



RESEARCH ARTICLE

Alleviating Effect of Exogenous Methionine on Growth and Antioxidant Defense of Mechanisms Pepper Seedlings Under Drought Stress

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ARTICLE INFO

Article History

Received: 19.07.2025

Accepted: 18.08.2025

First Published: 30.12.2025

Keywords

Antioxidant enzyme activity

Drought stress

Methionine application

Pepper seedling



ABSTRACT

This study aimed to reduce the negative effects of drought stress on pepper seedlings by methionine application. In the experiment, which was organized according to the randomized plots experimental design as a pot study, two different irrigation levels (100% and 60% field capacity) and different methionine (M0: 0 mg/L (control), M10: 10 mg/L and M20: 20 mg/L) doses were applied to pepper (*Capsicum annuum* L.) seedlings. In the study, the effects of the applications on morphological parameters, chlorophyll content, antioxidant enzyme activities, and stress markers were evaluated. According to the findings, drought stress caused negative effects on plant development in pepper seedlings. However, methionine applications alleviated these effects. Although the effectiveness varied according to the methionine application dose, it was determined that both doses gave positive results in different parameters. In drought conditions, methionine applications increased plant growth and antioxidant enzyme levels and strengthened the plant's defense responses. As a result, it was determined that the negativities caused by water restriction in pepper seedlings were alleviated by methionine application and thus provided positive contributions at the morphological and physiological levels in pepper seedlings.

Please cite this paper as follows:

Pek, M., Yıldırım, E., & Ekinci, M. (2025). Alleviating effect of exogenous methionine on growth and antioxidant defense of mechanisms pepper seedlings under drought stress. *Journal of Agricultural Production*, 6(4), 217-225. <https://doi.org/10.56430/japro.1746243>

1. Introduction

Pepper (*Capsicum annuum* L.), which belongs to the Solanaceae family, is a widely consumed all over the world and is used as a fresh vegetable, spice, medicine and ornamental plant (Pandey et al., 2012; Yıldız & Özgüven 2011). Pepper, which is native to the American continent, can be cultivated as an annual or perennial in different parts of the tropical and subtropical regions of the world (Pandey et al., 2012). The presence of minerals such as vitamin C, potassium, phosphorus, calcium, magnesium, sodium, iron, zinc, copper and boron makes it a good food and a strong antioxidant against some diseases (Kantar et al., 2016). In pepper cultivation, it is

generally preferred that the soil is light, permeable and rich in nutrients, and sufficient water, heat and oxygen are required for seedling formation and plant development. As with other vegetable species, pepper plants are also significantly affected by the negative environmental factors in the environment in which they are grown. In this sense, pepper is a vegetable that is sensitive to abiotic stresses especially drought stress (Zhang et al., 2024).

Water stress, an abiotic factor, has been identified as an important factor affecting plant development. In response to drought stress conditions, plants exhibit various physiological and morphological changes, including leaf shedding, reduced

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root development and leaf growth, reduced stomatal movement, photosynthesis, and respiration (Farooq et al., 2009). In plants, stress management can be achieved by applying some substances exogenously to plants, causing changes in cell metabolism and activating defense mechanisms. One of these applications is methionine, which is widely found in nature and is the basic metabolite of plants. Methionine is a sulfur amino acid that is one of the basic building blocks of protein. It is known that methionine initiates mRNA translation and regulates cellular processes, regulates plant development and increases resistance to stress conditions such as drought and salinity in various plant species (Almas et al., 2021; El-Bauome et al., 2022). In this study, the effect of exogenous methionine application on increasing tolerance to drought stress and alleviating damage in pepper seedlings was investigated. There aren't enough studies in the literature on the effects of methionine on pepper plants against drought stress. Therefore, the results obtained from this study will be an important resource for the literature.

2. Materials and Methods

Experiment was carried out in semi-controlled greenhouses (25/18±2 °C temperature, 50 % humidity) using pepper (*Capsicum annuum* L.) seeds as plant material. First, pepper seeds were sown in a peat: perlite (2:1, v:v) mixture, then seedlings with 2-3 true leaves were transferred to 1.5 L pots containing soil: sand: peat (2:1:1, v:v:v) with one seedling in each pot. After the seedlings were planted in pots, different doses of methionine solution (M0: 0 mg/L, M10: 10 mg/L and M20: 20 mg/L) were sprayed on the leaves, and then irrigation applications were made at 2-3 day intervals, calculated with a moisture sensor as 100% and 60% of the field capacity. Applications were repeated three times at one-week intervals. NPK fertilizer was given to the plants along with irrigation every 15 days.

