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Research Article

Improvement of growth parameters, flowering, and fruit of tomato under water deficit stress using seed pretreatment with salicylic acid

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Abstract

Water deficit is one of the most important stresses that has limiting effects on growth and crop productivity. One of the best methods for facing drought is seed priming with optimal concentrations of plant growth regulators. Salicylic acid (SA) is an important signal molecule modulating plant response to stress, which enhances tolerance to multiple stresses in plants, including drought. To analyze the effects of various concentrations of salicylic acid on some physiological and morphological traits as well as flower and fruit production of tomato, Riugerand variety, under water deficit conditions, a factorial experiment in a complete randomized design with three replications was carried out in the greenhouse. Six levels of salicylic acid (untreated seeds or control, zero SA or hydro-prim, 500, 1000, 1500, and 2000 mM) and three levels of drought stress (including water holding at field capacity [FC] of pots and water stress at 70% and 50% FC) were used as experimental treatments. Results showed that priming with salicylic acid increased plant height, leaf area, relative water content, and chlorophyll index compared to untreated plants. However, drought stress drastically decreased all these measured traits. 500 mM SA led to an increase in the number of flowers and fruits per plant as compared to other water deficit treatments. Tomato fruit characteristics, including pH, Brix, and EC, were significantly increased by drought stress. However, the application of SA decreased these traits, especially at 500 mM which enhanced the quality of the fruit. In conclusion, seed priming with suitable concentrations of SA (500 to 1000 mM) can be used as an appropriate strategy for the improvement of growth and fruit parameters of tomatoes under water deficit conditions.

Keywords: Drought stress, Greening index, Growth factors, Priming, Seed germination

Introduction

Increasing demand for agricultural products, along with water limitations in most areas of the world as well as Iran, has forced agricultural politicians and programmers to optimize water consumption in agricultural fields. Drought stress, which has the most negative effects on agricultural products, is the most famous landmark for arid and Mediterranean areas resulting from the decrease in water precipitation and pro-harvest irrigation (Rastegar, 1992). Understanding the mechanisms employed by plants in response to drought stress has great value. Drought stress restricts the growth and production of plants as well as crops by decreasing the photosynthetic rate (Liang, *et al*., 2020; Zhang *et al*., 2018). A major factor that is responsible for decreased photosynthesis could be stomatal closure and decreased CO_2 assimilation (Varone *et al*., 2012). The rate of photosynthesis is also negatively affected by drought stress in plants, which leads to a decrease of yield production in crop plants (Ghotbi *et al*., 2014). The effects of these factors on plants under drought stress are highly dependent on the kinds of treatments used in an experiment, as well as the species, cultivar, age, and developmental stages of the plants being studied (Liang *et al*., 2020). Accumulation of osmotic substances, changes in membrane lipid composition, scavenging of reactive oxygen species (ROS), and regulation of protein induction and hormonal balance are some general responses of plants to cope with drought stress (Cuming *et al*., 2007).

Under drought conditions, one of the best ways to increase seed germination is seed pretreatment or priming (Demir Kaya *et al*., 2006). Priming includes the moistening of seeds in water, osmotic solutes with matric potential, growth regulators, and micronutrient solutions. This method, especially in disfavored

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conditions, increases the percent, rate, and uniformity of seed germination and greening indices of plants at a wide range of high temperatures and drought conditions. Since the primed seeds have a great potential tolerance against plasmolyzation, they can improve the quantity and quality of plant products, specifically under drought conditions (Reed, 2007).

One of the most commonly used growth regulators for seed priming is salicylic acid (SA) which can affect many physiological processes in plant cells. It is also involved in regulating many growth and development processes such as stomata movements, seed germination, ion assimilation, and the polarity of plant embryos (Zhang *et al*., 2003). SA plays an important role as a non-enzymatic antioxidant substance in regulating physiological processes. It is also involved in plant defense responses against drought, cold, and heat stresses (Arfan *et al*., 2007). Regulatory roles in the activity of antioxidant enzymes and quenching of reactive oxygen species (ROS) are being considered as some molecular mechanisms that have been proposed for SA (Shi and Zhu, 2008).

Tomato (*Lycopersicum esculentum*) from the *Solanaceae* family, has occupied second place in the crop-cultured area and consumption scales in Iran's farmlands. Tomatoes contain high fiber and lycopene content without any cholesterol and have great value in the human diet and health. Lycopene is among the most important antioxidant substances, which can protect cells from free radical damage and also prevent cancer in humans (Puah *et al*., 2021). However, low water availability and precipitation remain a big threat to tomato cultivation, especially at the seed germination stage. Some photosynthetic indices, such as light saturation point, light saturated net photosynthetic rate, light compensation point and some other factors, were measured in tomato plants under different soil drought stress conditions. It was shown that all the above indices declined in tomato plants under drought stress treatments (Liang *et al*., 2020).

