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Combined kinetin-morphactin application delays petal senescence in cut flower sticks of *Polianthes tuberosa*

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Abstract

To assess the role of kinetin (KN) and morphactin (MOR) separately and also in combination in the regulation of petal senescence, cut flower sticks of *Polianthes tuberosa* were placed in various holding (vase) solutions like sucrose (0.1M), kinetin (KN, 20 pM), morphactin (MOR, 20 pM), KN (20 pM) + MOR (20 pM) and sucrose (0.1M) + KN (20 pM) + MOR (20 pM). Holding solutions were taken in sterilized conical flasks and the whole set up was placed under fluorescent tubes (40 Lux light intensity). A combination of sucrose + KN + MOR was the best followed by KN + MOR in partly controlling the loss of moisture content and flower turgidity, as well as a rapid decline in the fresh weight of flower sticks during 4-day as witnessed in treated and control sets. It was also noticed that both plant growth regulators (PGRs) when applied together and when sucrose was included, became more effective in maintaining relatively higher values of flower diameter. Investigation on selected biochemical changes revealed a sharp increase in reducing and total sugars of petals of untreated flower sticks, and KN treatment was found to be the best in arresting sugar accumulation. For the retention of protein, holding solution having sucrose + KN + MOR was the best while lipid peroxidation was arrested by KN+MOR but sucrose application alone was more effective in reducing this process. This study revealed a sharp increase in proline content in untreated tuberose petals, while reduced to some extent by sucrose and further reduction by MOR and KN. Sucrose was found to be the least effective in lowering the proline level.

Key words: Combined application, flower sticks, kinetin, morphactin, *Polianthes tuberosa,* senescence.

Introduction

Senescence is characterized by various deteriorative changes which are not only morphological but also physiological and biochemical in nature. These diverse changes are responsible for metabolic failure that will decrease the functional capacities of plants or plant organs. Flower senescence represents the last stage of floral development exhibiting wilting or abscission of whole flowers or flower parts (Stead and van Doorn, 1994). It is generally rapid and continuous process similar to whole plant or leaf senescence (Voleti *et al.,* 2000).

A cut flower may be defined as flower or an inflorescence containing more than one floral unit in the opened or unopened state which is harvested and marketed for ornamental purpose. Freshness and turgidity are lost rapidly in cut flowers than in uncut ones as the supply of water and nutrients become a limiting factor. The degradation of carbohydrates, proteins, lipids and nucleic acids are common biochemical changes observed in cut flowers and petals (van Doorn and Woltering, 2008; Khokhar *et al.,* 2018). Various holding solutions like sugars, biocides, essential elements and plant growth regulators (PGRs) have been used to increase the longevity of cut flowers (Ma *et al.*, 2018; Singh *et al.*, 2018; Mukherjee and Mukherjee, 2020).

Sugars like sucrose can maintain the flower quality and extend vase life as they are the source of carbon and energy (Kuiper *et al.,* 1995; Monteiro *et al.,* 2002; van Doorn, 2004; Mukherjee and Mukherjee, 2017). Sucrose can increase the osmotic concentration of petal cell sap and maintain the turgidity in cut flowers when present in the vase solution (O' Donoghue *et al.,* 2002). Sugars are known to delay senescence by suppressing ethylene synthesis. Ethylene is a naturally occurring PGR associated with petal senescence besides its other effects (Woltering and van Doorn, 1988). PGRs like abscisic acid (ABA), cytokinins, auxins, salicylic acid, morphactins (MOR), gibberellins, polyamines, etc. are also involved in the regulation of petal senescence (Kaur *et al.,* 2017; Khokhar *et al.,* 2018).

In general, cytokinins delay petal senescence either by inhibiting ethylene synthesis or by decreasing the sensitivity of the cells to ethylene (van Staden and Dimalla, 1980; Saha *et al.,* 1985). Enhanced cytokinin level in *Petunia* and tobacco delays flower senescence (Zubko *et al.,* 2002). Longevity of harvested *Grevillea* inflorescences was increased with 6-benzylaminopurine treatment (Setyadjit *et al.*, 2004). In cut *Eustoma* flowers, the vase life was improved by reducing weight loss and anthocyanin degradation and ethylene production in presence of benzylandenine (Asil and Karimi, 2010). However, vase life of *Bougainvillea glabra* bracts was much greater in presence of sucrose as compared to control and kinetin (Moneruzzaman *et al.*, 2010). The combined application of KN with sucrose made *Matricaria* flowers more turgid and fresh by lowering starch degradation, lipid peroxidation

and lipoxygenase activity (Mukherjee and Mukherjee, 2017; Kaur *et al.,* 2017).

Morphactins, all of which are flurene derivatives, developed by Schneider (1970) as a new group of synthetic PGR are associated with various investigations in the field of morphology, physiology and biochemistry (Schneider, 1970; Khokhar and Mukherjee, 2010, a,b). In recent years, methylchlorflurenol (MCF, a morphactin, MOR) has been reintroduced by Repar Corp., Maryland, USA. As the corresponding author has been working with this PGR since long, it was thought to use this PGR to assess its effect on the longevity of petals of cut sticks of tuberose (*Polianthes tuberosa*), a very important flower worldwide for its sweet and pleasant fragrance. Tube rose flowers are commercially used in India for decoration and extraction of essential oil (Singh and Singh, 2013).

Present study has been undertaken with *P. tuberosa* selecting two PGRs *viz.*, MCF - a morphactin (MOR) and kinetin (KN) - a cytokinin; both are known to delay senescence. Attempt has been made here not only to compare effectiveness of MOR and KN in delaying petal senescence but also to find out their performance when both are included in the same holding solution and when sucrose was present along with KN + MOR.

