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POST HARVEST REGULATION OF PETAL SENESCENCE IN *GAILLARDIA PULCHELLA* FOUG. SCAPES BY SUCROSE, 8-HYDROXYQUINOLINE AND METHYLCHLORFLURENOL INDIVIDUALLY AND IN COMBINATION AS VASE SOLUTION

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Using sucrose, methylchlorflurenol (a morphactin, MOR) and 8-hydroxyquinoline (8-HQ) individually as vase solutions and also in combination of two and three of these chemicals, regulation of petal senescence was investigated in scapes of *Gaillardia pulchella*, an important ornamental plant. Based upon prior experiments on other plants, the selected concentrations were 0.1M sucrose, 20µM MOR and 1.5 mM 8-HQ. It was interesting to note that individually sucrose and 8-HQ behaved differently in comparison to the situation when both were present in the same holding solution. A combination of three (sucrose + MOR + 8-HQ) could effectively reduce the decline in flower diameter and membrane stability index (MSI) of petals. It could also minimize protein degradation and lipid peroxidation. Higher fresh weight and cumulative uptake of solution in scapes could be maintained by the combination of sucrose + 8-HQ in vase solution. However, individual application of MOR with the selected concentration was not effective in *Gaillardia* in this study.

Key words: Biocide, guaiacol peroxidase, lipid peroxidation, petal senescence, plant growth regulator, protein

Like all plant organs, flowers also grow, develop and mature. They enter into senescence stage thereafter. In the senescent phase, various physiological and biochemical changes of flower parts can be noticed. Petals undergo various alterations like shrinkage, dehydration, appearance of wrinkles, inrolling or outrolling, wilting, etc. which can be seen within few days after cutting the flower stalk from the main plant. A large number of deteriorative changes like greater release of reactive oxygen species (ROS), H_2O_2, hydrolytic enzymes can also be witnessed at this stage. These are responsible for membrane disintegration; and degradation of starch, proteins, lipids, nucleic acids, etc. Petal senescence has been considered as developmental programmed cell death (PCD, van Doorn and Woltering 2008).

Cut flowers have shorter life span. Since they have detached, they get limited supply of reserved food materials. In case of scapes (flower stalks from which leaves have been removed), degradation of macromolecules becomes faster and there is a need of sugars in the vase solution as they are the source for carbon and energy (van Doorn 2004). Osmotic concentration of cell sap increased in petals due to translocation of sugars. More water is absorbed by petals to maintain turgidity (O'Donoghue *et al.* 2002). Sugars are also needed to carry out respiration and to suppress ethylene synthesis.

Cut scapes also face the attack by microorganisms at the cut surface. Bacterial multiplication and synthesis of extra cellular polysaccharides are responsible for vessel blockage. Release of pectinases and toxic compounds are also responsible for ethylene synthesis which in turn accelerates flower senescence (Jowkar *et al.* 2012). Attack by microbes can be prevented by using biocides that are often used in floriculture industry. Biocides like 8-hydroxy quinoline (8-HQ), 8-hydroxyquinoline citrate (8-HQC) and 8-hydroxyquinoline sulphate (8-HQS) are being used for this purpose. They can lower pH of the holding solution preventing vascular blockage in stems of many flowers including cut roses (van Doorn and Perik 1990).

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Experiment with cut lily flowers has indicated positive role of sucrose, 8-HQC and GA_3 when a combined treatment of these three chemicals was given to extend the longevity (Rabiza-Swider *et al.* 2012). Since, chemicals like sucrose, biocides and those plant growth regulators (PGRs) which delay senescence have important role to play, it was thought to use sucrose, 8-HQ and a synthetic PGR (methylchlorflurenol, a morphactin, MOR) in the regulation of petal senescence. Morphactins were developed by Schneider (1970) and many workers were associated with the research to find out their role in morphology, physiology and biochemistry of different plants (Schneider 1970). Among various important roles, they are also known to delay senescence (Schneider 1970, 1972, Jain and Mukherjee 1980, Khokhar and Mukherjee 2010). Recently methyl chlorflurenol (a MOR) has been reintroduced by Repar Corp., Maryland, USA. As work was going on to improve longevity of some important ornamental flowers such as *Calendula, Gaillardia, Matricaria, Chrysanthemum,* etc. the present study was undertaken to find out effects of these chemicals (sucrose, MOR and 8-HQ) on the longevity of *Gaillardia pulchella* flowers when they are present alone and when in combination in vase solution. Further, it was also important to know how MOR could regulate petal senescence as fewer reports are available with this PGR.

