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Araştırma
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Effects of Different Putrescine and Salicylic Acid Applications on Germination, Plant Growth, Quality Properties and Nutrient Content of Lettuce (*Lactuca sativa* L.) under Saline Conditions*

Ousseini KIEMDE [1], Beyhan KİBAR [1*]

[1]Bolu Abant İzzet Baysal University, Faculty of Agriculture, Department of Horticulture, Bolu/Türkiye

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Sorumlu yazar: Beyhan KİBAR, e-posta: beyhan.kibar@ibu.edu.tr

Abstract

Objective: This study was conducted to determine the effects of putrescine and salicylic acid applications at different doses on germination, plant growth, quality properties and nutrient contents of lettuce under saline conditions.

Materials and Methods: In the study carried out in climatic chamber conditions, a total of 15 different applications using two different salt levels (100 and 200 mM), two different putrescine doses (0.5 and 1.0 mM) and two different salicylic acid doses (0.5 and 1.0 mM) were investigated. The effects of putrescine and salicylic acid separately and together with salt were examined.

Results: According to the findings obtained from the research, it was detected that salinity negatively affected the germination, plant growth, quality properties and nutrient contents of lettuce. In general, it was determined that putrescine applications under saline conditions increased germination and plant growth parameters and significantly reduced the negative effects of salt stress. Furthermore, it was found that the 1.0 mM Putrescine+200 mM NaCl application, in which salt and putrescine were used together, increased germination rate by 75.57%, plant height by 42.49%, plant fresh weight by 58.89% and number of marketable leaves by 43.20% compared to the 200 mM NaCl application.

Conclusion: It was concluded that 0.5 mM Putrescine+100 mM NaCl, 1.0 mM Putrescine+100 mM NaCl and 1.0 mM Putrescine+200 mM NaCl applications were the most effective applications against salt stress in lettuce among the applications examined in the study, and these applications could be recommended as an alternative application method for lettuce cultivation in areas with salinity problems.

Keywords: Germination, Growth, *Lactuca sativa* L., Putrescine, Salicylic acid, Salt stress

Farklı Putresin ve Salisilik Asit Uygulamalarının Tuzlu Şartlarda Marulun (*Lactuca sativa* L.) Çimlenme, Bitki Gelişimi, Kalite Özellikleri ve Besin Elementi İçeriği Üzerine Etkileri

Öz

Amaç: Bu çalışma, marulda tuzlu şartlarda farklı dozlarda putresin ve salisilik asit uygulamalarının çimlenme, bitki gelişimi, kalite özellikleri ve besin elementi içeriği üzerine etkilerini belirlemek amacıyla yapılmıştır.

Materyal ve Yöntem: İklim odası koşullarında yürütülen çalışmada 2 farklı tuz seviyesi (100 ve 200 mM), 2 farklı putresin dozu (0.5 ve 1.0 mM) ve 2 farklı salisilik asit dozunun (0.5 ve 1.0 mM) kullanıldığı toplam 15 farklı uygulama ele alınmıştır. Putresin ve salisilik asidin tek başına ve tuzla birlikte etkileri incelenmiştir.

Araştırma Bulguları: Araştırmadan elde edilen bulgulara göre tuzluluğun marulda çimlenme, bitki gelişimi, kalite özellikleri ve besin elementi içeriğini olumsuz etkilediği belirlenmiştir. Genel olarak tuzlu şartlarda putresin uygulamalarının çimlenme ve bitki

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gelişim parametrelerinde artış sağladığı ve tuz stresinin meydana getirdiği olumsuz etkileri önemli ölçüde azalttığı tespit edilmiştir. Ayrıca tuz ve putresinin birlikte kullanıldığı 1.0 mM Putresin+200 mM NaCl uygulamasının 200 mM NaCl uygulamasına göre çimlenme oranını %75.57, bitki boyunu %42.49, bitki yaş ağırlığını %58.89 ve pazarlanabilir yaprak sayısını %43.20 oranında artırdığı saptanmıştır.

Sonuç: Çalışmada ele alınan uygulamalar arasında 0.5 mM Putresin+100 mM NaCl, 1.0 mM Putresin+100 mM NaCl ve 1.0 mM Putresin+200 mM NaCl uygulamalarının marulda tuz stresine karşı en etkili uygulamalar olduğu ve bu uygulamaların tuzluluk sorunu olan alanlarda marul yetiştiriciliğinde alternatif bir uygulama yöntemi olarak önerilebileceği sonucuna varılmıştır.

Anahtar Kelimeler: Büyüme, Çimlenme, *Lactuca sativa* L., Putresin, Salisilik asit, Tuz stresi

Introduction

Salinity is one of the most important abiotic stress factors that negatively affect growth, development and yield of plant and cause crop loss (Allakhverdiev et al., 2000). Around the world, approximately 20% of agricultural lands and 50% of irrigated lands are affected by salinity problem (Zhu, 2001). It is reported that about half of the crop potential in agriculturally important plants is lost due to salinity (Kreps et al., 2002). Nowadays, soil salinity is considered as an important threat in terms of the future of crop production in many fertile regions of the world (Serrano and Rodriguez, 2002). Salinity limits agricultural production, particularly in developing countries (El-Tayeb, 2005). Generally, seed germination is inhibited, growth rate slows down, yield and quality decrease under salty conditions (Dölarslan and Gül, 2012). This situation can lead to great economic losses.

