



EFFECT OF SALT STRESS ON MORPHOLOGICAL CHARACTERISTICS AND SECONDARY METABOLITES OF SOME FORAGE PEA CULTIVARS

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Abstract: Forage pea (*Pisum sativum* L.) is an annual legume forage crop grown in various regions of Türkiye. It is high in protein, carbohydrate, and digestible matter and contains minerals such as phosphorus, calcium, and vitamins A and D. Salinity stress is an important problem in the cultivation of forage peas. Salinity reduces the osmotic potential of soil solutes, making it difficult for the roots to absorb the water. This study aimed to determine some parameters of two registered forage pea cultivars at different concentrations of two salt types. The effects of these salts on the morphological characteristics and biochemical components of two different registered cultivars of pea, cv. Ateş and cv. Töre were investigated in the present study. The trials were conducted in pots and Na₂SO₄ and CaCl₂ were applied at concentrations of 0, 50, 100 and 150 mM. As a result of the trials, the morphological characteristics like fresh and dry weights and lengths of roots and shoots were investigated along with the biochemical properties like total antioxidant activity and total phenolic content. The study was performed in 2 replicates to determine the effect of different salt types and concentrations. The critical salt concentration values for the change in shoot and root fresh weight among morphological traits were determined as 100 and 150 mM for secondary metabolites. While the cv. Töre forage pea showed the highest salt resistance in shoot and root fresh weights in the presence of Na₂SO₄ the cv. Ateş forage pea showed the lowest salt resistance in the presence of CaCl₂. In terms of shoot and root dry weights, the cv. Töre forage pea showed the least resistance at 50 mM Na₂SO₄ concentration. As for plant length, the cv. Ateş forage pea cultivar showed the least resistance in shoot length at 150 mM CaCl₂ concentration, while it showed the highest resistance in root length at this value. The highest total antioxidant activity for the cv. Ateş forage pea and the highest total phenolic content for the cv. Töre forage pea were determined at 150 mM CaCl₂ concentration. The lowest total phenolic content value was estimated in the cv. Töre forage pea cultivar at 150 mM Na₂SO₄ salt concentration.


Keywords: Salt stress, Morphological properties, Total phenolic content, Radical scaving activity

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1. Introduction

Forage pea (*Pisum sativum* L.) is an annual legume fodder plant known as "külür" or "kürül" in various regions of Türkiye. It is highly suitable for feeding all livestock. In European Union countries, it is an alternative to soybean due to its protein content (21-25%). In addition, it is suitable for consumption as livestock feed due to its high level of carbohydrates and digestible substances (86-87%) (Ouafi et al., 2016; Çağan et al., 2018). Forage peas have the ability to bind nitrogen (5-15 kg ha⁻¹) from the air to the soil and thus the need for excess nitrogen fertiliser in agriculture is decreased. In addition to minerals such as phosphorus and calcium, it also contains vitamins A and D (Tekeli and Ateş, 2003; Uzun et al., 2012; Ouafi et al., 2016). Salinity is an important problem especially for forage peas (Arslan et al., 2013). It is the primary factor that will directly affect crop yield nowadays and in the future (Mohamed and Aly, 2008;

Kang et al., 2010; Bu et al., 2015; Korkmaz and Durmaz, 2017). Currently, more than 6 per cent of the world's land area and 20 per cent of the world's irrigated land are affected by salinity. Salinity, even in well-watered soils, causes water deficit by reducing the osmotic potential of solutes in the soil, thus making it difficult for roots to draw water from the surrounding medium (Rhoades, 1988; Izadi et al., 2014). According to FAO's 2018 world soil salinity data, the soil salinity rate is increasing and it is thought that the salinity increase rate will reach up to 50% by 2050 (Kang et al., 2010; Tiryaki, 2018; Demirkol et al., 2019).

It was reported that Australia, China, Egypt, India, Iraq, Iraq, Mexico, Pakistan, Russia, and Syria as well as our country are facing salt stress (Rhoades, 1988). Approximately 1.5 million hectares of our country are under salinity stress. Of the 230 million hectares of irrigated land, 45 million hectares are affected by salt



stress. It is thought that a salt problem of this size will cause great economic losses in parallel with the loss in crop yield and quality (Bu et al., 2015; Dogru and Canavar, 2020). Salinity is naturally present in arid and semi-arid climate zones, and insufficient rainfall and high evaporation, poor drainage, improper agricultural practices and soil properties induce the salinity problem (Roy et al., 2014; Tiryaki, 2018; Zambani and Aşçı, 2020).

