

Impact of Drought on Osmotic Adjustment, Antioxidant Enzymes and Yield in Contrasting Genotypes of Tomato (*Solanum Lycopersicum*)

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ABSTRACT

The effect of drought stress on osmotic adjustment, antioxidant enzymes activity and yield of tomato (*Solanum lycopersicum*) genotypes was investigated under pot culture conditions in rainout shelter. The drought condition was created at 15 days after transplanting based on field capacity of soil. Experiment was laid out with eighteen genotypes by adopting completely randomized design with three replications and two treatments *viz.*, 100% and 50% field capacity. As the stress increased from 100 per cent field capacity to 50 per cent field capacity, reductions in relative water content (RWC), osmotic potential and increased malondialdehyde (MDA) content, superoxide dismutase (SOD), catalase activity were noticed at all the growth stages. The genotypes LE 114, LE 57, LE 118 and LE 27 which showed significantly less reduction in the RWC, osmotic potential and less increment of MDA, higher SOD and catalase activity during drought were considered as drought tolerant. Genotypes LE 1, LE 3 and LE 20 which recorded the lowest relative water content, osmotic adjustment and antioxidant enzymes activity and ultimately poor yield were considered as drought susceptible.

Keywords: Drought, RWC, Osmotic adjustment, MDA, SOD, Catalase, Yield

1. INTRODUCTION

Drought stress is one of the severe environmental issue affecting plant growth, development and yield. It stimulates various physiological and biochemical adaptations in plants. It has been estimated that up to 45% of the world agricultural lands are subjected to drought (Bot *et al.*, 2000). Water deficit leads to the perturbation of most of the physiological and biochemical processes and consequently reduces plant growth and yield (Boutraa, 2010). RWC is considered as a reliable indicator that reflects the water content in relation to maximum water content, therefore it indicates the level of hydration (Rosales *et al.*, 2004).

The increase in osmotic pressure is considered a potential cellular mechanism of drought tolerance as it enables turgor maintenance and growth continuation (Bajji *et al.*, 2000). Osmotic adjustment is a key mechanism by which plants adapt to water shortages resulting from an increased solute concentration of cells in order to maintain the water potential gradients needed to ensure continued uptake of water during the stress period. In addition, osmotic adjustment allows cell to maintain the turgor, which is essential for plant growth and various other physiological processes (Nahar *et al.*, 2011). RWC and osmotic adjustment have been suggested as selection criteria for assessing drought tolerance.

Cell membrane lipid peroxidation can be assessed by measuring the amount of malondialdehyde (MDA), a product

of unsaturated fatty acid peroxidation (Heath and Packer, 1968). During water stress, fluidity status of cell membrane is altered. As a result a compound called MDA is accumulated in the cell. Drought-induced overproduction of ROS increases the content of malondialdehyde. The content of MDA has been considered as an indicator of oxidative damage created by various stresses (Moller *et al.*, 2007).

Plant cells are protected against the detrimental effects of ROS by a complex antioxidant system comprising of the non-enzymic as well as enzymic antioxidants (Noctor and Foyer, 1998). Among the enzymes, catalase (CAT) is an important and most powerful antioxidant enzyme under abiotic stress condition to nullify the effect of H₂O₂ and protects the plants under stress condition. This enzyme is generally regarded as H₂O₂ scavenger involved in the reduction of damage by oxidation function (Reddy *et al.*, 2004). The SOD activity in both tolerant and sensitive tomato cultivars increased in drought condition, but the increase of SOD activity was larger in tolerant cultivars than in sensitive one (Rahman *et al.*, 2002).

The productivity of the crop under drought may be related with relative water content, osmotic adjustment and activity of antioxidant enzymes. Higher RWC and osmotic adjustment indicates better growth and development, which in turn depends on leaf area. Rapid early growth and maintenance of RWC at reasonably higher level during growth period greatly influences the yield (Haloi and Baldev, 1986).

Tomato (*Solanum lycopersicum*) is one of the most popular and widely grown vegetables in the world. Considering the potentiality of this crop, there is plenty of scope for its improvement, especially under the drought situation. Water is a scarce resource for irrigation. Although the concept of drought tolerance has been viewed differently by molecular biologist, biochemist, physiologists and agronomists, the major concern is to enhance the biomass and yield under limited input of water, which is a characteristic feature of rainfed agriculture. Therefore, some of the adoptive mechanisms of plants to drought stress, which do not decrease plant yield to a greater extent, assume greater importance.

