

1 **ACCEPTED ARTICLE**

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

4
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13
14 **Running title: Different responses in tomato to water deficit irrigation cultivars**

15
16 **Abstract**

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

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

37 **Introduction**

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

46 Plants responded to water deficit by making morphological, physiological, and metabolic changes
47 (Faghani *et al.*, 2022). Some studies have shown that stress due to water deficit leads to a lack of
48 growth in various parts of the plant, including roots, shoots, leaf area, height, and dry weight. A
49 decrease in stomata closure during photosynthesis, and a decline in the levels of chlorophyll have
50 been observed in drought-stress (Hung *et al.*, 2005). Drought changes the metabolic process and
51 function of some enzymes in plants and makes some changes on the anatomical and morphological
52 levels (Zhang *et al.*, 2020). One of the biochemical changes which occur due to the placement of
53 plants in drought conditions is an increase in the production of free radicals of oxygen (ROS)
54 (Mostajeran and Rahimi-Eichi, 2008). Their toxic effects are neutralized by the plant's antioxidant
55 system (enzymatic and non-enzymatic). The degree of sensitivity to oxidative stress relies on the
56 proportion of agents producing ROS and the production of antioxidants in the plants (Nadarajah,
57 2020). ROS is reactive and would destroy the natural metabolism of plants in the absence of any
58 defensive mechanism by oxidative damage to lipids, proteins, and other macromolecules (Rout and
59 Shaw 2001).

60 The structure of catalase (CAT) includes a tetrameric protein, porphyrin iron, and is considered
61 one of the most important antioxidant enzymes. CAT is found in all living organisms, including
62 plant cells, animal cells, and aerobic microorganisms (Sarker and Oba, 2018). CAT performs a
63 vital function in neutralizing H₂O₂, which is produced as a result of various processes such as
64 electron flow in the mitochondrial electron transport chain, beta-oxidation of fatty acids, and
65 oxidation during photorespiration (Mura *et al.*, 2007). CAT in animals is only coded by one
66 particular gene, whereas in plants, a small gene family codes the catalase enzyme. In *Arabidopsis*,

67 a small family of proteins including *CAT1*, *CAT2*, and *CAT3* is coded by the *CAT* gene (Du *et al.*,
68 2008).

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

75 76 **Materials and Method**

77 **Plants material**

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

84 85 **Irrigation treatments**

86 The experiment was conducted as a randomized complete block design with five replications. The
87 four irrigation levels were calculated based on field capacity (FC):100% FC as a control, 75%,
88 50%, and 25% FC. Irrigation of the samples was done three times a week (for four weeks). The
89 amount of water was determined based on field capacity by weighing the pots. The plants were
90 harvested after four weeks of drought stress applied and utilized for various studies.

91 92 **Morphological and anatomical studies**

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

99

100 RNA expression analysis by real-time RT-qPCR

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

109

110 Co-expression study

111 GPL4741 was obtained from the geodatabase containing 47 series and 744 samples. This particular
112 GPL belongs to the [Tomato] Affymetrix Tomato Genome Array. Within the 47 series, four were
113 associated with salinity, drought, and heat stressors on the tomato plant. Samples were further
114 subdivided based on plant sensitivity, tolerance, or applied stressor, which resulted in the creation
115 of 10 datasets. Weighted gene co-expression network analysis (WGCNA) was utilized in deriving
116 co-expression networks, followed by implementation in the R *WGCNA* package. **The Kin**
117 **parameter is derived from the amount of hub gene and descriptions of the gene. The genes are**
118 **arranged according to the amount of sub, meaning the difference between the kin CI and kin MS.**
119 **Kin CI and kin MS are related to resistant and sensitive varieties respectively.** The power of beta
120 = 12 was chosen based on the scale-free topology criterion.

