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DROUGHT AND BIOSTIMULANT TREATMENTS AFFECTED ORGANIC ACID CONTENT OF TOMATO SEEDLINGS

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Abstract: Water stress causes significant problems in plant growth and development in arid and semi-arid regions in the world. Water stress symptoms especially appears during the seedling period of plants in many crops. However, the tolerance of plants to water stress can be increased with some exogenous biostimulant applications. The present study investigated the effect of exogenous biostimulant application on organic acid content of tomato seedlings under water stresses conditions. The study was conducted as pot experiment under controlled greenhouse conditions. Drought stress treatments was applied in two different levels; full irrigation (100%) and 50% of the field capacity in the study. 1% Zn, *Bacillus subtilis*, *Bacillus megaterium*, *Azosprillum* and B*acillus amyloliquefaciens* (1x10^9cfu/ml) mixture were used as a biostimulant treatment. The biostimulant solutions were prepared at a ratio of 1/10 and 1/5 and given to the tomato seedlings three times with one-week intervals as root drench. The effects of water stress and the solutions on organic acid content of tomato seedlings were determined in the study. The results were differed depending on the organic acid type under water stress and non-stress conditions. However, depending on the application doses; the negative effect of lower irrigation level on the organic acid content was alleviated. The results of the biostimulant application doses found statistically significant. In most of the organic acids, the application dose makes massive differences on the content of the organic acids. As a result, it is thought that the effect of lower irrigation level on tomato seedlings in terms of organic acid content can be improved by exogenous biostimulant applications.

Keywords: bacteria, drought, organic acid, stress, tomato

Introduction

Drought effects normal growth, alters water relations, and reduces water use efficiency in plants. However, plants response with a variety of physiological and biochemical responses at cellular and whole organism levels (Farooq et al., 2012). Organic acids are the one of the responses of the plants under different stress factors. Adverse environmental conditions such as salinity and drought, which are among the most important abiotic stress factors, cause a decrease in yield at varying rates depending on the duration and severity of the stress; there is an increase in quality parameters such as soluble substance contents, vitamine C and organic acids that determine the taste (Kiran et al., 2018). Plants react with basic chemical properties of these organic acids for their growth and survival under stress. As well as they are an important part of metabolism, they also have an essential significance for metabolic processes (Salisbury and Ross, 1997). Multiple genes inorganic acids pathways are either up-

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regulated or down regulated to fine-tune the adaptation to these adverse conditions (Panchal et al., 2021). Many environmental stresses stimulate the biosynthesis of organic acids and their release from the roots. Webb et al. (1995) stated that oxalic acid was effective in increasing tolerance to drought conditions. Similarly, Greene et al. (1993) mentioned that malonic acid increases plant tolerance to water deficit conditions by modulating osmotic potential. All organic acids play an important role in plant development and growth, as well as in many metabolic activities, both in normal and in a wide variety of stress conditions. Organic acids are also essential for the tolerance and endurance of plants under both biotic and abiotic stress conditions (Zolman et al., 2008; Rivas-Ubach et al., 2012; Song et al., 2012; Gupta et al., 2016).

Although studies are carried out to prevent the negative effects of drought stress in plants by breeding and genetic engineering studies of tolerant varieties, the complexity of abiotic stress tolerance mechanisms makes it difficult to identify new tolerant varieties. Therefore, research has focused on increasing tolerance to drought stress with various applications, one of which is to promote stress tolerance by using various biostimulants. One of them is plant growth promoting bacteria (PGPR). In recent years, the use of plants promoting plant growth bacteria has become widespread to improve tolerance for drought stress in plants (Ruzzi and Aroca, 2015). They can positively affect the capabilities of tolerance of plants under drought stress, increasing water use efficiency, regulating physiological process such as enzyme activity, phytohormone, organic acid, amino acid and developing various mechanisms (Backer et al., 2018, Marasco et al., 2012). The importance of organic acids and their role in stress response of plants is crucial as well as it is a complex phenomenon. In the current research we focused on the organic acid content of the tomato plant that treated with different doses biostimulant under drought conditions to investigate plant organic acid response to drought and to evaluate the effect of biostimulant treatment under stress.

