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APPLICATIONS OF SALICYLIC ACID ON SEEDS AND SEEDLINGS OF PEPPERS UNDER SALINITY CONDITIONS

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Abstract

As a result of the global drought, improper irrigation and excessive fertilizer applications; salt accumulation in agricultural lands can cause production to stop. It is not possible to wash the soil in large lands and it is a difficult practice to rehabilitate the salty soils formed. In order to regain these soils for production, researchers have different applications; rotation and fallow practices, different irrigation methods, using of fertilizers and plant growth regulators etc. can be performed. In addition, with the changing and developing technologies, studies on production models that are not dependent on soil (soilless farming, rapid plant cultivation with LED lights in different aggregate cultures in artificially controlled environments) are being made on new models every day. This study was carried out to determine the effect of salicylic acid applications on pepper seeds and seedlings in saline conditions. Çermik pepper genotype, which is a local variety belonging to Diyarbakir, was used as plant material. Commercial rock salt was used as NaCl. While the pepper seeds were treated by keeping in 0.1mM and 0.5mM salicylic acid solution dosages, the seedlings were treated in pots containing the same doses of water culture + Hoagland nutritional solution. In order to identify the physiological and morphological changes in control and subjected to treatment plants, Chlorophyll-a, Chlorophyll-b, Total Chlorophyll content, Caretonoid content, 0-5 damage index of plants, plant fresh and dry weight (without roots), root fresh weight, plant fresh weight, leaf fresh weight, root length, stem length and stem diameter values were measured at the end of the research. When the statistical data obtained at the end of the study were examined, it was determined that both doses of Salicylic Acid increased the resistance of the plant to NaCl compared to the untreated seedlings. Furthermore, it has been determined that the application of 0.1 and 0.5mM doses of salicylic acid to the seeds and the subsequent application of SA to these seeds increases the resistance to NaCl that will be given to the environment.

Key words: *salicylic acid, salinity, pepper, seed, seedling.*

INTRODUCTION

All kinds of extreme good or bad conditions that may occur in optimum environmental conditions in plant breeding can limit the growth and development of plants. Stress is the broad term used to describe these undesired negative situations (Gürel and Avcıoğlu, 2001).
Stress is grouped into abiotic (salinity, drought, low and high temperatures, deficiency or excess of nutrients, heavy metals, air pollution, radiation) and biotic (disease-causing fungi, bacteria, viruses and pests), which leads in low productivity and might result in losses in manufacturing output (Yağmur, 2008; Kuşvuran, 2010; Tuna and Eroğlu, 2017).
It is stated that 71% of abiotic stress and 29% of other stress factors are effective in the reduction of agricultural production due to stress (Boyer, 1982; Mahajan and Tuteja, 2005).
Salinity, one of the abiotic stress factors, negatively affects soils in agricultural production; it causes many negativities in plants grown in these soils (Bora and Deveci, 2015; Yılmaz et al., 2011; Paul and Nair, 2008). More than 800 million hectares of land in the world is affected by salinity (Munns, 2002). Approximately 1.5 million hectares of Turkey's land is faced with salinity problems (Kalefetoğlu and Ekmekçi, 2005).
This global salinity problem can cause economic losses in national incomes of countries by causing product yield and quality in crop production (Mahajan and Tuteja, 2000; Shabala and Cuin, 2008).
Considering the main cause of salinization in soils; it may result from insufficient precipitation, the presence of underground salt rocks, the intensive use of salty groundwater in agricultural production, high air temperatures and high evaporation, and unbalanced and

excessive use of salty fertilizers (Saruhan et al., 2008; Uygan et al., 2006; Ekmekçi et al., 2005). Moreover, the increase in salinity in agricultural soils, it negatively affects the water intake of the plant due to high osmotic pressure, and also disrupts the metabolism and organelles such as chlorophyll in the plant, reducing the uptake of nutrients such as K^{+} , Ca^{+2} , Mn^{+2} and $NO3^{-}$. If any of these situations persist, the plant's growth and development will regress, its yield will drop, and it will eventually perish. (Ashraf and Bhatti, 2000; Hasegawa et al., 2000; Yıldız et al., 2010). In an effort to lessen the negative consequences of salinity, researchers are always conducting new studies. The most important and permanent one among the searches is the selection of resistant genotypes and its recommendation to the farmers or the development of new varieties via breeding studies. Studies on the resistance of genetic resources in agricultural regions where salt issues exist are considered as the most permanent solution (Daşgan et al., 2006).