High salinity causes both ionic and osmotic stresses, leading to secondary stresses such as oxidative stress and nutritional disorders. Moreover, with increasing salt concentration in the soil, plant water uptake becomes difficult and plant growth slows down due to the deterioration of soil structure (Arslan et al., 2013; Bu et al., 2015).

There are also different plant mechanisms against salt stress such as osmotic effect, ion excretion and tissue tolerance. Osmotic effect decreases the water availability of plants as a result of increased salt concentration in the soil. The ion excretion mechanism reduces the accumulation of toxic salts in the leaves. Tissue tolerance is the growth retardation observed in plants in the face of salt stress (Arslan et al., 2013; Roy et al., 2014; Tiryaki, 2018).

Plants produce secondary metabolites as a defense mechanism against pathogens and insects. These metabolites can also be formed under different environmental stress conditions (e.g. salinity) and they contain the majority of plant antioxidants. Phenolic compounds are one of the secondary metabolites produced in plant tissues to scavenge free radicals and/or inhibit their production through hydroperoxide decomposition (Mohamed and Aly, 2008; Boughalleb et al., 2020). Salinity, one of the abiotic stress factors, increases the synthesis of phenolic compounds such as phenolic acids, flavonoids, proanthocyanins, and anthocyanins in plants (Tetiktabanlar et al. 2020). Phenolic compounds can chelate heavy metals with hydroxyl and carboxyl groups. They can prevent lipid peroxidation by capturing lipid alkoxy radicals (Michalak, 2006). Many studies have shown that peroxidase and polyphenol oxidase enzymes involved in the synthesis of phenolic compounds increase under biotic and abiotic stress conditions (Ruiz et al., 2003). Kıpçak et al. (2019) found that total phenolic content in green parts of bean cultivars treated with different concentrations of salt decreased significantly compared to control plants. In addition, reactive oxygen species are formed in plants under oxidative stress in saline conditions. These reactive oxygen species cause serious problems in plants such as inactivation of proteins and enzymes, injury to plant metabolism, change in the structure of photosynthetic components, and lipid peroxidation (Amirjani, 2010). Phenolic compounds neutralize these reactive oxygen species thanks to their antioxidant activities (Kıpçak et al., 2019).

This study aimed to evaluate the morphological and biochemical responses of two different Turkish forage

pea cultivars to salinity stress induced by Na_2SO_4 and CaCl_2 salts during early growth.

2. Materials and Methods

2.1. Material

In this study, Töre and Ateş forage pea cultivars developed by Namık Kemal University constituted the material of the experiment. The study was carried out as a pot experiment at Bilecik Şeyh Edebali University, Faculty of Agriculture and Natural Sciences.

Soil picked up a depth of 0-30 cm was used as the growing medium. The 4 kg of soil was dried and passed through a 4 mm sieve before being filled into plastic pots measuring 8×8×9 cm in size and 4 cm in height. Twenty-five seeds of each genotype were sown in pots at a depth of 2.5 cm and watered with solutions. The pots were then placed in a greenhouse to germinate and grow.

2.2. Method

2.2.1. Salt concentrations of irrigation water

Four different concentrations of Na_2SO_4 and CaCl_2 (0, 50, 100 and 150 mM) solutions were used in the study.

2.2.2. Morphological measurements

The roots and shoots of the plants cultivated in pots were trimmed using a scalpel after the third week. The lengths of the roots and shoots were assessed using a caliper. Each root and shoot were weighed by analytical balance to determine their fresh weights. Then, each root and shoot were subjected to drying in an oven at 65°C for 48 hours, and their dry weights were determined gravimetrically.

2.2.3. Extraction of plant fractions

In the extraction step, 1-2 grams of dried sample were weighed and extracted with 30 mL of pure methanol for 1.5 hours in an ultrasonic bath (Bandelin Sonorex Digiplus). The samples were filtered through a silk sieve into an amber bottle and stored under refrigerated conditions until analysis.

