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Role of salicylic acid in decreases of membrane senescence in Cut Carnation Flowers

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Cut flowers of carnation were treated with Salicylic acid (0,1.5, 3 mM) and sucrose (0, 3%).The effects of Salicylic acid on the ACC-oxidase activity, bacteria populations in vase flower preservative solution, anthocyanin leakage, Membrane stability, malondialdehyde and ACC-oxidase activity of cut flowers of carnations (*Dianthus caryophyllus L. cv.White*) were investigated. The experimental results showed that SA treatment cause descrease MDA content and ACC-oxidase activity, reduced the membrane permeability and peroxidation of lipids.also, Results showed that The best treatment involved 1.5 mM SA+ sucrose 3% . The vase solution containing 1.5 mM Salicylic acid + sucrose 3% significantly descreased MDA content, ACC-oxidase activity and bacteria populations in vase flower preservative solution and increased the vase life and membrane stability of carnation cut flower compared to the control. Results suggested that Salicylic acid increases membrane stability by descrease MDA content and ACC-oxidaseactivity, bacteria populations in vase flower preservative solution of the carnation cut flowers.

Key words: Salicylic acid ,carnation, Membrane stability, MDA,vase life

Introduction

Carnation is one of the world's most popular flowers to produce cut flowers. The flower of Carnation is highly ethylene sensitive and the longevity of the cut flower is very short (Brandt,1992). Short postharvest vase life is one of the most important problems on the cut flowers. Some workers have shown that reduced vase life is associated with increased concentration of microbes in the vase solution (Burdett, 1970; Dansercau *et al.,* 1975) the media used to determine microbial concentrations in vase solutions may not have been suitable for growth of microbial taxa adversely affecting flower vase life, also Zagory and Reid (1986) found that some bacteria from vase water produced ethylene. In carnations, senescence of the petals is associated with a

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climacteric-like increase in ethylene production during the final stages (Brandt, 1992). Evidence for an increase in membrane permeability during senescence of several flower species(Nichols, 1968; Hanson, 1971). (Beutelmann, 1977 and Suttle, 1980) were noted a strong correlation between membrane leakiness and phospholipid breakdown in senescing flowers. Ethylene accelerates the onset of membrane leakiness and phospholipid deterioration in petals. Senescing carnation flowers exhibit a climacteric-like rise in ethylene production. Ethylene production increases sharply with senescence while exogenous application of ethylene enhanced flower senescence and wilting (Halvey,1979), increased permeability of petal cells (Suttle,1980), and accelerated the decrease in cell membrane fluidity (Thompson,1982) and increase production of ROS. ROS cause chlorophyll degradation and membrane lipid per oxidation and increase malondialdehyde (MDA) product (Reezi *et al.,* 2009). To scavenge ROS, plants posses specific mechanisms, which include activation of antioxidant enzymes (Jaleel, *et al.,* 2006) and non- enzymatic antioxidants such as, carotenoids , ascorbic acid and Phenolic compounds (Mittler, 2002). The effects of ethylene and ROS can be reduced by inhibitors of ethylene biosynthesis and increase enzyme antioxidant activity. SA is a well known phenol that can prevent ACC-oxidase activity that is the direct precursor of ethylene and decrease ROS with increase enzyme antioxidant activity. also Salicylic acid seems to act by germicide the decrease of bacteria, which block the xylem vessels in the cut region and interfere with the normal flux of water through the stem (Nowak and Rudnicki, 1990). Mei-hua *et al.,* (2008) showed that SA can extending the vase life of cut flowers with decrease ROS and ethylene. SA acid with increases the enzyme antioxidant activity cause delay the onset of hydrolysis of structural cell components, decrease ROS production, ACC-oxidase activity and sensitivity. SA acid decreased the permeability of plasma membrane of floret cells and improved the structure of chloroplasts which were badly damaged by ethylene. This research was designed to investigate the role of SA in alleviating membrane lipid per oxidation and MDA content in Cut flowers of Dianthus caryophyllus.

Materials and methods

Plant material

Carnations (*Dianthus caryophyllus* L.) were grown in the greenhouse standard production methods (pakdasht, Tehran, Iran). The experimental site was in horticulture laboratory of agriculture faculty of University Azad Karaj, Tehran, Iran. Flowers were weighed initially immediately after harvest and used for setting treatments. The experiment was arranged in a factorial test with

complete randomized design with 4 replications. The factors were three levels of salicylic Acid (0, 1.5, 3mM), and two levels of sucrose (0,3%). The flowers were individually placed in bottles containing 250 ml of preservative solution and were held at ambient temperature (19 ±5°C). Analysis of variance was performed on the data collected using the general linear model (Proc GLM) procedure of the SPSS ver 16 software. Where a significant F-test was observed, treatment means were separated using the Duncan at P= 0.05.

