Yüzüncü Yıl Üniversitesi
Tarım Bilimleri Dergisi
(YYU Journal of Agricultural Science)

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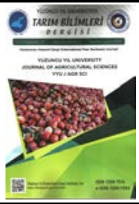

Araştırma Makalesi (Research Article)

Putrescine, Spermine and Spermidine Mitigated the Salt Stress Damage on Pepper (*Capsicum annum* L.) Seedling

Melek EKİNCİ[1], Ertan YILDIRIM[*1], Atilla DURSUN[1], Noor S. MOHAMEDSRAJADEN[1]

[1]Atatürk University, Agriculture Faculty, Department of Horticulture, 25240, Erzurum, Turkey
*Corresponding author e-mail: ertanyil@atauni.edu.tr

Article Info

Received: 09.05.2019
Accepted: 14.06.2019
Online Published 28.06.2019
DOI:10.29133/yyutbd.562482

Keywords
Pepper,
Polyamine,
Plant growth,
Salt stress,
Seedling

Abstract: In order to evaluate the effects of polyamines on plant growth, physiological and biochemical characteristics of pepper seedlings grown under salt stress (0, 50 and 100 mM NaCl), putrescine (Put), spermine (Spr) and spermidine (Spd) were foliarly applied to the seedlings under controlled greenhouse conditions. The effects of polyamines on plant height, number of leaves, stem diameter, chlorophyll reading value (CRV), stoma conductance (SC), tissue electrical conductivity (TEC), leaf relative water content (LRWC), enzyme activity of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) of pepper seedlings were significant under salt stress. As salt concentration increased, plant height, stem diameter, number of leaves, CRV, plant and root fresh and dry weight, and LRWC lowered but an increase in TEC occurred. However, polyamine treatments improved the parameters investigated under salt stress. In the study, it has been determined that the negative effects of salt stress can be mitigated with exogenously polyamine applications to the pepper seedlings.

Putresin, Spermin ve Spermidin Uygulamalarının Biber (*Capsicum annum* L.) Fidesinde Tuz Stresi Zararını Hafifletici Etkisi

Makale Bilgileri

Geliş: 09.05.2019
Kabul: 14.06.2019
Online Yayınlanma 28.06.2019
DOI: 10.29133/yyutbd.562482

Anahtar kelimeler
Biber,
Poliamin,
Bitki büyümesi,
Tuz stresi,
Fide

Öz: Poliaminlerin tuz stresi altında (0, 50 ve 100 mM NaCl) yetiştirilen biber fidelerinde bitki büyümesi, fizyolojik ve biyokimyasal özellikleri üzerine etkilerini belirlemek için, kontrollü sera koşullarında putresin (Put), spermin (Spr) ve spermidin (Spd) fidelere yapraktan uygulanmıştır. Poliaminlerin tuz stresi altındaki biber fidelerinde bitki boyu, yaprak sayısı, gövde çapı, klorofil değeri, stoma iletkenliği, doku elektrik iletkenliği, yaprak bağıl su içeriği, süperoksit dismutaz (SOD), katalaz (CAT) ve peroksidaz (POD) enzim aktivitesi üzerine etkisi istatistiksel olarak önemli olmuştur. Tuz konsantrasyonu arttıkça, bitki boyu, gövde çapı, yaprak sayısı, klorofil değeri, bitki ve kök taze ve kuru ağırlığı ve yaprak bağıl su içeriği azalmış, ancak doku elektrik iletkenliğinde bir artış meydana gelmiştir. Bununla birlikte, poliamin uygulamaları tuz stresindeki biber fidelerinde incelenen parametreleri iyileştirmiştir. Araştırmada, tuz stresinin olumsuz etkilerinin biber fidelerine dışarıdan yapılan poliamin uygulamasıyla hafifletilebileceği belirlenmiştir.

1. Introduction

Salinity is one of the abiotic stress factors that cause important problems in crop production. Salinity starts to show its effect from seed germination, and when the plant is over this stage, it can cause full damage to the plant if the salinity effect increases. For this reason, it is essential to know the responses of crops to salinity in terms of continuity of production. Improving the tolerance of plants to unfavorable conditions is a major issue for agricultural productivity and also important for environmental sustainability because plants with low stress tolerance use more water and fertilizer (Zhu, 2016).