2.2.4. Determination of total phenolic content with folin ciocalteu metod

The Folin-Ciocalteu method, a colorimetric method for total phenolic analysis, has been modified (Shahidi et al., 2001). First, 1 mL of the extracts was taken and mixed with 1 mL of Folin-Ciocalteu solution. Then, 3 mL of 20% Na_2CO_3 was added and the resulting mixture was vortexed and kept at 25 °C in the dark for 2 hours. Absorbance values were measured in a UV-spectrophotometer at 765 nm against pure methanol as a blind. Total phenolic contents were expressed in terms of gallic acid equivalent, (GAE) (standard curve equation: $y = 14.574x + 3.5652$, $R^2 = 0.9898$), mg of GAE g of extract. The experiment was repeated two times at each concentration.

2.2.5. Determination of DPPH radical scavenging activity

Free radical activities were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH), which is a known free radical (Ayaz Seyhan, 2019). For the determination of DPPH radical scavenging activity, the concentration was

prepared by dissolving 0.004 mg DPPH in 100 mL methanol. Dilutions were prepared from the extracts in different concentrations from stock solution. For each sample, 4 mL of DPPH radical and 1 mL of extract solutions of different samples were added. After incubating in the dark for 30 minutes at room temperature, absorbance was measured at 517 nm (blind MeOH) on a spectrophotometer device. The calibration curve was expressed in mg mL⁻¹ with the equation: $y = 0.0539x - 0.0168$, $R^2 = 0.9976$. The DPPH stock solution was prepared with a concentration of 0.004 mg mL⁻¹. Calibration curve evaluation concentrations were selected between 0.0005 and 0.004 mg mL⁻¹.

2.2.6. Statistical analysis

The data obtained in the study were analyzed by Jump Statistical Analysis and the differences between the averages were determined with the Duncan Multiple Comparison Test. The correlations of the characteristics and path values were also calculated (Sokal and Rohlf, 1981).

3. Results

The shoot fresh weight decreased with increasing in salt concentration of irrigation water, irrespective of the salt type and the pea cultivar. Compared to the control group, the highest decrease was determined at 150 mM CaCl₂ concentration in both cv. Ateş and cv. Töre (Figure 1). As a result of the increase in CaCl₂ concentration, the shoot

fresh weight in the control group was 0.55-0.59 g, while at 150 mM it was 0.35-0.41 g. With the increase in Na₂SO₄ in irrigation water, the shoot fresh weight in the control group was 0.50-0.57 g, while it was 0.39-0.45 g at 150 mM.

The root fresh weight decreased with increasing salt concentration regardless of salt type and forage pea cultivar. When compared with the control group, the highest decrease was determined in 150 mM CaCl₂ concentration in both Ateş and Töre forage pea cultivars, cv. Ateş and cv. Töre (Figure 2). As a result of the increase in CaCl₂ concentration, the root fresh weight in the control group was 0.08-0.09 g, while it was 0.040-0.045 g at 150 mM. As a result of the increase in CaCl₂ concentration, the highest decrease in root weight was observed in the cv. Töre. While the root fresh weight of the control group was 0.09 g, it was 0.04 g at 150 mM.

There was no significant change in shoot dry weight depending on salt type and concentration. Compared to the control group, both Ateş and Töre forage pea cultivars, cv. Ateş and cv. Töre showed a decrease at 150 mM concentrations (Figure 3). As a result of the increase in CaCl₂ concentration, shoot dry weight in the control group was 0.045-0.048 g, while it was 0.039-0.047 g at 150 mM. As a result of the increase in Na₂SO₄ concentration, the shoot dry weight of fever forage pea decreased the most. While the shoot dry weight of the control group was 0.045 g, it was 0.039 g at 150 mM.

