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Effect of salycilic acid and sodium nitro proside on the pomegranate aril browning disorder

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ABSTRACT: Pomegranate (*Punica granatum* L.) is one of the important fruits of the Middle East. The fruit is prone to a disorder called aril browning. This disorder threatens production, consumption, and exports of pomegranates, because affected fruit cannot be externally distinguished from healthy fruit. This study designed to evaluate the effects of foliar application of salicylic acid 0, 10-3, 10-4M (SA) and sodium nitro proside 0, 10-3, 10-4M (SNP) on the reduction of the disorder. Results indicated that foliar application of SA and SNP significantly reduced percentage of aril browning in 10-4M concentration. Also, SA and SNP significantly increased total anthocyanin content, ascorbic acid, ascorbate peroxidase activity (APX) and superoxide dismutase activity (SOD). All in all it seems that application of these growth regulators is a good approach to reduce aril browning damage in the pomegranate fruit.

Keywords: Anthocyanin, aril browning, ascorbate peroxidase, superoxide dismutase.

INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to Punicaceae family and is one of the oldest known edible fruits. It is some when called Chinese apple (Mars 1994). Pomegranate fruits are mainly grown for fresh consumption of arils (botanic exact term is seed) or juice, although in different countries they are produced for the food and drink industry as seasoning and coloring agents (Gil *et al*. 2000).

The edible part of pomegranate has notable amounts of acids, sugars, vitamins, polysaccharides, polyphenols, and important minerals (Mirdehghan & Rahemi 2007). Pomegranate is considered due to nutritional, chemical and antioxidants characteristics. One of the characteristics that affect the quality of the fruits are anthocyanins and responsible for the color of pomegranate arils (Miguel *et al*. 2004). Recently, occurrence physiological disorder under the title aril browning or aril paleness is threatened the value of the fruit. First time this physiological disorder reported in Ferdows region of Iran in. Affected arils are soft, light creamy-brown to dark blackish-brown, deformed, acidic and possess unacceptable off-flavor and are unsuitable for consumption. The extent of damaged arils could vary from a few to all in a fruit. Pomegranate fruits having this disorder do not show outward signs, and only after the cut fruits, defective arils appear. Sometimes consumers do not show reluctant to buy these fruits because of the hidden brown arils. In many cases, ripe fruits show more than 50% aril browning, which severe reduce of quality. Prior researchs demonstrated that this disorder is affected by various factors, such as fruit size, variety, pruning, season growth, genetic background, harvest time and pathogens (Jalikop *et al*. 2010; Shivashankar *et al*. 2012), but until today, the main reason for this disorder unknown.

Shivashankara *et al*. (2004) has been attributed browning of arils in pomegranate to the oxidative damage of membranes leading to higher activities of certain enzymes such as polyphenol oxidase and peroxidase. Biochemical studies suggest that pomegranates defective show soluble solids (TSS), pH, ascorbic acid, calcium, phosphorus and low catalase activity, instead, starches, tannins, nitrogen, magnesium, boron, potassium, polyphenol oxidase and peroxidase enhancements (Shete & Waskar 2005). Perhaps the problem is due to a decrease in the activity of antioxidant system of plant and and substances such as salicylic acid and sodium nitroprusside can boost the activity of antioxidant enzymes such as ascorbate peroxidase and suoeroxide dismutase.

Salicylic acid (SA) is plant hormone. This hormones affect the growth and development of plants. Salicylic acid is endogenous signal molecule, playing pivotal roles in regulating stress responses and plant developmental processes such as thermogenesis, photosynthesis, transpiration, ion uptake and transport, disease resistance, seed germination, crop yield, plant stress resistance and glycolysis.The effect of Salicylic acid is more on stress active, but Salicylic acid has many effects on stress bioactive such as drought, salinity, UV and chill (Hayat & Ahmad 2007).

Nitric oxide is involved in many different physiological processes, such as plant responses to pathogens (Siddiqui *et al*. 2011).

