Indian J. Plant Physiol., Vol. 15, No. 4, (N.S.) pp. 303-309 (Oct.-Dec., 2010)

INOSITOL PREVENTS SENESCENCE OF GLADIOLUS FLOWERS

V.K. VIKAS, AJAY ARORA AND V.P. SINGH*

Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi 110 012

Received on 6th April, 2010, Revised on 4th Dec., 2010

SUMMARY

An experiment was conducted to study the effect of inositol on the vase life of cut flowers of gladiolus variety Snow Princess. Vase life of gladiolus was increased when treated with inositol (75 mM). Fresh weight, membrane stability index, total soluble protein content and activities of antioxidant enzymes, superoxide dismutase, catalase, glutathione reductase and ascorbate peroxidase were also increased by the treatment in comparison to control. On the other hand, level of lipid peroxidation in terms of thiobarbituric acid reactive substances (TBARS) and the activities of lipoxygenase enzyme were reduced by inositol treatment. This suggests that inositol mediates the scavenging of free radicals, hence decrease their interaction with proteins and lipid, delaying the process of lipid peroxidation and consequently senescence.

***Key words*: Gladiolus, inositol, senescence, antioxidants**

INTRODUCTION

Flowers are highly perishable, hence any effort to improve their vase life by regulating senescence, either through chemical or genetic means will reduce the post harvest losses. Senescence as defined by Watada *et al*. (1984) is "those processes that follow physiological maturity and lead to death of a whole plant, organ tissue or cell. Senescence represents the sequence of metabolic events occurring in the final stage of development and ultimately culminating in the programmed death. It is an actively ordered process that involves the synthesis of new RNA and proteins and results in highly coordinated changes in the metabolism. Senescence is determined genetically and governed by environmental factors during development (Buchanan–Wollaston *et al*. 2003).

The senescence of flower petals is associated with a series of highly regulated physiological and biochemical processes (Mayak and Halevy 1980). These include an increase in hydrolytic enzyme activity, degradation of macromolecules, increased respiratory activity and loss of membrane integrity and cellular compartmentation. Of the various postulates concerned with the initiation of senescence in plant tissues. The involvement of reactive oxygen species (ROS) has attracted considerable attention (Dhindsa *et al*. 1981). Activated oxygen species such as O_2^- or H_2O_2 and their interaction product, hydroxyl radical (OH^-) react with and degrade proteins, lipids and nucleic acids leading to senescence (Arora *et al*. 2002). Antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase, glutathione reductase, peroxidase and catalase are involved in scavenging of reactive oxygen radicals (Asada 1992, Foyer 1993). In recent years molecular biological approaches have been utilized to identify genes that may be involved in the initiation and regulation of the senescence. The identification and characterization of these senescence genes have begun to provide us with an understanding of the process of senescence.

*Corresponding author, E-mail: drsinghvp@yahoo.com

Gladiolus is an important commercial cut flower. Like other flowers with spike inflorescence, it is normally harvested with relatively few open florets and the like flower is a function of both the life of the individual florets and of the post harvest expansion and opening of the floret remaining on the spike. Gladiolus undergoes senescence independent of the controlling effects of ethylene (Woltering and van Doorn 1988). Tepals/petals from this species therefore, were used in the present study as a system to investigate the events associated with ethylene independent floral senescence. The typical vase life of individual florets in just 4-5 days and the senescent florets remain at the bottom of the spikes after the opening of the upper florets. Exogenous ethylene and ethylene inhibitors have no effect on petal senescence of gladiolus. However, several saccharides and polyols do affect the vase life of gladiolus. Sucrose has been shown to extend the vase life of rose, carnation, gladiolus etc. Arora and Singh (2006) reported that treatment with polyols, particularly inositol prolongs the vase life of cut gladiolus spike. Polyols are polysaccharide alcohols which serve arrange of function in plants. They may serve as carbon storage and translocation compounds. Acyclic polyols are often as important as sucrose in phloem translocation from source leaves. Of the compound tested, myo-inositol was the most effective closely followed by sorbitol and mannitol. Present study was conducted to test effect of inositol on regulation of flower senescence.

