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EFFECT OF POLY ETHYLENE GLYCOL INDUCED WATER STRESS ON MORPHOLOGICAL AND BIOCHEMICAL PARAMETERS IN TOMATO (*SOLANUM LYCOPERSICUM* L.) AT SEEDLING STAGE

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ABSTRACT

Among the abiotic stress, drought stress is one of the major threats to agricultural crop yield around the world. Selection and development of drought stress tolerant cultivars through effective screening method is one of the important strategies to overcome the drought associated problem. To evaluate the impact of drought stress we screened fifteen germplasm of tomato (*Solanum lycopersicum*) and seeds were germinated at control, 10% and 20% concentration of polyethylene glycol (PEG6000) and allowed for growth twenty five days. Root length; shoot length, fresh weight, dry weight, Relative water content (RWC) and proline parameters were evaluated. The results revealed that the fresh weight, dry weight (mg) and relative water content (%) were reduced with increasing drought stress. The proline (µ.moles/fw) content was varied in different germplasm at different drought stress condition. The relative water content of the plants grown in control conditions was 93.29% and it reduced to 67.73% under stress conditions. The average proline content of all germplasm was estimated 17.39 µ moles $g^{-}1$ fresh weight in controlled condition and under stress conditions it was noted 34.3 µ moles $g^{-}1$ fresh weight. The results revealed that the variety NB4 showed best tolerance followed by NB6, NB7 and NB13.

INTRODUCTION

Drought is a global problem which limits the growth, survival and yield of the crops. The effect of stress depends on the severity and duration of the stress as well as it depends on the development stage of the plant. Usually, seed germination and seedling stage are considered as the critical and the most sensitive stage in the life cycle of the plants when they exposed to drought stress. Drought stress not only influence the seed germination (Basha *et al.,* 2015), but also increase the germination mean duration in plants (Willenborb *et al*., 2004). Basha *et al.,* 2015, reported that different morphological characters were affected by PEG induced drought stress included reduction in plant height, root length as well as plant biomass. Drought stress results in reduction of Relative Water Content (RWC) and chlorophyll content which in terms associated with reduced photosynthesis (Kumar *et al.,* 2011). Reddy *et al.,* 2004 also reported that reduced photosynthesis and severe metabolic disturbance and plant death was associated with severe drought stress conditions. In addition, some evidences have indicated that water stress causes considerable decrease in seed yield components and seed oil content of sunflower (Alahdadi *et al*., 2011; Stone *et al*., 2001).

Several authors stated that use of Poly Ethylene Glycol (PEG) is one of the appropriate procedure to induce dehydration condition to screen the germplasm and several drought tolerance indices such as plant height, root length and biomass have been proposed as methodology to discriminate the genotypes for drought stress (Basha *et al.,* 2015; Mohammed *et al*., 2002; Dutta and Bera, 2008; Ahmad *et al*., 2009). Usually most of the tomato germplasm are affected by drought stress at all developmental stages of plant including seed germination and seedling growth (Foolad *et al.,* 2003; Nuruddin *et al.,* 2003). Rauf *et al*., 2007 sated that drought sensitivity not only critical in seed germination and seedling growth and the drought induced parameters were more important to study the drought stress response of germplasm in-term of yield factor. In view of the above information, the present investigation carried out to study the effect of drought stress on seedling stage of fifteen tomato germplasm using polyethylene glycol 6000. The germination studies, growth parameters, RWC and proline

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parameters were studied to identify the effects of drought stress on tomato germplasm.

MATERIALS AND METHODS

Plant material

To evaluate the effect of drought stress by PEG, we selected fifteen variety of tomato (*Lycopersicon esculentum L.*) germplasm NB1, NB2, NB3, NB4, NB5, NB6, NB7, NB8, NB9, NB10, NB11, NB12, NB13, NB14 and NB15. The germplasm were obtained from National Bureau of Plant Genetic Recourses (NBPGR), Rajendranagar, Telangana, INDIA.

Seedling growth conditions

Seeds were surface sterilized with 4% (v/v) sodium hypochlorite for 8-10 min and washed thoroughly with several times with sterile distilled water. The seeds were inoculated in coconut peat and applied 0, 10% and 20% concentrations of PEG and allowed for incubation at 16 hours light (70 µ mol M2 s-1) and 8 hours dark conditions at 28^0C temperature for twenty five days.

