**International Journal of Botany Studies**
**www.botanyjournals.com**
**ISSN: 2455-541X**
**Received: 15-03-2022, Accepted: 31-03-2022, Published: 16-04-2022**
**Volume 7, Issue 4, 2022, Page No. 88-96**

# Drought induced morpho physiological responses in tomato *Lycopersicon esculentum* (L.) genotypes

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## Abstract

Understanding the impact of drought stress in tomato (*Lycopersicon esculentum* L.) genotypes is essential under such global climate change situations which will facilitate drought tolerance potential information and crop improvement in future. The study assessed the morpho-physiological variability of four tomato genotypes in response to drought stress. Drought treatment caused an average reduction to about 45% in plant height in all genotypes. Similarly there was a rapid decrease in the root length under stress, which can be considered as negative aspect towards tolerance. Apart from this, there was also a drastic reduction seen in the leaf number and leaf area, which indirectly indicate the decline in growth attributes. The water stress of the crop under depleting soil moisture status resulted in declined RLWC to about 25% in the entire maximum reduced RLWC (about two fold) under drought stress. Drought stress altered the concentration of Chlorophylls, Carotenoids and Xanthophylls which in turn altered the CSI. Correlating MSI with these parameters, variety ML which showed better adaptation also proved to be less injured under stress with a minimal injury index. Additional to this membrane injury, the lipid Peroxidation also proved the damage levels under stress, where MP showed highest (0.62) Peroxidation, where MR recorded the lowest (0.46). Hence the response to drought stress in genotypes involved some modifications in morphology, physiology, and growth.

**Keywords:** *Lycopersicon esculentum* L., RWC, morphological attributes, membrane injury, lipid peroxidation, CSI

## Introduction

Tomato (*Lycopersicon esculentum* L.,) belongs to the family Solanaceae, and is one of the most important vegetable and economically important crops around the world (Burton & Sesso, 2014) [6]. India is the second largest producer of tomato accounting for 10.58% of the World's production. The production of tomato in India is about 168.26 lakh tonnes in 2011 (NHB Database 2017). Tomatoes are important constituents of human diets, they contain about 94% water, 2.5% total sugars, 2% total fibers, 1% protein, and other nutritional compounds (acids, lipids, amino acids and carotenoids) (John *et al* 2004) [12].

They also contains high levels of other bio active compounds such as phenolics, vitamin C and pro vitamin A which are thought to protect and possibly prevent cancer (Vinha *et al.*, 2014) [4]. Lycopene is a fat soluble compound existing as small globules in the peripheral pericarp and B carotene is mainly associated with the pectin fraction. It has strong *in vitro* and *in vivo* antioxidant properties (Lenucci *et al.*, 2016). The other active compounds in tomatoes include total phenolic contents, ascorbic acid, carotenoids, and total flavonoids have interested by many researchers because of their biological and physicochemical properties, especially their natural antioxidant compounds and human health benefits. They are highly sensitive to environmental factors such as temperature, light and changes in irrigation throughout the growth of the plant (Murshed *et al.*, 2013) [20].

Drought stress is one of the most important factors limiting plant growth and crops production worldwide more than any other biotic or abiotic stress (Almeselmani *et al.*, 2011). It is an ever – growing problem that harshly limits the crop production and result in important agricultural losses especially in arid and semiarid areas (Boyer, 1982) [11]. The response of plants to drought stress is very complicated and they manage stress avoidance approaches that depend on genotype. Stress conditions may decrease net photosynthesis rate and transpiration; these physiological responses are common in zones where the evaporative demand is very high (Shakeel *et al* 2011) [24].

Drought stress and its impacts on crops is one of the most serious drawbacks of maximum crop production around the world. The search for varieties with improved resistance to a biotic stresses is a major goal of plant breeders and researchers all over the world. Under water deficit conditions the crops are exposed to a combination of stresses including a large number of climatic factors.

Drought tolerance is a complex trait involving several interacting physiological and biochemical mechanism for escape, avoidance, resistance and recovery. Drought reduces plant productivity by inhibiting growth and photosynthesis.

Tomato production in the tropical and subtropical Asia tends to be seasonal. Peak production occurs in the early dry season, when slightly lower temperatures and moderate rainfall favor tomato growth in the low lands

[<200m above sea level]. Declining tomato supply to markets in summer, results in drastic price increases. Promoting low land summer tomato production may help to reduce tomato prices and alleviate pressure to open highland areas for the production of vegetables including tomato.

It is essential to carryout research to understand morpho-physiological responses of tomato inbred lines to water stress at their early and late growth stage. Therefore, the present study has taken up to investigate water stress effects on morphological, growth, and physiological changes in tomato under water stress.

## Materials and Methods

A pot experiment was conducted utilizing four tomato (*Lycopersicum esculentum* L.), hybrid varieties ML, AR, MR and MP and sown in three replicates. Water stress was imposed by withholding water during the 60 – 90 days for a period of 10 days, the morphological data were recorded and samples were collected and the physiological studies were done.