MATERIAL AND METHODS

Plant material: *Gaillardia pulchella* Foug. plants were grown in the experimental beds maintained in the garden of Botany Department, Kurukshetra University, Kurukshetra. (Fig. 1). Uniformly developed flowers of the same physiological age and almost similar diameter were identified and flower twigs were cut under water in a bucket and brought to the laboratory. Twigs with flowers were recut in the laboratory to have an uniform length of 14 cm. Leaves were also removed from these twigs and placed in 100 ml Borosil-make conical flasks having 30 ml holding solution in each of them. Five conical flasks were maintained for each holding solution and three scapes were placed in each flask. Holding solutions were: double distilled

Figure. 1: General view of *Gaillardia pulchella* plants being grown in the experimental beds of the garden.

Figure 2: *Gaillardia pulchella* scapes of various stages with different holding solutions like (a) 0-day control (distilled water, DDW), (b) to (h) 2-day stage, (i) to (0) 4-day stage. (b) and (i) untreated control (DDW); (c) and (j) sucrose (0.1M) (d) and (k) morphactin (MOR, 20µM); (e) and (l) 8-hydroxyquinoline (8-HQ, 1.5 mM); (f) and (m) sucrose (0.1M) + MOR (20µM); (g) and (n) sucrose (0.1M) + 8-HQ (1.5 mM) and (h) and (o) sucrose (0.1M) + MOR (20 µM) + 8-HQ (1.5 mM).

water (DDW, control), sucrose (0.1M), methylchlorflurenol (a morphactin, MOR, 20µM), 8-hydroxyquinoline (8-HQ, 1.5 mM), sucrose (0.1M) + MOR (20µM), sucrose (0.1M) + 8-HQ (1.5 mM) and sucrose (0.1M) + MOR (20µM) + 8 HQ (1.5 mM). Experiment was set up at room temperature under day and night light intensity of 2.24µmol $m^{-2}s^{-1}$ and 1.13µ mol $m^{-2}s^{-1}$, respectively. Flower diameter and external appearance were noted at 0, 2 and 4-day. Petal samples were collected in triplicates to find out fresh weight of scapes, dry weight, total soluble protein, guaiacol peroxidase (GPOX) activity, lipid peroxidation (MDA content) and membrane stability index. The experiment has been repeated once to confirm the trend of results.

Fresh weight of scapes, flower diameter and moisture content: Fresh weight of scapes was recorded individually on the initial (0-day) and final day (4-day) of experiment and percent difference was calculated as the difference between two. Flower diameter of individual scape was determined as the mean of two perpendicular measurements across a flower. Data related to fresh weight of scapes and flower diameter were based upon 15 replicates. One hundred milligram fresh sample (in triplicates) was placed in an oven at 80^0C for 2-day to find out dry weight and moisture content.

Total soluble protein: One hundred mg petal sample was used for an extraction. The sample was dropped in 10ml of 80% boiling ethanol in a test tube placed in a water bath for 1 min. It was cooled and homogenized in same ethanol using pestle and mortar. The extract was centrifuged in a Remi centrifuge at 2124 RCF (5000 rpm) for 15 min. Supernatant was discarded and the residue was re-extracted with 10ml of 5% perchloric acid and centrifuged again at 2124 RCF for 15 min. Supernatant was discarded again and 5 ml of 1 N NaOH was added to the residue and collected it in a test tube. The residue in the alkali was tried to dissolve by shaking and putting in water bath at a temperature of 40-50 ^{0}C for 20 min. It was centrifuged again at 2124 RCF 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 double distilled water (DDW) was added.