121

122 Statistical analysis

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

125

126 Results

127 Effects of drought stress on morphometric characteristics of tomato cultivars

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

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

138 **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±0.115de
	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

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

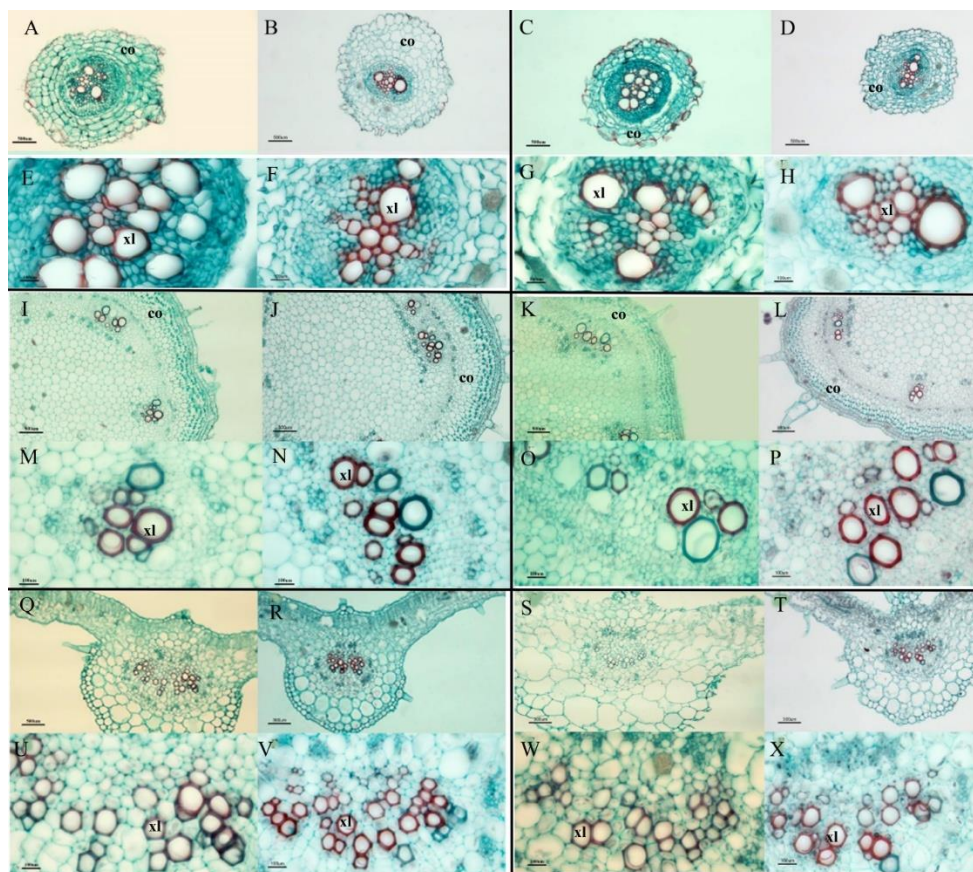
140 Effect of drought stress on anatomical characteristics of roots, stems and leaves in cultivars of tomato

141 Only control (100% FC) and 25% FC (high level) treatment samples were compared in both
 142 cultivars. Anatomical studies of roots showed that the diameter of the root does not change
 143 considerably in both control and treated plants (CaljN3 and Superstrain B) (Figure 1 A-D). In
 144 drought-stressed plants (Superstrain B), cells within the cortex appeared disordered (Figure 1 D).
 145 In general, the diameter of the vascular cylinders in the roots of control plants (both cultivars) was
 146 more extended than what was observed in drought-stressed plants (Figure 1 E-H). The diameter of
 147 metaxylem elements within the control plants of both cultivars was greater than that observed in
 148
 149