In this regard, by the researchers, especially cultivation using high-quality seeds; methods such as the use of plant growth regulators, the supplementation of various fertilizers (increasing the amount of humus in the soil by using organic fertilizers), and the application of different cultural cultivation approaches are viewed as applications to increase the plant's resistance systems under stress situations (Senaratna et al., 2000).

In agricultural lands with salinity problems, strengthening the plant's water intake, external Ca and K applications that will improve the nutrient balance, and applications that prevent the transport of Na and Cl to the upper part increase the salt resistance of the plant (Kaya and Tuna, 2005; Tuna et al., 2007; Amjad. et al., 2016; Estañ et al., 2004). The use of some healers applied externally to the plant during plant development has also been tried in recent years, and it has been reported by researchers that the applications using Salicylic Acid (SA) can have an effect on increasing the stress tolerance of the plant on various plants.

When applied externally to plants, SA plays a protective role against different abiotic stresses such as metal toxicity, high and low temperature conditions, drought and salinity, as well as curative effects on seed germination and seedling growth (Yalpani et al., 1994; Senaratna et al., 2000; Borsani et al., 2001; Rajjou et al., 2006; Alonso-Ramirez et al., 2009, Lee et al., 2010; Özdener and Kutbay, 2008; Ekinci et al., 2011). It also acts as an endogenous growth regulator of phenolic compounds (Shakirova et al., 2003). Several researches have shown that salicylic acid plays an active part in the plant's defence mechanism, pathogen resistance, flowering and flower vitality, and systemic resistance mechanism, in addition to its potential to boost plant development (Aslantaş, 2013). In a study, Salicylic acid is not required for germination under normal conditions; however, it has been reported that it promotes germination by reducing oxidative damage in high salinity environments (Lee et al., 2010).

In this study, it was aimed to determine the changes in some physiological parameters of the pepper plant in seedling period, which is moderately sensitive to salinity, by cultivating SA applied to the seeds of the pepper plant, seed + seedling and seedlings obtained from seeds that were not treated with anything. It is expected that Çermik pepper's, which is the local genotype, tolerance to salinity will be determined, and will contribute to the longevity of this genotype.

MATERIALS AND METHODS

During the spring vegetation period of 2022 the research was carried out in a plant growing cabinet belonging to Dicle University Faculty of Agriculture Department of Horticulture where the temperature, light, and humidity were all controlled.

Materials

In the study, seedlings obtained from the seeds of the Çermik pepper genotype that have been selected by us as a result of previous selection studies and are considered appropriate to work on were used as plant material.

Methods

Salicylic acid concentrations of 0.0 (control), 0.10, and 0.50 mM, as well as salt concentrations of 0.0 (control), 50, 100, and 150 mM, were utilized in the research.

Designing the trial

The following were the study subjects used in the trial:

1. Seed + control,
2. Seed + SA 0.1 mM,
3. Seed + 0.5 mM,
4. Seedling + control,
5. Seedling + SA 0.1 mM,
6. Seedling + SA 0.5 mM,
7. Seed + Seedling SA 0.1 mM,
8. Seed + Seedling SA 0.5 mM.

For salicylic acid application, pepper seeds were sown in viols containing peat: perlite (3:2) after seeds were kept in SA solutions at different doses for 12 hours. The seeds of the control group and the seeds to be applied during the seedling period were kept in a tap containing water for 12 hours; it was permissible to be planted all seeds at the same time. Two seeds were left in each cell for possible problems in seed germination; Thinning was done after germination. Seedlings consisted of SA-treated and untreated seeds were rinsed under tap water with the solids in roots 45 days after sowing and planted in 2.6 lt pots including water but no drainage. All seedlings taken into aquaculture were then supplemented with Hoagland nutrient solution, air was given to the water with aquarium pumps in order to increase the amount of oxygen in the water, and the water was completely drained once a week, and then fresh water and nutrient solution were added. Two distinct dosages of SA were administered to the pots that had been determined again at the end of the second week, and SA treatments were finished. One day following the SA application, salt was applied, and a total of 150 mM NaCl was gradually supplied to the medium over three days, 50 mM each day. Two weeks after the last salt application, the trial terminated and measurements and observations: Chlorophyll a, Chlorophyll b, Total Chlorophyll content, Caretonoid content, 0-5 damage index of plants, plant fresh and dry weight (without roots), root fresh weight, plant fresh weight, leaf fresh weight, root length, stem length and stem diameter were collected.
The experiment consisted of three replications and was carried out with 20 plants per application in each replication in accordance with the randomised plots trial design. The results were then submitted to an analysis of variance using the JUMP statistical programme.

