Anatomical changes and *Catalase* gene expression response of tomato (*Solanum lycopersicum* L.) to water deficit irrigation

Hanieh Mohajjel Shoja[1], Taha Khezriani[1], Maryam Kolahi[2*], Mina Kazemian[1], Elham Mohajel Kazemi[1], and Milad Yazdi[2]

1. Department of Plant Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Islamic Republic of Iran.
2. Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Islamic Republic of Iran.
***Corresponding author; e-mail: m.kolahi@scu.ac.ir**

Running title: Different responses in tomato to water deficit irrigation cultivars

Abstract

Drought alters plant metabolic processes resulting in some changes at the anatomical and morphological levels. Experiments were conducted to determine the morphologic and anatomic response of two cultivars of tomato (*Solanum lycopersicum* L.- CaljN3 and Superstrain B) under different irrigation regimes (100%, 75%, 50%, and 25% of field capacity). *Catalase* 1 (*CAT*1) gene expression was investigated by real-time RT-qPCR and protein interaction studies in tomatoes were utilized. Drought stress caused an increase in number of vessels in roots and stems of both cultivars. The diameter of vascular cylinders in roots of control plants (both cultivars) was more extended. Expression of the *CAT1* gene did not show any significant difference in the CaljN3 cultivar under drought conditions. Whereas, expression of the *CAT1* gene indicated a significant increase in Superstrain B cultivar at the 50% and 25% FC levels of treated conditions. The gene network showed that this protein interacts with superoxide dismutase, acyl-CoA oxidase, and glutathione peroxidase. CaljN3 cultivars showed more tolerance than Superstrain B at all levels of drought treatment. Therefore, Superstrain B is considered a susceptible cultivar under drought conditions. It suggested that the defense against oxidative stress may initiate one step before the activity of antioxidant enzymes. Thus, the tomato plant tries to fight the stress factor by activating proteins, especially channels, pumps, and some cellular messengers.

Keywords: Abiotic stress, Anatomy, Protein interaction, Tomato**.**

Introduction

Water deficit stress is considered one of the main barriers to the production of crops around the world, especially in arid and semi-arid areas such as the Middle East. Aridity is one of the most critical environmental stresses which affect morphological, physiological, and molecular processes, causing a lack of growth in plants (Mesgaran *et al*., 2017). Tomato (*Solanum lycopersicum* L.) is the chief agricultural product in many countries and is an essential contributor to human health. The fruits are rich in vitamins A, C, and fiber and is cholesterol-free. It also has a considerable amount of lycopene, which is an essential carotenoid antioxidant protecting the cell from deleterious free radicals and preventing cancer (Sangeetha *et al*., 2023).

Plants responded to water deficit by making morphological, physiological, and metabolic changes (Faghani *et al*., 2022). Some studies have shown that stress due to water deficit leads to a lack of growth in various parts of the plant, including roots, shoots, leaf area, height, and dry weight. A decrease in stomata closure during photosynthesis, and a decline in the levels of chlorophyll have been observed in drought-stress (Hung *et al*., 2005). Drought changes the metabolic process and function of some enzymes in plants and makes some changes on the anatomical and morphological levels (Zhang *et al*., 2020). One of the biochemical changes which occur due to the placement of plants in drought conditions is an increase in the production of free radicals of oxygen (ROS) (Mostajeran and Rahimi-Eichi, 2008). Their toxic effects are neutralized by the plant's antioxidant system (enzymatic and non-enzymatic). The degree of sensitivity to oxidative stress relies on the proportion of agents producing ROS and the production of antioxidants in the plants (Nadarajah, 2020). ROS is reactive and would destroy the natural metabolism of plants in the absence of any defensive mechanism by oxidative damage to lipids, proteins, and other macromolecules (Rout and Shaw 2001).

The structure of catalase (CAT) includes a tetrameric protein, porphyrin iron, and is considered one of the most important antioxidant enzymes. CAT is found in all living organisms, including plant cells, animal cells, and aerobic microorganisms (Sarker and Oba, 2018). CAT performs a vital function in neutralizing H_2O_2, which is produced as a result of various processes such as electron flow in the mitochondrial electron transport chain, beta-oxidation of fatty acids, and oxidation during photorespiration (Mura *et al*., 2007). CAT in animals is only coded by one particular gene, whereas in plants, a small gene family codes the catalase enzyme. In *Arabidopsis*,

a small family of proteins including *CAT1*, *CAT2*, and *CAT3* is coded by the *CAT* gene (Du *et al.*, 2008).

Selection of drought-tolerant plants and finding mechanisms that increase plant tolerance to drought stress are essential. The purpose of the current study is to measure changes in the morphological and anatomical characteristics of two cultivars of tomato (drought-susceptible and drought-tolerant). Morphological and anatomical changes due to stress and how the genes were expressed in different cultivars were evaluated. This research seeks to study the effects of drought stress on the expression of catalase through real-time PCR.

Materials and Method

Plants material

Seeds of tolerant and susceptible tomato cultivars (*Solanum lycopersicum* cv. CaljN3 and cv. Superstrain B) were sown in pots containing sterilized sand. The sand was hydrated with distilled water every few days to prevent dehydration. After the emergence of early leaflets (20 days), seedlings were transferred to pots containing coco peat and perlite mix (70%-30%) which were washed with distilled water and wholly dried at ambient temperature before plant transfer. Leaflets were illuminated with a light (16 h, 21 ˚C)/darkness (8 h, 18 ˚C) cycle (humidity 65%).