Morphological and Relative Water Content (RWC) parameters measurement

Seedling morphological parameters such as root length (cm), shoot length (cm), shoot weight and root weight were recorded after twenty five days of inoculation. Leaf material (100 mg) were weighed and immediately placed in double distilled water in petri-dishes to saturate them with water for 24 h in dark. The leaves were blotted on filter paper and turgor weight was measured. Dry mass of these leaves were obtained after drying them in oven at 80°C for 72 hrs. Relative Water Content (RWC) was calculated according to following formula: RWC= (fresh weight - dry weight)/ (turgor weight - dry weight) X 100 (Turner 1981).

Proline estimation

Proline was estimated following the protocol described by Bates *et al.,* (1973) with minor modification. One gram of leaf tissue was homogenized in 5 ml of 3% sulfosalicylic acid and the homogenate was centrifuged at 3000 rpm for 20 min. To the 0.5 ml of supernatant 2 ml of reagent mixture (30 ml glacial acetic acid, 20 ml water and 0.5 g ninhydrin) was added and the mixture was boiled for 1 hour in a boiling water bath. After cooling the mixture to room temperature, proline was extracted with 6 ml toluene and the absorbance was taken at 546 nm using spectrophotometer. Proline content was estimated using a proline standard curve prepared by dissolving the known quantities of proline standard in 3% sulfosalicylic acid.

RESULTS AND DISCUSSION

The results revealed a significant variation among germplasm at different stress conditions induced by different PEG-6000 concentrations. Twenty five days old seedlings were used to analyse the germplasm for drought stress. Results of fresh weight data are presented in Table 1 and the results indicated that different germplasm exhibited varied response to the stress.

Root data analysis

Initial and fast elongation of roots is a vital sign for resistance germplasm towards drought resistance and capability of continuation of root underneath situation of water strain is an important parameter to distinguish the germplasm as tolerant and sensitive. When germplasm exposed to 10% PEG induced stress NB6 showing highest root elongation upto 7.3 cm second highest root producer is NB9 as 7 cm and the NB4, NB7 & NB 14 are the next highest root producers. At 10% PEG stress NB2 and NB12 showed reduced root length (Table 1). At 20% PEG induced drought pressure, NB13 germplasm exhibited 4.4 cm lengthy roots and next highest root lengths was observed in NB4 (4.3 cm) followed by NB6 (3.9 cm). While the germplasm NB2 had least root length i.e, 1.3 cm. Germplasm such as NB12 (1.5 cm), NB11 (1.6 cm) and NB1 (1.7 cm) developed less root elongation (Table 1). Highest root weight change recorded by means of germplasm NB4 (35.6 mg), followed through NB6 (33.8 mg), NB7 (33.7 mg) and NB13 (29.8 mg) whereas lowest weight was noted in NB2 (10.4 mg), NB1 (12.4 mg), NB12 (11.7) and NB11 (16.7 mg) when examined at higher drought circumstance (20% PEG). Resistant germplasm will possess the potential to keep root growth at higher stress conditions and sensitive one cannot keep the growth. Liu *et al.,* 2011 reported that drought stress decreases shoot and root dry weight in Asian purple sage (*Salvia miltiorrhiza* Bunge), nevertheless the consequence become bigger intense on shoots, which better the shoot dry weight ratio. There are reports among sunflower, wheat, sorghum and maize that the increase in their root length under drought stress is related to osmotic stress (Voetberg and Sharp 1991; Tangpremsri *et al.,* 1991; Morgan 2000; Chimenti *et al.,* 2006; Rauf and Sadaqat 2008). In rain fed rice germplasm osmotic adjustment conserved a lower allocation of biomass to roots in comparison with non-osmotic adjustment germplasm (Wang *et al*. 2009). The similar phenomenon is observed in irrigated as opposed to rain fed rice sorts (Babu *et al*. 1999, 2001). Mutant derivatives and hybrids featured more ability of roots to extract water in water deficient conditions by altering the water potential in the cells. Similar type of results was reported by Tyagi *et al*. (1995).

