Indian J. Plant Physiol., Vol. 14, No. 4, (N.S.) pp. 384-391 (Oct.-Dec., 2009)

PHYSIOLOGICAL AND MOLECULAR ANALYSIS OF EFFECT OF SPERMINE ON SENESCING PETALS OF GLADIOLUS

G. SIVAPRAKASAM, V.P. SINGH* AND AJAY ARORA

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

Received on 9 July, 2008, Revised on 28 Dec., 2009

SUMMARY

Convincing evidences suggest the involvement of polyamines in the process of senescence. In order to unravel the mechanism underlying polyamines (spermine) regulated senescence in gladiolus, the present study was undertaken. Spermine at 5µM delayed flower senescence in ethylene-insensitive gladiolus by three days, along with increased fresh weight which was retained for longer period and increased vase solution uptake as compared to control. Activities of total SOD as well as that of its copper/zinc and iron isoforms were significantly enhanced by spermine. Qualitative analysis revealed one Fe SOD and two Cu/Zn isozymes, Cu/Zn SOD1 and Cu/Zn SOD2. Cu/Zn SOD1 appeared to be constitutively expressed but at a higher level than Cu/Zn SOD 2, though the later is more responsive to spermine treatment. Spermine reduced the expression of *GgCyP* while upregulating *GgSOD*. Spermine is thus effective in delaying senescence. It increased the activity of all the isoforms of SOD thereby, reducing oxidative stress and prolonging the vase life. Spermine affected gene expression of *GgCyP* and *GgSOD* without any impact on *GgERS1a,* while slightly affecting the expression of *GgERS1b*. The differential impact of spermine is related to the differential role of these genes in senescence.

***Key words: GgCYP, GgERS1a, GgERS1b, GgSOD,* SOD, spermine**

INTRODUCTION

Flower is one of the most aesthetic creations of life. The longevity of many flowers is quite short, as their biological function of reproduction is transient in nature. Flowers are thus highly perishable and it is estimated that nearly 30% are rendered unmarketable because of post harvest quality loss. Senescence is an active process and a highly regulated developmental event, often associated with the end of the useful life of an organ relative to the reproductive process. In floriculture, delaying the onset of senescence is an important area of research so as to extend the useful life of flowers (Woodson and Jones 2003). Inevitably, plant hormones like auxin (Cheng and Zhao 2007), gibberllic acid (GA) (Naveen *et al.* 2008), ethylene and cytokinin (Eason 2006) play an important role in regulation of senescence as in other developmental processes. Putrescine, spermine and spermidine are the major polyamines, a new class of plant hormones influencing plant growth and development (Kusano *et al.* 2007). Spermine was found to be more effective in delaying senescence in gladiolus (Singh 2005, Singh *et al.* 2005). But their mechanism of action during senescence and other developmental processes in plants is still not clear (Kusano *et al.* 2007). Superoxide dismutase (SOD) is represented by a diverse group of isozymes (Alscher *et al.* 2002) belonging to three isoforms, namely, copper/zinc SOD (Cu/Zn SOD), iron SOD (Fe SOD) and manganese SOD (Mn SOD). SOD has been reported to be upregulated by polyamines in delaying senescence in gladiolus (Singh 2005, Singh *et al.* 2005). Polyamines are known to act at gene

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

expression level of senescence associated genes (SAGs) like those coding for cysteine proteases, ethylene receptors and antioxidant enzymes during flower development (Eason 2006). So far most studies have been concentrated on genome wide expression of the SAGs with little focus on the response of SAGs to polyamines in general and spermine in particular. In order to unravel the mechanism underlying spermine regulated senescence in gladiolus, the present study was undertaken.

MATERIALS AND METHODS

Experiments were conducted with gladiolus var. Snow Princess to understand the role of polyamines in senescence of gladiolus flower in the Division of Plant Physiology, Indian Agricultural Research Institute (IARI), New Delhi, during 2005-2007. Healthy bulbs were planted in the experimental field of Division of Plant Physiology, and all the recommended packages of practices were adopted during growing period of plants. The gladiolus spikes were harvested in the morning when the lower florets started unfolding petals. Spikes were cut just above ground level and brought to laboratory wrapped in moistened filter paper. Just before placing in different vase solutions, the spikes were cut to a uniform length of 25 cm and all leaves were removed except one bract like leaf below the florets. The tubes were kept in the laboratory at a room temperature of 20±2°C and relative humidity 70±5 per cent and under continuous illumination range (400-700 µm) of 20 Wm^{-2}.

