

## ARTICLE

# THE EFFECT OF SALICYLIC ACID FUNCTION ON VASE LIFE AND ACTIVITY OF THE PHENYLALANINE AMMONIA LYASE ENZYME OF CUT FLOWERS (ROSA HYBRID ACV. BLACK MAGIC)

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## ABSTRACT

Ornamental products such as cut flowers have a limited display life so the effect of some effective chemical holding treatments on vase life of Ross (*Rosa hybrida* L.) cv. Black magic cut flower was studied. In this research conducted the effects of two levels salicylic acid (100 and 200ppm) treatment along with sucrose (3%) was conducted in a factorial arrangement, carried out in a completely randomized design. The recorded traits included: vase life, fresh weight, dry weight, ion leak, water absorption, anthocyanin content, total chlorophyll content and phenylalanine ammonia lyase enzyme activity. The results showed that the greatest delay in senescence was obtained in cut rose treated with salicylic acid 200 mg/l (7/8 Day) as compared to control treatment (distilled water, DW) in addition, the application of salicylic acid due to the impact of the Phenol cycle and Antioxidant role, reduces the activity of the Phenylalanine ammonia lyase enzyme.

## INTRODUCTION

In Iran, more than any other flower, the rose has a long history, European travelers who come to Iran (Orient) were named Iran as the source of Roses. Rose is one of the most important Ornamental and commercial plants in the world plants and is belongs to the Rosaceae family and is Rosoideae subcategory and as semi-permanent green to decidua, with a wide range of growth habits can be found in Asia, North Africa, North America and Europe. Within the Rosaceae family, there are 100 kinds and about 3100 species. Black magic flower is the hybrid of tea rose that has a dark red color and sweet fragrance.

Rose flower pot life is usually short. Flowers wilting and axis of flowers in such a way that is bend just below the neck. Develop or grow is one of symptoms that have been affected by vascular occlusion and inhibit water supply. Many methods published to extend the life of cut flowers and keep them fresh for a longer duration and provide freshness. Therefore further research needs to be done in the field of maintenance of cut flowers and its different varieties. Four major factor play a role in determining the storage and longevity of products, which are: 1 (Temperature, 2 (water relations, 3 (carbohydrate supply and 4 (growth regulators.

## Salicylic acid

Salicylic acid (SA) is considered an important signaling molecule that involved in immune response against biotic and abiotic stresses. Salicylic acid, in a period of time, was the best salable pharmaceutical composition in the world that were produced in Germany in 1898 (Raskin, 1992) although, John Buchner in 1928, isolated salicylic alcohol glucoside from willow bark in Munich, but later Rafacle Piria in 1938 was named it salicylic acid (SA). The word salicylic acid (SA) derived from the Latin word "Salix" means willow tree and the reason is that in all the whole plant kingdom (Raskin, 1992) SA has dedicated diverse role in regulating metabolism in plants (PoPova et al., 1997).

In terms of chemical structure, SA belongs to the diverse group of plant phenols which have aromatic ring with a hydroxyl group or a function of its derivatives (Figure 1). Released SA is a crystalline powder and can melt at a temperature of 157-159Centigrade. SA relatively is soluble in water and polar organic solvents that have greater solubility and its PH in aqueous solution is 2.4. Aspirin, has similarity with SA, and the result of a spontaneous hydrolysis is aqueous solution. Scientists largely believe that (ortho-hydrobenzic acid) is a natural derivative of cinnamic acid, is an intermediate in path of Shikmic acid and affecting factors in Synthesis of Phenolic Compounds. However, is provided two possible ways in this direction. [Fig. 2]. Salicylic acid to form compounds is known with the number of Glycosylation molecules and less often by esterification. Compare the effect produced by 22 phenolic compounds indicate that SA and ASA are prevented ethylene production. SA also ethylene enzyme inhibitor has dinitrophenyl (Leslie and Romina, 1980).