Materials and methods

Plant material: Fresh sticks of *Polianthes tuberosa* L. cv. Calcutta Single having almost similar diameter and morphological appearance of flower buds and open flowers were collected from Ma Santoshi Nursery, Burdwan and cut ends were maintained in water inside the bucket. All flowering sticks had about 8-9 open flowers on the lower side and 5-6 flower buds on the upper (apical) side. The cut ends of sticks were recut under water to get an uniform length of 16 cm. They were transferred to 250 mL Borosil conical flasks, each one having 50 mL of holding solution. Five conical flasks were maintained for a specific solution and each flask had 2 flower sticks. Holding solutions selected for the experiment were double distilled water (DDW, control), sucrose (SUC, 0.1M), methylchlorflurenol (a morphactin, MOR, 20 µM), kinetin (KN, 20 µM), MOR (20 µM) + KN (20 µM) and sucrose (0.1M) + MOR (20 µM) + KN (20 µM). The experiment was set up in continuous light under 1 fluorescent tube of 40 Lux light intensity at 30±2 °C for 4 days. Weight of flower sticks was recorded individually on initial and after 4-day. Volume of holding solutions in each conical flask was measured again after 4-day. Flower diameters were recorded at 0, 2 and 4-day. Petal samples were collected in triplicates to find out moisture content, dry weight, reducing and total sugars, protein content, amount of proline, MDA content and guaiacol peroxidase (GPOX) activity. Cumulative uptake of all holding solutions by flower sticks was also determined. Experiments were repeated to confirm the results.

Fresh weight of flower sticks, flower diameter and moisture content: Fresh weight of each tuberose stick was recorded at initial and final *i.e.*, 0-day and 4-day stages. Percent difference between the two readings was also noted to find out the effectiveness of specific treatment. Diameter of individual flower was determined as mean of two perpendicular measurements across a flower. Data of fresh weight and diameter were based upon 5 replicates. To determine the moisture content of flowers, 100 mg of fresh samples in triplicate were placed in an oven at 80 °C for 48 h.

Cumulative uptake of holding solutions: Volume of holding solutions in each conical flask was measured on the initial and final day. Difference between the two gave an idea regarding the uptake of the solution by scapes (tuberose sticks).

Reducing and total sugars: Anthrone reagent was used to estimate reducing and total sugars as recommended by Hart and Fisher (1971). For each extraction, 100 mg of petal sample was homogenized in 10 mL of double distilled water (DDW). It was centrifuged at 5000 rpm for 10 min. in a centrifuge (Remi). The supernatant was collected and final volume was raised to 50 mL with DDW. The pH of the extract was noted. Three milliliter of the extract was taken in triplicate in test tubes and kept in water bath at 100 °C for 5 min. Six milliliter of anthrone reagent was added slowly to these test tubes. Contents were mixed properly by gentle shaking, resulting blue-green colour. Test tubes were then cooled in ice water taken in a beaker. Blank set was prepared by mixing 3 mL of DDW and 6 mL of anthrone reagent in the same manner as already described. The absorbance was recorded at 600 nm wavelength in a spectrophotometer.

From the remaining aqueous extract, 35 mL was hydrolyzed with 10 mL 50 % HCl for a day at room temperature and next day the earlier pH was reset with 6 N NaOH. It was followed by raising the volume to 100 mL with DDW. Now three aliquots of 3 mL each were taken in test tubes followed by addition of 6 mL of anthrone reagent in the same manner as described for reducing sugars. After cooling in ice water, the absorbance was recorded in each case at 625 nm. Amount of reducing and total sugars were estimated in this manner. Amount of non-reducing sugars was determined by subtracting the amount of reducing sugars from that of total sugars.

Total soluble protein: Petal sample weighing 100 mg was dropped in 10 mL of 80 % boiling ethanol in a test tube placed in a water bath at 100 °C for 1 min. It was brought down to room temperature and homogenized in same ethanol using pestle and mortar. The extract was centrifuged in a Remi centrifuge at 5000 rpm for 15 min. Supernatant was discarded and the residue was re-extracted with 10 mL of 5 % perchloric acid and centrifuged again at 5000 rpm for 15 min. Supernatant was discarded again and 5 mL of 1N NaOH was added to the residue and collected it in a test tube. The residue in the alkali was dissolved (as far as possible) by shaking and putting it in to the water bath at a temperature of 40-50 °C for 20 min. It was centrifuged again at 5000 rpm for 15 min. and supernatant was collected separately for protein estimation by the method of Bradford (1976). To 0.3 mL of protein extract 0.7 mL of DDW was added. Further, 5 mL of Coomassie brilliant blue G-250 reagent was added and shaken well at room temperature. Blank was prepared by mixing 1 mL DDW and 5 mL of this reagent. Absorbance was recorded at 595 nm in a spectrophotometer. The protein content of samples were calculated against a standard curve of bovine serum albumin (BSA, Sigma, USA).

Proline content: Proline content was estimated following the method as described by Bates *et al* (1973). Two hundred milligram of petal sample was homogenized in 2 mL of 3 %

sulfosalicylic acid. The homogenate was centrifuged for 15 min at 10000 rpm in a Remi centrifuge (R-8C). Free proline in the supernatant was treated with 2 mL of 3 % glacial acetic acid (GAA) and 2 mL of acid ninhydrin. Reaction mixture in the test tubes was kept in boiling water bath for 1 h at 95 °C. Reaction was terminated in the ice bath and colour complex was extracted in 4 mL of pure toluene. The absorbance was recorded at 520 nm in a spectrophotometer. Proline was quantified against a standard curve of proline following the same procedure.

Lipid peroxidation (MDA content): The level of lipid peroxidation of petal sample was estimated in terms of MDA content (Heath and Packer, 1968). Two hundred milligram petal sample was homogenized in 2 mL of 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 10000 rpm for 20 min in a Remi refrigerated centrifuge. To 0.5 mL aliquot of the supernatant, 2 mL of 0.5 % thiobarbituric acid (TBA) in 20 % trichloroacetic acid (TCA) was added. The mixture was heated at 90 °C for 30 min in the water bath and then quickly cooled in an ice water bath. After centrifugation at 10000 rpm for 10 min, the absorbance of the supernatant was recorded at 532 nm. The value for nonspecific absorption of each sample at 600 nm was also recorded and subtracted from the absorption recorded at 532 nm. The concentration of MDA, an end product of lipid peroxidation was calculated in accordance to its extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as n mol g^{-1} FW.

