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PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF POLYETHYLENE GLYCOL (PEG) INDUCED DROUGHT STRESS CONDITION IN FOUR VARIETIES OF INDIAN TOMATOES(*LYCOPERSICON ESCULENTUM*).

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ABSTRACT

The present work deals with the study of Poly ethylene glycol (6000) induced drought stress condition and analysis of its physio-biochemical effects in 4 genotypes of Indian tomato (*Lycopersicon esculentum*).The genotypes, Deep-953, cultivar 327, 326and Aditya-955 were treated with 15% poly ethylene glycol. The parameters like rate of germination, relative water content, leaf area, days of maturity, osmolite content and content of photosynthetic pigments were analysed. The significant reduction in seed germination was as low as 60% in Deep-953, 40% in cultivar 327, 10% in cultivar 326 and 40% inAditya-955.The reduced leaf area, tissue water content and change in physiological characteristics in stress sensitive plants Deep-953, cultivar 327 and Aditya-955.Analysis of variance showed significant difference ($P<0.05$) in the mean proline content in tomato varieties subjected to stress. Result indicates treatment dependent variations in proline content. However, the tomato cultivar 326 was able to express best tolerance to induced drought.

KEYWORDS: Tomato varieties; Poly ethylene glycol (6000); proline; physiological parameters; biochemical parameters.

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INTRODUCTION

Drought is the effect of major water deficit due to lack of rain over the extended period of time affected various human activities and lead to problems like widespread crop failure, replenished ground water resources, depletion in reservoirs, scarcity of drinking water and, reduced fodder availability. Agriculture is the immediate victim of drought disaster – impacting crop area and crop production.Water deficits may develop into an agricultural drought, where plant water stress reduces yield and biomass.Drought adversely affect the field area leaving large portions of agricultural lands as barren. Mid-season droughts result in feeble crop growth and shortage in yields. 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 abiotic stresses is a major goal of plant breeders and researchers all over the world. Under water deficit condition the crops are exposed to a combination of stresses including a large number of climatic factors. Drought and heat stress are linked together and this condition is caused by high temperature, water shortage, excessive irradiance or low water potential. Low grade soil conditions also lead to stress, especially if the soil is resistant to the root penetration. 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 [21]. A positive correlation between photosynthesis rate and crop yield is commonly found. Alteration of growth patterns in plants contributes to survival under water depletion conditions [20]. A raise in root to shoot ratio is found commonly in physiological studies on the effects of drought condition on plants. Growth suspension can be considered as a medium by which plants can preserve carbohydrates for sustained metabolism, prolong energy supply and recovery faster after stress relief [18.] On the other hand, continuation of root growth increases the exploratory capacity of plants in deeper more humid soil layers [7]. Polyethylene glycol, a non-penetrable and non-toxic osmotic, lowers the water potential of the medium and has been used to simulate drought stress.PEG-6000 based in-vitro screening of drought tolerant germplasm is proven to be a significant method with good accuracy. Exposure to PEG-6000 has been effectively mimic drought with various metabolic interference. The role of induced drought by PEG is seen in germination stage to maturity of the test plants compared with the control. Tomato is one of the widely grown vegetables .It is an important vegetable crop of Asia. Declined water contents tend to reduce leaf area in tomato genotypes [6] which in turn results in reduced shoot lengths [22, 2]. Lack of tolerance to high temperature in most tomato cultivars presents a major limitation for growing in regions where the temperature reaches to 38 ° C during the growing season [7, 16]. Essential to carry out research to understand the physio-biochemical responses of tomato inbred lines to water stress at their early and late growth stage [21]. Therefore, in the present study, the effect of water stress condition was investigated using PEG 6000 on growth parameters in four tomato inbred lines from their germination stage to maturity.

