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EFFECTS OF BIOSTIMULANT TREATMENTS ON AMINO ACID CONTENT OF TOMATO SEEDLING UNDER WATER DEFICIT

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Abstract: In this study, the effect of biostimulant treatment against water stress in tomato seedling period was investigated. In the study conducted in the greenhouse conditions, water stress was applied in two different levels; full irrigation (100% of the field capacity) and 50% of the field capacity. The product used as a biostimulant has 1%Zn, *Bacillus subtilis*, *Bacillus megaterium*, *Azosprillum* and *Bacillus amyloliquefaciens* ($1x10^9$ cfu/ml) content. The solutions prepared at a ratio of 1/10 and 1/5 and were given to the plants three times with one-week intervals. The effects of water stress and biostimulant treatments on amino acid content in tomato seedlings were investigated. As a result of this study, some of the amino acid content in the plant leaf with has increased and some have decreased under different water availability conditions according to the amino acid type. However, depending on the application doses, it was seen that the negative effect of water stress on the amino acid content was alleviated. As a result of the current study, the effect of applications in reducing the damage caused by water stress on tomato seedlings may also be important in terms of amino acid content.

Keywords: amino acid content, drought stress, tomato seedling, biostimulant

Introduction

Plants are exposed to many environmental factors that limit or stop their growth and development. Abiotic stress factors caused by salinity, drought, low or high temperature, heavy metals, etc. in parallel with the increasing global climate change have been emphasized in agricultural production in recent years. Drought, which is probably one of the most important of these factors, is taken into account by many researchers due to the decreasing water resources in the world. Drought causes deterioration in plant growth, decrease in plant water use efficiency, and causes various physiological morphological, biochemical, and molecular reactions at plant cellular and whole organism level. Drought causes stomatal closure and therefore a decrease in photosynthesis (Farooq et al., 2012; Salehi-Lisar and Bakhshayeshan-Agdam, 2016).

Researchers are working on applications that increase tolerance against factors that negatively affect plant growth and development, such as drought stress. These applications are various hormones, fertilizers, different compounds etc., which increase plant growth and development. They also support tolerance mechanism in plants under abiotic stress. One of them are called biostimulators. These compounds which they secrete are effective in the formation of important biostimulants and modulate plant stress responses (Backer et al., 2018). A biostimulant is organic material and/or microorganism

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that increases nutrient uptake, promotes growth, improves stress tolerance or crop quality (Van Oosten et al., 2017).

Plant growth promoting rhizobacteria (PGPR) based formulations have attracted attention in the agricultural industry as microbial biostimulant. A group of microorganisms are plant growth promoting rhizobacteria (PGPR), which are also considered biostimulant and whose physiological modes of action are well known (Ruzzi and Aroca, 2015). PGPRs have functions such as hormone secretion or hormonal changes in the plant, production of volatile organic compounds, improving nutrient uptake and increasing tolerance to abiotic stresses (Ruzzi and Aroca, 2015). Rhizobium microorganisms increase plant growth by providing nutrient uptake and assimilation, secretion and modulation of extracellular molecules such as hormones, secondary metabolites, antibiotics and various signaling compounds (Backer et al., 2018). It has been stated that it may be important in the application of microbial-based formulations for sustainable agriculture and food production due to the metabolic events caused by the biostimulant in plants (Lephatsi et al., 2022). Additionally, it has been determined that the application of PGPR to plants that promote plant growth can be an effective strategy to promote plant growth and improve their tolerance to abiotic stresses such as drought and salinity (Backer et al., 2018). PGPR ensure plant survival under stressful conditions due to their response to water shortage (Marasco et al., 2012). Due to these features, studies on obtaining multifunctional PGPR-based formulations have gained importance in order to minimize the use of synthetic fertilizers and agricultural chemicals (Backer et al., 2018).

Plants respond to stress with changes in various metabolites and osmoprotectans in plant metabolism under drought. One of them is amino acids. Amino acids are involved in important functions in the plant. Although they are used in protein biosynthesis, they take place as building blocks for many biosynthesis pathways and are also effective in signaling processes and plant stress response (Hildebrandt et al., 2015). Many environmental stresses, including drought, cause an increase in free amino acids and amines in plant cells, possibly due to decreased electron transport chain (Reggiani et al., 2000). Therefore, in this study we have done, the changes in the plant amino acid content of the drought stress in tomato seedlings were investigated. In addition, the effects of the application of bacteria with biostimulant effect on the plant were investigated in terms of amino acids and their changes in drought stress were examined.