Guaiacol peroxidase (GPOX) activity: For GPOX activity, the methodology of Maehly (1954) was followed. Petal sample weighing 100 mg was homogenized with 10 mL of ice cold 0.1 M KH_2PO_4 - Na_2HPO_4 buffer of pH 7.0 and centrifuged in a Remi centrifuge at 5000 rpm for 15 min. Supernatant was collected and raised to 10 mL with the above ice cold phosphate buffer. Reaction set was prepared by mixing 2 mL of enzyme extract, 2 mL of phosphate buffer (pH 7.0), 2 mL of guaiacol (20 mM) and 2 mL of H_2O_2 (10 mM) in a sequence. Blank set contained 2 mL of enzyme extract, 2 mL of phosphate buffer (pH 7.0) and 4 mL of DDW. After 10 min the absorbance was recorded at 420 nm in a spectrophotometer. Specific GPOX activity was expressed in terms of milligram protein per 10 min. Protein was estimated from the enzyme extract using Coomassie brilliant blue reagent as mentioned earlier (Bradford, 1976).

Statistical analysis: Treatments were arranged in a randomized block design with at least three replicates in case of biochemical estimations and each replicate had three aliquots being analyzed. Some of the data were statistically analyzed using analysis of variance (ANOVA) by using SPSS ver. 23. The treatment means were analyzed by Duncan's multiple range test (DMRT) at $P<0.05$.

Results and discussion

Morphological variations in *P. tuberosa* cut flower sticks have been recorded in Table 1. All flower sticks remained fresh up to 1.5 day but after 2 days several changes could be noticed. All flowers were in the blooming stage but it was better in the treated ones. Combined application of KN+MOR exhibited best blooming of tuberose. Senescence symptoms like loss of turgidity, appearance of wrinkles, rapid moisture loss and dryness of petals could be noticed at this time. Senescence was more in the control than in the treated sets. Four day stage was characterized by drooping of sticks and abscission of flowers in control sets. Presence of PGRs could partly check these phenomena and the condition of flower sticks was the best in sucrose + KN + MOR> KN + MOR> MOR > KN > sucrose > control after 4-day.

Table 1. *Polianthes tuberosa* L. showing various physical changes in flower sticks maintained in double distilled water (DDW, control), sucrose (0.1M), morphactin (MOR, 20μM), kinetin (KN, 20 μM), MOR (20 μM) + KN (20 μM) and sucrose (0.1M) + MOR (20 μM) + KN (20 μM).

Day	Observations
1.5	All flower sticks remained fresh.
2.0	No drooping and abscission of petals. Blooming in all flowers. Better blooming in treated flowers. Best blooming in KN+MOR followed by KN. Senescence symptoms appeared. Degree of senescence greater in control sticks.
4.0	Drooping and abscission in control flowers; control flower buds dried. Drooping of one of the sticks in KN-treated sets while no drooping in MOR - treated ones. Flower drooping and abscission in sucrose treated sets.

Fresh weight changes of scapes having different vase solutions exhibited a sharp decline during 4 days (Fig. 1a). Scapes getting distilled water and maintained as control showed 62.17 percent decrease while some reduction was achieved in fresh weight loss by both PGRs and sucrose. Kinetin application was the best in minimizing this reduction when effectiveness of individual compounds was compared. It was interesting to note that combined holding solution containing sucrose (0.1M) + MOR (20 μM) + KN (20 μM) was the best with minimum loss in fresh weight of *P.tuberosa* scapes.

Moisture content of tuberose petals was very high (94.65 %) initially and the percent decline was relatively lower during 0 to 2-day irrespective of treatments. However, the decline was lowest when vase solution contained MOR, KN and sucrose not only at 0 to 2-day but also at other stages. The effectiveness in retaining moisture was in the order of sucrose + MOR + KN > MOR + KN> MOR > KN > Sucrose > control (Fig. 1b).

While working with cut flowers of *Calendula officinalis,* decline in fresh weight of scapes was noticed in the control and treated cut flowers during 6 days. Morphactin (MOR) treatment of 20 μM concentration was able to reduce this decrease and combined application of sucrose (0.1M) + MOR (20 μM) + 8 - hydroxy quinoline (8-HQ, 1.5 mM) was unique in showing slight increment (Singh *et al.,* 2018). Morphactin, a novel synthetic PGR, known for keeping the tissues hydrated (Schneider, 1970) has also been found to maintain moisture at higher level in *Calendula* (Khokhar *et al.,* 2018; Singh *et al.,* 2018) as also in the present study.

Combined applications of sucrose + MOR + KN as well as MOR + KN were able to reduce % decline in flower diameter appreciably during 0 to 2-day in comparison to control but at later stage it showed higher percent decline irrespective of treatments. However, on the whole, the combined applications of two PGRs with or without sucrose partly maintained flower diameter and moisture content (Figs. 1b and 2). Turgidity and freshness of flowers could also be maintained in their presence. This study has clearly revealed the effective role of sucrose + MOR + KN when they were present in the same conical flask collectively as the decrease in flower diameter was minimized. Role of PGRs like MOR and kinetin; metabolite like sucrose and biocide like 8-HQ