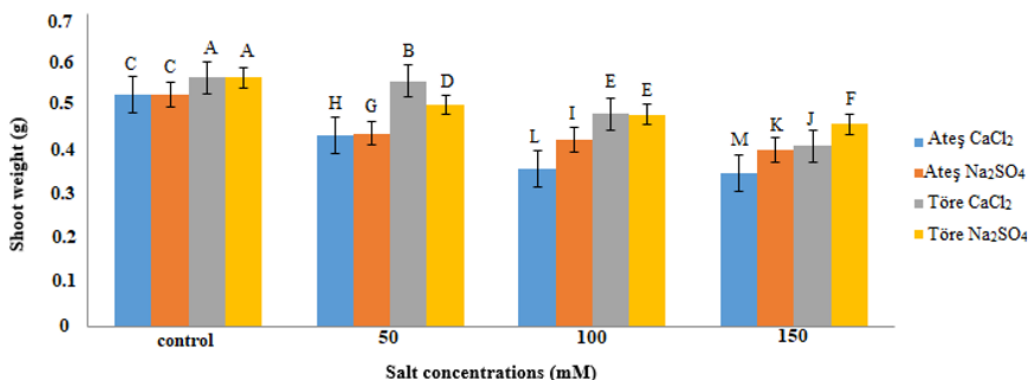


Figure 1. Change in shoot weight of Ateş and Töre forage pea varieties depending on CaCl₂ and Na₂SO₄ concentrations in irrigation water.

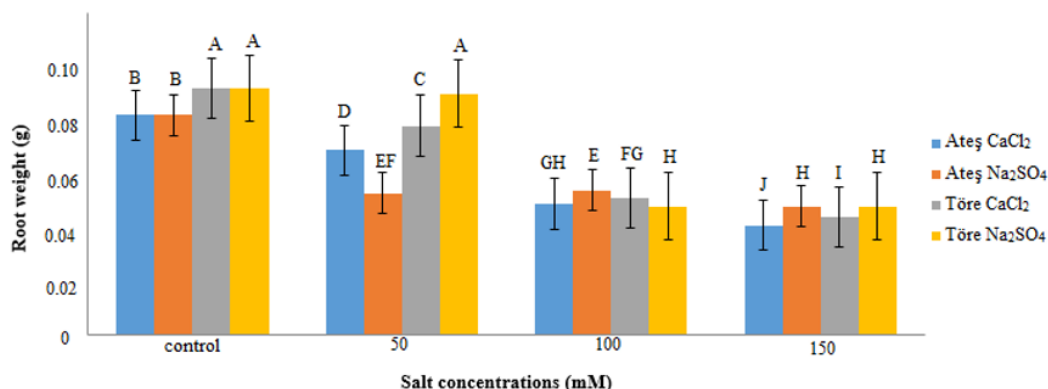


Figure 2. Change in root weight of Ateş and Töre forage pea varieties depending on CaCl₂ and Na₂SO₄ concentrations in irrigation water.

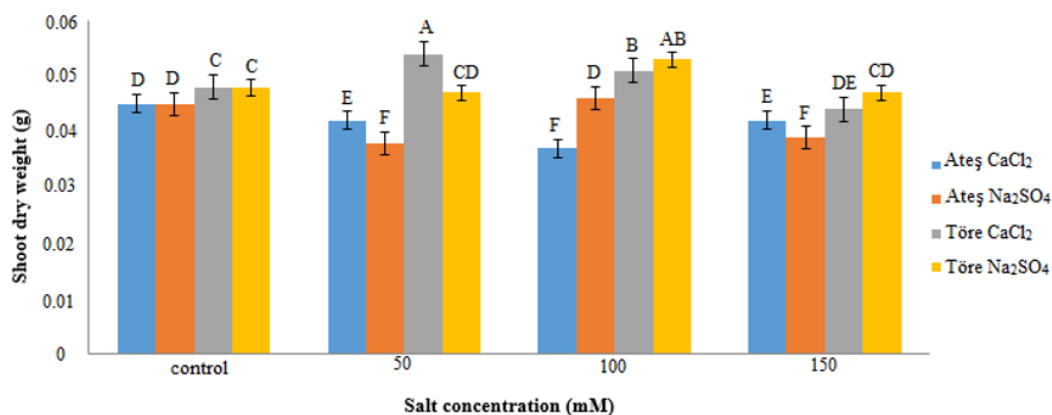


Figure 3. Change in shoot dry weight of Ateş and Töre forage pea varieties depending on CaCl₂ and Na₂SO₄ concentrations in irrigation water.

