

## **Phytochemical and Quality Attributes of Strawberry Fruit under Osmotic Stress of Nutrient Solution and Foliar Application of Putrescine and Salicylic Acid**

**Masoud Haghshenas<sup>1</sup>, Mohammad Javad Nazarideljou<sup>2\*</sup>, and Akbar Shokoohian<sup>1</sup>**

1. Department of Horticulture, Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran

2. Department of Horticultural Sciences, Faculty of Agriculture, Mahabad Branch, Islamic Azad University, Mahabad, Iran

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### **Abstract**

The moderating role of salicylic acid (SA) and putrescine (PUS) as plant growth regulators (PGRs), on the growth parameters and phytochemical and qualitative characteristics of strawberry fruit 'Selva' under osmotic stress was investigated under soilless culture. The osmotic potential (salinity) of the nutrient solution containing different NaCl concentrations (0, 7.5, 15, 30 and 45 mM) and foliar application of PUS (0 and 1.5 mM) and SA (0 and 1.5 mM) were studied. The results showed a significant decrease in plant leaf area (79.6%), total chlorophyll content (48%), fruit yield (73.5%), leaf relative water content (33%), total protein (33.4%), total phenol (7.8%), and vitamin C content (24.5%) under osmotic stress. Moreover, peroxidase (POD) and superoxide dismutase (SOD) enzymes activity, leaf ion leakage, and soluble carbohydrate and proline content increased significantly under osmotic stress. Application of PGRs had a significant effect on all the studied traits (except for SOD activity). Interactive effects of salinity and PGRs were significant on all the traits except for leaf ion leakage, POD activity, soluble carbohydrates, and protein. The highest total phenol and vitamin C contents were obtained with 15 mM salinity along with foliar application of PGRs. In conclusion, foliar application of PUS and SA ameliorate negative effects of salt stress on growth, yield, and quality of strawberry fruit.

**Keywords:** Antioxidant, salinity, yield, soilless culture.

**Abbreviations:** Peroxidase (POD); Plant growth regulators (PGRs); Putrescine (PUS); Salicylic acid (SA); Superoxide dismutase (SOD).



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### **Introduction**

Strawberry, as an important greenhouse crop, has a significant economic and nutritional value due to its high vitamin content and antioxidant capacity (Giampieri et al., 2012). Soilless

production of strawberry is progressively increasing (Palha et al., 2010). Among different production factors, osmotic potential of nutrient solution directly affects growth and quality of the fruits produced under soilless systems. Strawberry is susceptible to osmotic stress.

\* Corresponding Authors, Email: [nazarideljou@yahoo.com](mailto:nazarideljou@yahoo.com)

Negative effects of salinity on strawberry growth, yield, and quality have been reported in numerous studies (Karlıdag et al., 2009; Eshghi et al., 2017; Zahedi et al., 2020).

Salinity stress directly affects plant growth through osmotic effects, specific ion toxicity, and ionic imbalances, which result in oxidative stress, and thereby an increase in the production of free radicals or reactive oxygen species (Mahajan and Tuteja, 2005). This increase in the production of free radicals can damage cell biomolecules such as membrane lipids, proteins, nucleic acids, and photosynthetic pigments, resulting in a significant decrease in photosynthetic capacity (Jiang et al., 2017). The plant's response to stress appears with a range of morphological, physiological, biochemical, and molecular changes, which are controlled by a large number of stress-responsive genes (Liu et al., 2014).

In the recent decades, several chemicals have been tested to increase the resistance of plants to osmotic or salinity stress, such as growth regulators and osmotic protectors. Polyamines are a new group of natural growth regulators with low molecular weight and aliphatic polycations that is found in living organisms such as bacteria, animals, and plants (Hussain et al., 2011). Putrescine (PUS), spermidine (SPD) and spermine (SPM) are the three sources of polyamines that are involved in a wide range of physiological processes such as embryogenesis, cell division, leaf development, and relative resistance to environmental stresses (Pedraza et al., 2007). The interaction between polyamines and stress may be important for improving plant defense mechanisms (Groppa and Benavides, 2008). The most important characteristic of polyamines is the positive charge of the amino group, as it allows electrostatic interactions with several macromolecules (proteins, lipids, and nucleic acids) in response to biotic and abiotic stresses (Menendez et al., 2012).

The PUS acts as an antioxidant and increases relative resistance to salt stress through decreased peroxidation of lipids and degradation of macromolecules as well as increase in the amount of glutathione and carotenoids (Tang and Newton, 2005). Sun et al. (2018) studied tomato farms and found that PUS increases antioxidant enzyme activity, root activity, nitrogen metabolism, and chlorophyll and proline contents and decreases malondialdehyde content.

Another PGR with intrinsic and natural phenolic structure in plants is salicylic acid (SA). It regulates plant physiological processes such as growth, photosynthesis, nitrate metabolism, ethylene production, flower production, and resistance to biotic and abiotic stresses such as salinity (Hayat et al., 2010). The mechanism of salicylic acid-induced resistance in plants under salinity stress consists of activating biochemical pathways such as regulating and balancing reactive oxygen species (Lee et al., 2010), stabilizing photosynthetic capacity (Miura and Tada, 2014), and stabilizing potassium homeostasis and sodium uptake and distribution (Hayat et al., 2010). Samadi et al. (2019) reported that foliar application of SA (100  $\mu$ M) in strawberry causes improved performance, induction of compatible osmolytes, phenol metabolism, and decreased membrane damage under saline stress. Application of 1 mM SA has the best effect on the grape performance under saline conditions (Roustakhiz and Saboki 2017). Studies by Shen et al. (2014) also showed that SA significantly increases the activity of antioxidant enzymes under salinity stress.

Considering sensitivity of strawberry to salinity, expansion of strawberry soilless cultivation, and the impact of osmotic potential of nutrient solution on the yield and quality, and given the limited information that we have about PUS and SA interaction and their effects on the growth and fruit quality, the main objective of this experiment was to study the effects

of PUS and SA on physiological and qualitative attributes of strawberry fruit under osmotic stress in soilless culture.

