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EFFECT OF DROUGHT STRESS ON PROLINE AND CHLOROPHYLL CONTENTS IN SOME TOMATO GENOTYPES

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Abstract

Drought is the most important factor affecting yield loss in global agriculture. Drought stress negatively affects the physiological, genetic, biochemical, and morphological characteristics of plants. The objective of this study was to determine whether there are differential responses to drought stress on proline and chlorophyll content in some tomato genotypes. Drought deficit was induced by polyethylene-glycol (PEG 6000) solution using a control and two variants with different osmotic pressures (-2.72 Bars, -4.48 Bars). The determination of proline and chlorophyll content was evaluated after periods of 7/14 and 21 days after the induction of drought stress. Comparing the biological material studied, it can be observed that different concentrations of PEG 6000 influenced differently the analysed genotypes. High levels of proline content during drought stress were noticed also in Pontica, Viorica, Darsirius, and Buzau 47. The chlorophyll content of the leaves decreased proportionally with drought induction. The lowest chlorophyll content was recorded (22,965 SPAD units) after a longer period of water stress. The obtained results will be useful to serve in plant breeding programs.

Key words: *tomato genotypes, proline and chlorophyll content, drought stress.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important horticultural crop widely spread all over the world and, too, this is a model plant of theoretical and practical significance in research (Arie et al., 2007; Guoting et al., 2020; Arshad et al., 2023; Dobrin et al., 2019).

In recent times, drought has occurred more frequently and more severely under the influence of global climate change. Most regions and countries are threatened by drought to varying degrees. (Fullana-Pericàs et al., 2018; Lehner et al., 2006; Lesk et al., 2016). Because drought is the most important factor affecting in global agriculture, drought stress may adversely affect growth and productivity, leading to final low fruit yields (Patanè et al., 2020, Liu et al., 2021). Water is an important factor to enhance the crop growth and productivity and it is essential for all living organisms including plants. Drought among various abiotic stresses, is one of the basic factors for restricting crops production (Vallivodan and Nguyen, 2006; Demidchik, 2018). It is predicted that one third of world population will be threatened by water shortage in year 2025 (Mahlagha et al., 2012). Various photosynthesis mechanisms and metabolic activities require water (Oo et al., 2020). Additionally, to maintain their growing performance, maximum amount of water is required by the plants (Tátrai et al., 2016).

Drought stress negatively affects the physiological, genetic, biochemical, and morphological characteristics of plants. (Torres-Ruiz et al., 2015). Among several physiological mechanisms developed by plants to counteract the adverse effects of drought, osmoregulatory factors, such as proline, are accumulated by plants to maintain the osmotic balance under stressful environments without damaging cellular activities (Isah, 2019). Practically, compatible osmolytes are potent osmo protectants that play a role in counteracting the effects of osmotic stress.

Proline is one of the most common compatible osmolytes in water-stressed plants. The accumulation of proline in dehydrated plants is caused both by activation of the biosynthesis of proline and by inactivation of the degradation of proline. The metabolism of proline is inhibited when proline accumulates during dehydration and it is activated when rehydration occurs (Isah, 2019; Yoshiba et al., 1997). A lot of data suggests a positive correlation between proline accumulation and plant stress (Shamsul Hayat, 2012). Proline may act as a signaling molecule able to activate multiple responses that are component of the adaptation process. Its accumulation in leaves and mainly in roots is considered a salt-sensitive trait in tomatoes, which can be used to select plants with different degrees of tolerance (Albino et al., 2002; Kavir et al., 2005). Detecting tomato plant drought stress is vital for optimizing irrigation and improving fruit quality. Moreover, there is a study it has demonstrated that proline acts as an osmo protectant and that overproduction of proline results in increased tolerance to osmotic stress. Genetically engineered crop plants that overproduce proline might acquire osmo tolerance, the ability to tolerate environmental stresses such as drought and high salinity. Drought stress restricts plant growth by decreasing photosynthetic rate. Regarding photosynthesis in leaves, chlorophyll fluorescence reflects the intrinsic characteristics of this. There are some studies have been carried out on the photosynthesis of tomato under drought stress but are not comprehensive (Guoting et al., 2020; Brix, 2010; Jangid, et al., 2016). Chlorophyll fluorescence technique is useful as a non-invasive tool in eco-physiological studies and has extensively been used in assessing plant responses to environmental stress (Parry et al., 2006). Therefore, these parameters have been widely used in plant stress-tolerance physiology, crop breeding, and agronomy. Keeping in view of the increasing drought stress concerns upon tomato crop productivity, the objective of this study was to investigate whether there are differential responses to drought stress on proline and chlorophyll content in some tomato genotypes. The results obtained will be useful to serve in plant breeding programs for the development of tomato genotypes able to cope with actual and future climatic changes.