Salt stress is defined as the presence of various salts in water or soil in concentrations that can inhibit plant growth. The most common salt form in nature is sodium chloride (NaCl) (Zhu, 2003). Plants are divided into two groups as halophytes (salt-tolerant) and glycophytes (salt-sensitive) according to their response to salt (Yılmaz et al., 2011). Most of the economically valuable plants are sensitive to salt stress. In general, vegetables are more sensitive to salinity than other cultivated plants. In vegetables, a decrease in yield begins at salinity around 1.0-3.8 dS m^{-1}. Under salt stress, some physiological events in plants such as respiration, photosynthesis, transpiration, osmotic pressure, protein synthesis, enzyme activity and water intake are adversely affected. Although there is sufficient water in the soil, the plant roots cannot take the water in the soil due to the high osmotic pressure with the increase of salt concentration in the soil and physiological drought occurs (Esechie, 1994). Accordingly, the transport of nutrients to the plant also reduces (Güneş et al., 2010). It is reported that salt stress in plants causes stunting, reduction in root growth, small leaves and fruits, formation of necrotic spots, wilting and drying of the leaves, decreases in shoot growth, seed yield, number of leaves, chlorophyll amount, plant fresh and dry weight (Munns, 2003). These negative effects of salinity may vary depending on the plant species and variety, development period of the plant, amount of salt, type of salt and duration of salinity (Çulha and Çakırlar, 2011). Salinity is one of the biggest problems encountered in Turkey as well as all over the world. The areas affected by salinity in our country are constantly increasing. There is a salinity problem in about 1.5 million ha of the land in Turkey (Okur and Örçen, 2020). Therefore, considering the limited agricultural lands in Turkey, successful strategies are needed to increase salt tolerance in agriculturally important plants. The improvement of saline soils and the development of salt-resistant varieties is time consuming and expensive. Therefore, the external applications of plant growth regulators such as polyamines and salicylic acid are seen as rapid and alternative approaches to reduce the negative effects of salt stress on market value crops (Senaratna et al., 2000). The efficiency of polyamines and salicylic acid depends on many factors such as species, variety, plant growth period, environmental conditions, application method, duration and concentration. Polyamines occur naturally in plants and are among plant growth regulators. Polyamines are low molecular weight organic compounds. Polyamines play an important role in resistance to many abiotic stresses such as salinity, drought, extreme temperature, acid stress, oxygen deficiency and heavy metals (Gupta et al., 2013). It is reported that exogenous polyamine applications greatly improve growth and development of plant under different stress conditions (Xu et al., 2011). In plants, polyamines can be effective in many physiological events such as germination, root and shoot growth, flowering, ripening, aging, nucleic acid and protein synthesis (Kusano et al., 2008). Polyamines have anti-stress and antisenescence effects (Zhao and Yang, 2008). Polyamines accumulate internally in response to stress in plants under many abiotic stress conditions and function as stress messengers (Xu et al., 2011). The most common polyamines in plants are putrescine, spermine, and spermidine (Takahashi and Kakehi, 2010). Among the polyamines, putrescine is usually present in the highest proportion (Kalac and Krausova, 2005). In recent years, it has been stated that polyamines can be used to increase the salt tolerance of plants under salty conditions and to reduce the negative effects of salt stress on germination and plant growth (Singh and Gautam, 2013).

Salicylic acid is an endogenous plant growth regulator naturally found in many plants and it is a safe compound. Salicylic acid is a phenolic compound produced by plants (Rivas-San Vicente and Plasencia, 2011). Since salicylic acid activates the plant's defense mechanisms under biotic and abiotic stress conditions and protects the plant against stress, researchers have focused on exogenous salicylic acid applications to plants, especially under stress conditions (Senaratna et al., 2000). Salicylic acid plays important role in the regulation of many physiological events such as seed germination, plant growth and development, respiration, photosynthesis, transpiration, opening and closing of stomas, flowering, resistance to diseases, enzyme activity, senescence and nutrient uptake (Hayat et al., 2010; Rivas-San Vicente and Plasencia, 2011). Salicylic acid is a signal molecule that is very important in plant defense responses against many biotic and abiotic stress factors (Miura and Tada, 2014). Accordingly, it is reported that salicylic acid can increase the tolerance of plants under stress conditions (Hayat et al., 2010).

Lettuce (*Lactuca sativa* L.) is an annual winter vegetable. It belongs to the Compositae family. Lettuce has been cultivated for many years in the world, can be found in markets throughout the year. Fresh leaves of lettuce are used as vegetable. Lettuce is one of the most produced and consumed winter vegetables in Turkey and it has high economic value. It can be grown in the open field or greenhouse all over the country throughout the year. In Turkey, total lettuce production was 540.569 tons in 2021 (TÜİK, 2022). Lettuce is among the vegetables sensitive to salinity (Güneş et al., 2010; Dölarslan and Gül, 2012).

Studies are needed to determine the effects of exogenous putrescine and salicylic acid applications on germination and plant growth in order to improve the yield and quality of lettuce under salty conditions. Considering the threat posed by salinity for our country and the contribution of lettuce to the country's economy, the importance of such studies is increasing.

The objective of this study was to investigate the effects of exogenous putrescine and salicylic acid applications at different doses under saline conditions on germination, plant growth, quality properties and nutrient contents in lettuce, which is widely grown in our country and is one of the vegetables sensitive to salinity.

Material and Methods

The research was conducted in the climate room and laboratories of Bolu Abant Izzet Baysal University, Faculty of Agriculture, Department of Horticulture in 2021.

Material

Curly lettuce (*Lactuca sativa* L. var. *crispa* cv. Arapsaçı) was used as plant material in the study. Salt (NaCl) used as chemical material in the study was obtained from Isolab company, putrescine and salicylic acid were obtained from Sigma-Aldrich company.

Salt, Putrescine and Salicylic Acid Applications

In the study, a total of 15 different applications using two different salt levels (100 and 200 mM), two different putrescine doses (0.5 and 1.0 mM) and two different salicylic acid doses (0.5 and 1.0 mM) were investigated (Table 1).

