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Morphological and some biochemical aspects of flower development and senescence in *Narcissus poeticus* cv. Pheasant's Eye

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ABSTRACT

A study involving the morphological and some biochemical changes during flower development and senescence was undertaken to understand the basic mechanism of tepal senescence in *Narcissus poeticus* cv. Pheasant's Eye. For studies related to senescence, flower development has been divided into easily recognizable six morphological stages (Stages I to VI) and changes in some important morphological and biochemical parameters including fresh and dry mass of flowers, electrical conductivity of ion leachates from tepal discs, soluble protein content, α-amino acids and soluble solid content (sugars and total phenols) were recorded. SDS-PAGE (Sodium dodecyl sulphate-Polyacrylamide Gel Electrophoresis) of crude protein extract was also performed at different stages of flower development and senescence. The results of present study indicates that the loss of floral fresh and dry mass, loss of membrane permeability, decline in the tissue content of soluble proteins with concomitant increase in α-amino acid content and changes in the soluble solid content (sugar status and total phenols) may be considered as important parameters of tepal senescence in this flower system.

***Keywords*: Membrane permeability, Phenols, Senescence, Soluble proteins, Sugars**

1. INTRODUCTION

Senescence is the final stage in the overall development of an organism and has been found to involve a developmentally controlled and programmed sequence of events that ultimately culminates in the death of plant cells, tissues, organs and even the entire organism. At the cellular level, senescence is regarded synonymous to programmed cell death (PCD) which involves many events like degradation of membrane components, lipid peroxidation, upregulation of many catalytic enzymes like proteases, nucleases, oxidative enzymes and remobilization of essential nutrients [1-4]. Flowers provide an excellent system to study senescence as it (flower senescence) being rapid, and can be easily documented by distinct morphological and biochemical changes concomitant with the progression of senescence to its ultimate end [5]. Of the different floral whorls, the petals/tepals carry much significance being the most attractive part of the flower on one hand (horticultural aspect) and attractant for pollinators on the other hand (biological aspect). *Narcissus poeticus* is commonly known as Poet's Daffodil, Pheasant's Eye, Findern Flower and Pinkster Lily. It was one of the first daffodils to be cultivated and is frequently referred to as the *Narcissus* of the ancient times. The earliest mention of Poet's Daffodil is in the botanical writings of Theophrastus who wrote about a spring-blooming *Narcissus* that was later on identified as *Narcissus poeticus*. Poeticus is fragrant, with a ring of pure white tepals and a short corona of light yellow with a distinct reddish edge (Fig. 1). It is the last to flower of all the charming species from the genus *Narcissus* and its snowy blooms announce the arrival of approaching summer. Poet's Daffodil is native to north Meditteranean and has been found extensively from Spain to Greece. It has also been widely naturalized in North America and Europe. In Kashmir, Poet’s Daffodil is found growing wild on hills and is being cultivated in homes also. In the present study an attempt was made to understand the pattern and mechanism involved in flower development and senescence in *Narcissus poeticus* cv. Pheasant’s Eye.

II MATERIALS AND METHODS

Flowers of *N. poeticus* growing in the University Botanic Garden were used for the study. Flower development and senescence was divided into six stages. These stages were designated as tight bud stage (I), loose bud stage (II), half open stage (III), fully open stage (IV), partially senescent stage (V) and senescent stage (VI) (Fig. 2). Visible changes were recorded throughout flower development and senescence at periodical intervals. Floral diameter, fresh and dry mass were determined at each stage. Changes in membrane permeability were estimated by measuring the conductivity of leachates (µS) in tepal discs (5mm in diameter) punched from outer regions of perianth of five different flowers and incubated in 15ml glass distilled water for 15 h at 20^{o}C. For the estimation of tissue constituents 1g 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 were used for the estimation of reducing sugars, non-reducing sugars, total sugars, α- amino acids and total phenols. Non-reducing sugars were calculated as the difference between total and reducing sugars. The estimation of sugar fractions, α-amino acids and total phenols were made using the method of Nelson [6], Rosen [7] and Swain and Hillis [8] respectively. Soluble proteins were extracted from 1 g of the tepal tissue drawn separately from five different flowers at each of the six stages and suitable aliquots were used for the estimation. The soluble protein content of perianth tissue was estimated by the method of Lowry et al. [9]. Electrophoretic profiles of crude protein extract from tepal samples at various stages of flower development and senescence were obtained by the method of Ausubel [10]. 80 µL of the SDS- denatured protein extract was loaded into each lane. Each value represented in the tables corresponds to the mean of five to ten independent replicates. The data have been analyzed statistically by computing standard deviation.

