FLOWER DEVELOPMENT AND SENESCENCE IN *Ranunculus asiaticus* L.

Waseem Shahri and Inayatullah Tahir

Department of Botany, University of Kashmir, Srinagar- 190006, INDIA
Running title: Petal Senescence
e-mail: waseem.bot@gmail.com

(Received November 24, 2010/Accepted July 7, 2011)

A B S T R A C T

Flower development of *Ranunculus asiaticus* L. growing in the University Botanic Garden was divided into six stages (I – VI): tight bud stage (I), loose bud stage (II), half open stage (III), open flower stage (IV), partially senescent stage (V) and senescent stage (VI). The average life span of an individual flower after it is fully open is about 5 days. Membrane permeability of sepal tissues estimated as electrical conductivity of ion leachates (μS), increased as the development proceeded through various stages. The content of sugars in the petal tissues increased during the flower opening period and then declined during senescence. The soluble proteins registered a consistent decrease with the simultaneous increase in specific protease activity and α-amino acid content during different stages of flower development and senescence. The content of total phenols registered an initial increase as the flowers opened, and then declined during senescence.

Keywords: α-amino acids, flower senescence, membrane permeability, protease activity, soluble proteins, *Ranunculus asiaticus*, tissue constituents

INTRODUCTION

Senescence comprises those processes that follow physiological maturity leading to the death of a whole plant, organ or tissue, at the macroscopic level as well as microscopic level. It is a dynamic, closely regulated developmental process which involves highly coordinated changes in gene expression and requires active gene transcription and protein translation (Yamada et al., 2003; Hoeberichts et al., 2005; Jones, 2008). Flower petals are ideal tissues for cell death studies as they are short lived. Flower tissue is relatively homogenous, thus chemical manipulation can be applied without substantial wounding. Petal senescence

has been found to be accompanied by increase in the activity of catabolic enzymes, ion leakage, and nuclear fragmentation. This is all directed towards mobilization of nutrients from petals to other parts of the plant e.g. developing ovary (Halevy and Mayak, 1979; Xu and Hanson, 2000; Zhou et al., 2005; Chapin and Jones, 2007; van Doorn and Woltering, 2008). Ethylene has been shown to modulate senescence in a number of flowers (Woltering and van Doorn, 1988; van Doorn, 2001; Shahri and Tahir, 2011a). The present investigation has been undertaken on *Ranunculus asiaticus* to understand the changes occurring during flower development and senescence, with the aim to improve the postharvest performance of this flower. It is an ethylene-insensitive perennial geophyte that has a tuberous root and segmented leaves characteristic of the family Ranunculaceae (Kenza et al., 2000). It is commonly known as the Persian butter cup. The plants have bowl-shaped or cup to saucer shaped flowers with a range of beautiful colours, borne singly or in cyme-like panicles. The plant requires a mild climate. Flowering occurs during spring and early summer in outdoor gardens or during the winter and spring in greenhouses (Fig. 1).

MATERIAL AND METHODS

Flowers of *Ranunculus asiaticus* growing in the Kashmir University Botanic Garden were used. Flower development and senescence was divided into six stages. These stages were deciphered as the: tight bud stage (I), loose bud stage (II), half open stage (III), open flower stage (IV), partially senescent stage (V) and senescent stage (VI). Visible changes were recorded throughout flower development and senescence (Fig. 3 a).