Materials and Methods

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. These bacterial solutions prepared at a ratio of 1/10 (T1) and 1/5 (T2) were given to the plants three times with one-week intervals. Water stress applied in two different levels; full irrigation (100% of the field capacity) 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). Irrigation times were decided according to soil moisture level 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.

Approximately 50 days after seed sowing, organic acid analyzes were performed on fresh leaf samples taken from plants. Organic acid content of tomato seedling was made according to Siddiqui et al. (2015). At the end of the experiment, the data obtained were averaged and Duncan Multiple comparison test was used for statistical analysis (SPSS, 2010).

Results and Discussion

It has been reported that the amount of organic acid plays an important role in the tolerance of abiotic stress conditions in plants (Bucio et al., 2000). Distributions of organic acids under drought stress of tomato were given in Figure1 and Figure 2.

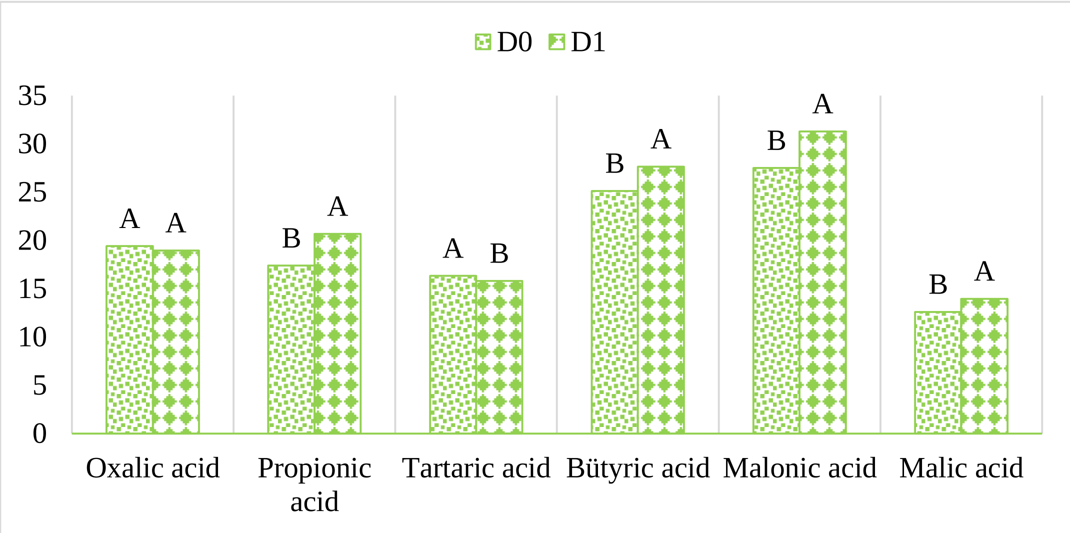

Figure 1. The contents of organic acids in leaves of tomato under drought. Mean with the same letters are not statistically different according to DMRT (p< 0.001). D0: full irrigation; 100% field capacity, D1: irrigation with 50% of field capacity.

While the content of tartaric acid, lactic acid, fumaric acid and succinic acid is higher under well-watered conditions, propionic acid, butyric acid, malonic acid, malic acid, and maleic acid content were higher under drought stress. The content of oxalic acid and citric acid is found statistically insignificant.

