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Research Article

# In Vitro Screening of Chilli (*Capsicum Annuum* L.) Cultivars for Drought Tolerance

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**Abstract**

Chilli (*capsicum annuum* L.) is an important vegetable crop and its area under production is limited by water scarcity. An effort was made to screen eight cultivars of *Capsicum annuum* through hydroponics under controlled conditions using polyethylene glycol (PEG) with five concentrations (0%, 5%, 10%, 15% and 20%). Important growth parameters like shoot length, root length, No. of leaves, No. of internodes, leaf area, shoot dry weight, root dry weight, root to shoot dry weight and proline were observed. Evaluated growth parameters resulted from PEG imposed drought conditions revealed the variability in drought tolerance among *Capsicum annuum* cultivars. Among eight cultivars Arka Lohit showed significant increase in proline accumulation and root to shoot dry weight. These results suggested that Arka Lohit may consider as drought tolerate cultivar.

**Keywords:** *Capsicum annuum*, Drought Stress, Poly ethylene glycol, Proline

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## Introduction

Chilli (*Capsicum annuum* L.), the green or the dried ripe fruits of pungent forms of Capsicum species, is one of the important members of the family Solanaceae. “This cultivated species has its unique place in the diet as a vegetable cum spice crop [1]”. “Chilli is the largest spice item exported from India it occupies first position in terms of value. During 2015-16, chilli exported 24.21 per cent by value of the total exports of spices from India [2]”. In global market, India has the highest share of 25%, followed by China with 24%. Though India has the substantial share in the world hectarage under chilli crop, the productivity (1.74 t/ha) is low when compared to the other hot pepper growing countries like Korea and Indonesia where it ranges from 2-3 t/ha. In India production of dried chillies is 1605000 MT from 760000 ha area and green chillies 678000 MT from 43000 ha area (NHB 2014-15). The main reason for low productivity is the majority of chilli cultivating area (~ 50%) is under rainfed conditions [3]. Drought is one of the major abiotic stresses which results in significant reduction in morphological traits such as plant height, plant spread and dry matter accumulation [3-6] affecting the physiological process, thereby causing considerable economic yield loss in peppers [7-12]. Genetic variability within a species is a valuable tool for screening and breeding for drought tolerance.

“Field experiments related to water stress have been difficult to handle due to significant environmental or drought interactions with other abiotic stresses [13]”. “An alternative approach is to induce water stress through polyethylene glycol (PEG) solutions for the screening of the germplasm [14-17]”. “Polyethylene glycol with the molecular mass of 6000 and above is non-ionic, water soluble polymer which is not expected to penetrate intact plant tissues. This solution interferes with the roots to absorb water due to the reduction of osmotic potential [18] and [19]”. This synthetically created water-stress environment is used to provide the opportunity in selecting superior genotypes. On the basis of these facts, the present attempt was made to categorize chilli germplasm against drought stress to select suitable cultivars for drought tolerance.

## Materials and Methods

This study was conducted in Growth and Development laboratory, Department of Horticulture, Sikkim University, Gangtok, India from 2015 to 2016. The Experimental material comprised of eight cultivars of *Capsicum annuum*, out of which, Five cultivars (LCA-334, LCA-353, G4, LCA-625, CA-960) were collected from Regional Agriculture Station, Lam farm, Guntur, Andhra Pradesh and two cultivars (Arka Lohit and Arka Mohini) from Indian Institute of Horticulture Research, Bangalore, India and one cultivar (Dallae Khursani) was collected from Sikkim. The seeds were sterilized with 70% ethanol for 1 min., followed by soaking in 0.1% $HgCl_2$ for 3 min. and thoroughly washed with sterile distilled water for three times. Seeds were germinated in perlite media by using protrays and seedlings were transferred at the age of 14 days into a hydroponic system where, trays were filled with modified Hoagland's nutrient solution containing different concentrations of PEG-6000 *viz.* 0%, 5%, 10% 15% and 20% for imposing drought conditions. The roots of seedlings were directly submerged in aerated growth solution and the shoots were supported to grow above the solution. Solution was changed once in every 7 days. Plants of control treatment were maintained in Hoagland's nutrient solution for same period of time and aerated throughout the duration of the experiment. Whole hydroponic culture system was maintained under optimum culture conditions at 16 hours photoperiod (70 μ mol $M^{-2}$ $s^{-1}$) at $28^{o}$ C temperature. After 30 days of treatment, measurements were recorded at five different stress levels for growth parameters like shoot length root length, No. of leaves, No. of internodes, leaf area, shoot dry weight, root dry weight and root to shoot dry weight. Shoot length was measured with help of meter scale and leaf area was measured using leaf area meter (model: 211, Systronics, India). For calculating fresh and dry weight, gravimetry was used. Fresh weight was measured immediately after removal from hydroponics and dry weight was recorded after plants were dried at 70°C for 72h in hot air oven. Proline was estimated spectrophotometrically following the method of [20]. The leaves weighing 250 mg were homogenized with 3 % sulphosalicyclic acid. The homogenate was centrifuged at 10,000 rpm for 10 minutes and supernatant collected. 2ml supernatant was reacted with 2 ml of freshly prepared ninhydrin (1.25 g of ninhydrin dissolved in a mixture of 30 ml glacial acetic acid and 20 ml of 6 molar orthophosphoric acid with warming and stirring) and 2 ml of glacial acetic acid in a test tube and then was kept in a boiling water bath at 1000C for 1 hour. The reaction was terminated in an ice bath and then shifted to room temperature. Thereafter, the reaction mixture was extracted with 4 ml of toluene, mixed vigorously with test tube stirrer for 15-20 seconds. The chromophore containing toluene was aspirated from aqueous phase and absorbance read at 520 nm using toluene as a blank. The proline concentration was determined from the calibration curve. The experiment was designed in factorial completely randomized design with two factors. The first factor was the cultivars and the second one was the external water stress treatments. Data were analysed with ANOVA, and means were separated by least significance difference (LSD) using $P < 0.05$.