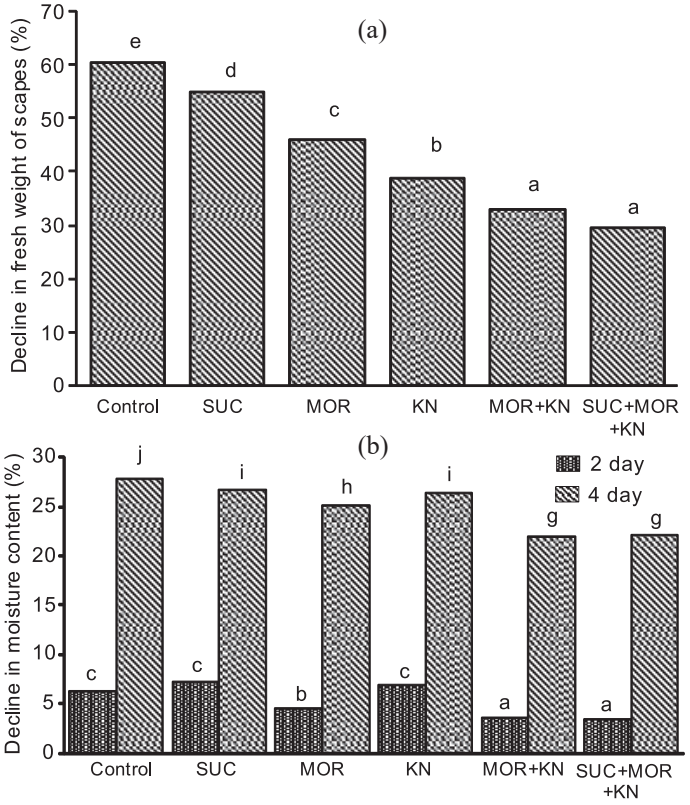

Fig. 1. *P. tuberosa* L. showing (%) decline in (a) fresh weight of scapes during 0-4 day and (b) moisture content of flower petals (during 0-2 day and 0-4 day) when scapes were maintained in double distilled water (DDW), sucrose (0.1M), morphactin (MOR, 20 µM), kinetin (KN, 20 µM), MOR (20 µM) + KN (20 µM) and sucrose (0.1M) + MOR (20 µM) + KN (20 µM). Bars followed by different letters show significant differences at $P < 0.05$ between treatments according to Duncan's multiple range test (DMRT).

are known to bring down the shrinkage of petals and decrease in flower diameter (Mukherjee and Mukherjee, 2017; Khokhar *et al.,* 2018; Singh *et al.,* 2018; Mukherjee and Mukherjee, 2020).

Table 2 showed that cumulative uptake of vase solution was maximum in control sets having distilled water and minimum in 0.1M sucrose solution. Between MOR and KN, the uptake was higher in the latter. However, when both were combined, the uptake increased markedly; whereas inclusion of sucrose reduced the process. Dry weight change in tuberose petals exhibited highest increment when sucrose was present as a holding solution as compared with control. Percent dry weight increase between 0 to 4-day was slightly lower in case of MOR than control. This value decreased further when both PGRs were included in vase solution with or without sucrose (Fig. 3).

Table 2. *P. tuberosa* L. showing cumulative uptake of holding solutions (in mL) by scapes when they were maintained in double distilled water (DDW), sucrose (0.1M), morphactin (MOR, 20 µM), kinetin (KN, 20 µM), MOR (20 µM) + KN (20 µM) and sucrose (0.1M) + MOR (20 µM) + KN (20 µM).

Holding solutions	Volume of holding solutions		Cumulative uptake during 4-Day
	0-Day	4-Day	
Control (DDW)	50	6.0	44.0
Sucrose	50	43.4	6.6
Morphactin	50	30.8	19.2
Kinetin	50	24.8	25.2
MOR + KN	50	14.0	36.0
Sucrose + MOR + KN	50	29.2	20.8

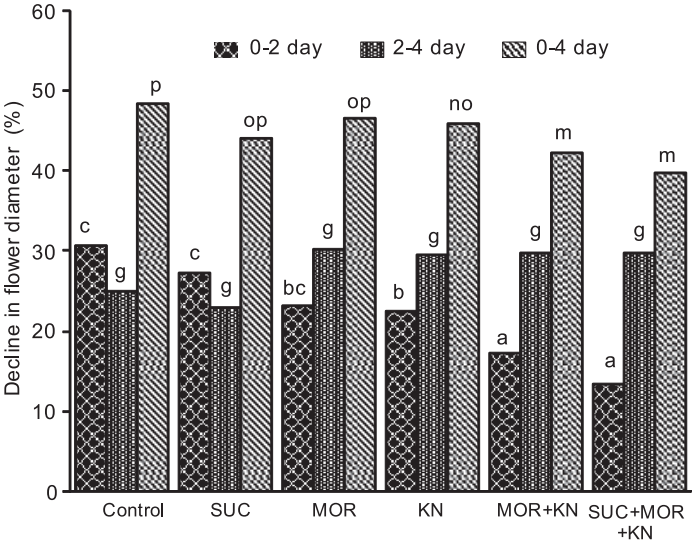

Fig. 2. *P. tuberosa* L. showing (%) decline in flower diameter (in cm) between 0-2, 2-4 and 0-4 day period when scapes were maintained in double distilled water (DDW), sucrose (0.1M), morphactin (MOR, 20 µM), kinetin (KN, 20 µM), MOR (20 µM) + KN (20 µM) and sucrose (0.1M) + MOR (20 µM) + KN (20 µM). Data are means ± standard error (S.E.) of five replicates. Bars followed by different letters show significant differences at $P < 0.05$ between treatments according to DMRT.

Alteration in the amount of reducing and total sugars can be seen in Fig. 4. Petals showed 23.78 mg per 100 mg total sugars at 0-day before any treatment in which the amount of reducing sugars was much lower than non-reducing sugars. This trend was maintained even at 2 and 4-day stages. Petals of cut sticks of *P. tuberosa* exhibited a little higher value of total sugars at 2-day stage in all cases except in the inflorescence twigs held in KN solution. However, the reducing sugar concentrations at 2-day were lower than that of 0-day. A considerable reduction was recorded in the amount of total sugars at 4-day in comparison to 2-day; the decline was highly significant in non-reducing sugars. Among all holding solutions, KN was the best in maintaining much lower values of both reducing and non-reducing sugars at both stages. Maximum amount of total sugars recorded in petals of the inflorescence held in sucrose followed by control, MOR+KN, etc. and minimum was found in KN vase solution at 4-day. Relatively higher amount of total sugars in all petals (except those inflorescence maintained in KN solution) at 2-day than 0-day stage might be due to greater starch degradation as observed earlier (Khokhar *et al.*, 2018). Unlike flowers like Calendula, Gaillardia, etc. which are solitary in nature, tuberose sticks have flowers at various developing stages at different nodes. Younger unopened flower buds occupy apical portion. The drastic reduction in the sugar content at 4-day might be due to transport of sugars from open flowers to these flower buds.

