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Delaying petal and leaf senescence in Yellow Star chrysanthemum using ascorbic acid

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ABSTRACT

Effect of non-enzymatic antioxidant ascorbic acid on leaf and petal senescence was studied in chrysanthemum cv. Yellow Star. The experiment was laid out in completely randomized design with eight treatments replicated thrice having three stems per replication. Spraying ascorbic acid at a concentration of 50 and/or 100 ppm significantly enhanced vase life of cut flowers accompanied by minimum change in fresh weight, higher solution uptake, no leaf wilting and also preserved maximum chlorophyll content (a, b and total chlorophyll) as compared to control. Maximum superoxide dismutase activity (10.51 units/mg protein/min) and peroxidase activity (25.54 millimol/mg protein/min) was recorded in flowers sprayed with 150 ppm ascorbic acid. Catalase activity (7.86 millimol/mg protein/min) was significant in flowers sprayed with 100 ppm ascorbic acid. Minimum H_2O_2 content (1.13 µmol/g) was observed in flowers sprayed with 50 ppm ascorbic acid as compared to control. Thus, rise in the level of superoxide dismutase, catalase, peroxidase antioxidant enzymes was observed which indicated quenching of hydrogen peroxide released by the chrysanthemum petals thus delaying leaf and petal senescence.

Key words: *Chrysanthemum* × *morifolium*, antioxidants, oxidative stress, preservative, vase life.

INTRODUCTION

Chrysanthemum (*Chrysanthemum* × *morifolium* Ramat.) is one of the most popular flower crops grown for cut flower, loose flower and pot plant purpose. It is the second largest cut flower grown all over the world. In India, it is cultivated in an area of 16.63 thousand hectares with a production of 179.37 MT (Saxena *et al.*, 14). The economic value of flower crops depends on their visual appearance. Post-harvest quality of cut flowers in chrysanthemum is based on freshness of both petals and leaves. Chrysanthemum cut flowers have relatively longer vase life, but during postharvest life premature foliage discoloration in terms of yellowing, browning and wilting is a matter of concern (Jain *et al.,* 7). Spontaneous leaf discoloration of cut stems prior to the onset of petal senescence reduces the quality as well as vase life (Doi *et al*., 4). Leaf wilting is the major cause of poor quality or appearance in chrysanthemum cut flowers. Antioxidants such as ascorbic acid, glutathione, carotenoids, anthocyanin etc. play a pivotal role in the physiological processes like photosynthesis, photo-protection, cell division, plant growth, stress responses and regulation of senescence. Use of antioxidants as chemical preservatives in delaying leaf and petal senescence is entirely new approach. Information regarding use of antioxidants as preservatives in controlling leaf discoloration is very meager. Therefore, an effort was directed to study the ability of non-enzymatic antioxidant ascorbic acid as a chemical preservative to delay both leaf and petal senescence in chrysanthemum.

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MATERIALS AND METHODS

The present study was conducted at the Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi during 2016. Cut stems of chrysanthemum cv. Yellow Star were harvested during morning hours from the divisional research farm when they were fully open before anthesis. Harvested stems were immediately placed in a bucket containing clean water to keep them turgid and were brought to the laboratory. These stems were cut back to uniform length of 60 cm and the leaves from the lower 1/3rd portion of the stem were removed and flowers were kept in distilled water. The experiment was laid out in completely randomized design with eight treatments replicated thrice having three stems per replication and the data were subjected to analysis of variance. The basal portion of the cut stems (2cm) was re-cut under water and these cut stems were kept in test tubes (100ml) containing distilled water or preservative solutions as per treatment combinations. Keeping T_1 as control (Distilled water spray), the cut stems were uniformly sprayed with freshly prepared solutions of 50 ppm ascorbic acid (T_2), 100 ppm ascorbic acid (T_3) and 150 ppm ascorbic acid (T_4) at alternate days

till termination of vase life while, the cut flowers of T_5, T_6, T_7 and T_8 were kept continuously in test tubes containing vase solutions (T_5- 4% sucrose, T_6- 50 ppm ascorbic acid + 4% sucrose, T_7 - 100 ppm ascorbic acid + 4% sucrose and T_8- 150 ppm ascorbic acid + 4% sucrose).The open space between stem and test tube rim was covered using non-absorbent cotton to avoid moisture loss through evaporation. Physiological parameters were recorded under conditions having illumination of fluorescent lights up to 16hours and temperature of 20±2^{0}C.