The effects of drought stress on seed yield, seed quality and the growth of tomato plants were analyzed under greenhouse conditions. Results showed that drought stress did not have a significant effect on the total number of seeds and seed weight on plants (Pervez *et al*., 2009). To investigate the effects of drought stress on 17 race lands of tomato in Iran, a screening method was carried out using poly-phasic fluorescence transient, relative water content (RWC), electrolyte leakage (EL) and vegetative growth parameters based on Duncan’s multiple range test (DMRT) for all the studied parameters and drought factor index (DFI) (Sousaraei, *et al*., 2021).

As drought stress negatively affects seed germination and the growth rate of tomatoes at the field level, finding new strategies for the improvement of plant tolerance against drought stress seems to be more essential. In the present study, to improve the drought stress tolerance of tomatoes, different concentrations of SA were used for seed priming at the germination stage. In many previous studies in this field, only the growth or physiological parameters have been analyzed, however, in this study project, we have analyzed plant yield, flower and fruit parameters in addition to growth and physiological characteristics. The effects of SA on plant growth parameters, physiological characteristics, and flower and fruit indices of tomatoes under water deficit conditions were investigated to determine the most efficient SA concentration for alleviating the negative impacts of drought stress on tomatoes.

Materials and methods

In order to analyze the effects of SA priming on some physiological and morphological characteristics of tomatoes under water deficit, a factorial experiment was performed at a completely randomized design with three replications in greenhouse conditions. Two kinds of treatments were applied in this experiment. Water deficit treatments at three levels include soil water content at field capacity (FC) as control, 70% of FC, and 50% of FC. Other treatments regarding different concentrations of SA for seed pretreatments were hydro-prime (untreated seeds) as a control, 0, 500, 1000, 1500, and 2000 mM. For seed pretreatment with SA. Seeds of tomato (Riogrand variety) were subjected to different concentrations of SA for 24 hours. Seeds were desiccated at room temperature for 48 hours. After sterilization using vita wax as a fungicide, seeds were cultured in plastic pots at the greenhouse. 2.5-liter capacity pots were filled with soil, which was a mixture of clay, sand, and humus (2, 1, and 1 respectively). Totally, 54 pots were used and in each pot, 10 seeds at 2-3 cm depth from soil level were cultured and finally, three uniform seedlings remained alive. Pots were being irrigated at the three-day interval. Two months after growth, physiological traits were measured. The gravimetric method was applied to the determination of the field capacity in each pot (Ehyaei, and Behbehanizade, 1993).

Statistical design and data analysis: A factorial experiment was performed in a completely randomized design with three replications for each treatment. The collected data was analyzed using MSTATC and SPSS 18 as statistical software. For comparing data averages, Duncan's method was used at 1% or 5% significance. The drawing of charts and graphs was carried out using Excel software.

Measurement of growth parameters, shoot length, leaf number, leaf area, and chlorophyll index: Shoot length was measured from the cotyledonal zone to the top of the plant and the average length of all plants in each pot was considered for shoot length. The number of leaves was calculated based on the average number of leaves per plant in each pot. A leaf area meter instrument was used for measuring the leaf area. The chlorophyll content index was measured using a Manual chlorophyll meter (CCM-200).

Relative water content: Three leaves from three plants in each pot were removed, then their fresh weights were rapidly measured, after which the leaves were kept in distilled water at room temperature for 24 hours. Then the leaves were dried out with tissue paper and their saturated weight was measured carefully. The dry weight of the leaves was measured after keeping them at 70 ˚C in the oven for 48 hours. The RWC was calculated as follows:

RWC = (Fresh weight – Dry weight) / (Saturated weight – Dry weight) × 100

Number of flowers: After the beginning of flowering, the number of flowers at each pot was counted at each three-day interval (No. of flowers 1 to 4 in Table 2) until no new flowers were produced. The average number of flowers from three plants in each pot was considered as the number of flowers for each treatment.

Weight of fruits at each treatment: Red fruits were harvested from each plant and weighted rapidly. This process was continued to the end of the experiment, when there was not any new fruit production. As there were three pods at each pot the average weight of fruits of three pods was considered the fruit weight for each treatment.