III RESULTS

The greenish buds of *N. poeticus* open into bright white flowers with a small yellow cup shaped corona at the centre surrounded by tepals and the flower senescence is marked by the turgor loss in the perianth followed by complete wilting. The average life span of an individual flower after it opens fully is about 4 days. Flower diameter increased as the flower development progressed up to stage V and declined thereafter as the floral development progressed to senescence up to stage VI (Fig. 3A). Fresh mass, dry mass and water content of flowers increased with flower development up to stage IV and registered a decline thereafter as the senescence progressed through stages V and VI (Fig. 3B). Membrane permeability estimated as electrical conductivity of leachates (µS) from tepal discs increased as the flower development and senescence progressed through various stages, however almost steady increase in the ion leachates was registered up to stage IV after which the leachates increased considerably during senescence phase (stages V and VI) (Fig. 3C). The tissue content of reducing sugars increased during flower development from stage I to stage V and declined thereafter during senescence stage VI. The tissue content of non-reducing sugars was by and large maintained up to stage III followed by a decrease up to stage V after which a sharp increase was noticed in the non-reducing sugar content. The total soluble sugars decreased slightly during stage I to II and thereafter increased steadily up to stage V, a sharp decline was noticed thereafter during senescence at stage VI (Fig. 3D). The concentration of soluble proteins decreased as the flower development and senescence progressed from stage I to stage VI. The α-amino acid content registered an initial decrease during flower development from stage I to stage III after which a consistent increase was registered in the amino acid content as the flower development and senescence progressed from stage III to VI (Fig. 3E). The concentration of total phenols decreased as the flower development progressed from stage I to stage III and increased thereafter up to stage V followed by a sharp decline at stage VI (Fig. 3F). The SDS-PAGE of tepal proteins at various stages of flower development and senescence showed that most of the polypeptides were consistent from stage V and they almost disappeared at the last stage (stage VI) particularly polypeptides of molecular Weights of 83.1, 60.2 and 31.6 kDa (Fig. 4).

IV DISCUSSION

The flowers of *Narcissus poeticus* showed wilting type of perianth senescence in which tepals develop water soaked areas and lost turgidity prior to severe wilting. According to Chapin and Jones [11] and Jones [12], one of the important aspects of flower wilting is the nutrient remobilization from the senescing floral organs (petals/tepals) to other floral organs (ovary). Fresh as well as dry weight of flowers decreased as the development proceeded towards senescence. The decline in fresh and dry weight has been reported in other flower systems as day-lily, *Alstroemeria* and *Consolida* [5, 13-15]. The decrease in fresh weight may be attributed to the reduction in the water content of flowers as they progressed towards senescence. As far as the decline in dry weight is concerned, it may be due to the decline in the sugar status and soluble protein content of tepal tissues as both soluble protein content and sugar status (reducing and total sugars) declined during senescence. The sugar status (reducing and total) registered an initial increase upto the anthesis and it has been suggested to be the active involvement of sugars in petal expansion [16]. There was an increase in the electrical conductivity of ion leachates from the tepal samples as the flowers progressed through the designated stages of development. The increase in conductivity has been correlated to the loss of membrane permeability which has been reported to be an important feature of petal/tepal senescence [2].The tissue content of soluble proteins from tepals of flowers undergoing senescence showed a decline in the soluble proteins throughout the development (from small bud stage to senescent stage). The decrease in the perianth protein levels have been reported in other flower systems and have been suggested to be due to either accelerated proteolytic activity or slowing down of protein synthesis. The protein degradation has been reported to be brought out by proteasome-dependent and proteasome-independent (proteases) pathways [2-4, 15]. Moreover, the increase in the tissue content of α-amino acids was concomitant with the decrease in the soluble protein levels which may be suggestive of the fact that active protein degradation occurs at the onset of senescence with the resulting amino acids being the degradation products. van Doorn and Woltering [2] reviewed that these amino acids play an important role in remobilization process. The decline in the protein levels is also confirmed by the SDS-PAGE results of the tepal protein extract which showed a differential expression of proteins that corroborates the findings on other flower systems [3, 15, 17-18]. The tissue content of phenols from tepals has been found to be maintained more or less during flower development but as the senescence approaches there was a sharp increase and a decline thereafter. The increase in the phenolic content of tepal tissues has been related to their antioxidant properties and free-radical scavenging properties [19].