The plants develop different mechanisms against salt stress in order to protect both their osmotic order and the ion balance. High salt concentration causes a high osmotic stress and intracellular ion imbalance in plants, leading to secondary effects and damage. Osmotic adjustment is achieved by accumulation of compounds such as proline, glycine betaine and some sugar alcohols (Yokoi et al., 2002). Due to salt stress, antioxidant enzymes, chloroplast and cytosol enzyme activities have been found to be important changes in plants (Hernandez et al., 2001; Saha et al., 2015).

Studies on the use of some external applications to decrease the negative impacts of the salinity have been carried out. One of them has been the treatment of plants with polyamines. It has been determined by many researchers that polyamines have positive influence on plants grown under salinity depending on the application dose (Jiuju and Shirong, 2005; Duan et al., 2007; Liu et al., 2007; Zapata et al., 2008; Khan et al., 2012; Li et al., 2013).

It is known that the naturally occurring polyamines in plant metabolism have important effects on the tolerance mechanism to abiotic stress in plants. It has been observed in several studies that in the stress conditions, external polyamine applications can have the same effect as the tolerance mechanism induced by the internally occurring polyamines against stress (Meloni et al., 2003; Zeid, 2004; Jiuju and Shirong, 2005; Duan et al., 2007; Roychoudhury et al., 2011).

Polyamines such as putrescine (Put), spermidine (Spd) and spermine (Spr) are low-molecular organic cations found in a wide variety of organisms, from bacteria to plants and animals. Polyamines in plants can participate in various physiological processes such as growth, development, aging and stress reactions. Intracellular polyamines such as Spr, Spd and Put naturally occur and have important effects on the tolerance to abiotic stress in plants. In many abiotic stress conditions, three basic polyamines were deposited, and they tolerated to drought and salt stress. The presence of polyamines provides a great advantage in improving yield under unfavorable conditions. In recent years, the importance of polyamines has been taken into account in improving different environmental abiotic stresses (Gupta et al., 2013, Saha et al., 2015). There is not much information about the influences of different polyamines on growth, physiological and biochemical characteristics of pepper under salt stress. This study was conducted in order to determine the effects of different polyamines (Spr, Spd and Put) on growth, physiological and biochemical characteristics of pepper under salt stress, and evaluate if polyamines mitigate the deleterious effect of salinity.

2. Materials and Methods

In the study, the seeds of pepper (*Capsicum annum* L. cv. Yalova) were used as plant material. Pepper seeds were sown multi-celled trays filled with peat: perlite (3: 1 / v: v), 20 days after seedlings were transferred to pots containing garden soil: peat (2: 1 / v: v). Temperatures ranged from 27°C to 33°C during the day and 18°C to 22°C during the night, and the relative humidity was 40-60%.

Three different amounts of NaCl (0, 50 and 100 mM of NaCl) were added to irrigation water and their electrical conductivities were 0.57, 5.28, and 7.28 dS m^{-1} respectively. Salinity treatments were initiated after planting of seedlings with a daily increase of 25 mM NaCl to avoid an osmotic shock for plants.

Spr, Spd and Put solutions were prepared at levels of 0 (control), 0.1 and 2.5 mM in ultrapure water with Tween 20. The solutions were sprayed on leaves a day before planting of the seedlings and then every five days the seedlings were sprayed regularly until harvest. There were seven polyamine treatments; control, Spr 1 (0.1 mM), Spr 2 (2.5 mM), Spd 1 (0.1 mM), Spd 2 (2.5 mM), Put 1 (0.1 mM) and Put 2 (2.5 mM).

After 35 days of seedling transplantation, seedling height, number of leaves, stem diameter, leaf relative water content (LRWC), tissue electric conductance (TEC), chlorophyll reading value (CRV) and stomal conductance (SC) were measured. Plant samples were taken for dry weights and fresh samples kept for chemical analysis at -80 ^{o}C.

CRV was determined as leaf chlorophyll reading value (SPAD) with a chlorophyll meter.

SC (mmol (H_2O) m^{-2} s^{-1}) was measured before noon on the youngest fully expanded upper leaf, along the right abaxial side of the leaf lamina using a porometer (Sc-1 Porometer, Decagon Devices Inc., WA, USA).

TEC and LRWC measurements were performed on leaf samples taken from intact plants (Kaya et al., 2003).