## Results and discussion

Present study had revealed that all the observed growth parameters had shown highly significant variation between treatments as well as among cultivars (**Table 1**), (**Figure 10**). Drought stress affects most of the functions of plant growth, this effect depends on the level of drought stress, length of time to which plants subjected to water stress and genotypes of the plant species. In general drought stress reduced all phenotypic expressions such as shoot length, number of leaves, number of internodes, leaf area and dry matter of the plants. Similar type results were observed in the present experiment where all growth parameters were negatively affected by water deficient. Severity of drought stress was more in T5 (20%) PEG condition. In this condition LCA-353 could survive only seven days, this might be due to dehydration induced desiccation of the plant tissues lead to cellular death [21] or stomatal closer to prevent dehydration causes photosynthetic uptake of carbon to diminish and the plant starves as a result of continued metabolic demand for carbohydrates [22] leading to plant death.

**Table 1** Mean squares of 8 chilli cultivars for various plant traits under control and PEG stress conditions

| Characters | Cultivars (G) | treatment(T) | Interaction (GxT) | Error |
|---|---|---|---|---|
| D.F | 7 | 4 | 28 | 78 |
| Shoot length | 40.311** | 197.15** | 2.2037** | 0.6914 |
| Root length | 62.0109** | 1112.8** | 3.68002** | 1.20352 |
| No. of leaves | 41.729** | 129.28** | 2.9548** | 1.5536 |
| No.of Internodes | 8.2655** | 49.304** | 0.8994** | 0.4085 |
| Leaf area | 964.51** | 23383** | 160.62** | 60.978 |
| Shoot dry weight | 17956** | 275995** | 2144.7** | 432.31 |
| Root dry weight | 7329.2** | 59106** | 634.65** | 118.22 |
| Root to shoot dry weight | 0.094645** | 0.139446** | 0.010313** | 0.002021 |
| Proline | 120162.9** | 995877.1** | 44490.21** | 806.4331 |

Shoot length was significantly reduced with increased drought stress in all cultivars compared to control (**Figure 1**). The mean shoot length was varied from 4.084cm (LCA-353) to 8.94cm (Arka Lohit). In all drought stress conditions, Arka Lohit performed better and showed least reduction of shoot length. At highest concentration of PEG (20%), cultivars Arka Lohit, LCA334, G4 recorded 6.5cm, 5.4cm, and 5.1cm of shoot length respectively, which were higher than other cultivars. These results indicate that Arka Lohit, LCA334 and G4 showed better performance under drought stress as far as shoot length was concerned. Similar results reported by [15] and they observed that drastic reduction in shoot growth in tomato with increased PEG concentration, which was considerably lower in mutant derivatives and hybrid which were resistant [23] and [24] also found similar results in pearl millet in drought induced by polyethylene glycol.