V CONCLUSIONS

In conclusion, these results indicate that the loss of floral fresh and dry mass, decrease in membrane permeability, decline in the soluble proteins and changes in the soluble solid content (sugar status and phenolics) might act as important indicators of flower senescence in *Narcissus poeticus*.

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REFERENCES

[1] Y. Xu, and M.R. Hanson, Programmed cell death during pollination-induced petal senescence in *Petunia*. *Plant Physiology*, *122*, 2000, 1323-1333.

[2] W.G. van Doorn, and E.J. Woltering, Physiology and molecular biology of petal senescence, *Journal of Experimental Botany*, *59*, 2008, 453-480.

[3] W. Shahri, and I. Tahir, Flower senescence: strategies and some associated events, *Botanical Review*, *77*, 2011a, 152-184.

[4] W. Shahri, I. Tahir, Flower senescence: some molecular aspects, *Planta*, *239*, 2014, 277-297.

[5] R.L. Bieleski, Onset of phloem export from senescent petals of daylily, *Plant Physiology*, *109*, 1995, 557-565.

[6] N. Nelson, Photometric adaptation of Somogy's method for the determination of glucose, *Journal of Biological Chemistry*, *153*, 1994, 375-380.

[7] H. Rosen, A modified ninhydrin colorimetric method for amino acids, *Archives for Biochemistry and Biophysics*, *67*, 1957, 10-15.

[8] T. Swain, and W.E. Hillis, The phenolic constituents of *Prunus domestica* L. The quantitative analysis of phenolic constituents, *Journal of Food Science and Agriculture*, *10*, 1959, 63-68.

[9] O.H. Lowry, N.J. Rosenbrough, A.L. Farr, and R.J. Randall, Protein measurement with folin phenol reagent. *Journal of Biological Chemistry*, *193*, 1951, 265-275.

[10] F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.C. Seidman, and K. Struhl, *current protocols in molecular biology* (John Wiley and Sons, New York, 1989).

[11] L.J. Chapin, and M. Jones, Ethylene regulates phosphorus remobilization and expression of a phosphate transporter (PhPT1) during petunia corolla senescence, *Journal of Experimental Botany*, *60*, 2009, 2179-2190.

[12] M.L. Jones, Mineral nutrient remobilization during corolla senescence in ethylene-sensitive and -insensitive flowers, *AoB Plants (SPECIAL ISSUE: Ethylene 2012)*, *5*, 2013, 1-11.

[13] M. Lay-Yee M, A.D. Stead, and M.S. Reid, Flower senescence in daylily (*Hemerocallis*), *Physiologia Plantarum 86*, 1992, 308-314.

[14] D.E. Collier, Changes in respiration, protein and carbohydrates of tulip tepals and *Alstroemeria* petals during development, *Journal of Plant Physiology*, *150*, 1997, 446-451

[15] W. Shahri, and I. Tahir, Physiological and biochemical changes associated with flower development and senescence in *Consolida ajacis* Nieuwl cv. Violet blue, *Frontiers of Agriculture in China*, *5*, 2011b, 201-208.

[16] A. Trivellini, A. Ferrante, P. Vernieri, G. Carweighti, and G. Serra, Spatial and temporal distribution of mineral nutrients and sugars throughout the lifespan of *Hibiscus rosa-sinensis* L. flower. *Central European Journal of Biology*, *6*, 2011, 365-375.