MATERIALS AND METHODS

The biological material used in this study was represented by six tomato genotypes ('Buzau 47', 'Viorica', 'Pontica', 'Darsirius', 'Coralina', 'Carisma'). The experiment was conducted under normal (Vo - 0 Bars), and drought stress (V1 - 2.72 Bars, and V2 - 4.48 Bars) conditions created with Polyethylene-glycol (PEG6000) using the method suggested by Michael and Kaufman (1973). The plants were grown in culture pots filled with soil mixture. Plants were grown in greenhouse using a 14/10 h day/night photoperiod at 20/22ºC night/day temperature. Distilled water or PEG solution was added to each culture pot under normal and drought stress conditions, respectively, after every 2 days. The data for proline and chlorophyll contents was recorded on 7, 14 and 21 days after the induction of drought stress. Proline content was estimated according to the method proposed by Bates et al. [29] using L-proline as standard. A one-gram leaves sample was homogenised in 5 mL of aqueous sulfosalicylic acid (3%) and centrifuged at 14,000 g for 15′. Two mL of supernatant was added to 2 mL of mixed acetic acid and ninhydrin, and heated for 1 h at 100°C. The mixture was cooled rapidly in an ice-bath and added to 4 mL of toluene. Absorbance was read in a spectrophotometer at 520 nm. Leaf chlorophyll content was measured by SPAD-502 portable chlorophyll meter (Konica Minolta, Osaka, Japan), a non-destructive method for measuring optical absorbance of chlorophyll (Sala, 2021). Measured values are expressed in SPAD units and there is a direct link between the total content of chlorophyll in the leaf and measured values. Data analysis was statistically performed using ANOVA and Tukey test for a three-way factorial design (Ciulca, 2006).

RESULTS AND DISCUSSIONS

Given the results in table 1, we can observe that the study genotypes exhibited different behaviours during the experiment. Thus, a decrease in chlorophyll content can be observed after a longer period of drought stress. A significant decrease was recorded after the 14-day period, followed by the 21-day period after stress induction (Table 1). Under drought conditions, reduction of chlorophyll content, a typical symptom of oxidative stress, may result from degradation of chlorophyll and photo-oxidation of the pigments.

Table 1. The influence of stress period on chlorophyll content (SPAD) in tomato

	Periods			
Genotypes	**7days**	**14days**	**21days**	**Mean G**
BUZAU 47	22.58 c	22.08 c	21.34 c	22.00 D
VIORICA	19.80 d	18.47 d	17.99 d	18.75 E
PONTICA	33.26 a	32.07 a	31.61 a	32.31 A
DARSIRIUS	27.04 b	25.24 b	24.90 b	25.73 B
CORALINA	26.33 b	24.15 b	24.20 b	24.90 C
CARISMA	19.08 d	18.29 d	17.75 d	18.37 F
Mean	24.68 X	23.38 Y	22.96 Y	23.68

In each column, means with different letters are significant according to Tukey's test at $p \leq 0.05$.
In the last row, means with different letters are also significant at $p \leq 0.05$.