Two possible mechanism for the role of nitric oxide is proposed to deal with stress: Firstly, nitric oxide may direct way act as an antioxidant. Secondly, nitric oxide can act as a signal molecule and be changed in expression of defense genes (Lamattina *et al*. 2003). Nitric oxide increases the activity of antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, peroxidase, catalase, and that increases plant resistance (Tanou *et al*. 2009).

This investigation was performed to evaluation the role of salicylic acid and sodium nitroprusside on quality of pomegranate.

MATERIALS AND METHODS

A. Sample collection

In this study nitric oxide and salicylic acid was foliar in the summer. At the end of each season, pomegranate fruits of cv. Malase - Yazdi were collected from orchard in the Yazd agriculture research center and natural resources and immediately transported to the horticulture laboratory at the University of Tabriz, Iran. Fruits were cooled immediately after harvest and then transported to the laboratory by a refrigerated car. To prevent physical damage cotton placed between of the fruits.

B. Total anthocyanin content

The anthocyanins (TAC) were extracted from 0.5g fresh fruits with methanol acidified with 0.1M HCL. The extract was diluted with acidified methanol, and the absorption was measured at 530nm using a spectrophotometer the according the methodology of Fuleki & Francis (1968) with some changes.

C. Ascorbic acid measurement

Ascorbic acid was extracted and assayed by the method of Weaver and Charley (1974), which measures the extent to which ascorbic acid discolours a solution of 2,6-dichlorophenol indophenols. The amount of vitamin C expressed in milligrams per 100 gram of juice.

D. Percent of Arils Browning

Pomegranate seeds tested, completely apart and then the number of arils healthy and brown in each fruit was counted. The ratio was calculated as the total number of Arils.

E. Measurements of ascorbate peroxidase (APX) activity

The activity of APX is determined by the measurement of the diminution of the absorbance of oxidized ascorbate at 290 nm (using quartz cuvets) according to (Nakano & Asada 1981). The volume of the reaction is 1 ml containing 0.5 mM potassium phosphate, 0.2 mM of ascorbate, 0.1 mM EDTA, 0.1 mM H_2O_2, and protein extract to be tested for enzyme activity The reaction was started by adding the enzyme or hydrogen peroxide, and the absorbance decrease was recorded 10 to 30 sec after this addition. Usually no correction for the oxidation of ascorbate in the absence of hydrogen peroxide was necessary, which shows the lack of or very low activity of ascorbate oxidase (EC 1.10.3.3) in spinach leaves. Correction was done for the low, non-enzymatic oxidation of ascorbate by hydrogen peroxide. Activity was calculated using the extinction coefficient (2.8 mM^{-1} cm^{-1} at 290 nm) for ascorbate.

F. Measurements of Superoxide Dismutase (SOD) activity

Total SOD activity was determined according to Beyer and Fridovich (1987) by monitoring the inhibition of nitroblue tetrazolium (NBT) photoreduction using the following colorimetric assay; The reaction mixtures (1 mL) contained 50 mM potassium phosphate buffer, pH 7.8, 9.9 mM l-methionine, 58 ?M NBT and 2.4 ?M riboflavin (as the source of superoxide radicals). After adding riboflavin, the reaction mixtures were irradiated for 10 min at 25°C, and the absorbance at 560 nm was measured, using a non-irradiated reaction mixture as a blank. One SOD unit was defined as the amount of enzyme that causes 50 % inhibition of NBT photoreduction under assay conditions.

G. Hydroperoxide Assay

In most experiments, solutions containing the hydroperoxide were mixed with the appropriate volume of reagents to give final concentrations of 25 mM H2SO4, 100 mM XO, and 100-250 mM ferrous ammonium sulfate in a volume of 2 ml. The final pH was 1.8 6 0.5. For hydroperoxides insoluble or unstable in sulfuric acid, the solvent was 90% methanol and 10% 250 mM sulfuric acid or a 1:1 solution of glacial acetic acid and water. After 30 min in the dark, the absorbance was read at 560 nm with XO as blank. For high accuracy, a correction was made to the blank for the amount of XO removed by complexing with the Fe31 produced in the reduction of the hydroperoxide. Absorption coefficient was 2.9 × 104 M^{-1} cm^{-1} (Jessup *et al*. 1994).