Irrigation treatments

The experiment was conducted as a randomized complete block design with five replications. The four irrigation levels were calculated based on field capacity (FC):100% FC as a control, 75%, 50%, and 25% FC. Irrigation of the samples was done three times a week (for four weeks). The amount of water was determined based on field capacity by weighing the pots. The plants were harvested after four weeks of drought stress applied and utilized for various studies.

Morphological and anatomical studies

Morphological parameters such as plant height, root length, root and shoot fresh and dry weight and leaf area were measured. The seedlings was embedded in an alcohol-formalin-acetic acid solution (18:1:1, v/v/v) and dehydrated in a series of alcohols, and after paraffin penetration in samples, sectioning (8 μm) was done for microscopy analysis. Different parts of the plant such as the internodes, roots, and leaves (sixth internode, middle or apical leaflet in the seventh leaf, for roots two centimeters from the root cap) after sectioning were stained with safranin-fast green.

RNA expression analysis by real-time RT-qPCR

Total RNA was extracted utilizing YTzol (Pure RNA isolation reagent) (Yekta Tajhiz Azuma Co., Iran). Sequences of sense and antisense primers (Bioneer, Seoul, South Korea) for *CAT1* and *ACTIN* (*ACT*) were designed utilizing Primer Express 3 software (ABI, USA). The sequence of the primers was as follows: *CAT1*: 5'- GCGACCAAGGATCTTTACGA -3', reverse: 5'-CAACACCAATCGACCAACTG -3', *ACT*: 5'-ATGCCTATGTTGGTGACGAG-3' and 5'-CTCTGGAGCCACACGAAGT -3'. qRT-PCR results were analyzed based on the ΔΔCt method, utilizing the Step One software 2.1. Relative quantification was performed according to the comparative 2ΔΔCt method.

Co-expression study

GPL4741 was obtained from the geodatabase containing 47 series and 744 samples. This particular GPL belongs to the [Tomato] Affymetrix Tomato Genome Array. Within the 47 series, four were associated with salinity, drought, and heat stressors on the tomato plant. Samples were further subdivided based on plant sensitivity, tolerance, or applied stressor, which resulted in the creation of 10 datasets. Weighted gene co-expression network analysis (WGCNA) was utilized in deriving co-expression networks, followed by implementation in the R *WGCNA* package. The Kin parameter is derived from the amount of hub gene and descriptions of the gene. The genes are arranged according to the amount of sub, meaning the difference between the kin CI and kin MS. Kin CI and kin MS are related to resistant and sensitive varieties respectively. The power of beta = 12 was chosen based on the scale-free topology criterion.

Statistical analysis

One-way ANOVA (SPSS 20.0 software) was used to test differences between various means, followed by the post hoc Tukey test ($P < 0.05$).

Results

Effects of drought stress on morphometric characteristics of tomato cultivars

Morphological results from the application of drought stress to different cultivars of tomatoes showed significant changes in plant height and fresh and dry weight of shoots. In both cultivars, plant height decreased considerably due to the drought conditions. The most decrease was observed at the highest level of drought treatment (25% FC). Furthermore, drought levels resulted in a

decrease in leaf surface area for both cultivars. The results indicated that drought stress reduced the fresh and dry weight of roots in CalJN3. Whereas, the fresh and dry weight of roots in Superstrain B decreased to 1.0 and 0.07 g, respectively. Moreover, drought stress decreased stem weight in both cultivars (Table 1). CaljN3 cultivar showed more tolerance than Superstrain B at all levels of drought treatment. Therefore, Superstrain B is considered a susceptible cultivar during drought conditions.

Table 1. Effect of different irrigation levels on tomato root and shoot growth.

Parameters	cultivars	100% FC	75% FC	50% FC	25% FC
Shoot length (cm)	Calj N3	33.5±0.8a	30.7±1.9a	23.9±2.5b	16.8±0.9cd
	Super strain B	24.2±2.8b	18.4±0.4c	17.2±1.6c	13.6±1.5d
Root length (cm)	Calj N3	32.0±1.6a	27.9±1.5a	22.7±2.6b	18.5±2.2c
	Super strain B	23.0±2.1a	19.9±1.7b	14.9±2.1c	10.9±0.4c
Shoot fresh weight (g)	Calj N3	13.5±1.3a	13.1±1.2a	9.6±0.9b	6.5±0.8cd
	Super strain B	10.0±1.6b	8.1±1.0bc	7.3±0.6c	4.8±1.21d
Root fresh weight (g)	Calj N3	5.5±1.3a	4.2±1.0ab	3.7±0.8bc	2.3±0.55cd
	Super strain B	4.1±0.9ab	3.2±0.5bc	2.3±1.0cd	1.06±0.17d
Shoot dry weight (g)	Calj N3	1.2±0.17a	1.1±0.1a	0.8±0.0bc	0.5±0115de
	Super strain B	0.9±0.1b	0.7±0.1bcd	0.6±0.05cd	0.4±0.1e
Root dry weight (g)	Calj N3	0.5±0.1a	0.4±0.01a	0.3±0.1ab	0.1 ±0.07b
	Super strain B	0.3±0.8a	0.2±0.07ab	0.2±0.1bc	0.07±0.02c
Leaf area (mm^2)	Calj N3	4018±194.6a	3993.6±273.8a	2873±313.7b	1875±232.2c
	Super strain B	3602±276.0a	2541.3±232.5b	2426±297.5b	1767.3±246.3c
Seedlings fresh / dry weight	Calj N3	11.3±0.04a	11.2±0.07a	12.8±0.05b	14.2±0.05c
	Super strain B	10.4±0.02b	10.3±0.06b	11.7±0.02b	13.3±0.06c

Values with different letters are statistically significantly different at P< 0.05.