There are several physiological and biochemical traits contributing to the drought tolerance of horticultural crops.

However, large number of tomato genotypes have not been screened for drought tolerance or exploited for their cultivation under drought situation. To breed drought tolerant genotypes, it is necessary to identify physiological traits of plants, which contributes to drought tolerance. Therefore, the present investigation was carried out to study the physiological traits to facilitate the screening and selection of tomato genotypes for drought tolerance.

2. METHODS

The study was undertaken to find out effect of drought on osmotic adjustment, antioxidant enzymes activity and yield of tomato genotypes in the pot culture experiment at Rainout Shelter of Crop Physiology Department, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu during 2011-12. The experiment was conducted with 18 tomato genotypes viz., LE 1, LE 3, LE 5, LE 13, LE 14, LE 18, LE 20, LE 23, LE 27, LE 57, LE 100, LE 114, LE 118, LE 125, CO 3, PKM 1, TNAU THCO 3 and COTH 2 and two treatments viz., 100% FC and 50% FC with three replications. Seeds of selected genotypes were sown in trays filled with vermicompost for nursery. Uniform size (38 cm width and 32 cm height) pots were filled with 25 kg of soil and saturated with water and the field capacity of the soil was recorded. Twenty five days old seedlings were transplanted and one plant was maintained in each pot. Drought was imposed at 15 days after transplanting onwards based on field capacity, 50% field capacity for drought stress and 100% field capacity for control pots were maintained by weighing and watering each pot at regular interval. Crop was supplied with fertilizers and other cultivation operations including plant protection measures as per recommended package of practices of Tamil Nadu Agricultural University, Coimbatore. All the observations were recorded on third leaf from top at 30, 60 and 90 DAT. The experiment was laid out in completely randomized block design with three replications.

2.1. Estimation of RWC

The relative water content (RWC) was estimated according to Barrs and Weatherly (1962). Fifty uniform leaf discs were used

and fresh weight (Fw) was recorded. The leaf discs were floated in water for one hour to attain full turgid and turgid weight (Tw) was recorded. Then the leaf discs were kept in hot air oven at 80°C for 48 hours and the dry weight (Dw) was recorded. The relative water content (RWC) was calculated by using following formula

$$\text{RWC} = \frac{[(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight})] \times 100}$$

2.2. Measurement of osmotic potential

Leaf samples were thawed, centrifuged for 5 min at 18000 ppm, and osmotic potential of the expressed sap was recorded by using vapour pressure osmometer (VAPRO, 5520).

2.3. Calculation of osmotic adjustment

Osmotic adjustment was calculated by using following formula according to the method described by Flower and Ludlow (1986)

Osmotic adjustment (OA) = Drought leaf Ψ_s 100 – Irrigated leaf Ψ_s 100

$$\Psi_s100 = (\Psi_s \times \text{RWC}) / 100$$

2.4. Estimation of MDA content

The amount of MDA derived from unsaturated fatty acid peroxidation of membrane lipids was measured according to the method of Sese and Tobita (1998). 250 mg leaf sample was weighed and homogenized with 5 ml of 0.1% TCA. 1ml supernatant was taken and 4 ml of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95°C for 30 min. The content was cooled in an ice bath and again centrifuged at 10000 rpm for 10 min. The absorbance of was measured at 532 nm and the result was expressed in nmol g^{-1} .

2.5. Estimation of catalase activity

Catalase activity was assayed as per the procedure adopted by Gopalachari (1963) and expressed as $\mu\text{g H}_2\text{O}_2 \text{ g}^{-1}\text{min}^{-1}$. Phosphate buffer (51 ml of 0.2 M monobasic + 49 ml of 0.2 M dibasic and made up to 200 ml) was used to homogenize the leaf sample and 1.5% sodium perborate used as substrate and H donor. The reaction was allowed for one minute and the enzyme action was stopped by using 2 N sulphuric acid. The

solution was titrated by using 0.05 N potassium permanganate and the remaining H_2O_2 in the solution was calculated by taking one ml of KMnO_4 consumes 0.85 microgram of H_2O_2 .