Assays of antioxidant enzyme activity were performed according to Agarwal and Pandey (2004), Angelini et al. (1990), Gong et al. (2001) and Yordanova et al. (2004).

The study was designed according to randomized plot design with three replications. Variance analysis was performed by means of the data obtained from the results of the study and the differences between the applications were determined by Duncan multiple comparison test.

3. Results

The effects of polyamine on plant growth and CRV of pepper seedlings were found to be statistically significant ($p < 0.001$). As NaCl concentration increased, the decrease in plant height, stem diameter and chlorophyll were observed in the pepper and the highest decreases occurred in 100 mM salt stress (Figure 1).

The highest plant height (12.08 cm) was obtained with Spd 2 application in the absence of salt stress (0 NaCl), while Spr 2 application (10.19 cm) at 50 mM NaCl conditions and Put 2 application (8.95 cm) at 100 mM NaCl conditions. The highest leaf number was obtained from Spd 2 under the control conditions (7.17) and 50 mM NaCl (6.42) while Put 2 gave the maximum leaf number (6.17) at 100 mM NaCl. Polyamine treated plants had greater stem diameter at 0 mM NaCl than the control plants except for Spr 1. Whereas Put 1and Put 2 increased statistically the stem diameter compared to the control at 100 mM salt stress. Polyamine treatments did not affect statistically CRV under 0, 50 and 100 mM NaCl conditions. It has been determined that exogenous polyamine applications comforted the salt stress on plant growth and chlorophyll reading values (Figure 1).