Effect of drought stress on anatomical characteristics of roots, stems and leaves in cultivars of tomato

Only control (100% FC) and 25% FC (high level) treatment samples were compared in both cultivars. Anatomical studies of roots showed that the diameter of the root does not change considerably in both control and treated plants (CaljN3 and Superstrain B) (Figure 1 A-D). In drought-stressed plants (Superstrain B), cells within the cortex appeared disordered (Figure 1 D). In general, the diameter of the vascular cylinders in the roots of control plants (both cultivars) was more extended than what was observed in drought-stressed plants (Figure 1 E-H). The diameter of metaxylem elements within the control plants of both cultivars was greater than that observed in

stressed plants (Figure 1 E-H). The cell volume of the cortex layer in drought-stressed plants for both cultivars showed an increase in comparison to control plants. Comparative data showed that the epidermis in transversal sections of the internodes of CaljN3 and Superstrain B cultivars was made of one cell layer in both the control and drought-stressed plants (Figure 1 I-L). The number of trichomes was higher in the treatment plants rather than control (both cultivars). The number of vessels and vascular bundles in the treated plants increased significantly (Figure 1 M-P). The diameter of vascular pores was wider in control plants of both cultivars relative to the treated plants. On the other hand, the thickness of the transversal wall of vessels in the treated plants was higher than that of the control due to high levels of lignin deposition (Figure 1 M-P). The pith area of the treated and control plants was the same in both cultivars.

Anatomical studies of the leaves indicated that mesophyll tissue contained one layer of palisade parenchyma followed by spongy parenchyma tissue in both cultivars (Figure 1 Q-T). The lower epidermis, which covers the lower surface of the leaf blade, contains trichomes. A comparison of a transversal section of control leaves and treated leaves of both cultivars implied that the vascular system in midrib and secondary veins were diminished (Figure 1 L-X). In both cultivars, the diameter of vascular pores in treated plants was smaller (Figure 1 V, X).