The shoot and root lengths of the plants were measured using a meter-scale.. Numbers of leaves were counted separately from selected plants and then the average numbers of them were computed. Leaves from 10 plants in each genotype were cut and leaf area was measured in cm2 by green leaf area meter (OSK- Model GA-5).

The relative water content was measured in controlled and water stressed leaf samples following the method of Weatherly (1950) and was calculated using the formula

$$\text{RWC} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

## Membrane Injury (Cellular membrane thermostability)

Leaf discs (0.5 cm diameter) weighing 0.2 g were cut and washed with distilled water and infiltrated in 20 ml of distilled water in test tubes covered with plastic wrap. The control tubes were kept at $10^{o}$C for 15 to 22 hours. An identical set of tubes were treated with 43% poly ethylene glycol (PEG) which gives an osmotic stress of about -18 bars and kept separately for 24 hours. Later PEG solution was drained off and the discs were washed and immersed in deionized water for 24 hours for electrolyte leakage. The initial leakage was measured through EC meter and the discs were heated at 110 $^{o}$C for 20 minutes and the final leakage was measured and the membrane thermostability was calculated using the formula of (Martineau *et al*., 1979).

$$1-[(1-T_1/T_2) / (1- C_1/ C_2)] \text{ x } 100$$

Where T and C refer to the treatment and control samples, while the subscripts 1 and 2 denote the initial and final electrical conductivities, respectively.

## Chlorophyll stability index (CSI)

0.2 g of fresh leaf discs were immersed in 10ml distilled water in test tubes. One set was kept at room temperature and the other set was incubated in a water bath at $60^{o}$C for one hour. The water was decanted and the tissue was extracted for chlorophyll in 80% acetone and centrifuged. After centrifugation the supernatant containing the pigments (dissolved in acetone) was saved and its absorbance was read at 663 nm against acetone blank. The difference between the control and treatment was calculated as the chlorophyll stability index (Kaloyereas, 1958) [13].

## Lipid Peroxidation

Fresh leaf material (0.5g) was ground with 10 ml of distilled water, filtered through 4 layers of muslin cloth and centrifuged at 7000 rpm for 10 minutes. The supernatant was collected and an aliquot of 2 ml was taken to which 5 ml of 0.5 % (w/v) thiobarbituric acid (TBA) in 20% (w/v) trichloroacetic acid (TCA) was added and the mixture was heated at $100^{o}$C for 30 minutes. The samples were cooled and the absorbance was read at 532 nm and 600.nm using a UV spectrophotometer (Shimadzu Corporation, Japan). The manlondialdehyde (MDA) was quantified by utilizing the extinction coefficient of 155mM$^{-1}$ cm$^{-2}$ (Heath and Packer, 1968) [10] and expressed in nmol g f wt$^{-1}$.

## Result and Discussion

Tomato (*Lycopersicum esculentum* L.) is one of the most important vegetable plants in the world. Drought is one of the major abiotic stresses which adversely affect crop growth and yield. Drought stress results when the plant’s water content is reduced enough to interfere with normal plant process and when water loss from the plant exceeds there are many morphological and physiological effects of drought which leads to lowered metabolic and structural capacity.

### Morphological Attributes

### Shoot Length

The symptoms in plants during drought stress are one of the major issue when related to the plant height or shoot length. The shoot length gets invariably reduced under drought stress in the tomato cultivars. In further, a rapid change is noted in four different varieties of tomato under drought stress. The shoot length was reduced uniformly under water stress (14.96: ML, 17.96: AR, 14.03: MR, 12.21: MP). The variety MP recorded a higher reduction (12.21) in the shoot length under water stress, while variety AR recorded a least reduction (17.13). Variety MP maintained a high Shoot length than others under stress (Table 1 & Figure 1).The decrease in shoot length may be either due to decrease in cell elongation The reduction of shoot length may protect the loss of water by mechanisms of migirate drought stress. Ehsan *et al.* (2017) [7] outlined the reduction in the plant height during the investigation on the Physiological and Morphological Responses of Almond Cultivars under *In Vitro* Drought Stress. Kohler *et al*., (1982) also observed that stalk elongation as expressed by plant height in drought stressed plants was less than 80% of the plants in well – watered plots. In the case of sugarcane, the reduction in shoot height indicated the reduction in final size. The cell expansion rather than the cell division appeared to be sensitive to water stress, which might be the cause for reduction in plant height (Venkataramana and Ramanujam, 1999) [28].