2.6. Estimation of superoxide dismutase activity (SOD)

SOD activity was determined by using nitro blue tetrazolium (NBT) salt as described by Champ and Fridovich (1971) and expressed in enzyme units mg^{-1} protein. 500 mg leaf sample was weighed and macerated with 10 ml HEPES-KOH buffer containing 0.1 mM EDTA. The contents were centrifuged at 15000 ppm for 15 min and 1 ml of enzyme extract was mixed with 3 ml of reaction mixture. One unit SOD activity was defined as the amount of enzyme required to 50 per cent inhibition of the rate of NBT reduction at 560 nm.

2.7. Estimation of yield

The fruit weight per plant was recorded in control and stressed plants in each picking and fruit yield (kg per plant) was calculated as fresh weight of fruits in all the pickings.

2.8. Statistical analysis

The data on various parameters were analyzed statistically as per the procedure of Gomez and Gomez (1984).

3. RESULTS

3.1. Decreased RWC under drought

Tomato genotypes responded differentially to water deficit in the form of changes in various parameters used in this study. Relative water content (RWC) decreased under water deficit stress (50% FC) compared to control (100% FC). Among the genotypes, LE 118, LE 114, LE 57 and LE 27 recorded the highest RWC, while LE 20, LE 23, COTH 2, LE 125, LE 5 and LE 1 recorded the lowest RWC at 50% FC during 60 DAT (Table 1). Among the genotypes, LE 114 showed comparatively less reduction (12.3%) in RWC at 50% FC, followed by LE 118 (12.8%), LE 57 (14.4%) and LE 27 (14.7%). Whereas the highest reduction per cent of 21.7 was registered by the hybrid COTH 2 followed by LE 1 (19.8%) and LE 125 (19.7%).

3.2. Decreased osmotic potential under drought

The osmotic potential was lowered under drought condition (50% FC) contrasted to the control (100% FC). Among the genotypes, the highest reduction of osmotic potential was observed in LE 118 (63.56%) followed by LE 114 (61.54%) and LE 57 (58.97%) in 50 per cent FC compared to 100 per cent FC. Osmotic adjustment of plant under stress condition is more vital for endurance by keeping up the tissue water potential. In the present study, the highest osmotic adjustment value was recorded by the genotype LE 118 followed by LE 114 while the lowest value by LE 1 (Table. 1). However, the genotypes LE1, LE3, LE 13 and LE18 were shown the negative osmotic adjustment values indicated that the poor osmotic adjustment under drought condition.

3.3. Increased MDA content due to drought

Accumulation of MDA at significant level could be noticed under water deficit condition and the increase was 62 per cent over control. The genotypes LE 57 and LE 118, however, showed lesser accumulation of MDA with 32 per cent increase, whereas a higher level of 94 and 86 per cent was noticed in LE 1 and LE 125 respectively (Table 2). Water deficit condition stimulates the catalase activity at various levels due to genotypic variations to stress tolerance.

3.4. Increased antioxidant enzymes activity under drought

The elevation in enzyme activity was about 65 and 58 per cent in LE 57 and LE 118 respectively (Fig. 1). However, the genotypes, LE 5 (27.3%), LE 20 (31.6%), LE 100 (32.50%) and LE 125 (35.6%) were showed lowest increment of catalase activity under drought condition. Therefore, high CAT activity in these genotypes could be related to its role in preventing the formation of ROS like H_2O_2 , and therefore the appearance of excessive damage by oxidative stress, achieving better water-deficit tolerance. In the present study, it could be observed that, drought stress triggered the SOD activity, which enables the plants to acquire tolerance at various levels. The genotype LE 57 and LE 118 showed elevated SOD activity with 88 and 85 per cent increase over control respectively at 60 DAT (Table 2). However, the genotypes LE 100, LE 1 and LE 125

were able to enhance the activity up to only 26, 29 and 32 per cent over control respectively.

3.5. Reduced fruit yield up to 83% due to drought

The fruit yield showed significant differences among the genotypes and treatments. Decrease in fruit yield was observed at 50% FC level compared to 100% FC. LE 114 recorded higher fruit yield, followed by LE 118, LE 57 and LE 27 (Fig 2.). The percentage yield reduction under drought over control has been suggested in the most important parameter for assessing drought tolerance than fruit yield. The highest percentage reduction in yield under drought was recorded in LE 125 (83%), followed by LE 5 (80%), LE 23 (76%) and COTH 2 (71%). The least reduction in fruit yield under drought was observed in LE 57 (18%), LE 114 (20.6%), LE 27 (21%) and LE 118 (27%).

