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# Effect of deficit irrigation on physiological and biochemical traits of tomato (*Solanum lycopersicum*) genotypes

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#### Abstract

The frequency of drought periods influences the yield potential of crops where the change in climatic condition force researchers to find drought tolerant genotypes. The aim of the study was to screen thirteen tomato (*Solanum lycopersicum*) genotypes under mild and severe drought conditions in order to identify those genotypes with best performance. As a result of the analysis, it shows that genotypes showed decrease in pigment concentration, relative water content and soluble protein under both drought conditions compared with the control. In contrast, the proline concentration and MDA were found to increase. The antioxidant enzyme (SOD, CAT and POD) activity was in increasing pattern in all genotypes and among them SOD exhibits good correlation with drought stress compared to other enzymes. Conclusively genotypes SL CBE 106, SL CBE 126 and SL CBE 115 could be considered as drought tolerant and could be used as an excellent donor in genetic improvement program.

Keywords: Drought, tomato, pigment, antioxidant, genetic improvement

#### 1. Introduction

Abiotic stress is considered the biggest challenge facing the world's agriculture (Mitler, 2006; Raja *et al.* 2020) <sup>[1, 2]</sup>. About 50% of yield reduction in most of the crops is a direct result of abiotic stress (Rodríguez M, 2005) <sup>[3]</sup>. Water scarcity is one of the major abiotic stresses, which influences plant growth and development. Soil is said to be droughted when the soil water potential and plant's turgor drop below a threshold level which perturbs the plant's normal function (Kamanga, Rowland M, 2020) <sup>[4]</sup>. The incidence of drought is becoming more severe especially in arid and semi-arid regions not only due to increasing temperature but also to enormous and irrational depletion of natural resources (Wakchaure, G.C, 2020) <sup>[5]</sup>. The response of plants to drought stress may cause several reversible and irreversible changes in their physiology and metabolism which is more specific. The ability of a plant to produce an economic product with minimum water loss under drought conditions is referred as drought tolerance (Mitra J 2001) <sup>[6]</sup>. To develop water saving and drought resistant crop, it is necessary to obtain knowledge on the fundamental science of drought resistance.

Tomato (*Solanum lycopersicum*) belongs to the family Solanaceae, one of the commercially important crops grown worldwide. It is rich in vitamin A, B,C and antioxidants which has been shown to prevent several diseases. Inspite of its nutritive value and high yielding nature, tomatoes are most sensitive to water deficiency particularly at fruit setting and intensive fruit development periods (Nemeskéri, E, 2019)<sup>[7]</sup> which results in a 25 to 50% decrease in yield (Pires, 2011)<sup>[8]</sup> and during early flowering which causes flower shedding and lack of fertilization (Bahadur, 2011)<sup>[9]</sup>

To cope up with the damage caused by drought, plants enhance the defense mechanism by reducing leaf size and number, increasing root length, developing thick cuticles, and maintain cell turgor by accumulating compatible osmolytes (Harb, 2010)<sup>[10]</sup>. Another strategy involved in plant's drought response is by the ROS scavenging antioxidant mechanisms (Apel K, Hirt H, 2004)<sup>[11]</sup>. This mechanism can be divided into enzymatic such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and non-enzymatic such as carotenoids, ascorbic acid and tocopherol (Zgallaï,2006)<sup>[12]</sup>. They work in concert to control the cascade of uncontrolled oxidation and protect plant cells by scavenging ROS from oxidative damage (Mittler, 2004)<sup>[13]</sup>.

Despite large variability of tomato genotypes and landraces, global tomato production relies on modern cultivars which is high-yielding but significantly sensitive to water stress (Tieman *et al.*, 2017) <sup>[14]</sup>. To utilize the wild genotypes of tomato in breeding for drought resistance it is necessary to increase knowledge on the response of plants to water deficit. Therefore from the economic point of view, effective screening indices should be developed for identifying genotypes/genotype which is bale to provide unwavering yield under water deficit condition.