RESULTS AND DISCUSSIONS

Salinity stress is considered to be a serious and pervasive environmental issue as well as damaging plant growth, development, yield, and other essential functions. Researchers are working to find solutions to the problems of drought and salinity, which are becoming more evident with the increasing global climate crisis, and new applications are made in aquaculture with new breeding strategies (Flowers and Yeo, 1995). One of these applications is recognized as SA. The study was terminated in the seedling period by applying 0.1 and 0.5 mM doses to the seeds of pepper plants whose regulators are sensitive to salt, which many researchers work with.
Chlorophyll-a, chlorophyll-b, total chlorophyll content, carotenoid content and damage scale in plants are presented in Table 1. The applications have been found to be statistically significant in all parameters shown in Table 1. As seen in the table, the application of SA during the seedling period caused a decrease in terms of chlorophyll a. During the seedling period, SA treatment led to a reduction in chlorophyll a content. It was discovered that the chlorophyll a content of SA applied to the seed increased in comparison to the control group. The maximum chlorophyll a content was found with SA and salt administered both to the seed and later in the seedling stage. The seed + SA 0.5 + SA 0.5 + NaCl treatment had the highest chlorophyll a content, with an increase of 66.7% over the control group. The plants in the control + salt group had the lowest chlorophyll-a concentration, with a drop of 56.86% in comparison with the control group. Similar findings were reported when the table was evaluated in terms of chlorophyll b level. With an increase of 96.72% relative to the control group, Seed + SA 0.5 + SA 0.5 + NaCl treatment had the greatest chlorophyll b concentration. In comparison to the control group, the control + salt condition had the poorest chlorophyll b level, dropping by 47.54%. When the table was examined in terms of total chlorophyll content, a similar picture emerged. While the highest value of total chlorophyll content was obtained from the seed + SA 0.5 + SA 0.5 + NaCl application with an increase of 131.6% compared to the control group plants, the lowest

value was obtained from control + salt application with a decrease of 27.4%. As a result of SA and NaCl applications, when the carotenoid content of the pepper plant seedlings was evaluated, it was discovered that this content decreased in all applications in contrast to the control group. The highest Carotenoid content was found as 1.97 in the control group; the lowest value was obtained in the control + salt application (0.76), representing a 61% drop. On the other hand, the degree of the plant damage was scored using a scale from 0 to 5. Plants with low damage severity were assessed using a 0 scale, whereas 5 scale was used for plants with high damage severity. In general, with the presence of salt given to the medium, it was determined that damage occurred in all applications compared to the control group. In the study, the best plants were determined as the plants belonging to the control group; the worst plants were obtained from control + salt application and SA + salt application given to medium afterwards, as in chlorophyll and carotenoid content. If Table 1 is summarized in general; while the doses of SA applied to the seed and the doses of SA + salt applied later in the seedling period had positive effects on the chlorophyll and carotenoid content, it was revealed that the SA + salt applications applied during the seedling period caused more damage to the plants. Tuna and Eroğlu (2017) applied SA to pepper seedlings under salty conditions in their study. Investigated the photosynthetic pigment content. Researchers stated that the total chlorophyll content of the plants in the control group was 12.51, and the chlorophyll content decreased to 5.41 as a result of NaCl application. It was stated that the total chlorophyll content was 8.72 despite the presence of salt in the medium with the presence of SA. It has also been reported that a similar situation was observed in chlorophyll a and b parameters. In the same study, in terms of carotenoid content, it was stated that while the total carotenoid content was 3.05 in the control group, it decreased to 1.81 with NaCl application. It has also been reported that the carotenoid content is 2.34, together with the presence of SA + NaCl in the medium. It is stated that the increase in salinity in photosynthetic tissues causes the breakdown of chlorophyll (Ashraf and Harris, 2004). High salinity disrupts the molecular structure of chlorophyll and reduces its amount (Deveci and Tuğrul, 2017). Bora and Deveci (2015) reported in their study to determine the physiological, morphological and chemical changes caused by different salt concentrations in pepper that the damage index of plants increased as the salinity increased.

