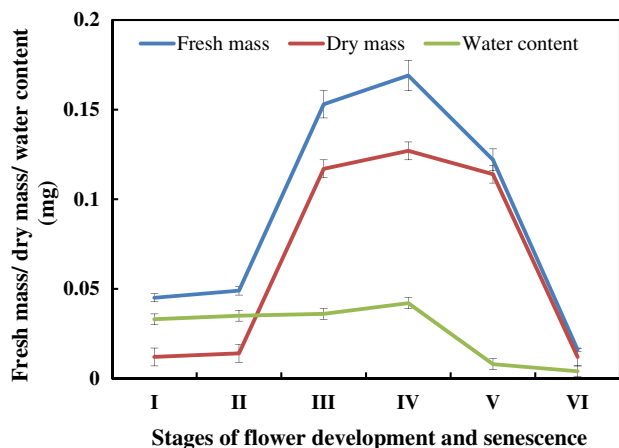


Fig. 3 Changes in fresh and dry mass and water content during various stages of flower development and senescence

Materials and methods

Plant materials

Flowers of *D. chinensis* growing in (KUBG) were selected for the present study. Flower development and senescence

was divided into six stages (stage I–VI). These stages were designated as I: tight bud stage, II: mature bud stage, III: paint brush stage, IV: fully open/bloom stage, V: partially senescent stage and VI: senescent stage (Fig. 1). Visible changes were recorded throughout flower development and senescence. (Figs. 2, 3).

Determination of fresh and dry mass, water content and floral diameter

Ten flowers were taken for the determination of fresh mass at each stage. The flowers were then kept in paper bags and oven dried at 70 °C for 48 h. The material was put in a desiccator for 24 h before recording the dry mass. Water content was determined as the difference between fresh and dry mass. Flower diameter was recorded as the mean of two perpendicular measurements across the flower.

Fixation of plant material

1 g of finely chopped petal tissue was fixed in hot 80 % ethanol in triplicate. The material was macerated in a glass pestle and mortar and centrifuged at 3,000 \times g for 20 min. The supernatants were pooled and made to volume. A suitable aliquot from the supernatant was used for the determination of α - amino acids and sugar fractions.

Estimation of α -amino acids and sugar fractions

α -amino acids were estimated by the method of Rosen (1957) using glycine as the standard. Reducing sugars were determined by the method of Nelson (1944) using glucose as the 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.

Proteins and specific protease activity

For the estimation of proteins 1 g petal tissue was homogenized in 5 ml of 5 % sodium sulphite (w/v) and

0.1 g of polyvinyl pyrrolidone (PVP) and centrifuged at 4,000 g for 20 min in a refrigerated centrifuge. Proteins were precipitated from a suitable volume of supernatant with equal volume of 20 % trichloroacetic acid (TCA) and centrifuged at 2,000 g for 15 min. The pellet was re-dissolved in 4 ml of 0.1 N NaOH and protein content was estimated from a suitable aliquot by the method of Lowry et al. (1951).

For the estimation of protease activity, 1 g of pre-chilled petal tissue was homogenized in 15 ml chilled 0.1 M phosphate buffer (pH 6.5) in a pre-cooled glass pestle and mortar. Protease activity was estimated by the modification of the method as described by Tayyab and Qamar (1992). One ml of enzyme extract was mixed with 1 ml of reaction mixture (0.1 % BSA dissolved in 0.1 M phosphate buffer, pH 6.5). The enzyme activity has been expressed as μg tyrosine equivalents liberated per mg protein per minute ($\mu\text{g tyr. mg protein}^{-1} \text{ min}^{-1}$).

Procedure for SDS-page

At each stage 1 g of petal tissue was homogenized in 1 ml of 0.1 M phosphate buffer (pH 7.2–7.4), containing 0.1 g of PVP. The mixture was centrifuged at 5,000 g at 5 °C in a refrigerated centrifuge (Remi K-24) for 15 min. The supernatant was collected in eppendorf tubes and used for SDS-page. The extracted protein mixture was denatured by mixing equal volumes of protein aliquot with 2X sample loading buffer (0.5 M Tris pH 6.8, 10 % SDS, 10 % glycerol, 5 % β -mercaptoethanol, 0.1 % bromophenol blue). The mixture was incubated in boiling water for 5–7 min. The concentration of protein was determined in both the original extracts and the TCA precipitated samples by the method of Lowry et al. (1951) using BSA as the standard. One dimensional vertical gel electrophoresis was carried out according to the method as described by Ausubel et al. (1989). Slab gels 0.7 mm thick containing 12 % resolving gel [(acrylamide + bisacrylamide), (1.5 M Tris pH 8.8), 10 % SDS, TEMED and 10 % ammonium persulphate] and 3 % stacking gel [(acrylamide + bisacrylamide), (0.5 M Tris pH 6.8), 10 % SDS, TEMED and 10 % ammonium persulphate] were used. SDS-denatured protein extract (80 μl) was loaded into each lane. Electrophoresis was carried out at room temperature with a constant voltage of 50 V during stacking and 150 V during running. GENEI molecular weight standards were used for determining approximate molecular weights (myosin, rabbit muscle 205,000; phosphorylase-b 97,400; bovine serum albumin 66,000; ovalbumin 43,000; carbonic anhydrase 29,000; aprotinin 6,500; insulin (α and β chains) 3,000). Following electrophoresis the gel was stained overnight in 0.25 % coomassie brilliant blue in 45 % methanol: 10 %

