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Morphological Changes of Salicylic Acid Application on Pepper (*Capsicum annuum* L.) Seedling under Cold Condition

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Abstract: This project was developed to investigate the contribution of salicylic acid (SA) to the development of pepper seedlings grown in low temperature (0 ^{0}C) conditions. The research was carried out in the controlled plant growing cabinet in the research- investigation area of the Department of Horticulture, Faculty of Agriculture, Siirt University. As a vegetable material, Urartu F1 pepper type (capia) which is used in greenhouse cultivation has been used. As a dose of different salicylic acid; 0.01 and 0.05 mmol doses were applied. The dose of 0 mmol salicylic acid was used as a control group. Application frequency; It was applied 1 time, 2 times and 3 times.3 different cold application times were also investigated; 24 hours, 48 hours and 72 hours. The experiment was designed in randomized plots and 3 replications. In the pepper seedlings Rate of Lost Seedling Weight (ROLSW) and Rate of Lost Seedling Length (ROLSL) were investigated. At the end of the research; Both SA applications increased the ROLSW rate according to the control. The application of 0.01 ppm was the SA application with the highest ROLSW rate. The application of 0.01 ppm SA also increased the ROLSL rate compared to the control. The highest ROLSW and ROLSL rates were obtained from 24-hour cold application. There was no statistically significant difference between the frequencies of application.

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1. INTRODUCTION

Temperature is a major factor in abiotic stress and to determine agricultural productivity and crop productivity. The rate is reduced and the amount of absorption of water and nutrients from cold stress, leading to cell drying and starvation and called extreme forms of cold stress stresses frozen and cause the formation of ice in the cell fluid, which leads to dehydration and death in plants. The low temperature (LT) is the environmental stress that affects crop production and quality. Regulates the expression of several proteins, metabolites and many genes [1].

Pepper plants are initially from tropic areas and require high-temperature conditions for their advancement. Subsequently, the ideal development temperature is in the vicinity of 25 and 30 °C, such that temperature changes influence an assortment of physiological capacities and

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morphological improvement. At the point when temperature diminishes underneath 15 °C, pepper development is decreased, and sprout and organic product generation stop [2].

Salicylic acid's activities incorporate practicing a thermogenic impact [3], expanding thermotolerance [4], empowering extrinsic root arrangement [5], demonstrating herbicides impact [6], lessening leaf shed [7], giving protection against pathogens [8], manages ethylene biosynthesis [9-10] and changing the quality and amount of proteins [11]. It has been asserted [12] that SA and comparative phenolic mixes practice their impact of giving protection against various anxiety factors in plants corrosive [13] and cytokinins [12]. These perceptions and reports on numerous other physiological impacts achieved by SA conjured in a few scientists this substance may be another plant development controller [14]. The experiment was designed to study the effect of different concentrations of salicylic acid on resistance to low temperature. The aim of this study to find out the answer to these questions; which morphological changes occur at different low-temperature periods. And just how do doses of salicylic acid effect at cold condition on pepper seedlings' morphological features?

2. MATERIAL and METHODS

This study was carried out in the horticulture department, agriculture faculty of Siirt University in 2018. Siirt is a province and located between the 41˚- 57˚ East longitude and 37˚ -55˚ northern latitudes in as its geographical location. In Siirt province, it dominates the continental climate which is the most important feature of the four seasons, continental climate prevails. The summers are hot and arid, with no precipitation in June and October [15].

2.1. Plant Materials

A capia pepper variety (Urartu F1) was used for plant material. This pepper variety could be grown at the cool climate in the greenhouse. Pepper seedlings were grown at a growing chamber.

2.2. Methods

0.01 mmol, 0.05 mmol, and 0 mmol doses of salicylic acid were applicated before applying cold stress. For control groups only distilled water was applicated each time. After 0 ^{0}C temperature application; did cold application create a non-irreversible wilting to seedlings, do all seedlings' have cold damage on their leaves and stems? Are there differences in seedlings weight and lengths? Also, salicylic acid was 1, 2 and 3 times applicated for each dose. Polysorbate [16] (Twin 20) was also used enough quantity for adhesive. For easier penetration of salicylic acid thin holes in the leaves were made by small needles.

2.2.1. Seedling Weight (gr)

Before and after cold application, 5 randomly seedlings per application were selected and then measured by scale sensitive to 0.01 g (Figure 1).