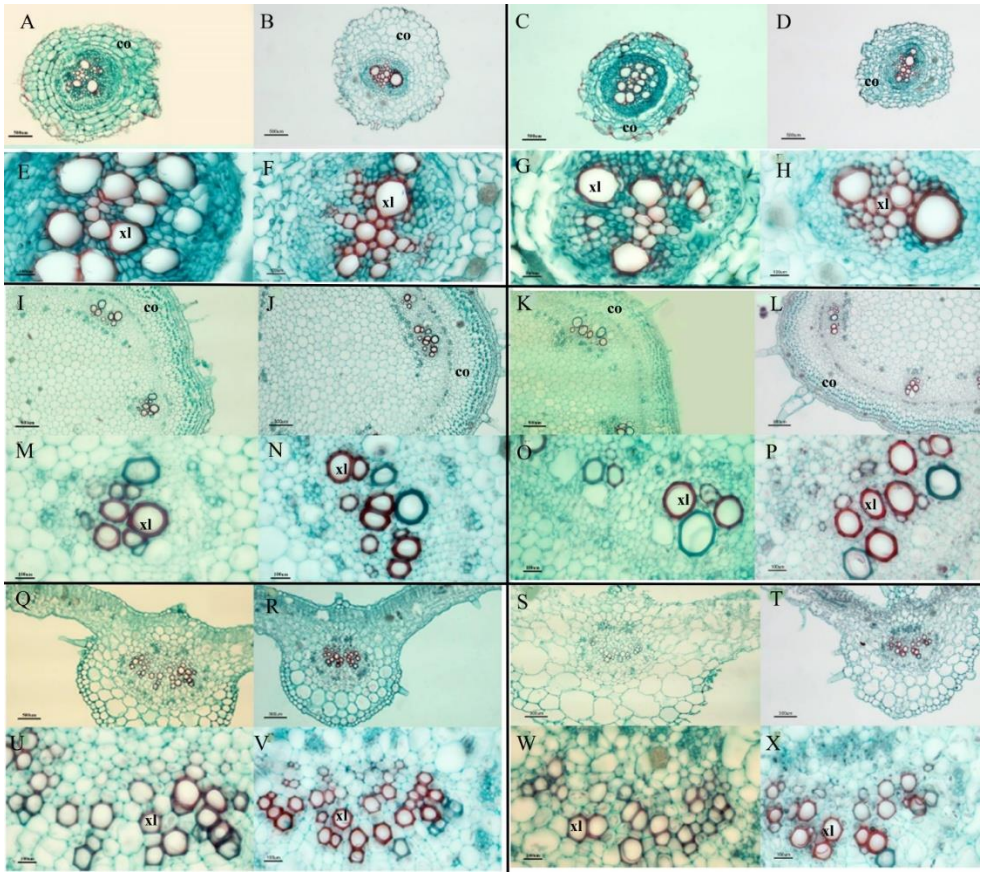

Figure 1. Drought stress effects on root, stem, and leaf anatomy of two tomato cultivars (CaljN3 and Superstrain B. (control plant of CaljN3 (**A, E, I, M, Q, U**); Stressed plants of CaljN3(B,F,J,N,R,V) ; control plant of Superstrain B (**C, G, K, O, S, W**) ; Stressed plants of SuperstrainB (D,H,L,P,T, X)). **A**, **B**, **C**, **D**: root. **E**, **F**, **G**, **H**: xylem and Phloem of root. **I**, **J**, **K, L**: stem. **M**,**N**, **O**, **P**: xylem and Phloem in stem. **Q**, **R**, **S, T**: leaf. **U**, **V**, **W**, **X**: xylem and Phloem in leaf. (Scale bars, 500µm in A-D, I-L, Q-T and Scale bars, 100µm in E-H, M-P, U-X). co: cortex, xl: xylem.

Effect of drought stress on CAT1 gene expression in tomato cultivars

A study of the relative expression of the CAT1 gene in two cultivars of tomatoes in drought conditions was conducted (Figure 2). A comparison of the expression level of the CAT1 gene in the two cultivars revealed that the expression of the CAT1 gene in CaljN3 and SuperstrainB is similar in control conditions (100% FC). The relative expression of the CAT1 gene did not show any considerable difference as the 75% FC level of stress in both cultivars. Likewise, expression of the gene CAT1 indicated a significant increase in Superstrain B cultivars at the 50% and 25% FC levels of treated samples (Figure 2). Whereas, the relative expression of the CAT1 gene did not show any considerable difference in the 25% FC level of stress in the CaljN3 cultivar.

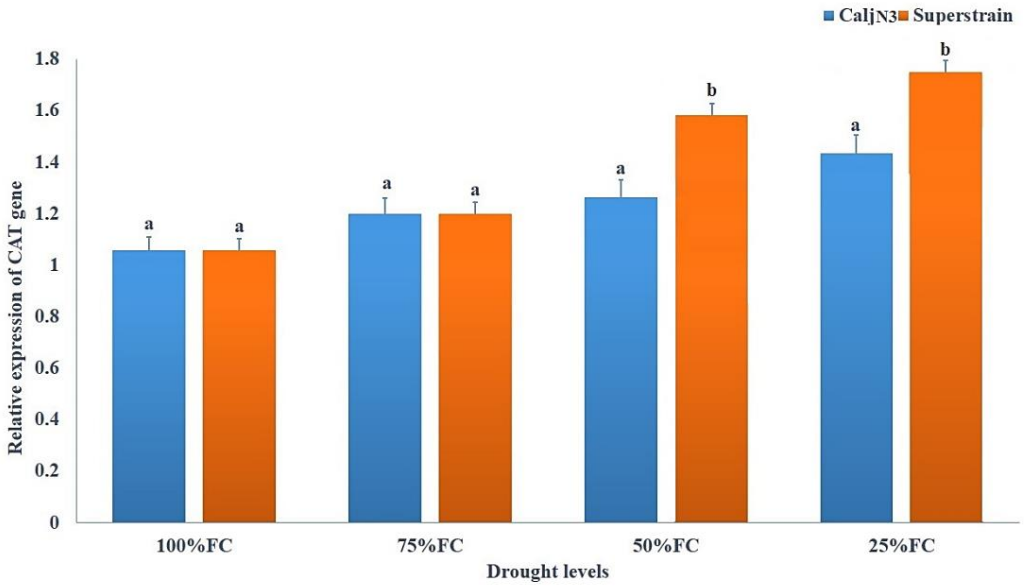

Figure 2. Comparison of relative expression of CAT1 gene in CaljN3 and Superstrain B cultivars. Values with different letters are statistically significantly different at $p< 0.05$.

Bioinformatics study of CAT1 gene utilizing microarray analysis

In the current study, probe Id (Les.3098.1.S1_at) was selected as indicative of CAT1 in *Solanum Lycopersicum* with Gene ID 543990. In study groups that were divided based on cultivars and type of stress, the probe did not show any significant log fold change (Table 2). Gene enrichment did not show any pathway with a significant *p*-value for the cluster. Although the CAT1 gene was not involved in specific biochemical pathways during drought stress, the gene network showed that this protein interacts with superoxide dismutase, acyl-CoA oxidase, and glutathione peroxidase (high score) (Figure 3). The results indicated that genes representing the hub gene changed between the two tolerant and susceptible states in different clusters. The Kin parameter was derived from the number of hub genes and gene descriptions. Kin CI (0.41) and kin MS (0.53) are related to the tolerant and susceptible cultivars, respectively.

Table 2. CAT gene expression in microarray studies of different tomato cultivars under different stress conditions.