MATERIALS AND METHODS

The experimental material varieties, Deep-953, cultivar 327, cultivar 326 and Aditya-955 were procured from Namdari seeds Pvt. Ltd, Pune and the experiment was conducted in the Department of Botany, Institute of science, Mumbai.The seeds of selected varieties were treated with polyethylene glycol (PEG-6000) for imposing drought condition. Ten (10) seeds of each variety were placed on the moist whatman germination papers in Petri dishes and 5ml of 15% PEG solution was applied on each day up to seven days along with control [22.]For control the sprinkling of distilled water was continued to maintain moisture in the petri plate. First counting of germinated seeds after 48 hours of incubation period in their respective petri dishes then continue every day at the same time for 5 days. After 7 days of treatment, the percentage of germination and morphology of the root was noted down from each replica of control and PEG treated seeds of selected varieties of Indian tomatos.The germinated seeds were transferred to green house for overall physio-biochemical study. The crop

was maintained in the green house condition using conventional agronomic practices to keep crop in healthy condition and data was recorded.

Physiological evaluation of Tomato varieties

The physiological parameters were noted down in the selected varieties Deep-953, cultivar 327, cultivar 326 and Aditya-955 under stressed and controlled conditions. The data recorded for characters like percentage of germination, leaf area, days of maturity, relative water content and chlorophyll estimation (Table 1 & 2).

Percentage of germination

Germination rate was calculated by the formula followed by Li., (2008) [14]

GR=A/BX100

Leaf Area

The following equation was used to calculate the leaf area non-destructively using the formula, Leaf area (cm^2) = x/y, where x is the weight of the graph paper covered by the leaf outline (g) and y is the weight (g), of the cm^2 area of the graph paper. In addition, areas of ten leaves each from five species were measured using the leaf area meter while still attached to the plants.

Days to maturity

This was done by counting the days from planting date to flowering date.

Relative water content *(RWC)*

RWC of the flag leaf was measured following the method of Turner, (1981) [23].Relative water content of a plant tissue is expressed by RWC (%) = [(FM - DM)/(TM - DM)] * 100, where, FM, DM, and TM are the fresh, dry and turgid masses, respectively, of the tissue. Outline of these attached leaf samples were also drawn on the millimetre graph paper.

Chlorophyll estimation

Chlorophyll was estimated using the protocol of Holden (1960) [4]. Each of the plant samples were weighed 100 mg and homogenized separately in a mortar in the presence of excess of 80% acetone until all the color was released from the tissue. CaCO3 was added to prevent pheophytin formation and this was then centrifuged at 5000 rpm for 10 minutes at room temperature. The clear supernatant was collected and then made up to a known volume (10ml). The test tubes were wrapped with black paper to prevent chlorophyll degradation. UV visible spectrophotometer was adjusted in 663 nm for chlorophyll 'a' and 645 nm for chlorophyll 'b' set at 100% transmittance using 80% acetone as blank before taking the readings of the sample respectively.

The optical density was measured and the chlorophyll contents in the original extract was estimated using the formula

$$\text{Total Chlorophyll (mg/L)} = 20.20A645 + 08.02\ A663 \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll 'a' (mg/L)} = 12.70A663 - 02.69\ A645 \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll 'b' (mg/L)} = 22.90A645 - 04.68\ A663 \times \frac{V}{1000 \times W}$$

Total Chlorophyll = a + b

Biochemical evaluation of tomato varieties

Proline

Proline was determined calorimetrically following Bates *et al* (1973) [3] based on proline's reaction with ninhydrin. The fresh leaves (0.5 g) were hand-homogenised in 5ml of 3% (w/v) sulfo-salicylic acid. The homogenate was filtered through Whatman No. 2 filter paper. In another test tube, 2 ml of acid ninhydrin and 2 ml of glacial acetic acid was added to 2 ml of the filtrated extract. The resulting mixture was incubated at 100° C in a hot water bath. The reaction was then stopped using an ice bath, and the contents was extracted with 4 ml of toluene and mixed vigorously using a test tube stirrer for 15-20 seconds. The chromophore

containing toluene was aspirated from the aqueous phase and thawed to room temperature, and the absorbance of the solution was measured at 520 nm using a UV-visible spectrophotometer. The proline concentration was determined from a standard curve prepared with L-proline. Proline content was expressed as µg−1 FW (Fresh weight).