4. DISCUSSION

4.1. Maintenance of RWC under drought by the tolerant genotypes

Genotypes, which showed higher RWC ensure more favourable internal water relations of tissue and showed better drought tolerance capacity (Srinivas Rao and Bhatt, 1992). Similar results were obtained in the present study in tomato. Maintenance of high RWC by the tolerant genotypes might be due to the accumulation of osmolytes in the cells which cause increase of root length leads to absorption of more water from deep soil layer. The data on osmotic pressure increased under drought over control. The highest increment of 63.6 per cent was recorded by LE 118 in response to drought while the lowest increment was noted by LE 1 and LE 3 (15.8%) (Table 1). The increase in osmotic pressure is considered a potential cellular mechanism of drought tolerance as it enables turgor maintenance and growth continuation (Bajji *et al.*, 2000).

In the present study, LE 118 and LE 114 exhibited high osmotic pressure and thus it turned to be a better drought tolerant genotype than others. However, it also presents a metabolic cost due to the synthesis and compartmentation of osmolytes (Bajji *et al.*, 2000). Many important physiological and morphological processes, such as leaf enlargement,

stomatal conductance and photosynthetic activity are directly affected by leaf turgor potential. During osmo regulation, solutes accumulate in the leaf. As a result, decreasing the osmotic potential, leads to up take of water for maintaining turgor. In the present study, lowering in osmotic potential was observed under water stress. The genotype, which maintained higher turgor, was tolerant to drought (Ashraf *et al.*, 1994). The same finding was obtained in the present study.

4.2. High osmotic adjustment recorded by the tolerant genotypes

The osmotic adjustment results from the accumulation of solutes which lowers the osmotic potential and helps in maintaining turgor of plants experiencing water stress (Ashraf *et al.*, 1994). It is reported that decrease in osmotic potential is essential to maintain the potential differences to allow water uptake by the root. The present study confirmed that, with the fall in leaf water potential in terms of RWC due to soil water deficit simultaneous fall in osmotic potential was observed. An increasing number of reports provide evidence on the association between high rate of OA and sustained yield or biomass under water-limited conditions across different cultivars of crop plants.

Since OA helps to maintain higher leaf relative water content at low leaf water potential, it is evident that OA helps to sustain growth while the plant is meeting transpirational demand by reducing its LWP. Osmotic adjustment sustained turgor maintenance and hence the yield-forming processes during moderate and severe water stress (Ali *et al.*, 1999). Increased deep-soil moisture extraction has been found to be a major contribution of OA in sorghum (Wright and Smith, 1983). Beyond the effect on cellular hydration, other putative roles of OA have been recently assembled under the vague term of 'Osmo protection' (Rontein *et al.*, 2002). Such a possible role for cell compatible osmolytes in protecting enzymes against heat inactivation was indicated a while ago (Paleg *et al.*, 1981). In the present study, the superior osmotic adjustment made by LE 118 and LE 114 might be due to the synthesis of compatible osmolytes is an imperative trait for tolerance.

4.3. Low MDA content under drought was favorable for tolerance

The generation of reactive oxygen species (ROS) is one of the earliest biochemical responses of eukaryotic cells to abiotic stresses. Being highly reactive, ROS can seriously damage plants by increasing lipid peroxidation, protein degradation, DNA fragmentation and ultimately cell death. Drought induces oxidative stress in plants by generating reactive oxygen species (Farooq *et al.*, 2009). The ROS such as O_2^- , H_2O_2 and OH^* radicals, can directly attack membrane lipids and increase lipid peroxidation (Mittler, 2002). MDA is a product of lipid peroxidation created by any stress. Less accumulation of MDA under stress is favorable for tolerance. Hence, estimation of MDA is an important trait to assess drought tolerant capacity of crop plants. In this sense, low concentrations of MDA have been associated with water-stress tolerance in pea plants and wheat (Sairam *et al.*, 2000).