GSE ID	Cultivar/Genotype	Type of stress	Other characterization	Log fold change
GSE16401	Moneymaker	salinity	susceptible	-0.339588916
GSE16401	PI365967	salinity	tolerant	-0.235236336
GSE22304	Is not mentioned	drought	susceptible	0.840218131
GSE22304	Is not mentioned	drought	tolerant	0.75971927
GSE39894	*S. lycopersicum*	drought		0.399835513
GSE39894	*S. pimpinellifolium*	drought		0.182271858
GSE97045	*S. lycopersicum*, cv. P73	drought		0.143336524
GSE97045	*S. pennellii* (Sp) (acc. PE47)	drought		0.189479176
GSE22304	Is not mentioned	heat	susceptible	-0.22713333
GSE22304	Is not mentioned	heat	tolerant	0.97014

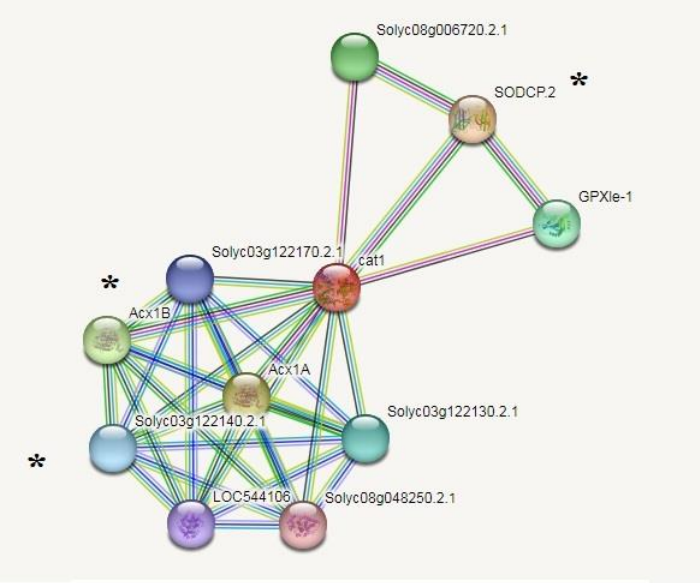

Figure 3. Interaction of CAT1 with different proteins. Empty nodes: Proteins of unknown 3D structure. SODCP.2: Superoxide dismutase, Acx1A and Acx1B: acyl-CoA oxidase, Solyc08g006720.2.1: glutathione peroxidase family, GPXle-1: phospholipid hydroperoxide glutathione peroxidase, LOC544106: Glycolate oxidase. * Significant interaction.

Discussion

The study revealed that under drought stress there was a considerable decrease in length, fresh weight, and dry weight of the roots for both cultivars. Research has shown that with sufficient moisture, root growth increased significantly. In low levels of irrigation, less moisture is around the root, consequently, this results in mechanical resistance of the soil against root development and a reduction in the length and density of the root in common irrigation treatments (Navarro *et al*. 2008). With sufficient irrigation, water is more reserved in the root area and the plant by

condensing its roots makes better use of water (Faghani *et al*., 2022). Factors limiting photosynthesis like light and water, in addition to decreasing plant function also decrease root growth. Plants in dry environments prefer to deposit their photosynthetic production in the root and not in the stems and shoots as the plant can preserve its ability to absorb more amounts of soil water (Halo *et al*., 2020). Tomato is susceptible to drought stress, and therefore, when applying water stress, its vegetative growth and function decrease considerably. Miguel and Francisco (2007) also reported a reduction in root growth, fresh weight, and dry weight in tomatoes. Plant growth under stress usually depends on the root's ability to absorb water from the soil and transfer it to stems (Navarro *et al*. 2008). Root length is an index for absorbing water from deep layers of soil. Therefore, the intensity of root growth affects the shoot of a plant (Franco *et al*. 2011).

The results indicated that drought stress caused a reduction in leaf area in both CaljN3 and Superstrain B cultivars. The production and expansion of leaves are very susceptible to water deficit because of the essential need for cellular division and growth (Hernandez-Espinoza *et al*., 2020). Drought has a profound impact on the growth, production, reduction of leaf expansion, reduction in stomata pores and the quality of the plant. The crucial impact caused by stress is a loss of turgor pressure, which affects the speed of cell expansion and final cell size (Kumar and Purohit, 2001). The reduction of leaf growth induced by drought stress could be considered an adaptation response. Furthermore, drought stress restricts leaf area and ultimately transpiration (Sikuku *et al*., 2010). The typical reaction of a plant to drought stress includes reducing stem growth and the size of the whole plant (Mostajeran and Rahimi-Eichi, 2008). A decrease in leaf area causes a reduction into receive of light and photosynthesis (Ourcut and Nilsen, 2000).

Results of the current study showed that under stress conditions, shoot weight in susceptible cultivars was lower relative to tolerant cultivars, which can be used as an index for the selection of susceptible and tolerant cultivars. The decrease in shoot growth and weight probably occurred due to the decrease in photosynthesis, the production of inhibitory substances, and the decline in the level of hormones during drought stress (Hayat and Ahmad, 2007). It is suggested that under water deficit conditions, the absorption of nutritional substances decreases and consequently transpiration might reduce. These processes cause a reduction in the growth and expansion of shoots in the plants (Kirnak, 2001). The level of production of essential metabolites in plants has a strong co-relation with leaf area and absorbed light. A reduction of each one of these indexes can reduce the fresh

and dry weight of the plant. Consequently, the continuous loss of water in the soil causes a decrease in leaf size and surface (Hernandez-Espinoza *et al*., 2020).