The present work was undertaken to study the degree of tolerance among different genotypes of *Solanum lycopersicum* under drought stress and identify the tolerant genotype among them. Physiological changes in pigment content, membrane stability, relative water content, proline content, lipid peroxidation and the activity of enzymatic antioxidants were analysed in thirteen tomato genotypes under water deficit condition and the variation among them will be discussed.

#### 2. Materials and Methods

#### **2.1. Planting materials and Growing conditions**

Thirteen tomato genotypes were used in the study. The seeds were surface sterilized with 1% Sodium Hypochlorite solution for 15 mins and washed thoroughly with distilled water to avoid sediments of NaOCl<sub>2</sub> on the seed surface. The seeds were grown in seedling tray containing cocopeat and vermicompost mixture as substrate. Twenty-five days old seedlings were transplanted to plastic pots and soil mixture was prepared by mixing tank silt and vermicompost. Two water treatments were induced by adjusting the pot water capacity as 100% for control and 50% for stress treatment and were maintained by watering the plants with required amount of water as per the weight recorded.

#### 2.2. Experimental design

The experimental material was arranged in Factorial Completely Randomized Design (FCRD) with three replicates of each genotype. Drought stress was imposed on 14 days after transplanting. The plant materials were harvested at three time frames, including 0, 5 and 10 days during water stress and the daily irrigated plants were also harvested at same time frames. Fully grown top most leaves were harvested and submerged in liquid nitrogen for biochemical and antioxidant enzyme analysis.

#### 2.3. Pigments

Pigments including Chlorophyll a(Chl a), Chlorophyll b (Chl b) and carotenoids were extracted from leaf tissue by following the method described by (Lichtenthaler, H. K., & Wellburn, A. R.,1983) <sup>[15]</sup> Fresh leaf tissue (250mg) was homogenized with 10ml of 80% acetone and centrifuged at 3000rpm for 20 mins. The supernatant was collected and made up to 20ml with 80% acetone and OD value was absorbed at 480nm, 510nm, 640nm and 665 nm and expressed as mg/g.

#### 2.4. Relative water content

Leaves with same developmental stage were used as that of the method described by (HD Barrs, PE Weatherley,1962) <sup>[16]</sup>. The fresh weight ( $w_1$ ), turgid weight ( $w_2$ ) and dry weight ( $w_3$ ) of the leaves were measured and Relative water content was calculated as follows:

#### $RWC = W1 - W2/W2 - W3 \times 100$

#### 2.5. Malonaldehyde (MDA)

Lipid peroxidation induced by drought stress is indicated by the decomposition product MDA as a result of oxidative damage, was measured according to Heath and packer (1968) <sup>[17]</sup> methodology. 0.5 g of leaf tissue was extracted in 2ml of 0.1% TCA and centrifuged at 13,000 rpm for 20 mins. 1.5ml of 20% TCA containing 0.5% TBA was added to 0.5ml aliquot of supernatant. The mixture was placed in hot water bath at 95°C for 30 mins and cooled by placing in water bath. The absorbance was read at 532nm and 600nm and was calculated using the extinction coefficient 155m/M/cm.

#### 2.6. Proline determination

The proline content was determined according to the method described by Bates *et al.*, (1973) <sup>[18]</sup>. 0.5g leaf samples homogenised with 10ml of 3% Sulphosaliciylic acid and centrifuged. A mixture of 2ml of Supernatant, 2ml of Ninhydrin and 2ml of Glacial acetic acid was boiled at  $100^{\circ}$  C for one hour. After cooling, 4ml of toluene was added, stirred and the fraction were separated. Absorbance was measured at 520nm and expressed in µmoles / g tissue.