Measurement of the quality of fruits: For this purpose, the fruit juices were extracted from harvested fruits. This juice was used for analyzing Brix (total soluble solids of Juice) and electric conductance (EC) as the main indices of fruit quality.

Measurement of EC: Measuring soluble minerals of fruit juice is the main goal for measuring EC which was done by injecting fruit juice into an EC meter instrument and calculated using a modified method of Ehyaei and Behbahanizade (1993).

Measuring of brix: A refractometer instrument was used for measuring Brix. Two drops of fruit juice were put on a sensitive glass of the instrument. After closing the protective cap the amount of Brix was read.

Results and discussion

Leaf area: Water deficit and salicylic acid pretreatment of seeds had a significant effect on leaf area at 1% significant level (Table 1). No salicylic acid treatment (hydroprime treatment) resulted in the highest effect on leaf area under normal conditions, with a 33.33% increase in comparison with control. Leaf area decreased at 70% and 50% of field capacity treatments as compared to normal conditions. The highest amount of leaf area at two water deficit treatments was observed in 500 mM SA pre-treated seeds (Figure 1) which had no statistical difference to 1000 mM SA at this water deficit treatment. The decrease of cellular turgor under water deficit conditions, which consequently decreases cell division rate in leaves, has been mentioned as an important cause of the decrease in leaf area (Nielson and Nielson, 1998). It is suggested that priming of seeds with SA increases the rate of both seed germination and the growth of seedlings in such a way that these plantlets establish very quickly in the soil compared to unprimed seeds. Thus, the primed seeds have more time to use minerals and water to grow and extend their leaf area. More leaf area conservation under drought stress has great value for taking more CO_2 for photosynthesis (Porwanto, 2003).

Height of plantlets: The maximum plant height was achieved from seeds that had been pretreated with 500 or 1000 mM SA. Although this difference was not statistically significant among SA treatments at 100% FC (no drought stress treatments). At 500 mM SA, the plant height increased 35.4% more than other SA treatments at average (50% FC) and severe (50% FC) drought stresses (Figure 2). The difference between 500 and 1000 mM SA was not significant. It has been reported that SA increased the cell division rate in the meristem of wheat plantlets which improved plant growth (Shakirova *et al*., 2003). It is likely that in our experiment, SA had the same effect on tomato plants by increasing their height. It has been shown that SA is involved in the regulation of important plant physiological processes such as photosynthesis, nitrogen metabolism, proline (Pro) metabolism, production of glycinebetaine (GB), antioxidant defense system, and plant-water relations under stress conditions and thereby provides protection in plants against abiotic stresses (Khan *et al*., 2015), so it can confer noticeable resistance against drought tolerance, which leads to prevent decrease in plant height in SA treated plants. Exogenously sourced SA applied to stress plants, either through seed soaking, adding to the nutrient solution, irrigating, or spraying, was reported to induce major abiotic stress tolerance-mechanisms (Anwar *et al*., 2013). Additionally, SA causes the accumulation of abscisic acid (ABA) and also prevents a decrease in indol 3- acetic acid (IAA) and cytokinin content, which subsequently reduces the inhibitory effects of drought stress on plant growth (Prabha and Negi, 2014).

Chlorophyll index: There was a significant difference between treatments in the chlorophyll index (Figure 3). Seeds that had been primed by 500 mM SA at the control and water deficit stress conditions, had the highest chlorophyll index. Under normal conditions, the 2000 mM SA treatment showed the lowest chlorophyll index. Reduction of specific lamellar proteins (light harvesting protein chlorophyll a/b) at drought stress conditions (Alberte and Thorner, 1997), increasing chlorophyllase (Majumdar *et al*., 1991) and peroxidase (Ashraf *et al*., 1994) enzyme activity have been mentioned as important factors that are involved in decreasing chlorophyll at water stress conditions, which corroborate our findings. Consistent with our results, the reduction of leaf chlorophyll under drought stress was improved by using SA in wheat (Singh and Usha, 2003). Sobhani-Jahani (2011) reported that despite the reduction in chlorophyll content under drought stress in tomatoes, SA application increased the chlorophyll index of leaves under both non-stress and stress conditions.