## Phenylalanine ammonia lyase

Plant produces a large group of secondary products that contains a phenolic group. Phenolic according to their chemical diversity in plants play a diverse role. In plants most secondary groups of Phenolic compounds by removing an ammonia molecule derived from phenylalanine into cinnamic acid. This reaction catalyzed by phenylalanine ammonia lyase (PAL). PAL activity increases by environmental factors such as lack of food, light and fungal contamination. Control appears in the transcription initiation. During invasive fungal being targeted which encoding messenger RNA to PAL therefore PAL is increased in plant and then phenyl synthesis is stimulated. There is a positive correlation between the activities of PAL, total

## KEY WORDS

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phenol and total flavonoids and there is a negative correlation between activities of PAL and antho cyanins content in leaves and flowers. Phenylpropanoid is derivatives of trans-cinnamic acid that of L-phenylalanine in a reaction catalyzed by the enzyme has been established L- phenylalanine ammonia lyase. (Vogt, 2010)

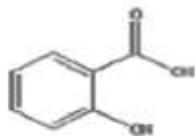


Fig. 1: Salicylic acid structure.

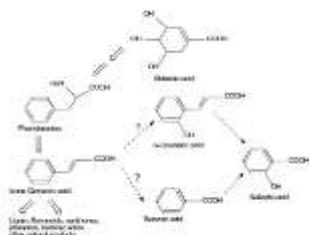


Fig. 2: Synthesis of Phenolic Compounds.

Cinnamic acid is a white crystalline organic compound, which is slightly soluble in water. It's Biosynthesis done by the phenylalanine ammonia lyase enzyme. PAL has central role in the synthesis of phenol and many reports has presents on the relationship between genetic PAL / protein expression / activity and increase phenolic compounds in response to different stimuli. The Phenyl propanoid direction includes a wide range of polyphenols with low molecular weight. These compounds and their derivatives are the largest and most important group of polyphenols. Biochemical studies in plants using the isotope show that the biosynthesis of SA in plant, from the cinema (the phenylpropanoid) and another way of isomerism to form SA. Phenyl propanoid course, will be the main course of the production of secondary metabolites in plants, which ultimately led to the production of different kinds of combinations, such as anthocyanins, flavonoids, guards UV rays, anti-microbial keromaryn furan, Iso flavonoides, Finno auxin, lignin and phenolic esters. Cinnamic acid hydroxylase (C4H) trans-cinnamic acid that produced by the PAL activity of the precursor material of Phenylalanine converts to Kumarat [Fig. 3].

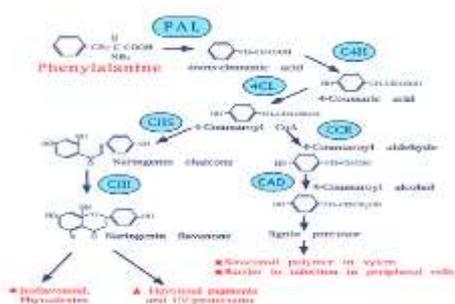


Fig. 3: phenylalanine ammonia lyase Cycle.

### MATERIALS AND METHODS

In this research cut flowers rose of Black magic type, from the greenhouse of Mr. Hussein banaian that has standard conditions and provided in the paktasht city and in favorable conditions transported to the laboratory. The present research was conducted in the October, 2012 in the garden laboratory of Islamic Azad University of Garmsar in Khak Behin Azma laboratory. During experiment, the laboratory temperature setting on  $2 \pm 22^{\circ} \text{C}$ , relative humidity on 55 to 75 percent and light cycle to 12 hours of light and 12 hours of darkness. Cut flower rose to minimize experimental error again in terms of uniform the stem length and after cut flowers stems diagonally and first we have the flowers with a similar stem and height and second if air bubbles in the vessels created during the harvesting which can impede water movement to the ends of the flowers and this will remove bubbles and water will more easily transfer within. This research in order to investigate long-term treatment effect of salicylic acid on two levels of 100 and 200 ppm with 30 mg distilled water and 3% sucrose was used as control. This experiment with four treatments and three replicates and each replicates containing five experimental units (Table 1) was conducted as the

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factorial experiment in a completely randomized design. Assessment and characteristics of sampling were performed on zero, 2, 4 and 6 days.

**Table 1:** Diseases abbreviations list

Abbreviation	Treatments name	.No
Control Dw	Distilled water control	1
Sucrose	Sucrose	2
SA100	Salicylic acid 100 ppm	3
SA200	Salicylic acid 200 ppm	4

### Indicators of longevity

Durable cut flowers which is one of the most important factors of post-harvesting were calculated as the number of days of post-harvesting from the chemical treatments to appearance of symptoms such as wilting petals, bending the flowers neck or petals change color or petal fall. The life of cut flowers, which is considered as the number of days from harvest to aging flower, is one of the important characteristics to assess the effect of different treatments and new varieties and species.