The study was completed approximately 40 days after planting the seedlings and plant height, stem diameter, leaf area, plant fresh weight, plant dry weight, root fresh weight and root dry weight were determined while the plants were in the pot and after they were removed from the pot. In addition, the chlorophyll contents of the plants (a, b and total) were measured spectrophotometrically at 645 and 663 nm wavelengths and calculated as mg/g fresh weight (Witham et al., 1971).

Frozen leaf tissues were ground, homogenized with trichloroacetic acid (TCA), and centrifuged at 12,000 × g for 15 minutes. The resulting supernatant was used for H₂O₂ measurement by adding a mixture of potassium phosphate buffer and potassium iodide. Leaf samples were homogenized in TCA and centrifuged at 10,000 g for 15 minutes at 4°C. A

mixture of TCA and thiobarbituric acid (TBA) was added to the resulting supernatant. The mixture was then kept waiting in hot water and then rapidly cooled in an ice bath. In the analysis performed on fresh leaf samples, it was determined after measuring H₂O₂ at 390 nm and MDA at 532 and 600 nm in the spectrophotometer (Shams et al., 2019).

The leaf sample was homogenized in phosphate buffer containing polyvinyl polypyrrolidone (PVPP). The solution was then centrifuged at 15,000×g for 15 minutes, after which the supernatant was used for CAT, SOD and POD analysis. The CAT enzyme activity was measured at 240 nm (Gong et al., 2000), and the POD enzyme activity was measured at 470 nm (Angelini et al., 1990; Ye et al., 2003). SOD enzyme activity was measured at 560 nm by determining the amount of enzyme that causes 50% inhibition of NBT reduction. The results were given as eu/g (Agarwal & Pandey 2004; Yordanova et al., 2004).

The study was organized according to the randomized plots experimental design, variance analysis of the obtained data was performed using the SPSS 20 program and the differences between the applications were determined by Duncan multiple comparison test.

3. Results

The effect of the applications on pepper seedlings was found to be statistically significant and the results are given below.

The highest plant fresh weight (9.43 g) was obtained in M20 application at full irrigation (100%) level, while it was determined as 9.08 g in M10 application and 7.40 g in M0 application. At 60% irrigation, the highest value was seen in M20 application with 5.75 g, and the highest decrease in plant fresh weight was detected in M0 application. Under full irrigation conditions (%100), the highest plant dry weight was obtained with 1.34 g in M10 application. In water restricted (%60) seedlings, the highest dry weight value was seen with 0.78 g in M20 application and 0.76 g in M10 application. M0 application showed the lowest plant dry weight value with 0.59 g (Figure 1).

At full irrigation rate, the highest root fresh weight was determined as 5.82 g in M10 application. In water restriction application, the highest root fresh weight value was reached as 3.59 g in M10 application, 3.40 g in M20 application and 2.97 g in M0 application. In the study, the highest root dry weight values were determined in M10 application with 0.35 g at 100% irrigation level and in M10 application with 0.24 g at 60% irrigation level. The lowest root dry weight value was determined in M0 application (0.16 g) in water restricted (60%) application (Figure 1).