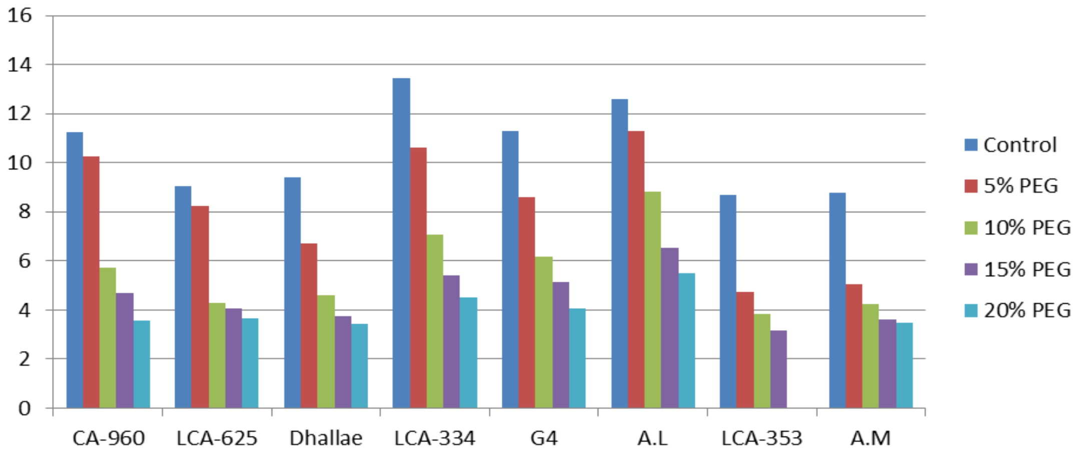


**Figure 1** Shoot Length (cm) of different genotypes at different concentration of PEG

In the present experiment, compared to control root length was significantly reduced with increased drought stress in all cultivars (**Figure 2**). The mean root length was varied from 8.07 cm (LCA-353) to 14.24 cm (Arka Lohit). In all the stress condition it was observed that Arka Lohit showed higher mean root length and also shown least root length reduction at different water deficient conditions, followed by LCA 334. Comparable results found in tomato by [15] and in pearl millet by [23] and [24]. These results indicated that Arka Lohit produced more root length in drought stress condition which was most important character for drought tolerance. "Early and rapid elongation of root was important indication of drought tolerance. A root system with longer root length at deeper layer is useful in extracting water in upland conditions [25] and [26]".