### Fresh weight

Changes in fresh weight of flowers have a role in determining the durability of cut flowers because decreases the fresh weight causes wilting petals and ultimately reduce the ornamental value. In this experiment, fresh weight weighed by a digital scale, with 0/01 precision and was expressed based on percentage (Celice, 2002).

### Dry weight

Flowers after the end of their life weighed inside the oven with 62 temperature for 96 hours were dried and then by a digital scale with 0/05. By subtracting the dry weight can be achieved as well as the amount of water content (Jones et al, 1993).

### Absorbance

Obstruction of the vessels stems for various reasons, such as the enter of air bubbles during the cutting or microbial growth in the dilution is created due to the presence of sucrose and lead to the reducing water absorption and ultimately accelerated wilting flowers. In this research, the volume uptake solution was measured by measuring the decrease in volume of the solution in the flask containing flowers and was expressed as mL (Zamani et al, 2011). To measure this feature, then put the flowers in the vase solution in the graduated cylinder, the entrance of the container will be completely blocked to prevent evaporating the solution and reduce the amount of solution in the container is only due to its absorption by flowers. The following formula was used to calculate the amount of absorption solution:

**(Formula 1)**  $(\text{ml} \cdot \text{day}^{-1} \cdot \text{stem}^{-1}) = (V_T - V_1)$  the amount of uptake solution

Where  $V_T$  is solution size in measured days and  $V_1$  is the volume of the solution in the day before (Hetarachchi, 2005).

### Anthocyanin leakage

To measure the petals of anthocyanins was used (Sankhla et al, 2005) method so that 1.0 grams of fresh flower petals weighing and turned into small pieces and then pulverized in a mortar and to extract anthocyanins to each sample was added 5 cc of the extraction solution that containing a mixture of methanol and hydrochloric acid (1%) and the samples were on overnight at 4 ° C, and finally in the absorption solution was read using spectrometers at wavelengths of 530 and 657 nm and then were the following formula (Sevelius, 2003).

**(Formula 2)** Anthocyanin =  $A_{530} \frac{1}{4} A_{657}$

### Leaf chlorophyll

For the measurement of chlorophyll was used the Arnon (1949) method. Thus, 1 gram of fresh leaf was pulverized in a porcelain mortar with 10 ml of 80% acetone and the clear solution after centrifugation was measured by light absorption spectrophotometry at a wavelength of 645 and 663 nm and finally the chlorophyll content was measured based on milligrams per gram of tissue by the following formula.

**(Formula 3)** chlorophyll a =  $(12/7 \times A_{663}) - (2/69 \times A_{645})$

**(Formula 4)** chlorophyll b =  $(4/68 \times A_{663}) - (22/9 \times A_{645})$

**(Formula 5)** total chlorophyll =  $(8/02 \times A_{663}) - (20/2 \times A_{645})$

Ionic leakage

Measurement of ion percentage can be a good indicator in determining the stability of the membrane in water with high EC. Salinity stress, leads to the oxidative stress, osmotic stress and ion toxicity. To assess the extent of damage to the membrane ion leakage, were used the Ben Hamed et al, 2007 method. In this way, 5.0 g of flower petals were placed into Falcon 10 ml and added distilled water. Then put the Falcon in 30 minutes in a hot water bath (Ben Murray) with a temperature of 60 degrees and then examples electrical conductivity (EC1) were measured using the EC meter model Metrhom (Switzerland). The Falcon at 120 ° C for 20 minutes and then after cooling to a temperature of 25 degrees, re-measurement the electrical conductivity of the samples (EC2) and ion leakage was calculated by the following formula (Kazemi et al, 2012).