Relative water content (RWC): Seed pretreatment

Table 1. Analysis of variances of some physiological and morphological traits of tomato, var: Riogrand, under water deficit stress and seed pretreatment with salicylic acid at seedling stage

Source of variation	Degree of freedom	Average of squares						
		Leaf area	Plant height	Leaf No.	1 leaf FW	RWC	Chl. Index	Stomata area
Salicylic acid	5	275.55**	21.98**	5. 89**	3. 52**	39.92**	643.97**	167.68**
Water deficit	2	876.82**	159.12**	3. 35**	4. 39**	647.60**	470.09**	1713.91**
Interaction effects	10	23. 72**	1.66 ns	0. 55 ns	0. 265 ns	6. 36ns	63.01**	19.63**
Experimental error	36	5. 72	1.85	0. 35	0. 373	4. 05	2.52	2.21
Coefficient of variations	-	5. 66	11.81	12. 51	16.77	2.53	3	2.28

ns: not significant, * P<0.05, ** P<0.01. Abbreviations: FW: Fresh Weight, RWC: Relative Water Content, Chl: Chlorophyll

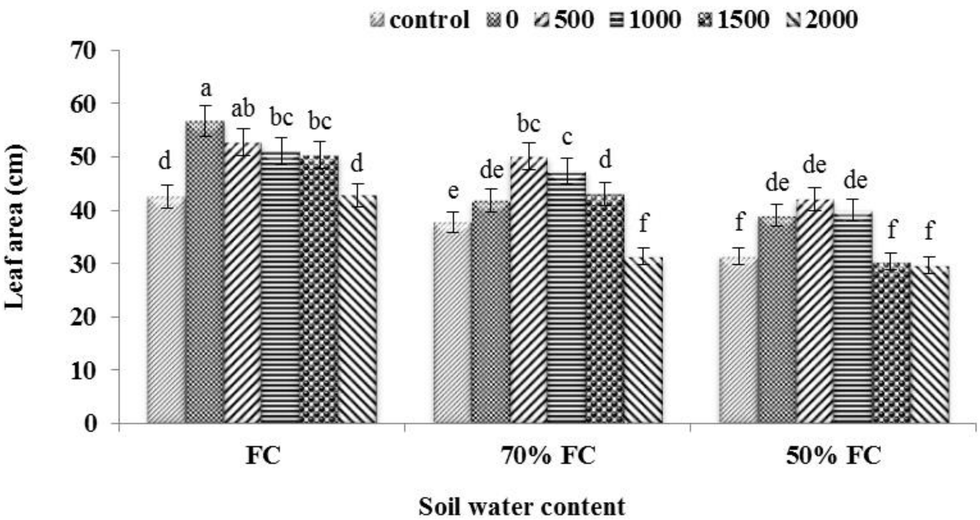

Fig. 1. The intraction effect of water deficit stress and SA pretreatment on leaf area of tomato. Different letters show statistical significant difference at 5% level according to Duncan test.

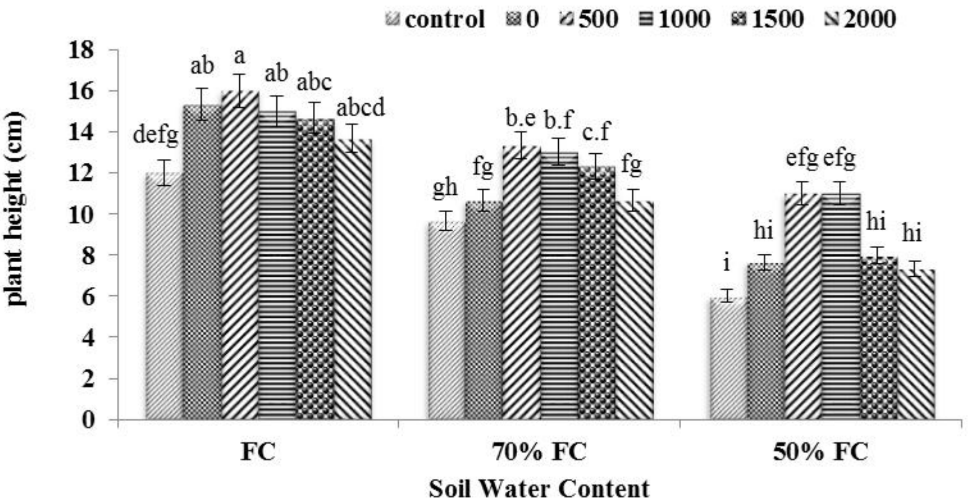

Fig. 2. The intraction effect of water deficit stress and SA pretreatment on the height of tomato plants. Different letters show statistical significant difference at 5% level according to Duncan test.

by SA and water deficit treatments showed a significant effect on RWC (Table 1). The highest amount of RWC was achieved at 500 mM SA (Figure 4).

