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Influences of ascorbic acid and salicylic acid on vase life of cut flowers rose (*Rosa hybrida* cv. black magic)

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Abstract

Vase life of cut flower plays important economic values in flower production industries. In this study two levels of ascorbic acid (10 and 20 mg l^{-1}) and two levels of salicylic acid (100 and 200 mg l^{-1}) along with sucrose (3%) were carried out in a complete randomized design on 60 rose cut flowers cv. black magic in horticulture laboratory of agriculture faculty of Islamic Azad University, Garmsar, Iran.The recorded traits included: vase life, total chlorophyll content, anthocyanin and phenylalanine ammonialyase (PAL) content, ion leakage, superoxide dismutase content and water absorption. The results showed that salicylic acid treatments increased cut-flower water absorption, fresh weight and vase life, while delay of senescence. Maximum flower vase life was recorded in treatment 200 mg l^{-1} salicylic acid.

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Introduction:

Roses *(Rosa hybrida)* of Rosaceae family are recognized highly valuable for economical benefits being the best source of raw material to be used in agro-based industry especially in the cosmetics and perfumery. Additionally, roses play a vital role in the manufacturing of various products of medicinal and nutritional importance. However, a very peculiar aspect of rose production is to get the cut flowers, which greatly deals with the floricultural business [1]. The cut flowers of different cultivars of rose show variation in their attitude regarding vase life due to gene differences. The use of sucrose with or without certain additive and also the use of some chemicals to the pulsing solutions could be of practical significance for prolonging the life of many cultivars of cut roses. Such preservatives to extend flower life might be used effectively at all levels of handling the crop that would be beneficial both for producers and consumers. The vase life of cut roses is generally short. Short vase life of cut flowers is related to wilting, ethy-lene production and vascular blockage by air and micro-organisms [2]. Preservative solutions are generally required to supply energy source, reduce microbial build up and vascular blockage, increase water uptake of the stem, and arrest the negative effect of ethylene [3]. Incorporation of different chemical preservatives to the holding (vase) solution is recommended to prolong the vase life of cut flowers [4]. Salicylic acid (SA) is an endogenous signal molecule, involved inregulating stress responses as well asmany processes regarding plantgrowth and development [5]. SA is considered as a plant hormone,inhibiting ethylene biosynthesis and delaying the fruit senescence [6]. It has been shown that postharvest treatment of various cut flowers by SA could improve their vase life [7,8]. Ascorbic acid (vitamins C) is a product of D-glucose metabolism in higher plants which affect on plant growth and development, and play a role in electron transport system [9]. Ascorbic acid also has been associated with several types of biological activities in plants such as in enzyme co factors, antioxidant, and as a donor / acceptor in electron transport at the plasma membrane or in the chloroplast [10]. A high level of endogenous ascorbate is essential effectively to maintain the antioxidant system that protects

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plants from oxidative damage [11]. Nahed *et al.*, [12] refer that the best results for flowering parameters of gladiolus plants were obtained by application ascorbic acid at 200 ppm showed a stimulatory effect on all chemical constituents. Therefore, the main objective of this study was to evaluate the interactive effects of ascorbic acid and salicylic acid on chlorophyll content, anthocyanin and phenylalanine ammonia-lyase (PAL) content, ion leakage, superoxide dismutase content, and also to examine changes in membrane stability, possible change of vase life, relative water content and flower quality of rose cultivar "black magic".

Materials and methods

Cut flowers (*Rosa hybrida* cv. black magic) were harvested in open stage in the morning from a local commercial greenhouse (Pakdasht, Tehran, Iran), and transported with appropriate covers immediately to Laboratory (horticulture laboratory of agriculture faculty of Islamic Azad university, Garmsar Branch). Stems were recut to 40 cm length. In this study, two levels of ascorbic acid (10 and 20 mg l^{-1}) and two levels of salicylic acid (100 and 200 mg l^{-1}) along with sucrose (3%) were carried out in a complete randomized design on 60 rose cut flowers cv. black magic. After recording the fresh weight, each flower was placed in a 500-ml bottle containing preservative solutions. The flowers were held at ambient temperature (22 ±2 ℃). The experiment was started in season 2012-2013 and chlorophyll content, membrane stability, SOD content and PAL activity were measured on the last day of vase life for each flower. Vase life was determined as the number of days to wilting of flowers. The flowers were checked once a day for signs of deterioration. Chlorophyll index was measured by Arnon [13] method.