Regarding the influence of PEG concentration on chlorophyll content, it can be seen from (Table 2) that the highest mean chlorophyll content was recorded for variant V0 (24.31 SPAD) and the lowest chlorophyll content was recorded for variant V1 (23.14 SPAD). Control variants have a SPAD value between 20.12 for Carisma and 31.27 for Pontica genotype. Treatments applied to induce drought stress resulted in significant reductions in chlorophyll content in proportion to the differences between these treatments. These results are in accordance with results obtained by (Tomescu D., 2015).
The genotypes studied showed mean values of this character between 18.37 SPAD in 'Carisma' and 32.31 SPAD in 'Pontica' genotypes. The largest decrease was found in 'Carisma' and 'Viorica' cultivars. These results showed that these two genotypes suffered a large decrease in their ability to capture light energy, which would have influenced their photosynthesis. The total chlorophyll reduction under drought conditions is thought to be related to the decrease in relative water content (Makbul et al., 2011). Osmotic adjustment can also be achieved by increasing the production of organic compounds (Sakya et al., 2018).

Table 2. The influence of PEG concentration on the chlorophyll content (SPAD)

	Drought stress			
Genotypes	**0 Bars**	**-2.72Bars**	**-4.48Bars**	**Mean G**
BUZAU 47	22.91 cd	20.77 d	22.32 cd	22.00 D
VIORICA	21.61 d	13.07 f	21.57 d	18.75 E
PONTICA	31.27 a	33.78 a	31.88 a	32.31 A
DARSIRIUS	23.15 c	29.75 b	24.28 b	25.73 B
CORALINA	26.80 b	24.35 c	23.53 bc	24.90 C
CARISMA	20.12 e	17.08 e	17.92 e	18.37 F
Mean V	24.31 X	23.14 Y	23.58 Y	23.68

In each column, means with different letters are significant according to Tukey's test at $p \leq 0.05$.
In the last row, means with different letters are also significant at $p \leq 0.05$.

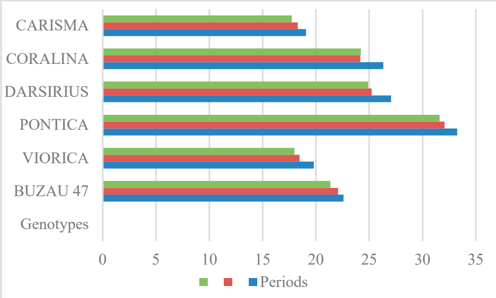

Figure 1. The influence of stress period on chlorophyll content (SPAD) in tomato

The proline content of the six genotypes under drought conditions was extremely diverse, ranging from 0.75 to 1.14 mg/g fresh weight (Table 3).
The proline content in 'Pontica' (1.14 mg/g) followed by 'Viorica' (1.13 mg/g) and 'Darsirius' (0.97 mg/g) genotypes were higher than others. This indicates that genotypes attempted to survive in drought conditions by increasing the proline content.
To survive in drought conditions, plants accumulate osmolyte compounds such as amino acids, proline, and organic acids (Behnamnia et al., 2009; Zhang et al., 2006; Ashraf et al., 2007; Hamim et al., 2009). However, not all plants exhibit osmotic adjustment through high proline accumulation.
Regarding the influence of stress period, a mean increase in proline content in tomato

leaves can be observed from 0.91 mg/g fw after 7 days to 0.98 mg/g fw after 21 days (Table 3).

Table 3. The influence of stress period on proline content in tomato

	Period			
Genotypes	7 days	14 days	21 days	Mean G
BUZAU 47	0.83 c	0.84 c	0.86 c	0.84 C
VIORICA	1.10 a	1.12 a	1.16 a	1.13 A
PONTICA	1.08 a	1.13 a	1.21 a	1.14 A
DARSIRIUS	0.93 b	0.95 b	1.03 b	0.97 B
CORALINA	0.72 d	0.73 d	0.79 d	0.75 D
CARISMA	0.82 c	0.83 c	0.84 cd	0.83 C
Mean P	0.91 Z	0.94 Y	0.98 X	0.94

In each column, means with different letters are significant according to Tukey's test at $p \leq 0.05$.
In the last row, means with different letters are also significant at $p \leq 0.05$.