**Table 1:** Changes in Shoot Length (cm) in four cultivars of tomato during stress

| Genotype | Control | Treatment |
|---|---|---|
| ML | 19.40 ± 1.03 | 14.96 ± 0.54 |
| AR | 18.87 ± 0.47 | 17.13 ± 0.50 |
| MR | 24.79 ± 1.37 | 14.03 ± 0.39 |
| MP | 30.96 ± 1.21 | 12.21 ± 0.19 |
| SEd | 0.65424 | |
| CD (p<0.05) | 1.38694 | |

Values are mean ± SD of three samples

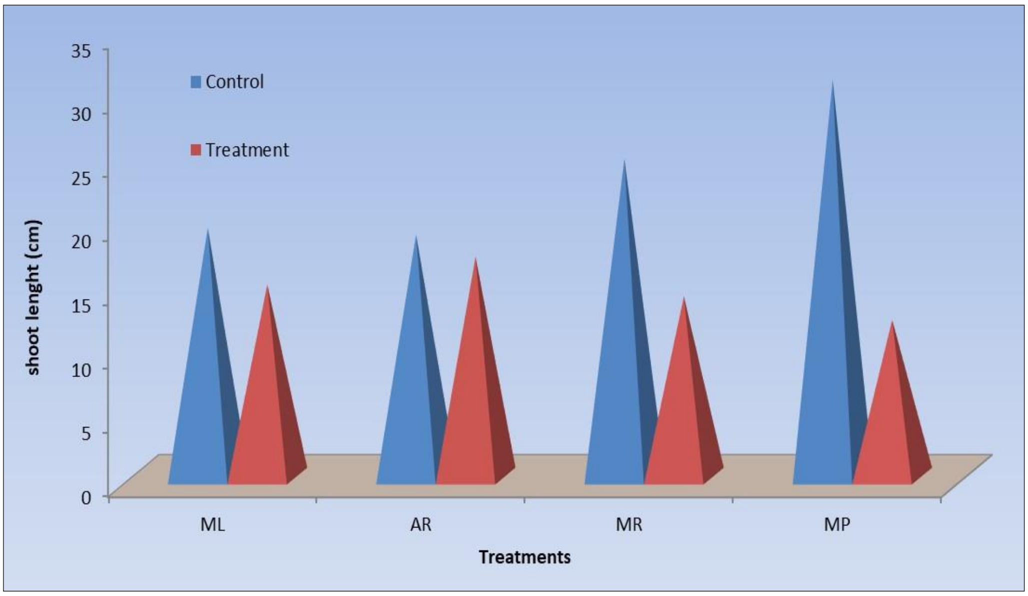

**Fig 1:** Changes in Shoot Length (cm) in four cultivars of tomato during stress

### Root length

In plants, drought stress leads to a rapid decrease or increase in the root length. Comparatively, the root length becomes less affected under drought stress as compared with its shoot length. The root length gets invariably reduced under drought stress in the tomato cultivars. In further, a rapid change is noted in four different varieties of tomato under drought stress. In control plants, the root length ranges from 4.41cm (ML) to 14.55cm (MP). Roots penetrated deep in response to stress and a typical rope-type system and a positive association between shoot-root ratio and growth and yield were also demonstrated (Venkataramana and Naidu, 1989) Which was not noticed in this study.

**Table 2:** Changes in Root Length (cm) in four cultivars of tomato during stress

| Genotype | Control | Treatment |
|---|---|---|
| ML | 4.41 ± 0.47 | 3.74 ± 0.20 |
| AR | 12.62 ± 0.32 | 8.67 ± 0.29 |
| MR | 7.92 ± 0.36 | 6.74 ± 0.35 |
| MP | 14.55 ± 0.15 | 6.44 ± 0.05 |
| vSEd | 0.24528 | |
| CD (p<0.05) | 0.51997 | |