MATERIALS AND METHODS

Experiments were conducted with a gladiolus variety, Snow Princess to understand the role of polyols in the senescence of gladiolus flower. Gladiolus was grown in the fields of the Indian Agricultural Research Institute, New Delhi, India, during first week of October adopting standard cultural practices. The spikes were harvested when the lower most floret started showing colour or unfolding petals. The spikes were cut to uniform length of 20 cm and all leaves were removed to observe the actual potential of polyol compounds except one bract like leaf below the florets. Spikes were placed in test tubes (25 mm dia) containing 30 ml of vase solution of inositol (75 mM). Vase solution was changed after every 24 hours and the volume of remaining solution was recorded. The optimum concentrations inositol (75 mM) was arrived at from observations of a preliminary experiment involving a range of concentrations. Each treatment was replicated thrice. The tubes were plugged with non-absorbent cotton to prevent evaporation losses form the surface of vase solution. The tubes were kept at a room temperature for 20±2°C, relative humidity 70±5% under continuous illumination (range 400 -700 nm) of 20 Wm^{-2}.

Post harvest growth of gladiolus in vase solution is divided into 5 stages *viz* 1st- bud stage, 2nd half opened stage, 3rd fully opened stage, 4th incipient senescence stage and 5th senescence stage. Observations were recorded on changes in fresh weight, vase life, membrane stability index (MSI), total soluble protein, activities of superoxide dismutase (SOD), Catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (AP), lipoxygenase (LOX) and lipid peroxidation in terms of TBARS concentration. Observations on fresh weight were recorded daily. All other parameters were studied at five different stages of flower development *viz.*, 1st to 5th stage.

The end of vase life was defined as when the fifth floret from the bottom started senescing. More precisely, when the fresh weight of the floret spikes declined below its initial fresh weight then the floret spikes were said to have lost their shelf life quality. The rate of change in fresh weight with respect to the initial fresh weight was calculated and expressed as percentage increase/ decrease over initial fresh weight. Membrane stability index of petals was determined by recording the electrical conductivity of leaches in double distilled water as given by Bailley *et al.* (1996). Conductivity of the solution was measured using Conductivity Bridge (CM 180 conductivity meter, Elico Pvt. Ltd., Hyderabad, India), lipoxygenase (lox) enzyme assay was done according to the method of Doderer *et al.* (1992). The level of lipid peroxidation was measured in terms of TBARS concentration was measured by Heath and Packer (1968) method. The protein concentration of the supernatant was estimated using the method of Bradford (1976). Superoxide dismutase activity was estimated according to the method of Dhindsa *et al.* (1981). Catalase activity was assayed by measuring glutathione reductase activity was assayed by measuring the disappearance of H_2O_2 according to Teranishi *et al.*

(1974). Glutathione reductase activity was assayed as per the method of Smith *et al*. (1988). Ascorbate peroxidase activity was assayed according to the method described by Nakanoy and Asada (1981). Standard error was calculated and has been given in figures.

RESULTS AND DISCUSSION

Fresh weight of the spikes kept in inositol remain higher than initial fresh weight up to eight days after treatment, while spikes kept in control (distilled water) started to loose fresh weight from 5th day onwards (Fig.1). Initial increase in fresh weight of spikes in all the treatments and control can be attributed to the increased requirement of spikes for opening of flowers. Later reduction in the fresh weight is caused by decreased water uptake and increased respiration rate (Ezhilmathi *et al.* 2007). In the absence of current photo assimilates, reserve carbohydrates are used in respiration causing depletion in fresh weight. Decline in fresh weight can also be attributed to ion leakage due to membrane deterioration. These results are in agreement with the work of Bieleski and Reid (1992), Borochov *et al*. (1995) and Serek *et al*. (1995) in different species of flowers.