The levels of lipid peroxidation in leaves increased two to four fold with an increase in drought stress and this was highly correlated with protein peroxidation (Moran *et al.*, 1994). It could also be explained that the increment of MDA content might be due to the membrane damage and lipid peroxidation by the reactive oxygen species produced under drought. These observations corroborate the findings of the present study. The damage to cell membranes may be caused by high H_2O_2 levels, which could accelerate the Haber-Weiss reaction, increasing the formation and therefore prompting lipid peroxidation (Mittler, 2002). In the present investigation, maintenance of low level peroxidation (denoted by the MDA concentration) in the genotypes LE 118, LE 57, LE 27 and LE 114 indicating the ability of these genotypes to endure the stress effect more efficiently.

4.4. High antioxidant enzymes activity under drought was constructive for tolerance

Production of ROS like superoxide and H_2O_2 have been found to be stimulated in plants under a variety of environmental stresses (Sgherri *et al.*, 1996). These ROS are easily captured by the antioxidant enzymes like SOD and catalase. Catalase is highest turnover rate enzyme which efficiently nullifies the effect of H_2O_2 and superoxide by SOD. Low rate of enzyme

activity coupled with higher accumulation of superoxide and H_2O_2 indicates the susceptible nature of the genotype to drought. Catalase enzyme plays an important role in lowering the ROS levels and helping avoid oxidative stress (Rao *et al.*, 2012). This view corroborates with present investigation. However, decreased CAT activity under water stress has been observed in sunflower (Quartacci and Navari, 1992), wheat (Zhang and Kirkham, 1994) and tomato (Tahi *et al.*, 2008).

Other authors demonstrated that, high activity of CAT enzyme conferred tolerance to water deficit in several species of plants such as Allium (Egert and Tevini, 2002) and Kentucky grass (Wang and Huang, 2004). Present study corroborates the earlier findings. Superoxide Dismutase (SOD) is a key enzyme to nullify the effect of super oxide which is produced by Haber-Weiss reaction. PEG induced drought stress to plants, significantly increased the activity of SOD at both the stress level of -0.45 MPa and -1.22 MPa (Kumar *et al.*, 2011). SOD activities play an important role in drought tolerance of tomato at various plant ages, and suggest that, SOD activity could be used as a criterion for selecting drought tolerance in tomato cultivars. Maintenance of higher level of anti-oxidative enzyme activities may contribute to drought tolerant induction by increasing the capacity against oxidative damage induced by various stresses (Sharma and Dubey, 2005). The present results strongly support the earlier findings.

Maintenance of fruit yield under drought by the genotypes LE 57, LE 114, LE 118 and LE 27 may be attributed to their

ability to maintain higher RWC, osmotic adjustment and antioxidant enzyme activity.

5. CONCLUSION

From the perusal of results obtained for RWC, osmotic adjustment, MDA, SOD and catalase activity and yield, it can be inferred that genotypes LE 114, LE 57, LE 118 and LE 27 performed better under drought conditions and could be categorized as drought tolerant genotypes compared to genotypes LE 1, LE 3 and LE 20, which can be categorized as drought susceptible ones. However, further studies are required to confirm the results by molecular evidence. The tolerant genotypes could be utilized for further breeding programme to evolve new tomato genotype for better drought tolerance with higher yield.

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S. No.	Genotypes	Relative water content (%)			Osmotic potential (-MPa)			Osmotic Adjustment
		100% FC	50% FC	Mean	100% FC	50% FC	Mean	
1	LE 1	68.22	54.74	61.48	1.14	1.32	1.23	-0.055
2	LE 3	68.20	54.97	61.59	1.15	1.33	1.24	-0.053
3	LE 5	65.89	54.67	60.28	1.14	1.42	1.28	0.025
4	LE 13	71.16	59.38	65.27	1.16	1.36	1.26	-0.018
5	LE 14	68.90	58.10	63.50	1.15	1.47	1.31	0.062
6	LE 18	70.61	58.93	64.77	1.17	1.38	1.28	-0.013
7	LE 20	64.87	52.81	58.84	1.13	1.40	1.27	0.006
8	LE 23	66.50	53.68	60.09	1.15	1.52	1.34	0.051
9	LE 27	72.20	61.62	66.91	1.18	1.80	1.49	0.257