acetic acid. Gel was destained in 45 % methanol: 10 % acetic acid, followed by in 7 % methanol: 5 % acetic acid.

Statistical analysis of the data

The values depicted in the figures represent the mean of several independent replicates. Standard deviation has been computed as:

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$$

Scanning electron microscopic studies

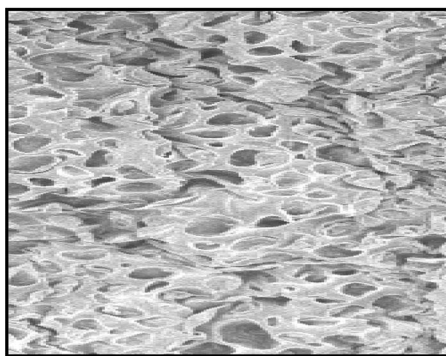
For scanning electron microscopy, the specimens were dried by simple air drying technique. Samples were slowly allowed to dry at room temperature. After complete air drying samples were kept in desiccator for 24 h. The petal tissue was coated with gold and loaded on the plate of the scanning electron microscope (Model Hitachi S3000H). A constant voltage of 5 kV was maintained during the analysis of the petal tissue.

Results and discussion

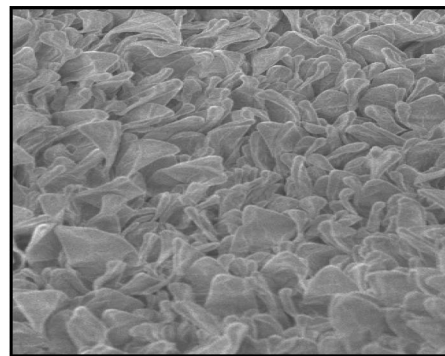
The present study provides insight into the turnover of various physiological and biochemical aspects crucial for the developmental shift from bud until death of the flower. The greenish buds of *D. chinensis* opened into flowers of various colors ranging from white to pink. Flower senescence is preceded by anthesis and is characterized by the loss of petal turgidity and color change, followed by the inrolling of corolla. The average life of individual flower on the sprays after it had opened fully was about 4 days. A significant increase in the ovary size was registered towards flower senescence.

Floral diameter, fresh mass, dry mass and water content increased as the flower development progressed towards opening and a continuous decrease in these parameters was registered with senescence. Our results corroborate with the earlier studies on *Narcissus*, *Nerine*, rose, *Iris* and *Consolida* (Evans and Reid 1988; van Doorn et al. 1991; Gul and Tahir 2009; Shahri and Tahir 2011; Gul and Tahir 2012). Decrease in the fresh and dry mass can be attributed to the fact that flowers act as source during senescence for ecological benefits of the plant (Zhou et al. 2005; Shahri and Tahir 2011; Shahri et al. 2011). During the final developmental stage the resources are reallocated to the developing parts of the plant. Thus nutrient recycling seems to be the basic phenomenon for getting rid of the flowers once pollination has taken place (ten Have and Woltering 1997; Tripathi and Tuteja 2007). Increase in the

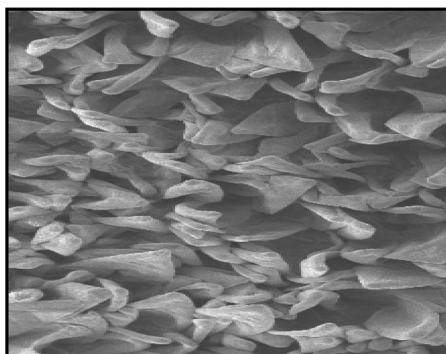
Fig. 4 Scanning electron micrographs of different stage of flower development and senescence in *Dianthus chinensis*. The photographs have magnification of 400X. Note the turgidity of cells at stage IV (fully open stage) and cellular disorganization at stage V and VI (partially senescent stage and senescent stage)