#### 2.7. Activity assay for protein and antioxidant enzymes

For Protein and antioxidant assay, 0.5 g of frozen leaf samples were taken from control and stress induced plants and were ground to fine powder in liquid nitrogen and homogenized with 2ml of extraction buffer (pH 7.0) containing 2mM EDTA, 1% PVP. The homogenate was centrifuged at 13,000 rpm at  $4^{0}$  C for 10 mins and supernatant was used for assay. The Soluble protein concentration was determined by the method of Lowrey's *et.al* using Bovine serum albumin as standard.

Catalase (CAT) activity was determined by the method of Aebi (1984) <sup>[19]</sup>. The rate of decomposition of  $H_2O_2$  was followed by decrease in absorbance at 240nm in a reaction mixture containing 1.5ml phosphate buffer, 1.2ml hydrogen peroxide and 300µl of enzyme extract. One unit of CAT activity was defined as absorbance change of 0.01 units per min.

Peroxidase (POD) was estimated according to the method of Putter (1974). The reaction mixture comprises of 1ml enzyme extract, 3ml pyrogallol and 0.5ml  $H_2O_2$  and read at absorbance of 430nm.

Superoxide dismutase was measured based on the inhibitory effect of SOD on the reduction of nitro blue tetrazolium (NBT) by superoxide radicals, that are generated by the autooxidation of hydroxylamine hydrochloride. The reduction of NBT was read at 540nm according to the method of Kono (1978) <sup>[20]</sup>. The reaction mixture comprises of 1.3m buffer, 500µl NBT,100µl Triton X-100, 100 µl of hydroxylamine hydrochloride and 70 µl of enzyme extract.

#### 2.8. Statistical analysis

The stress related data were normalized with the control by the following formula  $(Tn \times T0/Cn)$  given by (Aghaie P.*et al.*, 2018)<sup>[21]</sup> where Cn and Tn were the parameters taken at n days after stress for control and treated respectively, and T0 is the base measured before imposing stress. All experiments were repeated at least three times and analysed using SPSS package (version 16).

#### 3. Results and Discussion

### 3.1. Effect of water deficit on Pigment content

The photosynthetic pigments play significant role in harvesting photons for energy metabolism and under stressful environments chlorophyll measurements are deliberated as an imperious tool to evaluate the effects on plants. In the present study, the pigment concentration was found to decrease in a linear manner by increasing drought intensity (Table.1). Significant variations was observed for Chlorophyll a, Chlorophyll b, total chlorophyll and carotenoids among all the tomato genotypes. The highest mean reduction of Chl a and Chl b was observed in SL CBE 130 by 50.45% and 32.43% respectively, under severe drought stress condition compared with the control. Whereas no significant difference was observed in genotypes SL CBE 106 and SL CBE 126 under severe drought level. The maximum reduction of total carotenoid (21.4% and 24.5% compared with control) was also observed in SL CBE 130 and PKM-1. Overall, maximum reduction in the pigment concentration was observed in SL CBE 130 and PKM-1 under both drought conditions compared with the control. The reduction may occur due to the stress induced damages in the pigment biosynthesis pathway. This might also occur due to the enhanced increase in synthesis of compatible solutes like proline, since both these compounds are synthesized from same precursors (LG Paleg et al., 1981)<sup>[22]</sup>. The genotype SL CBE 130 which is having the highest reduction might be considered as sensitive and in contrast genotype SL CBE 106 and SL CBE 126 having high pigment concentration may be considered as tolerant. Carotenoids that are located together with chlorophyll in leaves plays protective function during drought stress (M Egert, M Tevini, 2002) <sup>[3]</sup>. The decrease of  $\beta$ carotene is often synchronized with degradation of chlorophyll pigments which is in conscience with our results which shows a positive correlation between carotenoid and chlorophyll content in both drought stress levels. Therefore, a decrease in total chlorophyll eventually results in lower light harvesting capacity under stressful conditions.