The amount of free proline was calculated by following formula.

$$\text{Proline (µmoles/g fresh weight)} = \frac{(\text{µg proline/ml} \times \text{ml toluene})}{[115.5\ \text{µg}/\mu\text{mole}]/\ [(\text{g sample}/5]}$$

Statistical Analysis

Analysis of variance (ANOVA) was carried out to test for significant differences in the mean proline content in selected tomato varieties exposed to PEG induced stress.

RESULTS AND DISCUSSION

Physiological characteristics of tomato varieties under controlled and treatment condition

Polyethylene glycol (PEG) is known to create water stress in the plants and act as asimulant. In this study the PEG-6000 was used for creating osmotic stress in plants .

Percentage of germination

The observation regarding the germination percentage of selected varieties namely, Deep-953, cultivar 327, cultivar326 and Aditya- 955 under stress condition induced by PEG (6000) and control germination .The value of germination was same recorded in all tomato varieties under control treatment. While in water stress treatment the variety 326 gave maximum germination and tolerance (Table-1, Fig-1a). Essential parameter to access the survival success of crops to the induced stress. Low moisture acts as a limiting factor during germination [2]. It is essential to understand the germination of seeds under drought condition. Seed germination undergoes considerably varies under low moisture environment. Germination in polyethylene glycol measures survival or growth of seedlings which is influenced by stress, have been suggested for drought screening.

Leaf Area

Among drought treated varieties, Deep-953, cultivar 327, cultivar 326 and Aditya- 955, the cultivar 326 showed reduced leaf area compared to the control (Table-1, Fig-1b). Plants have a number of ways to reduce the water loss that occurs through transpiration. One is to simply reduce leaf area. When water stress is an issue, large leaf areas can be detrimental to growth and survival because there is more surface area from which water can be lost. Therefore, drought-tolerant plants will often have small leaves. Leaf expansion in several species has been shown to be sensitive to water stress [4] [13]. 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 [25].One way in which stress can enhance plant performance is by lowering the leaf area and growth rate of plants, thereby decreasing the rate at which soil water content is depleted and thus enhancing the longevity of the plants [17].

Days for maturity

The maturity period of studied varieties was not uniform. However among other studied varieties, it is notable feature of variant 326 to escape drought condition (Table -1, Fig-1c). Plants have a definite temperature requirement before they attain certain phonological stages. The accumulative heat units and system was adopted for determining the dates to flowering/heading and maturity of different field crops [12]. However, different phonological stages differ in their sensitivity to drought and high

temperature stress, and this depends on plant species and genotype as there are great inter and intraspecific variations [8].Stress indicators, such as stay green leaves or after physiological maturity, have been proposed as a way to identify genotypes with better stress tolerance like late-season drought and heat stress [9].

Table 1
Physiochemical characteristics of control and PEG treated varieties.

S.No	Genotypes	Rate of germination (%)		Leaf are(cm^2)		Days of maturity	
		C	T	C	T	C	T
1	Aditya-953	100	40	26.2	52.0	60	65
2	Cultivar-327	100	60	25.4	23.5	61	68
3	Cultivar-326	100	90	20.4	22.1	63	62
4	Deep-955	100	60	40.0	28.0	62	60

C=Control, T-Treated

Relative water content

The higher RWC was estimated after PEG treatment in Deep-953 and cultivar 326 and thus can be considered as drought tolerant .The lowest RWC estimated after PEG treatment was obtained in cultivar 327 and Aditya-955 and can be considered as drought sensitive (Table-2,Fig-1e). Water stress levels significantly affected on relative water content (RWC).The relative water content indicates the water condition of the cells and exhibits important correlation with biotic and abiotic stress tolerance. The relative water content was estimated under controlled condition and PEG treatment. Our observation reveals significant differences in RWC among four selected varieties of tomatoes [2].The variation may be due to the ability of tomato in the ability of tomato varieties to avoid stress by maintained turgor pressure osmotically[1].RWC may be used as selection criterion in breeding for improved drought resistance character [19].