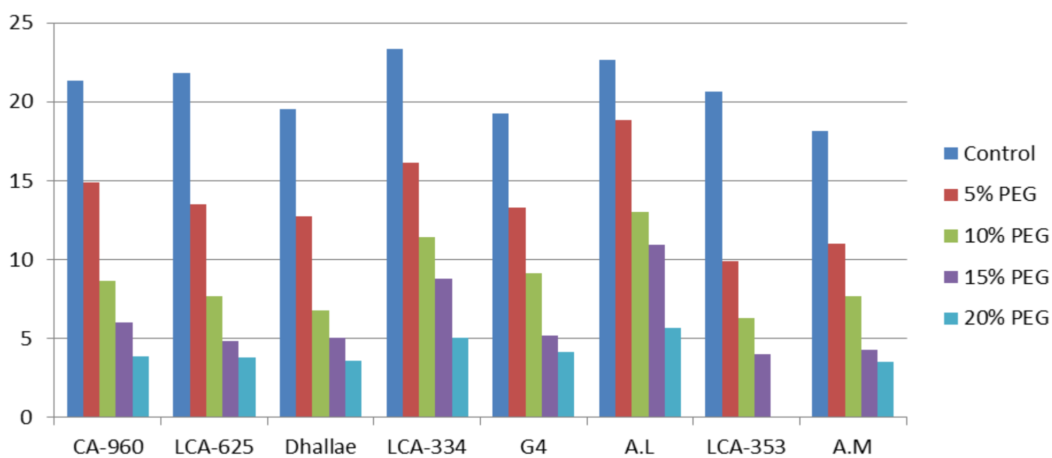


**Figure 2** Root Length (cm) of different genotypes at different concentration of PEG

An increase in drought stress reduced the number of leaves (**Figure 3**). Mean value of the number of leaves varied from 4.13 (LCA-353) to 8.60 (Arka Lohit). Arka Lohit (8.60), LCA 334 (8.07), G4 (7.33) were statistically at par with each other. Number of Internodes was also reduced by increasing drought stress. The highest number of internodes was recorded in Arka Lohit (4.67) where as lowest No. of Internodes was observed in LCA-353 (2.53) (**Figure 4**). A clear difference was observed in leaf area among seven cultivars when plants were growing in control condition (0% PEG). When comparing the effects of drought stress on leaf area, the highest leaf area was found in control, followed by T2 (5% PEG), while leaf area of plants of T5 (20% PEG) had the least leaf area among survived cultivars, suggesting that severe drought stress decreased leaf area. From mean analysis, the highest leaf area was observed in Arka Lohit (47.38cm$^2$) and lowest was in LCA-353 (24.15 cm$^2$) (**Figure 5**). In the present experiment there was reduction of number of leaves, number of internodes and leaf area with increasing drought stress. Similar results reported by other researchers during drought stress, chilli [27] and [28] cow pea [29]. Other workers had also shown that water deficit during the vegetative phase causes leaf and plant growth reductions [30]. This was due to onset of water deficient condition reduces the plant-cell's water potential and turgor, which elevate the solutes' concentrations in the cytosol and extracellular matrices. As a result, cell enlargement decreases, leading to reduction of leaf development and growth inhibition, which was reflected in shoot length, leaf area, number of leaves and number of internodes and other growth parameters [31]. Reduced leaf area through the early leaf senescence profoundly reduces the photosynthetic activity of the plant. Drought-tolerant cultivars maintain reasonable photosynthetic leaf area under stress comparing to drought-avoidant cultivars [32]. Hence we conclude that Arka Lohit was tolerating drought stress.