Figure 1. Flowers of *Ranunculus asiaticus* in full bloom

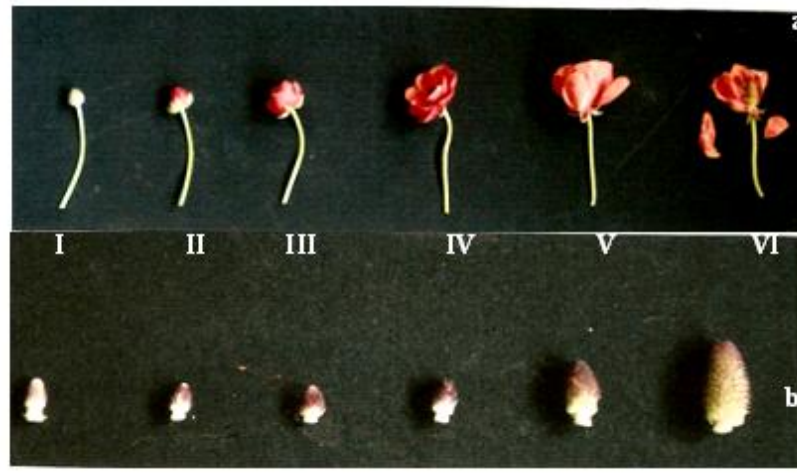


Figure 3. Stages of flower development and senescence in *Ranunculus asiaticus* (a) and increase in the pistil dimensions during various stages of flower development and senescence (b)

Floral diameter, fresh and dry mass of 10 flowers as well as pistils were determined at each stage. Dry mass was determined by drying the material in an oven at 70 ^{o}C for 48 h. Changes in membrane permeability were estimated by measuring the electrical conductivity (μS) of leachates of 5 petal discs per flower (5 mm in diameter) punched from outer regions of petals of five different flowers incubated in a 15 ml glass of distilled water for 15 h at 20 ^{o}C.

At each stage, 1 g of chopped material of the petal tissue drawn from 5 different flowers was fixed in hot 80% ethanol. The material was macerated and centrifuged three times. The supernatants were pooled and used for the measurement of the amount of sugars, amino acids and total phenols. 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. α-amino acids were estimated (Rosen method, 1957) using glycine as the standard. Total phenols were estimated by the method of Swain and Hillis (1959) using gallic acid as the standard.

Proteins were extracted from 1 g of petal tissue drawn separately from 5 different flowers. The tissue was homogenized in 5 ml of 5% sodium sulphite (w/v) adding 0.1 g of polyvinylpyrrolidone (PVP), and centrifuged. Proteins were precipitated from a suitable volume of the cleared supernatant with an equal volume of 20% trichloroacetic acid (TCA), centrifuged at 1000 x g for 15 minutes. The pellet was redissolved in

0.1 N NaOH. Proteins were estimated from a suitable aliquot by the method of Lowry et al. (1951) using Bovine serum albumin (BSA) as the standard.

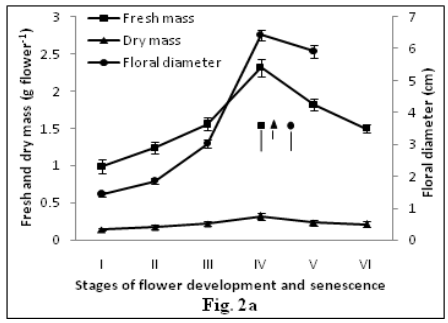
For protease activity determination, at each stage 1 g pre-chilled petal tissue (in 5 replicates) was homogenized in 15 ml chilled 0.1 M phosphate buffer (pH 6.5) in a pre-cooled glass pestle and mortar. The contents were squeezed through four layers of muslin cloth and centrifuged for 15 minutes at 5000 x g in a (Remi K- 24) refrigerated centrifuge at -5 ^{o}C. The supernatant was used for the assay of protease activity by the method of Tayyab and Qamar (1992), with modification. The reaction mixture comprised 1 ml of 0.1% BSA dissolved in a 0.1 M phosphate buffer (pH 6.5). The reaction was stopped by adding 2 ml of 20% cold TCA. Blanks in which TCA was added prior to the addition of the enzyme extract were run along with each sample. The contents were centrifuged and supernatants collected. Free amino acids were estimated (as tyrosine equivalents) in a suitable aliquot of the supernatant by the method of Lowry et al. (1951), using tyrosine as the standard. The specific enzyme activity was expressed as µg tyrosine equivalents liberated per mg of protein in the tissue extract.