Physiological observations such as vase life(days), change in fresh weight (%), solution uptake (ml), foliage wilting (%), foliage yellowing (%) were recorded at the termination of vase life of cut flowers. Biochemical parameters like chlorophyll content (chlorophyll a, b and total chlorophyll), hydrogen peroxide (H_2O_2) content, superoxide dismutase (SOD) activity, catalase (CAT) activity, peroxidase (POX) activity were measured at 0, 7, 14 days after harvest and at the termination of vase life and their mean values were calculated. Biochemical constituents such as chlorophyll (chlorophyll 'a' 'b' and total chlorophyll) pigments were extracted from the fully expanded leaves of cut stems by non-maceration method using Dimethyl Sulphoxide (DMSO). Chlorophyll 'a', 'b' and total chlorophyll were calculated by using formulae given by Arnon (1). Hydrogen peroxide content (H_2O_2) was analysed by estimating titanium-hydro peroxide complex which absorbs at 415 nm (Rao *et al.,* 12).For the determination of antioxidant enzyme activities (SOD, CAT, POX), enzyme extract was prepared by homogenizing the weighed amount of petal samples with extraction buffer. Protein concentration of enzyme extract was estimated by Bradford reagent method. SOD activity (units/mg protein/min) was assayed based on the formation of blue coloured formazone by nitro-blue tetrazolium and O_2^- radical, absorbed at 560 nm and the enzyme decreases the absorbance due to reduction in the formation of O_2^- radical. CAT activity (milli mol/mg protein/min) was estimated by recording the absorbance of H_2O_2 at 240 nm in UV-range. A decline in the absorbance was noticed over a time period. POX activity (milli mol/mg protein/min) was assayed based on increase in absorbance due to the oxidation of guaiacol to tetra-guaiacol.

RESULTS AND DISCUSSION

Effect of ascorbic acid treatment on postharvest parameters of chrysanthemum cv. Yellow Star is depicted in Table 1. It is evident from the data that the maximum vase life (23.00 days) was recorded in treatment T_7 i.e. vase solution containing ascorbic acid 100 ppm + sucrose 4% which was at par with T_2, T_4 and T_6. It has been reported that ascorbic acid enhanced flower tolerance to water deficit stress and increased vase life of cut rose flowers (Jin *et al.,* 8). Minimum change in fresh weight (17.53%) was observed when flowers were held in a solution containing 50ppm ascorbic acid (T_2) compared to control (26.57%) and it was statistically at par with ascorbic acid 100ppm+ sucrose 4% (T_7) (Table 1). Solution uptake was found maximum (105.60ml) when flower sprayed with 100ppm ascorbic acid (T_3) compared to control. Lesser change in fresh weight is due to free uptake of solution into cut stems and

Table 1. Influence of ascorbic acid on physiological parameters and leaf senescence of chrysanthemum cv. Yellow Star.

Treatments	Vase life (days)	Change in fresh weight (%)	Solution uptake (ml)	Leaf Wilting (%)	Leaf Yellowing (%)
T_1 - Distilled water (spray)	18.33	26.57 (5.16)	40.33	14.44 (2.65)	5.06 (8.39)
T_2- Ascorbic acid 50ppm (spray)	22.00	17.53(4.18)	81.67	0.00 (1.00)	6.17 (9.77)
T_3- Ascorbic acid 100ppm (spray)	19.67	48.02 (6.90)	105.60	0.00 (1.00)	9.85 (9.71)
T_4- Ascorbic acid 150ppm (spray)	20.33	32.20 (5.66)	48.00	0.00 (1.00)	7.44 (8.81)
T_5- Sucrose 4% (vase solution)	19.00	36.00 (5.99)	63.67	3.69 (1.71)	21.42 (16.95)
T_6- Ascorbic acid 50ppm + Sucrose 4% (vase solution)	22.67	36.67 (6.04)	67.67	37.79 (5.01)	0.00 (1.00)
T_7- Ascorbic acid 100ppm + Sucrose 4% (vase solution)	23.00	25.34 (5.00)	75.00	54.80 (6.64)	10.43 (10.03)
T_8- Ascorbic acid 150ppm + Sucrose 4% (vase solution)	19.33	29.39 (5.42)	62.67	0.00 (1.00)	0.00 (1.00)
CD (0.05)	2.914	0.891	11.05	0.60	2.82