Anatomical changes can occur in plants under water deficit. Some of these changes include increased lignification or suberin deposition in the cortex, endoderm cells, and cell layers that are near to cortex and medulla (Farooq *et al*., 2009). The reduction of vessel diameter, which is caused by an increase in lignification, shows the adaptability of a plant to stress conditions (Halo *et al*., 2020). Increased thickness of the transverse wall of vessels and a reduction in the diameter of the vessels, allow water to run through the vessels with greater speed (Jogawat *et al*., 2021). A secondary structure formation is a kind of defense response of plants against stressful conditions. It has been observed that the tonality rate of lignified areas is much lower than that of the control plants, which can be a result of increased polymerization of the lignin component (Jogawat *et al*., 2021). The number of layers and root volume of cortex cells in drought-stress plants for both cultivars increased as compared to the control plants (Granier *et al*. 2000). Tissues placed in water-deficit conditions usually demonstrate a decrease in cell size and the number of vascular tissues. Under these conditions, processes corresponding to cell elongation are more vulnerable compared to processes related to cell division (Nevo *et al*. 2000). The space between spongy parenchyma cells of leaves seems to be beneficial for the prevention of water loss. Reduction in blade thickness, palisade, and spongy parenchyma in some species of *Acacia auriculiformis* under water deficit stress was reported by Liu *et al*. (2004). A leaf is considered a responsive organ to environmental conditions and among environmental factors that could potentially affect the structure of a leaf, certainly drought stress is one of the most important ones (Nardini *et al*. 2005). Changes in leaf anatomy in plants under stress could be related to reducing transportation via the stomata. Moreover, a reduction of leaf expansion could be related to different mechanisms such as a reduction in cell division and firmness of the cell wall (Bouchabke *et al*., 2002).

Based on the results of the present study, drought stress did not have a significant effect on the expression of the CAT1 gene in the CaljN3 cultivar while the expression significantly changed in the Superstrain B cultivar. Changes in antioxidant enzyme function are a mechanism utilized by the plant to increase plant tolerance against stress (Daneshmand *et al*., 2014). Several reports have determined that drought stress, high temperature, and salinity cause an increase in superoxide dismutase and CAT activity in tolerant genotypes (Sairam *et al*., 2001). The level of antioxidant enzyme activity during drought stress is variable between plant species and even cultivars (Bacelar

et al., 2006 a). Moreover, changes in the expression of the catalase enzyme during stress are dependent on the species (Ufuk Demirel *et al*., 2020). In rice seedlings, water deficit stress has been found to increase the expression of all the antioxidant enzymes that remove ROS (Srivalli *et al*., 2003). A study of the impact of salinity on oxidative stress in two Faba bean cultivars did not show a significant effect on SOD activity in plant roots (Gaballah *et al*,, 2005).

A study of stress-tolerant and stress-sensitive potato genotypes under drought stress suggested that the plants responded to potential increases in oxidative stress by altering antioxidant metabolism and activities of key antioxidant enzymes (Rizhsky *et al*., 2002). A mechanism that maintains the balance between CAT and APX activity is considered a critical process for ROS suppression in the leaves of some drought-exposed tomato cultivars (Hasanagić et al., 2020).

Bioinformatics study of the catalase gene by microarray datasets showed no significant difference in catalase gene expression under salinity and drought stress. The results of the enrichment gene showed that this gene does not guide any significant cell pathways. Studies show that in tomato drought and salinity treatments, rather than activating the catalase pathway, the cell process activates the salt overly sensitive (SOS) pathway of cells, pumps, carriers, and cellular messengers until they have an enzymatic response (Sahni *et al*., 2016). Tomatoes seem to go one step further in response to stress oxidation and increased oxygen free radicals, activating enzymes other than catalase. Brassinosteroid signaling activation adjusts the expression of genes involved in cell wall biosynthesis and remodeling and cell wall homeostasis through cell expansion in response to environmental stress (Sahni *et al*., 2016). Apparently, in this plant, the fight against oxidative stress begins one step before the antioxidant enzymes and seeks to expel the stressor by activating proteins, especially channels, pumps, and cellular messengers.

Conclusions

Anatomic observations showed that drought stress causes a reduction in the diameter of vessels and increased thickness of transverse wall due to the deposition of lignin in leaves, internode, and root cells of both CaLjN3 and Superstrain B cultivars. Based on the morphological results the CaLjN3 cultivar is tolerant compared to Superstrain B as it had the lowest reduction in fresh and dry weight of root and shoot. CaLjN3 cultivar showed more tolerance concerning a reduction of height compared to other variables. Superstrain B is therefore considered the susceptible cultivar. Results obtained by quantitative real-time PCR showed that the CaLjN3 cultivar is considered the tolerant cultivar while the level of expression of the CAT1 gene increases in Superstrain B. Gene

enrichment did not show any pathway with a significant *p*-value for the cluster. It seems that in some cases tomatoes undergoing abiotic stress instead of activating the catalase pathway, the cell process activates other pathways. Apparently, in this plant, the fight against oxidative stress begins one step before the enzymes and seeks to expel the stressor by activating proteins, especially channels, pumps, and cellular messengers. The results reveal that the CaLjN3 cultivar is suitable for cultivation under drought-stress conditions rather than the Superstrain B cultivar.