Materials and Methods

This study was conducted under greenhouse with day/night temperature of 25/18 °C. In the study, tomato (*Solanum lycopersicum* L.) seeds were used as plant material. The product used as a biostimulant has 1%Zn, *Bacillus subtilis, Bacillus megaterium, Azosprillum* and *Bacillus amyloliquefaciens* ($1x10^9$ cfu/ml) content and irrigation of the plants was done in a way that the field capacity (100%) and 50% of the field capacity. First, tomato seeds were sown in viols containing peat:perlite, (2:1) and after about a month those seedlings were transplanted in 2.5 L pots containing soil:peat:sand (2:1:1). Bacterial solutions prepared at a ratio of 1/10 and 1/5 were given to the plants three times with one-week intervals.

Irrigation times were checked with a soil moisture meter (WET Sensor) at intervals of approximately 2 days. The study was conducted in 3 replications and 6 plants in each replication. After 30 days, samples were taken to determine the amino acids content in the leaves and roots of the plant.

After harvested of plants, amino acid analyzes were performed on leaf samples taken from plants as described by Aristoy and Toldra (1991) Antoine et al., (1999) and Henderson et al., (1999).

Data were subjected to variance analysis and the differences between the applications were determined by Duncan multiple comparison test with SPSS.

Results and Discussion

In this study amino acid content of tomato were determined under drought and biostimulant treatment and results were given in Figure 1-6. The effects of treatment, drought and treatment x drought interaction were statistically significant.

Drought effect on amino acid was differed from one to another. According to mean of drought values; content of aspartate, asparagine, threonine, tyrocine, cysteine, tryptophan, phenylalanine and proline content were decreased with drought, while other amino acids were increased (Figure 1). The decrease of aspartate, asparagine, glycine, threonine, tyrocine, tryptophan, phenylalanine and proline content under D1 condition (50%) were ratio of 17%, 9%, 16%, 2%, 18%, 25%, 14% and 9% compared to D0 (100%). On the other hand, under drought conditions (D1) increase of glutamate, serine, glutamine, histidine, arginine, alanine, cysteine, valin, methionine, isoleucine, leucine, lysine, hydroxyproline and sarcosine by ratio of 10%, 13%, 9%, 8%, 10%, 45%, 15%, 2%, 14%, 34%, 29%, 46%, 37% and 3% respectively, compared to D0 (100% irrigation) Figure 1.

The highest glutamate, serine, glutamine and histidine content were determined on T2D1 treatments, while the highest aspartate content was on T2D0 and the highest asparagine content was on T1D0 application. The lowest aspartate, asparagine, serine and glutamine content were obtained from T1D1, the lowest glutamate content from T2D0 and the lowest histidine content from T0D1 (Figure 2).

The highest glycine and tyrocine content obtained from T2D0 treatments, the highest alanine and cysteine content from T2D1, the highest threonine content from T1D1 and the highest arginine content from T1D0. The lowest glycine and threonine content were obtained from T1D1, the lowest alanine and cystine content from T0D1, the lowest arginine content from T0D0 and the lowest tyrocine content from T1D0 (Figure 3).