Anthocyanin content

For anthocyanin content, petals were cut into small pieces, ground to an even consistency in 5 ml 1% HCl in methanol, and kept at 4°C in the dark for a day. The absorbance of the solution, after suitable dilution, was measured at 530 nm [14].

Electrolyte leakage

The electrolyte leakage was determined as described by Ben Hamed *et al.*, [15]. Petals samples (0.5 g) were placed in test tubes containing 10 ml of double distilled water. The tubes were incubated in a water bath at 30 °C for 1 hr and the initial electrical conductivity of the medium (EC_1) was measured by an EC meter. The samples were autoclaved at 120°C for 20 min to release all the electrolytes, cooled at 25°C and then the final electrical conductivity (EC_2) of each was measured. The electrolyte leakage rate (EL) was then calculated by using the formula: EL= (EC1/EC2) × 100.

phenylalanine ammonia-lyase (PAL) content

The PAL activity was assayed according to the method of Wang *et al.*, [16]. Petals samples (0.3 g) were homogenized in 6.5 ml Tris-HCl buffer at pH 8.8 containing 15 mM of b-mercaptoethanol. The homogenate was centrifuged at 5,000 rpm for 30 min and the supernatant was used for enzyme activity assay. 1 ml of the extraction buffer, 0.5 ml of 10 mM L-phenylalanine (ME-1.07256), 0.4 ml of double distilled water and 0.1 ml of enzyme extract were incubated for 1 h at 37°C in a water bath and the reaction was stopped by adding 0.6 ml of 6 M HCl. The product was extracted with 15 ml ethyl acetate, followed by evaporation to remove the extracting solvent. The solid residue was suspended in 3 ml of 0.05 M NaOH and the amount of cinnamic acid was quantified spectrophotometrically (Biochrom WPA Biowave II) at 290 nm.

Activity of superoxide dismutase (SOD) content

The activity of superoxide dismutase (SOD) was measured using the method of Giannopolitis and Ries [17] by monitoring the inhibition of nitroblue tetrazolioum (NBT) reduction at 560 nm. The reaction mixture (3 ml) contained 50 mM phosphate buffer (pH 7.5), 50 mM carbonate sodium (pH 10.2), 0.1 mM Na-EDTA, 1 mM riboflavin, 12 mM L-methionine, 75 mM NBT and 50 µl enzyme extract. The reaction was carried out in test tubes at 25°C under the illumination of a fluorescent lamp (40-W). The reaction was allowed to run for 10 min and stopped by switching the light off. Blanks and controls were run in the same manner without illumination and enzyme, respectively. Under the experimental condition, the initial rate of reaction, as measured by the difference in the increase of absorbance at 560 nm in the presence and absence of extract, was proportional to the amount of enzyme. The unit of SOD activity was defined as the amount of enzyme that inhibits the NBT photoreduction by 50%. SOD activity values are given in units per mg of protein.

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Statistical analysis

Analysis of variance was performed on the data collected using the general linear model (GLM) procedure of the SPSS software (Version 16, IBM Inc.). The mean separation was conducted by Duncan´s analysis in the same software (p= 0.05).

RESULTS AND DISCUSSION

The results of the experiment revealed that treatments increased fresh weight and water absorption significantly compared to control (Table 1). Among the treatments, maximum fresh weight and water absorption were recorded in treatment 200 mg l^{-1} salicylic acid (Fig 1). This might be due to maximum uptake of water by the flowers as influenced by salicylic acid which helped in increased uptake of water and germicidal properties, in addition to inhibition of ethylene biosynthesis which resulted in gain in fresh weight. Microbial contamination was one of the most important factors causing vascular occlusion of cut flower [18]. Salicylic acid has been reported to act as an inhibitor of microbial growth [19].

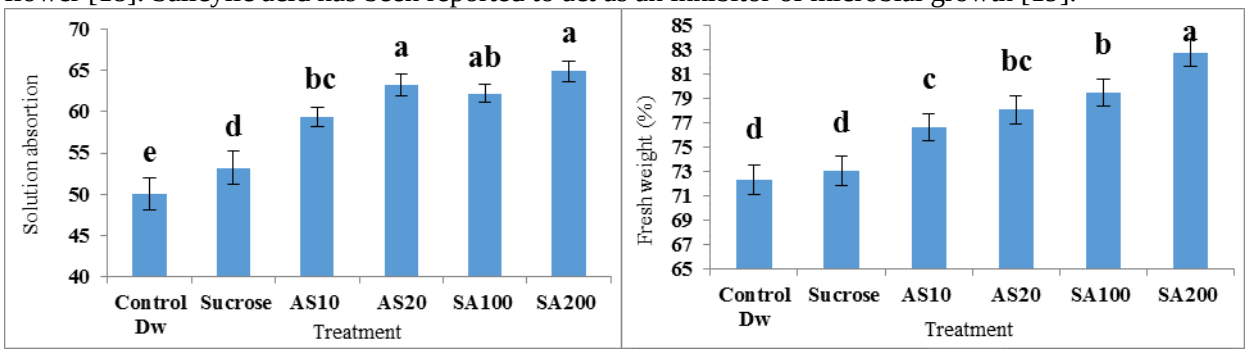

Fig (1) Effect of experimental factors on fresh weight and water absorption of cut flowers. The similar pattern among recorded variables is notable.