References

1. Bacelar, E.A., Santos, D.L., Moutinho-Pereira, J.M., Gonçalves, B.C., Ferreira, H.F., and Correia, CM. 2006. Immediate responses and adaptative strategies of three olive cultivars under contrasting water availability regimes: changes on structure and chemical composition of foliage and oxidative damage. Plant Sci., 170(3):596–605.
2. Bouchabke, O., Tardieu, F. and Simonneau, T. 2002. Leaf growth and turgor in growing cell to maize (*Zea mays* L.) respond to evaporative demand under moderate irrigation but not in water saturated soil. Plant Cell Environ., 19:10-15.
3. Daneshmand, F. 2014. Response of antioxidant system of tomato to water deficit stress and its interaction with ascorbic acid. I.J.P.B., 6(1): 57-72.
4. Demirel, U., Morris, W.L., Ducreux, L.J.M., Yavuz, C., Asim, A., Tindas, I. *et al*. 2020. Physiological, Biochemical, and Transcriptional Responses to Single and Combined Abiotic Stress in Stress-Tolerant and Stress-Sensitive Potato Genotypes. Front. Plant Sci., 11: 196.
5. Du, Y.Y., Wang, P.C., Chen, J., Song, C.P. 2008. The comprehensive functional analysis of catalase gene family in *Arabidopsis thaliana*. J. Integr. Plant Biol., 50:1318-1326.
6. Faghani, E., Kolahi, M., Kazemian, M., Goldson-Barnaby A. and Razzaghi MH. 2022. Effect of irrigation regimes on starch biosynthesis pathway, cotton (*Gossypium hirsutum*) yield and in silico analysis of ADP-glucose-pyrophosphorylase. Int. J. Environ. Sci. Technol. 19, 10809–10830. https://doi.org/10.1007/s13762-022-04281-x
7. Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, S.M.A. 2009. Plant drought stress: effects, mechanisms and management. Agron. Sustain. Dev., 29: 185-212.
8. Franco, J.A., Banon, S., Vicente, M. J., Miralles, J. and Martı´nez-Sa´nchez, J.J. 2011. Root development in horticultural plants grown under abiotic stress conditions- a review. J. Hortic. Sci. Biotech., 86: 543–556.

9. Gaballah, M.S. and Gomaa, A.M. 2005. Interactive effect of Rhizobium inoculation, sodium benzoate and salinity on performance and oxidative stress in two faba bean varieties. IJAB, 8530. 7-3.
10. Granier, C., Turco, O. and Tardieu, F. 2000. Co–orination of cell division and tissue expansion in sunflower, tobacco and pea leaves: dependence or independence of both processes?. Plant Growth Regul., 39145-54.
11. Halo, B. A., Al-Yahyai, R. A. and Al-Sadi, A. M. 2020. An endophytic *Talaromyces omanensis* enhances reproductive, physiological and anatomical characteristics of drought-stressed tomato. J. Plant Physiol., 249: 153163.
12. Hasanagić, D., Koleška, I., Kojić, D. *et al.* 2020. Long term drought effects on tomato leaves: anatomical, gas exchange and antioxidant modifications. Acta Physiol Plant, **42**: 121. https://doi.org/10.1007/s11738-020-03114-z
13. Hayat, S. and Ahmad, A. 2007. Salicylic Acid a Plant Hormone. Springer. P: 97-99.
14. Hernandez-Espinoza, L. H. and Barrios-Masias, F.H. 2020. Physiological and anatomical changes in tomato roots in response to low water stress. Sci. Hortic., 265: 109208.
15. Hung, S. H., Yu, C. W. and Lin, C. H. 2005. Hydrogen peroxide functions as a stress signal in plants. Bot. Bull. Acad., 46: 1-10.
16. Jogawat, A., Yadav, B., Lakra, N., Singh, A. K. and Narayan, O. P. 2021. Crosstalk between phytohormones and secondary metabolites in the drought stress tolerance of crop plants: a review. Physiol. Plant., 172(2), 1106-1132.
17. Kirnak, H., Kaya, C. Tas, I. and Higgs, D. 2001. The influence of water deficit on vegetative growth, physiology fruit yield and quality in eggplants. J. Plant Physiol, 27: 34-46.
18. Kumar, A. and Purohit, S.S. 2001. Plant Physiology Fundamentals and Applications. Second Enlarged Edition. Agrobios (India). P: 18-25.
19. Li Y. 2008. Kinetics of the antioxidant response to salinity in the halophyte *Limonium bicolor*. Plant Soil Environ, 54:493–497.
20. Liu, L.X, Xu, S.M and Woo, K.C. 2004. Deficit irrigation effects on photosynthesis and the xanthophyll cycle in the tropical tree species *Acacia auriculiformis* in north Australia. New Zealand .J. Botany, 42:949-957.