## Material and Methods

### *Plant Material and growth conditions*

To investigate the effect of PGRs on physiological and qualitative attributes of strawberry fruit under osmotic stress, a factorial experiment was carried out based on the completely randomized design with three replications in the soilless culture in the greenhouse. Greenhouse climate conditions were set to 25/18 °C average day/night temperature, 70 ± 5% relative humidity, and 10 h light duration.

Different levels of osmotic or salinity stress were applied by adding of sodium chloride (NaCl) to the nutrient solution at 0, 7.5, 15, 30 and 45 mM concentrations. All

pots were fertigated five times a day with 2 hours intervals (2 L/day). Foliar applications of both PGRs including SA and PUS at 1.5 mM, and distilled water for control treatment were applied once every two weeks following the establishment of strawberry transplants (*Fragaria × ananassa* Duch cv. Selva). Coco-fiber and perlite (V/V; 70:30) was used as the culturing substrate. Each replicate or experimental unit was containing three plants, and each treatment had three true replicates, and in total each treatment had 9 plants.

The fertilizer formulation used in this experiment (Table 1), is based on previous research (Caruso et al., 2011) on the role of electrical conductivity (EC) of nutrient solution on strawberries as well as previous experiments of this formulation by another report (Shirko et al., 2018).

**Table 1. Chemical composition of nutrient solution used in the experiment.**

Mineral sources [Amounts of salt in water (g 1000 L <sup>-1</sup> )]			
Macro-nutrients		Micro-nutrients	
Ca(NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O	58.928	H <sub>3</sub> BO <sub>3</sub>	0.114
MgSO <sub>4</sub> .7H <sub>2</sub> O	45.639	ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.725
KH <sub>2</sub> PO <sub>4</sub>	5.205	MnSO <sub>4</sub> .H <sub>2</sub> O	0.431
K <sub>2</sub> HPO <sub>4</sub>	10.21	CuSO <sub>4</sub> .5H <sub>2</sub> O	0.511
KNO <sub>3</sub>	7.298	Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.023
K-EDTA	11.812	Fe EDTA	2.462

To investigate the salinity effects under soilless conditions, recipe introduced by Caruso et al. (2018) with an EC of 1.3 dS/m was used to prepare the nutrient solution. Moreover, the pH of the solutions was adjusted to 5.5 to 6 and daily EC and pH of the nutrient solutions were carefully monitored until the end of the experiment.

During the growth period and at the end of the experiment, the number of leaves in each replicate was counted separately and the leaf area of each plant was measured. Photosynthetic pigments including chlorophyll a, b and total chlorophyll, was measured by sampling fully grown mature leaves and was prepared for acetone digestion. The absorbance of each sample was determined using a spectrophotometer (Perkin Elmer, Lambda UV/VIS 25) at

646.8, 663.2 nm for determination of chlorophyll a and b, respectively (Lichtenthaler, 1987). Moreover, the leaf relative water content was measured based on the method described by Ritchie et al. (1990).

In addition, the fruits harvested per plant was weighed separately and considered as fruit yield. Moreover, quantitative or weight losses of fruits was monitored daily and recorded during postharvest storage at 4°C.

### *Fruit phytochemical and quality indices*

#### • *Fruit TSS, TA and total phenol content*

The total soluble solids (TSS) of the fruits were measured using a refractometer (Atago Co. Ltd., Tokyo; Japan). The fruit titratable acidity (TA) was determined by

sodium hydroxide titration method (Hernandez-Munoz et al., 2008).

Total phenol content was measured by Velioglu et al. (1998) method. Briefly, 0.1 g of fresh fruit tissue (ripped fruit), with 5 mL of 80% methanol containing 1% hydrochloric acid incubated for 2 h at room temperature. Then samples were centrifuged at 10,000 rpm for 10 min. Total phenol measurement has been done by mixing 100  $\mu$ L of the supernatant with 750  $\mu$ L of Folin reagent, and the mixture was stored at room temperature for 5 min. Then 750  $\mu$ L of 6% sodium carbonate was added and after 90 min, absorbance of each sample was read at 725 nm. The total phenol content was calculated using the standard curve of gallic acid. Results were reported in milligrams of gallic acid per gram of fresh weight.

#### • *Vitamin C content*

To measure the vitamin C content of the fruit, 1 g of strawberry fruit was extracted using 5 mL of 10% trichloroacetic acid. The extract was centrifuged at 3500 rpm for 20 min. 0.5 ml of supernatant was removed and 1 ml of 6 mM ditrophenylhydrazine was added and placed in a 37 °C water bath for 3 h. After removing the samples from the water bath, 0.75 mL of 65% cold sulfuric acid was added and allowed to stand in the water bath for 30 min at 30 °C. After cooling the samples to room temperature, their absorbance was read at 520 nm using a spectrophotometer. Ascorbic acid content was measured according to the standard curve prepared with ascorbic acid and expressed as mg 100 g<sup>-1</sup> fresh weight (Omaye et al., 1979).

#### • *Enzymatic antioxidant activity (Superoxide Dismutase (SOD) and Peroxidase (POD))*

The activity of antioxidant enzymes was measured by scrapping 0.5 g of fruit tissue in liquid nitrogen with a 3 mL buffer of 50 mM tris-hydrochloric acid (pH 7) containing 3 mM magnesium chloride, 1

mM EDTA sodium in cold mortar. The resulting homogenates were centrifuged at 4 °C for 10 min at 10,000 rpm (Hermle Z216 MK, Germany). The supernatant was then stored in a freezer at -80°C to measure enzymatic activity (Dhindsa, 1981).