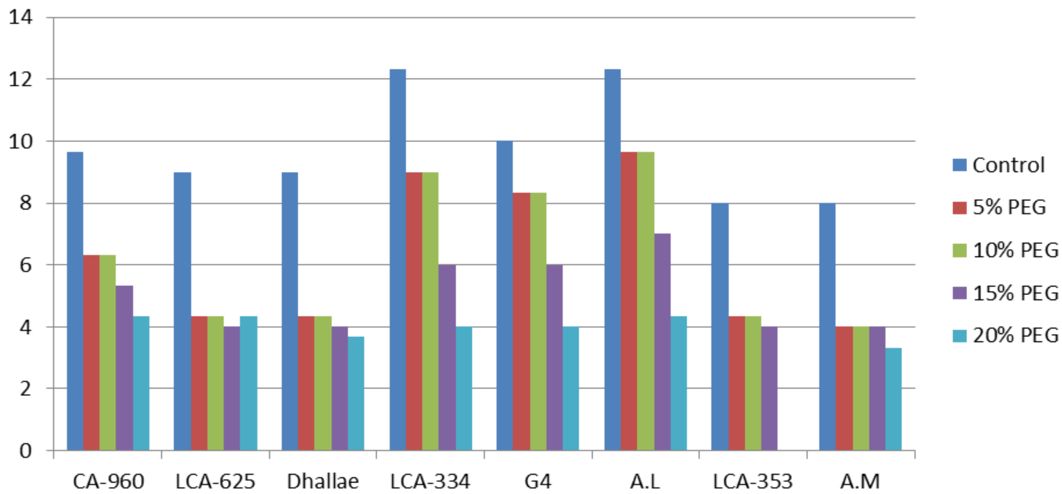


**Figure 3** No. of Leaves different genotypes at different concentration of PEG

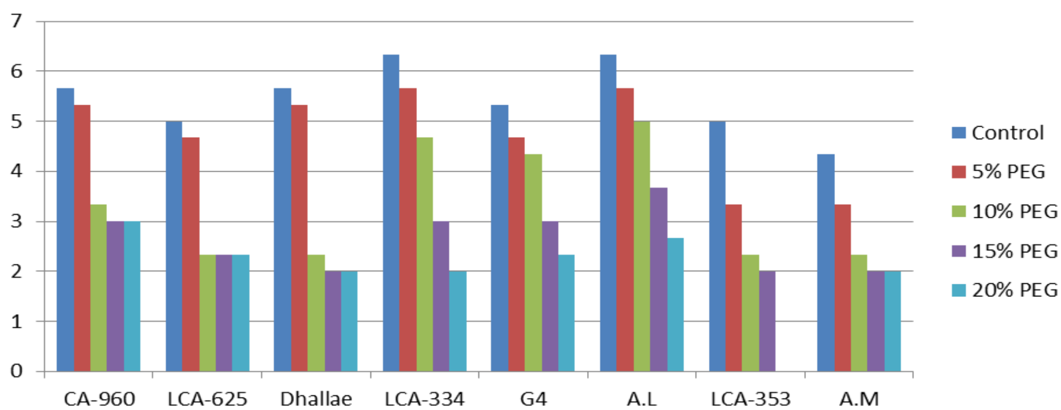


**Figure 3** No. of Leaves different genotypes at different concentration of PEG

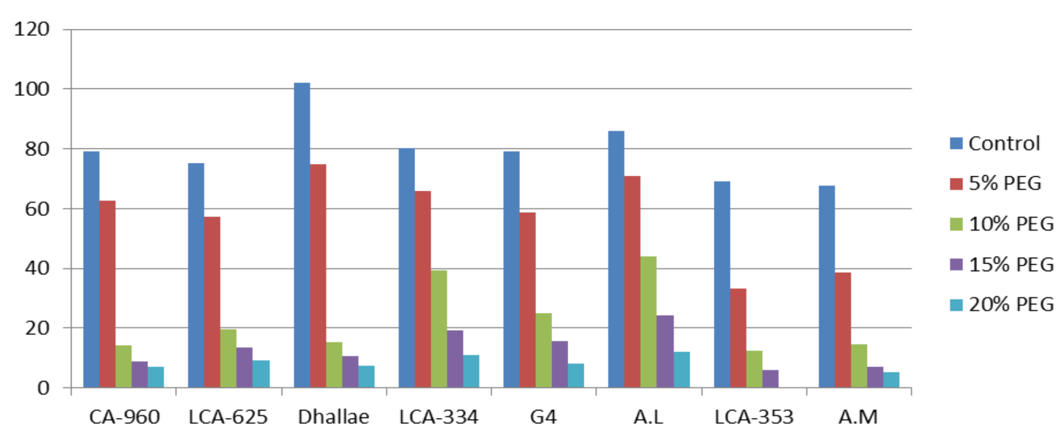


**Figure 5** Leaf area ($cm^2$) different genotypes at different concentration of PEG

When plants were subjected to drought stress, shoot dry weight decreased significantly in all treatments in all cultivars compared to control. Highest shoot dry weight was recorded in Arka Lohit (195.83 mg) followed by LCA 334 (174.68 mg) where as lowest dry weight was recorded in LCA-353 (95.98mg) (**Figure 6**). Similarly root dry weight was also reduced along with increasing drought stress. Highest root dry weight was recorded in Arka Lohit (100.67 mg) and lowest was recorded in LCA-353 (35.69 mg) (**Figure 7**). Among all cultivars, Arka Lohit root dry weight reduction was least and even at highest drought stress condition (20% PEG), root dry weight was recorded highest among all cultivars. Reduction leaf area results in reduced transpiration surface [33] and may be a drought avoidance strategy for the plants. On the other hand, the reduction of leaf area limits photosynthesis, and further decreases biomass production, this was the reason for the reduction of shoot dry weight and root dry weight along with increasing drought stress in this experiment. Comparable results found in tomato cultivars screening under Water Stress by [34] and [15].