# 3.2. Effect of drought on Proline

Proline is one of the compatible solutes and osmoregulatory in higher plants, which is believed to play important role in stress tolerance. (N Verbruggen, C Hermans, 2008) [24]. The result of the study shows a significant difference in proline content of cultivars before and after drought stress (Fig 1). Basal proline level in stressed tomato plants almost doubled the level observed on control plants. Proline content increased meaningfully in genotype SL CBE 106, SL CBE 123, SL CBE 124 and SL CBE 126. The highest ratio of proline was observed in SL CBE 106 and SL CBE 124 by 2.2 and 2.4-fold increase compared with control, whereas other cultivars showed a moderate increase in the proline level. In Several studies, the higher accumulation of proline under stress condition has been considered as a selection criteria for identifying drought tolerant genotypes. Accordingly, the genotypes SL CBE 106 and SL CBE 124 having increased proline level would be considered as tolerant. Several recent study supports the idea that Proline accumulation occurs under stress condition (W Claussen, 2005) <sup>[25]</sup> because of its property to stabilize subcellular structures and buffer cellular redox potentials (MI Dar et al., 2015)

# 3.3. Relative water content (RWC) under drought

Relative water content is a widely used index to display the

water status of plants (Siddique *et al*, 2000)<sup>[27]</sup>. Under stress conditions, plants maintain their physiological balance through higher RWC. The RWC of all genotypes showed higher levels (72% and 90%) under well-watered condition (control). RWCs significantly decreased in genotypes SL CBE 105, SL CBE 108, SL CBE 130 and PKM-1 by 29%, 35%,20% and 29% respectively. Meanwhile, in genotypes SL CBE 106, SL CBE 126 and SL CBE 115 RWCs showed less than 10% reduction in comparison with the control. Other genotypes showed a moderate reduction between 15-20% under severe stress condition. Present investigation is in accordance with earlier findings of Devendra and Minhas (1999)<sup>[31]</sup> in potato, Kirnak et al., (2001) in egg plant. Related to its protoplasmic permeability, plants with lower RWC are believed to be more sensitive to drought. Confirming with previous studies, which states, the maintenance of relatively constant RWC was considered as a criteria to classify cultivars as tolerant and sensitive. In our present study, the Genotype SL CBE 106, SL CBE 126 and SL CBE 115 which maintained constant RWC under both drought levels will be considered as most tolerant to water deficit condition.

# 3.4. Malondialdehyde (MDA)

Plants subjected to different stress condition results in accumulation of reactive oxygen species (ROS) which degrades polyunsaturated lipids thereby forming MDA and being the product of lipid peroxidation in cell membrane, which is directly proportional to the extent of lipid peroxidation and membrane injury(A Ayala, *et.al.*,2014). In this study, lipid peroxidation level shows significant increase under both drought levels compared with the control. The highest increase in MDA under severe drought stress was observed in genotypes SL CBE 124, PKM-1 and G 30 by 57%, 48%. 52% respectively. Whereas only 13.7%, 13.8% and 12% increase was seen in genotypes SL CBE 106, SL CBE 109 and SL CBE 126 respectively.

# **3.5. Soluble Protein under drought**

The soluble protein content of the leaf, being a measure of RuBP carboxylase activity is considered as an index for photosynthetic efficiency. Diethelm states that RuBisCO content per unit leaf area was positively correlated with that of leaf soluble protein content. As a result of present study, the soluble protein content significantly reduced upon increasing the severity of the stress. Changes in soluble protein content caused by drought stress were given in (Fig.2). The highest reduction was observed in genotype SL CBE 123 and SL CBE 130 by 38.8% and 38.9% respectively. Meanwhile the least reduction was observed in genotype SL CBE 108 and SL CBE 133 (19.6% and 19.5% respectively) and other genotypes fall between these two levels compared with the controls. The reduction of soluble protein might be due to the degradation of available soluble protein in plants and also due to reduction of synthesis of new protein. Maintenance of soluble protein in genotypes could be due to the higher RuBisCO activity which thereby lead to more carbon fixation and ultimately to higher photosynthetic efficiency under drought which act as a criteria for tolerance.