Figure 1. Seedling weight (before and after) application

2.2.2. Seedling length (cm)

5 seedlings per applications were randomly selected. The distance from the soil surface to the seedling's top was measured for each seedling by a ruler. All measurements were performed before and after the cold application (Figure 2).

Figure 2. Seedling length (before and after) application

2.2.3. Experimental Design

The experimental design used was a Randomized Complete Parcel Design (RCPD) with factorial. The treatment in each experiment had three replicates. Where was statistically analyze the data and compared means using "LSD"s Multiple Range Test at 0.05 and 0.01 levels.

3. RESULTS and DISCUSSION

The differences between the doses to Least Square (Sq.) Mean was significant. Least Sq. Mean of ROLSW ranged between -46.0 – - 63.1 percent. The average Least Sq. Mean of doses was -52.0 %. The highest Least Sq. Mean value of ROLSW was obtained from 0.01mmol dose as -46.0 %, and the lowest ROLSW's Mean value was obtained from the control concentration of salicylic acid as -63.1 %. The highest Least Sq. Mean value was obtained from frequency 1 as -47.8 %, and the lowest Least Sq. Mean value was obtained from frequency 2 as -54.3 %. The highest Mean of doses value of ROLSL was determined from 0.01 mmol dose as -52.0 % (Table 1).

Table 1. Effect of Doses, Time and Application Frequency of Salicylic Acid

	Doses (ppm)			Mean	Time (hour)			Mean	Frequency (times)			Mean
	0.01	0.05	Con.		24	48	72		1	2	3	
ROLSW	-46.0 a	-47.0 a	-63.1 b	-52.0	-13.8 a	-67.6 b	-74.6 b	-52.0	-47.8 a	-54.3 a	-54.1 a	-52.0
ROLSL	-52.0 a	-59.0 ab	-65.4 b	-58.8	-28.6 a	-60.2 b	-87.6 c	-58.8	-58.4 a	-57.9 a	-60.2 a	-58.8

ROLSW: Rate of Lost Seedling Weight, ROLSL: Rate of Lost Seedling length

The best value (-28.6 %) belong to 24-hour application in the ROLSL mean values. Three and two time frequency values have the same statistical group as the best frequency application (respectively -60.2 % and -57.9 %) for ROLSL (Table 1).

According to the Interaction of Time, doses and Frequency of salicylic acid; the highest Least Sq. Mean of ROLWS values were obtained from frequency 2 and time 24 h as -10.9 %, and the lowest Least Sq. Mean values was obtained from frequency 2 and time 72 h as -80.5 %. When the first three results are observed, 24 h applications was the best result and very different from the other applications. The highest Least Sq. Mean of ROLWS values was obtained from frequency 1 and dose 0.01mmol as -41.4 %, and the lowest Least Sq. Mean values was obtained from frequency 3 and control dose as -67.2 %. The differences between the frequencies, doses and times for Least Sq. Mean was significant. Least Sq. Mean ranged between -9.84 – -96.50 percent at Time x Doses x Frequency interaction of ROLWS. The average Least Sq. Mean of frequencies and doses and times was -52.02 %. The highest Least Sq. Mean values was obtained from frequency 2 and control dose and time 24 h as -9.84 %, and the lowest Least Sq. Mean values was obtained from frequency 3 and control doses and time 48 h as -96.50 %. In the Time x Doses interaction of ROLWS; the highest value was obtained from 24 h and dose 0.05 mmol interaction as -12.8 %, and the lowest Least Sq. Mean values was obtained from time 48 h and control dose as -92.80 %. (Table 2).

At the frequency x Time interaction of ROLSL; the differences between the frequencies and times to Least Sq. Mean were significant. The average Least Sq. Mean of frequencies and times was -58.8 %. The highest Least Sq. Mean values was obtained from frequency 2 and time 24 h as -24.6 %, and the lowest Least Sq. Mean values was obtained from frequency 2 and time 72 h as -89.1 %. It has been clearly observed that the first three results, 24 h applications was the best result and very different from the other applications, while the last three results have the worst results. Least Sq. Mean of the frequencies and the doses was significant; least Sq. Mean ranged between -43.8 – -68.8 percent. The average Least Sq. Mean of frequencies and doses were -58.8 %. The highest Least Sq. Mean values was obtained from frequencies 1 and dose 0.01 mmol as -43.80 %, and the lowest Least Sq. Mean values was obtained from frequency 2 and control dose as -68.8 %. At the point when watched the initial three outcomes, and two of the concentration at 0.01 mmol and one of the other at 0.05 mmol applications were the best outcome and altogether different from other applications. While the last three outcomes have fewer outcomes. The average Least Sq. Mean of frequencies and doses and times of ROLSL was -58.79 %. The highest Least Sq. Mean values was obtained from frequency 1 and dose 0.01 mmol and time 48 h as -19.31 %, and the lowest Least Sq. Mean values was obtained from frequency 2 and control dose and time 72 h -95.65. The highest Least Sq. Mean value of ROLSL was obtained from time 24 h and control dose as -21.2 %, and the lowest Least Sq. Mean values was obtained from time 72 h and control dose as -92.6 %.