Regarding the influence of PEG concentration on proline content in tomato (Table 4, Figure 2), the means values were between 0.81 mg/g fw for variant V0 (0 Bars H_2O) and 1.08 mg/g fw for variant V2 (-4.48 Bars PEG6000).
We observed that leaf proline increased significantly under mild and severe drought stress compared with the control in all genotypes. The treatments applied to induce osmotic stress resulted in a significant increase in proline content in proportion to the differences between these treatments.
The same results were reported by another researcher (Mahlagha et al., 2012).

Table 4. The influence of PEG concentration on the proline content mg/g in tomato

Genotypes	Drought stress			
	0 Bars	-2.72Bars	-4.48Bars	Mean G
BUZAU 47	0.76 b	0.83 c	0.94 b	0.84 C
VIORICA	1.03 a	1.10 a	1.24 a	1.13 A
PONTICA	1.03 a	1.15 a	1.25 a	1.14 A
DARSIRIUS	0.65 c	1.01 b	1.25 a	0.97 B
CORALINA	0.67 c	0.74 d	0.84 c	0.75 D
CARISMA	0.73 b	0.83 c	0.93 b	0.83 C
Mean V	0.81 Z	0.94 Y	1.08 X	0.94

In each column, means with different letters are significant according to Tukey's test at $p \leq 0.05$.
In the last row, means with different letters are also significant at $p \leq 0.05$.

In tomatoes, the production and accumulation of proline are based on the duration of stress, genetic potential, and stress conditions (Jureková, 2011; Cooper et al., 2006). Among osmoprotectants, proline is an essential amino acid that has excellent antioxidant capabilities, helping to prevent cell death (Bhardwaj and Yadav, 2012; Oguz, et al., 2022).

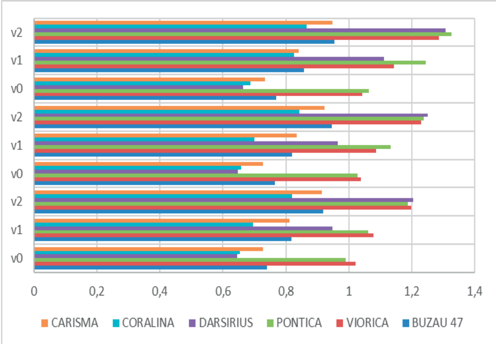

Figure 2. The influence of different drought stress treatments on proline content in tomato

Many scientists believe that the accumulation of proline due to stress serves as a biochemical indicator for the selection of resistant cultivars (Mwadzingeni et al., 2016).

CONCLUSIONS

Plant adaptive responses under drought, are mainly influenced by the timing, intensity, duration, and stress rate. Application of osmotic pressure caused significantly differences in terms of chlorophyll contents. Also, the biggest decrease was found in 'Carisma' and 'Viorica' cultivars. Treatments with PEG applied to induce osmotic stress determined significant increase of proline content in proportion to the differences between these treatments. The proline content in 'Pontica genotypes' was higher than others, this indicates that 'Pontica' followed by the 'Viorica' genotype attempted to survive in the drought conditions. The obtained results will be useful for tomato breeding programs, and also for seed production and tomato growers.

REFERENCES

Albino Maggio, Saori Miyazaki, Paola Veronese, Tomomichi Fujita, Jose I. Ibeas, Barbara Damsz, Meena L. Narasimhan, Paul M. Hasegawa, Robert J. Joly and Ray A. Bressan, (2002). Does proline accumulation play an active role in stress-induced growth reduction-*The Plant Journal* 31(6), 699±712.

Arie, T., Takahashi, H., Kodama, M. & Teraoka, T. (2007). Tomato as a model plant for plant–pathogen interactions *Plant Tissue Cult. Lett*. 24 135 -147.