Table 1. Applications used in the study, their contents and abbreviations

Application No.	Content	Abbreviation
1	Control	Control
2	100 mM NaCl	100 NaCl
3	200 mM NaCl	200 NaCl
4	0.5 mM Putrescine	0.5 Put
5	1.0 mM Putrescine	1.0 Put
6	0.5 mM Salicylic Acid	0.5 SA
7	1.0 mM Salicylic Acid	1.0 SA
8	0.5 mM Putrescine+100 mM NaCl	0.5 Put+100 NaCl
9	0.5 mM Putrescine+200 mM NaCl	0.5 Put+200 NaCl
10	1.0 mM Putrescine+100 mM NaCl	1.0 Put+100 NaCl
11	1.0 mM Putrescine+200 mM NaCl	1.0 Put+200 NaCl
12	0.5 mM Salicylic Acid+100 mM NaCl	0.5 SA+100 NaCl
13	0.5 mM Salicylic Acid+200 mM NaCl	0.5 SA+200 NaCl
14	1.0 mM Salicylic Acid+100 mM NaCl	1.0 SA+100 NaCl
15	1.0 mM Salicylic Acid+200 mM NaCl	1.0 SA+200 NaCl

The effects of putrescine and salicylic acid separately and together with salt were examined. Salt, putrescine and salicylic acid were not added to the control application.

Germination Study

Germination study was carried out in the laboratory to determine the effects of salt stress, putrescine and salicylic acid applications on germination parameters in lettuce seeds. Germination experiment was established in completely randomized design with 4 replications for each application and 50 seeds in each replication. The experiment was carried out in a germination cabinet at 22 °C for 10 days under dark conditions. Before germination, the seeds were sterilized in 5% sodium hypochlorite (NaClO) solution for 10 minutes and then the seeds were washed with distilled water. Afterwards, the seeds were dried on filter papers at room temperature. For germination, 9 cm diameter glass petri dishes were used. Seeds were placed on filter papers in petri dishes, with 50 seeds in each petri dish, and 15 ml of the prepared solutions were added to it. Then the petri dishes were closed and left to germinate in the germination cabinet. Only distilled water was added to the control application. When the moisture in the germination medium decreased and it started to dry, the equal amount of solution was added to petri dishes again. Seeds with 1-2 mm long radicles were considered sufficient for germination. Germinated seeds were counted at the same time each day and removed from the petri dish. At the end of the germination experiment (10th day), the parameters related to germination were determined.

Seed Sowing and Growing of Seedlings

The seedlings were grown in the climate room with 25±1°C temperature, 50-55% moisture and 14 hours light/10 hours dark period. Lettuce seeds were sown into plastic viols containing peat and perlite mixture in 3:1 ratio on 12 October 2021, and then irrigation was performed. Seedlings were ready for planting 25 days after sowing.

Growing of Plants

This experiment was conducted in the climate room with 20±1°C temperature, 50-55% moisture and 14 hours light/10 hours dark period. Plastic balcony pots (60 x 23 x 24 cm) were used for the cultivation of plants in the study. Each pot was filled with 19 liters of growing medium prepared with peat and perlite mixture (3:1, v/v). The experiment was established in completely randomized design with 3 replications. A total of 135 plants (15 x 3 x 3) were grown, with 3 plants in each replication. Lettuce seedlings were transplanted into pots with 3 seedlings in each pot at the 4-5 true leaf stage on 06 November 2021. After planting, the pots were placed on the shelves in the climate chamber. Life water was given immediately after planting the seedlings.

Putrescine, salicylic acid and salt applications were repeated 4 times at 1-week intervals 5 days after planting seedlings. Putrescine and salicylic acid solutions prepared at the doses discussed in the study were given to each plant as 10 ml in the 1st and 2nd applications, and 20 ml in the 3rd and 4th applications. Putrescine and salicylic acid solutions were applied to the plants by spraying in a way that the top and bottom of the leaves were thoroughly wet. Tween-20 (0.01%) was added into the solution to keep the solution on the leaf. The prepared salt solutions were given to each plant as 25 ml in the 1st and 2nd applications, and 50 ml in the 3rd and 4th applications. Tap water was used to irrigate the plants in the control application. After planting the seedlings, the water situation in the growing medium was checked and the plants were irrigated when needed. In the study, ammonium sulphate, triple super phosphate and potassium sulphate commercial fertilizers were applied as 15 kg N da^{-1}, 10 kg P_2O_5 da^{-1} and 15 kg K_2O da^{-1}. All of phosphorus and potassium fertilizers and half of nitrogen fertilizer were given at the time of planting, and the other half of nitrogen fertilizer was given two weeks after planting. The plants were harvested 34 days after planting on 09 December 2021. Harvested plants were brought to the laboratory of Department of Horticulture for necessary measurements and analyzes.

Measurements and Analyses Made in the Study

Germination rate was determined according to Ellis and Roberts (1981). Root collar diameter, radicle and plumule length were measured using a digital caliper. Plant height, root length, leaf length and width were detected by a ruler. Plant fresh weight and root fresh weight were determined by a precision balance. To determine plant dry weight and root dry weight, the samples were dried at 65 °C and then they were weighed by using a precision balance. The number of marketable leaves was detected by counting the edible leaves. The dry matter, ash and nitrogen contents of the samples were detected by using the procedures of AOAC (1990). The pH values of the samples were determined using a digital pH meter (Thermo Scientific, Orion Star A111). Electrical conductivity (EC) was detected using an EC meter (Thermo Scientific, Orion Star A212). The total soluble solid content was detected using a hand-held refractometer (ATC-1, Atago, Japan). The chlorophyll content of the leaves was determined with a chlorophyll meter (Apogee Chlorophyll Concentration Meter, MC-100). The color properties of the leaves (L*, a*, b*, C* and h°) were determined using a colorimeter (3NH NR60CP). To determine phosphorus, potassium, magnesium and calcium contents, dried samples were firstly ground by using a grinder (MC23200, Siemens, Germany) and then prepared for analysis according to wet digestion method. Element contents were detected using inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer, Optima 7000 DV) (Kacar and İnal, 2008).

Statistical Analysis

The data obtained in the study were subjected to variance analysis using the JMP 13.2 statistical program. Statistical differences among the means found to be significant in terms of the examined properties were determined by the Tukey HSD (Tukey's Honestly Significant Difference) multiple comparison test.