Earlier studies revealed higher requirement of sugars in plants undergoing stress (Koizuka *et al.,* 1995; Yakimova, 1997). Removing flower twigs from plant is to provide a kind of stress to them when the supply of both inorganic and organic nutrients become very much limited along with water. Untreated cut flowers accumulated greater amount of sugars as there was no check over starch degradation. No set pattern has been noticed regarding the relative amount of reducing and non-reducing sugars at different stages of flower development. In carnation flowers, reducing sugars were the main constituents at maturity whereas

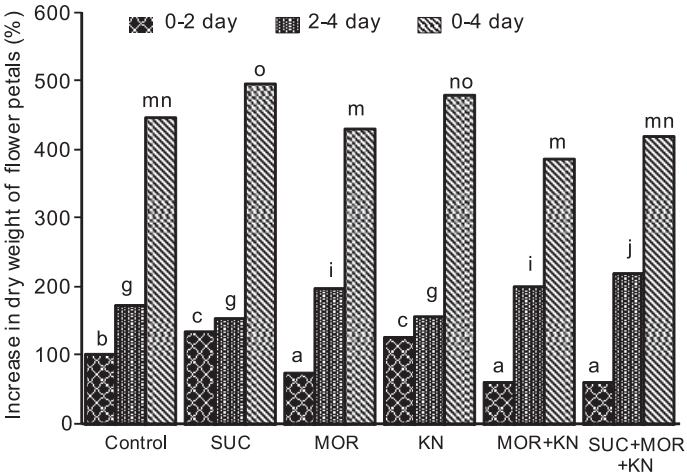

Fig. 3. *P. tuberosa* L. showing (%) increase in dry weight in petals between 0-2, 2-4 and 0-4 day period when scapes were maintained in double distilled water (DDW), sucrose (0.1M), morphactin (MOR, 20 µM), kinetin (KN, 20 µM), MOR (20 µM) + KN (20 µM) and sucrose (0.1M) + MOR (20 µM) + KN (20 µM). Bars followed by different letters show significant differences at $P < 0.05$ between treatments according to DMRT.

both reducing and non-reducing sugars declined at senescence (Nichols, 1973). In *Hibiscus rosa sinensis* petals, reducing sugars declined whereas sucrose increased at senescence stage (Trivellini *et al.,* 2011). Accumulation of both kinds of sugars at senescent phase has been reported in *Calendula officinalis* (Khokhar and Mukherjee, 2010 a, b; Kaur and Mukherjee, 2013), *Arctotis grandis* (Khokhar and Mukherjee, 2010b), *Chrysanthemum dendranthema grandiflorum* var. Chandrima (Kaur and Mukherjee, 2016) and *Matricaria parthenium* L. (Mukherjee and Mukherjee, 2017).

Protein content of flowers declined sharply between 0 to 2-day followed by a small decrease during 2 to 4-day in control. Treated holding solutions were able to reduce the loss (Fig. 5). Again, maximum reduction in protein loss was achieved by the combined application of sucrose + MOR + KN. The decrease in protein concentration precedes visible symptoms of senescence (Lay-yee *et al.,* 1992). It may be due to slowing down of protein synthesis and rapid degradation (Celikel and van Doorn, 1995). Soon after full development and maturation of flowers, protein degradation starts as also observed in our earlier studies on cut flowers *viz.* in *Chrysanthemum dendranthema grandiflorum, Chrysanthemum coronarium* and *Calendula officinalis* (Kaur and Mukherjee, 2014; Kaur and Mukherjee, 2016) and also in *Salvia splendens* (Kaur *et al.,* 2015). Uncut flowers also showed rapid breakdown of proteins with the onset of senescence (Kaur *et al.,* 2014).

Proline, a stress metabolite exhibited a gradual increase with time in various scapes. Applications of PGRs were highly effective in lowering the proline content. It was interesting to note that when KN and MOR both were present, they could further reduce the concentration of proline in petals (Fig. 5). Sucrose was very little effective in lowering the proline content. Sucrose was found to suppress the combined effect of MOR + KN resulting higher amount of proline in tuberose petals. Proline accumulation in many plants during stress is considered as an adaptive response to counteract the unfavourable stress conditions. Higher levels of proline have been measured in tissues with low water content in comparison to tissue with high water content (Chiang and Dandekar, 1995). However, it has also been noticed that flowers accumulate much higher proline inspite of the fact that they contain relatively large amount of water than leaves (Mattioli *et al.,* 2009). While working with the cut flowers of *Chrysanthemum,* a gradual decline has been noticed in proline content during 0 to 9-day stage (Kaur and Mukherjee, 2016). In *P. tuberosa,* however, a gradual increase has been recorded in this amino acid. Treatments with MOR and KN, however, brought down the amino acid level considerably, specially when both were combined. Proline level was found to rise in presence of sucrose.

One of the common observations during petal senescence is the rapid increase in lipid peroxidation; that was clearly visible in tuberose as well. Sucrose, KN and MOR were capable of reducing this process to some extent. Sucrose was found to be the best followed by MOR + KN application in controlling