Fruit SOD activity assay was performed based on the chemical shift of nitrobutetrazolium (NBT) according to Beauchamp and Fridovich (1971) method. Reactive mixture consisting of 50 mM sodium phosphate buffer at pH 7.8 (containing 0.17 mM NBT salt, 130 mM methionine, 0.1 mM EDTA, 20 mM riboflavin (riboflavin was added in the last step) ) and 1 mL of extract were placed under two 40-watt lamps at 30 °C for 45 min. After this time, the absorbance changes of each sample were read and recorded by a spectrophotometer at 560 nm. One unit of SOD enzyme activity was considered to be an enzyme that resulted in 50% inhibition of Nitroblue tetrazolium light reduction. SOD activity assay was calculated using the following equation.

$$\% \text{ Inhibition} = \frac{[\Delta A_{560\text{nm}}(\text{Control}) - \Delta A_{560\text{nm}}(\text{Test})]}{\frac{\Delta A_{560\text{nm}}(\text{Control})}{\text{minute}}} \times 100$$

To measure the POD activity, the absorbance of reaction mixture containing 2.5 mL of phosphate buffer (50 mM, pH 7, 1% guaiacol, 1% hydrogen peroxide) and 0.1 mL of fruit extract was read over one min at 420 nm wavelength and the activity was calculated using the following extinction coefficient (Upadhyaya, 1985).

$$\text{Units (mM/min)} = \frac{\frac{\text{doD}}{\text{min (slop)}} * \text{Vol. of assay (0.0001)}}{\text{Extinction coefficient (26.6)}}$$

#### • *Data analysis*

The SAS statistical software (SAS Institute, Cary, NC, USA) and Duncan's multiple range test were used to perform analysis of variance (ANOVA) and means comparisons, respectively.

## Results

### Growth parameters

Leaf area of strawberry plants was significantly affected by osmotic stress, PGRs and osmotic and PGRs interaction (Table 2). Leaf area per plant showed a decreasing trend with increasing of osmotic

stress of the nutrient solution. However, compared to the control, foliar application of PUS and SA, and especially their combined application in the highest stress level (45 mM) improved strawberry leaf area by 34, 50 and 55%, respectively (Fig. 1).

**Table 2. Analysis of variance of the effect of osmotic stress (NaCl), SA and PUS as plant growth regulators (PGR) and their interaction effects on some physiological traits of strawberry 'Selva' in soilless culture.**

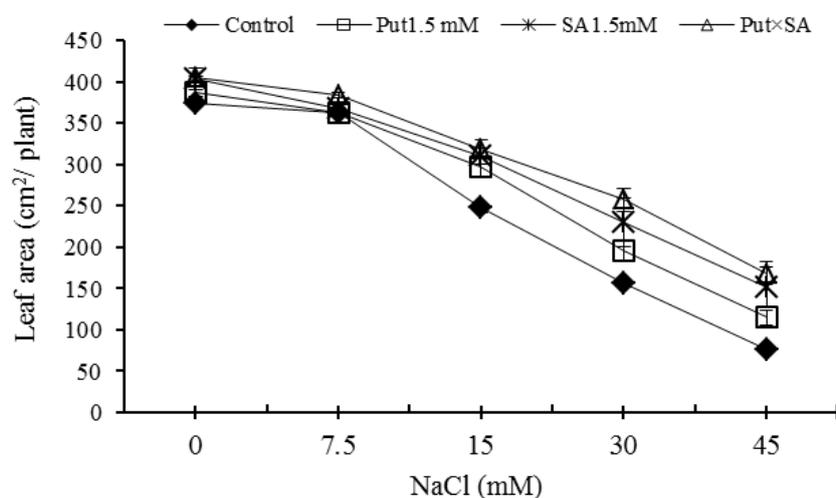
Source of variation	df	Mean Square					
		Leaf area	Total chlorophyll	Fruit yield	RWC	Ion leakage	Superoxide Dismutase
NaCl	4	146896.18 **	4.65 **	28752.50 **	937.85 **	2109.84 **	0.82 **
PGR	3	11478.31 **	2.53 **	3377.11 **	349.05 **	630.98 **	0.069 ns
NaCl×PGR	12	837.19 *	0.03 *	110.98 *	13.58 *	23.68 ns	0.077 *
Total Error	40	416.61	0.016	45.97	5.12	12.24	0.032
CV (%)		7.33	3.86	6.15	3.21	5.85	8.09

ns, \*, and \*\* represent: Non-significant, significant at the 5% and 1% levels probability, respectively. SA: salicylic acid; PUS: putrescine.

**Continued- Table 2. Analysis of variance of the effect of osmotic stress (NaCl), SA and PUS as plant growth regulators (PGR) and their interaction effects on some physiological traits of strawberry 'Selva' in soilless culture.**

Source of variation	df	Mean Square					
		Peroxidase	Soluble carbohydrate	Soluble protein	Proline	Total Phenol	Vitamin C content
NaCl	4	0.49 **	284.26 **	9.64 **	8.18 **	2.81 **	372.96 **
PGR	3	0.41 **	80.30 **	2.97 **	0.52 **	0.76 **	231.30 **
NaCl×PGR	12	0.068 ns	5.45 ns	0.059 ns	0.09 *	0.04 *	11.68 *
Total Error	40	0.035	4.30	0.030	0.05	0.02	5.76
CV (%)		9.69	6.62	3.18	9.19	7.56	6.23

ns, \*, and \*\* represent: Non-significant, significant at the 5% and 1% levels probability, respectively. SA: salicylic acid; PUS: putrescine.



**Fig. 1. Effects of different osmotic potentials of nutrient solution and foliar application of salicylic acid (SA) and putrescine (PUT) on leaf area of strawberry 'Selva'. The values are the means ± SE.**

### Total chlorophyll

The total chlorophyll was significantly affected by the treatments (Table 2). The highest total chlorophyll content (4.27 mg/g fresh weight) was obtained in control (without osmotic stress) and in the combination of SA and PUS. The results showed that at each level of osmotic stress, application of PGRs increased the total chlorophyll content compared to the control. Moreover, the most positive effect was observed in the combination of SA and PUS (Fig. 2).