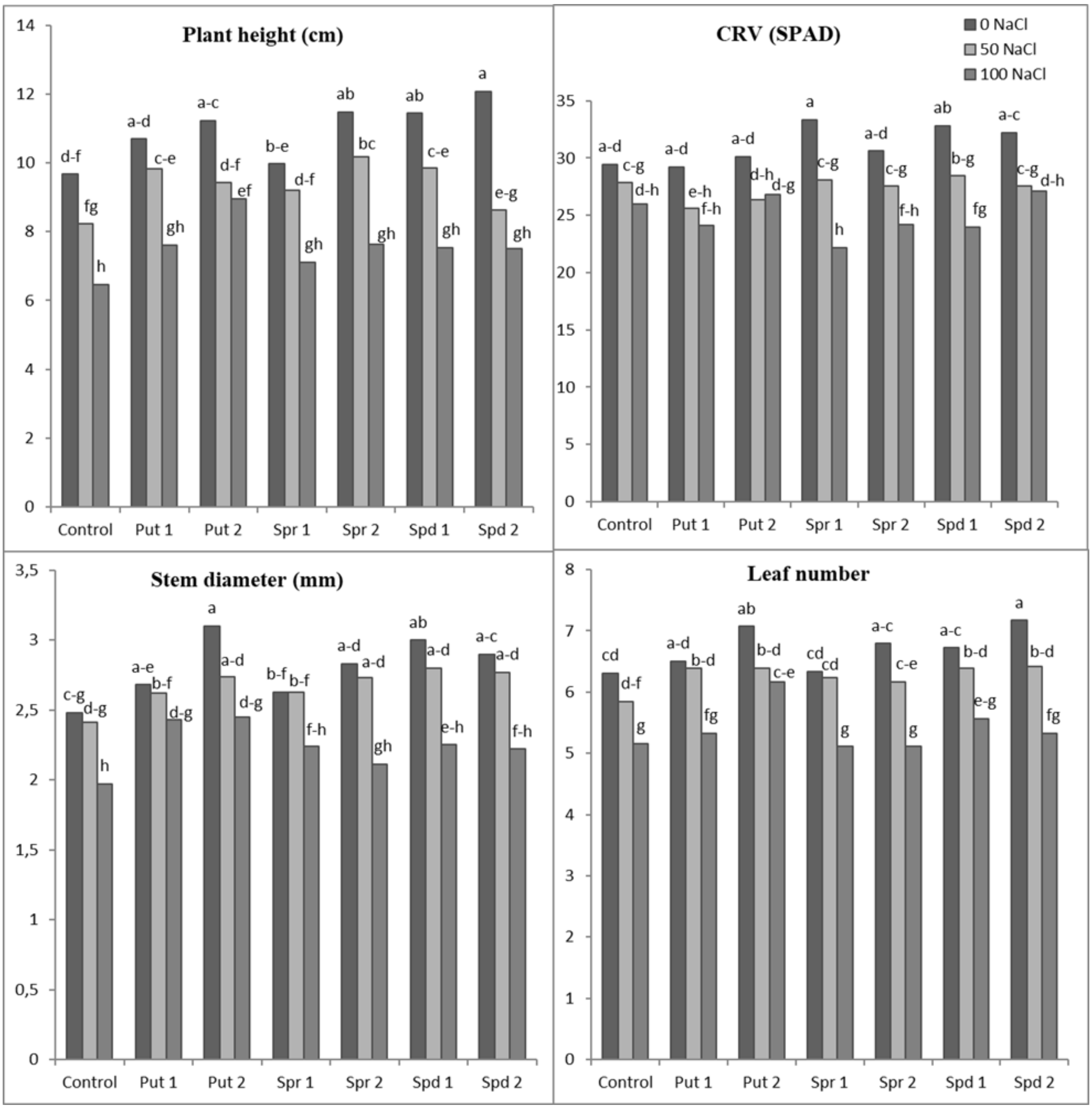

Figure 1. Effects of polyamine applications on plant height, CRV, stem diameter and leaf number of pepper seedlings under salt stress.

Put 1: 0.1 mM Putrescine, Put 2: 2.5 mM Putrescine, Spr 1: 0.1 mM Spermine, Spr 2: 2.5 mM Spermine, Spd 1: 0.1 mM Spermidine, Spd 2: 2.5 mM Spermidine
There is no difference between the averages shown on bars in the same letter

In the study, the effect of polyamine applications on fresh and dry weights was found to be statistically significant ($p < 0.001$). As NaCl concentration increased, plant and root fresh and dry weights of pepper seedlings decreased significantly, and the lowest values were observed at 100 mM NaCl. The greatest plant fresh weight was observed in Spd 2 treatment (1.91 g/plant) at 0 mM NaCl. There were no significant differences between treatments under 50 mM NaCl regarding to plant fresh weight. However, Put 2 increased the fresh weight compared to the control at 100 mM NaCl. In terms of plant dry weight, Spd 2 and Put 2 treatments were determined as the highest at 0 mM NaCl. Moreover, Spd 2 treated plants had statistically greater plant dry weight than the control at 100 NaCl.

The root dry and fresh weights were highest in the absence of salt stress. The application of Put 2 at 100 mM NaCl increased root fresh weight compared to other applications. Similarly, Put 2 treated plants gave higher root dry weight than the other treatments under 100 mM NaCl conditions (Figure 2).