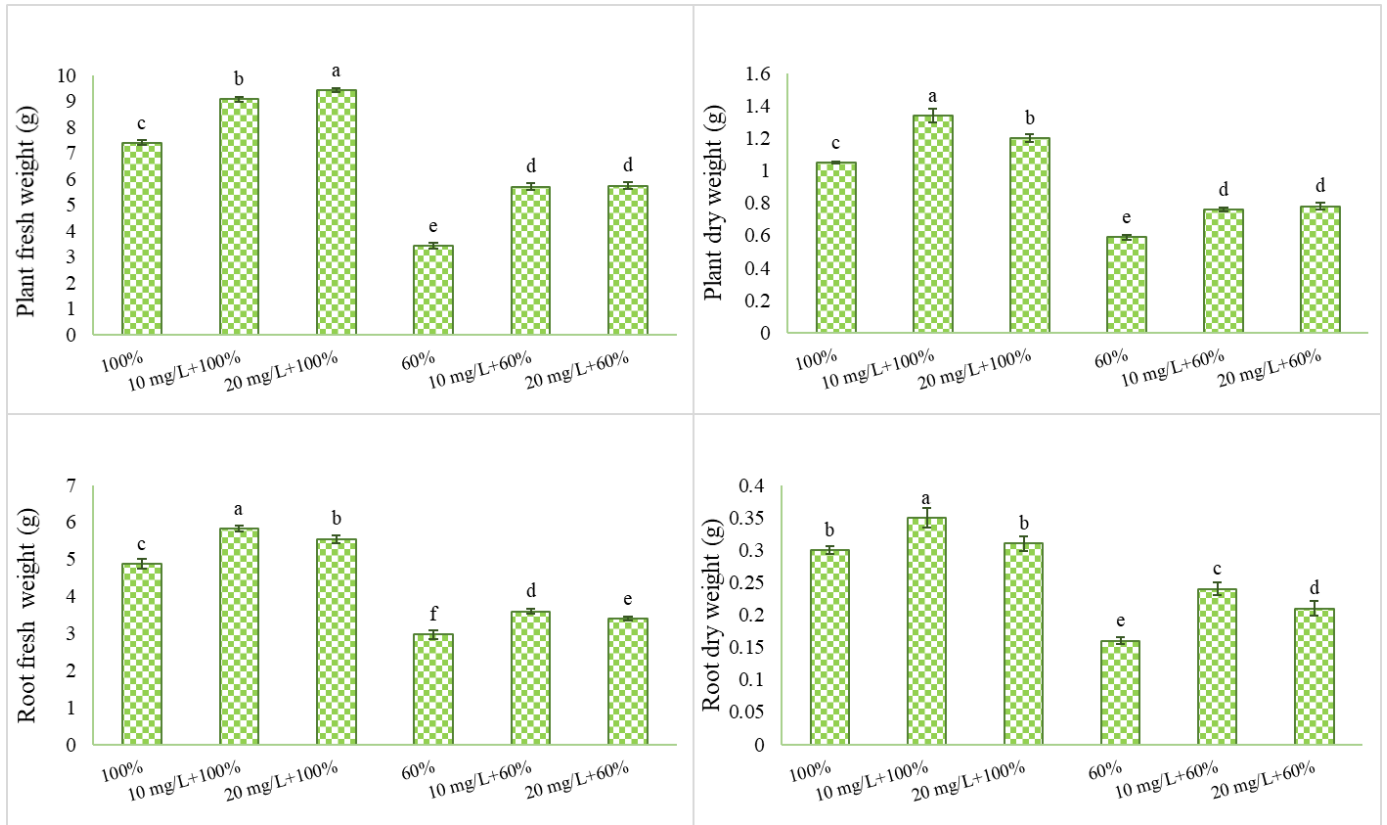


Figure 1. Changes in plant and root weights (fresh and dry) with applications. There is no statistical difference between means indicated with the same letter ($p < 0.001$).

In pepper seedlings, the plant height, which was 21.44 cm with full irrigation in control plants without methionine application, decreased to 16.06 cm with water restriction. The plant height, which was 22.63 cm and 22.97 cm in M10 and M20 applications at 100% irrigation, was measured as 18.90 cm and 18.52 cm, respectively, at 60% irrigation (Figure 2).

According to the results, the highest stem diameter was determined in the M10 (3.60 mm) application at full irrigation level. The lowest stem diameter was observed in the M0 (2.45 mm) application at water restriction. While the stem diameter

was measured as 3.55 mm in the M20 application and 3.24 mm in the M0 application at 100% irrigation, it was measured as 2.99 mm in the M10 application and 2.92 mm in the M20 application at 60% irrigation (Figure 2).

The highest leaf area was found in M10 application (250.59 cm²) at 100% irrigation level, while the lowest was found in M0 application (175.93 cm²). The lowest leaf area was found in M0 application with 94.23 cm² at 60% irrigation level, while the highest was found in M10 (156.02 cm²) and M20 (156.54 cm²) applications (Figure 2).