Determination of anthocyanin leakage and ACC oxidize activity

Anthocyanin leakage was used to assess membrane permeability and measured using Spectrophotometer. The procedure used was based on the method of Poovaiah (1979). Petal samples were cut into 1x1 cm segments and placed in individual tube containing 25 ml of deionizer water after two washes with distilled water to remove surface contamination.then 10ml distilled water added to samples and after 12 h at temperature 25^0C recorded anthocyanin leakage absorption at 525nm using Spectrophotometer (Perkin-Elmer- EZ-201). ACC oxidize activity was measured according to the method by (Moya-Leòn and John ,1994). For the measurement of in ACC oxidase activity, flesh slices of 1 mm thickness (approximately 1 g) were put into 40-mL Erlenmeyer flasks containing 2 mL of incubation buffer consisting of 1 mM ACC, 0.4 M mannitol, and 0.1 M Tricine (pH 7.5). ACC oxidase activity was determined both in the absence and in the presence of 30 mM sodium ascorbate, 0.1 mM FeSO4, and 20 mM NaHCO3. The flasks were incubated at 30°C for 1 h and the ethylene formed was determined as described above. The activity was expressed as ethylene (in nanomoles) produced per gram fresh weight per hour.

Assays of MDA content (Lipid per oxidation)

Oxidative damage to lipids was estimated by measuring the content of MDA in floret segment homogenates, prepared in 10% trichloroacetic acid containing 0.65% 2-thiobarbituric acid (TBA) and heated at 95°C for 25 min MDA content was calculated by correcting for compounds other than MDA, which absorb at 532 nm by subtracting the absorbance at 532 nm of a solution containing plant extract incubated without TBA from an identical solution containing TBA.

Chlorophyll (a+b)content measurement

Chlorophyll total(a+b) content was measured by Chlorophyll meter SPAD-502, Minolta Co. Japan which represented by SPAD value. The petal

was inserted into the meter and measured SPAD value 3 times from different spot of a single petal.

Microbe population

Test Microbe population were isolated from vase solutions of carnations. When the flowers had senesced (about 11 days), aliquots of the vase solutions were diluted 100-times, and 25 u.1 aliquots of the diluted solution were spread on sterile Nutrient Agar, in sterile Petri plates. The plates were allowed to incubate for 48 hr at room temperature, and individual colonies of microorganisms, representing the most common colony morphology types, then were picked off the agar media with a sterile loop and streaked on EMB medium for purification. Purified Microbe population were maintained axenically on EMB medium and transferred daily to fresh medium.

Vase life

Vase life was considered to be terminated when wilting occurred.

Results and discussion

Anthocyanin leakage and ethylene production

Result showed the effects of SA on the anthocyanin leakage of the petals of carnation under SA and SA+ SU treatment compared to the control (Table 1 and 2). Two treatments (SA 1.5 mM and SA+SU(SA1.5+SU3%) improved membrane permeability by decrease anthocyanin leakage(Table 1 and 3). Two treatments (3mM and 3mM+SU3%) impaired membrane permeability by increasing anthocyanin leakage and increase produce ethylene (Table 1 and 3). Addition of 1.5mM SA maintained membrane permeability.1.5 mM SA with or without SU could alleviate or decrease cell wall damages (Table 3). It is evident from the data presented in Table 1 that the maximum anthocyanin leakage was recorded in 3mM SA compare another treatments and control.

Table 1. Mean comparisons of chlorophyll content, Vase life, MDA, bacteria populations in vase flower preservative solution, Antocyanin leakage and ACC oxidase activity in SA treatment.

Treatment	Vase life(day)	Chlorophyll total (a+b) content (spad reading)	ACC Oxidase activity (nmol/gFW/h)	Membrane stability (Antocyanin leakage)	MDA (µmol/mg protein)	Microbe population (cfu)
Control	7b	2.71b	20.12b	224.17b	158.13b	32.42b
SA1.5Mmol	8.69a	4.22a	12.04a	156.75a	131.85a	20.08a
SA3Mmol	4.92c	0.96c	32.16c	401.08c	204.04c	21.75a

Means in each column followed by similar letters are not significantly different at 5% level using Duncan.