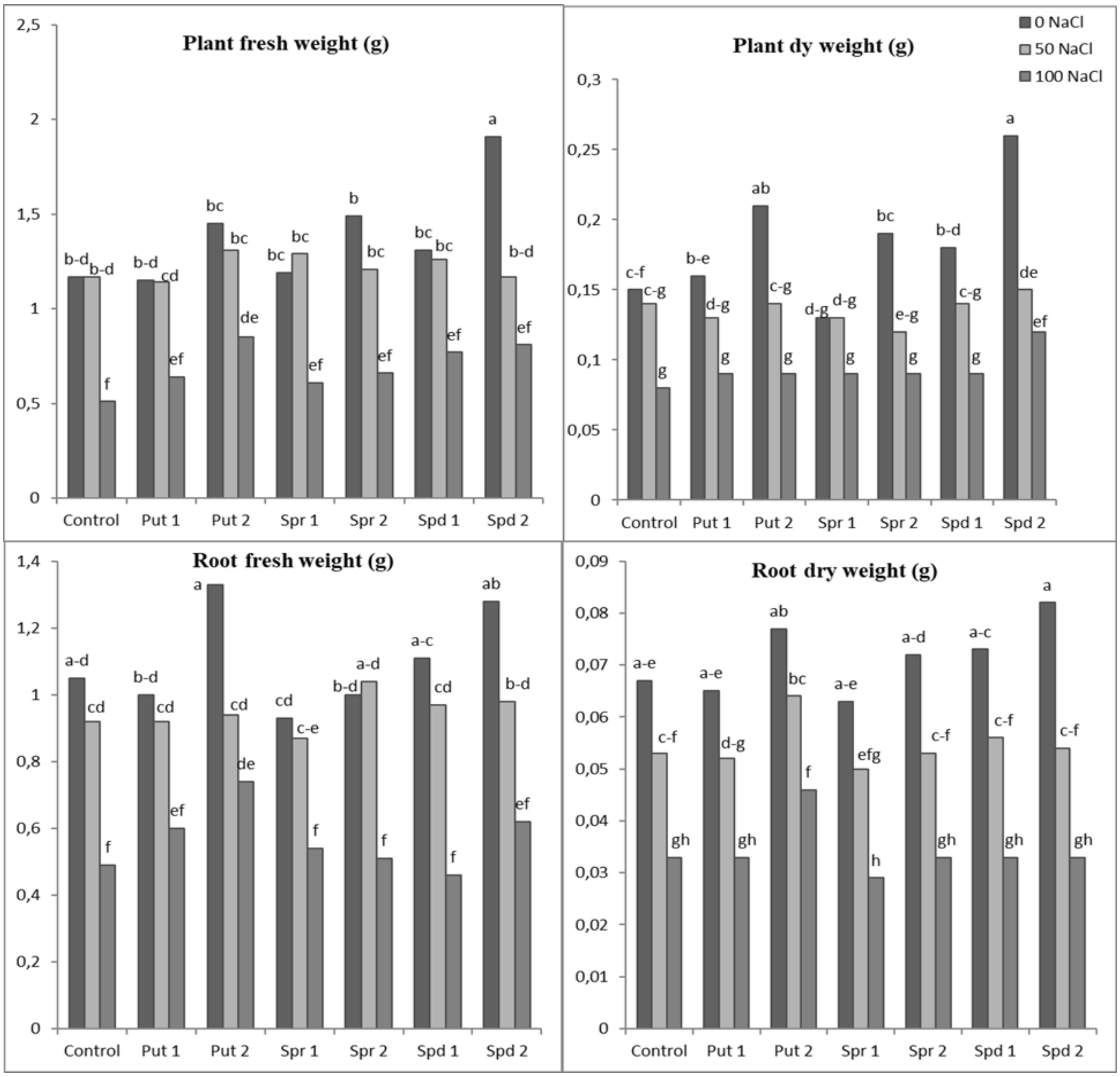

Figure 2. Effects of polyamine applications on plant fresh weight, plant dry weight, root fresh weight and root dry weight of pepper seedlings under salt stress

Put 1: 0,1 mM Putrescine, Put 2: 2.5 mM Putrescine, Spr 1: 0.1 mM Spermine, Spr 2: 2.5 mM Spermine, Spd 1: 0.1 mM Spermidine, Spd 2: 2.5 mM Spermidine
There is no difference between the averages shown in bars on the same letter.

SC and LRWC decreased with increasing salt stress. Polyamine applications generally had no significant effect on LRWC. The highest LRWC value was obtained from Spd 2 at 100 mM NaCl, but there were no significant differences between treatments.

Spd 2 treatment gave the highest SC in the absence of salt stress and at 100 mM NaCl while Spr 1 treated plants had higher SC values than the control at 50 mM NaCl. The TEC values increased significantly in seedlings grown under NaCl salt stress. However, Put 1, Put 2, Spr 2 and Spd 1 treatments decreased the TEC values under salt stress conditions compared to the control. Put 2 and Spr 1 decreased the TEC value at 100 mM NaCl salt stress compared to the control (Figure 3).

CAT activity was increased with salt stress and polyamine treatments, and Put 2 at 50 mM NaCl and Put 1 at 100 mM NaCl gave the highest values, respectively. The effects of the applications on POD enzyme activity in pepper seedlings were found to be statistically significant. Generally, POD content increased as salt concentration increased. In the highest salt stress (100 mM NaCl), POD activity was elevated with the application of Spd 2. The effects of the treatments on SOD enzyme activity in pepper seedlings were found to be statistically significant. The SOD activity rose with the application of Spr 2 at 50 mM NaCl compared to the control. In general, the salinity conditions caused the higher the SOD activity (Figure 3).