Spikes were treated in test tubes (25 mm dia) containing holding solutions of 100 ppm spermine and 4% sucrose with 4% sucrose solutions used as control. The mouth of the tube was plugged with non-absorbent cotton plug, which effectively prevented water loss. Fresh weight of the whole spike was measured daily till the end of their vase life and when the fifth floret from the bottom started senescing more precisely when the fresh weight of flower spikes declined below its initial fresh weight then the flower spikes were said to have lost their shelf life quality. The rate of change in fresh weight was calculated and expressed as percentage increase/ decrease over initial fresh weight. The amount of solution taken up by spike was measured daily and expressed as volume taken up in ml/day.

Flower petals were taken from the third floret from the base of the gladiolus spike during every sampling. Petals (0.5 g) were homogenized in ice cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA with prechilled pestle and mortar. The homogenate was transferred to centrifuge tubes and was centrifuged at 4^{O}C in Beckman refrigerated centrifuge (Model J2-21) for 15 minutes at 15,000 x g. The supernatant was transferred to 30 ml test tubes and referred to as enzyme extract. Superoxide dismutase activity was estimated according to the method of Dhindsa *et al.* (1981). For quantitative assay of SOD isozymes the reaction mixture contained 3 mM KCN and 5mM H_2O_2 (Yu and Rengel 1999). KCN was added to one of the tubes with incubating mixture while both KCN and H_2O_2 were added to another. One test tube was incubated without any inhibitors. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbance reading of samples to 50 per cent in comparison with tubes lacking enzymes.

The expression of SOD isozymes in flower petals of gladiolus of different stages taken from the control and treatment were analyzed using 10% polyacrylamide gel electrophoresis (PAGE) in Tris (8.9 pH) buffer as per the modified method of Scandalios *et al.* (1997). The enzyme extract was analyzed for protein content by Bradford method (1976). One sample was incubated in reaction mixture containing KCN (5 mM) while another was incubated in reaction mixture containing both KCN (5 mM) and H_2O_2 (10 mM) for 30 min in dark over a shaker. The gels were incubated in light for 15 min and destained with 10 per cent acetic acid for 1 min before washing in distilled water. The gel was visualized under illuminator in a gel documentation system (Flurochem Gel Documentation System).

The gene expression was studied in stages I, II, III, IV and V of gladiolus florets. The spikes 4 % sucrose was used as control and 100 ppm spermine + 4 % sucrose as treatment with three replications, each. The expression of the genes was done by two step RT-PCR using gene specific primers (Sambrook *et al.* 1989) under appropriate annealing temperatures. The amplified PCR products were subsequently resolved on 1.2 % (W/ V) agarose gel in 1X TAE buffer for 45 min at 80 volts

using power pack 1000 (BIO-RAD, USA) using 100-bp DNA ladder (MBI Fermentas, Germany) molecular weight marker. The gel was stained in ethidium bromide solution (1 µg/ml) for about 30 minutes and visualized under UV trans-illuminator in a gel documentation system (Flurochem Gel Documentation System).

RESULTS AND DISCUSSION

Spermine treated plants had significantly longer vase life contained more florets in stage III than that of control plants. The treated flowers remained fresh even after five days while control showed onset of senescence within three days after treatment (DAT) (Plate 1). The extended vase life of treated spikes was accompanied by higher fresh weight and longer retention of weight in them than control flowers, as was also shown earlier (Singh 2005, Singh *et al*. 2005). There was a sharp increase in the fresh weight of gladiolus flowers initially, that is, on the second day of treatment followed by a sharp decrease on the 3rd DAT and a gradual decline thereafter in both control and treated gladiolus spikes. Such a pattern in change of fresh weight was also observed in gladiolus by Ezhilmathi *et al*. (2007) in both control and 5-sulphosalicylic acid treatment. The decline in fresh weight after the peak from 2nd DAT was sharper in control than treatment indicating an early onset of senescence, accelerating at a faster rate in control.