10	LE 57	72.33	61.89	67.11	1.17	1.86	1.52	0.305
11	LE 100	67.79	55.17	61.48	1.14	1.42	1.28	0.011
12	LE 114	71.61	62.83	67.22	1.17	1.89	1.53	0.350
13	LE 118	74.13	64.62	69.38	1.18	1.93	1.56	0.372
14	LE 125	68.04	54.63	61.34	1.17	1.47	1.32	0.007
15	CO 3	71.88	57.01	64.45	1.13	1.75	1.44	0.185
16	PKM 1	70.35	57.45	63.90	1.21	1.70	1.46	0.125
17	THCO 3	67.75	54.68	61.22	1.17	1.66	1.42	0.115
18	CO TH 2	68.81	53.85	61.33	1.14	1.69	1.42	0.126
Mean		69.40	57.30	63.35	1.16	1.58	1.37	
SEd		0.27	0.36	0.31	0.008	0.004	0.011	
CD (0.05)		0.55	0.73	0.64	0.017	0.007	0.023	

Table 1. Effect of drought on RWC, osmotic potential and osmotic adjustment of tomato genotypes at 60 DAT

DAT – Days after transplanting; FC – Field capacity

S. No.	Genotypes	MDA content (nmol g ⁻¹)			SOD activity (Units mg ⁻¹ protein)		
		100% FC	50% FC	Mean	100% FC	50% FC	Mean
1	LE 1	8.12	15.73	11.93	223.2	287.8	255.5
2	LE 3	8.51	14.69	11.60	212.2	302.5	257.3
3	LE 5	8.73	14.68	11.71	216.3	298.4	257.3
4	LE 13	9.06	14.91	11.99	225.6	331.6	278.6
5	LE 14	8.56	14.42	11.49	221.1	343.5	282.3
6	LE 18	8.97	14.34	11.66	214.3	348.9	281.6
7	LE 20	8.89	15.06	11.98	210.0	293.4	251.7
8	LE 23	8.75	14.88	11.82	218.2	308.3	263.2
9	LE 27	8.82	12.41	10.62	224.0	367.7	295.8
10	LE 57	8.99	11.82	10.41	226.5	425.5	326.0
11	LE 100	8.81	14.94	11.88	227.2	287.1	257.1
12	LE 114	8.55	12.53	10.54	218.9	374.2	296.5
13	LE 118	9.14	12.09	10.62	223.6	412.5	318.0
14	LE 125	8.45	15.69	12.07	218.3	288.6	253.4
15	CO 3	8.72	14.23	11.48	223.8	336.3	280.0
16	PKM 1	8.84	14.09	11.47	221.5	312.5	267.0
17	THCO 3	9.09	14.47	11.78	226.0	308.4	267.2
18	CO TH 2	9.01	14.36	11.69	224.6	310.5	267.5
Mean		8.78	14.19	11.48	220.8	329.9	275.4
SEd		0.32	0.11	0.46	7.68	2.56	10.87
CD (0.05)		0.65*	0.22*	0.91*	15.32*	5.11*	21.66*

Table 2. Effect of drought on MDA content and SOD activity of tomato genotypes at 60 DAT