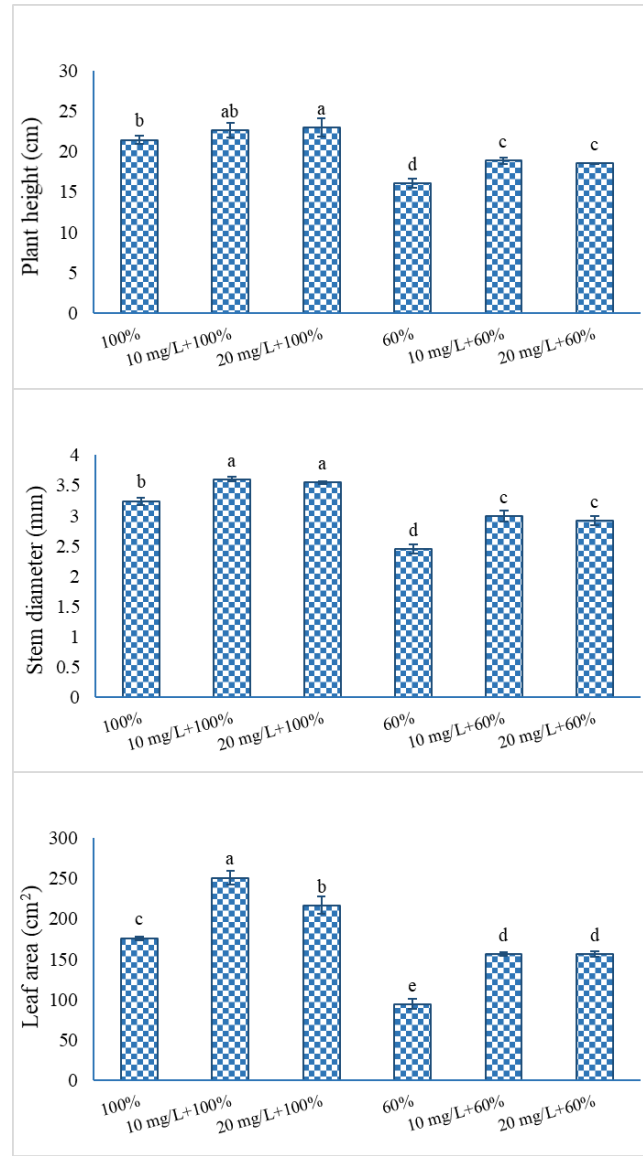


Figure 2. Change of plant height, stem diameter and leaf area of plant with applications. There is no statistical difference between means indicated with the same letter ($p < 0.001$).

It was determined that the highest chlorophyll a amount was 5.26 mg/g in M20 at 100% irrigation, and the lowest chlorophyll a amount was 1.69 mg/g in M0 at 60% irrigation application. It was observed that M20 (2.79 mg/g) and M10 (2.01 mg/g) applications at full irrigation level and M20 (2.40 mg/g) and M10 (2.14 mg/g) applications at 60% irrigation level gave the highest chlorophyll b amount. The lowest chlorophyll

b amount was determined in M0 (0.85 mg/g and 1.25 mg/g) application at both irrigation levels (100% and 60%). The effect on total chlorophyll was found to be highest in M20 (8.05 mg/g) application at full irrigation level. The lowest total chlorophyll amount was determined in M0 (2.54 mg/g) application at 60% irrigation level (Figure 3).