RESULTS

A. Total anthocyanin content

The results of the experiment showed that the interaction between nitric oxide and salicylic acid treatment was significant on anthocyanins, so that the highest concentration was for the interaction between nitric oxide and salicylic acid (10^{-4} mM and 10^{-4} mM). And the least amount was for control so that control fruit were found white pigments (Fig. 1).

B. Ascorbic acid

The results showed a significant difference there was between treatments, the highest amount of vitamin C was for interaction SNP and SA with the highest concentration and the lowest was in the control treatment (Fig. 2).

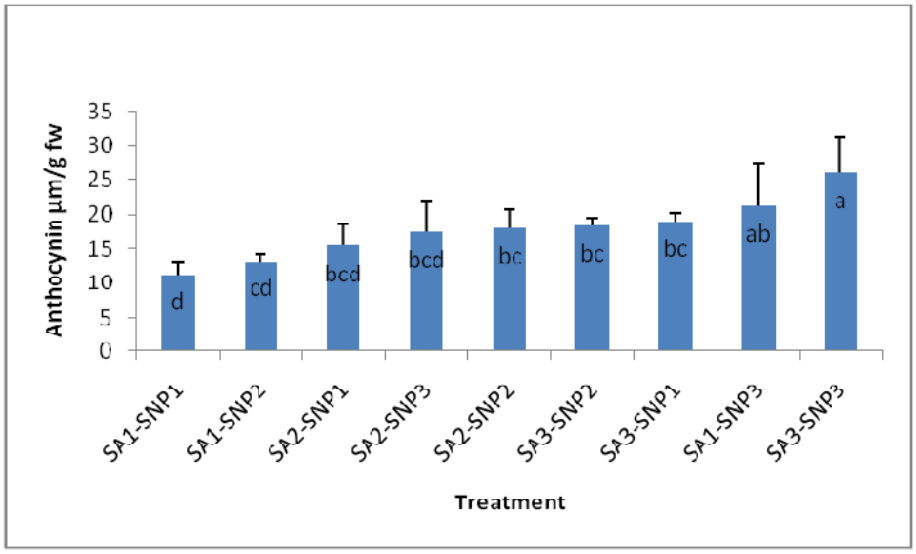

Fig. 1. The interaction between Sodium Nitro Proside and Salicylic Acid concentration on anthocynin of pomegranate juice. Data are means of three replicates. Different letters indicate significant differences between treatments according to Duncan mean separation test (P≤0.01).

*SA: Salicylic Acid, *SNP: Sodium Nitro Proside (SA1 and SNP1= 0, SNP2 = 10^{-3}M, SNP3 = 10^{-4}M, SA2 = 10^{-3} M, SA3 = 10^{-4}M)

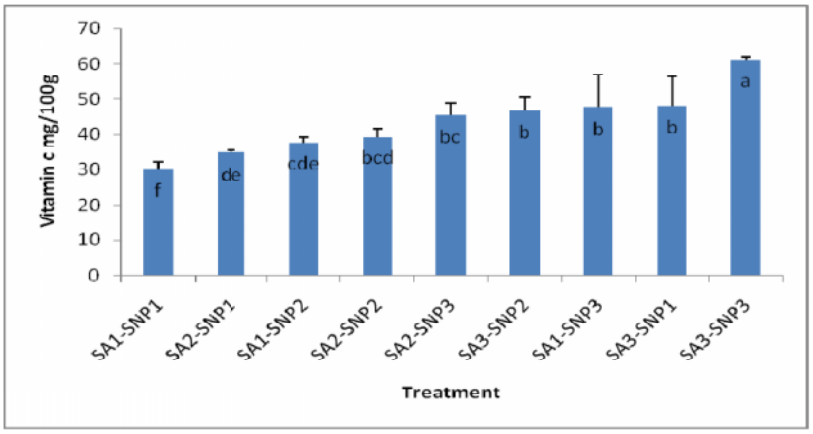

Fig. 2. The interaction between Sodium Nitro Proside and Salicylic Acid concentration on vitamin c (Ascorbic acid) of pomegranate juice. Data are means of three replicates. Different letters indicate significant differences between treatments according to Duncan mean separation test(P≤0.01).