Results and Discussion

Effects of putrescine and salicylic acid applications at different doses on germination rate, radicle length and plumule length of lettuce under saline conditions are presented in Table 2. The difference among the applications in terms of germination rate, radicle length and plumule length was significant at the $P < 0.01$ level. In the study, the germination rate of lettuce seeds varied between 55.25% (200 NaCl) and 98.50 (0.5 Put+100 NaCl). It was determined that the germination rate in 200 NaCl and 1.0 SA applications was significantly lower than that of other applications. Except for 200 NaCl and 1.0 SA applications, the other applications in the study were statistically in the same group and they possessed the highest germination rate. Salinity greatly inhibited germination of lettuce seeds. With the increase in salinity, the germination rate of lettuce seeds decreased. When compared to the control, 200 NaCl application decreased germination rate by 75.11%. 1.0 Put, 0.5 SA, 0.5 Put+100 NaCl, 1.0 Put+100 NaCl and 1.0 Put+200 NaCl applications increased the germination rate compared with the control. It was observed that especially putrescine applications under saline conditions had positive effects on germination rate. Furthermore, the 1.0 Put+200 NaCl application, in which salt and putrescine were used together, increased the germination rate by 75.57% compared to the 200 NaCl application. Radicle and plumule length depending on the applications ranged from 2.54 to 13.59 mm and 6.72 to 41.52 mm, respectively. The highest radicle length was recorded in 0.5 Put+100 NaCl, 1.0 Put, 1.0 Put+100 NaCl, 1.0 Put+200 NaCl and 0.5 Put applications, which they were not statistically different, while the lowest radicle length was detected in 200 NaCl, 1.0 SA, 1.0 SA+100 NaCl and 0.5 SA+100 NaCl applications. The highest values regarding plumule length were found in 1.0 Put+100 NaCl, 1.0 Put, 0.5 Put+100 NaCl and 1.0 Put+200 NaCl applications, which they were statistically in the same group. However, the lowest plumule length was observed in 200 NaCl application. Radicle and plumule length decreased with increasing levels of salt stress. On the other hand, it was found that especially salt + putrescine combinations provided significant increases on these germination parameters compared to the control and only salt applications (100 NaCl and 200 NaCl). The 0.5 Put+100 NaCl application, in which salt and putrescine were used together, increased the radicle length by 46.92% in comparison with the 100 NaCl application. In addition, it was determined that 1.0 Put+100 NaCl application increased the plumule length by 27.83% compared to the 100 NaCl application. In the 1.0 Put application, in which putrescine was used alone without salt stress, higher results in terms of germination properties were obtained compared with the control (Table 2). Similar to the results obtained in this study, negative effects of salt stress on germination have been reported in previous studies on different vegetables (Dadaşoğlu and Ekinci, 2013; Koç et al., 2014; Mena et al., 2015; Mohamedsrajaden, 2019; Kibar et al., 2020). It was determined that putrescine application alone without salt stress increased the germination rate, radicle and plumule length in pepper compared to the control, and putrescine was more effective especially at low concentrations (Khan et al., 2012). In the study carried out by Zeid (2004) on bean, germination rate increased with the external putrescine application under salty conditions. Likewise, in studies conducted in pepper (Koç et al., 2014), tomato (Mohamedsrajaden, 2019), fresh bean (Kibar et al., 2020) and pumpkin (Farsaraei et al., 2021), it was stated that salinity negatively affected germination and the harmful effects of salt stress on germination parameters can be reduced with putrescine application.

Table 2. Effects of putrescine and salicylic acid applications at different doses on germination rate, radicle length and plumule length of lettuce under saline conditions

Applications	Germination rate (%)	Radicle length (mm)	Plumule length (mm)
Control	96.75a**	10.52ab**	31.65b**
100 NaCl	94.50a	9.25abc	32.48b
200 NaCl	55.25b	2.54d	6.72d
0.5 Put	96.25a	12.75a	31.28b
1.0 Put	97.50a	13.18a	40.76a
0.5 SA	97.00a	6.07bcd	23.40c
1.0 SA	57.00b	3.04d	20.37c
0.5 Put+100 NaCl	98.50a	13.59a	39.25a
0.5 Put+200 NaCl	93.50a	9.00abc	31.83b
1.0 Put+100 NaCl	98.00a	12.93a	41.52a
1.0 Put+200 NaCl	97.00a	12.79a	39.23a
0.5 SA+100 NaCl	94.50a	3.43d	33.32b
0.5 SA+200 NaCl	94.00a	4.90cd	24.85c
1.0 SA+100 NaCl	92.00a	3.34d	23.09c
1.0 SA+200 NaCl	91.50a	7.03bcd	31.91b

Means followed by different letters within the same columns are statistically different according to Tukey's honestly significant difference test. **: Significant at $P < 0.01$

Ekinci et al. (2011) reported that the highest germination rate was obtained from 0.50 mM salicylic acid application in parsley, lettuce and carrot seeds, and 0.1 mM salicylic acid application in cabbage seeds, and that high doses of salicylic acid reduced the germination rate of seeds. In another study, it was reported that salt stress greatly inhibited germination in different radish cultivars, 0.50 mM salicylic acid application gave positive results in terms of germination percentage under salt stress. In the study, it was also stated that 1 mM salicylic acid application inhibited germination (Ulukapı et al., 2020). In studies conducted on different vegetables, it was found that as salinity increased, the germination rate decreased, and salicylic acid applications under salt stress increased the germination rate (Entesari et al., 2012; Dadaşoğlu and Ekinci, 2013; Akbıyık, 2019). Our findings agreed with the results of previous researchers.