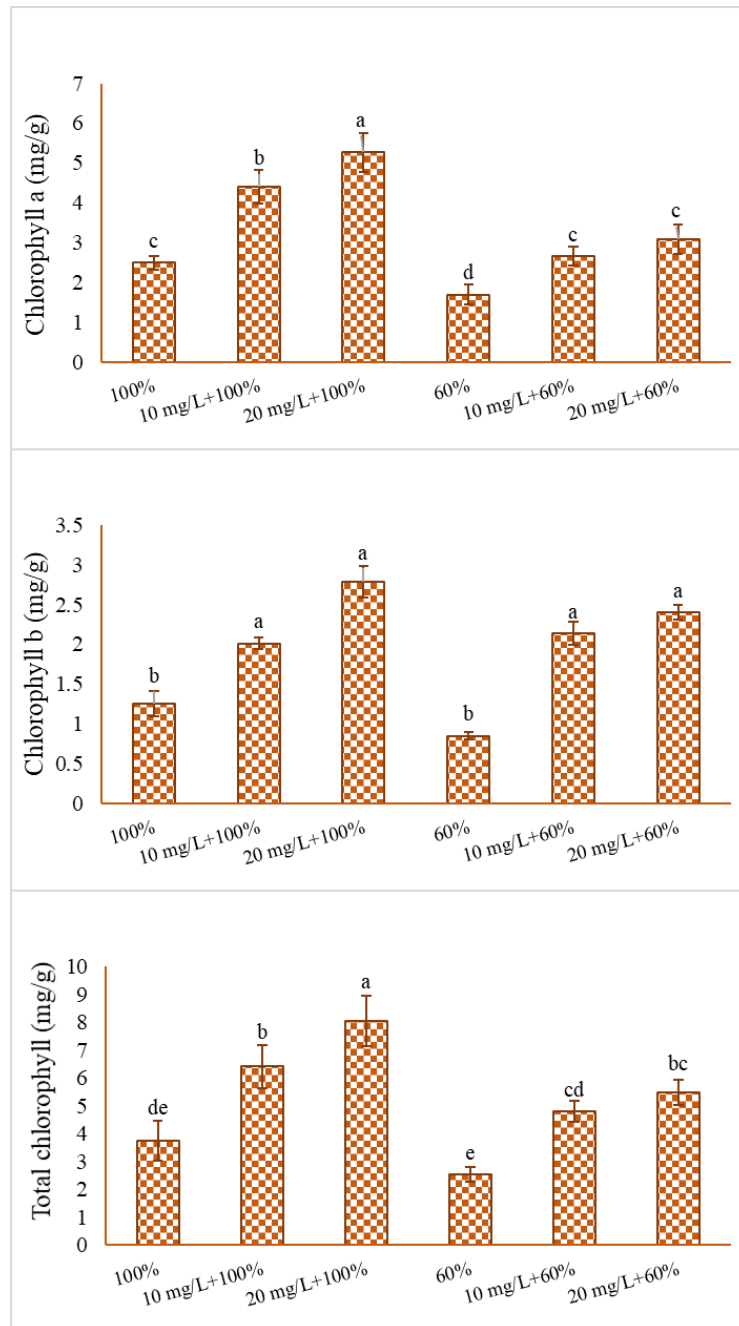


Figure 3. Change of chlorophyll content of plant with applications. There is no statistical difference between means indicated with the same letter ($p < 0.001$).

In the study, the highest H₂O₂ amount in pepper seedlings was determined in M20 application with 46.09 mmol/kg at 60% irrigation level. The lowest H₂O₂ amount was determined in M0 (12.09 mmol/kg) application at 100% irrigation level. According to the results obtained, the highest MDA amount at full irrigation capacity was seen in M20 (24.39 mmol/kg)

application, while the lowest MDA amount was found in M0 (7.82 mmol/kg) application. At the water restriction level, the highest MDA amount was found in M20 application with 44.51 mmol/kg. The lowest MDA amount was found in M0 (26.25 mmol/kg) application (Figure 4).

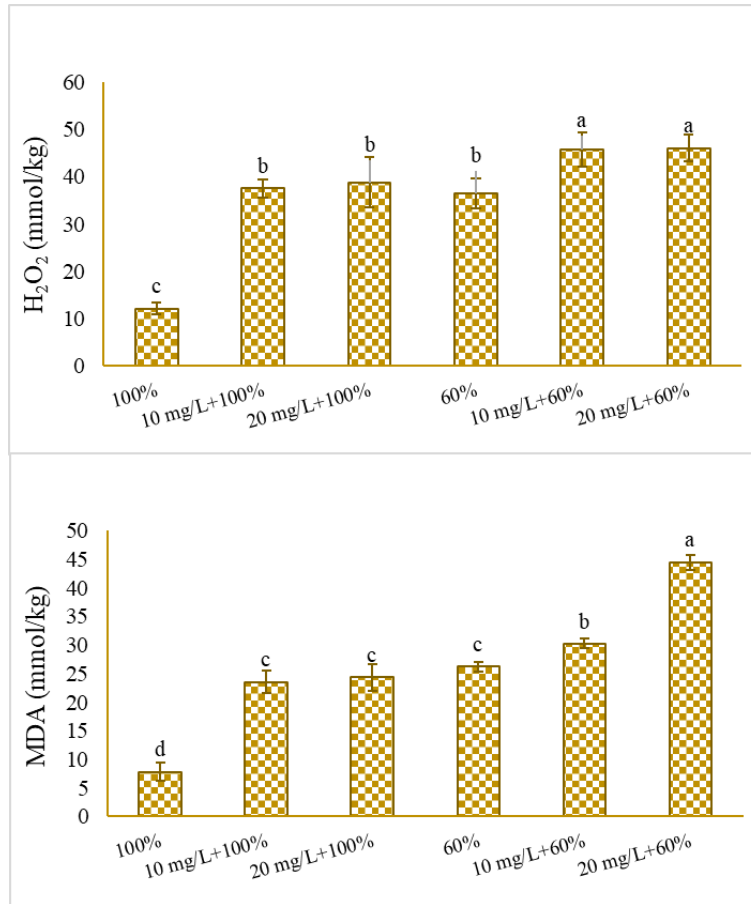


Figure 4. Change of H_2O_2 and MDA content of plant with applications. There is no statistical difference between means indicated with the same letter ($p < 0.001$).