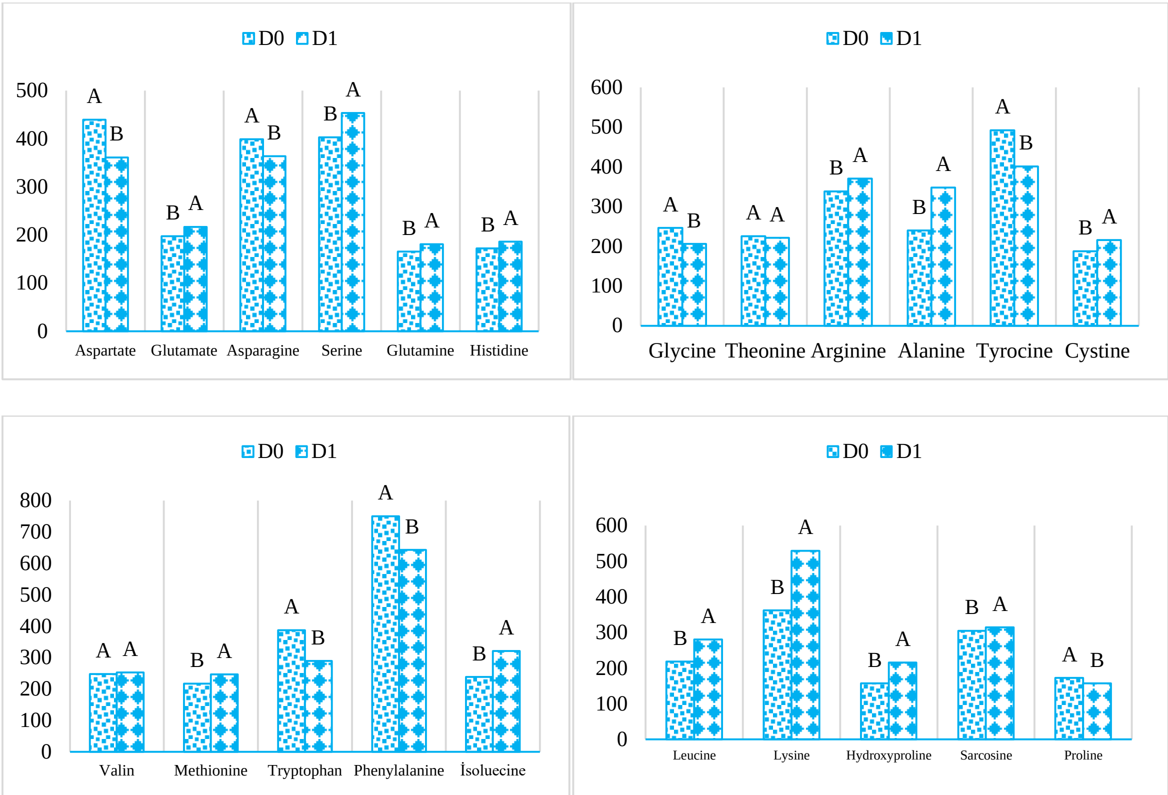

Figure 1. The effects of irrigation on amino acid content of tomato seedling. Mean with the same letters in column are not statistically different according to Duncan's multiple range test (p< 0.001). D0: 100% irrigation, D1. 50% irrigation of field capacity.