Each value represented in the tables corresponds to the mean ± S.E of five to ten independent replicates. The data has been analyzed statistically and LSD computed at $P_{0.05}$ using MINITAB (v 15. 1.2-EQUINOX_Softddl.net) software.

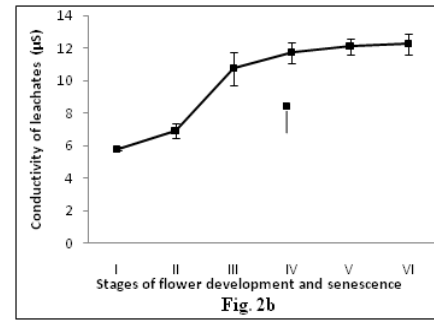
RESULTS

The greenish buds open into brilliant red flowers with a cluster of brownish stamens at the centre surrounding the pistil. The stamens turn blackish and abscise at senescent stage, whereas the petals showed a typical colour change from dark red to brick red. The petals loose shape, become flaccid and begin to wither. The withered petals finally abscise upon slight teasing and the pistils increase in dimensions and develop into fruit (etaerio of achenes). The average life span of an individual flower after it opens fully is about 5 days. Diameter, fresh mass, dry mass and water content of flowers increased as the flower development progressed up to stage IV, and then declined as the senescence progressed through stages V and VI (Fig. 2a). Membrane permeability estimated as electrical conductivity of ion leachates (µS) from petal discs, registered a gradual increase during various stages of flower development and senescence (Fig. 2b). Throughout flower development and senescence, the fresh and dry mass of the pistil increased, however, the increment of increase was pronounced during stages IV to VI (Fig. 3b and 2c).

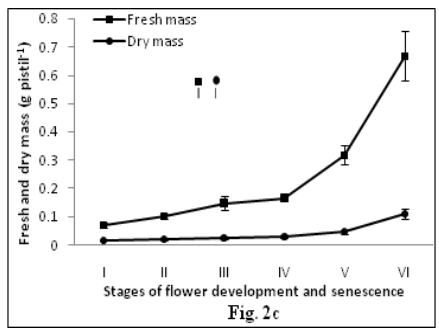
The tissue content of total and reducing sugars increased through stages I to V and then quickly declined during senescence (VI). The concentration of non-reducing sugars initially increased through stages I to III, decreased during stages IV and V but registered a slight increase at



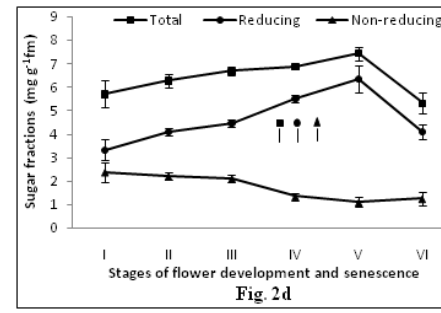
a) changes in fresh and dry mass of flowers



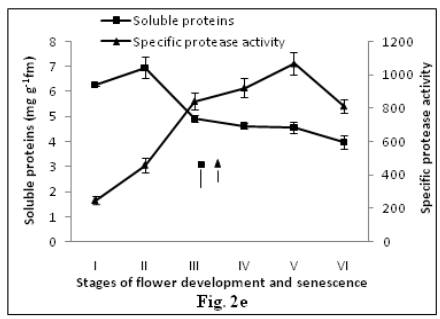
b) changes in conductivity of ion leachates