There is a close relationship between the turgor pressure of cells and RWC in plants. It means the more reduction of water potential in plants, the more reduction of cellular turgor. The greater the dependency of changes in cellular turgor of a plant to water

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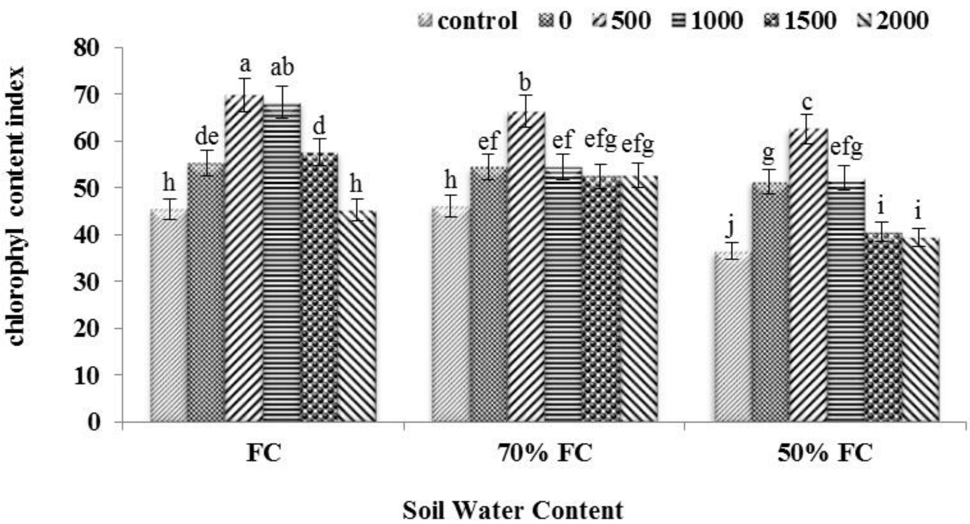

Fig. 3. The intraction effect of water deficit stress and SA pretreatment on leaf chlorophyll index of tomato. Different letters show statistical significant difference.

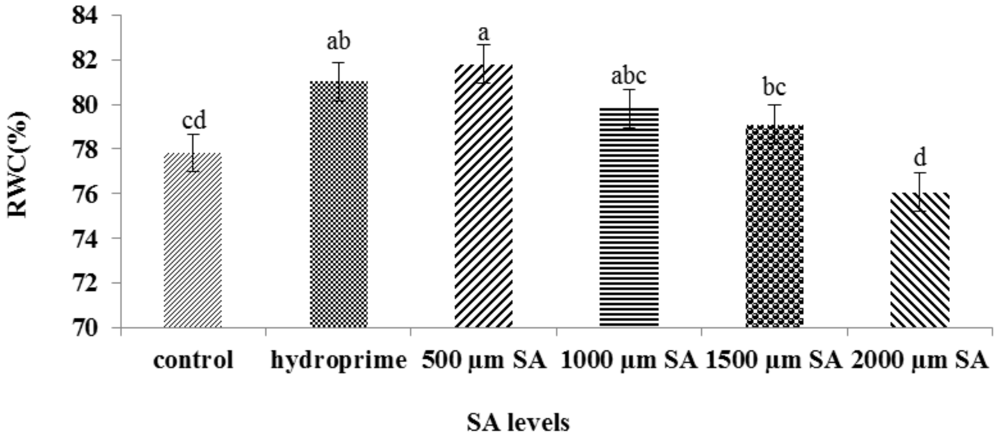

Fig. 4. The effect of SA pretreatment on the relative water content of tomato under different water conditions. Different letters show statistical significant difference at 5% level according to Duncan test.

potential, the more sensitive that plant to drought stress. Under drought and salt stress conditions, the low water potential of soil solutions leads to decreasing water potential in plant tissue, which subsequently leads to a drop in turgor pressure in plant cells (Bagheri *et al*., 2001).

The yield of flowers, fruit, and related traits, number of flowers: The effects of different levels of water deficit stress, SA pretreatment, and their interaction effects on the number of flowers were significant at all four counting stages (Table 2). The highest number of flowers was observed in unstressed conditions at all four counting stages. There was a reduction in the number of flowers by increasing the level of water deficit stress (Figure 5). Priming with SA caused an increase in the number of flowers under both stress and normal conditions. The maximum number of flowers per pod was observed at 500 mM SA under normal and water deficit stress conditions (Figure 5). A decreasing trend was observed in the number of flowers per pod by increasing SA priming levels. The number of flower bunches is an important component of tomato fruit yield, which is usually used for estimating tomato yield. Tomatoes are very sensitive to water deficits, especially at flowering and fruit development stages and terminal drought causes the abscission of flowers and fruits (Akbari Nodehi *et al*., 2013). Although pollen grain germination and flower fertility decreased under water deficit stress in our experiment, pretreatment by SA increased these traits. For this reason, seed pretreatment by SA can neutralize the negative effects of drought stress under water deficit conditions. Szepesi (2005) also reported that a 0.5 mM SA application can increase pollen grain germination and flower fertility in plants under drought stress.