The wet weights of leaves, stems and roots, root and stem length, and stem diameter data obtained in the study are presented in Table 2. As seen in the table, leaf fresh weights and stem fresh weights were found to be statistically significant (<0.005). While the highest values in terms of leaf fresh weight were obtained from plants belonging to the control group (1.25 g), the lowest value was obtained from plants belonging to control + salt treated pots with a decrease of 95% (0.06 g). When the table was examined, it was seen that the doses of SA applied to the seed in terms of leaf fresh weight decreased less than the doses applied to the seedling afterwards. No statistically significant difference was found in terms of root wet weights. While the highest root wet weight was detected in plants belonging to the control group (0.55 g), the lowest root fresh weight was obtained from control + salt treated plants with a decrease of 78% (0.12 g). As with the parameters of other applications, the doses of SA applied to the seed generally showed less change compared to the subsequent applications to the seedling. The highest weight in terms of stem fresh weight was obtained from plants belonging to the control group (1.67 g); It was determined that there were decreases in stem fresh weights in different doses of SA applied to plants and salt combinations. The lowest stem fresh weight was found in control + salt treatment with a decrease of 87% (0.21 g). It was recorded that treatments to be applied from seed changed less in terms of stem fresh weight than applications to be done later on seedlings. When the data on the length of the root lengths taken were examined, it was found that different applications were statistically significant on the plants (<0.001). It was observed that the longest plants in terms of root length belonged to the control group (20.32 cm). The shortest root length was obtained from plants treated with control + salt with a decrease of 75% (5.18 cm). As a result of the analyzes made in terms of

trunk length, it was seen that different applications were statistically significant (<0.001).
The longest stem length was obtained from plants belonging to the control group (15 cm); the lowest values were obtained from plants belonging to the control + salt treatment with a decrease of 60% (6.04 cm). Statistical analyzes of different applications were found to be significant (<0.005) as a result of measuring the body diameter with a digital caliper. The highest value was obtained from seed + SA 0.1 + NaCl with 1.96 mm and plants belonging to the control group with 1.92 mm.
The lowest value was obtained from control + salt application without SA application in the medium (0.90 mm). As with other parameters, it has been observed that the SA applications to be made from the seed will show less change than the SA applications to the seedling afterwards.
It has also been reported by many researchers that there are significant decreases in root fresh and dry weight, and leaf fresh and dry weight under salt stress (Tuna and Eroğlu, 2017; Erkılıç, 2005; Senaratna et al., 2000; Türkmen et al., 2002; Altunlu, 2019). Erkılıç (2005) reported that externally applied SA on pepper plants (*Capsicum annuum* L.) under salt stress had a positive effect on some physiological parameters such as fresh and dry weight of the plant. The researcher reported that there were significant drops in numerous parameters of the plants that just received salt treatment. Similar results were reported by Tuna and Eroğlu (2017) as well. To test the impact of several organic and inorganic substances on the antioxidant system in the pepper (*Capsicum annuum* L.) plant under salt stress, these two researchers treated the environment to SA.
Physiological disorders were experienced only in plants applied to salt; they stated that SA applications gave resistance to the plant. Şenay et al., (2005), in a study on wheat, reported that increasing salt concentrations reduced seedling length by 42.5% and root length by 74.4%. In their investigations on wheat, Shakirova et al. (2003) revealed that salt caused losses in the fresh and dry weights of the plants.
Yaylalı (2007) reported that the amount of salt in the irrigation water negatively affects the yield and quality characteristics of the tomato plant and the plant growth.
Aydın (2015), in his study examining the effects of salt stress on germination and seedling growth in some cultivated plants, stated that increased salt applications in all cultivars caused a significant decrease in stem and root lengths and fresh weight of the plant compared to the control.
In a study conducted to obtain the effect of SA application as an additive on salt stress, it was reported that when tomato plants are grown in SA and NaCl is added to the same medium after a certain period, salt damage to the plant can be prevented (Tari et al. 2002).

Table 1: The effects of different applications on chlorophyll, carotenoid and damage index of the plant

APPLICATIONS	Chlorophyll a	**% CHANGE**	Chlorophyll b	**% CHANGE**	T. Chlorophyll	**% CHANGE**	Carotenoid	**% CHANGE**	0-5 Scale
1. Seed + SA 0.1 + NaCl	0.67±0.00 c	**31.37**	0.96±0.00c	**57.38**	1.86±0.00 c	**95.79**	1.63±0.01 d	**-17.26**	0.40±0.31c
2. Seed + SA 0.5 + NaCl	0.75±0.00b	**47.06**	1.06±0.00b	**73.77**	2.01±0.02b	**111.58**	1.80±0.01b	**-8.63**	1.20±0.46bc
3. Seed + SA 0.1 + SA 0.1 + NaCl	0.74±0.01b	**45.10**	1.05±0.00b	**72.13**	1.98±0.01b	**108.42**	1.75±0.00 c	**-11.17**	1.00±0.29bc
4. Seed + SA 0.5 + SA 0.5 + NaCl	0.85±0.01 a	**66.67**	1.20±0.02 a	**96.72**	2.20±0.00 a	**131.58**	1.94±0.00 a	**-1.52**	0.40±0.27c
5. SA 0.1 + NaCl	0.29±0.00 f	**-43.14**	0.42±0.00 f	**-31.15**	0.85±0.00 f	**-10.53**	1.04±0.00 e	**-47.21**	1.40±0.24 bc
6. SA 0.5 + NaCl	0.36±0.00 e	**-29.41**	0.49±0.00 e	**-19.67**	1.04±0.00 d	**9.47**	0.89±0.00 f	**-54.82**	2.20±0.52b
7. CONTROL + NaCl	0.22±0.00 g	**-56.86**	0.32±0.01 g	**-47.54**	0.69±0.01 g	**-27.37**	0.76±0.01 g	**-61.42**	4.40±0.24 a
8. CONTROL	0.51±0.00 d		0.61±0.00 d		0.95±0.00 e		1.97±0.00 a		0.20±0.22c
CV	1,413889016		2.263746322		1,318101023		1,088409511		57,42914286
P	<,0001		<,0001		<,0001		<,0001		<,0001
LSD	0.0152		0.035		0.039		0.0317		1,587