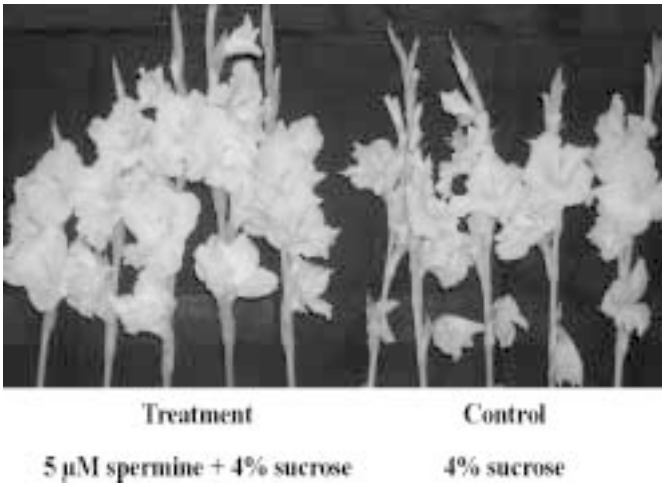

Plate. 1. Effect of spermine (5 µM) on the vase life of gladiolus flowers cv. Snow Princess

Concurrently, spermine treated spikes showed an initial increase in vase solution uptake followed by steady decline contrasting with the continuous and rapid decline of vase solution uptake in control. Singh *et al.* (2005) in a study on effect of polyamines on gladiolus flowers of two cultivars, namely, Snow Princess and Dhanwantari, extended the vase life by five to seven days along with increased fresh weight and vase solution uptake in treated spikes than control. The higher vase solution uptake and consequent increased fresh weight of polyamine treated flowers was attributed to reduced lipoxygenase activity and thus higher membrane stability index. Polyamines also increased the activities of antioxidant enzymes like SOD, CAT, glutathione reductase (GR) and ascorbate peroxidase (AP) thus, nullifying the impact of oxidative stress to significant extent and reduced lipid peroxidation (Singh 2005, Singh *et al.* 2005). Polyamines are known to bind to the negatively charged phospholipid components and affect membrane fluidity. Polyamines may thus modulate the activities of membrane associated enzymes indirectly (Slocum *et al*. 1984) which preserved the integrity and structure of intracellular compartments, increasing the transpiration rate and lowering the respiration in the treated spikes as compartmentalization of organelles helps them to adjust to variations in water potential.

SOD activity in gladiolus flowers increased significantly by 100-150 per cent upon treatment with spermine in comparison with control. The increase was consistent till 4th DAT in treated spikes falling thereafter till the spikes lose their fresh weight. In control, there is an initial fall in SOD activity (Fig. 1). This suggested a possible role for SOD in delaying of flower senescence by spermine. Singh *et al.* (2005) suggested a similar role for SOD in reduction of O_2^- concentration in gladiolus flowers upon treatment with polyamines. Spermine was found to be more effective in increasing the activity of SOD in gladiolus flowers and thus delayed senescence longer than other polyamines, namely, putrescine and spermidine. They also reported that SOD activity increased initially before falling till the spikes reached terminal senescence stage. A significant decline in total SOD activity was also observed during the onset of withering in carnation petals (Sylvestre *et al*. 1989). Yamane *et al.* (1999) also reported that in gladiolus

flowers, SOD and catalase activity started declining along with increased peroxidase activity in the wilting perianth after first day of unfolding.

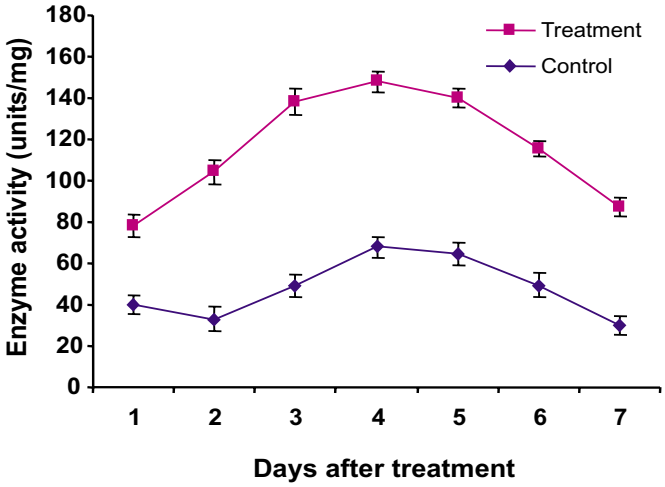

Fig. 1. Effect of spermine (5 µM) on superoxide dismutase (SOD) activity (units mg^{-1}) at various developmental stages in petals of Snow Princess cultivar of gladiolus