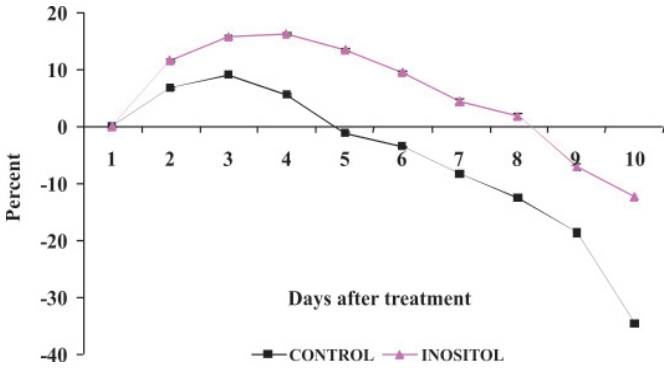

Fig. 1. Effect of inositol on percent change (increase/ decrease) in fresh weight of Snow Princess variety of gladiolus spikes

The senescence of gladiolus florets was delayed following treatments with inositol. The vase life of flower spikes was significantly increased by treatment with inositol, 8.27 days compared to 4.75 days in control and other treatments (Fig. 2). The extended vase life in inositol treated flower spikes is associated with increased water uptake, and improved water balance.

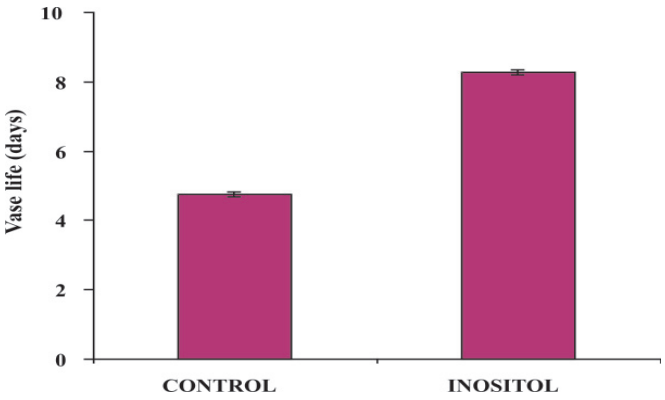

Fig. 2. Effect of inositol on the vase life (days) of Snow Princess variety of gladiolus

Electrolyte leakage was delayed or reduced by the use of inositol (75 mM). The flowers kept in inositol (75 mM) showed the least leakage while flowers kept in control showed the highest leakage (Fig. 3). Florets in first, second and third stage did not show significant difference in MSI in both control and treatment but florets in fourth and fifth stages showed significant difference in MSI. However inositol treated florets maintained high MSI than control in fourth and fifth stage. A decline in MSI represents change in membrane permeability, which leads to solute leakage. Similar results were recorded in gladiolus (Yamane *et al*. 1993, Singh 2005) and day lily (Bieleski and Reid 1992).

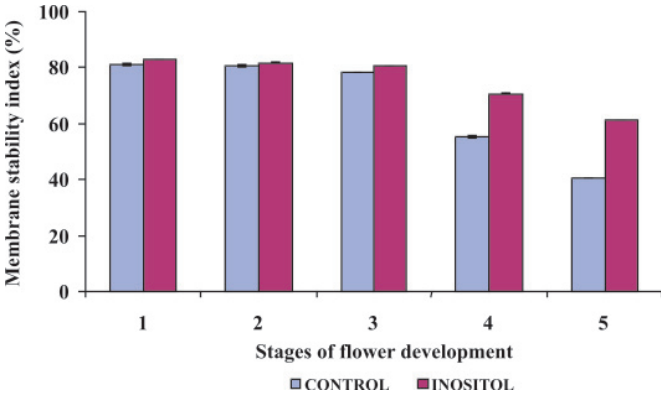

Fig. 3. Effect of inositol on the membrane stability index (MSI) (%) of Snow Princess variety of gladiolus

Progressive leakiness may be the consequence of free radical attack on lipids and protein - the constituents of bilayer membrane. The relationship between lipid peroxidation and solute leakage is already established by Dhindsa *et al*. (1981). Inositol treatment could alleviate MSI, as it was able to reduce lipid peroxidiation.