(Formula 6) ion leakage percentage= EC1/EC2 X 100

Phenylalanine Ammonia Lyase Enzyme (PAL)

3.0 g of fresh petal tissues weighed and in 5.6 ml of buffer Tris-HCl 50 mM(pH )8.8, that containing 15 mM beta-mercaptoethanol, pulverize and extract was placed for 30 minutes at 5000 g cm. The supernatant was used for identification. Mixture of 1 ml of extraction buffer, 5/0 mL of phenylalanine 10 mm, 4.0 ml water and 1.0 ml of enzyme extract were placed in hot water bath 37 ° C for one hour. Then the reaction was stopped by 0/6 ml of chloric acid in 6 M. Finally, the resulting solution was added 15 ml of ethyl acetate. Oil phase detached and remaining at put in laboratory to evaporate. then remaining, that is the same cinnamic acid solved in 3 ml of 0/05 molar and cinnamic acid concentration was obtained by measuring the absorption at a wavelength of 2 nm using the extinction coefficient of M<sup>-1</sup>cm<sup>-1</sup> 9500. The activity of this enzyme is determined based on the speed of converting phenylalanine to trans-cinnamic acid. A unit of PAL activity equivalent to 1 micromole of produced cinnamic acid in one minute. (Wang et al., 2006)

RESULTS AND DISCUSSION

Flavonoids and anthocyanins are remarkable and diverse group of secondary products with a wide range of biological functions, including protection under stress conditions. Poly phenolic compounds are in a wide variety of chemical compounds in plants that has significant physiological importance in plants. It is thought that the molecular basis for plant protection practice, are in the inhibitory properties of antioxidants and free radicals. Based on the above, salicylic acid caused antioxidant protection and stability of plant pigments [Fig. 1 and 2] and decrease electrolyte leakage due to the strength of the cell wall [Fig. 3].

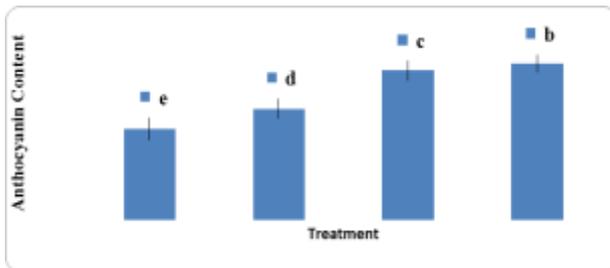


Fig. 1: Effect of experimental factors on anthocyanin petals of cut flowers.

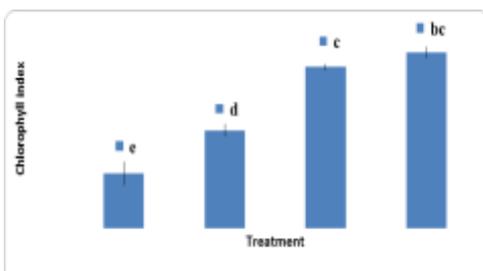


Fig. 2: Effect of experimental Factors on chlorophyll index of cut flower.

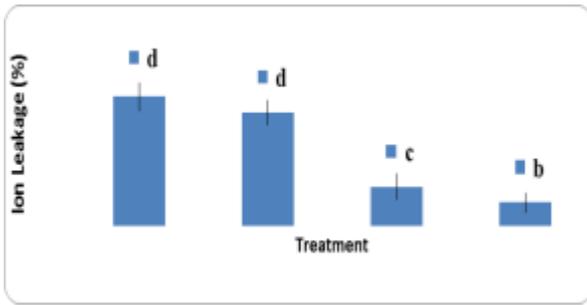


Fig. 3: Effect of experimental Factors on ion leakage of cut flower.

Plants in response to infections that cause by fungal or bacterial, to produce compounds phenylpropanoid and this combination will be largely responsible for plant defense mechanisms.

Salicylic acid due to the presence of acidic environment PH to control the growth of microorganisms and followed by its absorbance, increase relative fresh weight [Fig. 4 and 5].

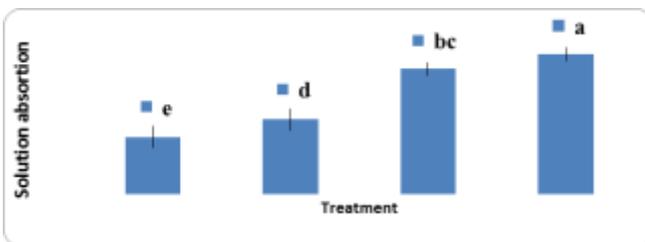


Fig. 4: Effect of experimental Factors on water absorption of cut flower.