According to Table 3, the difference among the applications in terms of plant fresh weight, plant dry weight and root length was significant at the $P < 0.05$ level, while the difference among the applications in terms of plant height was significant at the $P < 0.01$ level. On the other hand, no statistically significant difference was found among the applications in terms of root collar diameter. Plant height ranged from 20.38 (200 NaCl) to 29.66 (0.5 Put+100 NaCl) cm depending on the applications. In parallel with the increasing salinity level, a significant decrease in plant height occurred. In 200 NaCl application, plant height decreased by 31.11% compared with the control. Putrescine applications under saline conditions increased plant height. Additionally, the 1.0 Put+200 NaCl application, in which salt and putrescine were used together, increased the plant height by 42.49% compared to the 200 NaCl application. The highest values regarding plant fresh weight were found in 1.0 Put+200 NaCl and 0.5 Put+100 NaCl applications (238.57 and 231.91 g, respectively), which did not differ statistically between them. However, the lowest plant fresh weight was recorded in 200 NaCl application with 150.15 g. With the increase in salinity, plant fresh weight significantly decreased. In the study, 1.0 Put, 0.5 SA, 1.0 SA, 0.5 Put+100 NaCl, 0.5 Put+200 NaCl, 1.0 Put+100 NaCl, 1.0 Put+200 NaCl and 0.5 SA+200 NaCl applications had higher plant fresh weight values than the control. Putrescine applications under saline conditions increased plant fresh weight. It was determined that 1.0 Put+200 NaCl application increased the plant fresh weight by 58.89% compared to the 200 NaCl application. With respect to plant dry weight, the highest values were obtained from 0.5 Put+100 NaCl and 1.0 Put+200 NaCl applications (9.54 and 9.51 g, respectively), whereas the lowest value was observed in 200 NaCl application (6.05 g). As the salinity increased, plant dry weight decreased. The 0.5 Put+100 NaCl application, in which salt and putrescine were used together, increased the plant dry weight by 27.88% compared to the 100 NaCl application. When the effect of different applications on the root length of lettuce was examined, the highest value was found in 1.0 Put application with 16.82 cm, though the lowest value was detected in 100 NaCl application with 9.12 cm (Table 3).

Similar to our results, Ekinci et al. (2019) determined that as the salt concentration increased, plant height, stem diameter, plant fresh weight and plant dry weight decreased in pepper, and putrescine applications in pepper plants grown under salt stress improved the examined parameters. In studies conducted on bean (Zeid, 2004) and tomato (Mohamedsrajaden, 2019), it was reported that putrescine application under both salty and normal conditions increased plant growth. Salem (2021) found that plant height, plant fresh weight, plant dry weight, root length and root collar diameter decreased with increasing salinity in lettuce, and salicylic acid applications under salt stress increased the mentioned plant growth parameters. In the study, it was also detected that 2 mM salicylic acid under saline conditions was the optimum dose. Likewise, in the study conducted by Shama et al. (2016) on garlic, plant height, leaf fresh weight and leaf dry weight decreased with salt stress, and salicylic acid applications under salty conditions increased mentioned plant growth properties. In studies conducted on different vegetables, it was determined that salinity negatively affected plant growth, and salicylic acid applications under salty conditions had positive effects on plant growth parameters such as plant height, leaf length, plant fresh weight and plant dry weight (Qingmao et al., 2007; Yıldırım et al., 2008; Bukhat et al., 2020; Ulukapı et al., 2020). The results obtained in this study are in accordance with the findings in the literature.

Table 3. Effects of putrescine and salicylic acid applications at different doses on plant height, plant fresh weight, plant dry weight, root length and root collar diameter of lettuce under saline conditions

Applications	Plant height (cm)	Plant fresh weight (g)	Plant dry weight (g)	Root length cm)	Root collar diameter (mm)
Control	26.72a**	188.66ab*	7.60ab*	13.90ab*	18.09[ns]
100 NaCl	25.98a	176.47ab	7.46ab	9.12b	17.93
200 NaCl	20.38b	150.15b	6.05b	11.76ab	17.97
0.5 Put	25.16ab	177.84ab	7.97ab	14.32ab	21.72
1.0 Put	27.32a	198.38ab	8.38ab	16.82a	19.52
0.5 SA	27.42a	210.77ab	8.96ab	14.96ab	21.38
1.0 SA	25.66ab	192.93ab	7.89ab	12.34ab	18.60
0.5 Put+100 NaCl	29.66a	231.91a	9.54a	12.86ab	18.39
0.5 Put+200 NaCl	27.00a	192.34ab	7.00ab	14.60ab	17.49
1.0 Put+100 NaCl	29.10a	206.51ab	8.07ab	15.00ab	18.26
1.0 Put+200 NaCl	29.04a	238.57a	9.51a	12.32ab	19.03
0.5 SA+100 NaCl	26.82a	181.84ab	7.35ab	14.10ab	20.33
0.5 SA+200 NaCl	25.58ab	195.43ab	8.27ab	12.32ab	20.18
1.0 SA+100 NaCl	26.36a	187.30ab	7.53ab	14.22ab	17.10
1.0 SA+200 NaCl	26.56a	186.35ab	7.33ab	13.84ab	17.67

Means followed by different letters within the same columns are statistically different according to Tukey's honestly significant difference test. *: Significant at $P < 0.05$, **: Significant at $P < 0.01$, ns: non-significant.

As seen in Table 4, the effect of the applications on the number of marketable leaves of lettuce was significant at the $P<0.05$ level, and the effect on the root dry weight was significant at the $P<0.01$ level. On the other hand, there was no statistically significant difference among the applications with respect to root fresh weight, leaf length and leaf width. Root dry weight was the highest in 0.5 SA+100 NaCl application (1.44 g). On the contrary, root dry weight was the lowest in 100 NaCl application (0.70 g). The number of marketable leaves in lettuce varied from 25.00 to 35.80 depending on the applications. The highest value was recorded in 1.0 Put+200 NaCl application. However, 200 NaCl application was found to have the lowest value. Putrescine applications under salt stress significantly increased the number of marketable leaves compared to the control and only salt applications (100 NaCl and 200 NaCl). It was found that 1.0 Put+200 NaCl application increased the number of marketable leaves by 43.20% in comparison with 200 NaCl application (Table 4). In a study on tomato, it was determined that number of leaves, root fresh and dry weight significantly decreased with salinity, and putrescine applications under salt stress increased the mentioned properties, which was agreed with the findings of our study (Mohamedsrajaden, 2019). Similarly, in studies conducted on pepper (Ekinci et al., 2019) and corn (Biçer, 2016), salt stress reduced number of leaves, root fresh and dry weight, and putrescine applications under salty conditions improved the mentioned plant growth parameters. Zurnacı (2019) and Salem (2021) found that number of leaves, root fresh and dry weight decreased with increasing salinity in lettuce, and salicylic acid applications under salt stress increased these properties. Our findings were found to be compatible with previous studies. On the other hand, Karasakal (2020) stated that salicylic acid applications under unsalted conditions in lettuce reduced number of leaves, leaf length and leaf width.