Spermine increased the activity of Cu/Zn SOD in gladiolus flower by 50-100 per cent than control (Fig. 2). Woodson (1987) reported that among SOD isoforms Cu/Zn SOD decreased with ageing in carnation. Fe SOD activity in treatment was higher at 2-3 times than the activity in controls at all the stages of flower development

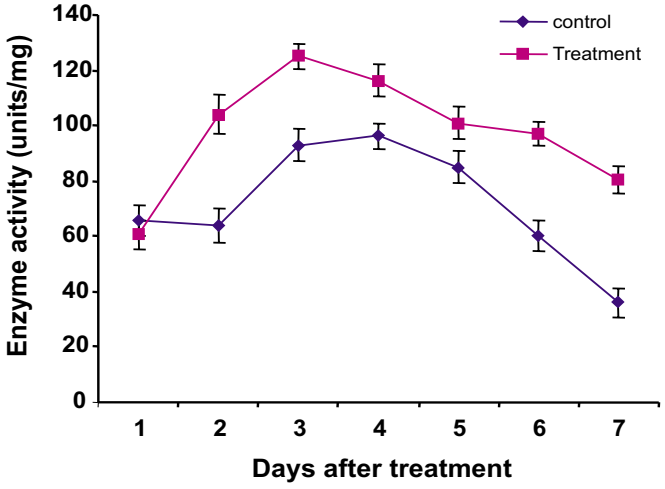

Fig. 2. Effect of spermine (5 µM) on Cu/Zn SOD activity (units mg^{-1}) at various developmental stages in petals of Snow Princess cultivar of gladiolus

and senescence (Fig. 3). Kumar *et al*. (2007) who reported that rose petal senescence was associated with higher production of superoxide radicals and different isoforms of SOD exhibited variable levels of activity, with Cu/Zn SOD being the most active followed by Mn SOD and Fe SOD. In gladiolus flowers, the fall in Fe SOD activity during senescence might explain the rapid senescence in control after three days. The activation of Fe SOD in gladiolus petals by polyamines might be thus related to a possible localization of Fe SOD in organelles other than chloroplast. Droillard *et al*. (1989) attributed the higher activity of Fe SOD in senescing carnation flowers to the peroxisomal location of the enzyme which is essential to maintain the structural integrity of the still active peroxisomes. In fact, Droillard *et al*. (1989) assigned a mitochondrial location to Fe SOD in carnation petals. Therefore, it appears that Fe SOD may have a wider distribution within plant cells than previously thought and thus, amenable to differential regulation by PGRs like polyamines.

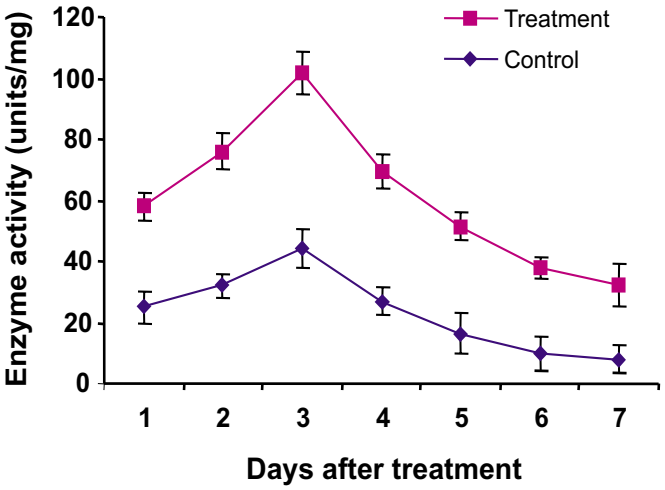

Fig. 3. Effect of spermine (5 µM) on Fe SOD activity (units mg^{-1}) at various developmental stages in petals of Snow Princess cultivar of gladiolus