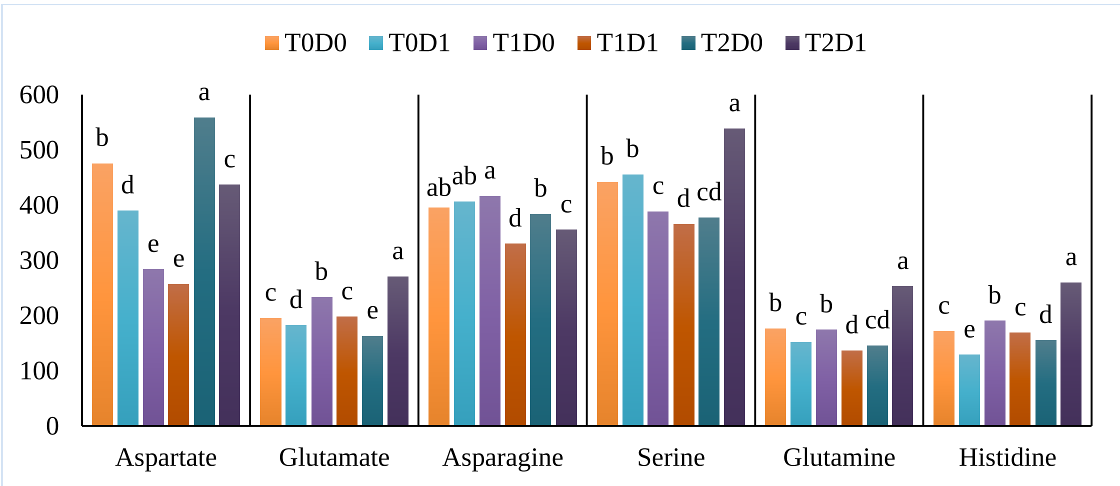

Figure 2. The effects of treatments x irrigation on aspartate, glutamate, asparagine, serine, glutamine and histidine content of tomato seedling. Mean with the same letters in column are not statistically different according to Duncan's multiple range test (p< 0.001). T0D0: No treatment and 100% irrigation, T0D1: No treatment and 50% irrigation, T1D0: Treatment with rate of 1/10 and 100% irrigation, T1D1: Treatment with rate of 1/10 and 50% irrigation, T2D0: Treatment with rate of 1/5 and 100% irrigation, T2D1: Treatment with rate of 1/5 and 50% irrigation

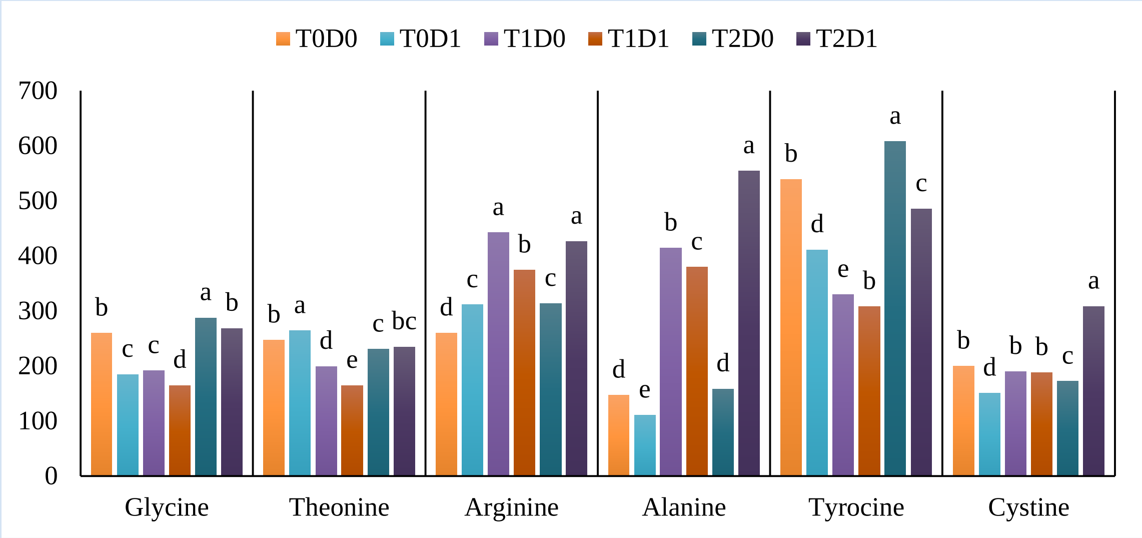

Figure 3. The effects of treatments x irrigation on glycine, theonine, arginine, alanine, tyrocine and cystine content of tomato seedling. Mean with the same letters in column are not statistically different according to Duncan's multiple range test (p< 0.001). T0D0: No treatment and 100% irrigation, T0D1: No treatment and 50% irrigation, T1D0: Treatment with rate of 1/10 and 100% irrigation, T1D1: Treatment with rate of 1/10 and 50% irrigation, T2D0: Treatment with rate of 1/5 and 100% irrigation, T2D1: Treatment with rate of 1/5 and 50% irrigation

The highest valin, methionine and isoluecine content were determined on T2D1 treatments, while the highest tryptophan and phenylalanine content were on T2D0 treatment. The lowest valin, methionine and isoluecine content were obtained from T0D1, the lowest tryptophan and phenylalanine content from T1D1 (Figure 4).

The highest leucine, lysine, hydroxyproline, sarcosine and prolin obtained from T2D1 treatment. The lowest leucine and hydroxyproline content were obtained from T2D0, the lowest lysine and sarcosine content from T0D1, the lowest hyroxyproline content from T2D0 and the lowest proline content from T0D0 (Figure 5).