In the study, the highest CAT activity in water limited and methionine applications in pepper seedlings was observed in M10 (275.92 eu/g) application at 60% water limited level, while the lowest CAT activity was determined as 21.83 eu/g in M0 at 100% irrigation level. According to the analysis results,

POD activity in pepper seedlings was highest in M20 application with 2992.69 eu/g at 60% irrigation level. According to the study results, SOD activity in pepper seedlings was observed most in M0 (325.07 eu/g) application at water restriction (60%) level (Figure 5).

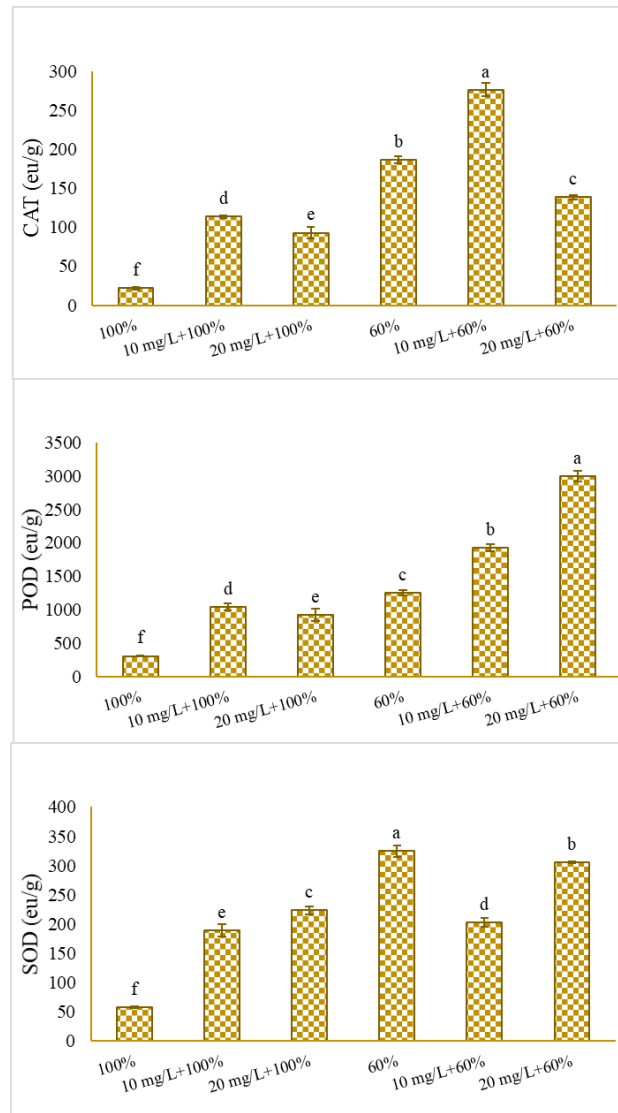


Figure 5. Change of antioxidant enzyme activity of plant with applications. There is no statistical difference between means indicated with the same letter ($p < 0.001$).

4. Discussion

Drought is an abiotic stress factor to which plants respond differently at physiological, biochemical and molecular levels. Especially in agricultural areas, this stress factor negatively affects plant growth, causing yield losses and becoming one of the most important restrictive factors threatening agricultural production. In studies conducted on pepper plants to date, it has been determined that they are negatively affected by drought stress and that this effect varies depending on the pepper variety and the severity of stress. Similarly, Kuşvuran et al. (2020) stated in their study that different pepper genotypes negatively affected plant growth and development under drought stress and that this effect varied among genotypes. However, in many studies, it has been determined that the negative effects of stress on plants can be reduced with various exogenous applications. One of these is methionine, which we used in our study.