### Fruit yield

Strawberry fruit yield was significantly affected by osmotic stress, PGRs, and their

interactions. According to the results with increasing osmotic stress, yield of strawberry decreased due to salt stress sensitivity, but SA and PUS foliar application ameliorated the negative effect of osmotic stress and consequently increased yield compared to control or without PGRs foliar application. The highest fruit yield (183.3 g/plant) was obtained in the plants treated with SA and PUS combination and in the NaCl-free treatment. As mentioned, this may be due to the high susceptibility of strawberries to salinity stress as well as the positive effect of growth regulators on improving the growth parameters and consequently on yield (Fig. 3).

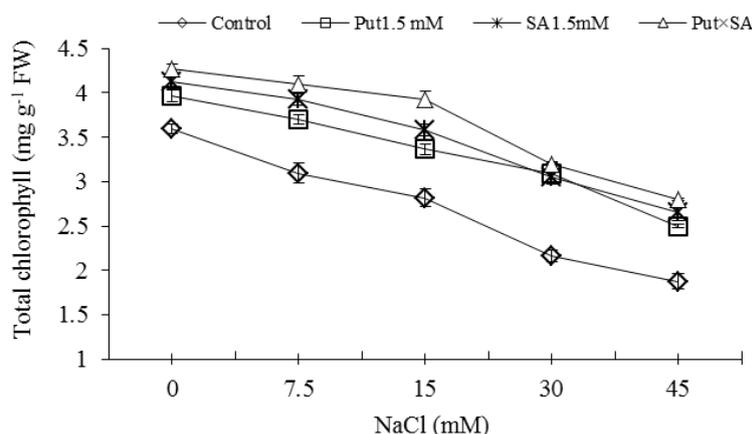


Fig. 2. The role of salicylic acid (SA) and putrescine (PUT) on the total chlorophyll content of strawberry 'Selva' under osmotic stress. The values are the means  $\pm$  SE.

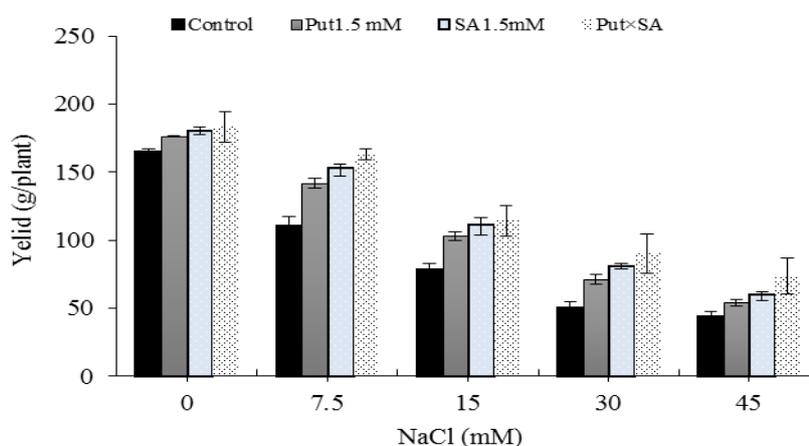


Fig. 3. The role of salicylic acid (SA) and putrescine (PUT) on strawberry fruit yield under osmotic stress induced by NaCl. The values are the means  $\pm$  SE.

**Leaf relative water content**

Increasing the concentration of NaCl in the nutrient solution significantly reduced the relative water content of strawberry leaves, but at each salinity level application of PGRs partially increased the leaf water content (Fig. 4).

**Cell membrane stability**

The leaf cell membrane stability or ion leakage of the strawberry leaf was reduced under osmotic stress and improved with the foliar application of the PGRs. Accordingly, at 45 mM NaCl, the cell membrane stability decreased by 42% compared to the control (Table 3).

**Antioxidant enzyme activity**

Superoxide dismutase (SOD) was significantly affected by salinity, and interaction effects of salinity and PGRs. SOD activity in control plants (without PGRs) was lower than other PGR treatments up to 15 mM NaCl salinity level, while in concentrations of 30 and 45 mM NaCl SOD activity was higher than other plants treated with PGRs (Fig. 5). Among the PGRs treatments, the combination of SA and PUS had a greater role in increasing enzymatic activity than their individual application.

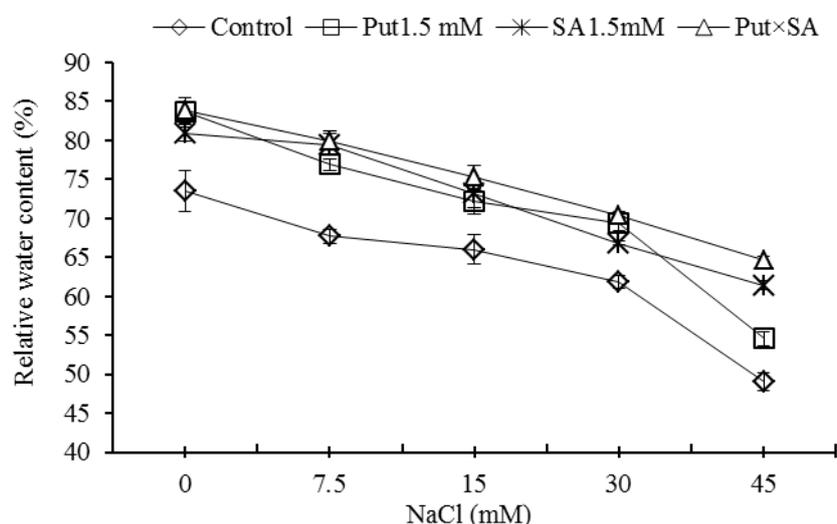
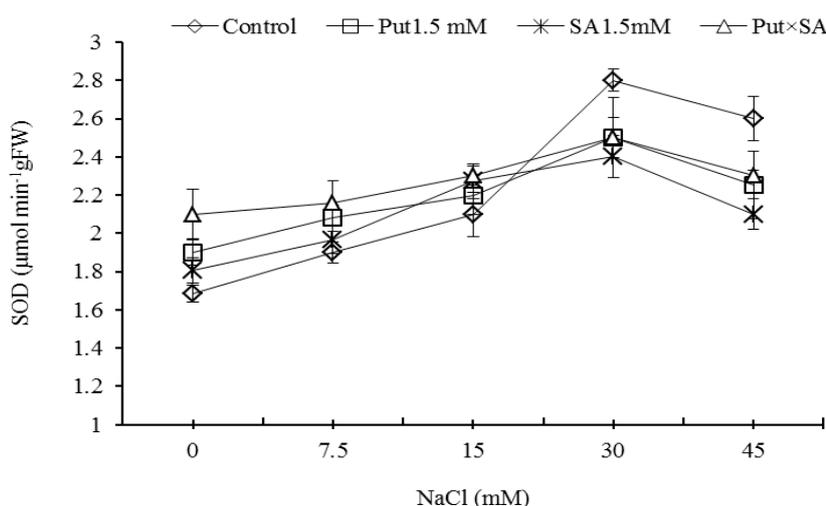