Values are mean ± SD of three samples

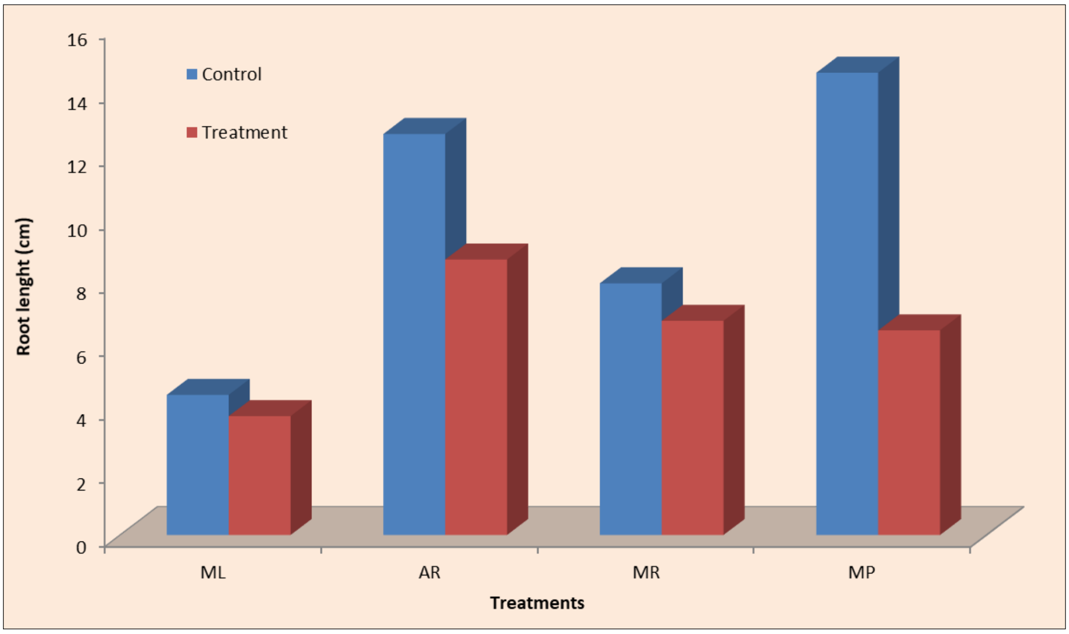

**Fig 2:** Changes in Root Length (cm) in four cultivars of tomato during stress

**Leaf Number**

The number of leaves gets reduced under drought stress. Comparatively, the leaf number becomes more affected under drought stress. The number of leaves per pot gets invariably reduced under drought stress in the four tomato cultivars. In further, a rapid change is noted in four different varieties of tomato under drought stress. In control plants, the leaf number ranges from 202.67(ML) to 385.00 (MP) per plot. The variety MP recorded a higher reduction (78.33) in the leaf number under water stress, while variety MR recorded a least reduction (99.33). Variety MP maintained a high Shoot length than others (Table 3 & Figure 3).Similar records of reduced leaf number under drought stress have been noted in sugar cane (Vasantha *et al*., 2005) [26]

**Table 3**: Changes in Leaf Number in four cultivars per plot of tomato during stress

| Genotype | Control | Treatment |
|---|---|---|
| ML | 202.67 ± 14.19 | 86.33 ± 3.21 |
| AR | 373.33 ± 49.17 | 79.67 ± 17.62 |
| MR | 385.00 ± 5.00 | 99.33 ± 21.57 |
| MP | 331.67 ± 74.22 | 78.33 ± 36.86 |
| SEd | 29.29211 | |
| CD (p<0.05) | 62.09734 | |

Values are mean ± SD of three samples

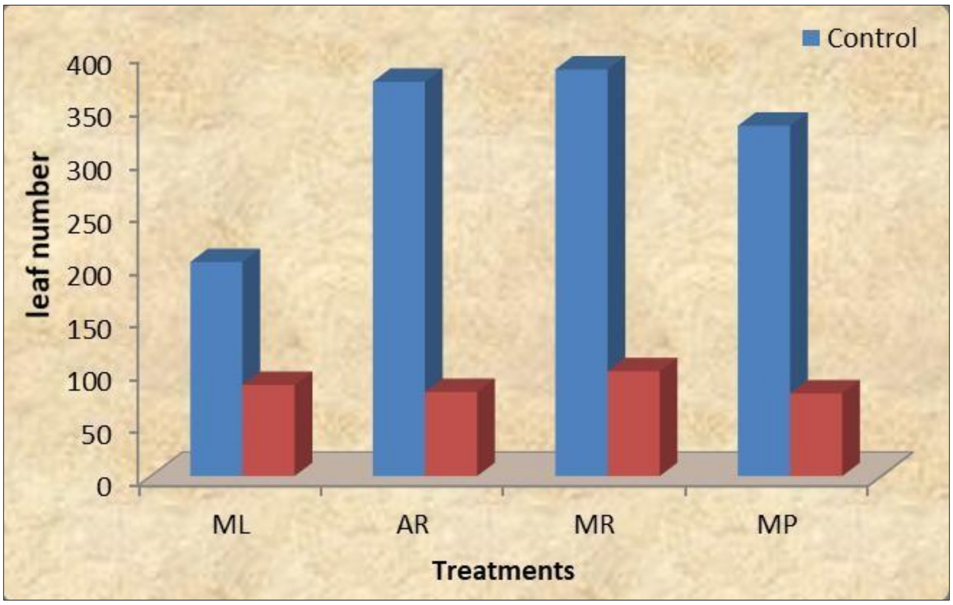

**Fig 3:** Changes in Leaf Number per plot in four cultivars of tomato during stress

### Leaf Area

Leaf area is an important variable for most eco physiological studies in terrestrial ecosystems concerning light interception, evapotranspiration, photosynthetic efficiency, fertilizers and irrigation response and plant growth (Zhan *et al.*, 2014) [29]. Among drought treated varieties, ML (15.17cm$^2$), AR(12.83cm$^2$), MR(13.20cm$^2$), MP(14.90cm$^2$) showed reduced leaf area compared to the control. The controlled plant varieties ranges from 26.30cm$^2$(MP) to 28.13cm$^2$(ML), while the stressed ones were ranging from 12.83cm$^2$(AR) to 15.17cm$^2$(ML). Therefore, drought stressed plants will often have small leaves as compared to the controlled cultivars of tomato (Table 4 & Figure 4).Plant water stress generally hastens leaf senescence (Asana, 1960) [5] and thus determines the effective leaf area. Similar reports of reduced leaf area was observed in Maize plant under drought. Plant water status caused the differences in elongation characteristics and was responsible for the differences in leaf size. Reduction in leaf area has been suggested to represent an important drought avoidance mechanism, which results in less water use or transpiration resulting from low leaf area (Turk and Hall, 1980) [25].