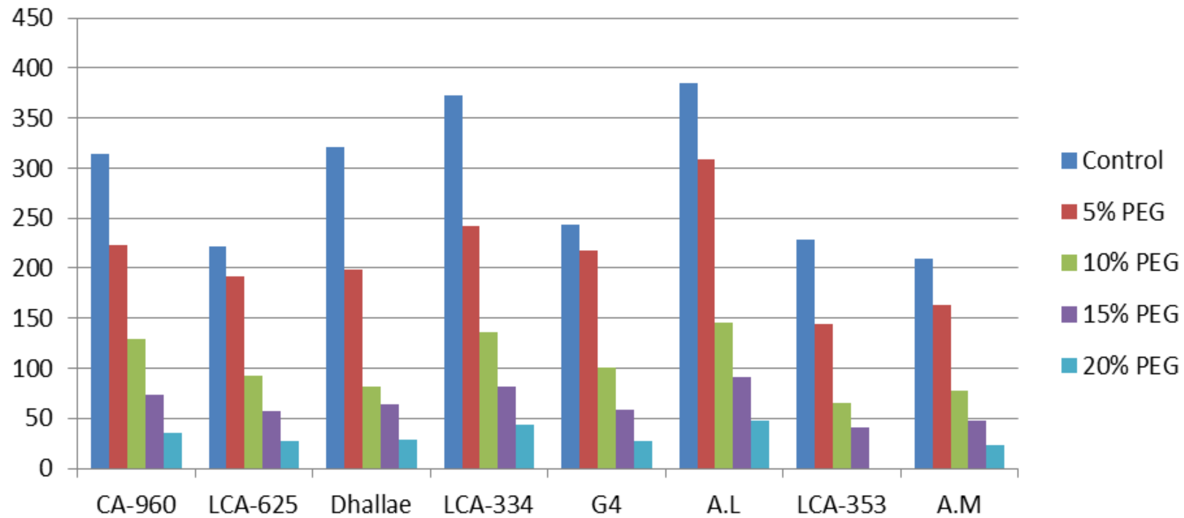


**Figure 6** Shoot dry weight (mg) different genotypes at different concentration of PEG

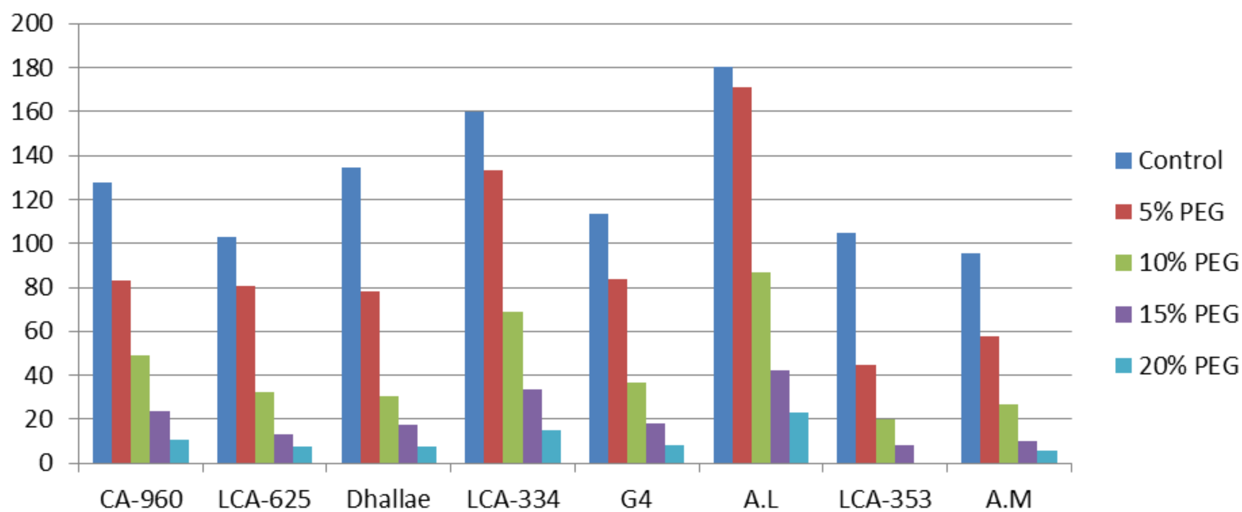


**Figure 7** Root dry weight (mg) different genotypes at different concentration of PEG

Root to shoot dry weight estimates the distribution of dry matter between the root and shoot systems and it is a good indicator for effect on roots and shoot dry weight. The results (**Figure 8**) showed that root to shoot dry weight was decreased in all cultivars except LCA-334 and Arka Lohit. In LCA-334, 0% PEG, 5% PEG, 10% PEG 15% PEG and 20% PEG drought stress conditions; root to shoot dry weight ratio was measured at 0.43, 0.56, 0.51, 0.41 and 0.35 respectively, indicating that moderate drought condition increased root to shoot dry weight and in severe drought conditions it decreased. Similarly, in Arka Lohit also root to shoot dry weight showed 0.49, 0.55, 0.59, 0.50 and 0.46. This result revealed that Arka Lohit till treatment 4 (15% PEG) root to shoot dry weight was increasing and again reduced in treatment 5 (20% PEG). It indicated that under moderate drought condition dry matter allocated to shoots was less compared to roots. Plants in dry condition often decreased biomass production and contribute more biomass to roots, maintaining a higher root to shoot ratio [35-38] as an adaptation to drought resistance.