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

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

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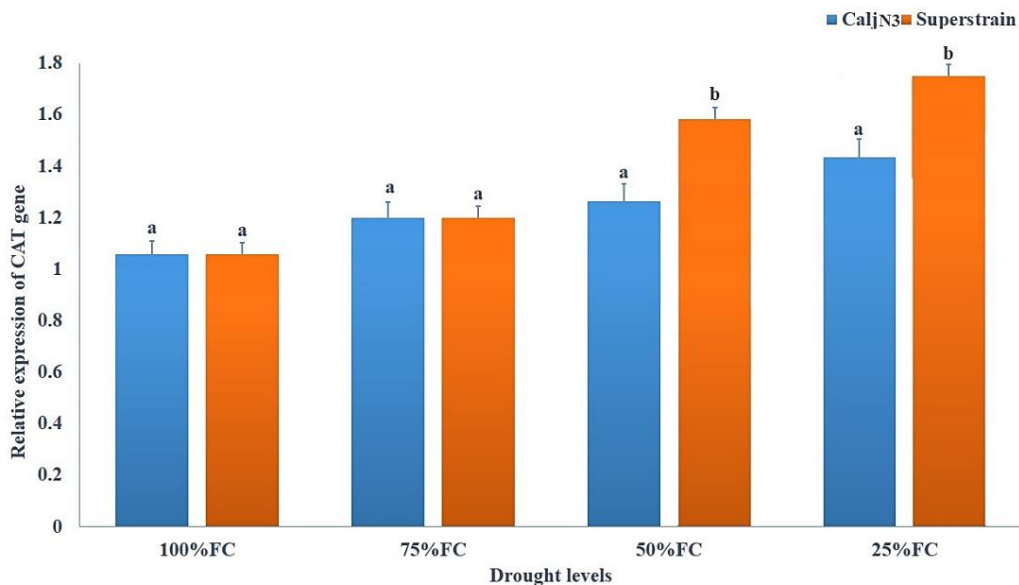


167
 168 **Figure 1.** Drought stress effects on root, stem, and leaf anatomy of two tomato cultivars (CaljN3
 169 and Superstrain B. (control plant of CaljN3 (A, E, I, M, Q, U); Stressed plants of
 170 CaljN3(B,F,J,N,R,V) ; control plant of Superstrain B (C, G, K, O, S, W) ; Stressed plants of
 171 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:
 172 stem. M,N, O, P: xylem and Phloem in stem. Q, R, S, T: leaf. U, V, W, X: xylem and Phloem in
 173 leaf. (Scale bars, 500µm in A-D, I-L, Q-T and Scale bars, 100µm in E-H, M-P, U-X). co: cortex,
 174 xl: xylem.

175
 176 **Effect of drought stress on CAT1 gene expression in tomato cultivars**

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

185



186
 187 **Figure 2.** Comparison of relative expression of CAT1 gene in CaljN3 and Superstrain B cultivars.
 188 Values with different letters are statistically significantly different at $p < 0.05$.

189
 190 **Bioinformatics study of CAT1 gene utilizing microarray analysis**

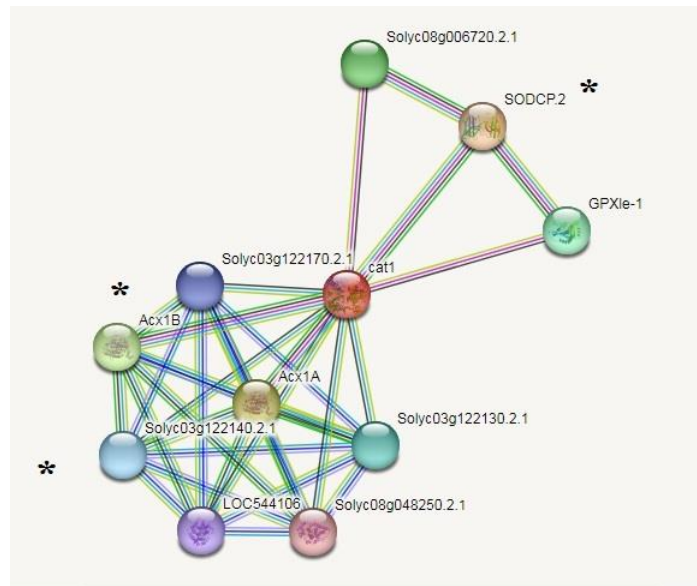
191 In the current study, probe Id (Les.3098.1.S1_at) was selected as indicative of CAT1 in *Solanum*
 192 *Lycopersicum* with Gene ID 543990. In study groups that were divided based on cultivars and type
 193 of stress, the probe did not show any significant log fold change (Table 2). Gene enrichment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