**Table 4:** Changes in Leaf Area (cm$^2$) in four cultivars of tomato during stress

| Genotype | Control | Treatment |
|---|---|---|
| ML | 28.13 ± 1.58 | 15.17 ± 0.25 |
| AR | 26.83 ± 1.45 | 12.83 ± 0.35 |
| MR | 26.63 ± 2.51 | 13.20 ± 0.30 |
| MP | 26.30 ± 0.95 | 14.90 ± 0.26 |
| vSEd | 1.00775 | |
| CD (p<0.05) | 2.13636 | |

Values are mean ± SD of three samples

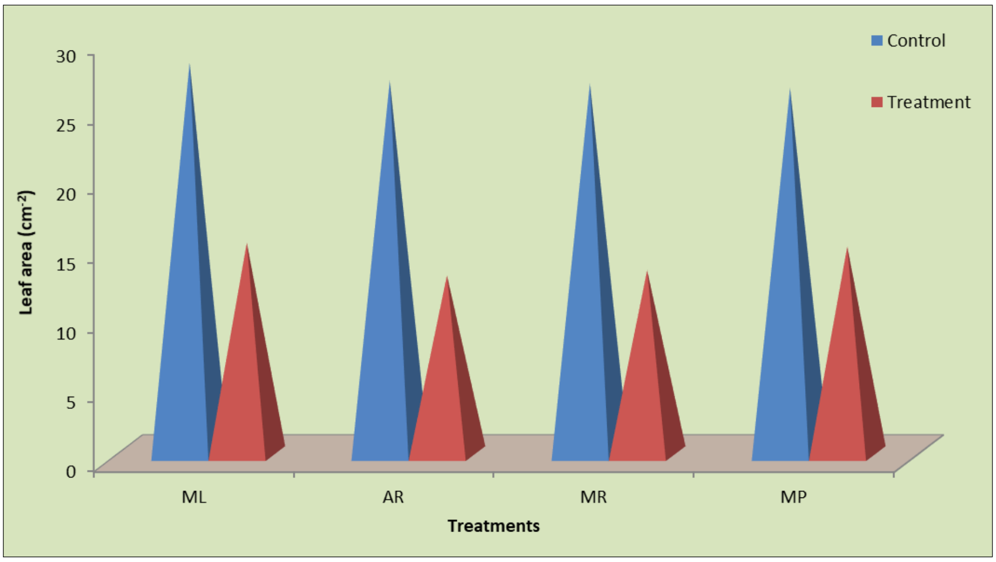

**Fig 4:** Changes in Leaf Area (cm$^2$) in four cultivars of tomato during stress

### Relative Water Content (RLWC)

One of the early symptoms of drought stress is the decrease of RWC which is considered to be the best integrated measure of plant water status, representing variation in the water potential, turgor potential, and osmotic adjustment in plant in plant tissues (Rampino *et al.*, 2006; Sanchez – Rodriguez *et al.*, 2010) [19]. The relative water content was reduced uniformly under drought in all four varieties. Variety AR recorded a higher reduction in RWC under water stress, while variety Co 06030 recorded a least reduction. Variety ML maintained a high RWC than others (Table 5 & Figure 5).Decrease in relative water content (RWC) was a main factor resulting in reduced growth in response to osmotic stress in pea (Alexieva *et al.*, 2001) [2]. Similar results showing reduced RWC in leaves of sugarcane under water stress by Ngamhui *et al.*, (2012) [18]. Rao. (2013) showed relative water content (RWC) significant decrease under black gram unirrigated condition and in (*Arachis hypogaea* L.) there was a significant reduction in REC with increase in the duration of water stress. The RWC indirectly relates to the tolerance level of any crop, when varieties which showed minimum reduction in RWC showed reduced pigments damage and increased levels of osmolytes which finally lead to tolerance and give maximum yield under drought.