Table 1 : *Gaillardia pulchella* showing changes in flower diameter (in cm S.E.) in scapes maintained in double distilled water (DDW, control), sucrose (0.1M), morphactin (MOR, 20µM), 8-hydroxyquinoline (8-HQ, 1.5 mM), sucrose (0.1M) + MOR (20µM), sucrose (0.1M) + 8-HQ (1.5mM) and sucrose (0.1M) + MOR (20µM) + 8-HQ (1.5mM).

				% Difference Between		
Holding Solutions	**0-DAY**	**2-DAY**	**4-DAY**	**0 to 2-DAY**	**2 to 4-day**	**0 to 4-DAY**
Control (DDW)	4.29 ±0.08	4.21 ±0.18	3.21 ±0.23	-1.86	-23.75	-25.17
Sucrose	4.24 ±0.08	2.02 ±0.10	1.75 ±0.07	-52.36	-13.37	-58.73
MOR	4.49 ±0.10	2.76 ±0.21	1.83 ±0.09	-38.53	-33.70	-59.24
8-HQ	4.26 ±0.13	4.43 ±0.10	2.12 ±0.18	+3.99	-52.14	-50.23
Sucrose + MOR	4.43 ±0.07	1.80 ±0.06	1.67 ±0.07	-59.37	-7.22	-62.30
Sucrose+8-HQ	4.60 ±0.07	4.29 ±0.11	3.50 ±0.22	-6.74	-18.41	-23.91
Sucrose + MOR + 8-HQ	4.50 ±0.07	4.29 ±0.13	3.57 ±0.19	-4.67	-16.78	-20.67

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 UV-Visible Spectrophotomaeter (Systronics, Double Beam Spectrophotometer 2203, India). The protein content of samples were calculated against a standard curve of bovine serum albumin (BSA, Sigma, USA).

Guaiacol peroxidase (GPOX) activity: The method of Maehly (1954) was followed to find out GPOX activity. Petal sample (100 mg for 1 sample) 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 2124 RCF (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 contains 2 ml of enzyme extract, 2 ml of phosphate buffer (pH 7.0) and 4µml of DDW. After 10 min. the absorbance was recorded at 420 nm in a Systronics Double Beam Spectrophotometer. Specific GPOX activity was expressed in terms of mg protein per 10 min. Protein was estimated from the enzyme extract using Coomassie brilliant blue reagent as mentioned earlier (Bradford 1976).

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 mg petal sample was homogenized in 2ml of 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 8497 RCF (10000 rpm) for 20 min in a Remi refrigerated centrifuge. To 0.5 ml aliquot of the supernatant, 2ml of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) was added. The mixture was heated at 90^0C for 30 min. in the water bath and then quickly cooled in an ice water bath. After centrifugation at 8497 RCF (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

Table 2 : *G. pulchella* showing changes in flower weight (in gm S.E.) of scapes and cumulative uptake of holding solutions (in ml) when scapes were maintained in double distilled water (DDW, control), sucrose (0.1 M), morphactin (MOR, 20 µM), 8-hydroxyquinoline (8-HQ, 1.5mM), sucrose (0.1M) + MOR (20µM), sucrose (0.1M) + 8-HQ (1.5 mM) and sucrose (0.1M) + MOR (20µ M) +8-HQ (1.5mM).

Holding Solutions	0-DAY	4-DAY	% Difference Between 0 to 4-DAY	Volume of Holding Solutions	Cumulative uptake during 4-DAY
Control (DDW)	0.875 ±0.019	0.732 ±0.047	-16.34	30	11.50
Sucrose	0.884 ±0.017	0.356 ±0.006	-59.72	30	12.00
MOR	0.875 ±0.031	0.372 ±0.016	-57.48	30	8.17
8-HQ	0.832 ±0.027	0.391 ±0.018	-53.00	30	17.17
Sucrose + MOR	0.890 ±0.033	0.363 ±0.014	-59.21	30	7.67
Sucrose + 8-HQ	0.873 ±0.031	0.897 ±0.046	+3.09	30	18.33
Sucrose + MOR + 8-HQ	0.899 ±0.028	0.934 ±0.056	+3.89	30	17.17

concentration of MDA, an end product of lipid peroxidation was calculated in accordance to its extinction coefficient of 155 per mM per cm.