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210 **Table 2.** CAT gene expression in microarray studies of different tomato cultivars under different
 211 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	<i>S. lycopersicum</i>	drought		0.399835513
GSE39894	<i>S. pimpinellifolium</i>	drought		0.182271858
GSE97045	<i>S. lycopersicum</i> , cv. P73	drought		0.143336524
GSE97045	<i>S. pennellii</i> (Sp) (acc. PE47)	drought		0.189479176
GSE22304	Is not mentioned	heat	susceptible	-0.22713333
GSE22304	Is not mentioned	heat	tolerant	0.97014

212



213

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

218

219 Discussion

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

226 condensing its roots makes better use of water (Faghani *et al.*, 2022). Factors limiting
227 photosynthesis like light and water, in addition to decreasing plant function also decrease root
228 growth. Plants in dry environments prefer to deposit their photosynthetic production in the root and
229 not in the stems and shoots as the plant can preserve its ability to absorb more amounts of soil water
230 (Halo *et al.*, 2020). Tomato is susceptible to drought stress, and therefore, when applying water
231 stress, its vegetative growth and function decrease considerably. Miguel and Francisco (2007) also
232 reported a reduction in root growth, fresh weight, and dry weight in tomatoes. Plant growth under
233 stress usually depends on the root's ability to absorb water from the soil and transfer it to stems
234 (Navarro *et al.* 2008). Root length is an index for absorbing water from deep layers of soil.
235 Therefore, the intensity of root growth affects the shoot of a plant (Franco *et al.* 2011).
236 The results indicated that drought stress caused a reduction in leaf area in both CaljN3 and
237 Superstrain B cultivars. The production and expansion of leaves are very susceptible to water
238 deficit because of the essential need for cellular division and growth (Hernandez-Espinoza *et al.*,
239 2020). Drought has a profound impact on the growth, production, reduction of leaf expansion,
240 reduction in stomata pores and the quality of the plant. The crucial impact caused by stress is a loss
241 of turgor pressure, which affects the speed of cell expansion and final cell size (Kumar and Purohit,
242 2001). The reduction of leaf growth induced by drought stress could be considered an adaptation
243 response. Furthermore, drought stress restricts leaf area and ultimately transpiration (Sikuku *et al.*,
244 2010). The typical reaction of a plant to drought stress includes reducing stem growth and the size
245 of the whole plant (Mostajeran and Rahimi-Eichi, 2008). A decrease in leaf area causes a reduction
246 into receive of light and photosynthesis (Ourcut and Nilsen, 2000).
247 Results of the current study showed that under stress conditions, shoot weight in susceptible
248 cultivars was lower relative to tolerant cultivars, which can be used as an index for the selection of
249 susceptible and tolerant cultivars. The decrease in shoot growth and weight probably occurred due
250 to the decrease in photosynthesis, the production of inhibitory substances, and the decline in the
251 level of hormones during drought stress (Hayat and Ahmad, 2007). It is suggested that under water
252 deficit conditions, the absorption of nutritional substances decreases and consequently transpiration
253 might reduce. These processes cause a reduction in the growth and expansion of shoots in the plants
254 (Kirnak, 2001). The level of production of essential metabolites in plants has a strong co-relation
255 with leaf area and absorbed light. A reduction of each one of these indexes can reduce the fresh