21. Mesgaran, M. B., Madani, K., Hashemi, H., and Azadi, P. 2017. Iran's Land Suitability for Agriculture. Sci. Rep., 7(1): 7670. https://doi.org/10.1038/s41598-017-08066-y
22. Miguel, A. and Francisco, M. 2007. Response of tomato s to deficit irrigation under surface or subsurface drip irrigation. J. Appl. Hortic., 9(2): 97-100.
23. Mostajeran, A. and Rahimi-Eichi, V. 2008. Drought stress effects on root anatomical characteristics of rice cultivars (*Oryza sativa* L.). Pakistan J. Biol. Sci., 11:2173–2183.
24. Mura, A., Pintus, F., Medda, R., Floris, G., Rinaldi, AC. and Padiglia, A. 2007. Catalase and antiquitin from *Euphorbia characias*: Two proteins involved in plant defense. Biochem., 72:501-508.
25. Nadarajah, KK. 2020. ROS Homeostasis in Abiotic Stress Tolerance in Plants. Int. J. Mol. Sci., 21(15):5208.
26. Nardini, A. and Salleo, S. 2005. Water stressinduced modifications of laef hydraulic architecture in sunflower: Co-ordination with gas exchange. J. Exp .Bot, 422:3093- 3101.
27. Navarro, A., Vicente, M. J., Martı´nez-Sa´nchez, J. J., Franco, J. A., Ferna´ndez, J. A. and Banon, S. 2008. Influence of deficit irrigation and paclobutrazol on plant growth and water statusin *Lonicera implexa* seedlings. Acta Hortic., 782: 299–304.
28. Nevo, E., Bolshakova, M.A., Martyn, G.I., Musatenko, L.I., Sytnik, K., Palieek, T. and Beharvan, A. 2000. Drought and light anatomical adaptive leaf strategies in three woody species caused by microclimatic selection at “Evolution canyon”. Isr. J. Plant Sci., 48:3346;
29. Ourcut, D.M. and Nilsen E.T. 2000 Salinity stress in: physiology of plants under stress. KA/PP p: 177-235.
30. Rizhsky, L., Hallak-Herr, E., Van Breusegem, F., Rachmilevitch, S., Barr, J.E., Rodermel, S., Inzé, D. and Mittler, R. 2002. Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. Plant J. Nov., 32(3):329-42.
31. Rout, N.P. and Shaw, B.P. 2001. Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes. Plant Sci., 160:415–423.
32. Sahni, S., Prasad B.D., Liu Q., Grbic V. *et al*. 2016. Overexpression of the brassinosteroid biosynthetic gene DWF4 in *Brassica napus* simultaneously increases seed yield and stress tolerance. Sci. Rep., 6: 28298-28298.

33. Sairam, R.K., Chandrasekhar, V. and Srivastava, G.C. 2001. Comparison of hexaploid and tetraploid wheat cultivars in their response to water stress. Biol. Plant., 44: 89-94.
34. Sangeetha, K., Ramyaa, R. B., Khaneghah, A. M., and Radhakrishnan, M. 2023. Extraction, characterization, and application of tomato seed oil in the food industry: An updated review. J. Agric. Res., 100529.
35. Sarker, U. and Oba, S. 2018. Catalase, superoxide dismutase and ascorbate-glutathione cycle enzymes confer drought tolerance of *Amaranthus tricolor*. Sci. Rep., 8: 16496. https://doi.org/10.1038/s41598-018-34944-0.
36. Sikuku, P.A., Netondo, G.W., Onyango, J.C. and Musyimi, D.M. 2010. Effects of water deficit on physiology and morphology of three varieties of NERICA rainfed rice (*Oryza sativa* L.). J. Agric. Biol. Sci., 5: 23-28.
37. Srivalli, B., Sharma, G. and Khanna-Chopra, R. 2003. Antioxidative defence system in an upland rice cultivar subjected to increasing intensity of water stress followed by recovery. Physiol. Plant., 119: 503-512.
38. Zhang, X., Yang, Z., Li, Z., *et al*. 2020. Effects of drought stress on physiology and antioxidative activity in two varieties of *Cynanchum thesioides*. Rev. Bras. Bot., 43: 1–10.

تغییرات آناتومی و پاسخ بیان ژن کاتالاز گوجه (Solanum lycopersicum L.) به آبیاری کم آبی فرنگی

چکیده

محصولات زراعی در مناطق خشک و نیمه خشک در معرض عوامل نامطلوب محیطی مانند خشکسالی قرار دارند. آزمایش‌هایی برای (Solanum lycopersicum L.- CaljN3 وSuperstrain B) تعیین پاسخ مورفولوژیکی و تشریحی دو رقم گوجه‌فرنگی تحت رژیم‌های آبیاری مختلف (100، 75، 50، و 25 درصد ظرفیت زراعی) انجام شد. بیان ژن کاتالاز با روش ریل تایم انجام گرفت و برهمکنش پروتئین نیز مورد بررسی قرار گرفت. نتایج حاکی از تغییرات مورفولوژیکی قابل توجهی در شرایط خشکی بود. تنش خشکی باعث افزایش تعداد آوند در ریشه و ساقه هر دو رقم گوجه فرنگی شد. قطر استوانه های آوندی در ریشه گیاهان شاهد بیشتر بود. بیان ژن کاتالاز در رقم کالج 3 تغییر معنی داری نشان نداد در حالیکه در رقم سوپراسترین بی افزایش معنی داری در سطح 50 و 25 درصد ظرفیت مزرعه ای دیده شد. شبکه ژنی نشان داد که این پروتئین با سوپراکسید دیسموتاز، آسیل کوآ اکسیداز و گلوتاتیون پراکسیداز تعامل دارد. رقم کالج 3 در تمامی سطوح آبیاری تحمل بیشتری نشان داد. بنابراین رقم سوپراسترین بی یک رقم حساس در شرایط خشکی محسوب می شود. بنظر می رسد که دفاع در برابر استرس اکسیداتیو ممکن است یک مرحله قبل از فعالیت آنزیم های آنتی اکسیدانی آغاز شود. بنابراین، گیاه گوجه فرنگی با فعال کردن پروتئین ها به ویژه کانال ها، پمپ ها و برخی پیام رسان های سلولی سعی در مبارزه با عامل استرس دارد.