Total soluble protein content increased upto third stage and then declined in both the treatment and control, however soluble protein was significantly higher in the treated florets than control (Fig. 4). The inositol treated florets maintained high and soluble protein content from first to fifth stages of floral development. Petal senescence is invariably associated with the loss of protein (Woodson and Honda, 1987). The protein content of petals in daylily flowers decreased rapidly due to little de novo synthesis and considerable protein degradation during senescence (Lay-Yee *et al*. 1992). Consistent with these results, there was gradual decline in total soluble protein until senescence in gladiolus (Singh and Jegadheesan, 2003). It is known that free radicals act directly on proteins, altering their conformation and causing them to be recognized by specific proteases (Thompson 1988). In the present study, flowers treated with inositol retained higher protein content that the control. It may be due to the function of the inositol as a free radical scavenger or inositol induced increase in the activity of SOD and CAT.

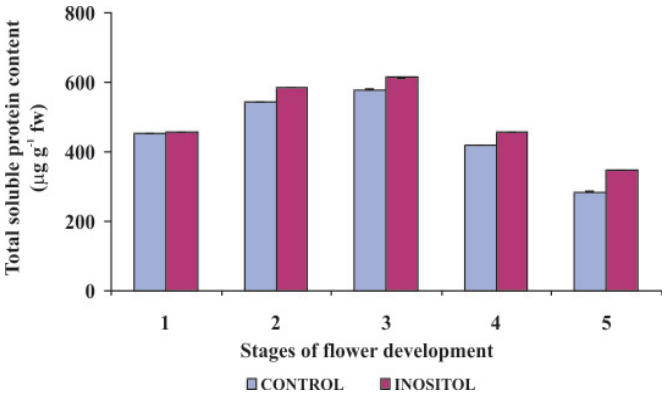

Fig. 4. Effect of inositol on total soluble protein content (ìg g^{-1} f.w.) in petals of Snow Princess variety of gladiolus at different stages of flower development

The TBARS content, an index of lipid peroxidation and lipoxygenase (LOX) activity which catalyzes the hydroperoxidation of polyunsaturated fatty acids especially in the membrane. TBARS content and LOX activity was gradually increased with the advancement of senescence, but the increase was less in the case of inositol treated florets than control (Fig. 5 and 6). As the senescence advances oxidative stress due to free radical production is increased, hence more deterioration of membranes. However, treatment of inositol decreased the TBARS content and LOX activity. Supplementation of vase solution with inositol reduced the production of free radicals during progressive senescence hence reduced the TBARS content as well as LOX activity and ultimately membrane damage. Similar results observed in tulips (Jones and McConchie 1995), rose (Fukuchi-Mizutani *et al*. 2000) and gladiolus (Singh 2005, Ezhilmathi *et al*. 2007, Arora and Singh 2006).

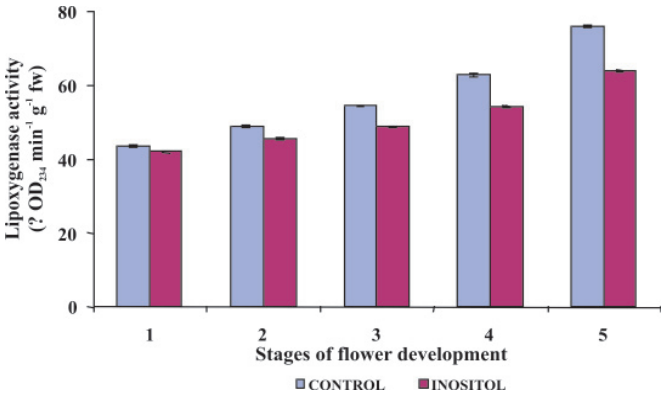

Fig. 5. Effect of inositol on lipoxygenase activity (LOX) (ÄOD234 min^{-1} g^{-1} f.w.) in petals of Snow Princess variety of gladiolus at different stages of flower development