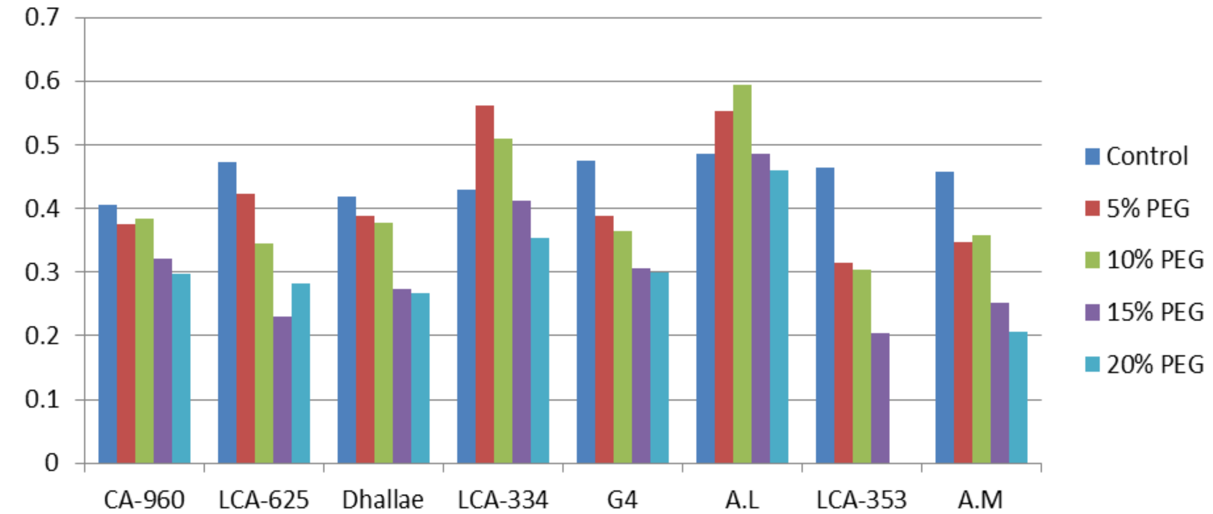


**Figure 8** Root to shoot dry weight different genotypes at different concentration of PEG

As proline accumulation is a common response of plants to drought, Proline has been estimated in the present study. The present experiment revealed that increased accumulation of proline has been observed in all the cultivars with increased PEG concentration. The mean proline accumulation was varied from 229 µg g–1FW (LCA-353) to 519 µg g–1FW (Arka Lohit) (**Figure 9**). In all drought stress conditions, Arka Lohit accumulated highest proline content than other cultivars whereas lowest was LCA-353 followed by Dalle Khursani. Genotypes which accumulate high proline concentration under stress environment are generally considered to be tolerant [39-41]. Similar type results reported by [42] in chickpea genotypes which performed better under drought showed significant levels of proline than that of genotypes which were sensitive under water deficit conditions. [43] also reported increased proline content in leaves and roots than control in Capsicum annuum Solan Bharpur during PEG and NaCl induced stress. These results indicated that Arka Lohit tolerates drought stress. This may due to Proline re-establishes cellular redox balance by removing excess levels of ROS