[17] S. Bai, B. Willard, L.J. Chapin, M.T. Kinter, D.M. Francis, A.D. Stead, and M.L. Jones, Proteomic analysis of pollination-induced corolla senescence in *Petunia*, *Journal of Experimental Botany 61*, 2010, 1089-1109.
[18] W. Shahri, I. Tahir, S.T. Islam, and M.A. Bhat, Physiological and biochemical changes associated with flower development and senescence in so far unexplored *Helleborus orientalis* Lam. cv. Olympicus. *Physiology and Molecular Biology of Plants*, *17*, 2011, 33-39.
[19] M. Cavaiuolo, G. Cocetta, and A. Ferrante, The antioxidant changes in ornamental flowers during development and senescence, *Antioxidants*, *2*, 2013, 132-155.

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Fig. 1: Scapes of Narcissus poeticus cv. Pheasant's Eye in full bloom

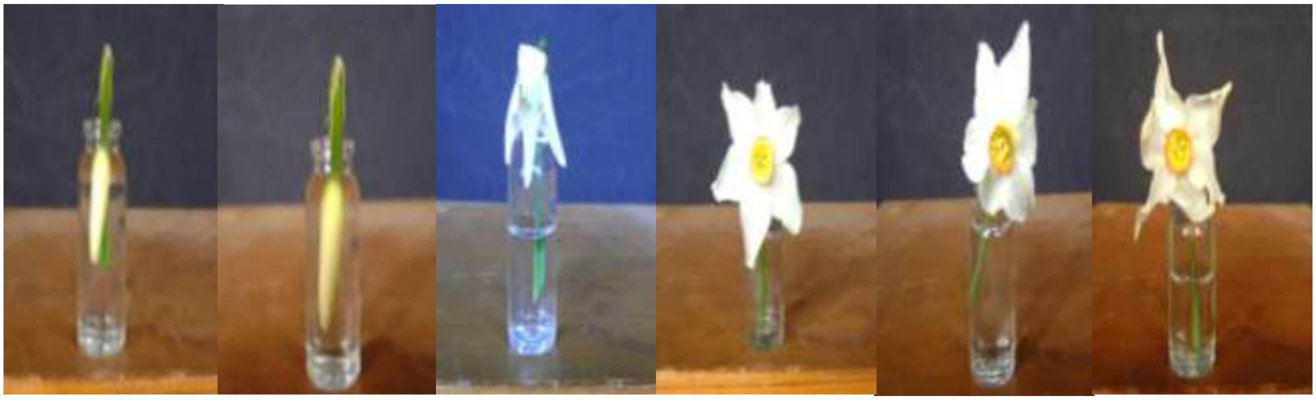

Fig. 2: Stages of flower development and senescence in *Narcissus poeticus* cv. Pheasant's Eye (From left to right are stages I to VI)