# 3.6. Enzymatic antioxidant system under drought

Drought stress is reported to induce or involve oxidative stress by increasing the production of reactive oxygen species (ROS) which can be toxic and inhibit metabolism and plant growth. The major source of ROS production in plant cell is the chloroplast where stress-induced changes lead to the formation of singlet oxygen, hydrogen peroxide and superoxide. Scavenging of ROS is achieved by an efficient antioxidant scavenging system comprising of enzymatic antioxidants (Noctor G.et al., 1998)<sup>[29]</sup>. There are several reports which highlight the relationship between enzymatic antioxidants and the rate of ROS production during water stress (Iturbe-Ormaetxe,1998)<sup>[30]</sup>. To overcome the oxidative stress, Superoxide dismutase (SOD) which converts the superoxide radical into H<sub>2</sub>O<sub>2</sub>, which is further reduced to H<sub>2</sub>O by catalase and Peroxidase (ozkur sci). Accordingly in the present study, significant difference was observed in SOD activity among all genotypes at both stress level. The highest increase in SOD was observed in genotype SL CBE 126, SL CBE 106 and SL CBE 115 by 37.6%, 37.1% and 30% respectively at severe stress compared with control. Meanwhile the lowest activity was seen in genotype SL CBE 130 and PKM-1(3.7% and 4.97% respectively) and other genotypes fall between the two levels. This was in accordance with the report in which increased SOD activity was observed at drought stress in tomato (Zgallaï, H. 2006) <sup>[12]</sup>. Kumar.et al., (2011)<sup>[9]</sup> reported significantly increased SOD activity by inducing stress with PEG (-0.45MPa and -1.22 MPa) in tomato plants and suggest that SOD activity could be used as

a criteria to screen genotypes for their tolerance to drought. Catalase (CAT) which is a key enzyme in glutathioneascorbate pathway involved in  $H_2O_2$  detoxification created by SOD (foyer bio). The result reveals significant difference in the activity of CAT(Fig.3). Among the genotypes, the CAT activity was high in SL CBE 106, SL CBE 110 and SL CBE 126 at both drought stress levels. Interestingly, the CAT activity significantly decreased in genotypes SL CBE 123, SL CBE 124 and SL CBE 130 under severe drought stress, which reflects the low-ROS scavenging capacity and increased damage in these genotypes under this condition.

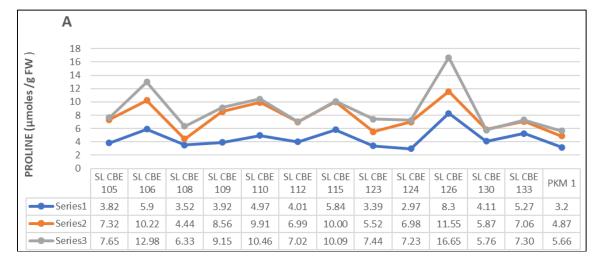
Peroxidase which catalyses  $H_2O_2$  dependent oxidative reactions. The results reveal a gradual increase of POD activity under both drought conditions. Significant difference was observed in genotype SL CBE 108, SL CBE 106 and SL CBE 126 having 1.6%, 1.59% and 1.5% increase at severe drought level compared with the control. In contrast to this, no significant difference was observed in genotype SL CBE 130, PKM-1 and SL CBE 123.

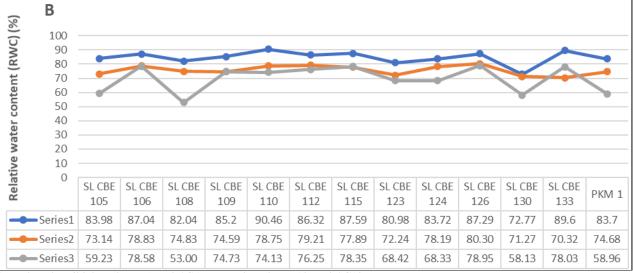
As a whole, our results suggest that compared to CAT and POD, SOD activity would be more a responsive character in drought stress of tomato and could be used as a reliable tool to screen for genotypes with drought tolerance.