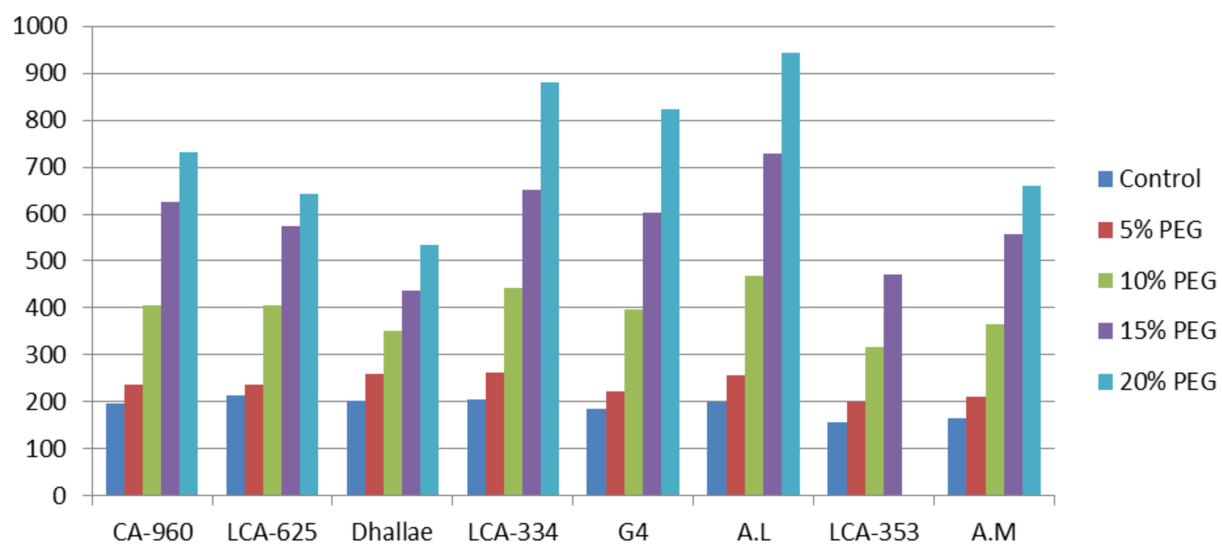


**Figure 9** Proline (µg g–1FW) different genotypes at different concentration of PEG

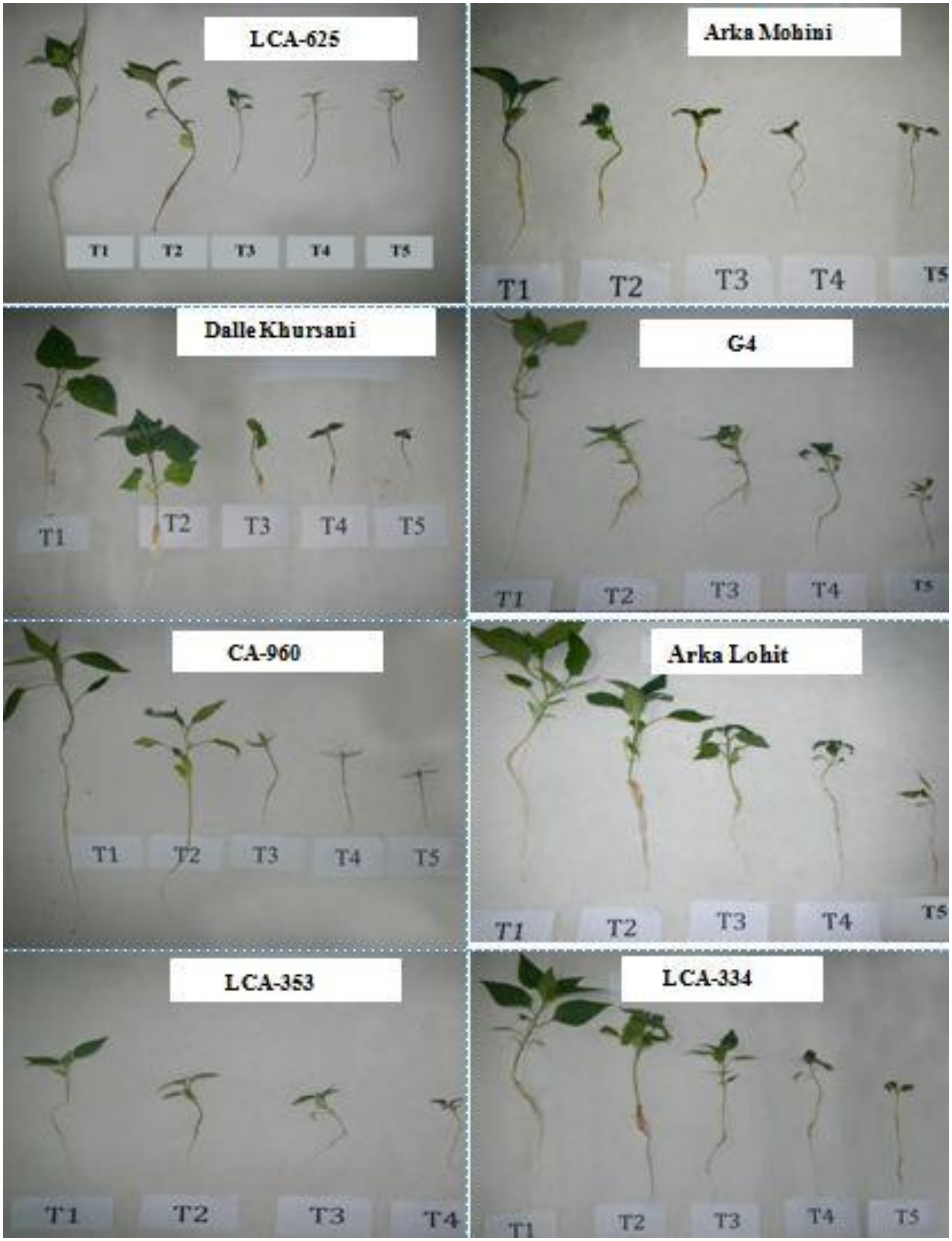


**Figure 10** Samples of different chilli cultivars under different drought stress in the study (T1=control 0%PEG, T2=5%PEG, T3=10%PEG, T4=15%PEG, T5=20%PEG)

## Conclusion

From the present experiment it has been concluded that performance of chilli cultivars subjected to different levels of stress showed significant differences **(Figure 10)** in all studied traits signifying the importance of the traits that are to be considered when selecting for drought tolerance. Among all the varieties studied, Arka Lohit showed high proline content, high root to shoot dry weight than other varieties, these may be considered as drought tolerant. Since LCA-353 could not survive at high concentration of PEG (20%) and remaining all treatments it showed least growth rate and low proline accumulation makes this cultivar considered as susceptible.

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