Table 2: The effects of different applications on the fresh leaf, root and stem weights (g), root and stem lengths (cm) and stem diameter (mm) of the plant

APPLICATIONS	Leaf Age weight	**% CHANGE**	Root Age Weight	**% CHANGE**	Body Wet Weight	**% CHANGE**	Root Length	**% CHANGE**	Body Length	**% CHANGE**	Stem Diameter	**% CHANGE**
1. Seed + SA 0.1 + NaCl	0.80±0.22 ab	**-36.00**	0.34±0.08 a	**-38.18**	1.07±0.25 ab	**-35.93**	18.08±2.36 ab	**-11.02**	12.90±1.08 ab	**-14.00**	1.96±0.19 a	**2.08**
2. Seed + SA 0.5 + NaCl	0.55±0.10 ab	**-56,00**	0.32±0.17 a	**-41.82**	0.72±0.14 ab	**-56.89**	8.28±2.17 cd	**-59.25**	10.84±0.68 bc	**-27.73**	1.72±0.15 ab	**-10.42**
3. Seed + SA 0.1 + SA 0.1 + NaCl	0.57±0.03 ab	**-54.40**	0.16±0.02 a	**-70.91**	0.78±0.03 ab	**-53.29**	9.40±1.21 bcd	**-53.74**	11.62±0.45 abc	**-22.53**	1.66±0.09 ab	**-13.54**
4. Seed + SA 0.5 + SA 0.5 + NaCl	0.77±0.12 ab	**-38.40**	0.34±0.08 a	**-38.18**	1.25±0.26 ab	**-25.15**	16.58±1.55 abc	**-18.41**	12.34±0.81 ab	**-17.73**	1.64±0.11 abc	**-14.58**
5. SA 0.1 + NaCl	0.36±0.12b	**-71.20**	0.16±0.07 a	**-70.91**	0.50±0.15b	**-70.06**	9.68±2.17 bcd	**-52.36**	11.88±1.07 abc	**-20.80**	1.26±0.22 abc	**-34.38**
6. SA 0.5 + NaCl	0.11±0.06b	**-91.20**	0.26±0.11a	**-52.73**	0.39±0.15b	**-76.65**	7.28±1.18 d	**-64.17**	8.08±0.90 cd	**-46.13**	1.14±0.08bc	**-40.63**
7. CONTROL + NaCl	0.06±0.05b	**-95.20**	0.12±0.03 a	**-78.18**	0.21±0.05b	**-87.43**	5.18±0.62 d	**-74.51**	6.04±0.37 d	**-59.73**	0.90±0.07c	**-53.13**
8. CONTROL	1.25±0.30 a		0.55±0.19 a		1.67±0.40 a		20.32±2.60 a		15.00±0.94 a		1.92±0.23 a	
CV	64,53751901		93,02381799		61.17061663		37,29478481		17,79143179		23.86546468	
P	0.0003		0.2295		0.0016		<,0001		<,0001		0.0005	
LSD	0.726		0.519		1,001		8,973		4,065		0.763	

CONCLUSIONS

The accumulation of ions such as Na+ and Cl- caused by salinization in agricultural lands can adversely affect plant growth. Soil salinity is one of the abiotic stress factors that limit crop production. Choosing resistant cultivars is the first of the measures that must be taken when cultivating in saline soils and it is followed by implementations for strengthening salt resistance.

Salicylic acid, which ensures the continuity of the nutrient and water intake of the plants to be grown under these conditions, has been tested on many plants to increase the resistance to stress conditions. Based on our findings, salicylic acid treatments, particularly from seed or later during the seedling stage, can be suggested to reduce the effects of salt damage. In addition, the use of salicylic acid in other crop plants under salt stress can be recommended.

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