Fruit yield: Two levels of water deficit stresses and seed pretreatment by SA had a significant effect on tomato fruit yield (Table 2). The fruit yield decreased with increasing drought stress levels. The least amount of fruit yield was observed at 50% FC treatment. Seed pretreatment by SA resulted in a significant increase in fruit yield. The highest amount of fruit yield was observed under normal conditions, which showed a 113.2% increase in comparison to the control (hydro-

Table 2. Analysis of variances of fruit yield of tomato and related traits under water deficit stress and seed pretreatment with salicylic acid

Source of variation	Degree of Freedom	Average of squares				
		Flower No. 1	Flower No. 2	Flower No. 3	Flower No. 4	Fruit yield
Salicylic acid	5	66.1**	104.47**	298.6**	272.24**	259.28**
Water deficit	2	56.79**	206.24**	356.01**	333.68**	136.09**
Interaction effects	10	2.92ns	8.59**	16.19**	8.01**	641.49**
Experimental error	36	1.98	2.18	3.44	2.77	1.83
Coefficient of variation	-	27.05	15.53	13.84	9.92	3.89

ns: not significant * P<0.05 ** P<0.01.

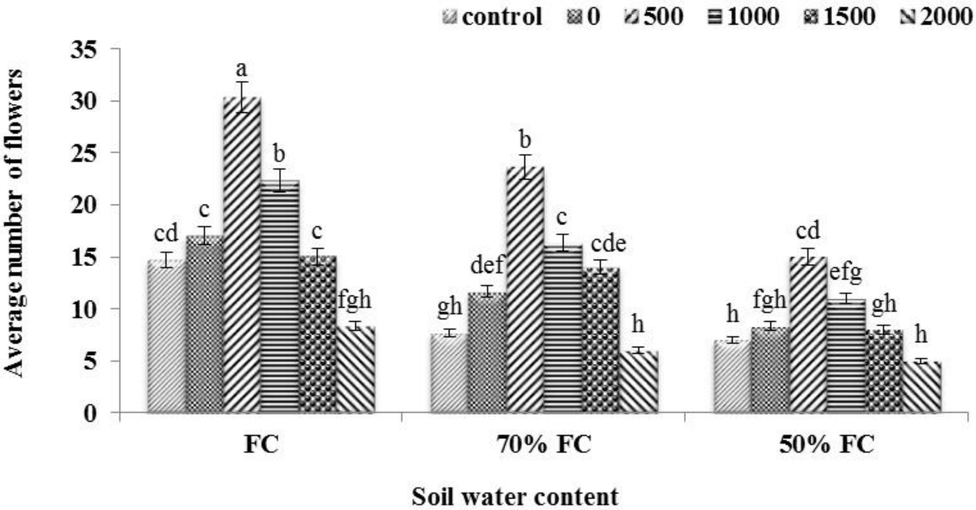

Fig. 5. The intraction effect of water deficit stress and SA pretreatment on a number of flowers in tomato. Different letters show statistical significant difference at 5% level according to Duncan test.