*Figures given in parentheses are transformed values

spontaneously reduced the transpiration losses from the leaves and petals. Spraying of ascorbic acid to the leaves reduced the cell sensitivity to postharvest stresses faced by the cut stems by preserving cell turgidity and constant supply of carbohydrates ensuring slower respiration rate. Ascorbic acid decreased pH of solution and improved transpiration rate thereby enhanced solution uptake. Therefore, the increase in vase life of chrysanthemum may be attributed to a minimum change in fresh weight and maximum solution uptake.

Results with respect to leaf senescence are presented in Table 1. It is clear from the table that no leaf wilting was observed in treatments T_2, T_3, T_4, T_5 and T_8 while maximum leaf wilting was observed in T_7. No leaf yellowing was observed in treatments T_6 and T_8 while maximum yellowing was recorded in T_5. Loss of quality of cut flowers was an outcome of leaf wilting due to impeded water movement in cut stems. It is well known fact that leaf senescence mainly caused due to the loss of chlorophyll pigments by natural stresses and prevention of chlorophyll degradation in cut flowers had been achieved by means of chemicals, with varying degrees of success. Addition of carbohydrate source such as sucrose to the holding solution may improve the vase life of flowers only when there is a controlled growth of microbes. Contrasting result with respect to T_7 might be due to xylem blockage at the end of cut stems. This might have hindered the movement of ascorbic acid in reaching the leaves. Elevated H_2O_2 concentration in leaves might have resulted in maximum leaf wilting as chloroplasts are main centers of its production. However, dissolved sugars in cells of petals have made cells become turgid utilizing hydrolyzed sugars for respiration (Ichimura and Hismatsu, 6). It has been emphasized that there is increased accumulation of sugars in the phloem tissues rather than leaf mesophyll during flower senescence due to active sugar metabolism in floral tissue to remobilize sugars to developing parts of the plant (Van Doorn and Woltering, 15). Exogenous ascorbic absorbed by the leaf stomata and epidermis, enter the cell organelles via cell membrane and gets accumulated in cytosol, chloroplasts and in cell walls. Ascorbic acid reacts rapidly with reactive oxygen species such as superoxide, hydrogen peroxide and singlet oxygen, thereby prevents oxidative damage to cell membrane and cell apparatus. It has been assumed that ascorbic acid regenerates α – tocopherol and acts as electron donor to promote photosynthesis. Thus, it is obvious from results that ascorbic acid stabilized the photosynthetic pigments (chlorophyll and carotenoids) against the action of ROS and delays leaf yellowing and wilting. Increased solution uptake and constant supply of carbohydrates in the form of sucrose may preserve chlorophyll content and finally turgidity of leaves thereby, delaying the foliage wilting and yellowing. Reyes-Arribas *et al*. (13) observed that floating of leaves in sucrose solution preserved the chlorophyll content and delayed leaf yellowing by 3-9 days in chrysanthemum. Spraying of antioxidants ascorbic acid and α-tocopherol to wheat flag leaves delayed leaf senescence by alleviating harmful effects of salinity (Farouk, 5).

Effect of exogenous application of ascorbic acid on chlorophyll content (chlorophyll 'a', 'b' and total chlorophyll) is depicted in the Table 2. Maximum chlorophyll 'a'(8.27mg/g fw), chlorophyll 'b'(2.38mg/g fw) and total chlorophyll (10.65mg/g fw) content was found when flowers held in 4% sucrose solution (T_5) followed by flowers sprayed with 50ppm ascorbic acid (T_2) however, it was statistically at par with T_4. Since, chloroplasts are the main platform in cells for all the processes related to chloroplast regeneration and degradation which decides the level of leaf senescence in plants. The degenerative processes such as loss of chlorophyll decrease in soluble protein content and changes in the ratio of chlorophyll a: b is due to leaf senescence (Munnre-Bosch. 9). The combination of ascorbic acid and sucrose might have helped in retention of chlorophyll content in leaves of chrysanthemum (Jain *et al*., 7). Similarly decreased leaf wilting and yellowing was observed in above mentioned treatments. Nahed *et al*. (10) reported that exogenous application of ascorbic acid in many plant species retard chlorophyll loss and protect plants against environmental stress, thus in present studies ascorbic acid helped to maintain freshness of leaves and flowers in chrysanthemum.