Table 4. Effects of putrescine and salicylic acid applications at different doses on root fresh weight, root dry weight, leaf length, leaf width and number of marketable leaves of lettuce under saline conditions

Applications	Root fresh weight (g)	Root dry weight (g)	Leaf length (cm)	Leaf width (cm)	Number of marketable leaves (number plant^{-1})
Control	4.35^{ns}	0.94efg**	22.08^{ns}	15.80^{ns}	30.20ab*
100 NaCl	3.26	0.70ı	21.70	14.60	27.80ab
200 NaCl	4.12	0.89fgh	21.14	14.23	25.00b
0.5 Put	6.38	1.38ab	21.72	15.86	27.20ab
1.0 Put	4.55	0.99efg	21.56	15.04	31.60ab
0.5 SA	5.87	1.28bc	22.14	18.20	30.20ab
1.0 SA	4.74	1.02efg	20.68	15.82	29.20ab
0.5 Put+100 NaCl	5.37	1.17cd	22.22	16.02	33.40ab
0.5 Put+200 NaCl	4.43	0.95efg	21.48	16.98	31.00ab
1.0 Put+100 NaCl	4.72	1.03def	22.86	15.42	33.20ab
1.0 Put+200 NaCl	4.85	1.04de	22.40	15.46	35.80a
0.5 SA+100 NaCl	6.60	1.44a	21.20	16.20	28.40ab
0.5 SA+200 NaCl	3.95	0.88gh	21.42	17.56	30.20ab
1.0 SA+100 NaCl	5.35	1.17cd	21.08	16.18	26.80ab
1.0 SA+200 NaCl	3.65	0.78hı	21.90	16.74	29.60ab

Means followed by different letters within the same columns are statistically different according to Tukey's honestly significant difference test. *: Significant at $P < 0.05$, **: Significant at $P < 0.01$, ns: non-significant.

While the difference among applications was statistically significant ($P<0.05$) only in terms of h° color value, the effect of applications on other color properties (L*, a*, b* and C*) was found to be insignificant. Depending on the applications evaluated in the study, the h° value varied between 109.45 (0.5 Put+200 NaCl) and 113.71 (1.0 Put+200 NaCl). In the study, the h° value was determined to be between yellow (90º) and green (180º) (Table 5).Color is one of the important quality characteristics of lettuce. Kibar et al. (2020) found that the differences among salt, putrescine and salt+putrescine applications were significant in terms of a*, b* and C* color values, while the differences among the applications were insignificant in terms of L* and h° color values in fresh bean.

Table 5. Effects of putrescine and salicylic acid applications at different doses on color properties of lettuce under saline conditions

Applications	L*	a*	b*	C*	h°
Control	55.16^{ns}	-14.72^{ns}	34.62^{ns}	37.62^{ns}	112.99ab*
100 NaCl	52.83	-12.97	32.31	34.82	111.85ab
200 NaCl	52.44	-14.17	33.62	36.46	112.87ab
0.5 Put	53.67	-12.07	31.78	34.02	110.60ab
1.0 Put	52.53	-12.59	31.37	33.82	111.83ab
0.5 SA	52.40	-14.87	35.21	38.23	112.95ab
1.0 SA	53.49	-14.12	33.51	37.97	112.86ab
0.5 Put+100 NaCl	53.58	-13.62	34.17	36.79	111.90ab
0.5 Put+200 NaCl	56.74	-12.88	36.03	38.29	109.45b
1.0 Put+100 NaCl	55.23	-13.64	36.31	38.80	110.72ab
1.0 Put+200 NaCl	53.74	-15.06	34.36	37.53	113.71a
0.5 SA+100 NaCl	54.54	-12.87	34.20	36.55	110.85ab
0.5 SA+200 NaCl	52.89	-14.20	32.88	35.78	113.16ab
1.0 SA+100 NaCl	56.23	-14.11	33.81	36.34	111.45ab
1.0 SA+200 NaCl	52.23	-14.32	35.05	37.87	112.25ab

Means followed by different letters within the same columns are statistically different according to Tukey's honestly significant difference test. *: Significant at $P < 0.05$, ns: non-significant.