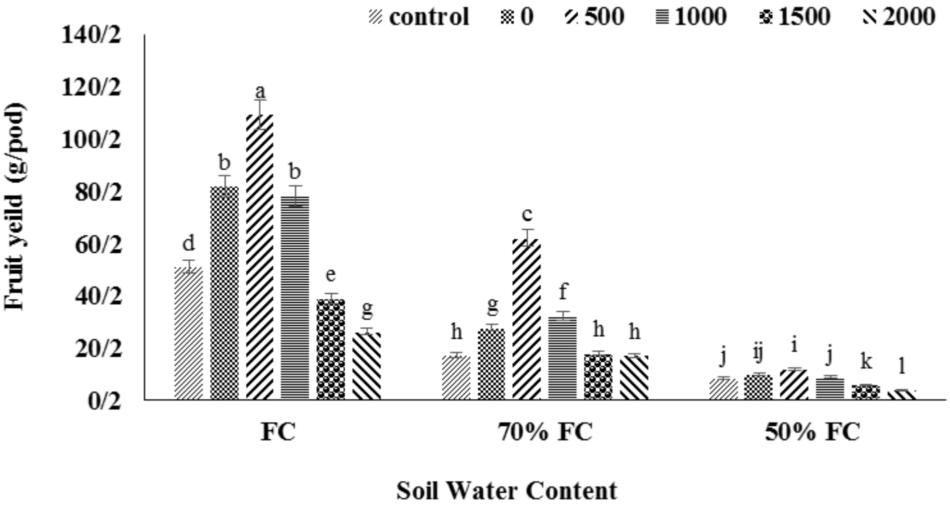

Fig. 6. The interaction effect of water deficit stress and SA pretreatment on the fruit yield of tomatoes. Different letters show statistically significant differences at the 5% level, according to the Duncan test.

primed seeds). 500 mM SA treatment also had the highest fruit yield at 50% and 70% FC treatments (Figure 6), although at 50% FC there was no statistical difference between 500 and 0 mM SA treatments. The sensitivity of tomatoes to drought stress, which causes a decrease in photosynthesis and a reduction in sugar transport towards fruits, has been mentioned as an important cause of the decrease in tomato fruit yield under drought stress (Lutforrahman *et al*., 1998). It has been reported that fruit yield is significantly affected by water stress, and the varieties with small or average fruit sizes were more resistant than other varieties. It has been well documented that a 50% reduction in the water supply of tomato plants resulted in a 44.7% decrease in

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Table 3. Analysis of variances in the quality properties of tomatoes under water deficit stress and seed pretreatment with salicylic acid

Source of variation	Degree of Freedom	Average of squares	
		Brix	EC
Salicylic acid	5	0.392**	2.383**
Water deficit	2	24.05**	63.268**
Interaction effects	10	0.045**	0.181*
Experimental error	36	0.01	0.079
Coefficient of variation	-	2.01	3.537

ns: not significant, * P<0.05, ** P<0.01. Abbreviations: EC: Electrical Conductivity

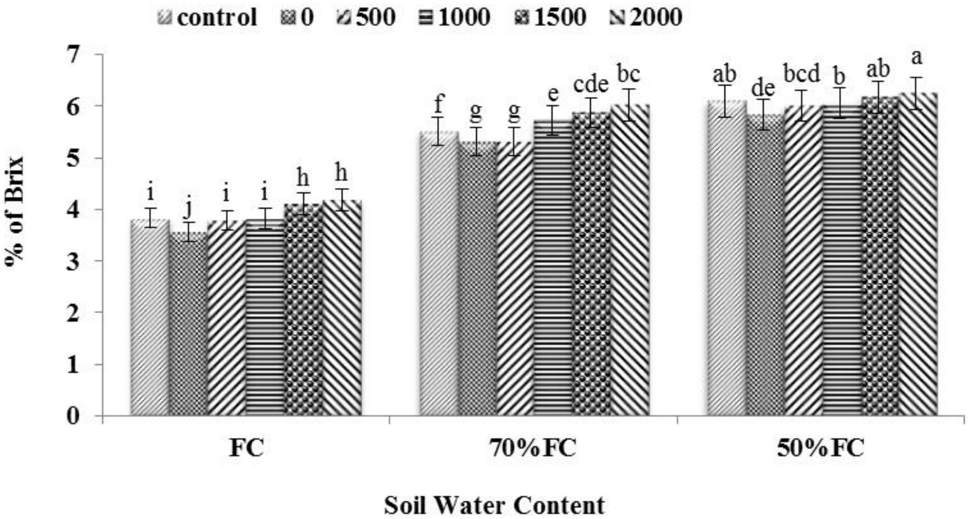

Fig. 7. The interaction effect of water deficit stress and SA pretreatment on the Brix of tomato fruit juice. Different letters show statistically significant differences at the 5% level, according to the Duncan test.