**Table 1:** The contents of Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids of thirteen tomato genotypes under control, mild andsevere drought stress condition. Values are the mean of three independent replicates. Mean difference is significant at p < 0.05, LSD – leastsignificant difference

	Genotype	chlorophyll a (mg/g FW)			chlorophyll b (mg/g FW)			Total chlorophyll (mg/g FW)			carotenoids (mg/g FW)			
		control	Mild	severe	contr	Mild	severe	contr	Mild	severe	contr	Mild	severe	
			drought	drought	ol	drought	drought	ol	drought	drought	ol	drought	drought	
	SL CBE 105	1.4533	1.31	0.95	0.58	0.55	0.53	2.42	2.21	1.71	0.48	0.43	0.39	
	SL CBE 106	1.4916	1.44	1.18	0.81	0.78	0.71	2.30	2.26	2.12	0.75	0.70	0.72	
	SL CBE 108	1.6001	1.36	0.88	0.55	0.48	0.46	2.87	2.52	2.48	0.50	0.43	0.42	
	SL CBE 109	1.2833	1.07	1.00	0.82	0.68	0.69	2.83	2.58	2.49	0.66	0.64	0.61	
	SL CBE 110	1.6483	1.40	1.24	0.73	0.70	0.67	2.30	2.03	1.87	0.58	0.50	0.53	
	SL CBE 112	1.4833	1.30	1.34	0.83	0.80	0.75	2.15	1.95	1.83	0.44	0.41	0.36	
	SL CBE 115	1.2237	1.14	0.84	0.58	0.49	0.43	1.80	1.72	1.63	0.44	0.42	0.38	
	SL CBE 123	1.923	1.70	1.74	0.79	0.66	0.73	2.78	2.38	2.35	0.69	0.64	0.65	
	SL CBE 124	1.866	1.66	1.59	0.70	0.64	0.63	2.57	2.34	2.30	0.53	0.50	0.46	
	SL CBE 126	2.486	2.36	2.12	0.99	0.96	0.93	3.46	4.09	3.08	0.77	0.73	0.75	
	SL CBE 130	0.8095	0.61	0.40	0.35	0.28	0.24	1.36	1.14	1.11	0.35	0.30	0.27	
	SL CBE 133	2.303	1.65	1.61	0.91	0.79	0.87	3.20	2.23	2.18	0.65	0.60	0.59	
	PKM 1	0.8938	0.76	0.44	0.39	0.36	0.32	1.28	1.21	0.97	0.35	0.26	0.27	
	G	0.09			0.08			0.23			0.27			
S.E (D)	D	0.05			0.04			0.11			0.13			
	C X D	0.16			0.14			0.39			0.47			
CD (0.05)	G	0.19				0.16			0.45			0.54		
	D	0.09				0.08			0.22			0.26		
	C X D	0.33			0.28			0.78			0.94			

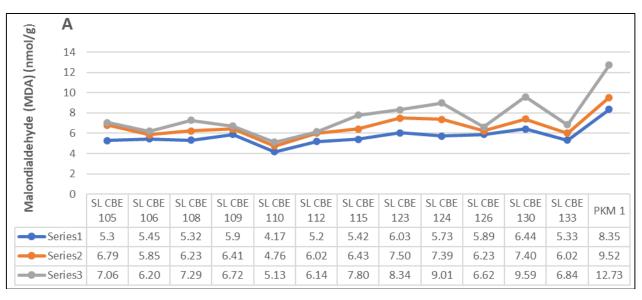
Control – T0; Mild drought – T5 x T0 / C5; severe drought – T10 x T0 / C10





Control – T0; Mild drought – T5 x T0 / C5; severe drought – T10 x T0 / C10

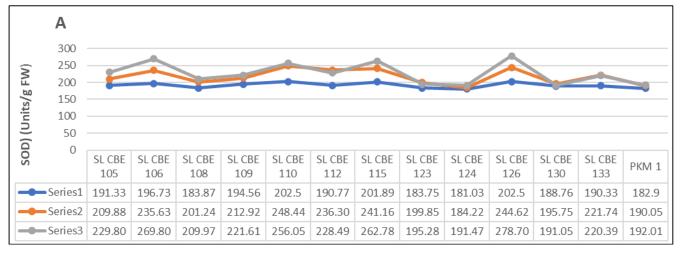
Fig 1: The activity of proline (A) and Relative water content (B) of thirteen genotypes under control, mild and severe drought stress conditions. Values are the mean of three independent replicates.