Arshad A., Jerca I.O, Chan S, Cîmpeanu S.M., Teodorescu R.I., Țiu J, Bădulescu L., Drăghici E., (2023) Study regarding the influence of some climatic parameters from the greenhouse on the tomato production and fruits quality, *Scientific Papers. Series B, Horticulture*. Vol. LXVII, No. 2, Print ISSN 2285-5653,

Ashraf M, and M R Fooland (2007) *Environmental and Experimental Botany* 59 206–16

Bates, L.S.; Waldren, R.P.; Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil*, 39, 205–207.

Bhardwaj, J., Yadav, S.K. (2012). Comparative study on biochemical parameters and antioxidant enzymes in a drought tolerant and a sensitive variety of horsegram (Macrotyloma uniflorum) under drought stress. *American Journal of Plant Physiology* 7:17–29.

Behnamnia, M., Kh. M. Kalantari and F. Rezanejad, (2009). Exogenous Application of Brassinosteroid Alleviates Drought-Induced Oxidative Stress In Lycopersicon esculentum L. *General and Applied Plant Physiol*. 35: 22- 34.

Brix, H. (2010). The effect of water stress on the rates of photosynthesis and respiration in tomato plants and loblolly pine seedlings. *Physiol. Plant*. 15:10–20.

Ciulca, S. (2006). *Experimental methodologies in agriculture and biology*. Timisoara, RO: Agroprint.

Cooper, M., van Eeuwijk, F., Chapman, S.C., Podlich, D.W., Löffler, C. (2006). Genotype-by environment interactions under water-limited conditions. In *"Drought Adaptation in Cereals*", (J-M. Ribaut, ed.). pp. 51-96. CRC Press, New York, NY, USA.

Demidchik, V. (2018). ROS-activated ion channels in plants: biophysical characteristics, physiological functions and molecular nature. *International Journal of Molecular Sciences* 19:1263

Dobrin A., Nedelus A., Bujor O., Mot A., Zugravu M., Bădulescu L. (2019) Nutritional quality parameters of the fresh red tomato varieties cultivated in organic system. *Scientific Papers. Series B, Horticulture, 63:1,* 439-446.

Fullana-Pericàs, M., Ponce, J., Conesa, M.A., Juan, A., Ribas-Carbó, M. & Galmés, J. (2018). Changes in yield, growth and photosynthesis in a drought-adapted Mediterranean tomato landrace (Solanum lycopersicum 'Ramellet') when grafted onto commercial rootstocks and solanum pimpinellifolium *Scientia Hort*. 233 70 77.

Guoting Liang, Junhui Liu, and Jingmin Zhang, (2020). Effects of Drought Stress on Photosynthetic and Physiological Parameters of Tomato, *J. Amer. Soc. Hort. Sci*. 145(1):12–17.

Hamim, Ashri K, Miftahudin and Triadiati (2008). Agrivita 30 201-10 Farooq M, Wahid A, Kobayashi N, Fujita D, and Basra S M A 2009 *Agron. Sustain. Dev*. 29 185-212

Isah, T. (2019). Stress and defense responses in plant secondary metabolites production. *Biol. Res*., 52, 39.

Yoshiba Y, Kiyosue T, Nakashima K, Yamaguchi-Shinozaki K, Shinozaki K, (1997). Regulation of levels of proline as an osmolyte in plants under water stress, *Plant Cell Physiol*, 38(10):1095-1020.

Jangid, K.K. and P. Dwivedi. (2016). Physiological responses of drought stress in tomatoes: A review. *Intl. J. Agr. Environ. Biotechnol*. 9:53–61.

Jureková Z, Németh-Molnár K, Paganová V, (2011). *Journal of Horticulture and Forestry* 3 294-300

Kavir Kishor, P. B., S. Sangam, R. N. Amrutha, P. Sri Laxmi, K. R., Naidu, S. Rao KRSS Rao, K. J. Reddy and N. Sreenivasulu. (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implication in plant growth and abiotic stress tolerance. *Curr. Sci*. 88(3): 424- 438.