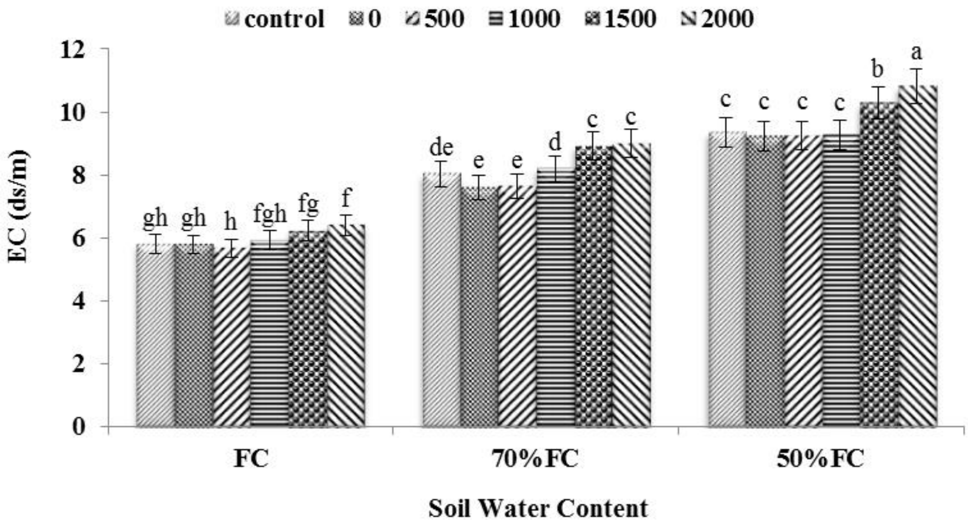

Fig. 8. The interaction effect of water deficit stress and SA pretreatment on EC of tomato fruit juice. Different letters show statistically significant differences at the 5% level, according to the Duncan test.

fruit yield (Akbari Nodehi *et al*., 2013). Our results strongly indicate that the fruit yield of tomatoes has been negatively affected by water deficit stress.

The quality properties of tomato, Brix of tomato fruit juice: Seed pretreatment by SA, water deficit treatments, and their interactions significantly affected the brix of tomato fruit juice (Table 3). In our research, drought stress led to an increase in the Brix of tomato fruit juice, which decreases the quality of fruits. Seed pretreatment by SA caused a decrease in the Brix of tomato fruits, and the increasing trend that was observed in drought stress treatments was inhibited by SA pretreatment levels, especially at 500 mM (Figure 7). Although there were no statistical differences between

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1000 and 1500 and also between 1500 and 2000 mM SA. The highest amount of brix (6.25%) was observed at 2000 and 1500 mM SA under 50% FC treatment. The lowest Brix (3.56%) was also achieved from hydro-prime treatment under normal conditions (Figure 7). Brix of fruit indicates the number of soluble solids in fruit juice. When the water absorbance decreases in roots, the percentage of minerals increases in the xylem stream, which results in an increase in soluble solids (Brix) in fruits. Drought stress decreases the water uptake by roots, which leads to a decrease in water in fruit juice, and this can increase the concentration of soluble solids in fruits and Brix. Increasing the fruit pH and Brix was also reported in tomatoes under progressive water stress (Mousavi Fazl and Mohamadi, 2005).

Electrical conductivity (EC) of tomato fruit juice: Different levels of SA priming and water deficit stress and their interaction effects had significant effects on the EC of tomato fruit juice (Table 3). The EC of tomato juice was increased by increasing water deficit stress. However, priming seeds with 500 mM SA decreased the EC of fruit juice. Increasing SA concentration above 500 mM led to an increase in EC under both normal and stress conditions (Figure 8). The least amount of EC of tomato juice (5.68 dsm^{-1}) was observed at 500 mM SA under normal conditions, but the highest amount (10.82 dsm^{-1} was achieved at 2000 mM SA at 50% FC. It has been reported that low water potential has an important effect on the EC of tomato fruit juice (Cuartero and Fernandez-Munoz, 1999). Priming seeds with 500 mM SA causes an increase in water uptake by roots, which can increase the water potential in fruit, which subsequently decreases the EC of fruit juice. It has been mentioned previously that salt stress increased the EC of tomato fruit juice, but SA decreased it under salt stress (Sobhani-Jahani, 2011); however, the involved mechanisms have not been determined yet.

Conclusion

Based on the results of this research, it can be concluded that priming of seeds by SA can be used as a practical strategy to improve growth, flowering, and fruit quality (Brix and EC) indexes in tomatoes under water deficit conditions. Drought stress decreased the fruit yield and quality of tomatoes in this experiment. However, the application of SA improved these traits in tomato plants under drought stress. The maximum yield of tomatoes was achieved from 500 and 1000 mM SA by treatment under normal conditions; however, application of SA above 1000 mM SA had negative effects on tomato plants. Therefore, high concentrations of SA are not advised. According to the results of this research, SA caused a better rate of vegetation indices and also green indices in treated plants, so SA treated plants get more advantages from this accelerated seed vegetation rate in future plant growth and yield. These plants would be strong enough to cope with drought stress. However, as we did not apply the concentration between 500 and 1000 mM of SA, the application of SA at this range is proposed for future experiments.

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