Membrane stability index (MSI): It was calculated on the basis of electrolytic leakage of petals, originally described for leaf tissue (Desmukh *et al.* 1991). The method has been described elsewhere (Khokhar *et al.* 2018).

Flower diameter and fresh weight change of scapes data are based upon 15 cut flower samples whereas biochemical data represent means of 6 values.

RESULTS AND DISCUSSION

Cut scapes of *Gaillardia pulchella* flowers appeared very fresh and turgid; without any sign of wilting, shrinkage and petal rolling at initial day (0-day) when they were placed in holding solutions (Fig. 2a). Fig. 2 also depicts alterations in external appearance of these leafless flowers subjected to individual (sucrose, 0.1M; morphactin, MOR, 20µM; and 8-hydroxyquinoline, 8-HQ, 1.5 mM) as well as combined treatments (sucrose + MOR, sucrose + 8-HQ and sucrose + MOR + 8-HQ) at 2 and 4-day.

How applied holding solutions affected the flower diameter of cut scapes getting constant light from fluorescent tubes in the laboratory has been shown in Table 1. Four day duration demonstrated a gradual decrease in diameter in all cut flowers irrespective of treatments except between 0 and 2-day in case of 8-HQ. Maximum decline was noticed after sucrose + MOR treatment followed by individual application of MOR, sucrose and 8-HQ. Application of sucrose + MOR + 8-HQ was the best in putting a break on the decreasing trend in flower diameter. It is interesting to observe different behaviour of sucrose and 8-HQ when they were present separately and when mixed together in holding solution. Since these flowers were already developed and experiment carried out in the last week of May during hot summer, petals experienced rapid dehydration and breakdown of reserved food including photosynthates. Decrease in flower diameter with time was also noticed earlier in *Calendula officinalis* cut flowers (Khokhar *et al.* 2018).

Difference in fresh weight of scapes during 4-day of experiment pointed out sharp decline in 4 cases like those having sucrose, sucrose + MOR, MOR and 8-HQ. However, in case of sucrose + 8-HQ and sucrose + MOR + 8-HQ, a slight increase was noticed in fresh weight (Table 2). Available data also revealed that above combinations and also 8-HQ caused considerable increase in cumulative uptake. Investigation on another plant (*Calendula*

Table 3 : *G. pulchella* showing changes in (a) total protein (in fresh and dry weight basis, mg/100mg ±S.E.) and (b) guaiacol peroxidase (GPOX) activity (per mg protein min.$^{-10}$) in flower petals of scapes maintained in control (DDW), sucrose (0.1M) + 8-HQ (1.5mM) and sucrose (0.1M) + MOR (20µM) + 8-HQ (1.5mM).
[Initial values: protein, 3.68mg ±0.13 / 100mg (fr. wt.), 21.88mg ±1.18 / 100mg (dry wt.); peroxidase activity, 0.044±0.002 per mg protein min^{-10}].

(a)	4-DAY		% Difference in Protein	
PROTEIN	**Fresh Weight**	**Dry Weight**	**0 to 4-DAY Fresh Weight**	**0 to 4-DAY Dry Weight**
Control (DDW)	1.85 – 0.14	8.02 – 0.99	-49.73	-63.35
Sucrose + 8-HQ	8.94–0.40	12.77–0.58	+142.93	-41.64
Sucrose+MOR+8-HQ	4.68–0.31	9.36–0.63	+27.17	-57.22

(b)		Percent Difference Between
GPOX-ACTIVITY	**4-DAY**	**0 TO 4-DAY**
Control (DDW)	0.024 – 0.001	-45.45
Sucrose + 8-HQ	0.021–0.001	-52.27
Sucrose + MOR + 8-HQ	0.011 – 0.001	-75.00

Table 4 : *G. pulchella* showing changes in (a) MDA content (in nM g^{-1} fresh weight ± S.E.) and (b) percent values of membrane stability index (MSI ±S.E.) in flower petals of scapes maintained in control (DDW), sucrose (0.1M) +8-hydroxyquinoline (8-HQ, 1.5mM) and sucrose (0.1M) + morphactin (MOR, 20µM) + 8-HQ (1.5 mM). [Initial MDA content, 132.66 ± 4.17, % MSI value, 66.86 ±0.76].