Fig. 4. Changes in relative water content of strawberry 'Selva' leaves treated with osmotic stress and foliar application of salicylic acid (SA) and putrescine (PUT). The values are the means  $\pm$  SE.

Table 3. Mean comparison of osmotic stress (NaCl), salicylic acid (SA) and putrescine (PUS) as plant growth regulators (PGR) on growth, yield and phytochemical attributes of strawberry 'Selva' in soilless culture.

Treatment	Membrane permeability (%)	POD ( $\mu\text{mol min}^{-1} \text{g FW}^{-1}$ )	Soluble carbohydrate ( $\text{mg g}^{-1} \text{FW}$ )	Protein ( $\text{mg g}^{-1} \text{FW}$ )	
NaCl (mM)	Control (0)	42.90 $\pm$ 1.17 <sup>d</sup>	1.64 $\pm$ 0.09 <sup>b</sup>	25.59 $\pm$ 0.97 <sup>c</sup>	5.76 $\pm$ 0.15 <sup>b</sup>
	7.5	49.15 $\pm$ 1.61 <sup>c</sup>	1.85 $\pm$ 0.07 <sup>ab</sup>	27.67 $\pm$ 0.92 <sup>c</sup>	6.30 $\pm$ 0.1 <sup>a</sup>
	15	63.19 $\pm$ 2.69 <sup>b</sup>	1.93 $\pm$ 0.08 <sup>ab</sup>	31.77 $\pm$ 0.63 <sup>b</sup>	5.87 $\pm$ 0.09 <sup>b</sup>
	30	70.34 $\pm$ 2.04 <sup>a</sup>	2.15 $\pm$ 0.04 <sup>a</sup>	37.83 $\pm$ 0.54 <sup>a</sup>	5.25 $\pm$ 0.15 <sup>b</sup>
	45	73.28 $\pm$ 2.19 <sup>a</sup>	2.09 $\pm$ 0.05 <sup>a</sup>	33.77 $\pm$ 1.07 <sup>b</sup>	3.96 $\pm$ 0.14 <sup>c</sup>
PGR (mM)	Control (0)	69.02 $\pm$ 3.97 <sup>a</sup>	1.70 $\pm$ 0.09 <sup>b</sup>	28.10 $\pm$ 1.47 <sup>b</sup>	4.85 $\pm$ 0.24 <sup>c</sup>
	PUS (1.5)	58.34 $\pm$ 3.14 <sup>b</sup>	1.95 $\pm$ 0.05 <sup>ab</sup>	31.78 $\pm$ 1.34 <sup>a</sup>	5.40 $\pm$ 0.22 <sup>b</sup>
	SA (1.5)	57.87 $\pm$ 3.31 <sup>b</sup>	2.02 $\pm$ 0.07 <sup>a</sup>	31.81 $\pm$ 1.14 <sup>a</sup>	5.52 $\pm$ 0.21 <sup>b</sup>
	PUS $\times$ SA	53.86 $\pm$ 2.69 <sup>b</sup>	2.07 $\pm$ 0.06 <sup>a</sup>	33.61 $\pm$ 1.13 <sup>a</sup>	5.93 $\pm$ 0.21 <sup>a</sup>

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



**Fig. 5. Leaf superoxide dismutase activity (SOD) of strawberry 'Selva' treated with osmotic stress and foliar application of salicylic acid (SA) and putrescine (PUT). The values are means  $\pm$  SE.**

The activity of peroxidase (POD) enzymes were affected by osmotic stress and PGRs, whereas the interaction of these two factors had no significant effect on the activity of these enzymes.

The results showed an increasing trend of POD activity with increasing osmotic stress, so that at 30 and 45 mM concentrations there was an increase of 23.72% and 21.53% compared to non-salinized treatment, respectively. (Table 3). POD activity increased by 17% with the use of PGRs, under the influence of SA and combination of SA and PUS (Table 3).

#### ***Soluble carbohydrates***

Soluble carbohydrate was significantly increased by osmotic stress and PGRs application, but the interaction of these two factors had no effect on soluble carbohydrate content (Tables 2 and 3).

#### ***Leaf soluble protein***

The leaf soluble protein content increased with increasing NaCl concentration from 0 to 7.5 mM, but by higher salt (45 mM) exposure, plants showed significantly lower protein content (25%) (Table 3). Moreover, the results showed the increasing effect of SA and PUS on the soluble protein content, while the combination of these two PGRs

had the highest effect on the protein content (Table 3).

#### ***Proline***

Leaf proline content was significantly affected by salinity, PGRs, and interaction effects (Table 2). Comparison of interaction effects showed that leaf proline content in control or non-saline treatment increased with the application of PGRs, but with increasing salinity concentration in the nutrient solution, PGRs foliar application caused higher production of proline in comparison with the control (no osmotic stress). This effect was greater when both SA and PUS were applied together. In total, PGRs alone or together caused accumulation of higher proline under osmotic stress (Fig. 6).

