Flower Development and Senescence-Related Changes in *Agapanthus africanus* (Aliaceae)

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Abstract

In this study it was determined that fresh weight changes, peroxidase activity, soluble protein and anthocyanin content and the changes in the membrane permeability in four different stages of flowering (developing flower buds, anthesis, mature flowers, senescing flowers) of *Agapanthus africanus*. It was seen that fresh weight amount in mature flowers stage is the highest when it was compared with senescing flowers. It was found a negative correlation between decrease of anthocyanin content and increase of membrane permeability in senescing flower stage because of losing of semi-permeability in membranes during senescence.

Keywords: Senescence, flower stage, *Agapanthus africanus*
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Introduction

Flowering is a complex biological process that is regulated by both environmental and developmental factors. Recently, extensive studies in Arabidopsis have revealed genetic and molecular mechanisms of the transition from vegetative growth to flowering (Mouradov et al. 2002). The modified ABC model explains the development of the petaloid perianth of *Agapanthus praecox* ssp. *orientalis* (Agapanthaceae) flowers (Nakamura et al. 2005). The observations of these researchers indicated that the flower developmental mechanism of *Agapanthus* followed the modified ABC model.

Senescence, like growth and reproduction, is a normal phase of plant development. Senescence is not a case of passive decay of structural, biochemical and molecular changes of cells. Rather it includes a decline in photoshyntesis; dismantling of chloroplast; degradation of macromolecules such as proteins, nucleic acids, and lipids; loss of chlorophyll; and mobilization of nutrients to developing parts of the plant (Buchanan-Wollaston 1997). The two senescent organs best studied are leaves and flowers. In leaves, this process can be reversed; however, in floral tissues it can not, indicating that a tightly controlled program for cell death exists (Rubinstein 2000). Flower petals are often the plant organ with the shortest life span, and as such provide useful tissue for studying the mechanisms underlying control of senescence (Borochow and Woodson 1989). Flower senescence, a genetically defined process associated with common morhological, physiological and biochemical changes (Xu and Hanson 2000). These changes during flower senescence include an increase in hydrolytic enzymes, degradation of macromolecules (Baumgartner et al. 1975). The interrelation between auxins and ethylene was recorded in

many cases. It is well established that ethylene is involved in the senescence of many plant flowers (Woltering and van Doorn 1988).

In this study was aimed to determine the flower development and senescence related changes in four different stages of flowering in *Agapanthus africanus*

Materials and methods

Plant Material. In this study, we used different stages of purple flower of *Agapanthus africanus* as a plant material (Figs. 1 and 2).