Shoot data analysis

Growth is a pattern of change in size, volume, or weight which comprises the stages of cell division, elongation, and differentiation which were affected under drought conditions due to reduced loss of turgor, less energy and reduced enzyme activities (Kiani *et al.,* 2007; Farooq *et al.,* 2009; Taiz and Zeiger 2010). Well-adjusted development was observed in drought resistant germplasm in comparison to sensitive germplasm. The germplasm under 10% PEG stress NB1, NB2, NB11 and NB12 showed great reduction in shoot length respectively 3.3 cm, 3.7 cm, 3.5 cm and 3.7 cm. The germplasm NB13 and NB6 showed 2.6 cm shoot length whereas the germplasm NB4 recorded 2.4 cm, drastic reduction in shoot growth was observed with increasing PEG stress in NB11 (0.8 cm), NB12 (0.9 cm), NB1 (0.9cm) and NB2 (1.1 cm) genotypes at 20% PEG stress (Table 1). Generally germplasm with indeterminate growth habit have showed better drought tolerance (Turner 1979). It specifies that determinate germplasm can be well suitable to grow in drought areas than germplasm which have the indeterminate growth habit. In terms of biomass, at 10% PEG NB4 (136.7 mg) and NB7 (128.6 mg) had highest shoot weight, whereas NB12 (73.6 mg) and NB2 (82.4 mg). Even at 20% PEG stress highest weight was noticed in NB4 (67.3 mg) whereas lower biomass was observed in NB12 (27.6 mg) germplasm. Among the evaluated germplasm, the mean weight for the resistant lines was noted as 66.3 mg while for susceptible lines the average weight was around noted as 31.35 mg. At the 20% PEG stress concentration, except drought resistant cultivars all other germplasm growth was found to be highly reduced (56.7 mg to 27.6 mg). Basha *et al.,* 2015, studies in tomato reported that significant negative correlation was observed between PEG induced water stress and root length & shoot length. Such kind of similar results were also reported by Rao and Bhatt (1991). Due to drought stress the comparable results such as reduction in shoot weight, flower fresh weight and dry weights of marigold (Tageteserecta L.) plants were reported (Asrar and Elhindi 2011). Reduction in leaf area due to loss of turgor and reduced leaf numbers were reported by Farooq *et al.,* 2010. Drought stress significantly decreases shoot and root dry weights in Asian red sage (Salvia miltiorrhiza L.).

accessibility of water from the root zone, portion of water that available in plants were used for transpiration functions to hold the minimum biological activities. In the present study it is noted that there were lower average relative water content of leaf material under drought stress in comparison to that of control plants (Fig 1). The average relative water content (RWC) was noted that 93.29% in control germplasm (Fig 1). Whereas under 10% and 20% PEG induced drought stress 88.81% and 61.73% RWC content was observed, respectively. Among all accessed germplasm at 10% PEG RWC noted in NB12 (80.7%) and highest RWC was found in NB4 (99.39%). But NB2 showed significantly less RWC (37.99%) followed by NB12 (42.67%), NB11 (42.91%) and NB1 (43.28%) at 20% PEG. The germplasm NB4 (87.75%), NB6 (86.44%) and NB13 (83.57%) showed better resistance to drought pressure was observed with higher RWC at 20% PEG stress (Fig 1). Nayyar *et al* (2005) also reported similar pattern of results with reduced relative water content in tomato germplasm under stress conditions. Desclaux and Roumet 1996 mentioned that deficient water supply triggers a signal to an early switching of plant progress from the vegetative to reproductive phase.

Table 1 Fifteen tomato germplasm Root length, Root weight, Shoot length and Shoot weight mean ± SE values under various PEG induced Drought conditions