It is evident from the Fig. 1 that the minimum hydrogen peroxide content (1.13 µmol/g) was observed in cut stems sprayed with 50 ppm ascorbic acid (T_2) compared to control and it was significantly different from all other treatments, however, maximum hydrogen peroxide content (3.72 µmol/g) was recorded in flowers sprayed with 100ppm ascorbic acid (T_3). Aged petals tend to contain higher amount of H_2O_2 due to oxidative stress in environment as H_2O_2 increase in the petals after treatments which accelerated during senescence and decreased when senescence is retarded by the antioxidant like sodium benzoate (Panavas and Rubinstein, 11). Since, ascorbic acid is an antioxidant therefore, may be responsible for reducing the H_2O_2 activity at onset of senescence.

It is clear from Fig. 2 that elevated superoxide dismutase activity (10.51 units/mg protein/min) was recorded in flowers sprayed with 150ppm ascorbic acid

Table 2. Influence of ascorbic acid on chlorophyll content (mg/g fw) of leaves in chrysanthemum cv. Yellow Star.

Treatments	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll
T_1 - Distilled water (spray)	6.58	1.93	8.51
T_2- Ascorbic acid 50ppm (spray)	8.03	2.30	10.33
T_3- Ascorbic acid 100ppm (spray)	6.83	2.14	8.97
T_4- Ascorbic acid 150ppm (spray)	7.96	2.38	10.33
T_5- Sucrose 4% (vase solution)	8.27	2.38	10.65
T_6- Ascorbic acid 50 ppm + Sucrose 4% (vase solution)	5.95	1.92	7.87
T_7- Ascorbic acid 100 ppm + Sucrose 4% (vase solution)	6.24	2.12	8.37
T_8- Ascorbic acid 150 ppm + Sucrose 4% (vase solution)	4.90	2.08	6.98
CD (0.05)	0.86	0.54	1.16

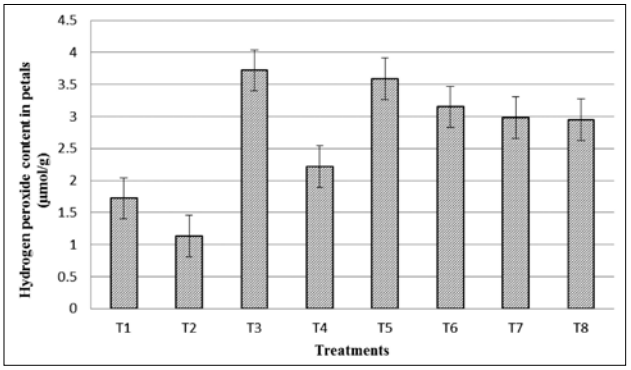

Fig. 1. Effect of ascorbic acid on hydrogen peroxide (H_2O_2) (µmol/g) content in chrysanthemum cv. Yellow Star.

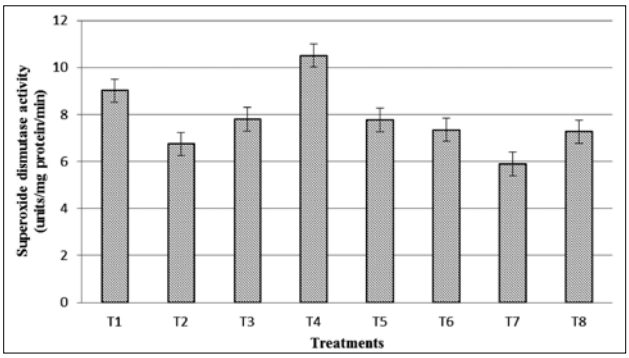

Fig. 2. Effect of ascorbic acid on superoxide dismutase activity (millimol/mg protein/min) in Chrysanthemum cv. Yellow Star.