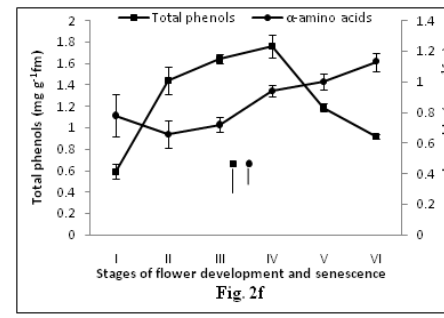
c) changes in fresh and dry mass of pistils



d) changes in the content of sugar fractions



e) changes in the content of soluble proteins and specific protease activity



f) changes in the content of á-amino acids and total phenols

Figure 2. Changes of same physiological parameters during various stages of flower development and senescence. Vertical bars with their corresponding markers represent LSD at $P_{0.05}$

stage VI. The concentration of non-reducing sugars was much lower compared to that of reducing sugars during stages III to VI (Fig. 3d). The concentration of soluble proteins registered an increase as the development progressed from stage I to II and a gradual decline thereafter dur-

ing senescence (2d). The specific protease activity registered an increase during stages I to V but decreased during stage VI. A sharp increase in protease activity was registered as the flower development progressed through stages I to IV (Fig. 2e).

The α-amino acid content showed a slight decrease from stage I to II and increased thereafter (Fig. 2f). The concentration of total phenols increased during stages I to IV and decreased during senescence, stage V and VI (Fig. 2f).

DISCUSSION

The results of our study suggest that the initial symptom of flower senescence in *Ranunculus asiaticus* is wilting (stage V), followed by abscission at the later stage (stage VI) and is marked by: 1) change in colour of petals from dark red to brick red, 2) change in colour of anthers from brown to black, 3) gradual separation of central cluster of stamens from the pistil and 4) an increase in the dimension of the pistil. The fresh and dry mass, water content and soluble carbohydrates showed an increase in the petal tissues during the process of floral development from bud to fully open bloom, after which a declining trend was found during senescence. The changes such as the decline of fresh mass, dry mass and soluble carbohydrates are often linked to PCD (Zhou et al., 2005). During the current investigation, it was revealed that fresh and dry mass of pistil increased consistently from bud to bloom and then sharply increased during senescence suggesting that the developing pistil becomes a strong sink during senescence. Flower maturation and senescence has been found to be accompanied by a decline in total soluble carbohydrate content in various flowers such as carnations, *Hemerocallis*, *Iris* and rose (Paulin and Jamain, 1982; Lukaszewski and Reid, 1989; Lay-Yee et al., 1992). Van Doorn and Woltering (2008) reported that sugar metabolism is active in senescent cells. This happens because many carbon skeletons that are remobilized from macromolecules are transported out of the petal, mainly as sucrose.

The petal senescence of *Ranunculus asiaticus* exhibited a general increase in ion leakage during different stages of flower development and senescence. The extent of leakage registered a sharp increase during the early stages of flower development (stage II to III). This increase suggests that the loss of membrane permeability occurs much earlier, before the visible signs of senescence become apparent. A consistent feature of senescence is the loss of differential permeability of cell membranes leading to the loss of ionic gradients and pumps. Membrane permeability has been shown to increase with age in various flowers (Celikel and van Doorn, 1995; Gulzar et al., 2005; Shahri and Tahir, 2011b; Shahri et al., 2011). Van Doorn and Woltering (2008) suggest that leakage may be rather an indicator of cell death and its increase is a measure of dead cells.

We observed a loss of proteins with a concomitant increase in amino acids as the flower senesced. An overall decrease in cell protein levels has been reported in various ethylene sensitive (*Consolida*, *Petunia*) and ethylene-insensitive (*Hemerocallis*) flower senescence (Jones et al., 2005; Lay-Yee et al., 2002; Shahri and Tahir, 2011b). An apparent increase in the amino acid content during the final stages of senescence may be due to the increase in protein degradation by various classes of proteases whose transcripts have been found to accumulate in senescing floral tissues (Jones et al., 2005). During the current study, a pronounced increase in the protease activity as the flowers progressed towards senescence was observed. The activity was commensurate with a drastic decrease in soluble proteins. The expression of transcripts encoding proteases is one of the earliest senescence-related gene changes (Eason et al., 2002). A marked increase in the protease activity during petal senescence has also been reported in various flowers (Pak and van Doorn, 2005; Jones et al., 2005; Shahri and Tahir, 2011b; Shahri et al., 2011). The increase in total phenols during flower opening has been suggested to be due to their antioxidant proprieties and the role scavengers play during senescence (Trivellini et al., 2007). Recently, the increment of antioxidants in broccoli flortets has been related to the increment of phenols in the tissues (Hasperuéa et al., 2011).