The results of this study, according to mean values of treatments; biostimulant treatment affected amino acid content of tomato seedling. Generally, treatment have increasing effect on amino acid content both D0 and D1 conditions. The aspartate, glutamate, serine, glutamine, histidine, glycine, tyrocine, cysteine, valin, methionine, tryptophan and phenylalanine content were increased by ratio of 15%, 15%, 2%, 21%, 38%, 25%, 15%, 37%, 14%, 45%, 3% and 7% compared to T0. On the other hand, T1 treatment have decreased effect on aspartate, serine, glutamine, glycine, threonine, tyrocine, tryptophan and phenylalanine, while have increase effect on arginine, alanine, isoleucine, leucine, lysine, hydroxyproline, sarcosine and prolin content of tomato seedling (Figure 6).

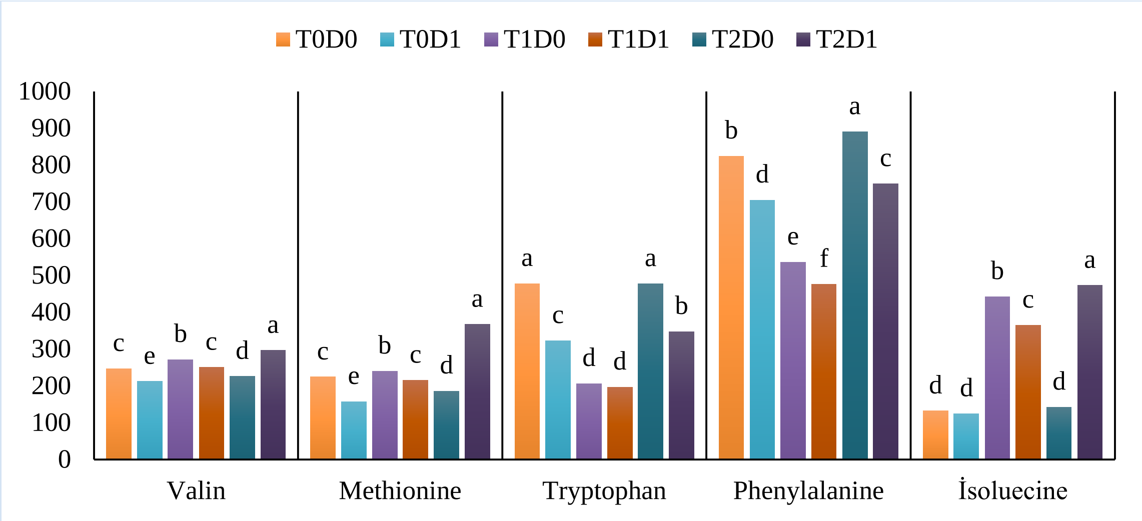

Figure 4. The effects of treatments x irrigation on valin, methionine, tryptophan, phenylalanine and isoluecine content of tomato seedling. Mean with the same letters in column are not statistically different according to Duncan's multiple range test (p< 0.001). T0D0: No treatment and 100% irrigation, T0D1: No treatment and 50% irrigation, T1D0: Treatment with rate of 1/10 and 100% irrigation, T1D1: Treatment with rate of 1/10 and 50% irrigation, T2D0: Treatment with rate of 1/5 and 100% irrigation, T2D1: Treatment with rate of 1/5 and 50% irrigation