* SA: Salicylic Acid * SNP: Sodium Nitro Proside (SA1 and SNP1= 0, SNP2 = 10^{-3} M, SNP3 = 10^{-4} M, SA2 = 10^{-3} M, SA3 = 10^{-4}M)

C. Percentage of seeds brown

Analysis of the data showed that there was significant difference between treatments and different levels of SNP and SA been influence on the amount Aril browning, so that the lowest percentage of browning related to treatment 4 mM SNP and SA and the highest browning related to control. There was significant difference well as between the rest treatments (Fig. 3).

D. Ascorbate peroxidase activity (APX)

The results showed that sodium nitroprusside and salicylic acid affected on ascorbate peroxidase enzyme activity, so that the highest enzyme activity APX related to 4 mM SNP and SA treatment and the lowest activity related to the control treatment (Fig. 4).

E. Superoxide dismutase activity (SOD)

Analysis of the data showed that sodium nitroprusside and salicylic acid is a significant increase in the activity of the enzyme superoxide dismutase, so that the maximum activity in the experiment related to treatment 4 mM sodium nitroprusside and salicylic acid and the lowest activity related to treatments SA1-SNP2, SA2-SNP1 and control (Fig. 5).

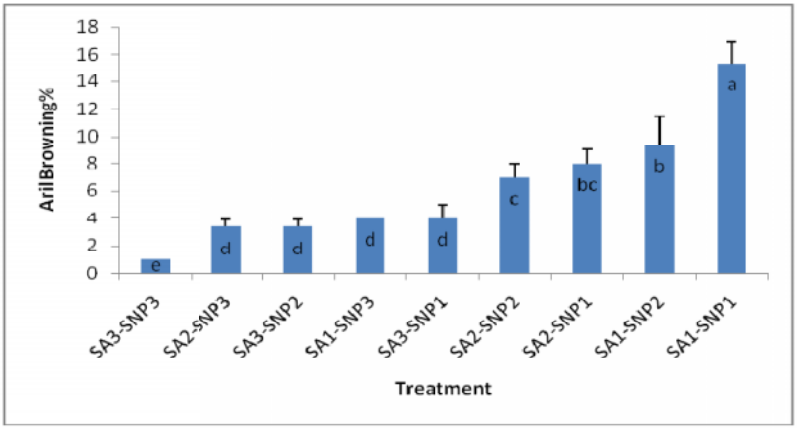

Fig. 3. The interaction between Sodium Nitro Proside and Salicylic Acid concentration on Percentage of seeds brown of pomegranate juice. Data are means of three replicates. Different letters indicate significant differences between treatments according to Duncan mean separation test($P \leq 0.01$).

*SA: Salicylic Acid, *SNP: Sodium Nitro Proside (SA1 and SNP1= 0, SNP2 = 10^{-3}M, SNP3 = 10^{-4}M, SA2 = 10^{-3} M, SA3 = 10^{-4}M)

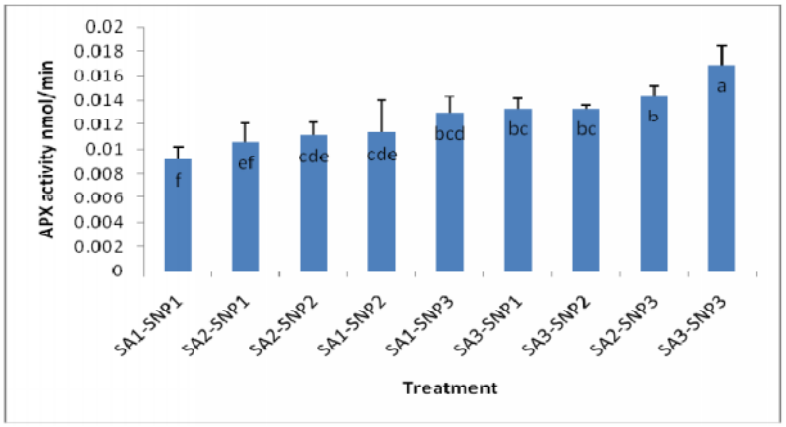

Fig. 4. The interaction between Sodium Nitro Proside and Salicylic Acid concentration on ascorbate peroxidase content of pomegranate juice. Data are means of three replicates. Different letters indicate significant differences between treatments according to Duncan mean separation test($P \leq 0.01$).