Chlorophyll estimation

It was observed that, the total chlorophyll content, chlorophyll *a* and *b* were found minimum in the PEG treated varieties and maximum in controlled condition. The lowest Chlorophyll was recorded after PEG treatment as observed in the cultivar 326(4.804µg) and Deep-955(5.347µg), as compared with respective control. (Table-3, Fig-1d). Osmotic stress generated by polyethylene glycol (PEG-6000) generally reduces the rate of photosynthetic. Exposure to the drought stress leads significant changes in Chlorophyll-*a* and Chlorophyll-*b* contents (Table-2). The drought stress condition produce relative oxygen species such as O_2 and H_2O_2, which contribute to the formation of lipid peroxidase and consequently changes green colours of the leafs into yellow [4].

Table 2
Changes in chlorophyll a and chlorophyll b concentration in the control and PEG treated varieties

Chlorophyll (mg/g1)FW	C1	C2	C3	C4	T1	T2	T3	T4
a	2.43	1.39	2.88	4.60	2.07	2.71	1.99	2.15
b	3.69	2.12	3.86	5.66	3.03	3.37	2.83	3.18

C=Control, T=Test

Proline estimation

The high proline content was recorded in the leaf of tomato cultivar 326 i.e. 1.18µg/ml compared with the proline content in the control, it is quite obvious as proline is known to be produced in higher amount under stress condition and it help in resisting the plants against stress (Table-3, Fig-1f).

Proline is the major organic osmolyte that accumulate in the plants in response to stress [10]. The proline accumulated in response to water stress in plants is primarily localized in the cytosol [11] [12]. It is able to protect cells from damage by acting as both an osmotic agent and a radical scavenger. The high capacity of accumulation of proline under stress suffers smaller cell membrane injuries [17]. Major function of proline during osmotic stress was to protect the structure and function of protein by being excluded from protein hydration sphere as suggested by Low (1985) [15].Analysis of variance (ANOVA) showed that the mean proline content of selected tomato cultivars exposed to PEG induced stress was statistically significant ($P<0.05$), which was not of chance but due to the treatment given by PEG.

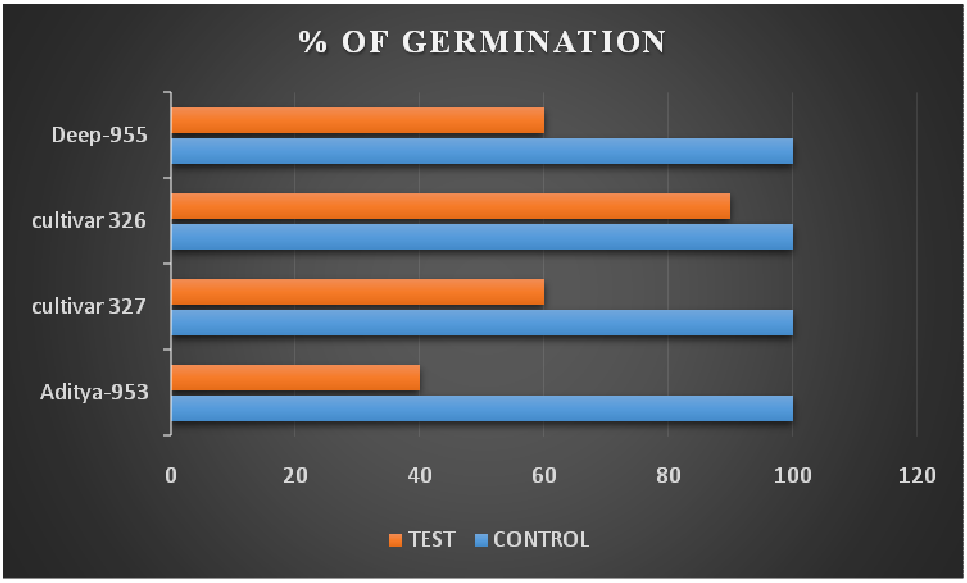
Table 3
Physio Biochemocal characteristics of control and PEG treated varieties.