Figure 1. *Agapanthus africanus*

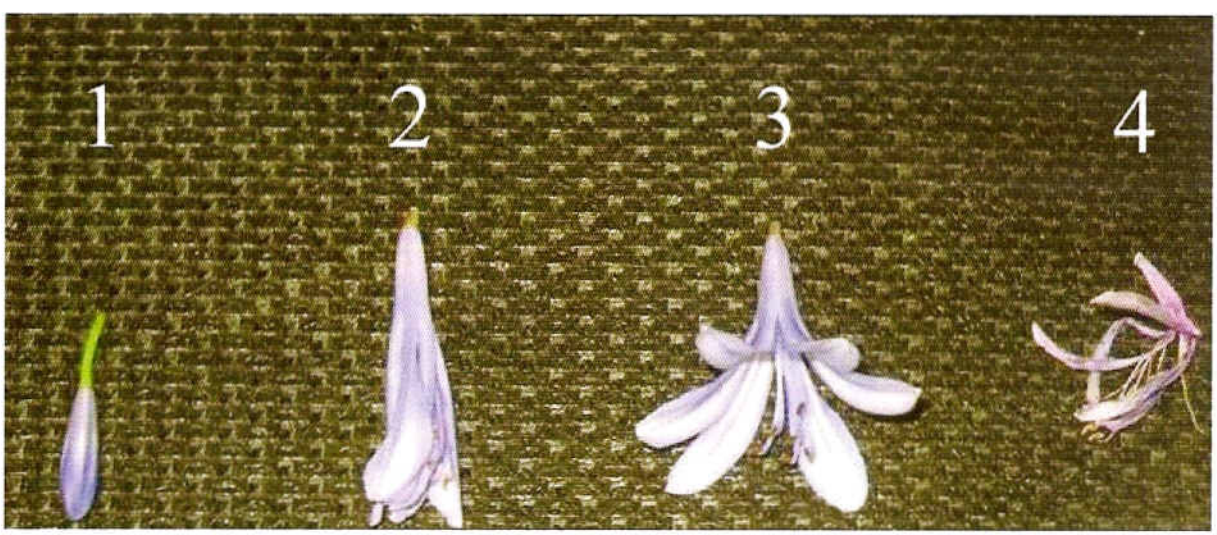

Figure 2. Four different flowering stages of *Agapanthus africanus*. 1. Flower Bud 2. Anthesis 3. Mature Flower 4. Senescing Flower

Extraction of protein. The bud and flower samples were homogenized with ice-cold 0.1mM sodium phosphate buffer (pH 6.8). The homogenates were then centrifuged at 13,000 rpm for 30 min at 4 °C and supernatants were used for determination of total soluble protein content and total POD enzyme assays. Protein content of the extracts was determined according to Bradford (1976) using bovine serum albumin as standard.

Peroxidase activity assay. The reaction mixture consisted of 0.25% (v/v) guaiacol in 1ml 0.1M sodium phosphate buffer, pH 7.0, containing 0.1% hydrogen peroxide. 60 µl of the crude enzyme extract were added to initiate the reaction which was measured spectrophotometrically at 470 nm due to the guaiacol oxidation was recorded for 2 min and defined quantitatively as ΔA/g.FW.min. (Birecka et al. 1973).

Changes in the permeability of the membrane: In order to measure the changes in the permeability of the membrane, fresh weights of all flowers and buds, they were placed in petri dishes lined with filter paper and containing 8ml of distilled water. Then they were placed in an oven at 25 °C for 24, 48 and 72 hours. Absorption values of samples which were removed from the liquid medium in petri dishes at the end of these periods were measured with spectrophotometer at 280 nm (Poovaiah and Leopold 1976).

Anthocyanin determination: After being thoroughly extracted in 3 ml methanol-HCl (1% HCl, v/v), the samples were left at 4 °C in the refrigerator for 2 days. Later on, the extract were filtered and the total anthocyanin content was measured by an UV visible spectrophotometer 530 nm wavelength, defined quantitatively as OD 530/ml. (Manchinelli, 1990).

Result and Discussion

Senescence, one type of programmed cell death (PCD) in plants, is a genetically controlled sequence of events comprising its final developmental stage. It is a highly regulated, ordered series of events in which organelles, membranes, and macromolecules are broken down and nutrients, i.e., amino acids, sugar and minerals, are reclaimed for export out of the senescing parts of the plants (Srivastava 2002).

When the flower development stages compared to the senescing flower stage, the highest fresh weight accumulation in the mature flowers (Fig. 3). Besides, fresh weights in developing flower buds, anthesis stage, and senescing flowers decreased by 36%, 21% and 95% , respectively, compared to the mature flowers stage. Similarly, Eason and Webster (1995) have reported that fresh weight decrease in senescence stage that it is last phase of flower development in *Sandersonia aurantiaca* (Eason and Webster 1995).

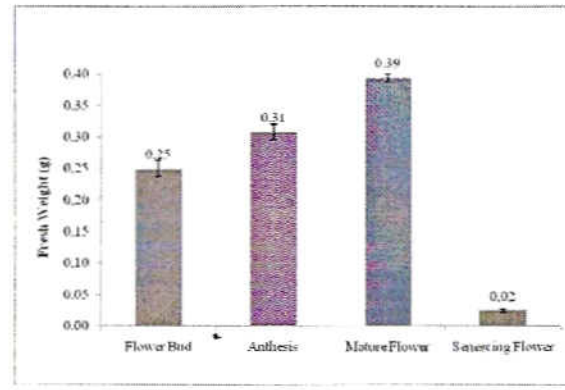

Figure 3. Comparison of the fresh weights of flowers in different stage of *Agapanthus africanus* ($P<0.05$)

It was seen that the anthocyanin content of senescing flower stage decreased by 29% compared to the mature flower stage (Fig. 4). Meng and Wang (2004) indicated that anthocyanin content increased with pigmentation during the flower development.