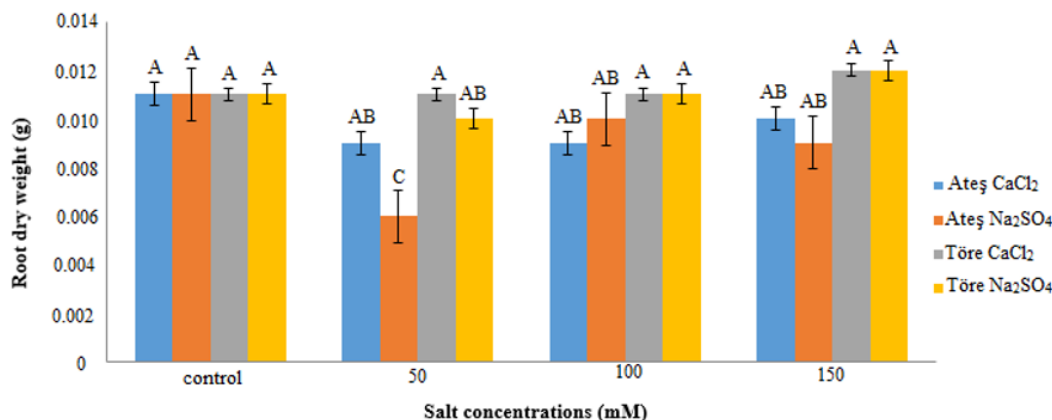


Figure 4. Change in root dry weight of Ateş and Töre forage pea varieties depending on CaCl₂ and Na₂SO₄ concentrations in irrigation water.

There was no significant change in root dry weight depending on salt type and concentration. Compared to the control group, both Ateş and Töre forage pea cultivars, cv. Ateş and cv. Töre showed a significant change at 150 mM (Figure 4). The highest decrease in root dry weight was estimated in 50 mM Na₂SO₄. While the shoot dry weight of the control group was 0.011 g, it was 0.006 g at 50 mM. When we compared the change in root dry weight with the control group, it was 0.011 g; while it was 0.010-0.012 g at 150 mM salt concentration. Shoot length decreased with increasing in salt concentration regardless of salt and forage pea cultivar. This decrease was clearer in 100 mM CaCl₂ concentration. When compared with the control group, the highest decrease was determined at 150 mM CaCl₂ concentration in both Ateş and Töre forage pea cultivars (Figure 5). As a result of the increase in CaCl₂ salt concentration, the shoot length in the control group was 41-43 cm, while it was 29 cm at 150 mM. In Na₂SO₄ irrigation water, the shoot length in the control group was 40-43 cm, while it was 32-34 cm at 150 mM. Root length generally decreased with increasing in salt concentration regardless of salt type and forage pea cultivar. When compared with the control group, the highest decrease was determined in 150 mM Na₂SO₄ concentration in both forage pea cultivars (Figure 6). As a

result of the increase in Na₂SO₄ concentration, root length in the control group was 7.6-8 cm, while it was 4.2-4.8 cm at 150 mM. With respect to CaCl₂ in irrigation water, the root length of the control group was 6.8-7.5 cm, while it was 5.8-6.2 cm at 150 mM. Kayın et al. (2022), an increase in root length was estimated in cv. Töre at 100 mM Na₂SO₄ concentration.

When the total antioxidant activity of forage peas against salt stress were estimated, the total antioxidant activity increased with the increase in salt concentrations. When compared with the control group, the highest increase was determined in 150 mM CaCl₂ concentration in both forage pea varieties (Figure 7). As a result of the increase in CaCl₂ concentration, the total antioxidant activity in the control group was 0.08-0.09 mg mL⁻¹, while it was 0.16-0.18 mg mL⁻¹ at 150 mM. As a result of the increase in Na₂SO₄ irrigation water concentration, while the total antioxidant activity in the control group was 0.08 mg mL⁻¹, it was determined as 0.14-0.16 mg mL⁻¹ at 150 mM.

When the total antioxidant activity of forage peas against salt stress were determined, total phenolic content also increased with the increase in salt concentrations. When compared with the control group, the highest increase was determined in 150 mM CaCl₂ concentration in both cv. Ateş and cv. Töre (Figure 8).