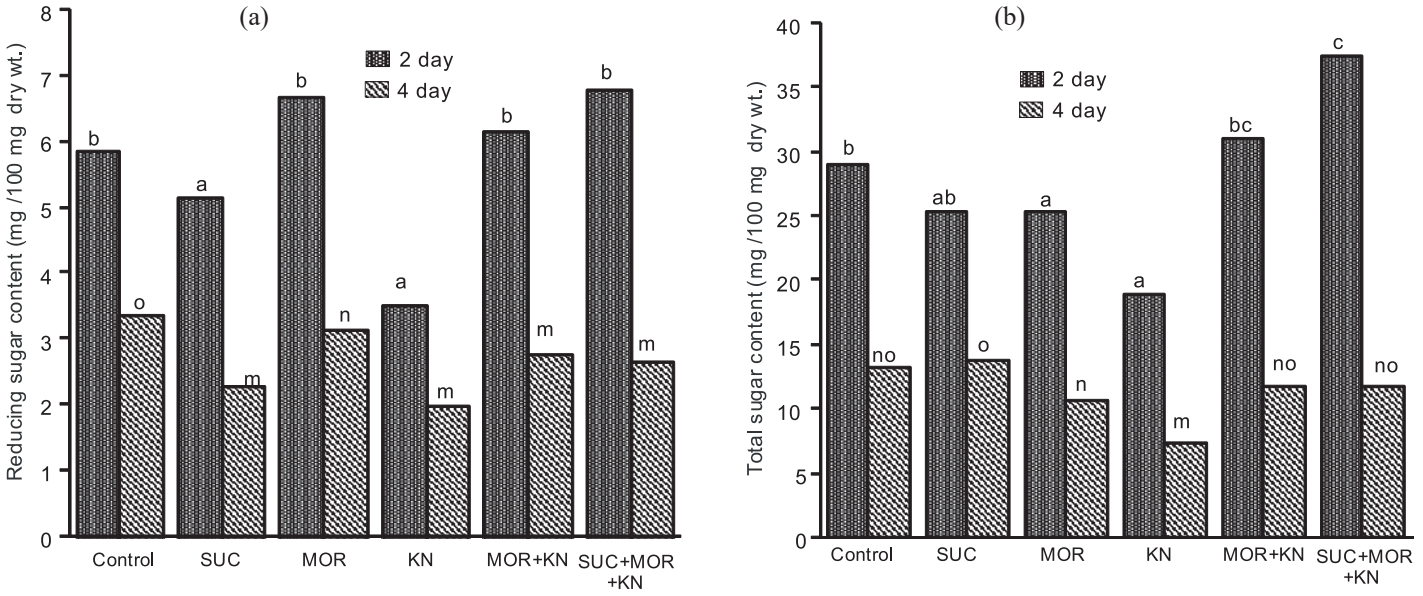

Fig. 4. *P. tuberosa* L. showing changes in (a) reducing sugars and (b) total sugars in petals of scapes maintained in double distilled water (DDW), sucrose (0.1M), morphactin (MOR, 20 µM), kinetin (KN, 20 µM), MOR (20 µM) + KN (20 µM) and sucrose (0.1M) + MOR (20 µM) + KN (20 µM). [Initial 0-day values of reducing sugars: 6.88mg ± 0.96, total sugars: 23.78mg ± 1.73]. Bars followed by different letters show significant differences at $P < 0.05$ between treatments according to DMRT.

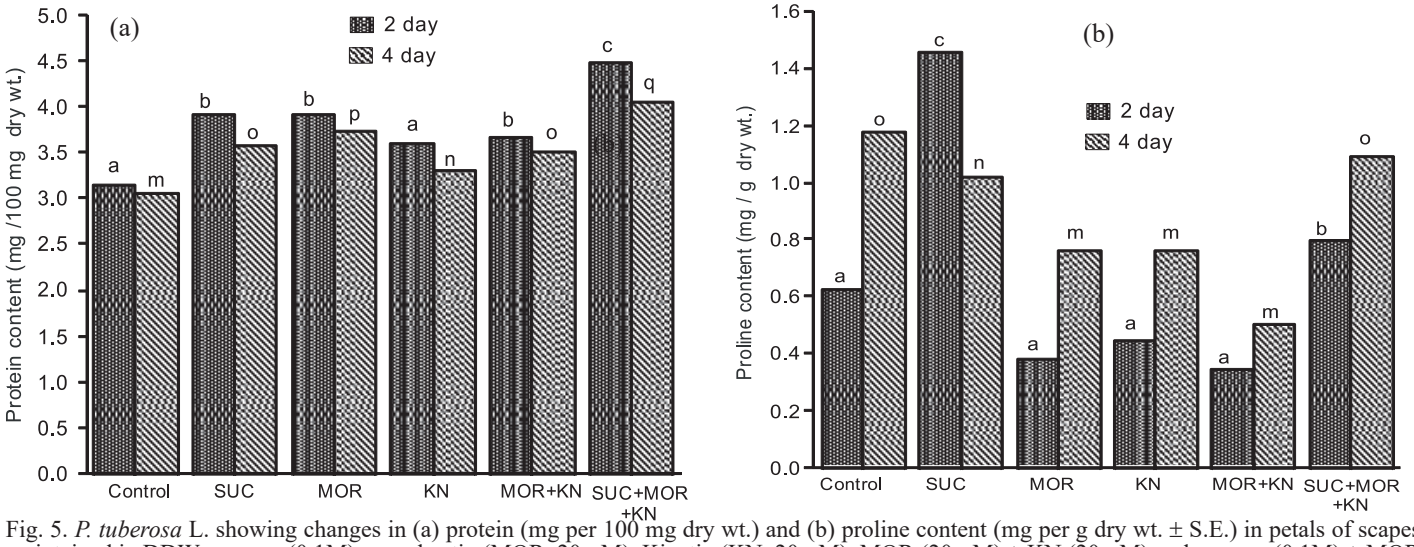

Fig. 5. *P. tuberosa* L. showing changes in (a) protein (mg per 100 mg dry wt.) and (b) proline content (mg per g dry wt. ± S.E.) in petals of scapes maintained in DDW, sucrose (0.1M), morphactin (MOR, 20 µM), Kinetin (KN, 20 µM), MOR (20 µM) + KN (20 µM) and sucrose (0.1M) + MOR (20 µM) + KN (20 µM). [Initial 0-day value of protein: 5.34 mg ± 0.28, initial 0-day value of proline: 0.127 mg ± 0.003]. Bars followed by different letters show significant differences at $P < 0.05$ between treatments according to DMRT.