Acknowledgements: The authors thank the Head of the Department of Botany for providing the facilities and Dr. A.Q. John (Professor Emeritus, SKUAST, Kashmir) for cultivar identification. Waseem Shahri thanks the University Grants Commission (India) for providing a Junior Research Fellowship under the NET (JRF) scheme.

REFERENCES

Celikel F.G., van Doorn W.G. 1995. Solute leakage, lipid peroxidation and protein degradation during senescence of *Iris* tepals. PHYSIOL. PLANT. 94: 515-521.

Chapin L., Jones M. 2007. Nutrient remobilization during pollination-induced corolla senescence in *Petunia*. ACTA HORT. 755: 181-190.

Eason J.R., Ryan D.J., Pinkney T.T., O'Donoghue E.M. 2002. Programmed cell death during flower senescence: Isolation and characterization of cysteine proteinases from *Sandersonia aurantiaca*. FUNCTIONAL PLANT BIOL. 29: 1055-1064.

Gulzar S., Tahir I., Amin I., Farooq S., Sultan S.M. 2005. Effect of cytokinins on the senescence and longevity of isolated flowers of daylily (*Hemerocallis fulva*) cv. Royal crown sprayed with cycloheximide. ACTA HORT. 669: 395-403.

Halevy A.H., Mayak S. 1979. Senescence and postharvest physiology of cut flowers - Part I. HORT. REV. 1: 204-236.

Hasperuéa J.H., Chavesa A.R., Martínez G.A. 2011. End of day harvest delays postharvest senescence of broccoli florets. POSTHARVEST BIOL. TECHNOL. 59: 64-70.

Hoeberichts F.A., de Jong A.J., Woltering E.J. 2005. Apoptotic like cell death marks the early stages of gypsophila (*Gypsophila paniculata*) petal senescence. POSTHARVEST BIOL. TECHNOL. 35: 229-236.

Jones M.L. 2008. Ethylene signalling is required for pollination-accelerated senescence in Petunia. PLANT SCI. 175: 190-196.

Jones M.L., Chaffin G.S., Eason J.R., Clark D.G. 2005. Ethylene sensitivity regulates proteolytic activity and cysteine protease gene expression in petunia corollas. J. EXP. BOT. 56: 2733-2744.

Kenza M., Umeil N., Borochov A. 2000. The involvement of ethylene in the senescence of *Ranunculus* cut flowers. POSTHARVEST BIOL. TECHNOL. 19: 287-290.

Lay-Yee M., Stead A.D., Reid M.S. 1992. Flower senescence in daylily (*Hemerocallis*). PHYSIOL. PLANT. 86: 308-314.

Lowry O.H., Rosenbrough N.J., Farr A.L., Randall R.J. 1951. Protein measurement with folin phenol reagent. BIOL. CHEM. 193: 265-275.

Lukaszewski T.A., Reid M.S. 1989. Bulb type flower senescence. ACTA HORT. 261: 59-62.

Nelson N. 1994. Photometric adaptation of Somogy's method for the determination of glucose. J. BIOL. CHEM. 153: 375-380.

Pak C., van Doorn W.G. 2005. Delay of Iris flower senescence by protease inhibitors. NEW PHYTOL. 165: 473-480.

Paulin A., Jamain C. 1982. Development of flowers and changes in various sugars during opening of cut carnations. J. AM. SOC. HORT. SCI. 107: 258-261.

Rosen H. 1957. A modified ninhydrin colorimetric method for amino acids. ARCH. BIOCHEM. BIOPHYS. 67: 10-15.