#### ***Phenol content***

Total phenol content showed a significant response to osmotic stress, PGRs, and interaction effects. Based on the interaction effects, with increasing NaCl concentration from 0 to 15 mM in each PGRs treatment, the total phenol content increased; while increasing NaCl level more than 15 mM led to lower phenol content production. In general, the results showed that at each level of osmotic stress, total phenol content increased using PGRs (Fig. 7).

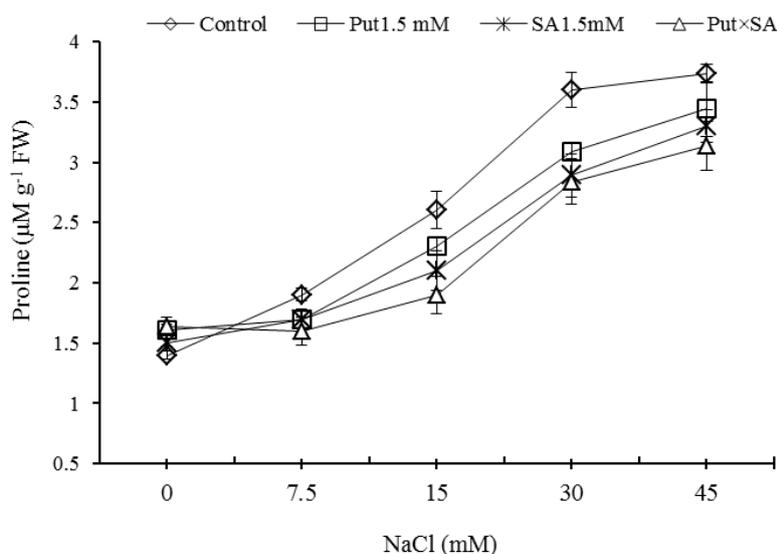


Fig. 6. Effect of osmotic stress and salicylic acid (SA) and putrescine (PUT) on leaf proline content of strawberry 'Selva'. The values are the means  $\pm$  SE.

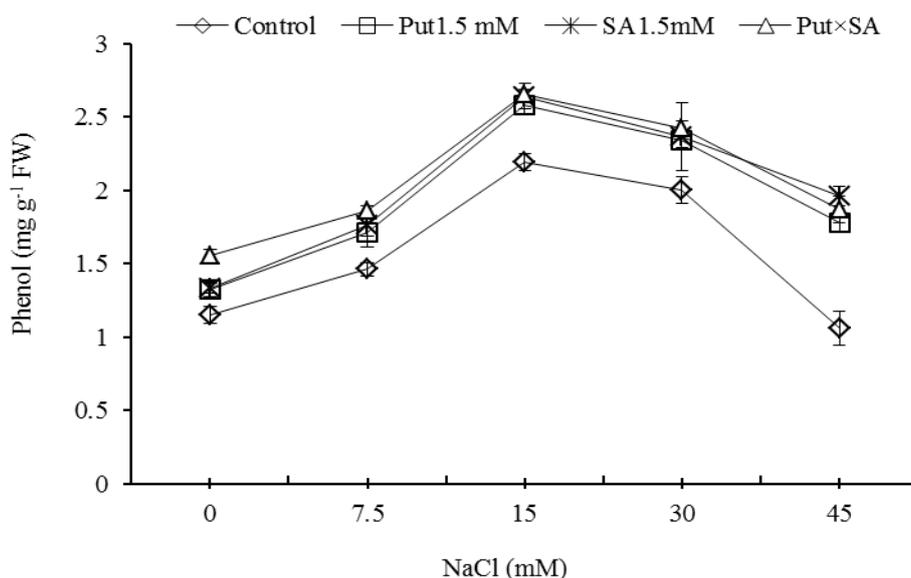
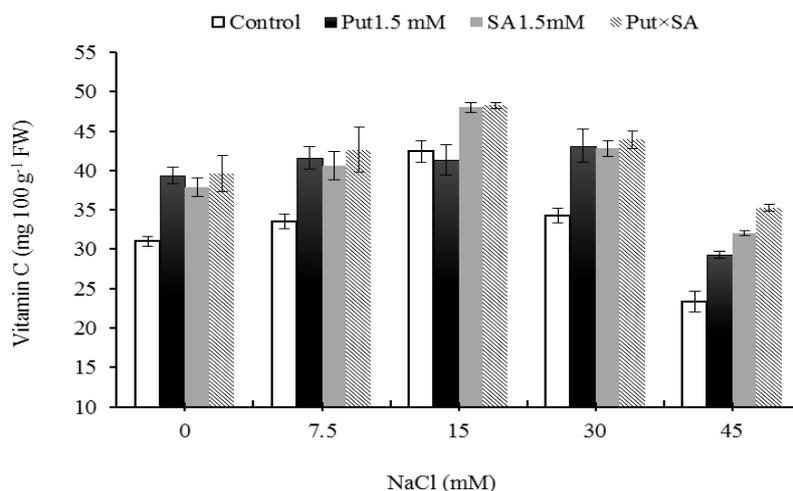


Fig. 7. Changes of total phenol content of strawberry 'Selva' as a result of osmotic stress and foliar application of salicylic acid (SA) and putrescine (PUT). The values are the means  $\pm$  SE.

### Vitamin C content

Vitamin C content of strawberry was significantly influenced by osmotic stress, PGRs, and their interaction effects. Accordingly, in all solutions containing NaCl, foliar application of PGRs increased the vitamin C content of the fruit compared to the control. The highest vitamin C content of fruit (48 mg/g fresh weight) was

obtained in strawberry plants sprayed with PUS plus SA as well as SA treatments. The results showed that combination of PGRs and SA treatments (except PUS), caused higher vitamin C production along with increasing salinity concentration from 0 to 15 mM while increasing of NaCl more than 15 mM, decreased the fruit vitamin C content (Fig. 8).