**Table 5:** Changes in Relative Water Content (percent) in four cultivars of tomato during stress

| Genotype | Control | Treatment |
|---|---|---|
| ML | 66.19 ± 2.62 | 56.19 ± 0.62 |
| AR | 56.89 ± 0.53 | 23.14 ± 1.54 |

| | | |
|---|---|---|
| MR | 69.11 ± 0.99 | 43.30 ±0.58 |
| MP | 46.81 ± 1.11 | 43.69 ± 1.28 |
| vSEd | 1.08356 | |
| CD (p<0.05) | 2.29708 | |

Values are mean ± SD of three samples

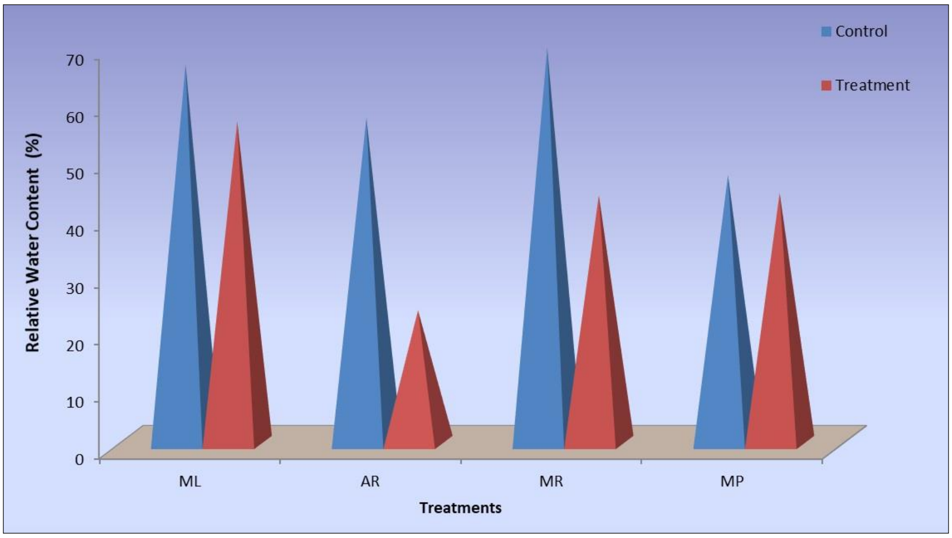

**Fig 5:** Changes in Relative Water Content (percent) in four cultivars of tomato during stress

### Chlorophyll Stability Index (CSI)

Chlorophyll stability index is a measure of integrity of membrane or heat stability of the pigments under stressed conditions (Kaloyereas, 1958) [13]. The high chlorophyll stability indices help the plants to withstand stress through better availability of chlorophyll. Chlorophyll Stability Index varied from 0.28 (MR) to 0.75 µg g dr/ (ML)in control plants, while in stressed plants it varied from 0.61µg g dr/wt (MR) to 0.76µg g dr/wt (ML).In responses to stress varieties ML (0.76)and AR(0.0.72) showed an increase in Chlorophyll Stability. (Table 6 & Figure 6).

**Table 6:** Changes in Chlorophyll Stability Index (percent) in four cultivars of tomato during stress

| Genotype | Control | Treatment |
|---|---|---|
| ML | 0.76 ± 0.02 | 0.75 ± 0.03 |
| AR | 0.72 ± 0.03 | 0.68 ± 0.06 |
| MR | 0.61 ± 0.02 | 0.28 ± 0.03 |
| MP | 0.68 ± 0.05 | 0.52 ± 0.02 |
| vSEd | 0.02918 | |
| CD (p<0.05) | 0.06186 | |