DAT – Days after transplanting; FC – Field capacity

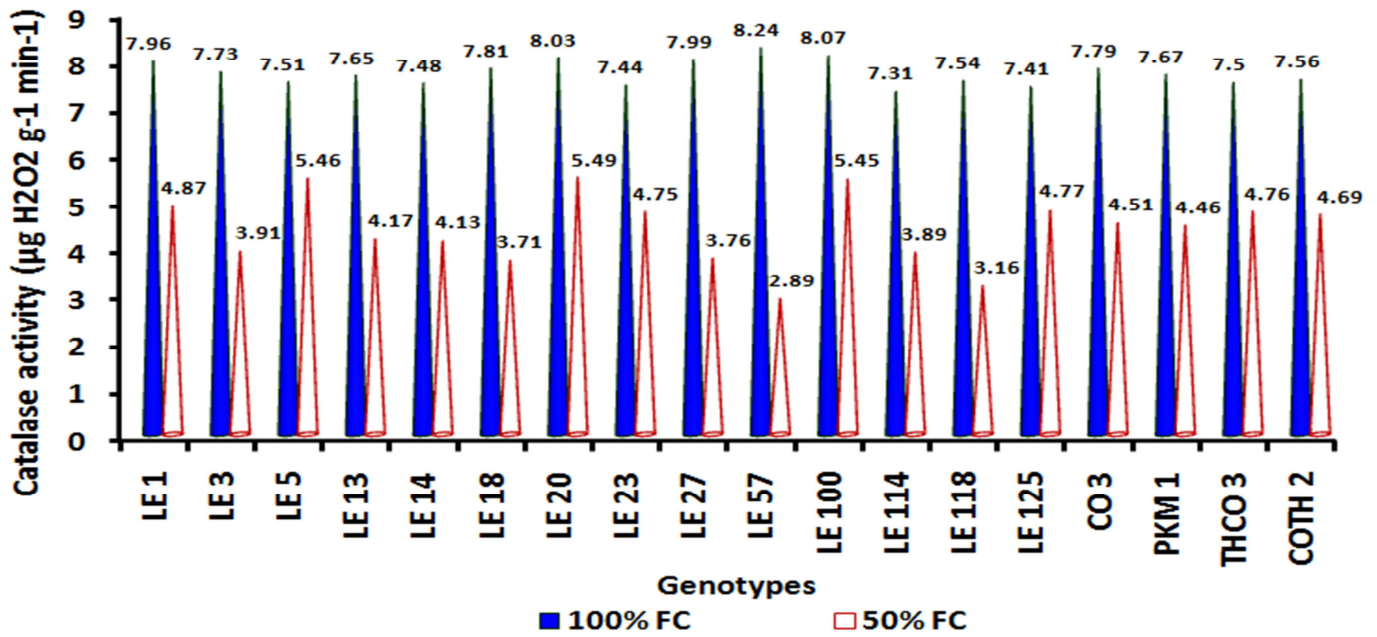


Fig 1. Effect of drought on catalase activity ($\mu\text{g of H}_2\text{O}_2 \text{ g}^{-1} \text{ min}^{-1}$) of tomato genotypes

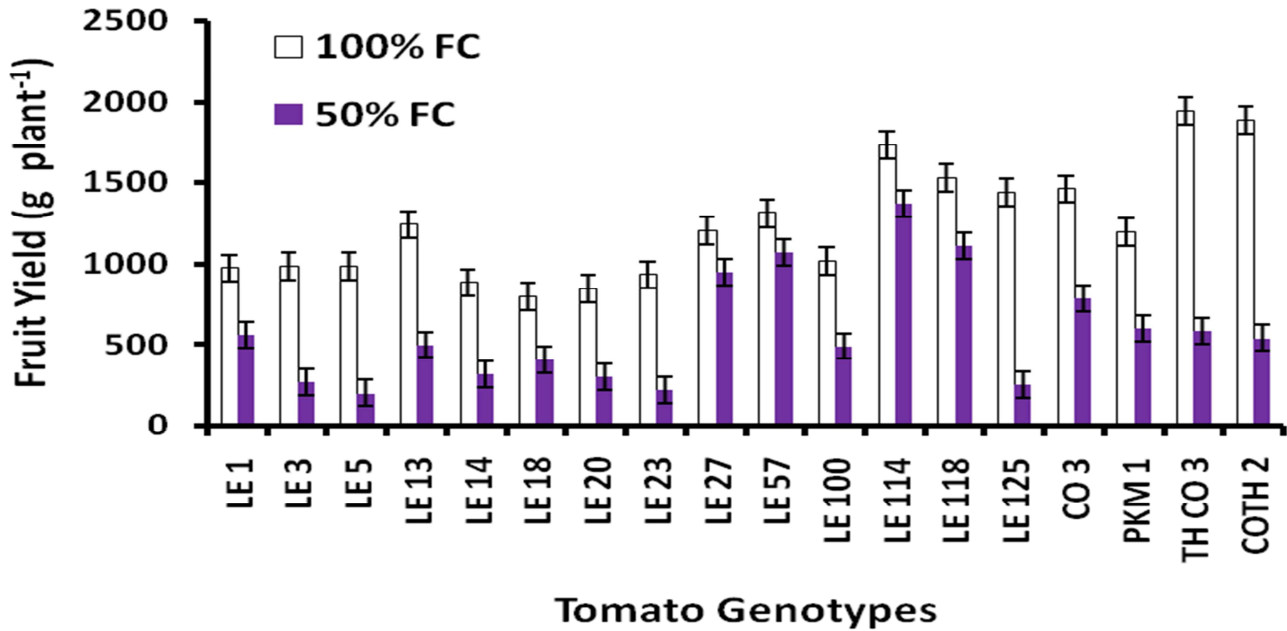


Fig 2. Effect of drought on fruit yield (g plant^{-1}) of tomato genotypes

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