**Fig. 8. Vitamin C content of strawberry 'Selva' fruit under different osmotic stress and foliar application of salicylic acid (SA) and putrescine (PUT). The values are the means  $\pm$  SE.**

## Discussion

The first response of the plant to osmotic stress is to limit leaf area and to stunted growth especially under intense stress levels (Parida and Das, 2005). By causing negative effects on photosynthetic processes, osmotic pressure regulation, and enzyme activity, osmotic stress decreases plant leaf development. However, PUS and SA have a positive effect in the form of controlling the destructive factors under stress conditions, which result in a higher growth rate, stability of leaf area (Amin et al., 2011; Ghaderi et al., 2015). In this paper, the applied PGRs (SA and PUS) ameliorated the adverse effects of osmotic stress through maintaining leaf RWC. They improved cell division and enlargement, and consequently increased growth and leaf area development of strawberry.

The decrease in chlorophyll content under stress conditions can be due to altered nitrogen metabolism in the biosynthesis of a compatible solute such as proline, which is usually induced by osmotic regulation (De La Rosa-Ibarra and Maiti, 1995). Increased proline production makes glutamate (a precursor of chlorophyll and proline) less involved in the chlorophyll biosynthesis pathway. Application of polyamines as nitrogenated compounds plays an important role in

ameliorating stress conditions, which reduces chlorophyll degradation. Polyamines also have a protective role over chlorophyll and protein and decrease membrane peroxidation (Unal et al., 2007). It seems that PUS has an antioxidant role that results in stabilizing the macromolecules and cell membranes under oxidative stress and consequently protects chlorophyll from stress induced damages. Mahgoub et al. (2011) confirmed the positive and significant effect of PUS on increasing chlorophyll content of dahlia, which is in agreement with the results of this study. Moreover, according to Yildirim et al. (2008), SA significantly increases leaf chlorophyll content in cucumber under the stress induced by NaCl, which is in agreement with the results of this study. The SA appears to have a positive effect on the biosynthesis of cytokinin, which is involved in inhibition of chlorophyll degradation (Bastam et al., 2013).

The decrease in strawberry yield under salinity stress would be due to a decrease in the number and weight of fruits. In addition, osmotic stress by chlorophyll degradation results in leaf chlorosis, leaf area reduction, and photosynthetic depression; thereby, reducing the production of carbohydrates needed for fruit production (Saied et al., 2005;

Aliniaiefard et al., 2016). This is in agreement with the results of the present study about the reduction of chlorophyll content under osmotic stress. The results of the present study showed that with and without osmotic stress, strawberry fruit yield increased by foliar application of PUS, SA, and particularly by combination of PUS and SA. These results are in line with the findings by Movahed et al. (2012), who showed the positive effect of polyamines on growth, flowering, and yield of strawberries produced in the soilless system. According to the Jamali et al. (2013), strawberry fruit yield of 'Pajaro' increases by foliar application of SA, which is in agreement with the results of present study. SA had a significant role in maintaining fruit yield by decreasing the harmful effects of osmotic stress. It seems that SA increased carbohydrate production or yield by increasing chlorophyll content and plant photosynthetic activity.

Studies have shown that the decrease in relative water content of leaves is affected by salinity (Sai Kachout et al., 2011). It seems that the accumulation of salt in the root zone, by reducing osmotic potential, hinders root water uptake and thereby decreases leaf water content (Warrence et al., 2002). The results of present study showed that the application of PUS and SA improved the relative leaf water content under salinity. According to other reports, SA increases the relative water content under salinity stress (Bastam et al., 2013), which is also in line with the results of the present study. The application of SA seems to decrease leaf transpiration by increasing leaf resistance to stress and consequently maintain relative water content of leaves. Moreover, polyamines decrease transpiration by reducing the number and size of stomata under osmotic stress and cause the stability of the cellular water status (Cavuşoglu et al., 2007). Increasing the relative water content under polyamine treatment may also be attributed to plant osmotic regulation and increased proline

content (Duan et al., 2008), which is confirmed by the correlation between polyamine and proline production under osmotic stress (Fig. 5).

Leaf ionic leakage is used to measure membrane permeability and its increase in tissue indicates stress and damage to the cell membrane of living tissue (Farkhondeh et al., 2012). Yildirim et al. (2008) showed that high concentrations of NaCl significantly increase the ionic leakage of cucumber leaves. It has been shown that PUS, as a polyamine, plays an effective role in preventing the destruction and peroxidation of membrane lipids and thereby stabilizing cell (Zhang et al., 2009). The positive role of SA in enhancing membrane stability is reported by other studies as well (Stevens et al., 2006). The reduction of membrane damage due to SA application under osmotic stress could be related to the motivation of antioxidant response or production of other compounds that protect the plant from oxidative damage (Zhao et al., 2008). Considering the role of SA and PUS in plants antioxidant potential under osmotic stress, probably is the reason for the improvement of the membrane stability through increased enzymatic (POD) and non-enzymatic antioxidant (phenol) potential under applied PGRs.

Under stress conditions, enzymatic activities such as SOD, ascorbate peroxidase, and glutathione reductase purify reactive oxygen species (free radicals) (Mishera et al., 2006). The results of the present study showed an increase in POD and SOD activities because of the increase in the NaCl concentration of nutrient solution or osmotic stress. Moreover, PUS foliar application increased the activity of antioxidant enzymes. Polyamines increased plant tolerance to environmental stresses by increasing the activity of antioxidant enzymes (Syed Sarfraz et al., 2011). The PUS reduces the harmful effects of free radicals by activating antioxidant enzymes and increasing the content of antioxidants

(Verma and Mishra, 2005). The antioxidant effect of polyamines is mainly related to their cationic properties, which causes the release of free radicals and inhibits lipid peroxidation. Moreover, SA has the same effects under osmotic conditions. According to Ma et al. (2017), and Shahmoradi et al. (2018) SA significantly increases the activity of antioxidant enzymes under salinity stress, which is in agreement with the results of this study.