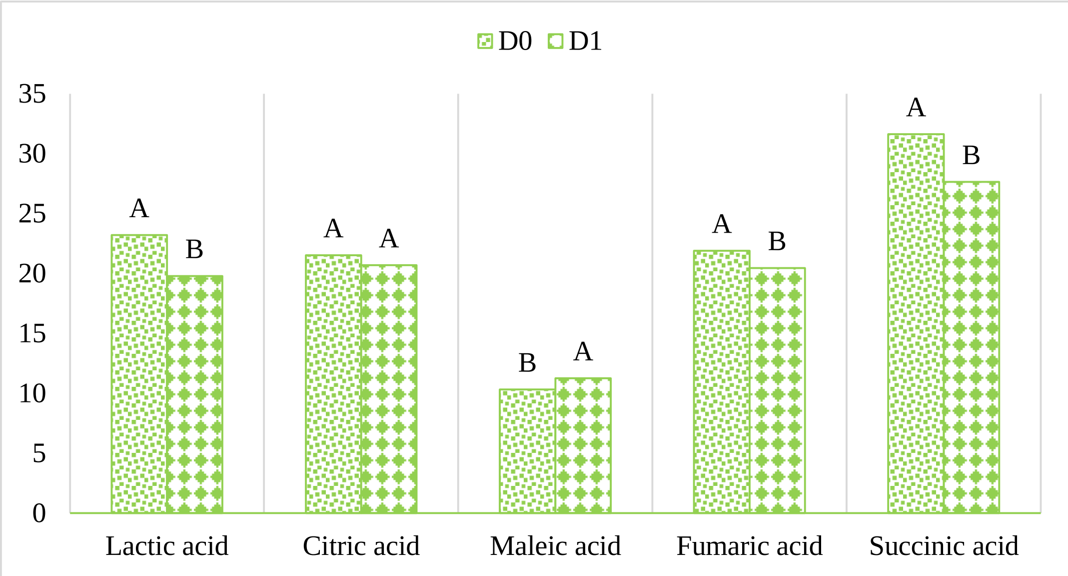

Figure 2. The contents of organic acids in leaves of tomato under drought. Mean with the same letters are not statistically different according to DMRT (p< 0.001). D0: full irrigation; 100% field capacity, D1: irrigation with 50% of field capacity.

Contents of tartaric acid, lactic acid, fumaric acid and succinic acid were negatively related with drought stress. Fumaric acid plays an important role under water stress. Fumaric acid can enrich the tolerance of the plant by modulating the osmotic balance of the plant under stress conditions (Song et al., 2012). Propionic acid, butyric acid, malonic acid, malic acid, and maleic acid contents were positively related with drought stress. The results show that tomato plants increase the content of propionic acid, butyric acid, malonic acid, malic acid, and maleic acid in the leaves as a defense mechanism to drought. Organic acids in the leaves of tomato are shown in Figure 1 and Figure 2. Butyric acid, malonic acid and succinic acids were the most three abundant organic acids in leaves. Under drought stress, butyric acid, malonic acid contents increased in leaves however succinic acid content decreased. Propionic acid content in leaves was promoted drought too.

The effect of biostimulant doses on contents of organic acids in leaves of tomato under drought were given in Table 1 and Table 2. The results show that the organic acids reacts differently to the applied biostimulant. While oxalic acid, propionic acid, tartaric acid, butyric acid, malonic acid, citric acid, and fumaric acid increased with highest dose application (T2), malic acid, lactic acid, maleic acid and succinic acid content decreased in the same treatment under control treatments (D0). The highest increase occurred in butyric acid content which increased by roughly 90% from control treatment to T2 treatment. It has been suggested that butyric acid plays an important role in many morphological, physiological and biochemical metabolic activities in plants (Zolman et al., 2008). The increase in butyric acid content supports plant tolerance under drought stress. Tartaric acid also increased roughly 65% from control treatment to T2 treatment. Rivas-Ubach et al. (2012) reported that enhanced tartaric acid content in water deficit stimulate the osmotic balance in the crops.

Table 1. The effect of biostimulant doses on contents of organic acids in leaves of tomato under drought. Mean with the same letters in column are not statistically different according to Duncan's multiple range test (p< 0.001). (D0: full irrigation; 100% field capacity, D1: irrigation with 50% of field capacity. T0: No treatment, T1: Treatment with rate of 1/10, T2: Treatment with rate of 1/5)