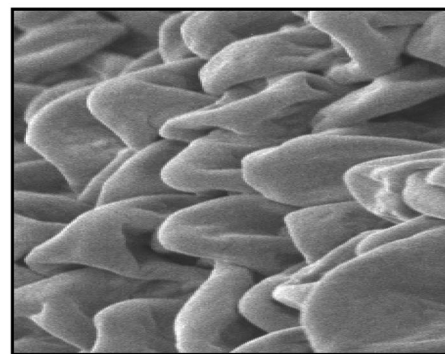
Stage I



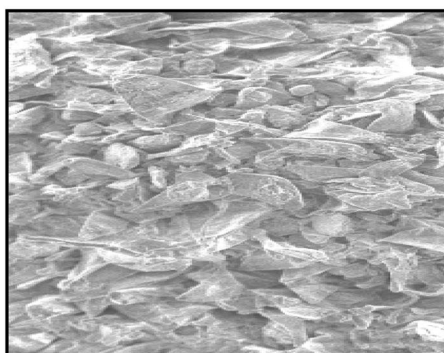
Stage II



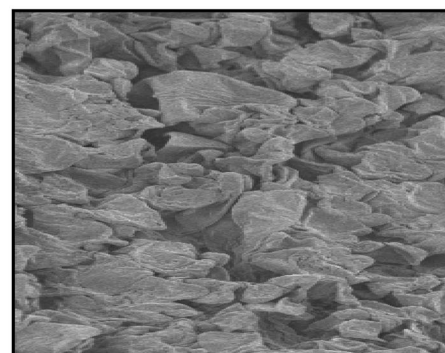
Stage III



Stage IV



Stage V



Stage VI

water content towards flower opening is attributed to the increase in cell turgidity, which is an important criterion for flower opening (Yamada et al. 2007).

The scanning electron microscopic (SEM) studies of the petal tissues revealed subtle changes in the cellular architecture at various stages of flower development and senescence. The study revealed that the cells maintain their integrity and gain turgor as the flower development progressed towards opening. At the open flower stage the cells were fully turgid but loss of turgidity and integrity was visualized as the flowers progressed towards senescence

(stage V–VI) (Fig. 4). The study also revealed that cellular decompartmentalization occurs during senescence. Moreover the cells lose their integrity as the middle lamella gets dissolved, thereby separating the adjacent cells. Our results are in agreement with the earlier studies on rose and carnation (Smith et al. 1992).

The concentration of soluble proteins from petal tissues increased as the flowers opened through stages I–IV and thereafter showed a continuous decrease as the senescence progressed through stages V and VI (Fig. 5). The specific protease activity showed a decrease from bud to bloom and

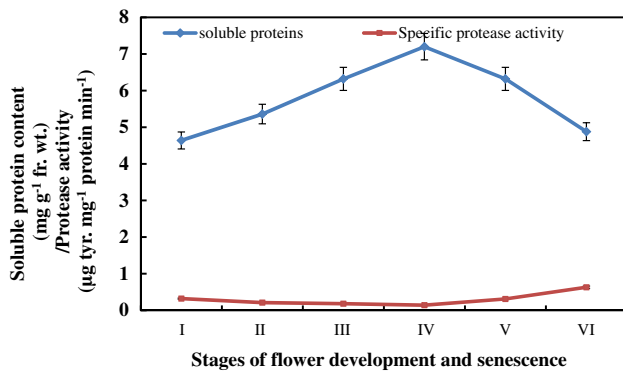


Fig. 5 Changes in soluble protein content and protease activity during various stages of flower development and senescence

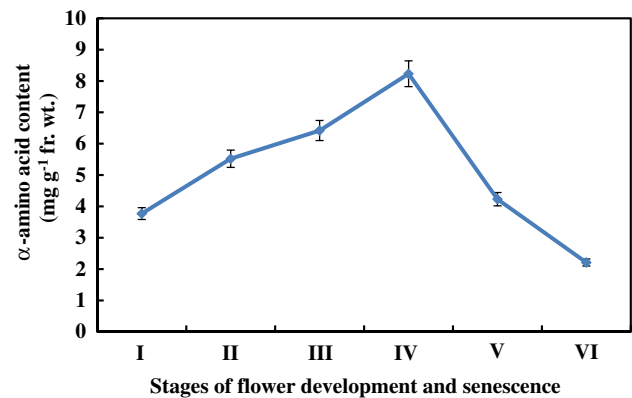


Fig. 6 Changes in α-amino acids content during various stages of flower development and senescence