Germplasm	Root length (cm)			Root weight (mg)			Shoot length (cm)			Shoot weight (mg)		
	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%
NB1	9.2 ± 0.35	4.2 ± 1.21	1.7 ± 1.5	104.3 ± 0.2	52.3 ± 0.24	12.4 ± 0.62	6.5 ± 2.01	3.3 ± 0.7	0.9 ± 1.25	207.1 ± 0.8	90.4 ± 0.51	33.4 ± 0.5
NB2	8.9 ± 0.48	3.8 ± 0.85	1.3 ± 2.14	100.8 ± 0.8	49.3 ± 0.6	10.4 ± 0.85	7.2 ± 0.85	3.7 ± 0.92	1.1 ± 0.51	202.6 ± 0.9	82.4 ± 1.1	28.6 ± 0.9
NB3	8.5 ± 0.41	6.8 ± 1.11	2.8 ± 2.3	99.3 ± 0.11	58.2 ± 0.7	23.7 ± 1.11	7.5 ± 1.11	4 ± 2.51	1.8 ± 0.21	203.4 ± 0.5	103.7 ± 1.2	38.9 ± 0.64
NB4	8.8 ± 0.24	6.9 ± 0.96	4.3 ± 0.74	89.5 ± 0.31	61.7 ± 0.16	35.6 ± 0.92	7.3 ± 0.27	4.7 ± 0.81	2.4 ± 0.52	210.4 ± 0.3	136.7 ± 1.3	67.3 ± 1.4
NB5	9.7 ± 0.17	6.5 ± 0.87	2.4 ± 0.88	101.7 ± 0.86	50.4 ± 1.12	25.4 ± 1.08	7.7 ± 1.04	4.1 ± 0.51	1.9 ± 1.14	223.4 ± 0.5	113.7 ± 0.8	43.6 ± 0.81
NB6	10.1 ± 0.41	7.3 ± 1.14	3.9 ± 1.25	89.3 ± 0.5	62.5 ± 0.81	33.8 ± 1.04	7.1 ± 1	4.5 ± 1.04	2.6 ± 0.21	200.7 ± 0.7	121.4 ± 0.9	65.7 ± 0.68
NB7	8.7 ± 0.31	6.9 ± 2.05	3.4 ± 0.76	82.3 ± 0.8	66.5 ± 0.7	33.7 ± 0.85	6.9 ± 0.85	4.1 ± 0.58	2.3 ± 0.57	206.7 ± 1.1	128.6 ± 1.4	70.5 ± 1.2
NB8	9.7 ± 0.52	5.9 ± 1.14	2.1 ± 0.58	92.7 ± 0.9	45.8 ± 1.11	19.3 ± 1.14	7.5 ± 1.14	3.8 ± 0.52	1.7 ± 0.67	205.6 ± 1.3	109.9 ± 1.7	46.8 ± 2.4
NB9	10.4 ± 0.2	7 ± 0.85	2.5 ± 2.01	102.7 ± 1.14	58.3 ± 0.8	22.7 ± 2.01	6.8 ± 0.85	3.3 ± 0.85	1.1 ± 0.81	210.7 ± 2.1	118.4 ± 0.61	51.2 ± 0.81
NB10	8.6 ± 0.51	6.7 ± 1.34	2.3 ± 2.04	85.6 ± 0.8	39.7 ± 1.04	24.7 ± 1.05	7.6 ± 0.62	4.1 ± 1.04	1.9 ± 0.42	196.5 ± 0.8	89.7 ± 0.5	37.9 ± 1.4
NB11	9.3 ± 0.47	5.1 ± 0.95	1.6 ± 0.81	100 ± 1.11	48.3 ± 0.52	16.7 ± 1.14	7.2 ± 1.04	3.5 ± 0.82	0.8 ± 0.85	203.7 ± 1.4	90.5 ± 0.53	31.5 ± 0.52
NB12	8.4 ± 0.52	3.8 ± 0.74	1.5 ± 1.12	84.3 ± 0.8	32.5 ± 0.63	11.7 ± 0.54	7.6 ± 0.85	3.7 ± 1.11	0.9 ± 1.04	186.5 ± 1.2	73.6 ± 0.57	27.6 ± 1.1
NB13	8.6 ± 0.34	6.6 ± 1.42	4.4 ± 2.41	93.7 ± 0.21	59.4 ± 1.04	29.8 ± 1.04	7.5 ± 0.9	4.2 ± 0.81	2.6 ± 0.82	201.7 ± 2.1	119.3 ± 1.1	66.8 ± 0.21
NB14	9.5 ± 0.15	6.9 ± 1.31	2.8 ± 2.72	87.8 ± 0.85	38.7 ± 0.5	20.1 ± 0.94	7 ± 1.24	3.2 ± 0.8	1.8 ± 0.31	210.7 ± 0.8	120.4 ± 1.4	56.8 ± 0.24
NB15	8.8 ± 0.25	6.4 ± 1.54	2.1 ± 0.76	101.5 ± 1.1	49.8 ± 083	20.4 ± 1.04	7.4 ± 0.51	3.3 ± 0.81	2 ± 0.57	203.8 ± 0.51	116.4 ± 0.8	53.4 ± 0.42