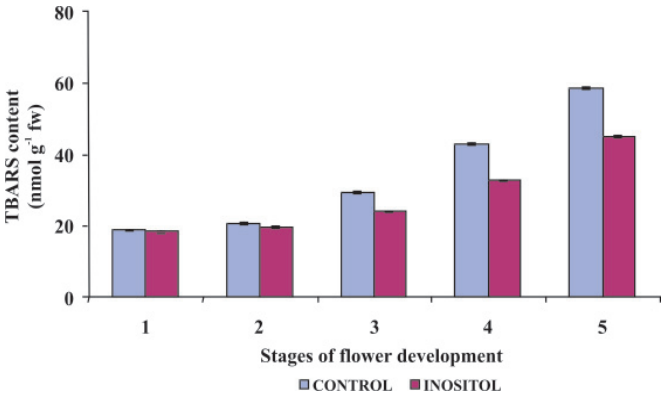

Fig. 6. Effect of inositol on lipid peroxidation in terms of TBARS content (nmol g^{-1} f. w.) in petals of Snow Princess variety of gladiolus at different stages of flower development

Various studies have demonstrated that vase life of flowers is modulated by antioxidants (Baker *et al*. 1978) suggesting the involvement of ROS in senescence. Dhindsa *et al*. (1981) had reported the participation of ROS in plant senescence. In the present study, SOD and catalase activity increased during early floral development i.e. upto 3rd stage and declined after the flowers were fully opened i.e. in 4th and 5th stages (Fig. 7 and 8). The decline in these enzyme activities is less in inositol treated florets than control. So the treatment with inositol reduced the rate of decline of SOD and catalase activity, thereby retained the higher activity for an extended period over control. These results are consistent with the pattern of SOD and catalase activity during senescence in gladiolus (Yamane *et al*. 1999, Singh and Jegadheesan 2003, Ezhilmathi *et al*. 2007), carnation (Droillard and Paulin 1987) and daylily (Panavas and Rubinstein 1998).

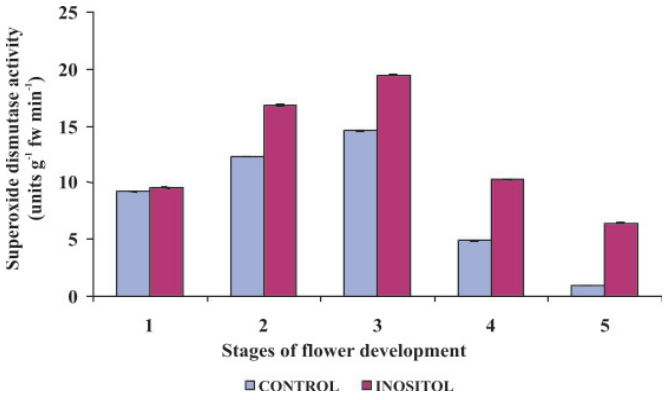

Fig. 7. Effect of inositol on superoxide dismutase (SOD) activity (units g^{-1} f.w. min^{-1}) in petals of Snow Princess variety of gladiolus at different stages of flower development