S.No	Genotypes	Chlorophyll(µg)		RWC (%)		Proline(µg/ml)	
		C	T	C	T	C	T
1	Aditya-953	6.132	5.104	30.0	42.3	1.15	1.41
2	Cultivar-327	3.517	6.087	22.0	23.0	0.77	1.11
3	Cultivar-326	6.752	4.804	21.7	29.0	0.65	1.18
4	Deep-955	10.268	5.347	34.7	20.8	3.24	0.46

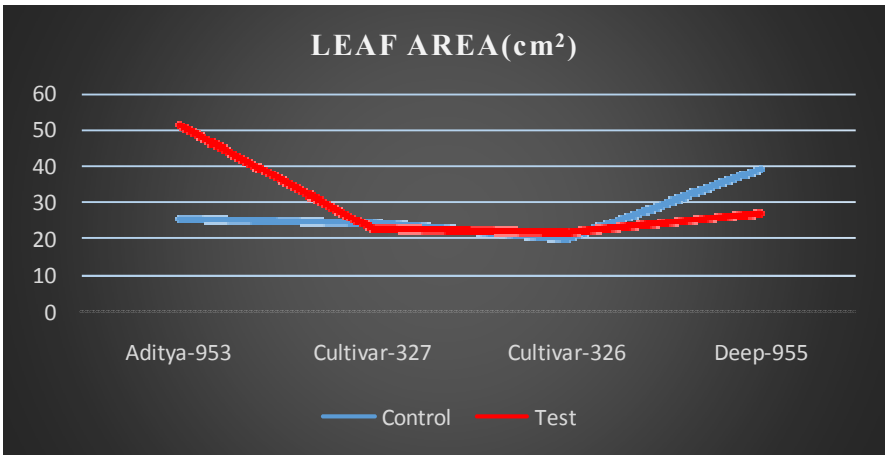
C=Control, T-Treated

Figure 1
Graphical representation of physio-biochemical characteristics, a) Percentage of germination b) Leaf areac) Days for maturity d) Chlorophyll content e) Relative water content f) Proline content, under control and PEG imposed stress condition.