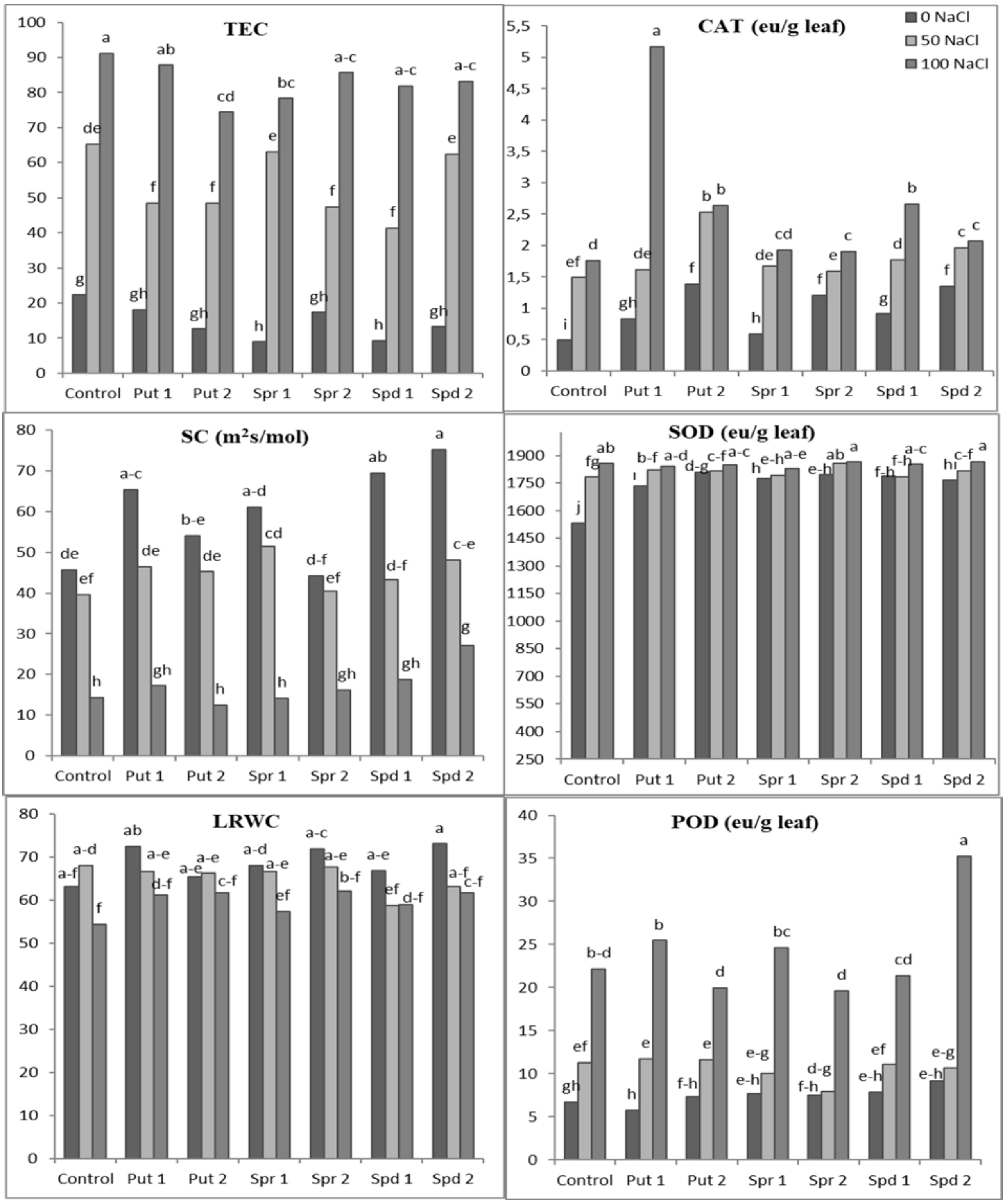

Figure 3. Effects of polyamine applications on TEC, SC, LRWC, SOD, POD and CAT enzyme activity of pepper seedlings under salt stress.

Put 1: 0.1 mM Putrescine, Put 2: 2.5 mM Putrescine, Spr 1: 0.1 mM Spermine, Spr 2: 2.5 mM Spermine, Spd 1: 0.1 mM Spermidine, Spd 2: 2.5 mM Spermidine
There is no difference between the averages shown in bars in the same letter.