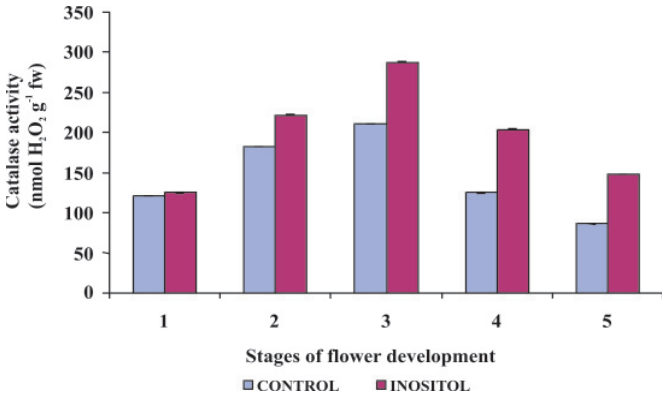

Fig. 8. Effect of inositol on catalase (CAT) activity (nmol H_2O_2 g^{-1} f.w.) in petals of Snow Princess variety of gladiolus at different stages of flower development

Glutathione reductase (GR) catalyzes the reduction of glutathione disulfide with the accompanying oxidation of NADPH. The enzyme is postulated to play an important role in plant protection against various forms of stress. In the present investigation, the activity of GR initially increased upto 3rd stage and then decreased as the senescence proceeds i.e. from 4th stage in both the treated and control spikes (Fig. 9). However, treatment of spikes with inositol reduced the rate of decline of GR activity and retained higher activity than control and other treatments. The same pattern of results was observed in gladiolus (Singh *et al*. 2005) and sunflower seeds (Bailley *et al*. 1996). Reduction in GR activity probably results in a decrease in the levels of reduced glutathione known to be an important factor in preventing oxidative injuries (Alscher 1989). Hence it can be proposed that inositol can postpone the senescence by increasing or restoring the GR activity.

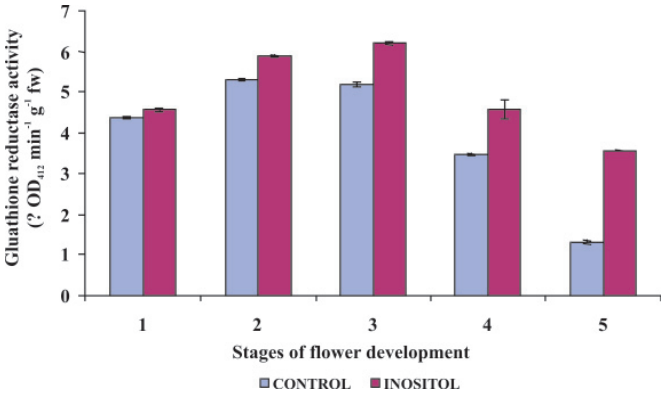

Fig. 9. Effect of inositol on glutathione reductase (GR) activity ("OD412 min^{-1} g^{-1} f.w.) in petals of Snow Princess variety of gladiolus at different stages of flower development

Membrane bound ascorbate peroxidase (AP) is found to scavenge the H_2O_2 which was produced by the action of SOD on the super oxide radical (O_2^-). The ascorbate peroxidase activity was found to be directly correlated with the reduction in free radical in reduced membrane damage (Nakano and Asada, 1981). In our study, AP activity was similar to that of GR activity. The

inositol supplemented gladiolus florets maintained increased AP activity over control and other treatments (Fig. 10). These results are on par with the results of Singh *et al*. (2005) in gladiolus.

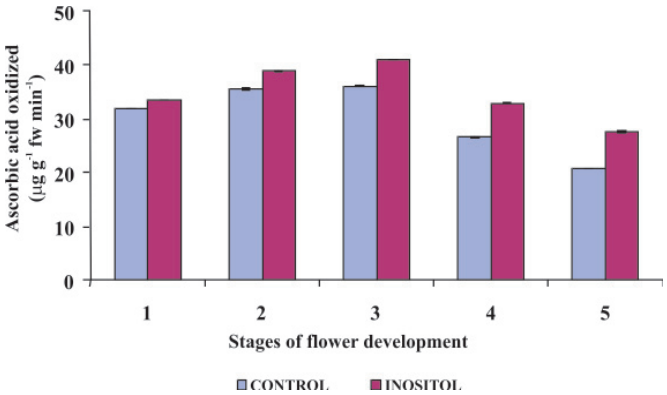

Fig. 10. Effect of inositol on ascorbate peroxidase activity in terms of ascorbic acid oxidized (ìg g^{-1} f.w. min^{-1}) in petals of Snow Princess variety of gladiolus at different stages of flower development