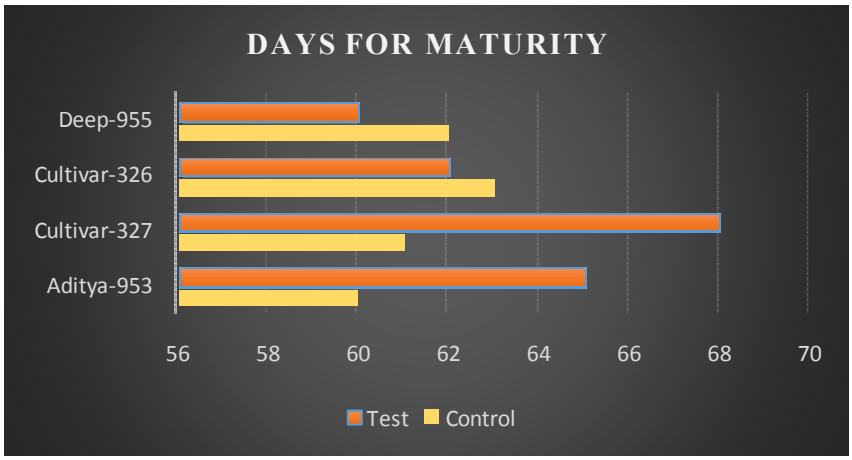
(a)
PERCENTAGE OF GERMINATION



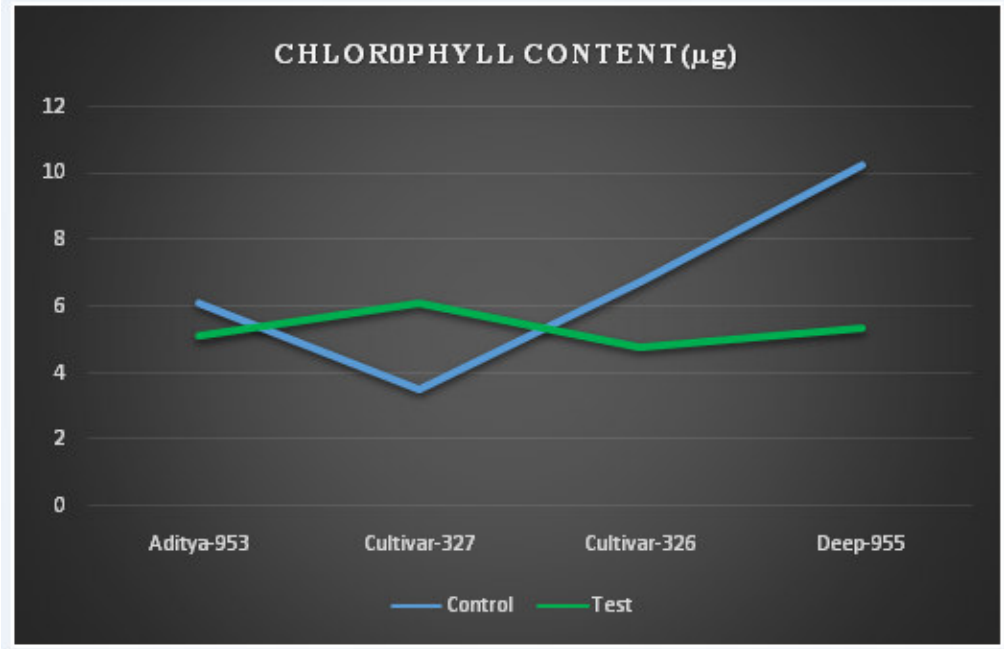
(b)
LEAF AREA



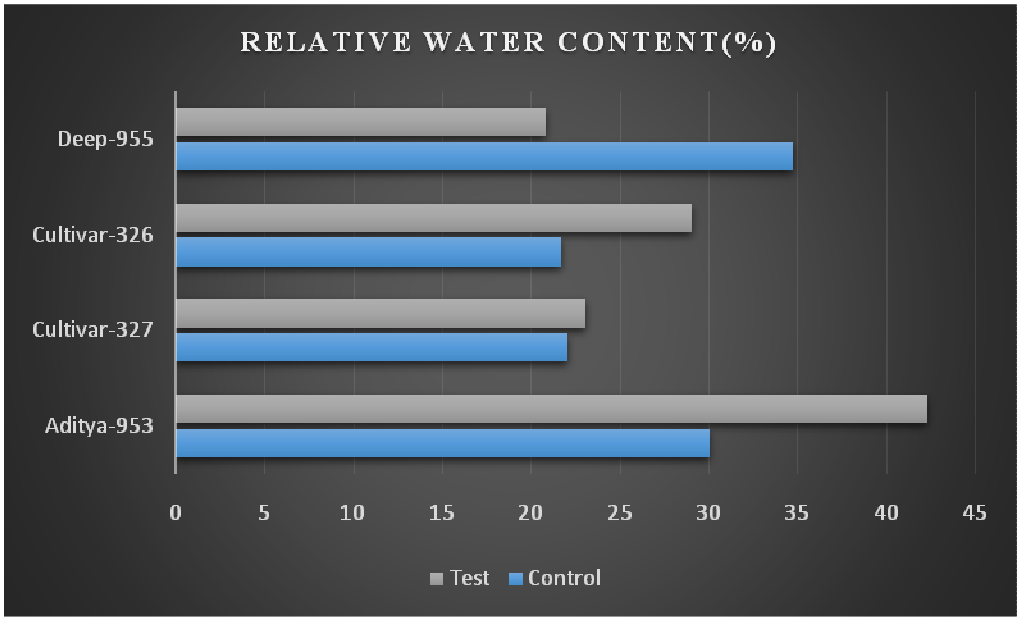
(c)
DAYS OF MATURITY



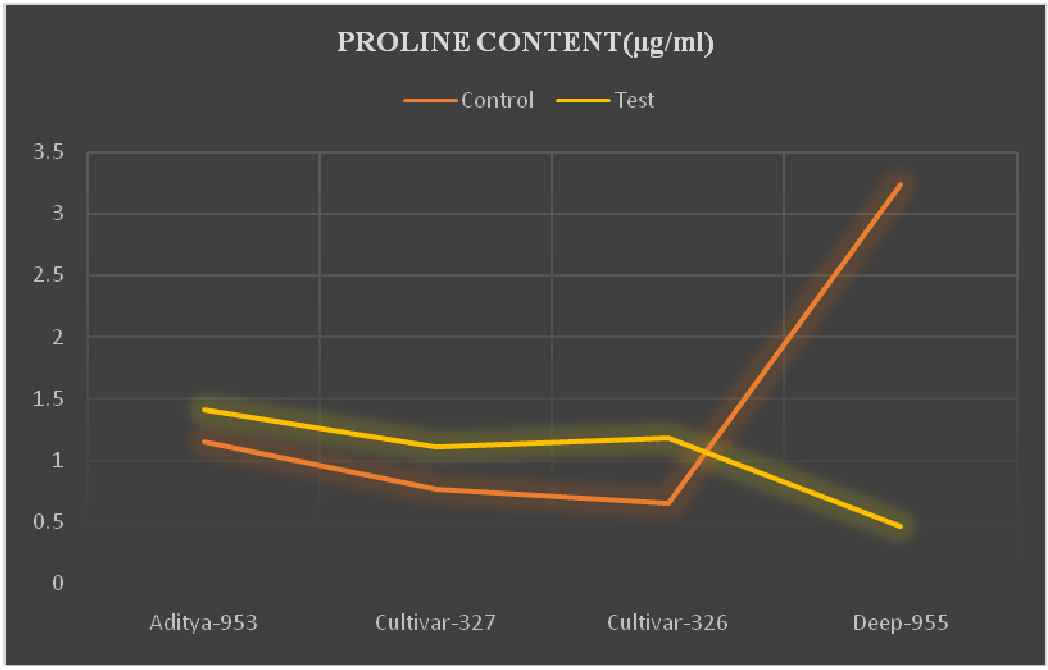
(d)
CHLOROPHYLL CONTENT



(e)
RELATIVE WATER CONTENT



(f)
PROLINE CONTENT



CONCLUSION AND RECOMMENDATION

Artificially imposed drought condition by PEG caused a substantial reduction in growth related attributes in Deep-953, cultivar 327 and Aditya-955. Over all the genotypes, cultivar326 shows least effect of PEG treatment in terms of percent of germination, leaf area, proline content and chlorophyll content, and can be considered as drought tolerant genotype. The genotypes which showed positive behaviour under drought conditions as compared with control may carry a kind of tolerance mechanism, which makes plants retaining a good turgor pressure and absolute water level even under stressed conditions.This study allowed us to recognize physiological characteristics that are associated with drought stress so as to screen out appropriate tomato genotype. Such genotype can be introduced in arid area to produce high yield in drought condition and further can be used in

breeding programs to produce a stress tolerant genotype.

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CONFLICT OF INTEREST

The authors declare that there was no conflict of interest.

ABBREVIATIONS

PEG-Polyethylene glycol, RWC-Relative water content, ANOVA (Analysis of variance)

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