The SOD activity of isoforms in various stages of gladiolus flowers closely followed the daily changes in activity as the progression of stages in gladiolus is determined chronologically. SOD activity, both of total (Fig. 1) and of the Fe SOD (Fig. 3) and Cu/Zn SOD (Fig. 4) broadly followed a similar pattern with increased activity in treated flowers, than in control. But a closer

look at the SOD the activity revealed significant differences. While, Cu/Zn SOD activation was higher in the initial stages of flower development, Fe SOD was activated more in the latter stages. This is in line with the study of Droillard and Paulin (1990) in carnation where they found that Fe SOD activity was retained, whereas Cu/Zn SOD activity declined in senescing petals. Sylvestre *et al.* (1989) also reported that all the isoforms of SOD including Fe SOD showed reduced activity at different stages of senescence of carnation flowers depending on the isozyme. They too suggested that these differences may be related to the sub-cellular localizations of the SOD isozymes and their relation with the loci where the superoxide anions regenerated.

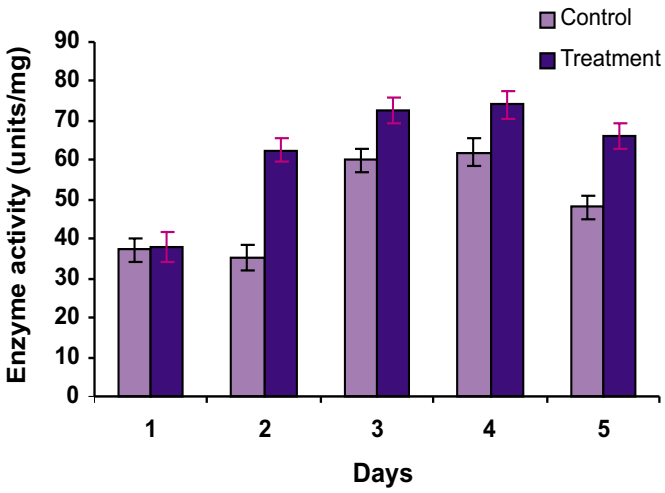

Fig. 4. Effect of spermine (5 µM) on total SOD activity (units mg^{-1}) at various developmental stages in petals of Snow Princess cultivar of gladiolus

The qualitative analysis of SOD isoforms revealed the presence of three isozymes of two isoforms with the Cu/Zn SOD having two types of isozymes. Fink and Scandalios (2002) attributed the occurrence of multigene SOD families and diverse roles played by these enzymes in plants. Fe SOD has been reported to be of a higher molecular weight than Cu/Zn SOD (Fink and Scandalios, 2002). The Fe SOD in gladiolus petals is also of relatively higher molecular weight thus confirmed the findings by earlier workers. The significant difference in the molecular weight of Cu/Zn SOD1 and Cu/Zn SOD2 suggested that both are cytoplasmic SODs as the chloroplastic SOD has just one amino acid extra than cytoplasmic SODs and chloroplasts are absent in petals. Moreover, no Cu/Zn SOD has ever been reported in mitochondria (Alscher *et al.*, 2002). But it has been reported in the peroxisomes of carnation petals by Droillard et *al*. (1989).

From a platform of equal activity in Stage I, a marked increase in activity of Fe SOD was observed in treatment than in control flowers (Fig. 5). The relatively high activity of Fe SOD in the treatment, particularly in stage IV showed that it was inducible by spermine and had a different subcellular location in petals of gladiolus from its normal chloroplastic localization. Hassan (1998) suggested that Fe SOD was a constitutive enzyme while Mn SOD and Cu/Zn SOD are inducible under a tight biosynthetic regulatory regime.

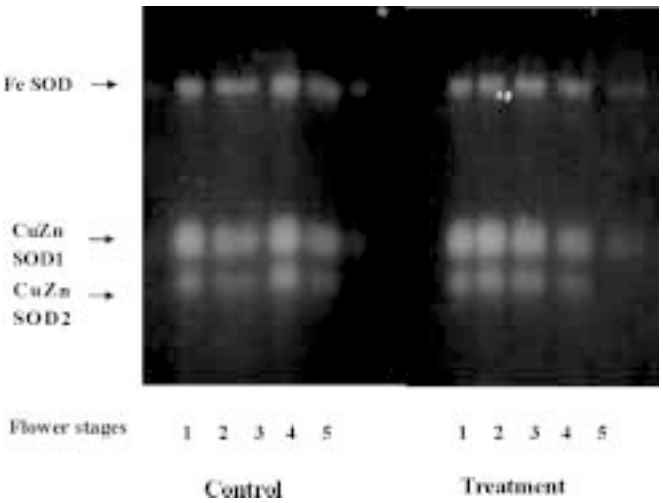

Fig. 5. Effect of spermine (5 µM) on activity of SOD isozyme at various developmental stages in petals of Snow Princess cultivar of gladiolus