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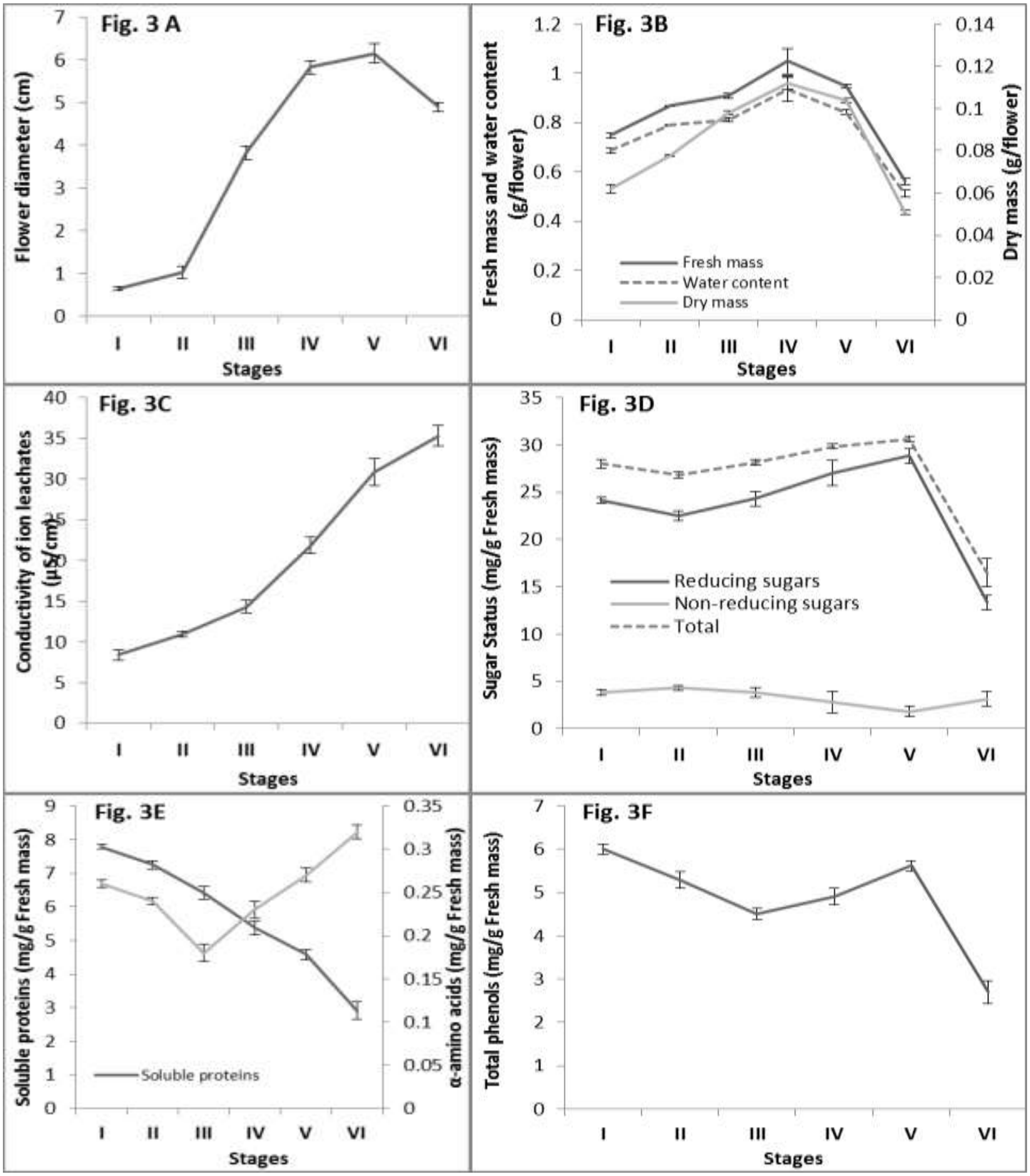

Fig. 3: Changes in some physiological and biochemical parameters during flower development and senescence in Narcissus poeticus cv. Pheasant’s Eye

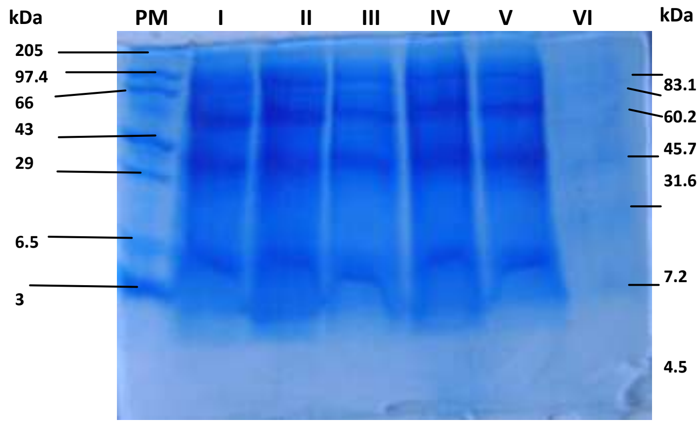

Fig. 4: SDS–PAGE of crude protein extract at various stages (I-VI) of flower development and senescence from tepal tissues of *Narcissus poeticus* cv. Pheasant's Eye. The gel was stained with coomassie blue. Number above the lanes corresponds to developmental stages. Molecular weight standards are indicated on the left (kDa) and approximate molecular weights of major polypeptides to the right of the gel (kDa).