Shahri W., Tahir I. 2011a. Flower senescence: strategies and some associated events. BOT. REV. 77: 152-184.

Shahri W., Tahir I. 2011b. Physiological and biochemical changes associated with flower development and senescence in *Consolida ajacis* Nieuwl cv. Violet blue. FRONT. AGRIC. CHINA 5: 201-208.

Shahri W., Tahir I., Islam S.T., Bhat M.A. 2011. Physiological and biochemical changes associated with flower development and senescence in so far unexplored *Helleborus orientalis* Lam. cv. Olympicus. PHYSIOL. MOL. BIOL. PLANTS 17: 33-39.

Swain T., Hillis W.E. 1959. The phenolic constituents of *Prunus domestica* L. – The quantitative analysis of phenolic constituents. J. FOOD. SCI. AGRIC. 10: 63-68.

Tayyab J., Qamar S. 1992. A look into enzyme kinetics: Some introductory experiments. BIOCHEM. EDU. 20: 116-118.

Trivellini A., Vernieri P., Ferrante A., Serra G. 2007. Physiological characterization of flower senescence in long life and ephemeral hibiscus (*Hibiscus rosa-sinensis* L.). ACTA HORT. 755: 457-464.

van Doorn W.G. 2001. Categories of petal senescence and abscission: a reevaluation. ANN. BOT. 87: 447-456.

van Doorn W.G., Woltering E.J. 2008. Physiology and molecular biology of petal senescence. J. EXP. BOT. 59: 453-480.

Woltering E.J., van Doorn W.G. 1988. Role of ethylene in senescence of petals – morphological and taxonomic relationships. J. EXP. BOT. 39: 1605-1616.

Xu Y., Hanson M.R. 2000. Programmed cell death during pollination-induced petal senescence in *Petunia*. PLANT PHYSIOL. 122: 1323-1333.

Yamada T., Takatsu Y., Manabe T., Kasumi M., Marubashi W. 2003. Suppressive effect of trehalose on apoptotic cell death leading to petal senescence in ethylene-insensitive flowers of gladiolus. PLANT SCI. 4: 213-221.

Zhou Y., Wang C.Y., Ge H., Hoeberichts F.A., Visser P.B. 2005. Programmed cell death in relation to petal senescence in ornamental plants. J. INT. PLANT BIOL. 47: 641-650.

ROZWÓJ I STARZENIE SIĘ KWIATÓW JASKRA AZJATYCKIEGO (*Ranunculus asiaticus* L.)

Waseem Shahri i Inayatullah Tahir

S T R E S Z C Z E N I E

Rozwój kwiatów jaskra azjatyckiego uprawianego w Uniwersyteckim Ogrodzie Botanicznym podzielono na sześć stadiów (I – VI): mocno zwinięty pąk kwiatowy (I), luźny pąk kwiatowy (II), kwiat w połowie otwarty (III), kwiat otwarty (IV), kwiat starzejący się (V) i kwiat stary (VI). Średnia długość życia poszczególnych kwiatów po pełnym ich otworzeniu się wynosiła około 5 dni. Przepuszczalność błony tkanek działki kielicha określona jako elektryczna przewodność jonowa (μS) zwiększyła się wraz z postępującym rozwojem na kolejnych stadiach. Zawartość cukrów w tkankach płatków zwiększyła się w okresie otwierania kwiatów, natomiast obniżyła się podczas ich starzenia. W poszczególnych stadiach rozwoju i starzenia się kwiatów liczba białek rozpuszczalnych stale wzrastała, czemu towarzyszył spadek aktywności proteaz oraz zawartości α-aminokwasów. Zawartość fenoli wzrastała wraz z otwieraniem się kwiatów, a następnie zmniejszała się podczas ich starzenia.

Słowa kluczowe: α-aminokwasy, starzenie się kwiatów, przepuszczalność błony, aktywność proteaz, białka rozpuszczalne, jaskier azjatycki, składniki tkanki