The strong activity of both isozymes of Cu/Zn SOD accounts for the higher activity of Cu/Zn SOD than Fe SOD reported in gladiolus (Fig. 5). Kumar *et al*. (2007) contend that Cu/Zn SOD is the most active followed by Mn SOD and Fe SOD in rose flower senescence thus, confirming the preeminent role of Cu/Zn SODs in reducing oxidative stress in floral tissues. The activity of Cu/Zn SOD1 was higher than that of Cu/Zn SOD2 in all stages of the flower with Cu/Zn SOD2 not showing any activity at Stage V. Perhaps Cu/Zn SOD1 is constitutively expressed. But the Cu/Zn SOD2 was more

responsive to spermine treatment than Cu/Zn SOD1, indicating that it is spermine inducible and accounted for the elevated activity of total SOD of treated flowers.

A comparison of the expression profile of Cu/Zn SOD gene (*GgSOD*) and enzyme activity in response to spermine treatment (Fig. 6a) indicated that polyamines acted at the transcriptional level rather than at the post transcriptional level. *GgSOD* mRNA expression levels of spermine treated flowers was far more intense than control, while the difference in Cu/Zn SOD enzyme activity level was relatively less intense. The inducible nature of Cu/Zn SOD2 as compared to the constitutively expressed Cu/Zn SOD1, suggests that it is encoded by the isolated partial fragment of *GgSOD* whose transcription is increased by spermine in gladiolus.

Expression of *GgSOD* was significantly higher in treatment than in control in all the stages of gladiolus flowers resulting in the higher activity of SOD in the gladiolus flowers, delaying the senescence (Fig. 6a). According to Yoda *et al*. (2006) H_2O_2 is generated when plants are treated with polyamines. The expression of various genes, including those encoding antioxidant enzymes and modulators of H_2O_2 production are regulated by H_2O_2 (Neill *et al.* 2002a, b, Geisler *et al.* 2006). In the Arabidopsis genome initiative H_2O_2 signaling was reported to induce the expression of all isoforms of SOD in Arabidopsis (Mittler *et al.,* 2004). Gene expression and enzyme activity of Cu/Zn SOD was linked to H_2O_2 linked with both xylem and phloem tissues (Walz *et al.* 2002).

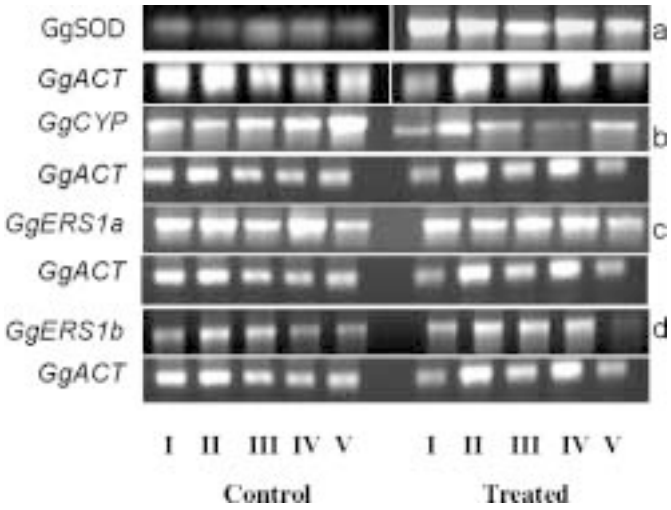

Fig. 6. RT-PCR analysis of *GgSOD, GgCyP, GgERS1a* and *GgERS1b* gene expression at different developmental stages in *Gladiolus* as affected by spermine (5 µM). RT-PCR was performed using total RNA (1.0 µg) as template from these stages and using gene specific/degenerate primers as probe to obtain products representing these gene transcripts. Gladiolus partial actin gene (*GgActin*) stained with ethidium bromide is shown as a charge control

Spermine reduced the expression of *GgCyP* (Fig. 6b) in all the stages of gladiolus flowers resulting in lower cysteine protease activity, thereby decelerating senescence. Such a delay in the senescence by reduced activity of cysteine protease activity has been earlier reported by the use of protease inhibitors and transgenics. Treatment of Sandersonia and Iris flowers with the cysteine protease inhibitors, leupeptin and E-64, respectively, was found to delay visible symptoms of senescence and decrease endogenous protease activity (Eason *et al*. 2002, Pak and van Doorn 2005). Eason *et al.* (2005) by silencing of the cysteine protease gene *(BoCP5)*, in broccoli flower tissues delayed senescence and the flowers contained significantly greater chlorophyll levels.