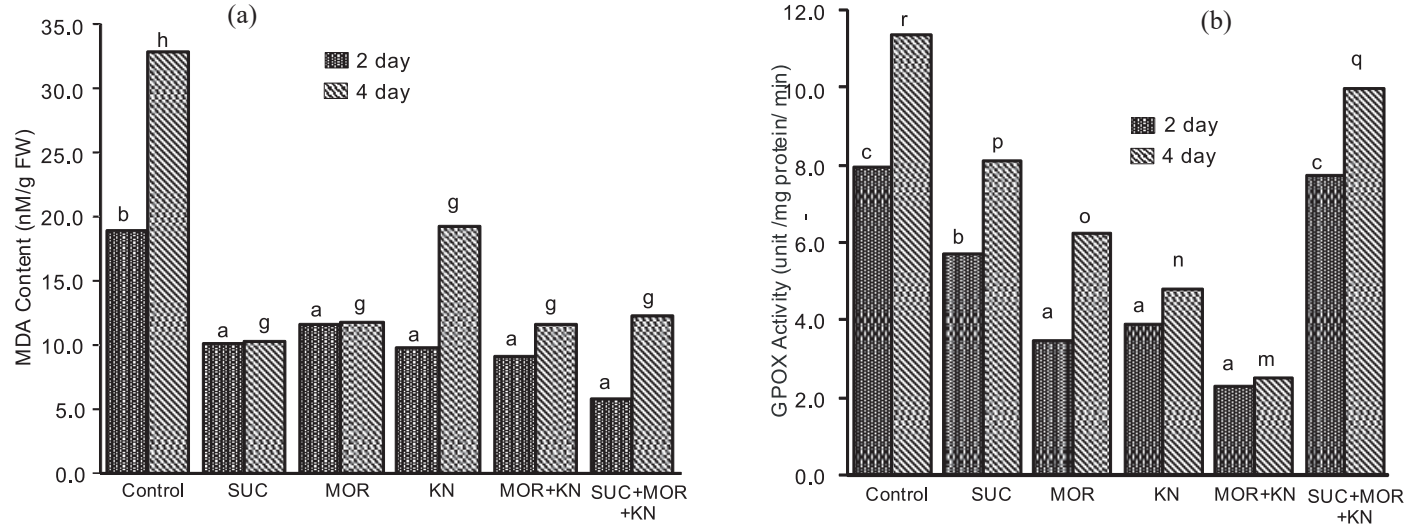

Fig. 6. *P. tuberosa* L. showing changes in MDA content (nM g^{-1} fresh weight ± S.E.) and guaiacol peroxidase activity (GPOX, unit mg^{-1} protein min^{-10} ± S.E.) of flower petals when scapes were maintained in DDW, sucrose (0.1M), morphactin (MOR, 20 µM), kinetin (KN, 20 µM), MOR (20 µM) + KN (20 µM) and sucrose (0.1M) + MOR (20 µM) + KN (20 µM). [Initial 0-day values of MDA : 3.45 nM g^{-1} fresh weight and GPOX 1.42-unit mg^{-1} protein min^{-10}]. Bars followed by different letters show significant differences at $P < 0.05$ between treatments according to DMRT.

the enhancement in lipid peroxidation (Fig. 6). Rise in lipid peroxidation and MDA - content during flower senescence has been noticed earlier also in tulips (Jones and McConchie, 1995), roses (Fukuchi-Mizutani *et al.,* 2000) and Gladiolus (Ezhilmathi *et al.,* 2007). Investigation carried out in our laboratory with other flowers also indicated such enhancement in MDA level due to speedy disintegration of various membranes and sub-cellular organelles (Kaur and Mukherjee, 2016). Most of the treatments were very effective in lowering this breakdown process.

As far as GPOX activity was concerned, the value registered an increase from 0 to 4-day petals of cut scapes. Among the various holding solutions, MOR + KN application resulted least activity of GPOX and addition of sucrose reduced it considerably. Values of all treatments were much lower than the value recorded in untreated petals. Like the observation in tuberose, some earlier studies carried out by us also indicated rapid increase in GPOX activity as in *Calendula officinalis* cut and uncut flowers with the advancement of development and senescence (Kaur *et al.,* 2015; Kaur and Mukherjee, 2015; Singh *et al.,* 2018). However, PGRs were very effective in arresting the rise in the activity of this enzyme. In the present study KN alone and KN + MOR in combination were most effective in maintaining the activity of GPOX.

A combination of sucrose, KN and MOR was found to be the best holding solution to decrease the loss of moisture and turgidity in petals. It was also useful in reducing the fresh weight loss of scapes, the shrinkage of petals and protein breakdown. Both PGRs were able to control the increase in reducing and total sugars. A combined application of MOR + KN was not only responsible for least increment in proline, MDA content and GPOX activity but also delayed senescence in cut flower sticks of tuberose (*P. tuberosa*).

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References

Asil, M.H. and M. Karimi, 2010. Efficiency of benzyladenine reduced ethylene production and extended vase life of cut *Eustoma* flowers. *Plant Omics Journal,* 3(6): 199-203.

Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water stress studies. *Plant and Soil*., 39: 205-207.

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

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

Chiang, H.H. and A.M. Dandekar, 1995. Regulation of proline accumulation in *Arabidopsis* during development and in response to dessication. *Plant Cell Environ.*, 18: 1280-1290.

Ezhilmathi, K., V.P. Singh, A. Arora and R.K. Sairam, 2007. Effect of 5-sulphosalicylic acid on antioxidant activity in relation to vase life of *Gladiolus* cut flowers. *Plant Growth Regul*., 51: 99-108.

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

Hart, F.L. and H.J. Fisher, 1971. *Modern Food Analysis*. Springer-Verlag, Berlin-Heidelburg-New York.

Heath, R.L. and L. Packer, 1968. Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.,* 125: 189-198.

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

Kaur, P. and D. Mukherjee, 2013. Senescence regulation by alcohols in cut flowers of *Calendula officinalis* L. *Acta Physiol. Plant.,* 35: 1853-1861.

Kaur, P. and D. Mukherjee, 2014. Tomato fruits regulating petal senescence and affecting levels of UV-B absorbing pigments and proline in cut flowers of *Calendula* and two species of *Chrysanthemum. International Journal of Agriculture Innovations and Research.,* 3(2): 485-491.

Kaur, P., Narender Singh and D. Mukherjee, 2014. Characterization of senescence processes in attached flowers of *Calendula officinalis* L. and *Aster novae belgii* L. *International Journal of Advanced Research.,* 2(4): 1195-1205.

Kaur, P. and D. Mukherjee, 2015. L-Serine and spermine delay petal senescence in cut flowers of *Calendula officinalis* L. *Life Sciences Leaflets.,* 69: 112-124.

Kaur, P., Narender Singh and D. Mukherjee, 2015. Regulation of membrane leakage and activities of some antioxidant enzymes in petals of cut flowers of *Calendula officinalis* and *Salvia. splendens* with metabolites and plant growth regulators. *J. Appl. Hort.*, 17(1): 31-39.

Kaur, P. and D. Mukherjee, 2016. Post-harvest physiology and vase life of cut *Chrysanthemum* flowers with sucrose, putrescine and spermidine as holding solutioins. *J. Indian Bot. Soc.,* 95(1&2): 105-115.

Kaur, P., S. Mukherjee and D. Mukherjee, 2017. Physiology of cut flowers and senescence regulation. *Trends in Biosciences.,* 10(45): 9226-9232.

Khokhar, M. and D. Mukherjee, 2010a. Role of plant growth regulator in petal senescence of *Calendula officinalis* L. and *Coreopsis lanceolata* L. *J. Indian Bot. Soc.,* 89: 37-43.