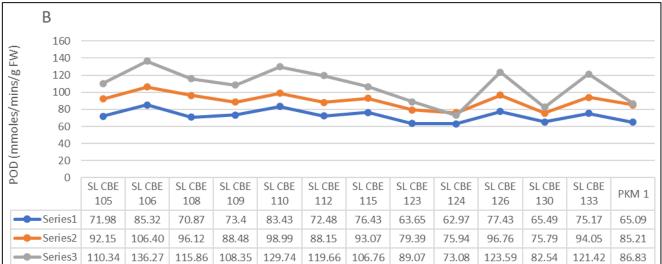


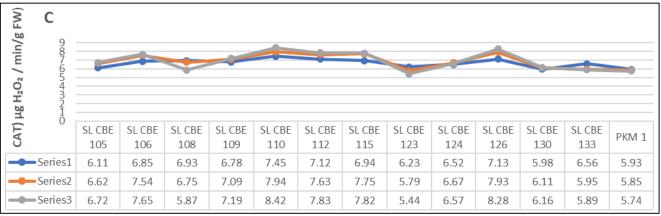


Control – T0; Mild drought – T5 x T0 / C5; severe drought – T10 x T0 / C1

Fig 2: The activity of Malondialdehyde (A) and soluble protein (B) of thirteen genotypes under control, mild and severe drought stress conditions. Values are the mean of three independent replicates.







Control – T0; Mild drought – T5 x T0 / C5; severe drought – T10 x T0 / C10

Fig 3: The activity of SOD (A), CAT (B) and POD (C) of thirteen genotypes under control, mild and severe drought stress conditions. Values are the mean of three independent replicates.

#### 4. Conclusion

In the present study, thirteen tomato genotypes were studied for their tolerance to drought stress by analysing the biochemical and antioxidant enzyme activity under mild and severe drought stress condition. As a result of the analysis, it could be concluded that genotype G 6, G 26, G 10 and G 15 which exhibited higher proline and maximum antioxidant enzyme activity, as tolerant to drought stress compared with other genotypes which were on par or even lower in their activity. Based on this evaluation, these above mentioned genotypes could represent excellent donors of genomic traits for genetic improvement.

#### 5. References

- 1. Mittler R. Abiotic stress, the field environment and stress combination. Trends in plant science 2006;11(1):15-19.
- 2. Raja V, Qadir SU, Alyemeni MN, Ahmad P. Impact of drought and heat stress individually and in combination on physio-biochemical parameters, antioxidant responses, and gene expression in Solanum lycopersicum. 3 Biotech 2020;10(5):1-18.
- 3. Rodríguez M, Canales E, Borrás-Hidalgo O. Molecular

aspects of abiotic stress in plants. Biotecnología Aplicada 2005;22(1):1-10.