*SA: Salicylic Acid *SNP: Sodium Nitro Proside (SA1 and SNP1= 0, SNP2 = 10^{-3} M, SNP3 = 10^{-4} M, SA2 = 10-3M, SA3 = 10^{-4}M)

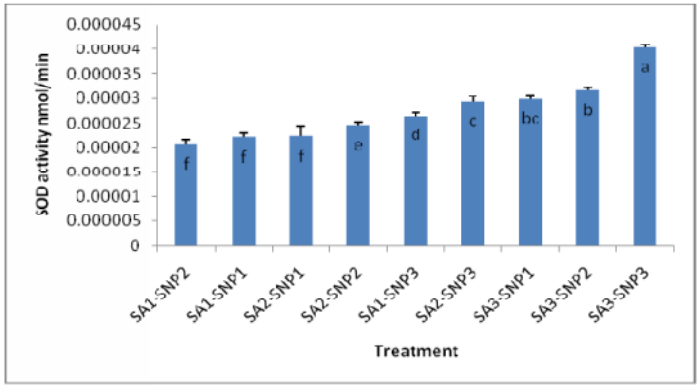

Fig. 5. The interaction between Sodium Nitro Proside and Salicylic Acid concentration on superoxide dismutase content of pomegranate juice. Data are means of three replicates. Different letters indicate significant differences between treatments according to Duncan mean separation test($P \leq 0.01$).

*SA: Salicylic Acid, *SNP: Sodium Nitro Proside (SA1 and SNP1= 0, SNP2 = 10^{-3} M, SNP3 = 10^{-4} M, SA2 = 10^{-3} M, SA3 = 10M).

F. The amount of hydrogen peroxide

The data showed that SNP and SA treatments affected the amount of hydrogen peroxide in plants, as the SNP and SA treatments significantly reduce the amount of hydrogen peroxide has been compared to the control plant. In this experiment, the maximum amount of hydrogen peroxide related to control and SA2-SNP1 and the lowest amount was related to the interaction between SNP and SA with the highest concentration (4 mM). There was a significant difference well as between the rest treatments (Fig. 6).

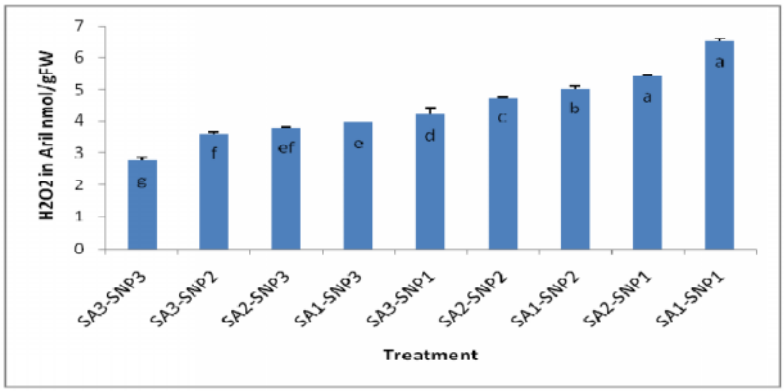

Fig. 6. The interaction between Sodium Nitro Proside and Salicylic Acid concentration on H_2O_2 content of pomegranate juice. Data are means of three replicates. Different letters indicate significant differences between treatments according to Duncan mean separation test($P \leq 0.01$).