Preservative solution had effect significant on chlorophyll index, ion leakage and anthocyanin petals of cut flowers (P<0.05). The results indicated that treatment 200 mg l^{-1} salicylic acid caused a significant decrease in ion leakage, while increasing chlorophyll index and anthocyanin content compared to other levels (Fig 2,3) (p<0.05).Similarity, Canakci [20] reported that treatment with salicylic acid significantly extends the vase life with increases chlorophyll content and anthocyanin petals. These findings are in agreement with reported by Kazemi and Shokri [21]. The effect of SA on senescence and vase life extension of cut flowers was reported earlier which is confirmed here was anticipated. In previous studies, pre-treatment with SA evidenced by a reduction in the level of lipid peroxidation and leakage of electrolytes from plant tissues as well as by more intensive growth processes as compared to the control plants [20, 21].

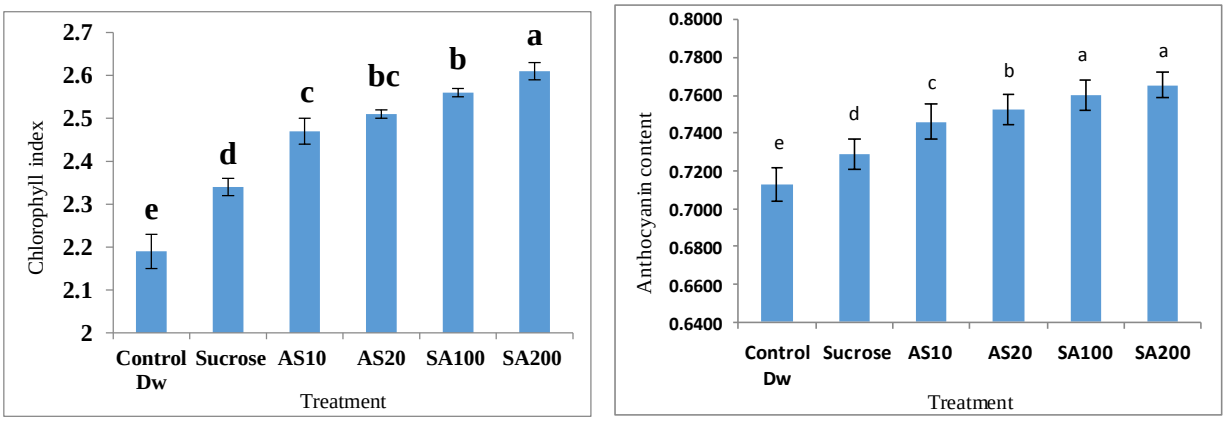

Fig (2) Effect of experimental factors on chlorophyll index and anthocyanin petals of cut flowers.

The results of the experiment revealed that treatments significantly increased SOD and PAL activity compared to control (Table 1). Among the treatments, maximum SOD and PAL activity were recorded in treatment 200 mg l^{-1} salicylic acid (Fig 4). An increase in antioxidant activity of SA-treated crop may beattributed to increase in total phenolics. When applied exogenously at suitable concentrations, SA was found to enhance the efficiency of antioxidant system in plants [22]. Zeng et al., [23] reported that salicylic acid treatment significantly enhanced PAL activity in grape berries. Relatively higher activities of ROS-scavenging enzymes have been reported in tolerant genotypes when compared to susceptible ones,

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suggesting that the antioxidant system plays an important role in plant tolerance against environmental stresses [24]. In many studies, it was found that the function of ASA alleviation of oxidative stress was attributed to the induction of various ROS-scavenging enzyme activities [25]. This effect was observed in the high concentration of ASA (1 mM).