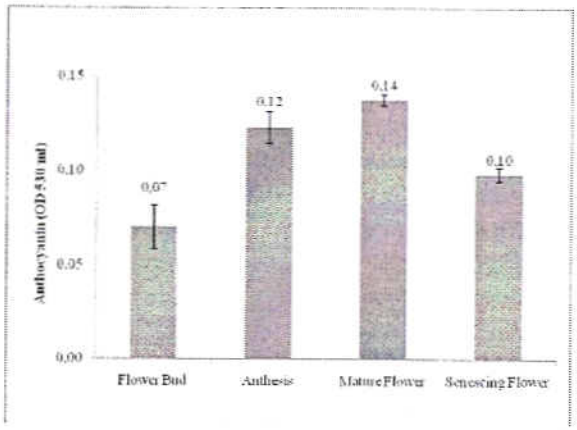

Figure 4. Comparison of anthocyanin content of flowers in different stage of *Agapanthus africanus* (P<0.05)

When total protein content was investigated in the stage of flower development, no differences determined (Fig. 5). Carnation sensescence is associated with derepression of specific genes, increased polyribosome activity, and major changes in patterns of protein synthesis (Reid and Wu 1992). There was little variation in either soluble or insoluble protein levels during flower development in *Chamelaucium uncinatum* (Olley et al. 1996).

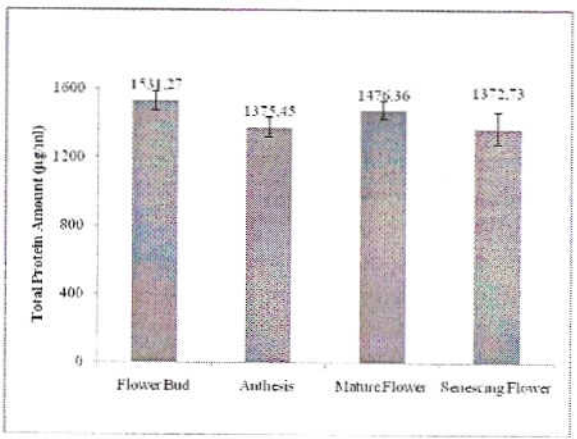

Figure 5. Comparison of the total protein amount of flowers in different stage of *Agapanthus africanus* (P<0.05)

Peroxidase activity that is criteria of senescence increased along the flower development (Fig. 6). Paliyath and Pinhero (2000) established that peroxidase activity increased during all flower development and especially senescence.

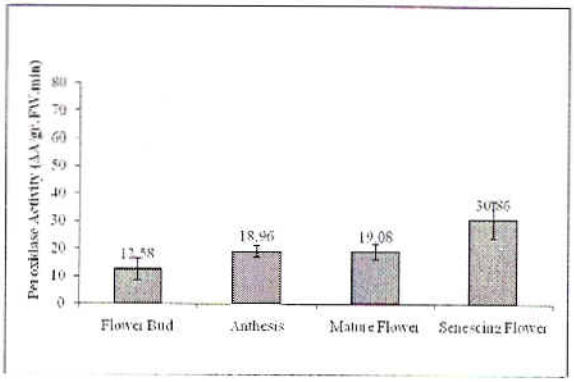

Figure 6. Comparison of the peroxidase activity of flowers in different stage of *Agapanthus africanus* (P<0.05)

The substances that leak from the cell membrane to outer medium is deemed to be a good parameter in senescence. The increase in leakage is even faster in the last phase of senescence (Nooden and Leopold 1988). The leakage values in stages of flowering (developing flower buds, anthesis and mature flowers) of *Agapanthus* are very similar to each other (Fig. 7). It was thought that the permeability of membrane did not change in these three phase of development. Leakage significantly increased during the senescence because of losing of membrane permeability.