Liu, Y., Wen, L., Shi, Y., Su, D., Lu, W., Cheng, Y., & Li, Z. (2021). Stress-responsive tomato gene SlGRAS4 function in drought stress and abscisic acid signalling. Plant Science, 304, 110804, https://doi.org/10.1016/j.plantsci.2020.110804.

Lehner, B., Döll, P., Alcamo, J., Henrichs, T. & Kaspar, F. (2006). Estimating the impact of global change on flood and drought risks in Europe: *A continental, integrated analysis Clim. Change* 75 273 299.

Lesk, C., Rowhani, P. & Ramankutty, N. (2016). Influence of extreme weather disasters on global crop production. *Nature* 529.

Mahlagha Ghorbanli, Maryam Gafarabad, Tannaz Amirkian and Bahareh Allahverdi Mamaghani (2012) Investigation of proline, total protein, chlorophyll, ascorbate and dehydroascorbate changes under drought stress in Akria and Mobil tomato cultivars, *Iranian Journal of Plant Physiology*, Vol (3), No (2)

Makbul S, Guler N S, Durmus N and Guven S (2011). *Turk J. Bot*. 35 369-77

Oo, A.T., van Huylenbroeck, G., Speelman, S. (2020). Measuring the economic impact of climate change on crop production in the dry zone of Myanmar: A ricardian approach. *Climate* 8:9.

Oguz, M.C., Aycan, M., Oguz, E., Poyraz, I., Yildiz, M. (2022). Drought stress tolerance in plants: interplay of molecular, biochemical and physiological responses in important development stages. *Physiologia 2*(4):180- 197. https://doi.org/10.3390/physiologia2040015

Parry, M.A.J., J. Flexas and H. Medrano. (2006). Prospects for crop production under drought:research priorities and future directions. *Ann. Appl. Biol*., 147: 211-226.

Patanè, C.; Corinzia, S.A.; Testa, G.; Scordia, D.; Cosentino, S. L (2020). Physiological and agronomic responses of processing tomatoes to deficit irrigation at critical stages in a semi-arid environment. *Agronomy*, 10, 800.

Sakya, A. T., Sulistyaningsih, E., Indradewa, D., Purwanto, B.H. (2018), Physiological characters and tomato yield under drought stress. IOP Conf. Series: *Earth and Environmental Science* 200 012043

Sala, F. (2021). Variation of photosynthetic pigments and chl/car ratio in wheat in relation to fertilization rate. *AgroLife Scientific Journal*, 10(2). https://doi.org/10.17930/AGL2021220

Shamsul Hayat, Qaiser Hayat, Mohammed Nasser Alyemeni, Arif Shafi Wani, John Pichtel, and Aqil Ahmad, (2012). Role of proline under changing environments, *Plant Signal Behav*. Nov 1; 7(11): 1456–1466.

Tátrai, Z.A., Sanoubar, R., Pluhár, Z., Mancarella, S., Orsini, F., Gianquinto, G. (2016). Morphological and physiological plant responses to drought stress in Thymus citriodorus. *International Journal of Agronomy* 4165750.

Torres-Ruiz, J.M., A. Diaz-Espejo, A. Perez-Martin, and V. Hernandez Santana. (2015). Role of hydraulic and chemical signals in leaves, stems and roots in the stomatal behaviour of olive trees under water stress and recovery conditions. *Tree Physiol*. 35:415–424.

Tomescu D. Sumalan R., Soran Loredana, Copolovici L. (2015). The influence of soil salinity to chlorophylls and β-carotene contents in Lycopersicon esculentum Mill., *Journal of Horticulture, Forestry and Biotechnology* Volume 19(2), 35- 38.

Vallivodan, B. and H. T. Nguyen. (2006). Understanding regulatory networks and engineering for enhanced drought tolerance in plants. *Curr. Opin. Plant Biol*. 9: 189-195.

Zhang, J., W. Jia, J. Yang, and A. M. Ismail, (2006). Role of ABA in integrating plant response to drought and salt stresses. *Field Crops Res*. 97: 111-119.