Treatment	Irrigation	Oxalic acid	Propionic acid	Tartaric acid	Bütyric acid	Malonic acid	Malic acid
ng µg-1							
T0	D0	19,06 c	17,14 c	14,15 d	20,62 d	25,34 d	14,06 b
	D1	22,77 b	23,81 a	12,11 e	25,84 c	27,84 d	16,52 a
T1	D0	15,03 d	16,33 c	11,62 e	16,57 e	24,96 d	10,68 c
	D1	17,89 c	19,24 b	18,93 b	24,76 c	28,66 c	11,09 a
T2	D0	24,16 a	18,69 b	23,20 a	38,19 a	32,21 b	13,05 b
	D1	16,21 d	18,99 b	16,38 c	32,36 b	37,37 a	14,26 b

Malonic acid content in the leaves increased roughly 28% from control to T2 treatment and 48% from control to T2 treatment under drought stress. Since malonic acid increases tolerance to water deficit by modulating osmotic potential in crops, the increase in malonic acid content indicates that plant tolerance to drought stress promoted by biostimulant application (Greene et al., 1993).

The reaction to the application doses also significant since T1 (1/10 concentration) application caused a certain decrease in almost all organic acids except citric acid. The results indicated that the effect of biostimulant application such as PGPR depends mostly on the application dose or concentration of the material. It is worth to notice that further research needs to focus on the application doses of the applied materials as well as the content of the biostimulant.

Table 2. The effect of biostimulant doses on contents of organic acids in leaves of tomato under drought. Mean with the same letters in column are not statistically different according to Duncan's multiple range test (p< 0.001). (D0: full irrigation; 100% field capacity, D1: irrigation with 50% of field capacity. T0: No treatment, T1: Treatment with rate of 1/10, T2: Treatment with rate of 1/5)

Treatment	Irrigation	Lactic acid	Citric acid	Maleic acid	Fumaric acid	Succinic acid
ng µg-1						
T0	D0	27,16 a	19,03 c	11,32 b	19,97 bc	37,66 a
	D1	19,64 c	20,48 c	19,10 a	21,83 b	25,83 c
T1	D0	18,29 c	20,98 bc	10,84 b	17,34 c	27,36 bc
	D1	17,60 c	18,56 c	7,61 cd	17,89 c	20,03 d
T2	D0	24,18 b	24,58 a	8,78 c	28,38 a	29,88 b
	D1	22,16 b	23,03 ab	7,06 d	21,56 b	37,07 a

The effects of biostimulant treatments on organic acid content of tomato seedling results were given in Figure 3 and Figure 4. The results indicate that the concentration of the biostimulant makes statistically different impact on organic acid content.