thereafter showed an increase with senescence (Fig. 5). The increase in protease activity with a concomitant decrease in proteins manifests the earliest signs of flower senescence related to gene changes (Eason et al. 2002). Increase in protease activity towards flower senescence has been reported in various flowers like *Hemerocallis*, *Iris*, *Petunia* and *Consolida* (Stephenson and Rubinstein 1998; Pak and van Doorn 2005; Jones et al. 2005; Shahri and Tahir 2011). During the present study, it was observed that there was a marginal increase in the α-amino acid content towards flower opening but showed a significant decrease towards senescence (Fig. 6). Our results are in agreement with the findings on the flowers such as *Hemerocallis* and *Consolida* (Beileski 1995; Shahri and Tahir 2011). The decrease in the α-amino acid content can be attributed to the fact that petals act as a source and the ovary as a metabolic sink during flower senescence and as such the amino acid pool is rapidly transported to the developing ovary. The present investigations revealed that the reducing, non-reducing and total sugar fractions registered a continuous increase up to flower opening and then showed a concomitant decrease as the senescence progressed through stages V and VI (Fig. 7). Flower maturation and senescence has been shown to be accompanied by a decline in the carbohydrate content in various flower systems like *Hemerocallis*, *Helleborus*, *Consolida* and rose (Nichols 1973; Paulin and Jamain 1982; Lukaszewski and Reid 1989; Lay-yee et al. 1992; Beileski 1993; Mwangi et al. 2003; Gulzar et al. 2005; Reid 2005; Shahri and Tahir 2011; Shahri et al. 2011). SDS-PAGE analysis of petal tissue revealed an overall decrease in both low and high molecular weight proteins at senescence. Structural patterns showed that a polypeptide with a molecular mass of ca 37.3 kDa was generally maintained throughout flower development and senescence. A low molecular weight protein having molecular mass of ca 14.3 kDa appeared

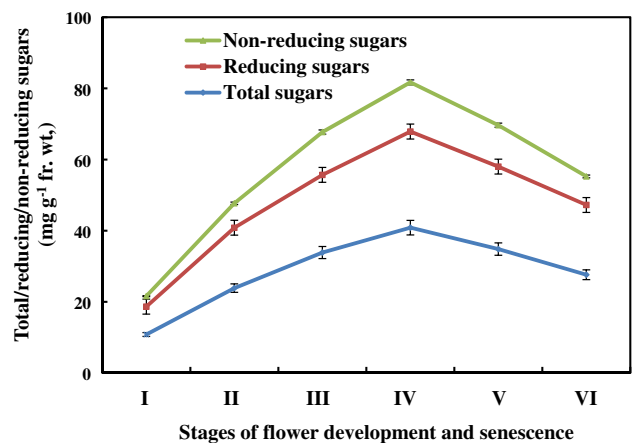


Fig. 7 Changes in sugar fractions during various stages of flower development and senescence

during anthesis (Fig. 8). Protein degradation started soon after the flower started showing visible signs of senescence like loss of petal turgidity. The protein content was maintained up to open flower stage and the decrease in the concentration of proteins with senescence could be attributed to the decreased protein synthesis or increased activity of proteolytic enzymes (Celikel and van Doorn 1995). In case of *Hemerocallis* flowers a decline in both high and low molecular proteins has been reported with senescence (Lay-Yee et al. 1992; Celikel and van Doorn 1995).

The present study revealed that protein turnover along with the changes in sugar fractions can be attributed as the key factors associated with flower development and senescence in *D. chinensis*. Further investigations on protein patterns are required that can offer clues to flower senescence and its modulation in this beautiful flower.

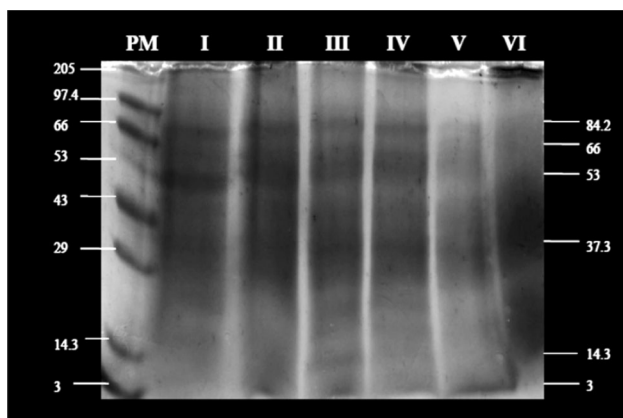


Fig. 8 Electrophoretogram depicting various stages of flower development and senescence in *Dianthus chinensis*

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