The difference among the applications in terms of chlorophyll content was significant at the $P < 0.05$ level, while the differences among the applications in terms of dry matter content, total soluble solid content, ash content, pH and electrical conductivity (EC) were significant at the $P < 0.01$ level. Among the applications, the highest chlorophyll content was determined in 1.0 SA+200 NaCl and 1.0 Put+200 NaCl applications (26.58 and 26.02 spad, respectively). Conversely, the lowest chlorophyll content was observed in 200 NaCl application (18.10 spad). In the study, higher chlorophyll values were obtained from the 1.0 SA, 1.0 Put+200 NaCl and 1.0 SA+200 NaCl applications compared with the control. The 1.0 SA+200 NaCl application increased the chlorophyll content by 46.85% compared to the 200 NaCl application. When the effect of different applications on dry matter content in lettuce was examined, 1.0 SA+200 NaCl application took the first place with 6.05% and 0.5 SA application followed closely it. On the other hand, 200 NaCl application had the lowest value with 4.30%. The 1.0 SA+200 NaCl application provided an increase of 40.70% in terms of dry matter content compared to the 200 NaCl application. The highest total soluble solid content was observed in 0.5 SA+100 NaCl application with 3.80%, though the lowest total soluble solid content was found in 0.5 Put+200 NaCl application with 2.30%. Ash content in lettuce plants varied between 21.01% and 25.38%, the highest value was observed in 0.5 SA+100 NaCl application, whereas lowest values were detected in 200 NaCl, 0.5 Put and 1.0 SA+200 NaCl applications which did not differ statistically among them. In the present study, the pH value of lettuce plants ranged from 5.90 (100 NaCl) to 6.25 (0.5 SA+100 NaCl). With respect to EC, 1.0 Put+200 NaCl application took the first place with 469 μS cm^{-1}, and it was closely followed by 200 NaCl application. The lowest EC was observed in control application with 307 μS cm^{-1}. It was found that the EC value in salt-containing applications was significantly higher than in the control. As the salinity level increased, the EC values also increased. Additionally, EC level in 1.0 Put+200 NaCl application was found to be 52.77% higher compared to the control, in which no salt was given (Table 6). Similar to the results of this study, it was determined that salinity significantly decreased chlorophyll content in studies performed on bean, tomato, corn and cucumber, and chlorophyll content increased with exogenous putrescine application (Zeid, 2004; Shu et al., 2012; Biçer, 2016; Mohamedsrajaden, 2019). In the study carried out by Eraslan et al. (2007) in lettuce, it was stated that EC values of the plant increased under salt stress. In another study, EC value of tomato plants significantly increased with the increase in salt concentration, and putrescine applications under both salty and unsalted conditions decreased EC values compared to the control (Mohamedsrajaden, 2019). In our study, the EC content of the plant in putrescine applications was found to be higher than the control. Kibar et al. (2020) found that as the salt concentration increased, chlorophyll content, dry matter content and pH values decreased, while the EC value increased in fresh bean. Researchers also determined that putrescine applications under salt stress increased dry matter content and chlorophyll content. In studies conducted on cucumber (Yıldırım et al., 2008), radish (Bukhat et al., 2020) and lettuce (Salem, 2021), chlorophyll content decreased with increasing salinity, and salicylic acid applications under salt stress increased chlorophyll content. It was found that salicylic acid applications in tomato did not affect the pH, but it increased the chlorophyll content and total soluble solid content (Yıldırım and Dursun, 2009). In a study conducted on garlic, it was detected that salicylic acid applications under salt stress increased the dry matter content (Shama et al., 2016). The findings obtained from this study are consistent with the results of other researchers.

Table 6. Effects of putrescine and salicylic acid applications at different doses on chlorophyll content, dry matter content, total soluble solid content, ash content, pH and electrical conductivity of lettuce under saline conditions

Applications	**Chlorophyll content (spad)**	**Dry matter content (%)**	**Total soluble solid content (%)**	**Ash content (%)**	**pH**	**EC (μS cm^{-1})**
Control	24.14ab*	5.26cd**	2.97bc**	23.94cd**	6.09bcd**	307g**
100 NaCl	22.18ab	4.47ef	2.83cd	23.64cd	5.90e	376ef
200 NaCl	18.10b	4.30f	2.93bc	21.14h	6.05cde	459ab
0.5 Put	22.56ab	5.35c	2.93bc	21.01h	6.17abc	396c-f
1.0 Put	21.18ab	5.42bc	2.57de	22.22f	6.16abc	430a-d
0.5 SA	21.60ab	5.96ab	3.17b	23.98c	6.24ab	360f
1.0 SA	24.86ab	5.47bc	3.07bc	21.71g	6.18abc	428a-d
0.5 Put+100 NaCl	24.00ab	4.71def	2.97bc	24.75b	6.11a-d	418b-e
0.5 Put+200 NaCl	22.66ab	4.50ef	2.30e	22.71e	6.07cd	433a-d
1.0 Put+100 NaCl	24.08ab	4.78def	2.97bc	23.50d	6.16abc	400c-f
1.0 Put+200 NaCl	26.02a	5.24cd	2.87c	24.67b	6.00de	469a
0.5 SA+100 NaCl	22.34ab	5.16cd	3.80a	25.38a	6.25a	373ef
0.5 SA+200 NaCl	22.62ab	4.92cde	3.20b	24.64b	6.06cd	443abc
1.0 SA+100 NaCl	23.30ab	5.35c	2.97bc	22.74e	6.10a-d	390def
1.0 SA+200 NaCl	26.58a	6.05a	2.87c	21.23h	6.11a-d	402c-f

Means followed by different letters within the same columns are statistically different according to Tukey's honestly significant difference test. *: Significant at $P < 0.05$, **: Significant at $P < 0.01$.

There were significant differences ($P < 0.01$) among the applications in terms of nitrogen, phosphorus, potassium, calcium, and magnesium contents of lettuce. Nitrogen content of lettuce plants belonging to different applications varied between 4.08% and 5.29%. Nitrogen content was the highest in 0.5 SA+100 NaCl application. However, 200 NaCl application was found to have the lowest nitrogen value. In 0.5 Put and 0.5 SA+100 NaCl applications, higher nitrogen values were obtained than the control. The maximum and minimum phosphorus contents were obtained from 1.0 Put+200 NaCl application (1487 mg 100 g^{-1}) and 200 NaCl application (1094 mg 100 g^{-1}), respectively. Phosphorus content in 0.5 Put, 1.0 Put, 0.5 SA, 1.0 SA, 0.5 Put+100 NaCl, 1.0 Put+100 NaCl, 1.0 Put+200 NaCl and 1.0 SA+200 NaCl applications was found to be higher than in the control. In the present study, it was determined that 1.0 Put+200 NaCl application increased the phosphorus content by 35.92% in comparison with the 200 NaCl application. The highest value regarding potassium content was found in 0.5 Put application (8775 mg 100 g^{-1}), while the lowest value was observed in 200 NaCl application (6873 mg 100 g^{-1}). In terms of potassium content, the higher values were obtained from 0.5 Put, 1.0 Put, 1.0 Put+100 NaCl and 1.0 Put+200 NaCl applications compared with the control. Among the applications examined in the study, the highest calcium content was determined in 1.0 Put+100 NaCl application (2277 mg 100 g^{-1}), though the lowest calcium content was observed in 100 NaCl and 200 NaCl applications (1531 and 1487 mg 100 g^{-1}, respectively), which they were statistically in the same group. Calcium content in 0.5 Put, 0.5 Put+100 NaCl, 0.5 Put+200 NaCl, 1.0 Put+100 NaCl, 1.0 Put+200 NaCl, 0.5 SA+100 NaCl, 0.5 SA+200 NaCl and 1.0 SA+200 NaCl applications was found to be higher than in the control. The 1.0 Put+100 NaCl application increased the calcium content by 48.73% compared to the 100 NaCl application. When the magnesium content of lettuce plants belonging to different applications was examined, the highest values were found in 0.5 Put+200 NaCl and 1.0 Put+100 NaCl applications (539 and 543 mg 100 g^{-1}, respectively), which did not differ statistically between them. Conversely, the lowest magnesium content was detected in 200 NaCl application with 379 mg 100 g^{-1}. When compared to the control, the higher values in terms of magnesium content were obtained from 1.0 Put, 0.5 SA, 0.5 Put+200 NaCl, 1.0 Put+100 NaCl and 1.0 Put+200 NaCl applications. Magnesium content in 1.0 Put+100 NaCl application was found to be 26.57% higher than in 100 NaCl application (Table 7).