Statistically significant differences existed among 1.5 mM SA compared another treatment and control. The minimum anthocyanin leakage was noted in 1.5 mM SA prevented control (Table 1). Results showed adding SA in vase water was prevent anthocyanin leakage by maintained PH vase solution. Adding SA was found to be positively correlated with anthocyanin leakage of the carnation cut flower (Table 2).

Table 2. Simple correlation lines between the SA treatment with other variables.

Treatment	Vase life (day)	Chlorophyll total (a+b) content (spad reading)	ACC Oxidase activity (nmol/gFW/h)	Membrane stability (Antocyanin leakage)	MDA(µmol/mg protein)	Microbe population (cfu)
SA	-0.458**	-0.367*	0.439**	0.526**	0.478**	-.207

* and **: Significant different at 5% and 1% level, repectively.

Table 3. Mean comparisons of chlorophyll content, Vase life, Antocyanin leakage, MDA content, bacteria populations in vase flower preservative solution and ACC oxidase activity in SA x SU treatment.

SU	SA	vase life (day)	Chlorophyll total (a+b)content(spad reading)	ACC Oxidase activity (nmol/gFW/h)	Membrane stability (Antocyanin leakage)	MDA (µmol/mg protein)	Microbe population (cfu)
0	0	7.1b	3.341b	17.827c	210.833c	157.38c	24.66b
	30	6.8c	2.098c	22.418ab	237.5ab	158.882c	40.16ab
1.5	0	8.7a	4.542a	11.645a	144.833a	129.63a	15a
	30	8.66a	3.905b	12.452a	168.667b	134.07b	27.16c
3	0	4.5ac	0.982ab	32.947ac	425.333ac	218.75	16.66a
	30	5.3ab	0.948ab	31.39ac	376.833abc	189.337ab	26.83c

Means in each column followed by similar letters are not significantly different at 5% level using Duncan.

It is indicated that with SA concentration increased, the anthocyanin leakage was increased. Mei-hua *et al.,* (2008) showed that SA can extend the vase life of cut flowers with increase membrane stability.The produce ethylene

is in petal cut flower harmful to cut flowers and their consequent accelerate flower senescence, anthocyanin leakage and cell death. Adding a suitable inhibit produce ethylene in vase water can prevent accelerate flower senescence , anthocyanin leakage and cell death. These results was in agreement with previous workers who reported decreased accelerate flower senescence , anthocyanin leakage and cell death of cut flowers when placed in solutions of a suitable inhibit produce ethylene (Mei-hua *et al.,* 2008). The data reported here provide good evidence that SA had an additive effect in decreased accelerate flower senescence , anthocyanin leakage and cell death. It seems that in high concentrations of SA, pH increased and affected vacuoles pH and resulted anthocyanin leakage (Table 1). Result demonstrated that produce ethylene stimulates anthocyanin leakage and electrolyte leakage from petals (Table 1 and 3). It is indicated that ethylene increased membrane permeability in an unspecific fashion. Therefore, since anthocyanins are localized within the vacuole of all cells. The action of ethylene in enhancing the rate of leakage of this pigment can be interpreted as an effect of the gas on senescence and the membrane permeability of cut flower. SA in suitable concentration (1.5mM) with sucrose can prevent negative effects of low pH that cause Anthocyanin leakage. Unfortunately, high salicylic acid concentration rapidly increased in anthocyanin leakage, cell death and accelerate flower senescence.

Lipid per oxidation, MDA content and Chlorophyll content

The effects of SA on the data for malondialdehyde (MDA) content are presented in Tables 1 and 3. The effect of 3 mM SA treatment MDA content increased significantly when compared to control (Table 1). Salicylic acid 1.5 mM and salicylic acid 1.5 mM+sucrose 3 % significantly reduced MDA content of cut flower in all treatments except 3 mM SA and SA+SU (SA 3 mM + SU 3 %) treatments compared to other treatment (Table 3). 1.5 mM SA treatment has a significant of difference with control and other treatments (Table 1). Addition of decrease SA produced this factor and showed better results compared to sucrose treatment and high SA concentration. Adding SA was found to be positively correlated with lipid per oxidation of the carnation cut flower (Table 2). This indicated that with SA concentration increased, the lipid per oxidation was increased. Per oxidation of membrane lipids is an indication of membrane damage and leakage under senescence conditions. A decrease in content of MDA suggested that oxidative damage induced by produce ethylene be alleviated by the addition of SA (Table 1). Cut flower under senescence is in good correlation with increased lipid per oxidation levels and this result was in good correlation with the increase in MDA content under senescence (Shakirova, 2007). A lower lipid per oxidation resulting from