Processing the pepper seedlings with salicylic acid for 16 hours, the results obtained, it was the negative effect on fresh weight increase, and dry weight increase in the application 5- and 10-mM SA. The result is different from this study because the times used are different from each other's (24, 48 and 72 hours in the test) [17]. In winter wheat leaves grow at low temperatures. It is sprayed with salicylic acid, the influence of external factors decreased and also the decreased freezing injury [18]. Salicylic acid sprinkled on coriander plant with at concentrations of 20 and 35 mg/L. got results on the significant increase in the soft weight of the vegetative group, and the number of flowers inflorescences, and the number of seeds per inflorescence, and weight 100 seed and production of the plant seeds [19]. It could be recommended that foliar spraying with salicylic acid at 100 ppm, to increase the final yield and fruit quality of sweet pepper plant during the low temperatures of autumn plantations [20]. The researcher found [21] the effect of salicylic acid on macrophomina and the evolution of fever disease on the Sun Flower plant. Results experience recommended that all pots concentrations may be affected significantly in reducing the percentage of injury. As for the concentrations of 200 and 250 mg /L salicylic acid effect of the dry weight increase total of vegetables and also an increase in the dry weight total of the roots. The study indicated that high concentrations of

Table 2. Effect of Interaction of Time, Doses and Frequency of Salicylic Acid

	Interaction	ROLWS	ROLSL		Interaction	ROLWS	ROLSL
Time x Doses	24 x 0,01	-14.9 a	-24.3 a	Time x Doses x Frequency	1 x 0,01 x 24	-21.61 a	-27.05 ab
	24 x 0,05	-12.8 a	-40.4 b		1 x 0,05 x 24	-14.16 a	-40.33 a-d
	24 x Con.	-13.7 a	-21.2 a		1 x Con. X 24	-16.20 a	-21.86 ab
	48 x 0,01	-51.5 b	-41.4 b		2 x 0,01 x 24	-12.40 a	-23.28 ab
	48 x 0,05	-58.5 bc	-56.8 c		2 x 0,05 x 24	-10.71 a	-28.21 a-c
	48 x Con.	-92.8 e	-82.4 d		2 x Con. x 24	-9.84 a	-22.27 ab
	72 x 0,01	-71.6 cd	-90.2 d		3 x 0,01 x 24	-10.57 a	-22.46 ab
	72 x 0,05	-69.7 cd	-79.9 d		3 x 0,05 x 24	-13.56 a	-52.53 c-g
	72 x Con.	-82.8 de	-92.6 d		3 x Con. x 24	-15.18 a	-19.43 a
	Mean	-52.0	-58.8		1 x 0,01 x 48	-23.01 a	-19.31 a
Frequency x Time	1 x 24	-17.3 a	-29.8 a		1 x 0,05 x 48	-66.60 b-e	-68.71 e-i
	1x 48	-58.8 b	-58.8 b		1 x Con. x 48	-86.87 d-f	-88.43 i-k
	1 x 72	-66.9 bc	-86.5 c		2 x 0,01 x 48	-65.86b-e	-44.75 b-e
	2 x 24	-10.9 a	-24.6 a		2 x 0,05 x 48	-53.43 b	-46.46 b-f
	2 x 48	-71.4 bc	-59.9 b		2 x Con. x 48	-95.04 f	-88.46 i-k
	2 x 72	-80.5 c	-89.1 c		3 x 0,01 x 48	-65.69 b-e	-60.26 d-h
	3 x 24	-13.1 a	-31.5 a		3 x 0,05 x 48	-55.32 bc	-55.28 d-g
	3 x 48	-72.5 bc	-61.9 b		3 x Con. x 48	-96.50 f	-70.19 f-j
	3 x 72	-76.5 c	-87.1 c		1 x 0,01 x 72	-79.44 c-f	-85.03 h-k
	Mean	-52.0	-58.8		1 x 0,05 x 72	-58.60 bc	-83.64 h-k
Frequency x Doses	1 x 0,01	-41.4 a	-43.8 a		1 x Con. X 72	-62.75 b-d	-90.83 i-k
	1 x 0,05	-46.5 a	-64.2 bc		2 x 0,01 x 72	-67.84 b-e	-91.50 i-k
	1 x Con.	-55.3 ab	-67.0 cd		2 x 0,05 x 72	-77.97 b-f	-80.08 h-k
	2 x 0,01	-48.7 a	-53.2 a-c		2 x Con. x 72	-95.71 f	-95.65k
	2 x 0,05	-47.4 a	-51.6 ab		3 x 0,01 x 72	-67.37 b-e	-93.91 jk
	2 x Con.	-66.9 b	-68.8 d		3 x 0,05 x 72	-72.56 b-f	-75.94 g-k
	3 x 0,01	-47.9 a	-58.9 bc		3 x Con. X 72	-89.83 ef	-91.37 i-k
	3 x 0,05	-47.2 a	-61.3 bc		Mean	52.02	-58.79
	3 x Con.	-67.2 b	-60.3 bc				
	Mean	-52.0	-58.8				