In this study, the effects of different irrigation levels and methionine applications on some morphological and physiological traits of pepper seedlings were investigated. In the study, the effects of different irrigation levels and methionine applications on pepper seedlings were investigated in terms of plant height, plant fresh and dry weight, root fresh and root dry weight, leaf area, stem diameter, chlorophyll content (chlorophyll a, b and total chlorophyll), MDA, H_2O_2 content, SOD, POD and CAT enzyme activities. There was a decrease in the morphological and physiological parameters examined with drought, and the effect of stress was greater with an increase in H_2O_2 , MDA and enzyme activity. Similarly, in a study, it was determined that moderate and severe drought caused significant decreases in leaf relative water content, relative growth rate, carotenoid, chlorophyll a, b and total chlorophyll content in pepper at the vegetative stage (Okunlola et al., 2017).

At full irrigation (100%) level, especially M20 application provided an increase in plant height, stem diameter, plant fresh weight, plant dry weight, chlorophyll a, b and total chlorophyll values. On the other hand, the M10 application increased leaf area, root fresh and root dry weight. Reducing effects of water restriction were observed in these parameters. However, methionine applications alleviated this negative effect of stress and the decrease was at a very low level. Similarly, Akram et al. (2020) stated in their studies that methionine applied from the leaves to bitter melon plants had positive effects on plant growth parameters. In a study, it was observed that externally applied phthalanilic acid (PPA) contributed to plant height, stem thickness, root/stem ratio, chlorophyll content and seedling quality in pepper seedlings under drought conditions. It has been stated that PPA has a reducing effect on drought-induced physiological damage in plants (Lu et al., 2023).

In our study, it was observed that methionine applications increased the contents of chlorophyll a, b and total chlorophyll in pepper seedlings. It was determined that methionine application was effective in maintaining photosynthetic activity under water stress by providing these increases. Farooq et al. (2009) stated that in addition to the mechanisms developed by plants to increase water intake and reduce transpiration loss against drought stress, amino acids and organic acids increased photosynthetic efficiency by maintaining chlorophyll stability.

As an indicator of stress, levels of H₂O₂ and MDA increase were significantly increased in pepper seedlings in both control and methionine applications. Additionally, increases in CAT and POD enzyme activities were detected under water restriction with methionine applications. Similarly, it was determined that drought stress in pepper caused a significant increase in MDA and antioxidant enzyme activity, although it varied according to genotypes (Kuşvuran et al., 2020). Hayat et al. (2012) stated in their studies that amino acids are versatile molecules that play a role in osmotic regulation, antioxidant defense and signal transduction. It has been reported that it reduces the harmful effects of reactive oxygen species (ROS) by maintaining intracellular water balance in abiotic conditions such as water stress. In a study, it was determined that methionine application in wheat plants under water stress increased both growth parameters and antioxidant enzyme activities (SOD, POD, CAT) and reduced oxidative damage (Maqsood et al., 2022). One of the most important effects of exogenous applications in providing stress tolerance in plants is their effects on oxidative and osmotic regulation. In their study, Iqbal et al. (2023) stated that external application of aminobutyric acid (GABA) to sweet pepper was effective in tolerance by increasing the physiological resistance of pepper under drought conditions, preserving the osmotic balance and increasing the effectiveness of the antioxidative defense system. In our study, it is thought that the increase in enzyme activity provided by methionine application in increasing drought stress tolerance in pepper plays a role in osmotic

balance function and photosynthetic activity as well as reducing the formation of reactive oxygen species. Also similarly, has been determined that exogenous methionine improves growth, photosynthesis and gas exchange properties in plant tolerance in drought in wheat and eggplant, contributes to the development of yield and defense systems and thus increases tolerance (Akram et al., 2024; Maqsood et al., 2022).

5. Conclusion

Drought is one of the most important problems of today and the future and is widely emphasized in production. Herein, it has been determined that this negative effect, which is the decrease in seedling growth in pepper due to water restriction, can be reduced with exogenous methionine application. Studies show that the effectiveness of exogenous applications to protect plants under current stress conditions is emphasized more because they give short-term results. In this study, two doses of methionine were used and only the effects during the seedling period were examined. In our study, although M20 seems to be better in terms of application dose, it is thought that the effectiveness may be higher at lower doses (M10) and this effect may vary depending on the plant type, stress severity and application time. However, it would be useful to conduct studies considering different doses, application methods and different plant development periods and to clarify the results.

Compliance with Ethical Standards

This study does not require ethical committee approval.

Acknowledgment

This study includes the results of Melike PEK's master's thesis.

Conflict of Interest

The authors declare no conflict of interest.

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