Khokhar, M. and D. Mukherjee, 2010b. Senescence regulation in cut flowers of *Calendula officinalis* L. and *Arctotis grandis* Thunb. with kinetin, salicylic acid and morphactin. *J. Plant Biol.,* 37: 215-222.

Khokhar, M., S. Mukherjee and D. Mukherjee, 2018. Senescence regulation in petals of *Calendula officinalis* L. cut flowers with sucrose, kinetin, salicylic acid and a morphactin. *Trends in Biosciences*, 11(9): 1938-1944.

Koizuka, N., Y. Tanaka and Y. Morohashi, 1995. Expression of α-amylase in response to wounding in mung bean. *Planta,* 195: 530-534.

Kuiper, D., S. Ribot, H.S. van Reenen and N. Marrissen, 1995. The effect of sucrose on the flower bud ripening of 'Madelon' cut roses. *Scien. Hort.,* 60: 325-336.

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

Ma, N., C. Ma, Y. Liu, M.O. Shahid, C. Wang and J. Gao, 2018. Petal senescence: a hormone view. *J. Exp. Bot.*, 69: 719-732.

Maehly, A.C. 1954. *Determination of Peroxidase Activity*. Inter Science Publisher Inc., New York 1: 385-386.

Mattioli, R., P. Costantino and M. Trovato, 2009. Proline accumulation in plants not only stress. *Plant Signaling and Behavior*., 4(11): 1016-1018.

Moneruzzaman, K.M., A.B.M.S. Hossain, N.B. Amru, M. Saifudin, H. Imdadul and S. Wirakarnain, 2010. Effect of sucrose and kinetin on the quality and vase life of *Bougainvillea glabra* var. Elizabeth Angus bracts at different temperatures. *Australian Journal of Crop Science,* 4(7): 474-479.

Monteiro, J.A., T.A. Nell and J.E. Barrett, 2002. Effects of exogenous sucrose on carbohydrates levels, flower respiration and longevity of potted miniature rose (*Rosa hybrida*) flowers during postproduction. *Post-harvest Biology Technology*, 26: 221-229.

Mukherjee, S. and D. Mukherjee, 2017. Additive effects of sucrose with kinetin and salicylic acid in delaying petal senescence of cut flowers of *Matricaria parthenium* L. *Int. J. Curr. Res. Biosci. Plant Biol.,* 4 (11): 86-95.

Mukherjee, S. and D. Mukherjee, 2020. Individual effects of sucrose, kinetin and salicylic acid and combined effects of kinetin + sucrose and salicylic acid + surcose in petal senescence regulation of *Aster novae belgii* L. *Int. J. Curr. Res. Biosci. Plant Biol.,* 7 (2) : 14-21.

Nichols, R. 1973. Senescence of cut carnation flowers : respiration and sugar status. *J. Hort. Sci.,* 48: 111-121.

O'Donoghue, E.M., S.D. Somerfield and J.A. Heyes, 2002. Vase solutions containing sucrose result in changes to cell walls of *Sandersonia* (*Sandersonia aurantiaca)* flowers. *Post-havest Biology and Technology.,* 26: 285-294.

Saha, S., P.K. Nagar and P.K. Sircar, 1985. Changes in cytokinin activity during flower development of *Cosmos sulfurous. Plant Growth Regul.,* 3: 27-35.

Schneider, G. 1970. Morphactins: Physiology and performance. *Ann. Rev. Plant Physiol.,* 21: 499 -536.

Setyadjit, D.C. Joyce, D.E. Irving and D.H. Simons, 2004. Effect of 6-benzylaminopurine treatments on the longevity of harvested *Grevillea* 'Sylvia' inflorescences. *Plant Growth Regulation*, 43: 9-14.

Singh, K.P. and M.C. Singh, 2013. Evaluation of double petalled cultivars of tuberose (*Polianthes tuberosa* Linn.) under Delhi condition. *The Asian Journal Horticulture*., 8(2): 512-514.

Singh, S., S. Mukherjee, S. Jakhar and D. Mukherjee, 2018. Interaction of sucrose, a morphactin and 8-hydroxyquinoline in the regulation of petal senescence of *Calendula officinalis* cut flowers. *Int. J. Curr. Res. Biosci. Plant Biol.,* 5(12): 28-38.

Stead, A.D. and W.G. van Doorn, 1994. Strategies of flower senescence - A review. In: *Molecular and Cellular Aspects of Plant Reproduction*. Cambridge University Press, Cambridge, UK, Scott, R.J., Stead, A.D. (Eds.). pp. 215-238.

Trivellini, A., A. Ferrante, P. Vernieri, A. Mensuali-Sodi and G. Serra, 2011. Effects of promoters and inhibitors of ethylene and ABA on flower senescence of *Hibiscus rosa-sinensis* L. *J. Plant Growth Regul.,* 30: 175-184.

van Staden, J. and G.G. Dimalla, 1980. The effect of silver thiosulfate preservative on the physiology of cut carnations: II. Influence on endogenous cytokinins. Z. *Pflanzenphysiol.,* 99: 19-26.

van Doorn, W.G. 2004. Is petal senescence due to sugar starvation? *Plant Physiol.,* 134: 35-42.

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

Voleti, S.R., V.P. Singh, A. Arora, N. Singh and S.R. Kushwaha, 2000. Physiology of flower senescence in floriculture crops. In: , *Advances in Plant Physiology.* Hemantranjan. A. (Ed.). Scientific Publishers, Jodhpur, India, pp. 423-439.

Woltering, E.J. and W.G. van Doorn, 1988. Role of ethylene in senescence of petals, morphological and taxonomical relationships. *J. Exp. Bot.,* 39: 139-145.

Yakimova, E. 1997. Regulation of senescence processes in semi postharvest flowers with aid of some phytoeffectors. Ph.D. Thesis. *Inst. Plant Physiol.,* Sofia.

Zubko, E., C.J. Adams, I. Machekava, J. Malbeck, C. Scollen and P. Meyer, 2002. Activation tagging identifies a gene from *Petunia* hybrid responsible for the production of active cytokinins in plants. *Plant J.,* 29: 797-808.

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