- 4. Kamanga RM. Screening and differential physiological responses of tomato (*Solanum lycopersicum* L.) to drought stress. Plant Physiology Reports 2020;25(3):472-482.
- 5. Wakchaure GC, Minhas PS, Meena KK, Kumar S, Rane J. Effect of plant growth regulators and deficit irrigation on canopy traits, yield, water productivity and fruit quality of eggplant (*Solanum melongena* L.) grown in the water scarce environment. Journal of environmental management 2020;262:110320.
- 6. Mitra J. Genetics and genetic improvement of drought resistance in crop plants. Current science 2001, 758-763.
- Nemeskéri E, Neményi A, Bőcs A, Pék Z, Helyes L. Physiological factors and their relationship with the productivity of processing tomato under different water supplies. Water 2019;11(3):586.
- Pires RCDM, Furlani PR, Ribeiro RV, Bodine Junior D, Sakai E, Lourenção AL *et al.* Irrigation frequency and substrate volume effects in the growth and yield of tomato plants under greenhouse conditions. Scientia Agricola 2011;68:400-405.
- 9. Bahadur A, Chatterjee A, Kumar R, Singh M, Naik PS. Physiological and biochemical basis of drought tolerance in vegetables. Vegetable Science 2011;38(1):1-16.
- Harb A, Krishnan A, Ambavaram MM, Pereira A. Molecular and physiological analysis of drought stress in Arabidopsis reveals early responses leading to acclimation in plant growth. Plant physiology 2010;154(3):1254-1271.
- 11. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol 2004;55:373-399.
- 12. Zgallaï H, Steppe K, Lemeur R. Effects of different levels of water stress on leaf water potential, stomatal resistance, protein and chlorophyll content and certain anti- oxidative enzymes in tomato plants. Journal of Integrative Plant Biology 2006;48(6):679-685.
- 13. Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. Reactive oxygen gene network of plants. Trends in plant science 2004;9(10):490-498.
- 14. Tieman D, Zhu G, Resende MF, Lin T, Nguyen C, Bies D, Klee H. A chemical genetic roadmap to improved tomato flavor. Science 2017;355(6323):391-394.
- 15. Lichtenthaler HK, Wellburn AR. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents 1983.
- 16. Barrs HD, Weatherley PE. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian journal of biological sciences 1962;15(3):413-428.
- 17. Heath RL, Packer L. Photo peroxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of biochemistry and biophysics 1968;125(1):189-198.
- Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and soil 1973;39(1):205-207.
- 19. Aebi H. Catalase *in vitro*. Methods in enzymology 1984;105:121-126
- 20. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Archives of biochemistry and

biophysics 1978;186(1):189-195.

- 21. Aghaie P, Tafreshi SAH, Ebrahimi MA, Haerinasab M. Tolerance evaluation and clustering of fourteen tomato cultivars grown under mild and severe drought conditions. Scientia Horticulturae 2018;232:1-12.
- 22. Paleg LG, Aspinall D. *Physiology and biochemistry of* drought resistance in plants. Academic Press 1981.
- Egert M, Tevini M. Influence of drought on some physiological parameters symptomatic for oxidative stress in leaves of chives (*Allium schoenoprasum*). Environmental and Experimental Botany 2002;48(1):43-49.
- 24. Verbruggen N, Hermans C. Proline accumulation in plants: a review. Amino acids 2008;35(4):753-759.
- 25. Claussen W. Proline as a measure of stress in tomato plants. *Plant science*, 2005;168(1):241-248.
- 26. Dar MI, Naikoo MI, Rehman F, Naushin F, Khan FA. Proline accumulation in plants: roles in stress tolerance and plant development. In Osmolytes and plants acclimation to changing environment: emerging omics technologies Springer, New Delhi. 2016, 155-166.
- 27. Siddique MRB, Hamid AIMS, Islam MS. Drought stress effects on water relations of wheat. Botanical Bulletin of Academia Sinica 2000, 41.
- 28. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative medicine and cellular longevity 2014.
- 29. Noctor G, Foyer CH. Ascorbate and glutathione: keeping active oxygen under control. Annual review of plant biology, 1998;49(1):249-279.
- Iturbe-Ormaetxe I, Escuredo PR, Arrese-Igor C, Becana M. Oxidative damage in pea plants exposed to water deficit or paraquat. Plant physiology 1998;116(1):173-181.
- 31. Kumar D, Minhas JS. Effect of water stress on photosynthesis, productivity and water status in potato. J of Indian Potato Association 1999;26(1, 2):7-10.