GgERS1a shows a constitutive expression profile in gladiolus irrespective of the stage of the flower upon spermine treatment (Fig. 6c) while it slightly elevated the expression of *GgERS1b* in almost all the stages (Fig. 6d). The difference between *GgERS1b* expression in control and treatment are insignificant. Arora *et al.* (2006) reported a decrease in *GgERS1b* mRNA level in petal with flower development whereas *GgERS1a* mRNA was found to be constitutive at higher expression level and suggested that the latter to be the probable cause of ethylene insensitiveness in petals of gladiolus. On the other hand, the level of ethylene insensitivity of gladiolus might be regulated by *GgERS1b* expression (Arora *et al.* 2006).

Thus, spermine regulates flower senescence by acting at gene level, though selectively. Kusano *et al.* (2007) suggested that polyamines regulate gene expression through three signal molecules namely, H_2O_2, Ca^{2+} and Nitric oxide (NO). But Yoda *et al.* (2006) showed that hypersensitive response cell death is partly mediated by H_2O_2 produced from catabolism of

spermidine but not spermine, suggesting that spermine may not act through H_2O_2 mediated signaling. Induction of nitric oxide (NO) production by spermine and spermidine has recently been reported in Arabidopsis (Tun *et al*. 2006).

Spermine is thus effective in delaying senescence. It increased the activity of all the isoforms of SOD thereby, reducing oxidative stress and prolonging the vase life. Spermine affected gene expression of *GgCyP* and *GgSOD* without any impact on *GgERS1a*, while slightly affecting the expression of *GgERS1b*. The differential impact of spermine is related to the differential role of the genes in senescence.

REFERENCES

Alscher, R.G., Eturk, N. and Heath, L.S. (2002). Role of superoxide dismutases (SODs) in cotrolling oxidative stress in plants. *J. Exp. Bot.* **53:** 1331-1341.

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

Arora, A., Watanabe, S., Ma, B., Takada, K. and Ezura, H. (2006). A novel ethylene receptor homolog gene isolated from ethylene-insensitive flowers of gladiolus (*Gladiolus grandiflora* hort.). *Biochem. Biophys. Res. Comm.* **351:** 739-744.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248-254.

Cheng, Y. and Zhao, Y. (2007). A role for auxin in flower development. *J. Integrative Plant Biol.* **49:** 99-104.

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.

Droillard, M.J. and Paulin, A. (1990). Isozymes of superoxide dismutase in mitochondria and peroxisomes isolated from petals of carnation (*Dianthus caryophyllous*). *Plant Physiol*. **94:** 1187-1192.

Droillard, M.J., Bureau, D. and Paulin, A. (1989). Changes in activities of superoxide dismutases during aging of petals of cut carnations (*Dianthus caryophyllus*). *Physiol. Plant* **76:** 149-154.

Eason, J. (2006). Molecular and genetic aspects of flower senescence. *Stewart Post harvest Rev.* **2:** 1-7.

Eason, J.R. Ryan, D.J., Pinkney, T.T. and O'Donoghue, E.M. (2002). Programmed cell death during flower senescence: isolation and characterization of cysteine proteinases from *Sandersonia aurantiaca*. *Func. Plant Biol.* **29:** 1055-1064.

Eason, J.R., Ryan, D.J., Watson, L.M., Hedderley, D., Christey, M.C., Braun, R.H. and Coupe, S.A. (2005). Suppression of the cysteine protease, aleurain, delays floret senescence in *Brassica oleracea*. *Plant Mol. Biol*. **57:** 645-657.

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 Reg.* **51:** 99-108.