(a)		% Change Between
MDA-Content	**4-DAY**	**0 to 4-DAY**
Control (DDW)	205.22 – 10.07	+54.70
Sucrose + 8-HQ	185.50– 8.12	+39.83
Sucrose+MOR+8HQ	167.96–9.60	+26.61

(b)		% Change Between
% MSI Value	**4-DAY**	**0 to 4-DAY**
Control (DDW)	24.08 – 0.67	+63.98
Sucrose + 8-HQ	58.61– 0.69	-12.34
Sucrose+MOR+8HQ	61.91–0.23	-7.40

officinalis) also showed similar pattern and effectiveness of 8-HQ, especially when present in association with sucrose or sucrose + MOR (unpub. data). Sucrose + spermine (Kaur and Mukherjee 2015) and sucrose + sulfosalicylic acid (Kaur *et al.* 2015) also helped in retaining fresh weight of scapes, moisture content and cumulative uptake of vase solution.

Table 3 showed changes in total soluble protein on fresh and dry weight basis; and also guaiacol peroxidase (GPOX) activity in the petals of flower scapes maintained in sucrose +8-HQ, sucrose + MOR + 8-HQ and control. Since above combinations were found to be very effective in minimizing the loss of flower diameter, maintaining fresh weight of scapes

and showing higher cumulative uptake of holding solutions, these samples were collected for analyses. Although, on fresh weight basis, protein content increased in petals having both treatments unlike control, a sharp decline was observed in all samples on dry weight basis. The least decline was found in those cases having sucrose + 8-HQ as holding solution. Since development process was already over in petals of cut scapes as they entered senescent phase, the degradation of reserved food material (including proteins) was inevitable as also noticed in earlier studies (Celikel and van Doorn 1995, Kaur and Mukherjee 2014, Kaur and Mukherjee 2016). Regarding GPOX activity, a sharp decrease was noticed in all samples. The maximum decline was recorded in petals received sucrose + MOR + 8-HQ treatment followed by sucrose +8-HQ and control.

A sharp increase in MDA content of *G. pulchella* petals was noticed between 0 and 4-day in control and treated sets. But combined treatment of sucrose + MOR + 8-HQ and sucrose + 8-HQ could bring down the value significantly (Table 4). Similar trend was also noticed in *Calendula officinalis* in our laboratory having identical combination of holding solutions (uppub. results). With quite high value of membrane stability index (% MS1) initially, it became much lower 4 days later in petals. Combined treatments of sucrose +8-HQ and sucrose + MOR + HQ were very effective in maintaining higher values. Various biochemical changes such as degradation of proteins, lipids, starch, nucleic acids, etc. are associated with petal senescence. Several studies covering various flowers indicate that lipid peroxidation and membrane damage are also accelerated with the onset and advancement of senescence (Jones and Mc Conchie 1995, Fukuchi - Mizutani et al. 2000, Ezhilmathi *et al.* 2007, Kaur and Mukherjee 2016).

CONCLUSION

Sucrose, MOR and 8-HQ when present individually and in combination as holding solutions behaved differently in maintaining flower petals fresh and hydrated; and with respect to membrane damage. A combination of sucrose, MOR and 8-HQ (a carbon source, a plant growth regulator and a biocide respectively) was the best in minimizing the reduction in flower diameter and % MSI value; also controlled lipid peroxidation and was most effective in retaining fresh weight of scapes. Sucrose + 8-HQ application was responsible for highest cumulative uptake of holding solution and least protein degradation.

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