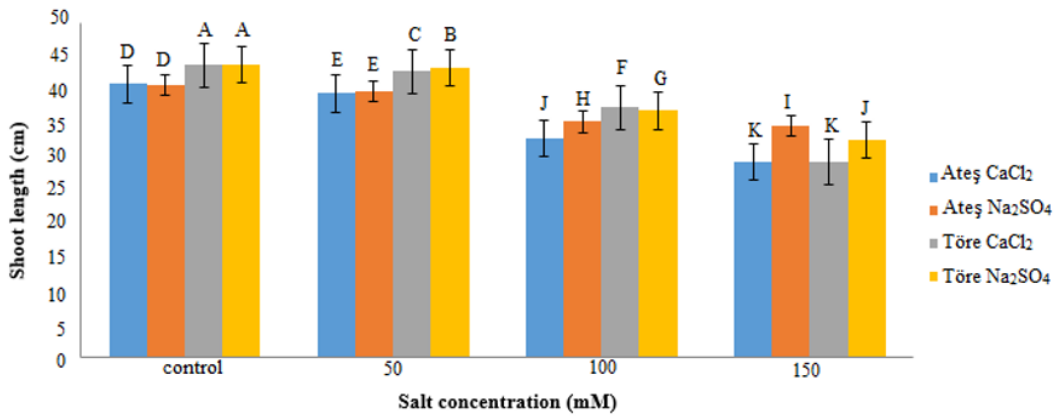


Figure 5. Change in shoot length of Ateş and Töre forage pea varieties depending on CaCl₂ and Na₂SO₄ concentrations in irrigation water.

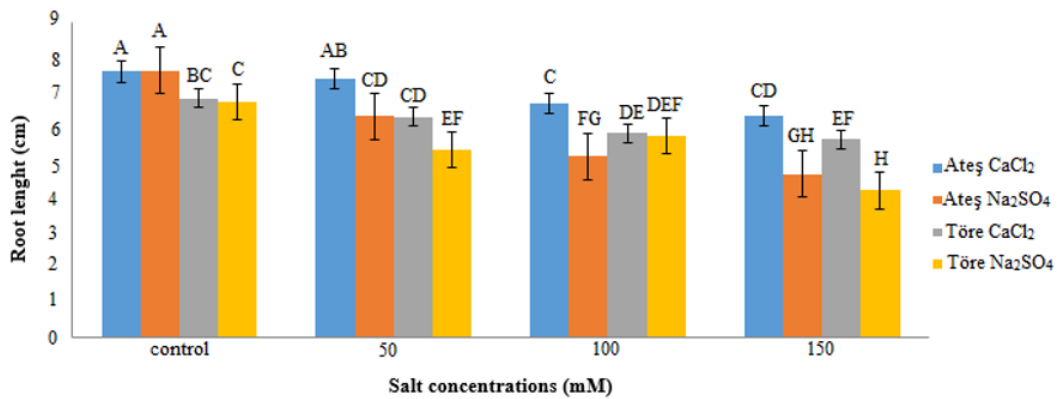


Figure 6. Change in root length of Ateş and Töre forage pea varieties depending on CaCl₂ and Na₂SO₄ concentrations in irrigation water.

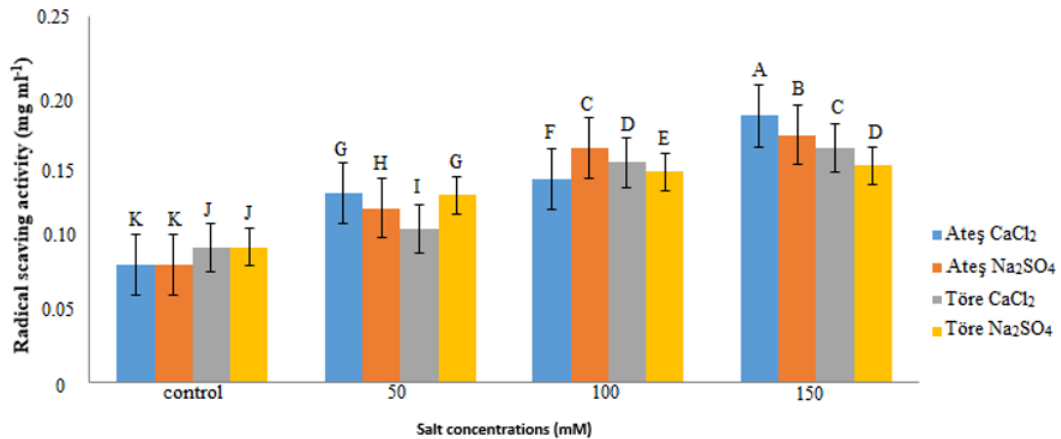


Figure 7. Change in total antioxidant activity of Ateş and Töre forage pea varieties depending on CaCl₂ and Na₂SO₄ at different concentrations.