(T_4) however, minimum superoxide dismutase activity (5.90 units/mg protein/min) was recorded in flowers held in 4% sucrose (T_5). Similar to our findings SOD levels were found to decrease with the progression of petal senescence in chrysanthemum (Bartoli *et al.,* 2). The significant increase in the activity of SOD, CAT, POX and APX (Ascorbate peroxidase) in the initial stages of senescence indicates that antioxidant defense triggered by coordinated mechanisms to control damage by aging in petals. Moreover, higher SOD activity due to increased oxidative stress may be attributed to increased membrane permeability and H_2O_2 generation.

It is clear from Fig. 3 that maximum (7.86 millimol/mg protein/min) catalase activity was recorded in flowers sprayed with 100ppm ascorbic acid (T_3) and it was significantly different from all other treatments, however, minimum catalase activity (4.05 millimol/mg protein/min) was recorded under control (T_1). Similarly, it was observed that catalase activity steadily increased during vase life and highest activity was observed 14 days after treatment before it declined at senescence (Chakrabarty *et al*., 3). Catalase activity was progressed towards

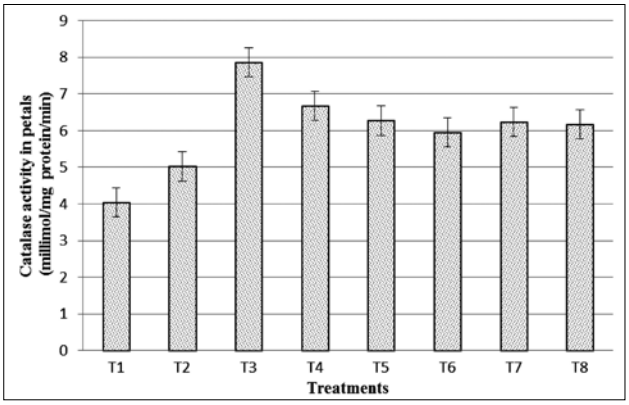

Fig. 3. Effect ascorbic acid on catalase activity (millimol/mg protein/min) in chrysanthemum cv. Yellow Star.

senescence because of higher H_2O_2 generation in petal tissues.

From Fig. 4 it is clear that maximum guaiacol peroxidase activity (25.54 millimol/mg protein/min) was recorded in flowers sprayed with 150ppm ascorbic acid (T_4) compared to control and other treatments. The present investigations are in close

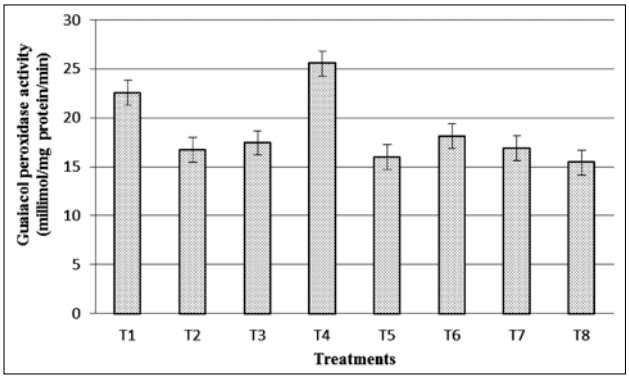

Fig. 4. Effect of ascorbic acid on peroxidase activity (millimol/mg protein/min) in chrysanthemum cv. Yellow Star.

conformity with the findings of Bartoli *et al*. (2) who reported that guaiacol peroxidase activity increases during senescence in carnation.

In this study, spraying flowers with 50ppm and 100ppm ascorbic acid has performed well with respect to physiological parameters like vase life, solution uptake and change in fresh weight. Lesser change in fresh weight and maximum solution uptake maintained osmotic gradient in vascular system of cut stems and balanced the turgidity to keep freshness of cut flowers. Leaf senescence was also delayed in cut flowers by maintaining stability in regeneration of chlorophyll content and constant supply of stored food reserves to cut flowers. No leaf wilting was observed when sprayed with 50ppm ascorbic acid with reduced level of hydrogen peroxide content. Substantial increase in levels of antioxidant enzymes after spraying with ascorbic acid indicates delayed senescence due to decreased hydrogen peroxide content in petal tissues. Therefore, it has been concluded from the present investigation that spraying ascorbic acid at 50 and/or 100 ppm helped in increasing vase life of chrysanthemum cv. Yellow Star cut flowers. Based on the results it can be concluded that spraying ascorbic acid may substitute vase solution for delaying leaf and petal senescence in chrysanthemum.

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