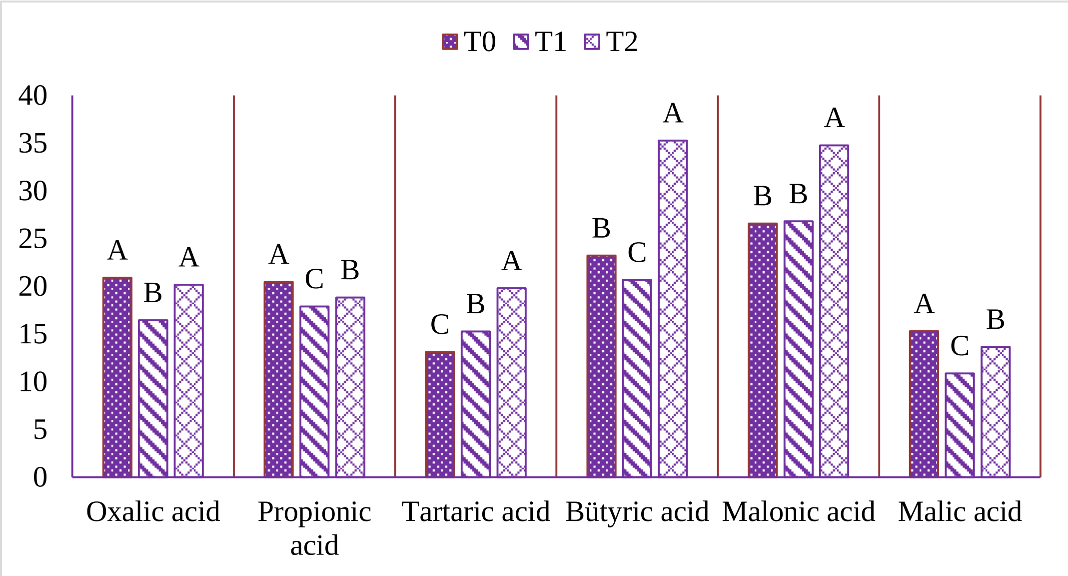

Figure 3. The effects of biostimulant treatments on organic acid content of tomato seedling. Mean with the same letters are not statistically different according to DMRT (p< 0.001). T0: No treatment, T1: Treatment with rate of 1/10, T2: Treatment with rate of 1/5

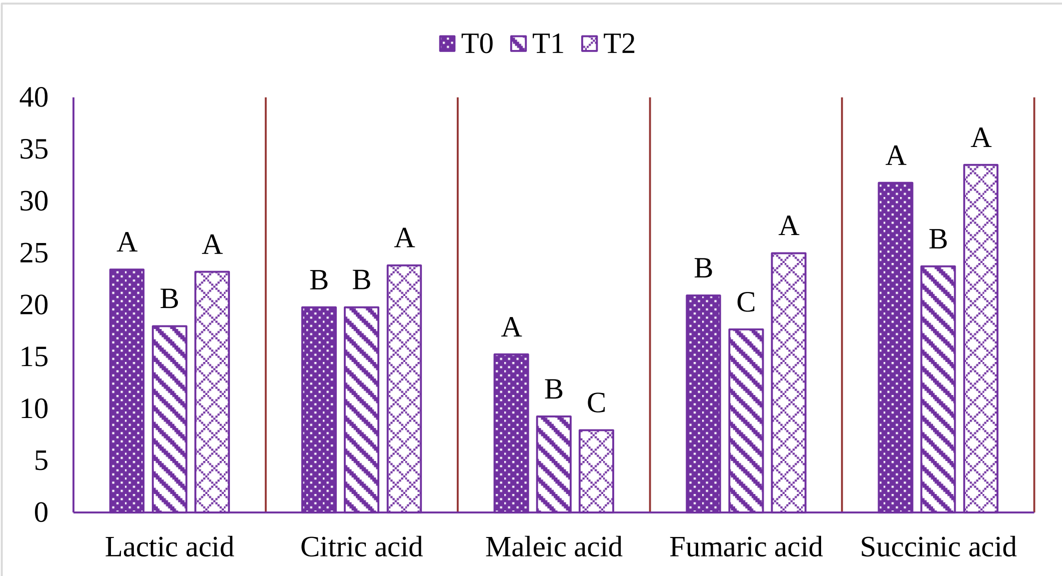

Figure 4. The effects of biostimulant treatments on organic acid content of tomato seedling. Mean with the same letters are not statistically different according to DMRT (p< 0.001). T0: No treatment, T1: Treatment with rate of 1/10, T2: Treatment with rate of 1/5.

The intense concentration of the biostimulant (T2) has higher impact in all organic acids except maleic acid as compared to T1 treatment. T2 treatments also have higher or statistically non-significant level of organic acid contents in most of the organic acid types except for propionic acid, malic acid, and maleic acid as compared to control treatments. Since these results are the mean values of D0 and D1 treatments, it mostly gives an idea about the concentration doses of the application material. Generally, the application dose makes massive differences on the content of the organic acids. The favorite effect of the biostimulant on the organic acid content is quite obvious if Figure 3 and Figure 4 assessed carefully.

Conclusion

The present study investigated the organic acid content of the tomato plant treated with different biostimulant under drought conditions to evaluate plant organic acid response to drought and to assess the effect of biostimulant treatment under drought stress. Study indicates that the content of tartaric acid, lactic acid, fumaric acid and succinic acid is higher under well-watered conditions. However, propionic acid, butyric acid, malonic acid, malic acid, and maleic acid content were higher under drought stress. The content of oxalic acid and citric acid is found statistically insignificant under drought. The results of the biostimulant application doses found statistically significant. In most of the organic acids, the application dose makes massive differences on the content of the organic acids. The intense concentration of the biostimulant (T2) has higher impact in all organic acids except maleic acid as compared to T1 treatment. It indicates that the effect of biostimulant application such as PGPR depends mostly on the application dose or concentration of the material. It is suggested that future research need to be focus on the application doses of the applied materials as well as the content of the biostimulant.