Values are mean ± SD of three samples

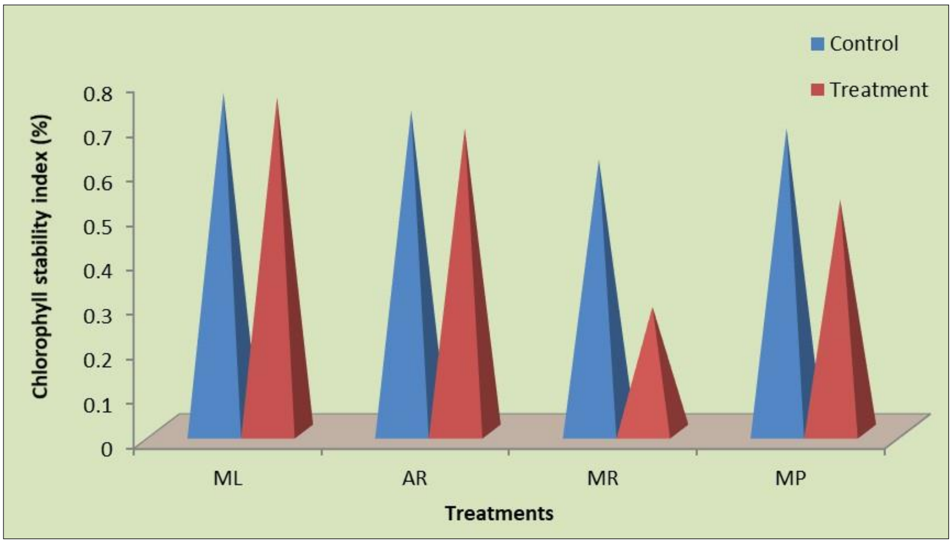

**Fig 6:** Changes in Chlorophyll Stability Index (percent) in four cultivars of tomato during stress

### Membrane Stability Index (MSI) and Lipid Peroxidation

After having imposed drought stress for a period of 10 days, the MSI of leaf tissues varied from 170.56(AR) to278.91%(ML).All the varieties showed an increase level of MDI content under contol, while it get reduced under stress. The variety ML accumulated maximum MDI of 222.79 while AR recorded minimum of 170.48%. An overall of 75% increase in MDI was noted. (Table 7 & Figure 7). Increased electrolyte leakage due to damaged cell membranes disrupts signaling processes and can lead to cellular dehydration and death (Nilsin and Orcutt, 1996). The membrane injury and content of lipid peroxidation products increased in prolonged water stress (Sairam *et al*., 1998) [23]. Sairam *et al*. (2002) [23] reported that genotypes that are sensitive to salinity show a significant reduction in their cell membrane stability index. The plant cells which are resistant against osmotic changes during drought stress have more cell membrane stability. (Saneoka *et al.*, 2004).Cell membrane stability (CMI) technique was used to screen stress tolerant and stress sensitive of many crops (Farooq and Azam, 2006) and in some cases higher membrane stability could be correlated with better field performance (Table 7 & Figure 7).The total MDA content increased with increased stress and it was found to be associated with membrane injury which possibly resulted in lesser affected metabolic activities (Kushwaha *et al*., 2003).

**Table 7:** Changes in Membrane Stability Index (percent) in four cultivars of tomato during stress

| Genotype | Control | Treatment |
|---|---|---|
| ML | 27.891 ± 51.84 | 22.279 ± 1.36 |
| AR | 17.056 ± 1.16 | 17.048 ± 0.60 |
| MR | 21.530 ± 4.43 | 19.185 ± 2.93 |
| MP | 24.812 ± 5.19 | 19.379 ± 1.81 |
| vSEd | 1.513678 | |
| CD (p<0.05) | 3.208898 | |