salicylic acid have been reduced by the number of stone bodies (sclerotia). While low concentrations of salicylic acid have significant differences with control treatment. The application of SA is different from this study, while application of SA was sprayed on the pepper plant at the low temperature. The researcher concluded [22] that spraying plants with low concentrations of salicylic acid can stimulate endure of vital and abiotic stresses such as cold and tolerance high-temperature. This is what we applied to our effects of the salicylic acid application on cold tolerance and gene expression in pepper seedling in the test. The researchers [23]'s study showed that plants did not show a saturated seed high concentration of 1 mM at the SA any change in tolerance iced, while the low concentrations of 0.1-0.5 mM at the SA encouraged tolerance to sedative stress in bean and tomato.

The results of the researcher [24] were that in the low temperatures showed cultivated plants decreased by 50–70 % in the number of leaves and the length and dry weight compared to the high-temperature system. It was also shown in the cold system of plants grown an increased number of shoots in the armpits. Also, the content of proteins and chlorophyll decreased in both temperature treatments. The total nitrogen content was slightly higher at low temperatures, but nitrate was lower. These results, are like the result that we obtained from the test, and an action the researchers' studies in the field, but we used the laboratory in the test. The researchers [25] concluded that Salicylic acid increases plant's resistance to inappropriate conditions especially against the stress that is the plant is exposed to stress (saline and drought), it works on the organization of some physiological processes of plant photosynthesis and transpiration. And the results of this conformity our results in the test while we used to tolerate pepper plants for low temperatures. The found in an experiment [26] results showed the superiority of cultivated plants in the first date in plant height, the leaf area, the dry weight of vegetative total and the dry weight of root total. The superiority of plants salicylic acid treatment with 100 mg/L concentration in the leaf area, the plant height, dry weight of vegetative growth and dry weight of roots, as well as the content of roots of *Arctium lappa* L. plant dry weight. Aziz et al. [27] found about the response of *narcissus* plants to spraying with plant growth regulators salicylic acid the characteristics of the vegetative growth and syphilis and bulbs characteristics of plant daffodils. The results increased at the 80 mg/L SA in the plant height, the number of branches/plant, and the ratio of chlorophyll and wet weight and flower diameter, weight wet and dry. The effect of overlap between the two studied factors is significant in most studied traits. The effect of water stress and the external application of SA on the growth and production of eggplant were investigated by Hameed et al. [28]. Results showed a significant increase for the first irrigation level W1 (water stress1) compared with level W2 (water stress2) transactions W3 (water stress3) for all indicators. Interacted treatment W1A2 (water stress1 x Applcation2) showed a significant increase compared with other treatments.

4. CONCLUSION

Three different doses of salicylic acid (SA) (0 mM, 0.01 and 0.05 mM) were given from the leaf. Each dose was administered in three different frequencies (1 time, 2 times and 3 times spraying). Each application was exposed to cold (0 C^0) in three different times (24 h, 48 h and 72 h). The best dose of SA was 0.01 mM in the Rate of Last Seedling Weight measurements, the worst result was obtained from the control group without SA treatment. Two (1 and 2 times) frequency applications applied to the seedlings exposed to low temperatures were the highest value in the Rate of Seedling Weight. Rate of Lost Seedling Lenght data, the best dose of 0.01 mM dose, the most appropriate frequency of applications 1 and 2 times were found to be.

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