References

Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S. & Smith, D. L. (2018). Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in Plant Science*, 1473.

Bucio, J.L., Jacobo, M.F.N., Rodríguez, V.R. & Estrella, L. H. (2000). Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. *Plant Science*, 160(1), 1-13.

Farooq, M., Hussain, M., Wahid, A. & Siddique, K.H.M. (2012). Drought Stress in Plants: An Overview. In: Aroca, R. (eds) Plant Responses to Drought Stress. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-32653-0_1

Greene, J.G., Porter, R.H., Eller, R.V. &Greenamyre, J.T. (1993). Inhibition of succinate dehydrogenase by malonic acid produces an 'excitotoxic' lesion in rat striatum. *Neurochemistry*, 61, 1151-1154.

Gupta, A., Dixit, S.K. & Senthil-Kumar, M. (2016). Drought Stress Predominantly Endures *Arabidopsis thaliana* to *Pseudomonas syringae* Infection. *Front Plant Science*, 7, 808.

Kiran, S., Kusvuran, S., Ates, C., Ellialtıoğlu, S.S. (2018). The changes of fruit quality parameters at using of different eggplant rootstock/scion combinations which growing under salt and drought stress. Derim, 2018/35(2):111-120 doi: 10.16882/derim.2018.427095

Marasco, R., Rolli, E., Ettoumi, B., Vigani, G., Mapelli, F., Borin, S., Abou-Hadid, A.F., El-Behairy, U.A., Sorlini, C., Cherif, A., Zocchi, G. &Daffonchio, D. (2012). A drought resistance promoting microbiome is selected by root system under desert farming. PLoS ONE 7, e48479.

Panchal, P., Miller, A. J., & Giri, J. (2021). Organic acids: versatile stress-response roles in plants. *Journal of Experimental Botany*, 72(11), 4038-4052.

Rivas-Ubach, A., Sardans, J., Perez-Trujillo, M., Estiarte, M. &Penuelasa, J. (2012). Strong relationship between elemental stoichiometry and metabolome in plants. Proceedings of the National Academy of Sciences, 109(11), 4181–4186.

Ruzzi, M. & Aroca, R. (2015). Plant growth-promoting rhizobacteria act as biostimulants in horticulture. *Scientia Horticulturae*, 196, 124-134.

Salisbury, F.B. & Ross, C.W. (1997). Plant Physiology, 4 th. Edition, Belmont, California, USA: Wadsworth Publishing Company.

Siddiqui, S. N., Umar, S. & Iqbal, M. (2015). Zinc-induced modulation of some biochemical parameters in a high- and a low-zinc-accumulating genotype of *Cicer arietinum* L. grown under Zn-deficient condition. *Protoplasma*, 252, 1335-1345.

Song, F., Han, X., Zhu, X. &Herbert, S.J. (2012). Response to water stress of soil enzymes and root exudates from drought and non-drought tolerant corn hybrids at different growth stages. *Canadian Journal of Soil Science,* 92, 501-507.

SPSS, 2010. SPSS Inc. 18.0 Base User's Guide. Chicago (IL): Prentice Hall, USA, 2010.

Webb, M.A., Cavaletto, J.M., Carpita, N.C., Lopez, L.E. & Arnott, H.J. (1995). The intravacuolar organic matrix associated with calcium oxalate crystals in leaves of Vitis. *Plant Journal*, 7*,* 633- 648.

Zolman, B.K., Martinez, N., Millius, A., Adham, A.R. & Bartel, B. (2008). Identification and characterization of Arabidopsis indole-3-butyric acid response mutants defective in novel peroxisomal enzymes. *Genetics*, 180, 237–251.