elevated activities of antioxidants was also reported on of Cut Gerbera Jamesonii Flower (Yuping *et al.,* 2009). The results of the present experiment were similar with the findings of Mei-hua (2008) in Gerbera which showed that added SA decreased the permeability of the plasma membrane of petal cells and decreased MDA level. It was reported that SA enhanced the stability of lipids in cell membranes of Gerbera when cut flower exposed to vase solution having SA. The evidence suggested that SA decreased the permeability of plasma membranes and membrane lipid per oxidation and maintains the membrane integrity. In this study, a significant increase in activities of the anti oxidative enzymes was observed in cut carnation. Increases in activities of these enzymes in response lipid per oxidation may be probably decreased the toxicity of ROS. Result showed that the expression levels of the anti oxidative enzymes increased after producing ROS. Two treatments (3mM and 3mM+SU3%) increased MDA content and decreased chlorophyll content in carnat cut flower, but SA at 1.5 mM decreased MDA content and prevent decreasing chlorophyll content in carnation cut flower (Table 1). SA can directly or indirectly affect enzyme antioxidant activity in plants . Excess concentrations of various SA can cause enzyme antioxidant activity inhibition and therefore alter metabolism or physiological function. Thus, the toxicity high concentrations of SA affect impair mechanisms in floret membrane lipid and pigment exists in cells. In this study, SA in high concentration (3 mM) showed that negative effects on low Lipid per oxidation and MDA content.

Chlorophyll contents were lower in both SA at 3 mM and SA+SU (SA 3 mM + SU 3 %) treatments compared to control values. SA at 1.5mM and salicylic acid 1.5 mM +sucrose 3% improved chlorophyll content in cut fower. Maximum increase was noticed when SA1.5 mM supplied (Table 1). In high concentrations of SA a significant difference was in Chlorophyll content between control. SA at 1.5 mM treatment significantly increased the total chlorophyll content to a larger extent when compared to control (Table1). Adding SA was found to be negativly correlated with Chlorophyll (a+b)content of the carnation cut flower (Table 2). This indicates that with SA concentration increased, the chlorophyll concentration compared to the control decreased. SA treatments lead to a considerable delay in degradation of chlorophyll total (a+b) compared to control (Table 1). Chlorophyll contents were lower when treated sucrose alone at 3 % in vase solution. Also, It is showed that in suitable concentrations of SA, a significant difference was in Chlorophyll total concentration compared to control.

Microbe population

The microbe population vase solution of carnation cut flowers was decreased by the concentration of salicylic asid 1.5 and 3 mM used (Table 1). The microbe population was lower in salicylic acid at 1.5 and 3 mM compared to salicylic combined with sucrose treatment and control (Table 3).The higher microbe population was attained when sucrose was used to compare with control (Table3). Means of microbe population on vase solution of cut flowers in various salicylic acid+sucrose containing vase solutions was slightly significantly than control. While microbe population on vase solution of Cut flowers in various salicylic acid containing vase solutions was higher significantly than control . Adding SA was found to be negativly correlated with microbe population vase solution of the carnation cut flower (Table 2).This indicated that with SA concentration increased, the microbe population vase solution was decreased.

Vase life

SA alone was capable of increasing longer vase life of carnation flowers than sucrose alone or together with SA (Table 1,3). It is evident from the data presented in Table 3 that the maximum vase-life (8.7 , 8.66 days) were recorded in SA at 1.5 mM and salicylic acid 1.5 mM +sucrose 3%, respective compared to other treatments and control.The minimum vase-life was noted in SA at 3mM compared to other treatments and control (Table 3). Mei-hua et al. (2008) showed that the treatment of salicylic acid extended the vase life and improved flower quality with reduced respiration rate delayed senescence and decreased lipid per oxidation, MDA content. Adding SA was found to be positively correlated with vase life of the carnation cut flower (Table 2). This indicated that with SA concentration increased, the vase-life was decreased.

Conclusion

It can be concluded that SA treatments significantly decreased bacterial population in vase flower preservative solution, produced MDA and ACC-oxidase activity, reduced the membrane permeability and per oxidation of lipids. The resulted in extended vase-life as compared to other treatments (Sucrose treatments and SA3mM+sucrose3%). SA at 1.5mM also proved more effective in delaying petal senescence and/or flower wilting. However, our results showed that SA treatments maintained the vase life of flowers for a longer period.

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