In the study conducted by Biçer (2016) on corn, it was reported that salt stress decreased calcium and potassium contents while it increased sodium content. Researcher also stated that putrescine application under salty conditions increased calcium and potassium contents, and the best result was found in 0.5 mM putrescine application.

Table 7. Effects of putrescine and salicylic acid applications at different doses on nitrogen, phosphorus, potassium, calcium and magnesium contents of lettuce under saline conditions

Applications	**N (%)**	**P (mg 100 g^{-1})**	**K (mg 100 g^{-1})**	**Ca (mg 100 g^{-1})**	**Mg (mg 100 g^{-1})**
Control	4.89b**	1296b-e**	8252cd**	2027de**	511abc**
100 NaCl	4.75bcd	1150fg	7513h	1531f	429de
200 NaCl	4.08e	1094g	6873k	1487f	379e
0.5 Put	4.94b	1365b	8775a	2181ab	471bcd
1.0 Put	4.59cd	1350bcd	8293cd	2018e	517abc
0.5 SA	4.84bc	1359bc	8041e	2017e	517abc
1.0 SA	4.74bcd	1304bcd	7667fg	1990e	490a-d
0.5 Put+100 NaCl	4.84bc	1336bcd	7736f	2097b-e	493abc
0.5 Put+200 NaCl	4.59cd	1273de	7051j	2081b-e	539a
1.0 Put+100 NaCl	4.76bcd	1323bcd	8306c	2277a	543a
1.0 Put+200 NaCl	4.87bc	1487a	8595b	2166abc	532ab
0.5 SA+100 NaCl	5.29a	1284cde	8168de	2085b-e	498abc
0.5 SA+200 NaCl	4.59cd	1218ef	7339ı	2067cde	492abc
1.0 SA+100 NaCl	4.54d	1217ef	7445hı	2000e	484a-d
1.0 SA+200 NaCl	4.84bc	1306bcd	7559gh	2134bcd	469cd

Means followed by different letters within the same columns are statistically different according to Tukey's honestly significant difference test. **: Significant at $P < 0.01$.

Similar to the findings obtained in this study, Salem (2021) determined that nitrogen, phosphorus, potassium, calcium and magnesium contents of lettuce decreased with increasing salinity, and salicylic acid applications under salt stress increased the mentioned element contents. Güneş et al. (2007) found that salicylic acid application in corn plants under salt stress decreased sodium, chlorine, phosphorus and potassium contents, while it increased nitrogen, magnesium, iron, manganese and copper concentrations. In the study conducted by Yıldırım et al. (2008) on cucumber, it was found that salt stress negatively affected the mineral uptake of plants, and salicylic acid applications under salt stress increased nitrogen, phosphorus, potassium, calcium and magnesium contents in the leaves and roots of plants, while it decreased sodium content. Researchers also stated that the highest values in terms of nutrient content were obtained from the 1 mM salicylic acid application. In another study carried out in lettuce, it was reported that as the salt dose increased, potassium and zinc contents decreased and sodium content increased, and salicylic acid application under saline conditions increased potassium, magnesium and calcium contents (Zurnacı, 2019).

Conclusion

The external use of growth regulators such as putrescine and salicylic acid in agricultural plants to protect from the damages caused by salt stress is among the effective approaches that enable plants to develop resistance to stress. In this study, the effects of exogenous putrescine and salicylic acid applications at different doses on germination, growth and quality of lettuce under saline conditions were investigated. The results indicated that the putrescine and salicylic acid applications evaluated in the study significantly affected germination, plant growth, quality properties and element contents in lettuce. It was found that salinity greatly inhibited germination of seeds and negatively affected plant growth and quality properties of lettuce. As the salt concentration increased, the germination rate, radicle length, plumule length, plant height, plant fresh weight and plant dry weight significantly decreased. Especially at the highest salt level (200 mM NaCl), the damage of salt stress was found to be at the highest level. Putrescine applications under salt stress generally increased germination and plant growth parameters and considerably reduced the negative effects of salt stress. It was detected that 0.5 Put+100 NaCl, 1.0 Put+100 NaCl and 1.0 Put+200 NaCl applications, in which salt and putrescine were used together, were determined as the most effective applications against salt stress, and these applications could be used to increase germination, growth and quality of lettuce under saline conditions. Additionally, the higher values in terms of germination properties, some plant growth parameters and element contents were obtained from the 1.0 Put application, in which putrescine was used alone without salt stress, compared with the control. When putrescine doses (0.5 and 1.0 mM) were evaluated, it was determined that 1 mM dose was more effective on germination and plant growth under both saline and normal conditions compared to 0.5 mM dose. Consequently, putrescine can be recommended as an alternative application method to reduce the negative effects of salt stress in lettuce cultivation in areas with salinity problems. The findings obtained from this study will be beneficial in terms of minimizing of salt damage, increasing of yield and quality, and reducing product losses under salty conditions.

Conflict of Interest

There is no conflict of interest between authors of the article.

Author Contribution Statement

OK: He contributed to the carrying out of the experiment and performing of laboratory studies. BK: She contributed to the design of the study, statistical analysis and writing of the manuscript.

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