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

258 Anatomical changes can occur in plants under water deficit. Some of these changes include
259 increased lignification or suberin deposition in the cortex, endoderm cells, and cell layers that are
260 near to cortex and medulla (Farooq *et al.*, 2009). The reduction of vessel diameter, which is caused
261 by an increase in lignification, shows the adaptability of a plant to stress conditions (Halo *et al.*,
262 2020). Increased thickness of the transverse wall of vessels and a reduction in the diameter of the
263 vessels, allow water to run through the vessels with greater speed (Jogawat *et al.*, 2021). A
264 secondary structure formation is a kind of defense response of plants against stressful conditions.
265 It has been observed that the tonality rate of lignified areas is much lower than that of the control
266 plants, which can be a result of increased polymerization of the lignin component (Jogawat *et al.*,
267 2021). The number of layers and root volume of cortex cells in drought-stress plants for both
268 cultivars increased as compared to the control plants (Granier *et al.* 2000). Tissues placed in water-
269 deficit conditions usually demonstrate a decrease in cell size and the number of vascular tissues.
270 Under these conditions, processes corresponding to cell elongation are more vulnerable compared
271 to processes related to cell division (Nevo *et al.* 2000). The space between spongy parenchyma
272 cells of leaves seems to be beneficial for the prevention of water loss. Reduction in blade thickness,
273 palisade, and spongy parenchyma in some species of *Acacia auriculiformis* under water deficit
274 stress was reported by Liu *et al.* (2004). A leaf is considered a responsive organ to environmental
275 conditions and among environmental factors that could potentially affect the structure of a leaf,
276 certainly drought stress is one of the most important ones (Nardini *et al.* 2005). Changes in leaf
277 anatomy in plants under stress could be related to reducing transportation via the stomata.
278 Moreover, a reduction of leaf expansion could be related to different mechanisms such as a
279 reduction in cell division and firmness of the cell wall (Bouchabke *et al.*, 2002).

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

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

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

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

309 310 **Conclusions**

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

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

325

326 **References**

- 327 1. Bacelar, E.A., Santos, D.L., Moutinho-Pereira, J.M., Gonçalves, B.C., Ferreira, H.F., and
328 Correia, CM. 2006. Immediate responses and adaptative strategies of three olive cultivars
329 under contrasting water availability regimes: changes on structure and chemical
330 composition of foliage and oxidative damage. *Plant Sci.*, 170(3):596–605.
- 331 2. Bouchabke, O., Tardieu, F. and Simonneau, T. 2002. Leaf growth and turgor in growing
332 cell to maize (*Zea mays* L.) respond to evaporative demand under moderate irrigation but
333 not in water saturated soil. *Plant Cell Environ.*, 19:10-15.
- 334 3. Daneshmand, F. 2014. Response of antioxidant system of tomato to water deficit stress
335 and its interaction with ascorbic acid. *I.J.P.B.*, 6(1): 57-72.
- 336 4. Demirel, U., Morris, W.L., Ducreux, L.J.M., Yavuz, C., Asim, A., Tindas, I. *et al.* 2020.
337 Physiological, Biochemical, and Transcriptional Responses to Single and Combined
338 Abiotic Stress in Stress-Tolerant and Stress-Sensitive Potato Genotypes. *Front. Plant Sci.*,
339 11: 196.
- 340 5. Du, Y.Y., Wang, P.C., Chen, J., Song, C.P. 2008. The comprehensive functional analysis
341 of catalase gene family in *Arabidopsis thaliana*. *J. Integr. Plant Biol.*, 50:1318-1326.
- 342 6. Faghani, E., Kolahi, M., Kazemian, M., Goldson-Barnaby A. and Razzaghi MH. 2022.
343 Effect of irrigation regimes on starch biosynthesis pathway, cotton (*Gossypium hirsutum*)
344 yield and in silico analysis of ADP-glucose-pyrophosphorylase. *Int. J. Environ. Sci.*
345 *Technol.* 19, 10809–10830. <https://doi.org/10.1007/s13762-022-04281-x>
- 346 7. Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, S.M.A. 2009. Plant drought
347 stress: effects, mechanisms and management. *Agron. Sustain. Dev.*, 29: 185-212.
- 348 8. Franco, J.A., Banon, S., Vicente, M. J., Miralles, J. and Martínez-Sánchez, J.J. 2011.
349 Root development in horticultural plants grown under abiotic stress conditions- a review.
350 *J. Hortic. Sci. Biotech.*, 86: 543–556.