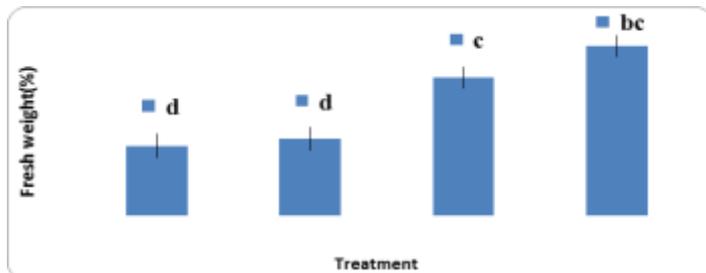


Fig. 5: Effect of experimental Factors on fresh weight of cut flower.

Phenylalanine ammonia lyase has central role in the production of phenolic compounds, which is responsible for defending in difficult environments in response to different stimuli. Usage of salicylic acid due to the impact of the phenolic cycle and antioxidant role, reduces the downward trend of Phenylalanine ammonia lyase enzyme [Fig. 6]. Improve all traits compared to the control group showed that reduced activity of microorganisms and thus extend the life of treated flowers with salicylic acid [Fig. 7].

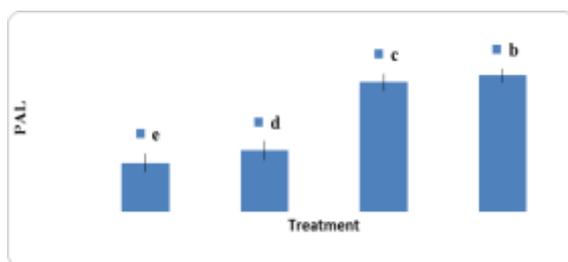
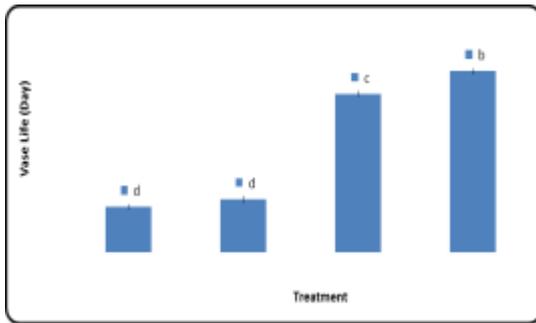


Fig. 6: Effect of experimental factors on PAL activity of cut flowers.



**Fig. 7:** Effect of experimental factors on vase life of cut flowers.

**CONFLICT OF INTEREST**  
 There is no conflict of interest.

**ACKNOWLEDGEMENTS**  
 None

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 None

## REFERENCES

- [1] Asni Ashari M, Zkaei Khosroshahi MR. [2008] After harvesting physiology and technology. Bu-Ali Sina University Press. 12-54.
- [2] Bijami A, Rezanejhad F, Hussein Ali S. [2010] UV-B radiation effect on some antioxidant combinations, PAL activity and total protein in *Lycopersicon esculentum* Mill after harvesting. *Plant Biology*, second year. 6:29-38.
- [3] Qasemi Ghehsareh M, Kafi M. [2005] Scientific and practical potting. Golbon Press.93- 523
- [4] Ghasemi Omran S, Qasem Nejhad M, Hatamzadeh A, Bkhshi D. [2011] Studies of seasonal variations in color, PAL activities and continuous quality of *Alstroemeria* cut flowers. *Iranian Horticultural Science Journal*. 43 (2): 189-198.
- [5] Kafi M, Borzooei A, Salehi M, Kamandi A, Mohammadi A, Nabati J. [2009] Physiology of environmental stresses in plants. Publications University of Mashhad. 429 - 453.
- [6] Momeni N, Javad arvyn MJ, Khajouie Nejhad GH, Daneshmand F, Keramat B. [2012] Effect of Sodium Chloride and Salicylic acid on antioxidant defense system in maize (*Zea mays* L). *Plant Biology*, Issue XIV, winter. 23-34.
- [7] Nabi Gul A. [2012] Compare Flowerpot life, soluble carbohydrate content and the ethylene production in cut flowers of rose varieties. *Science and Technology of Green house Culture*. 12: 117-123.