In general, as a result of the increase in concentration, the total antioxidant activity in the control group was 0.001-0.03 mg GAE g⁻¹, while 0.002-0.005 mg GAE g⁻¹ was determined at 150 mM. The highest increase in total phenolic content was observed when Töre forage peas were irrigated with 150 mM CaCl₂ solution.

In principal component analysis (PCA), the first component contains the most change, then the most variance is associated with the second component, and the last component has the least variance. PCA is a method often used to group cultivars, better interpret relationships, and determine the contribution of traits to

total diversity. The percentage variance of each factor expresses the importance of that feature in interpreting general changes in the data.

In this analysis, two independent factors explained a total of 81.84% of the data changes. The first factor explained 51.45% of the total data variance and its eigenvalue was 4.12. This factor includes shoot fresh weight, root fresh weight, shoot length, and root length (Figure 9). The variance of the second factor is 30.39% and its eigenvalue is 2.43. Shoot dry weight, root dry weight, DPPH radical scavenging activity and Total Phenolic properties are the second factors.

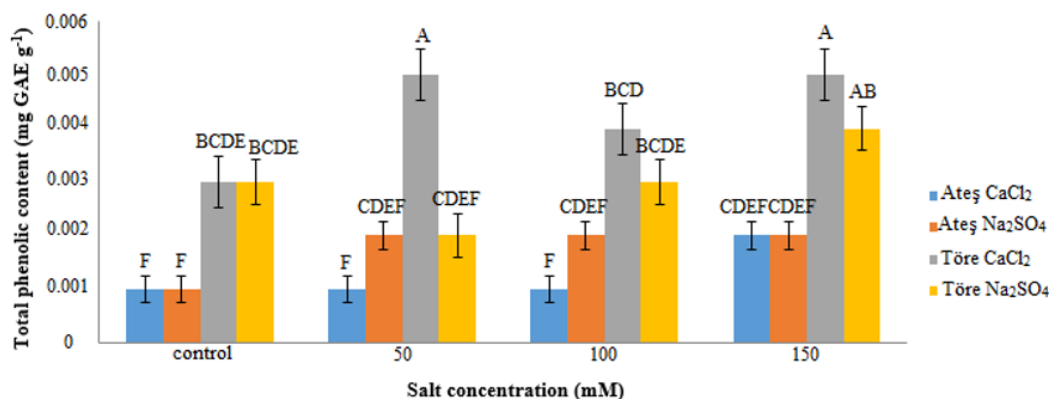


Figure 8. Change in total phenolic content of Ateş and Töre forage pea varieties depending on CaCl₂ and Na₂SO₄ at different concentrations.