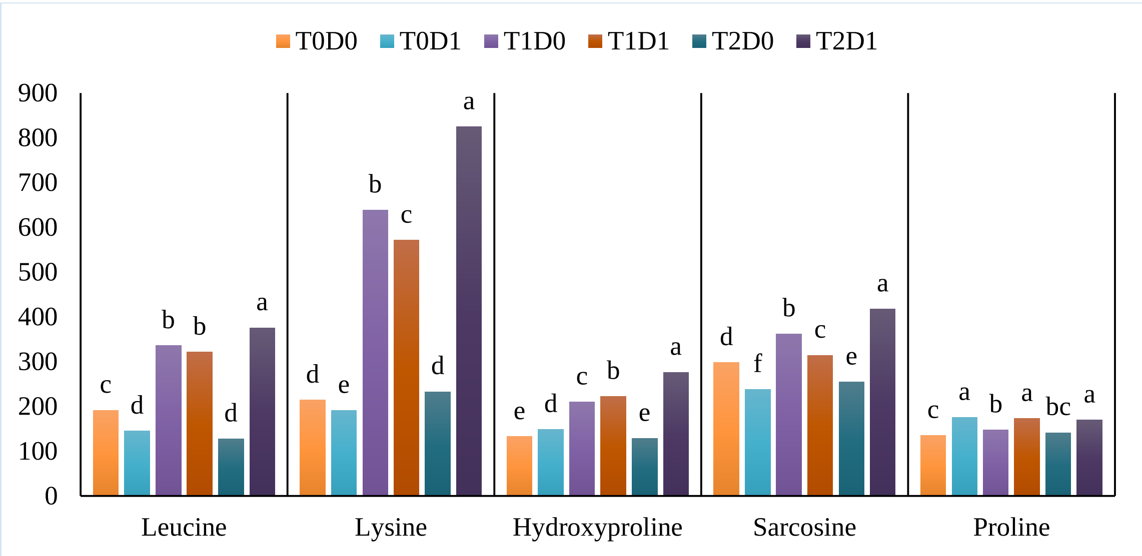

Figure 5. The effects of treatments x irrigation on leucine, lysine, hydroxyproline, sarcosine and proline content of tomato seedling. Mean with the same letters in column are not statistically different according to Duncan's multiple range test (p< 0.001). T0D0: No treatment and 100% irrigation, T0D1: No treatment and 50% irrigation, T1D0: Treatment with rate of 1/10 and 100% irrigation, T1D1: Treatment with rate of 1/10 and 50% irrigation, T2D0: Treatment with rate of 1/5 and 100% irrigation, T2D1: Treatment with rate of 1/5 and 50% irrigation

Low molecular weight osmolytes, including glycinebetaine, proline, amino acids, organic acids and polyols, are also effective in maintaining cellular functions under drought (Farooq et al., 2012). Drought affects plant amino acid metabolism (Sircelj et al., 1999). Stress reactions occur in the plant by increasing and decreasing the amino acid content of tomato seedlings with drought in this study. In plants, amino acids serve as the main source of mobilizable nitrogen (Moe, 2013). Amino acids can act as biological sources of carbon and nitrogen. Certain amino acids (glutamate and proline) and their betaines (glycine betaine) work as compatible solutes necessary for osmoregulation in plants and microbes. It has been determined that amino acids change the basic phenotypes related to plant root growth and microbial colonization, symbiotic interactions and pathogenesis in the rhizosphere (Moe, 2013). Proteinogenic free amino acids concentration increases were observed in drought resistant/non-tolerant cultivars, while significant decreases occurred only in stress resistant/tolerant cultivars. It has been stated that free amino acids may be markers for cultivars that have adapted differently to drought stress (Lanzinger et al., 2015).