4. Discussion and Conclusion

Salinity leads to a decrease in the growth of the plants and decrease in the yield and quality of the crops. As a matter of fact, studies have reported that salinity has a negative impact on growth in pepper (Shannon and Grieve, 1999; Asrhaf and Harris, 2004; Houimli et al., 2008; Houimli et al., 2010; Hussein et al., 2012). In plants grown under salinity conditions, water loss in the cell, deterioration in plasma membrane and free hydrolytic enzymes lead to degradation of the cytoplasm structure, resulting in a slowing of growth and reduction in turgor (Kusvuran et al., 2013). Osmotic and ion stresses under salt stress decrease the growth of plants. In previous studies, pepper has been reported to be salinity sensitive or semi-sensitive (De Pascale et al., 2003).

In recent years, studies on salt stress are aimed to obtain the least damage from the salinity on the plants. Both internal polyamines and exogenous treatments have positive effects on various plants under stress. Intracellular polyamines, such as Spr, Spd and Put, have been reported to occur naturally and have significant effects on the mechanism of resistance to abiotic stress in plants (Gupta et al., 2013). It has been reported that polyamines cause mitosis in the cell, leading to cell division and subsequent cell growth (Gallardo et al., 1996).

Salinity levels at 50 and 100 mM NaCl decreased plant weight, CRV, stem diameter, leaf number, plant height, plant dry weight, root fresh and root dry weight by 0-56%, 5-12%, 13-21%, 7-18%, 15-33%, 7-47%, 12-53% and 21-51%. However, depending on the doses, it was determined that exogenous polyamine treatments reduced negative effect of salt stress. Radhakrishnan and Lee (2014) pointed out that the Spd application increased plant height and dry weight of salt stress-grown cucumber (*Cucumis sativus* L.). There are a many studies on vegetable crops in which salinity and drought conditions negatively affected SPAD values (Esringu et al., 2011; Karlidag et al., 2011; Ekinci et al., 2015; Samancioglu et al., 2016). This could be attributed to the destruction of chlorophyll pigments, and a minimization of the vulnerability of the pigment-protein complexes and chlorophyll syntheses (Rasool et al., 2013; Ahmad et al., 2016). Furthermore, the researchers determined that the negative effect of salt stress on chlorophyll content was inhibited by Spd. Put has increased chlorophyll content, germination, root length, leaf area, leaf fresh and dry weight in beans under salt stress (Zeid, 2004). In another study, it was reported that exogenous Spr and Spd treatments reduced the negative effect of salt stress at different levels (Roychoudhury et al., 2011). Polyamines play an important role in many other physiological processes such as embryo formation, organ formation, flower formation and development, fruit development and maturation, leaf senescence, biotic and abiotic stress (Alcàzar et al., 2011; Alet et al., 2012).

Polyamine applications have the effect of protecting cell membrane, triggering expression of osmotic response genes, decreasing H_2O_2 levels, increasing antioxidant enzyme activity and reducing accumulation of Na and Cl ions in plant organs (Zhang et al., 2009; Terzi et al., 2014). Similarly, some polyamine treatments decreased TEC values and increased the antioxidant activity in this study. Shi et al (2013) found that polyamine applications significantly alleviate the effects of drought and salt stresses, and significantly increased antioxidant enzymes and several other stress-related proteins. The role of polyamines in reducing the adverse effects of stress conditions can probably be due to their direct interaction with membranes, reduction of oxidant activity, functioning as a compatible osmolite or ionic feature (Minocha et al., 2014). Polyamines are also reported to play a role in the regulation of H_2O_2 production in plants. Spd application has been reported to increase the tolerance to drought by controlling the production of H_2O_2 and the expression of antioxidant related genes (Li et al., 2015). Antioxidant enzyme activity in pepper seedlings varied according to salinity and polyamine treatments and concentrations. However, usually salt stress and polyamine treatments caused an increase in antioxidant enzyme activity (Figure 3). Similarly, studies investigating the effect of salt stress on SOD activity of various crops showed that salt-tolerant varieties produced more SOD activity than the sensitive ones under salt stress (Lin and Kao, 2000; Hernandez et al., 2001; Sudhakar et al., 2001; Bor et al., 2003; Meloni et al., 2003).

In the study, it was concluded that foliar polyamine applications during the seedling could mitigation effects on reducing negative effects of salt stress in pepper seedlings. In this study, as in previous studies, it is thought that this might be effective in reducing or preventing the effects of salinity, which is one of the most important problems of agricultural production.

Acknowledgment

We appreciate Atatürk University, Scientific Research Projects Foundation for generous financial support (Project Number FHD-2017/6153).

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