On the basis of the above results it is reasonable to propose that inositol has a role in the induction of antioxidant enzymes and/or might also be acting as a scavenger of ROS thus maintaining membrane integrity for extended periods. Thus petal wilting in gladiolus is associated with ROS induce lipid peroxidation enhanced LOX activity and decrease in ROS scavenging system in the form of SOD, CAT, GR and AP. Yamane *et al*. (1999) have also suggested the role of ROS in petal witling of gladiolus similar to that of ethylene sensitive carnation and ethylene insensitive daylily (Celikel and van Doorn, 1995).

REFERENCES

Alscher, R.G. (1989). Biosynthesis and antioxidant function of glutathione in plants. *Physiol. Plant.* **77:** 457-464.

Arora, A. and Singh, V.P. (2006). Polyols regulate the flower senescence by delaying programmed cell death in *Gladiolus*. *J. Plant Biochem. Biotech.* **15:** 139-142.

Arora, A., Sairam, R.K. and Srivastava, G.C. (2002). Oxidative stress and antioxidant system in plants. *Curr. Sci.* **82:** 1227-1238.

Bailley, C., Banamae, A., Corbineau, F. and Come, D. (1996). Changes in melondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. *Physiol. Plant* **97:** 104-110.

Baker, J.E. Wang, C.Y. Lieberman, M. and Harbenburg, R. (1977). Effects of two analogs of rhizobitoxine and sodium benzoate on senescence of snapdragons. *Horti. Sci.* **12:** 38–39.

Bieleski, R.L. and Reid, M.S. (1992). Physiological changes accompanying senescence in ephemeral Daylily flower. *Plant Physiol.* **98:** 1042-1049.

Borochov, A., Spiegelstein, H., Porat, R. and Field, T. (1995). Membrane lipids involved in the regulation of flower senescence. *Acta Hort.* **405:** 240-245.

Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Ann. Biochem.* **72:** 248-252

Buchanan-Wollaston, V. (1997). The molecular biology of leaf senescence. *J. Exp. Bot.,* **48**: 181-199.

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

Dhindsa, R.S., Plumb-Dhindsa, D. and Thorpe, T.A. (1981). Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.* **32:** 93-101.

Doderer, A, Kokkenlink, I., Vander Veen, S., Valk, B.E., Schram, A.W. and Douma, A.C. (1992). Purification and characterization of two lipoxygenase isoenzymes from germinating barley. *Biochem. Biophy. Acta,* **1120**: 97-104.

Droillard, M.J. and Paulin, A. (1987). Evolution of polar lipids and superoxide dismutase and catalase activities during the senescence of petals of cut carnations (*Dianthus caryophyllus* L. cv. Ember). *Comptos Rendes des Scances Del Academic des Sciences-III Sciences de la vie* **305:** 439-442.

Ezhilmathi, K., Singh, V.P., Arora, A. and Sairam, R. K. (2007). Effect of 5-sulfosalicylic acid on antioxidant activity in

relation to vase life of Gladiolus cut flowers. *Plant Growth Regul*. **51:** 99-108.

Foyer, C.H., Descourvieres, P. and Kunert, K.J. (1994). Protection against oxygen radicals : important defense mechanism studied in transgenic plants. *Plant, Cell and Env*. 17: 507-523**.**

Fukuchi-Mizutani, M., Ishiguro, K., Nakayama, T., Utsunomiya, Y., Tanaka, Y., Kusumi, T. and Ueda, T. (2000). Molecular and functional characterization of a rose lipoxygenase cDNA related to flower senescence. *Plant Sci*. **160:** 129-137.