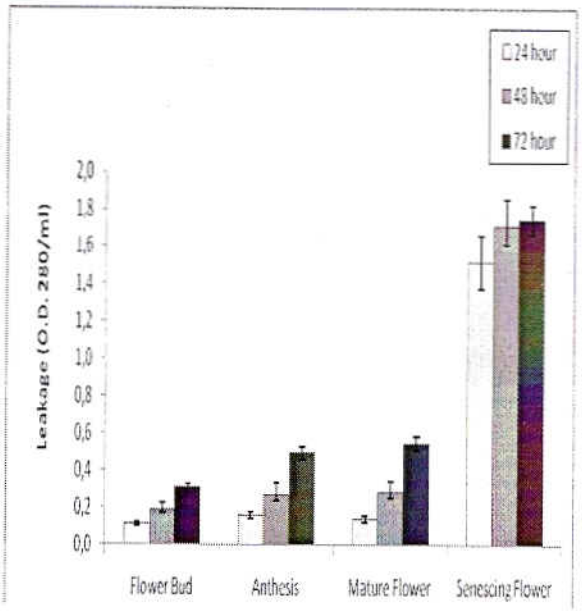

Figure 7. Comparison of the leaked subtances into the incubation medium from petal of flowers in different stage of *Agapanthus africanus*

According to these experiment results, we can say that leakage as well as peroxidase activity is a criteria of developmental stage and especially senescence phase in *Agapanthus africanus*.

References

Baumgartner B., Hurter J. and Matile P. (1975) On the fading of an ephemeral flower. *Biochem. Physiol. Pflanzen*, 168:299-306.

Birecka H., Briber K.A. and Catalfamo J.L. (1973) Comparative studies on tobacco pit and sweet potato root isoperoxidases in relation to injury, IAA, and Ethylene effects. *Plant Physiology*, 52:43-49.

Bradford R. (1976) A rapid and sensitive method fort he quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72:248-254.

Buchanan-Wollaston V. (1997) The molecular biology of leaf senecence. *Journal of Experimental Botany*, 48:181-199.

Eason J.R. and Webster D. (1995) Development and senescence of *Sandersonia aurantiaca* (Hook.) flowers. *Scientia Horticulturae*, **63:**113-121.

Manchinelli A.L. (1990) Interaction between light quality and light quantity in the photoregulation of anthocyanin production. *Plant Physiology*, 92:1191-1195.

Meng X. and Wang X. (2004) Regulation of flower development and anthocyanin accumulation in *Gerbera hybrida*. *Journal of Horticultural Science and Biotechnology*, 79: 131-137

Mouradov A., Cremer F. and Coupland G. (2002) Control of flowering time: interacting pathways as a basis for diversity. *Plant Cell*, 14: S111–S130

Nooden L.D. and Leopold A.C. (1988) Senescence and Aging in Plants., Academic pres, California, 0-12-520920-7.

Olley C.M., Joyce D.C. and Irving D.E. (1996) Changes in sugar, protein, respiration, and ethylene in developing and harvested Geraldton waxflower *(Chamelaucium uncinatum)* flowers. *New Zealand Journal of Crop and Horticultural Science*, 24: 143-150.

Paliyath G. and Pinhero R.G. (2000) Seasonal variation in peroxidase activity of carnation flower petals in response to ethylene. *Phyoiology and Molecular, biology of plants*, 6: 141-148.

Poovaiah B.W. and Leopold A.C. (1976) Effects of inorganic salts on tissue permeability. *Plant Physiology*, 58: 182-185.

Reid M.S. and Wu M.J. (1992) Ethylene and flower senescence. *Plant Growth Regulation*, 11: 37-43.

Srivastava L.M. (2002) *Plant growth and development, Hormones and Enviroment*, Academic Press, California, 0-12-660570-X.

Woltering E.J. and van Doorn W. G. (1988) Role of ethylene and senesence of petals: morphological and taxonomical relationships. *Journal of Experimental Botany*, 39: 1605-1616.