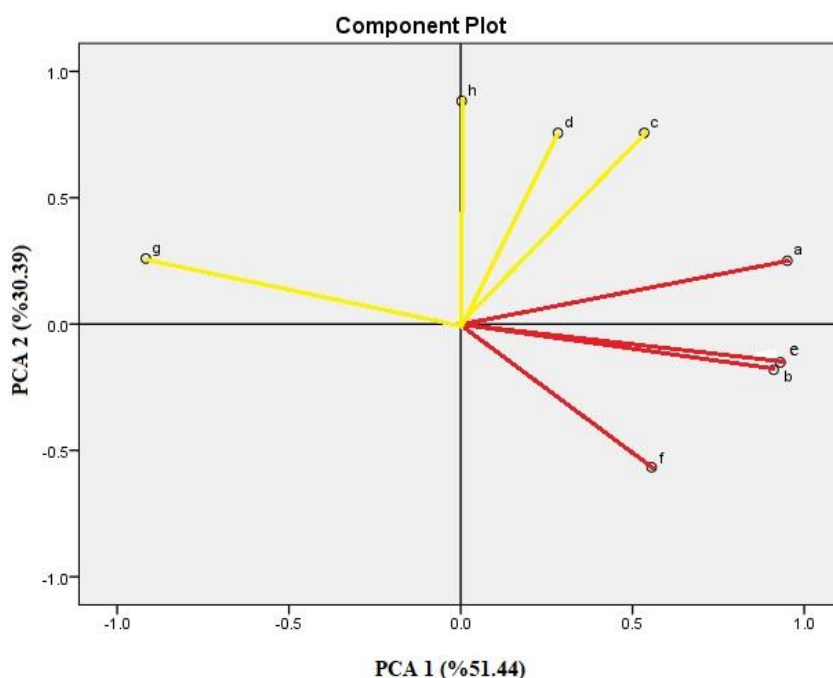


Figure 9. Distribution of investigated characters across the first two components based on PCA. a= shoot fresh weight, b= root fresh weight, c= shoot dry weight, d= root dry weight, e= shoot length, f= root length, g= total antioxidant activity, h= total phenolic content.

4. Discussion

In the study, a decrease in shoot fresh weight was observed as a function of salt concentration (Figure 1). Kayın et al. (2022) examined the effect of eight different NaCl concentrations on forage peas in their studies. They revealed that shoot fresh weight decreased in forage pea cultivars due to the increase in salt concentration. In addition, Avcı et al. (2018), Tsegay and Andargie (2018) and Demirkol et al. (2019) reported that the fresh weight of shoots decreased due to increasing salinity in pea cultivars. The effect of NaCl was evaluated in these studies. Similar results were observed when CaCl₂ and Na₂SO₄ were applied at increasing concentrations.

A significant decrease in root fresh weight was also observed, especially when CaCl₂ and Na₂SO₄ were applied at 100 mM concentration (Figure 2). Kayın et al. (2022) revealed that root fresh weight decreased in forage pea

cultivars due to the increase in salt concentration.

There was no significant change in shoot dry weight depending on salt type and concentration (Figure 3). Kayın et al. (2022) observed fluctuations in dry weight of shoots at different salt concentrations. NaCl 200 mM NaCl concentration did not show a significant change compared to control conditions. The results obtained are consistent with other studies. Avcı et al. (2018), Tsegay and Andargie (2018) and Demirkol et al. (2019) reported that shoot dry weights decreased in pea cultivars due to increased salinity.

No significant change was observed in root dry weight compared to the control group (Figure 4). Kayın et al., (2022) also observed a decrease in root dry weight with increasing NaCl salt concentration. In addition, no change in root dry weight was observed in cv. Urunlu and cv. Nany up to 175 mM salt concentration. In other forage

pea cultivars, various fluctuations were observed in root dry weight values depending on salt concentration. Various fluctuations were also observed in our study (Figure 4).

Shoot length decreased with increasing salt concentration regardless of salt type and forage pea cultivar. This decrease was clearer in 100 mM CaCl₂ concentration (Figure 5). Kayın et al., (2022) observed a decrease in shoot length with increasing NaCl concentration in their studies. The highest decrease in shoot length was observed in the cv. Guifredo, cv. Kurtbey and cv. Özkaynak. They also determined the critical salt concentration as 150 mM NaCl.

Root length generally decreased with increasing salt concentration regardless of salt and forage pea cultivar. Compared to the control group, the highest decrease was determined at 150 mM Na₂SO₄ in both forage pea cultivars (Figure 6).

When the total antioxidant activity and total phenolic content of forage pea were estimated against salt stress, total phenolic contents and total antioxidant activity increased with the increase in salt concentrations (Figure 7 and Figure 8). Kara et al., (2019) revealed that the total phenolic content and total antioxidant activity of *Echinacea purpurea* L. increased under salt stress. Boughalleb et al., (2020) revealed that the total phenolic content and antioxidant activity of *Polygonum equisetiforme* plants under different salt concentrations increased especially up to 300 mM.

5. Conclusion

It was determined that the increase in the concentrations of CaCl₂ and Na₂SO₄ salts increased the negative effect on the morphological characteristics of different cultivars of both forage peas used in the research. On the other hand, an increase in total phenolic content and antioxidant activity values was observed in secondary properties. This is thought to be due to the increase in the defense mechanism of forage peas against salt stress. Investigation of bound phenolic substances (flavonoids, tannins, etc.) as well as free phenolic substances and the use of different forage pea cultivars will enable a more comprehensive evaluation of the results.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	N.K.	A.A.B.	F.T.
C	100		
D	100		
S	50	25	25
DCP	80	20	
DAI	50		50
L	100		
W	60	20	20
CR	50	25	25
SR	100		

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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