Increased soluble carbohydrate content under salt stress has been reported in other studies on plant species such as apple (Liu et al., 2006) and rice (Pattanagule and Thitisakakul, 2008). In other words, the increase of carbohydrates during stress can be due to the accumulation of starch and an increase or change in the amount of glucose, sucrose, and fructose (Yin et al., 2009) as a result of osmotic adjustment under stress conditions. Compared to the control, soluble carbohydrates increased by application of SA and PUS. In support of these results, Samadi et al. (2019) showed higher soluble sugars production in strawberry by foliar application of 100  $\mu$ M SA under osmotic conditions. In the present study, the soluble carbohydrates under PUS foliar application increased significantly, which was previously reported by Abdel Aziz Nahed et al. (2009) in the gladiolus.

The decrease in protein content under salinity stress can be considered as a deleterious effect of sodium, which has a negative effect on the enzymes effective in protein synthesis and results in a decrease in protein content. Moreover, by decreasing nitrogen and ammonium uptake, salinity stress reduces nitrogen and ultimately reduces protein in the plant (Parvaiz and Satvawati, 2008). Researchers have found that the amount of soluble protein is affected by the SA application (Ashraf et al., 2010), which is consistent with the results of this study. The SA is effective in the production of defense proteins and different types of kinases and rubisco (Parvaiz and Satvawati, 2008) and

it can be involved in enhancing protein synthesis by affecting synthetic pathway enzymes or protein breakdown. Polyamines as nitrogenated organic compounds can play an important role in protein production. The increase in protein content under the influence of polyamines seems to be related to the role of these PGRs in eliminating reactive oxygen radicals to reduce protein degradation and as a result enhance protein biosynthesis.

According to the results of this study, leaf proline content increased with an increase in salinity stress. Increasing proline content under salinity conditions indicates the adverse effects of osmotic stress on the plant. This kind of plant reaction to osmotic stress has been reported in leaves of sunroot (Huang et al., 2013). Salinity stress seems to make glutamine, a co-precursor of chlorophyll and proline, less involved in the chlorophyll biosynthesis pathway and more likely to be used in proline synthesis (Mahajan and Tuteja, 2005; Hayat et al., 2010). According to the results, the foliar application of strawberry by SA and PUS may moderate the adverse effects of salinity and osmotic regulation of strawberry plants and consequently reduce the production of this amino acid in the plant. This is in line with the results of other reports on the reduction of proline content under SA treatment (Bastam et al., 2013).

According to the results of this study, phenolic compounds increased with 15 mM NaCl concentration; however, it decreased at concentrations greater than 15 mM. The accumulation of phenolic compounds in plants is a strategy for inhibiting the activity of free radicals and protecting the cell membrane and macromolecules from osmotic stresses that maintain photosynthetic capacity (Xu and Rothstein, 2018). According to the other findings, exposure to osmotic stress also increased the phenolic content of rice crop (Daiponmaka et al., 2010), which is in line with the results of this study. Therefore, the increased total phenol content of strawberry under the foliar

application of SA and PUS can be due to the plant defense mechanism triggered by PGRs in response to osmotic stress. Chen et al. (2006) reported that SA, as a phenolic stimulant compound, enhances phenol production in plants by affecting enzymes involved in the phenolic biosynthesis pathway, which is in line with the results of the present study. Increased levels of phenolic compounds under SA treatment have also been reported for salvia (Dong et al., 2010). Moreover, the PUS increased resistance to oxidative stress, via its antioxidant role, which is associated with an increase in phenolic compounds. The PUS increases the plant's antioxidant capacity, then prevents potential damage to the cell by neutralizing free radicals (Verma and Mishra, 2005). Increased phenolic compounds and activity of strawberry antioxidant enzymes under osmotic stress conditions and under the influence of PGRs indicate the important role of these compounds in the biosynthesis of compatible metabolites, activation of plant protection mechanisms, and mitigation of adverse effects of osmotic stress of nutrient solution.

The results showed that increasing the osmotic stress up to 15 mM resulted in a higher concentrations above 15 mM and less vitamin C content of the fruit. Jamalian et al. (2008) showed that salinity reduces the vitamin C content of strawberry, which is in line with the results of the present study. The decrease in vitamin C content of fruit at high salinity levels can be attributed to the decrease in carbohydrate (sugar) production caused by the decrease in photosynthesis required for vitamin C biosynthesis.

According to the results, at all levels of osmotic stress, fruit vitamin C content was increased by PUS and SA. There are reports on the effect of SA (Javaheri et al., 2012) and PUS (Mishra et al., 2016) on increasing vitamin C content, which confirms the results of this study. The PUS and SA play an important role in modulating the adverse effects of osmotic stress of nutrient solution

by improving plant adaptation responses to abiotic stresses through increased plant antioxidant capacity (vitamin C). However, no further increase in vitamin C content at high salinity levels (30 and 45 mM NaCl) is also likely due to the plant's inability to produce this active compound under extreme stresses. In other words, the gap between antioxidant compounds (vitamin C, total phenol, and etc.) and free radicals at high levels of oxidative stress is one of the most important causes of plant death under extreme stresses.

### Conclusion

Osmotic stress is one of the most limiting abiotic and oxidative stresses in crop production. It is more important in soilless culture conditions due to the initial salinity of irrigation water, as well as adding soluble salts for preparation of fertilizer formulations. Therefore, effective treatments should be used in strawberry production to increase the yield and overcome the harmful effects of salinity. According to the results of the present study, osmotic stress decreased the growth parameters, yield, and quality of strawberry fruit. However, foliar application of SA and PUS reduced the adverse effects of osmotic stress of the nutrient solution and increased plant resistance by producing compatible metabolites and antioxidant compounds (proline, total phenol and vitamin C). The results of this experiment showed that under osmotic conditions, SA, PUS, and especially their combination at 1.5 mM concentration increased the quality (Vitamin C content) and productivity of strawberry fruit, and attenuated the harmful effects of salinity. Based on the results of this experiment, further investigation on foliar application of the above-mentioned PGRs and their use in nutrient solution are needed to facilitate their application in soilless culture.

### Conflict of interest

The authors indicate no conflict of interest for this work.

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