Heath, R.L. and Packer, L. (1968). Photo-peroxidation in isolated chloroplast I.Kinetics and stochiometry of fatty acid peroxidation. *Arc. Biochem. Biophys.* **125:** 189-198.

Jones, R. and McConchie, R. (1995). Characteristics of petal senescence in non-climacteric cut flower. *Acta Hort.* **405:** 216-223.

Lay-Yee, M., Stead, A.D. and Reid, M.S. (1992). Flower senescence in Daylily (*Haemerocallis*). *Physiol. Plant* **86:** 308-314.

Mayak, S. and Halevy, A.H. (1980). Flower Senescence. In: K. Thimann (ed.), Senescence in Plants. CRC Press, Boca Raton, F.L., pp. 131-156.

Nakano, Y. and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* **22:** 867-880.

Panavas, T and Rubinstein, B. (1998). Oxidative events during programmed cell death of daylily (*Hemerocallis* hybrid) petals. *Plant Sci***. 133:** 125-38.

Serek, M., Jamari, G., Sisler, E.C. and Borochov, A. (1995). Inhibition of ethylene-induced senescence symptoms by 1-methyl cyclopropene, a new inhibitor of ethylene action. *Phyisol. Plant.* **94:** 229-232.

Singh, V.P. (2005). Deterioration of membrane during flower senescence in gladiolus and its amelioration with free radical scavenger. *J. Ornamental Hort*. **8:** 8-12.

Singh, V.P. and Jegadheesan, A. (2003). Effect of alpha lipoic acid on senescence in gladiolus flowers *Indian J. Plant Physiol*. (Special issue): 516-521.

Singh, V.P., Kiran, D. and Arora, A. (2005). Alleviation of antioxidants activity in gladiolus flowers during senescence by spermine and spermidine. *J. Ornamental Hort*. **8:** 167-172.

Smith, I.K. Vierheller, T.L., and Throne, C.A. (1988). Assay of glutathione reductase in crude tissue homogenous using 5, 5'-dithiobis (2-nitrobenzoic acid). *Ann. Biochem.* **175:** 408-413.

Teranishi, Y., Tanaka, A., Osumi, N. and Fukui, S. (1974). Catalase activity of hydrocarbon utilizing candida yeast. *Agri. Biol. Chem.* **38:** 1231-1216.

Thompson, J.E. (1988). The molecular basis for membrane deterioration during senescence. In: Senescence and ageing in plants, L.D. Nooden and A.C. Leopald (eds.), Academic Press, New York. pp. 51-83.

Watada, A.E., Herner, R.C., Kader, A.A., Romani, R.J. and Staby, G.L. (1984). Terminology for the description of development stages of horticultural crops. *Hort. Sci.* **19:** 20-21.

Woltering, E.J. and van Doorn, L.G. (1988). Role of ethylene in senescence of petals morphological and taxonomial relationship. *J. Exp. Bot.* **39:** 1605-1616.

Woodson, W.R. and Handa, A.K. (1987). Changes in protein patterns and *in vivo* protein synthesis during pre senescence and senescence of *Hibiscus* petals. *J. Plant Physiol.* **128:** 67-75.

Yamane, K., Abiru, S., Fujishige, N., Sakiyama, R. and Ogata, R. (1993). Export of soluble sugars and increase in membrane permeability of cut gladiolus florets during senescence. *J. Jap. Soc. Hort. Sci.* **62:** 575-580.

Yamane, K., Kawabata, S. and Fujishige, N. (1999). Changes in activities of SOD, catalase and peroxidases during senescence of gladiolus florets. *J. Jap. Soc. Hort. Sci.* **68:** 798-802.