Fink, R.C. and Scandalios, J.G. (2002). Molecular evolution and structure-function relationships of the superoxide dismutase gene families in angiosperms and their relationship to other eukaryotic and prokaryotic superoxide dismutases. *Arch. Biochem. Biophys.* **399:** 19-36.

Geisler, M., Kleczkowski, L.A. and Karpinski, S. (2006). A universal algorithm for genome-wide *in silico* identification of biologically significant gene promoter putative cis-regulatory-elements; identification of new elements for reactive oxygen species and sucrose signaling in Arabidopsis. *Plant J.* **45:** 384-398.

Hassan, H.M. (1988). Biosynthesis and regulation of superoxide dismutases. *Free Radical Biol. Med.* **5:** 377-385.

Kumar, N., Srivastava, G.C. and Dixit, K. (2007). Role of superoxide dismutase during petal senescence in rose (*Rosa hybrida* L.). *J. Hort. Sci. Biotech.* **82:** 673-678

Kumar, N., Srivastava, G.C. and Dixit, K. (2008). Flower bud opening and senescence in roses (*Rosa hybrida* L.). *Plant Growth Reg.* **55:** 81-99.

Kusano, T., Yamaguchi, K., Berberich, T. and Takahashi, Y. (2007). Advances in polyamine research in 2007. *J. Plant Res.* **120:** 345-350.

Mittler, R., Vanderauwera, S., Gollery, M. and van Breusegem, F. (2004). Reactive oxygen gene network of plants. *Trends Plant Sci.* **9:** 1360-1380.

Neill, S., Desikan, R. and Hancock, J. (2002a). Hydrogen peroxide signaling. *Curr. Opini. Plant Biol.* **5**: 388-395.

Neill, S., Desikan, R., Clarke, A., Hurst, R.D. and Hancock, J. (2002b). Hydrogen peroxide and nitric oxide as signaling molecules in plants. *J. Exp. Bot.* **53:** 1237-1247.

Pak, C. and van Doorn, W.G. (2005). Delay of Iris flower senescence by protease inhibitors. *New Phytol*. **165:** 473-480.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, vol. I. (2nd edn.). Cold Spring Harbor Laboratory Press, NY.

Scandalios, J.G. (1997). Oxidative Stress and the Molecular Biology of Antioxidant Defenses. Cold Spring Harbor Laboratory Press, NY.

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

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

Slocum, R.D. and Galston, R.W. (1984). Polyamine biosynthesis during tobacco ovary development. *Plant Physiol.* **75:** 152-159.

Sylvestre, I., Droillard, M.J., Bureau, J.M. and Paulin, A. (1989). Effects of the ethylene rise on the peroxidation of membrane lipids during the senescence of cut carnations. *Plant Physiol. Biochem.* **27:** 407-413.

Tun, N.N., Santa-Catarina, C., Begum, T., Silveira, V., Handro, W., Floh, E.I. and Scherer, G.F. (2006). Polyamines induce rapid biosynthesis of nitric oxide (NO) in *Arabidopsis thaliana* seedlings. *Plant Cell Physiol*. **47:** 346-354.

van Doorn, W.G., Balk, P.A., van Houwelingen, A.M., Hoeberichts, F.A., Hall, R.D., Vorst, O., van der Schoot, C. and van Wordragen, M.F. (2003). Gene expression during anthesis and senescence in Iris flowers. *Plant Mol. Biol*. **53:** 845-863.

Walz, C., Juenger, M., Schad, M. and Kehr, J. (2002). Evidence for the presence and activity of a complete antioxidant defence system in mature sieve tubes. *Plant J*. **31**: 189-197.

Woodson, W.R. and Jones, M.L. (2003). In search of eternal youth: The delay of postharvest senescence in flowers. *Acta Hort.* **624:** 305-314.

Woodson, W.R. (1987). Changes in protein and mRNA population during senescence of carnation petals. *Physiol. Plant.* **71:** 495-502.

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.

Yoda, H., Hiroi, Y. and Sano, H. (2006). Polyamine oxidase is one of the key elements for oxidative burst to induce programmed cell death in tobacco cultured cells. *Plant Physiol*. **142:** 193-206.

Yu, Q. and Rengel, Z. (1999). Waterlogging influences plant growth and activities of superoxide dismutases in narrow-leafed lupin and transgenic tobacco plants. *J. Plant Physiol.* **155:** 431-438.