*SA: Salicylic Acid *SNP: Sodium Nitro Proside (SA1 and SNP1= 0, SNP2 = 10^{-3} M, SNP3 = 10^{-4}M, SA2 = 10^{-4} M, SA3 = 10^{-4}M)

DISCUSSION

A. Total anthocyanin content

Sayyari *et al*. (2011) showed that the use of salicylic acid and calcium increase was pomegranate anthocyanin fruits. Also, Tian *et al*. (2004) stated that over time storage the polyphenol oxidase enzyme activity in cherry mesocarp increased due to the loss and degradation anthocyanins by the enzyme is.

In a trial by Bhamir and Mohammadkhani (2014) was demonstrated that nitric oxide protects the fruit anthocyanins in strawberries that these tests were in line with the experiment.

It seems that two of nitric acid and salicylic acid with increase of antioxidant systems, plant against destructive elements such as PPO, POD and H_2O_2 protection that these factors have played a major role in the degradation of anthocyanin.

B. Ascorbic acid

Sayyari *et al*. (2009) reported that the use of salicylic acid at a concentration of 2 mM, keep was the pomegranate vitamin C, also Abdollahi *et al*. (2010) demonstrated that treatment with nitric oxide and Putrescine on strawberry fruit cv. Selva were protects vitamin C compared to control fruit.

TSS, vitamin C, type and quantity of sugar, TA, pH and total antioxidant capacity is of the main factors the quality of horticultural products, that after harvesting of TSS and pH increase and TA and vitamin C decreases. The treatment with SNP and SA can delay decreasing of vitamin C.

C. Percentage of seeds brown

Sayyari *et al*. (2011) reported that treatment SA reduced browning of pomegranate fruit in the store 5°C. In this experiments showed that whatever concentration of SNP and SA is increased browning is reduced.

D. Ascorbate peroxidase activity (APX)

Plants to prevent of damage by ROS the benefit of development strategy antioxidant system, which can be enzymatically or non-enzymatically (Spinardi 2005). Antioxidants with giving electrons to ROS themselves oxidized and neutralize free radical. Ascorbate peroxidase is one of the important antioxidant enzymes and scavenges hydrogen peroxide (H_2O_2) using ascorbate (AsA) as an electron donor in the chloroplast and cytosol through the AsA-glutathione (GSH) cycle (Asada 1992). Wang *et al*. (2006) demonstrated that treatment with Salicylic Acid on Peach Fruit in a concentration of 1 mM significantly were increased antioxidant enzyme activity of APX and GR compared to control fruit. In experiments by Nasibi *et al*., (2015), it was observed that treatment with SNP on cut flowers of toberose (*Polianthes tuberosa* L.) was increased activity of antioxidant enzymes APX and GPX at a concentration of 1 and 10 µM.

E. Superoxide dismutase activity (SOD)

In a trial conducted by the Hung *et al*. (2007) reported that salicylic acid pretreatment on the pulp of Cara Cara navel orange (*Citrus sinensis* L. Osbeck) at different storage temperatures was intensified accumulation of $H_2O_2$and SOD activity.

Shi *et al*. (2007) the role of NO in reducing oxidative stress are related to the ability of NO and SOD enzyme induction convert superoxide to hydrogen peroxide and molecular oxygen ions, and states that if hydrogen peroxide produced when the cells are dissolved, superoxide anion reacts and produce OH radicals. Which are highly toxic and reactive. On the other hand NO by converting superoxide anion to hydrogen peroxide, this eliminates the anion from cells, and on the other hand, with induction of antioxidant enzymes such as APX reduces the toxicity of hydrogen peroxide (Kopyra & Gwozdz 2003).

F. The amount of hydrogen peroxide

It seems that hydrogen peroxide is one of the destructive of Aril browning disorder by SNP and SA reduced greatly.

CONCLUSIONS

This study indicated that SNP and SA increase pomegranate plant efficiency by the influence on physiological and biochemical processes. In this experiment, salicylic acid, and sodium nitroprusside increased anthocynin content and the activity of the Superoxide dismutase enzyme , causes loss of free radicals and prevent the aril browning. Also, our results showed that SA3-SNP3 treatment had positive effect on Ascorbate peroxidase activity, Ascorbic acid and hydrogen peroxide.

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