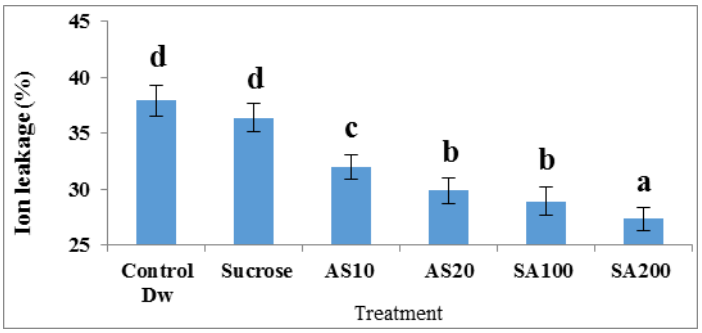

Fig (3) Effect of experimental factors on ion leakage of cut flowers.

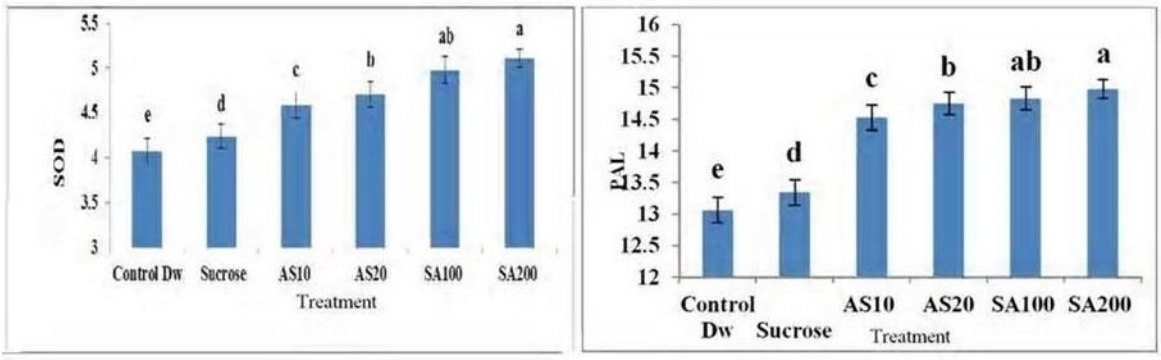

Fig (4) Effect of experimental factors on SOD and PAL activity of cut flowers.

The results indicated that 200 mg l^{-1} SA caused a significant increase in vase life compared to other levels ($p \leq 0.05$). These results are in agreement with those of [19, 20] who found that adding SA in vase water increased chlorophyll content cut flowers. Yuping [26] reported that treatment with salicylic acid significantly extends the vase life with increases the enzyme antioxidant activity and decreased ROS production.

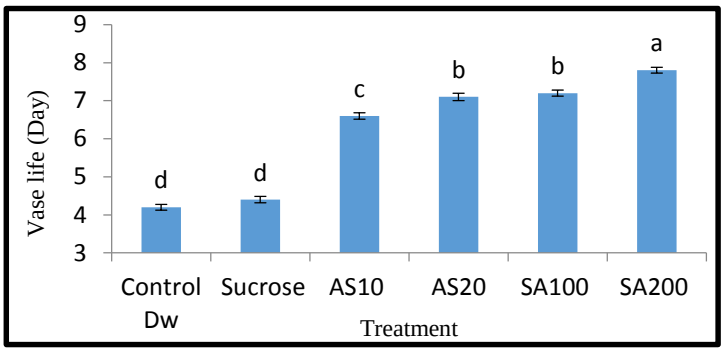

Fig (5) Effect of experimental factors on vase life of cut flowers.

In conclusion, based on results it could be stated that the utilization of SA may promote the vase life of rose cut flowers probably via inhibited microbial contamination, induced antioxidant enzymes, reduced oxidative stress, induced phenylpropanoid metabolism, prevented ethylene formation and decreased transpiration rates thereby delaying the senescence process.

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Table 1. Analysis of variances (ANOVA) of measured parameters in cut rose flowers under different treatments of ascorbic acid and salicylic acid.

Source of Variation (S.O.V)	Df	Fresh weight	Water absorption	Anthocyanin content	Ion leakage	chlorophyll content	PAL	SOD	Vase life
T	5	1469.820**	315.411**	0.009**	260.290**	0.466**	12.994**	2.296**	20.667**
time	3	18622.324**	1115.056 **	0.066**	809.597**	3.023**	69.654**	6.603**	--------
T.time	15	336.125**	19.233 ns	0.003 ns	58.908**	0.131 ns	4.059	0.467**	--------
Error	-	1.09	1.022	0.001	0.529	0.045	0.191	0.041	0.05
CV (%)	-	21.08	23.26	6.5	22.76	24.04	20.52	16.7	15.9

** Significant statistically at 1%.

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