- 351 9. Gaballah, M.S. and Gomaa, A.M. 2005. Interactive effect of Rhizobium inoculation,
352 sodium benzoate and salinity on performance and oxidative stress in two faba bean
353 varieties. IJAB, 8530. 7-3.
- 354 10. Granier, C., Turco, O. and Tardieu, F. 2000. Co-ordination of cell division and tissue
355 expansion in sunflower, tobacco and pea leaves: dependence or independence of both
356 processes?. Plant Growth Regul., 39145-54.
- 357 11. Halo, B. A., Al-Yahyai, R. A. and Al-Sadi, A. M. 2020. An endophytic *Talaromyces*
358 *omanensis* enhances reproductive, physiological and anatomical characteristics of drought-
359 stressed tomato. J. Plant Physiol., 249: 153163.
- 360 12. Hasanagić, D., Koleška, I., Kojić, D. *et al.* 2020. Long term drought effects on tomato
361 leaves: anatomical, gas exchange and antioxidant modifications. Acta Physiol Plant, 42:
362 121. <https://doi.org/10.1007/s11738-020-03114-z>
- 363 13. Hayat, S. and Ahmad, A. 2007. Salicylic Acid a Plant Hormone. Springer. P: 97-99.
- 364 14. Hernandez-Espinoza, L. H. and Barrios-Masias, F.H. 2020. Physiological and anatomical
365 changes in tomato roots in response to low water stress. Sci. Hortic., 265: 109208.
- 366 15. Hung, S. H., Yu, C. W. and Lin, C. H. 2005. Hydrogen peroxide functions as a stress signal
367 in plants. Bot. Bull. Acad., 46: 1-10.
- 368 16. Jogawat, A., Yadav, B., Lakra, N., Singh, A. K. and Narayan, O. P. 2021. Crosstalk
369 between phytohormones and secondary metabolites in the drought stress tolerance of crop
370 plants: a review. Physiol. Plant., 172(2), 1106-1132.
- 371 17. Kirnak, H., Kaya, C. Tas, I. and Higgs, D. 2001. The influence of water deficit on
372 vegetative growth, physiology fruit yield and quality in eggplants. J. Plant Physiol, 27: 34-
373 46.
- 374 18. Kumar, A. and Purohit, S.S. 2001. Plant Physiology Fundamentals and Applications.
375 Second Enlarged Edition. Agrobios (India). P: 18-25.
- 376 19. Li Y. 2008. Kinetics of the antioxidant response to salinity in the halophyte *Limonium*
377 *bicolor*. Plant Soil Environ, 54:493-497.
- 378 20. Liu, L.X, Xu, S.M and Woo, K.C. 2004. Deficit irrigation effects on photosynthesis and
379 the xanthophyll cycle in the tropical tree species *Acacia auriculiformis* in north Australia.
380 New Zealand .J. Botany, 42:949-957.