RELATIVE WATER CONTENT

Leaf water potential, osmotic capability and Relative water contents (RWC) are the essential parameters (Kirkham 2005), which has significant role under water stress. The Relative Water Content is one of the important traits used to assess the tolerance level of germplasm along with its biomass as they are critical for plant growth and retaining normal plant body temperature. Generally plant absorbs some instances larger water than the quantity integrated in cells and most of the excess water will be lost through the stomata during transpiration. The loss of water through transpiration has significance not be too warm to deactivate the enzymes of photosynthesis and respiration. When plants were under drought stress the normal phenomenon of maintaining cell activities were adversely affected. Due drought stress

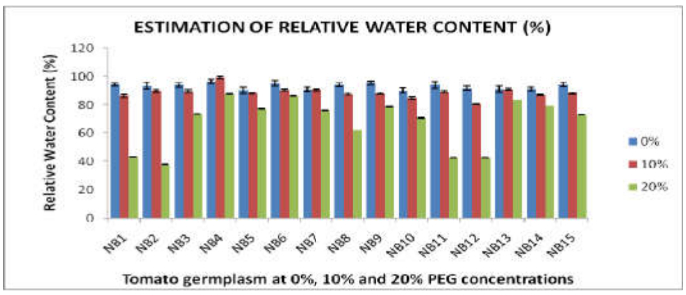

Figure 1 Effects of PEG induced water stress on Relative Water Content (RWC) in tomato germplasm. Each bar represents the mean (± SE) of five measurements ($P \leq 0.05$)

Proline estimation

The mean value of proline content is17.39 μ moles g^{-1} fresh weight in controlled conditions whereas it got increased to 26.3

µ moles g^{-1} and 34.3µmoles g^{-1} fresh weight at 10% and 20% PEG induced drought stress, respectively. This indicates that the proline content will increases against the lesser water availability. In the germplasm NB12 and NB1 grately increased in proline as 33.43 µ moles g^{-1} and 30.52 µ moles g^{-1} respectively at 10% PEG induced stress. The maximum proline concentration was noted in NB1 (43.15 µ moles g^{-1}) and lowest proline content was observed in NB6 (28.19 µ moles g^{-1}) at 20% PEG condition. It is noted that there were increased proline content of leaf material under drought stress in comparison to that of control plants (Fig 2). Osmotic adjustment is the important parameter that minimize the effect of drought induced damage in crop flowers (Blum 2005) by maintaining the leaf turgor and improves the stomatal conductance for effectual ingestion of CO_2 (Kiani *et al.,* 2007) followed by promoting the uptake of water (Chimenti *et al.,* 2006). Perez-Perez *et al.,* 2009 reported that proline is an important solute which accumulates in flowers exposed to dehydration pressure. Proline is one of the bio-molecule involved in osmoregulation and protects flowers from osmotic strain (Sankar*et al.,* 2007). Increased the level of endogenous proline levels enhance the antioxidant enzymatic activities (de Campos *et al.,* 2011).

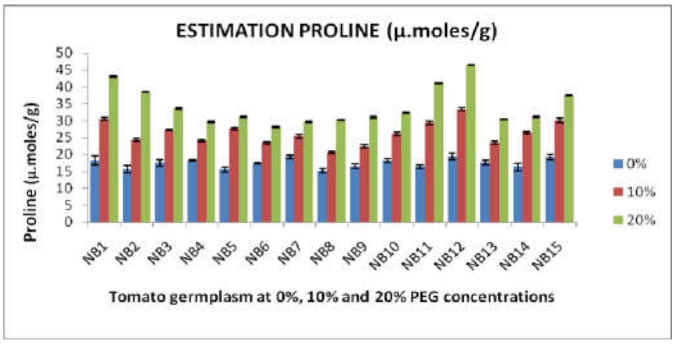

Figure 2 Comparative response of tomato varieties under control and PEG induced drought stress of proline; each bar represents the mean (± SE) of five measurements ($P \leq 0.05$).

CONCLUSIONS

The present study was carried out to evaluate the tomato germplasm under PEG induced drought stress. All the evaluated tomato germplasm showed strong negative correlation between drought stress and biomass and also similar negative relationship was noted with Relative Water Content (RWC).A total of four germplasm such as NB4, NB6, NB7 and NB13 showed better growth in comparison to other germplasm under PEG induced drought stress. These results highlight the importance of the PEG as an artificial stress inducer for drought tolerant germplasm screening in the laboratory conditions.

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