Values are mean ± SD of three samples

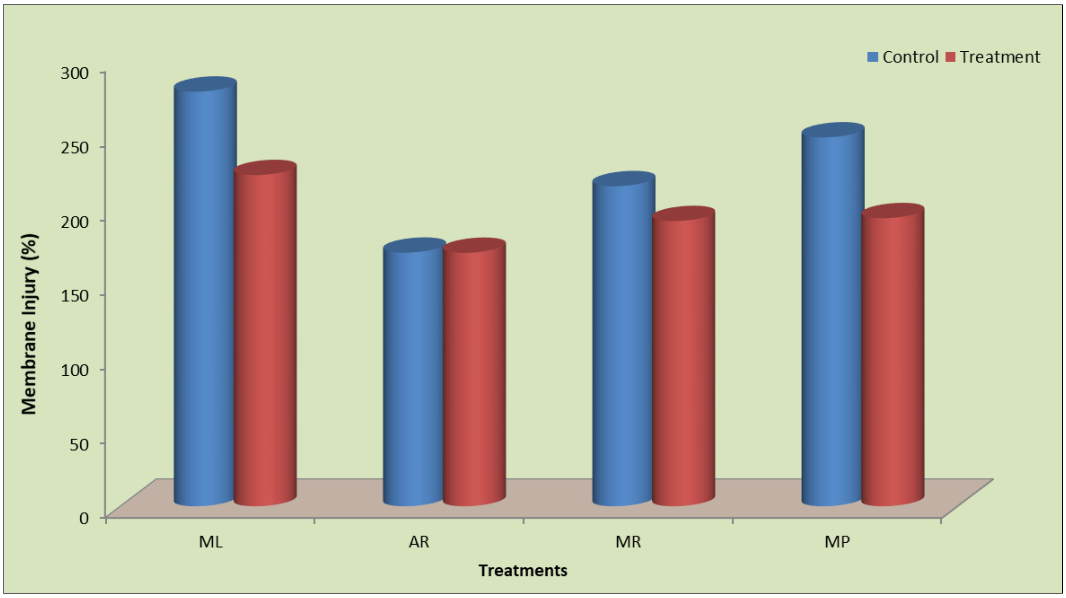

**Fig 7:** Changes in Membrane Stability Index (percent) in four cultivars of tomato during stress

### Lipid Peroxidation

The increased abiotic stress in tomato cultivars increase the MDA content under stressed varieties. The stressed cultivars invariably leads to an increase in the MDA content as compared to the normal plants. MDA content varied from 0.21nmol/g frwt (ML) to 0.45 nmol/g frwt (AR)in control plants, while in stressed plants it varied from 0.46 (MR) to 0.64 (AR).The average increase in MDA content is two – fold under stress. (Table 8 and Figure 8).The membrane injury and content of lipid peroxidation products increased in prolonged water stress (Sairam *et al*., 1998). Low MDA content was associated with water stress resistance MDA has been considered as an indicator of oxidative damage. Malondialdehyde is a final product of lipid peroxidation (Ge *et al.*, 2006).Water stress induces the overproduction of ROS and consequently increases the lipid peroxidation membranes measured as MDA content, which is the final product of lipid peroxidation and it is a well – known marker of oxidative damage (Moller *et al*., 2007). It is commonly considered as one of the best physiological components of drought tolerance in plants (Xu *et al*., 2008). Reduction in the peroxidation level have been noted by Mehri *et al*., (2009) [17] during the investigation on the effects of brassinosteroids on the induction of biochemical changes in *Lycopersicon esculentum* under drought stress.

**Table 8:** Changes in Lipid Peroxidation (n mol g/fw) in four cultivars of tomato during stress

| Genotype | Control | Treatment |
|---|---|---|
| ML | 0.21 ± 0.03 | 0.50 ± 0.02 |
| AR | 0.45 ± 0.07 | 0.64 ± 0.05 |
| MR | 0.28 ± 0.03 | 0.46 ± 0.12 |
| MP | 0.33 ± 0.06 | 0.62 ± 0.06 |
| SEd | 0.04787 | |
| CD (p<0.05) | 0.10148 | |

Values are mean ± SD of three samples

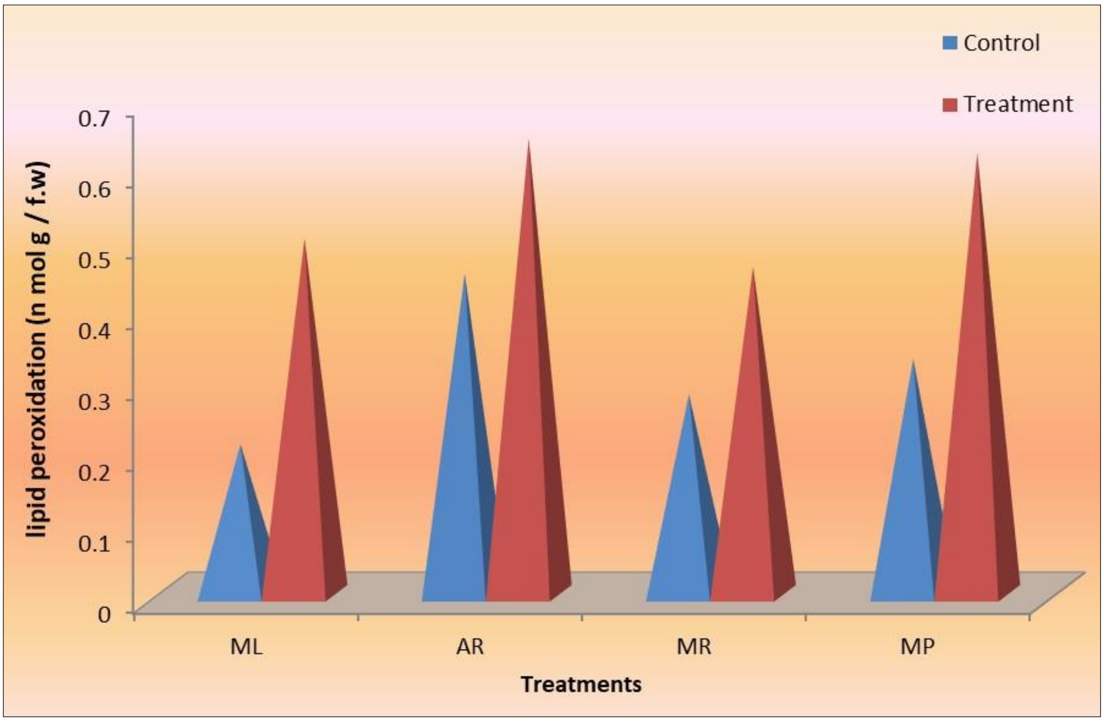

**Fig 8:** Changes in Lipid Peroxidation (n mol g/fw) in four cultivars of tomato during stress

## Conclusion

A pot experiment was conducted utilizing four tomato varieties (ML, AR, MR, MP) with an objective of assessing the influence of water stress on various morphological, physiological and biochemical parameters. The present study enlightened the drought induced changes at morphological and physiological levels and elucidates the growth patterns in concern to adaptive mechanism towards drought stress. The study will help the research on stress tolerance to evaluate the difference between the cultivars which can be important criteria for breeders towards production of efficient crop to cope with the current climatic global scenario. In this study attempt in being made to add to the knowledge on the response of Tomato under stress which in turn will be of prime importance, while engineering crops for abiotic stress tolerance.

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