- 381 21. Mesgaran, M. B., Madani, K., Hashemi, H., and Azadi, P. 2017. Iran's Land Suitability for
382 Agriculture. *Sci. Rep.*, 7(1): 7670. <https://doi.org/10.1038/s41598-017-08066-y>
- 383 22. Miguel, A. and Francisco, M. 2007. Response of tomato s to deficit irrigation under surface
384 or subsurface drip irrigation. *J. Appl. Hortic.*, 9(2): 97-100.
- 385 23. Mostajeran, A. and Rahimi-Eichi, V. 2008. Drought stress effects on root anatomical
386 characteristics of rice cultivars (*Oryza sativa* L.). *Pakistan J. Biol. Sci.*, 11:2173–2183.
- 387 24. Mura, A., Pintus, F., Medda, R., Floris, G., Rinaldi, AC. and Padiglia, A. 2007. Catalase
388 and antiquitin from *Euphorbia characias*: Two proteins involved in plant defense.
389 *Biochem.*, 72:501-508.
- 390 25. Nadarajah, KK. 2020. ROS Homeostasis in Abiotic Stress Tolerance in Plants. *Int. J. Mol.*
391 *Sci.*, 21(15):5208.
- 392 26. Nardini, A. and Salleo, S. 2005. Water stressinduced modifications of laef hydraulic
393 architecture in sunflower: Co-ordination with gas exchange. *J. Exp .Bot*, 422:3093- 3101.
- 394 27. Navarro, A., Vicente, M. J., Martí´nez-Sa´nchez, J. J., Franco, J. A., Ferna´ndez, J. A. and
395 Banon, S. 2008. Influence of deficit irrigation and paclobutrazol on plant growth and water
396 statusin *Lonicera implexa* seedlings. *Acta Hortic.*, 782: 299–304.
- 397 28. Nevo, E., Bolshakova, M.A., Martyn, G.I., Musatenko, L.I., Sytnik, K., Palieek, T. and
398 Beharvan, A. 2000. Drought and light anatomical adaptive leaf strategies in three woody
399 species caused by microclimatic selection at “Evolution canyon”. *Isr. J. Plant Sci.*,
400 48:3346;
- 401 29. Ourcut, D.M. and Nilsen E.T. 2000 Salinity stress in: physiology of plants under stress.
402 KA/PP p: 177-235.
- 403 30. Rizhsky, L., Hallak-Herr, E., Van Breusegem, F., Rachmilevitch, S., Barr, J.E., Rodermel,
404 S., Inzé, D. and Mittler, R. 2002. Double antisense plants lacking ascorbate peroxidase and
405 catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate
406 peroxidase or catalase. *Plant J. Nov.*, 32(3):329-42.
- 407 31. Rout, N.P. and Shaw, B.P. 2001. Salt tolerance in aquatic macrophytes: possible
408 involvement of the antioxidative enzymes. *Plant Sci.*, 160:415–423.
- 409 32. Sahni, S., Prasad B.D., Liu Q., Grbic V. *et al.* 2016. Overexpression of the brassinosteroid
410 biosynthetic gene DWF4 in *Brassica napus* simultaneously increases seed yield and stress
411 tolerance. *Sci. Rep.*, 6: 28298-28298.

- 412 33. Sairam, R.K., Chandrasekhar, V. and Srivastava, G.C. 2001. Comparison of hexaploid and
413 tetraploid wheat cultivars in their response to water stress. *Biol. Plant.*, 44: 89-94.
- 414 34. Sangeetha, K., Ramyaa, R. B., Khaneghah, A. M., and Radhakrishnan, M. 2023.
415 Extraction, characterization, and application of tomato seed oil in the food industry: An
416 updated review. *J. Agric. Res.*, 100529.
- 417 35. Sarker, U. and Oba, S. 2018. Catalase, superoxide dismutase and ascorbate-glutathione
418 cycle enzymes confer drought tolerance of *Amaranthus tricolor*. *Sci. Rep.*, 8: 16496.
419 <https://doi.org/10.1038/s41598-018-34944-0>.
- 420 36. Sikuku, P.A., Netondo, G.W., Onyango, J.C. and Musyimi, D.M. 2010. Effects of water
421 deficit on physiology and morphology of three varieties of NERICA rainfed rice (*Oryza*
422 *sativa* L.). *J. Agric. Biol. Sci.*, 5: 23-28.
- 423 37. Srivalli, B., Sharma, G. and Khanna-Chopra, R. 2003. Antioxidative defence system in an
424 upland rice cultivar subjected to increasing intensity of water stress followed by recovery.
425 *Physiol. Plant.*, 119: 503-512.
- 426 38. Zhang, X., Yang, Z., Li, Z., *et al.* 2020. Effects of drought stress on physiology and
427 antioxidative activity in two varieties of *Cynanchum thesioides*. *Rev. Bras. Bot.*, 43: 1–10.
- 428
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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 و Superstrain 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 سوپر استرین 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 گلوکاتایون پراکسیداز تعامل دارد. رقم کالج 3 در تمامی سطوح آبیاری تحمل بیشتری نشان داد. بنابراین رقم سوپر استرین 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.