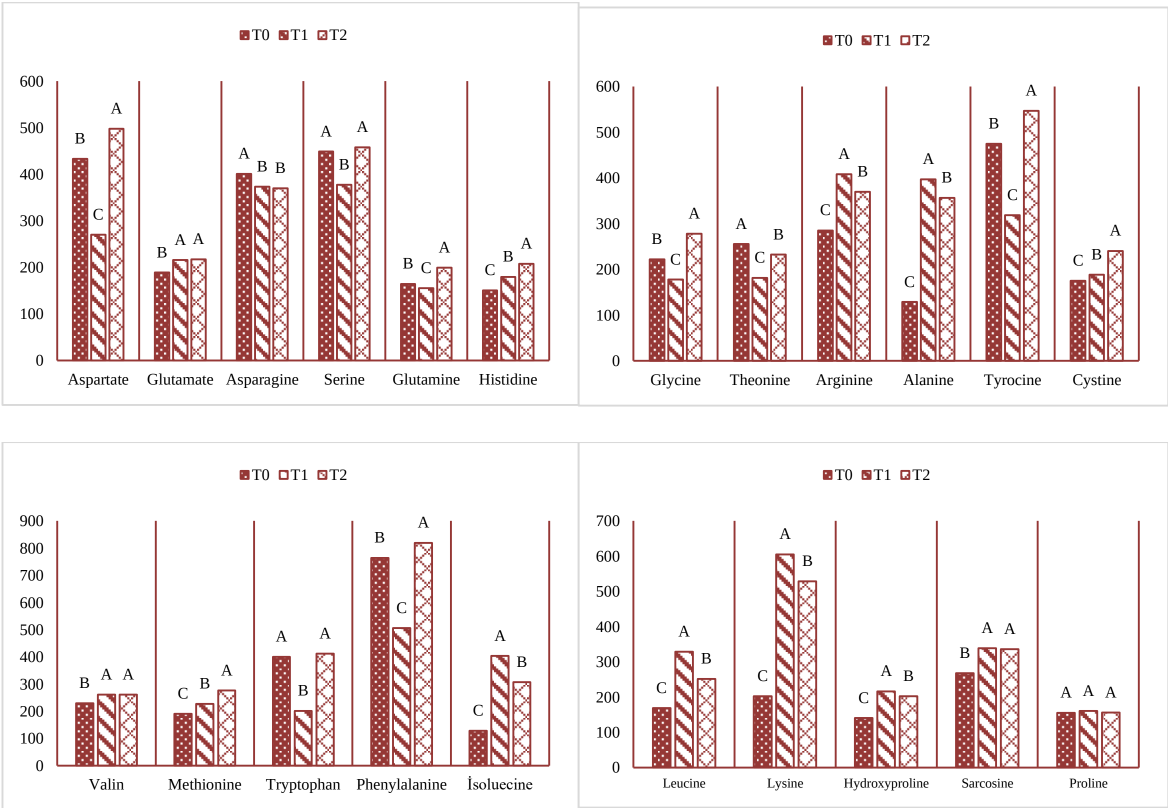

Figure 6. The effects of treatments on amino acid content of tomato seedling. Mean with the same letters in column are not statistically different according to Duncan's multiple range test (p< 0.001). T0: No treatment, T1: Treatment with rate of 1/10, T2: Treatment with rate of 1/5

In addition, it has been determined that the biostimulants used in this study support plant growth and development under stress conditions, and this is achieved by the changes in the plant amino acid content. PGPRs provide induced systemic tolerance to drought stress in plants with various mechanisms such as production of ACC-deaminase and phytohormones, nitrogen fixation, phosphate solubility, siderophore and exopolysaccharide production, improvement of antioxidant system, improved root and shoot system, increase in photosynthesis rate and carotenoid production (Ahluwalia et al., 2021). In a study, the application of microbial biostimulant provides drought resistance to maize plants by changing the basic metabolic pathways (redox homeostasis, osmoregulation, energy production and membrane restructuring) involved in drought resistance mechanisms (Ahluwalia et al., 2021; Nephali et al., 2021). Similarly, it is stated that there are differences in amino acid, phytohormone, flavonoid and phenolic acid levels in plants treated with biostimulant under well-watered, mild and severe drought stress conditions (Lephatsi et al., 2022). In a study, it was determined that water deficiency, silicon application and mycorrhiza inoculation caused changes in plant amino acids (Haghighi et al., 2022). Proline accumulation is one of the responses to drought stress, it was determined that proline increased with 100% irrigation by applying silicone without mycorrhiza vaccine, while the minimum proline was determined to be 40% irrigation with mycorrhiza inoculation without silicone application (Haghighi et al., 2022). The highest histidine and lysine values were obtained by irrigation with silicium solution and 80% field capacity water and mycorrhiza inoculation. It was stated that the minimum histidine and valine were obtained in 100% field capacity irrigation without silicon and mycorrhizae (Haghighi et al., 2022). The application of rhizobacteria (PGPR) to plants that promote plant growth can be an effective strategy to promote plant growth and improve their tolerance to abiotic stresses such as drought. Due to these features, multifunctional PGPR-based formulations have gained importance in order to minimize the use of synthetic fertilizers and agricultural chemicals (Backer et al., 2018).

Conclusion

In the study, the changes in the metabolism of tomato seedlings under drought stress were examined in terms of amino acid content, and the changes in the amino acid content of the plant formed a response to stress. In addition, it has been determined that biostimulant treatment can increase tolerance to stress by supporting plant growth and development with increases and decreases in these amino acid contents under stress conditions. PGPR biostimulants, the use of which has increased in recent years within the scope of sustainable agricultural practices, are also effective in increasing stress tolerance. We think that the biostimulant formulation used in this study has these effects and can be used to increase plant stress tolerance.

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