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**RESEARCH ARTICLE**

# EFFECT OF PEG INDUCED WATER STRESS ON GERMINATION AND SEEDLING DEVELOPMENT OF TOMATO GERMPLASM

**P. Osman Basha[1]*, G. Sudarsanam[2], M. Madhu Sudhana Reddy[1] and N. Siva Sankar[1]**

[1]Department of Genetics and Genomics, Yogi Vemana University, Kadapa, A.P., India
[2]Department of Botany, Sri Venkateswara University, Tirupati, A.P., India

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**ABSTRACT**

The effect of PEG-6000 induced water stress on germination and early seedling growth stages was studied. Five to mato germplasm were tested for drought tolerance using 0%, 2%, 4%, 6%, 8%,10%, 12%, 14% and 16% PEG-6000 and the data was recorded on various seedling parameters like germination rate, root length and shoot length.The experiment was carried out in three replicates under Complete Randomized Design. No significant difference was observed in germination rate between control and PEG induced drought stress up to 12% PEG concentration. After this critical point, a reduction in germination ratewas observed and all the varieties were unable to germinate at 22% of PEG solution. The shoot length increased significantly in Arka Rakshak, Arka Vikas and PKM-OP at 2% PEG stress condition in comparison to control. Subsequently noteworthy reduction was noticed in root and shoot length with the increasing PEG concentration. The longest shoot length (6.5 cm) was noticed in Arka Rakshak at 2% PEG in comparison to other germplasm. A positive correlation between shoot length and root length at all stress conditions was noticed. The results obtained in present investigation provided useful data in screening of drought tolerant germplasm using PEG.



## INTRODUCTION

Drought, flood, cold, chill, frost, elevated $CO_2$ level, heat and light are abiotic stress factors that severely affected the plant growth. The available literature and observations clearly indicated that "climate change" has a negative impact on agriculture production. A modest evaluation suggests that nearly 90% of global rural land area is affected by abiotic stress factors at some point throughout the growing period (Cramer *et al*, 2011). In general plants sense changes in climate and adjust their metabolism and growth within to their capacity. Generally plants tolerant to particular abiotic stress establish a metabolic homeostasis and carry on their growth without suffering stress-induced injuries. On the other hand sensitive plants unable to establish metabolic homeostasis that results in reduction in growth, ultimately leading to death (Jogaiah *et al*, 2013). Noteworthy developments were made in understanding the abiotic stress at molecular, biochemical, physiological, and agronomic scales. Especially the response mechanisms and potential targets for improving crop response to drought (Tester and Langridge, 2010; Chen Q *et al*, 2013), salt (Zhang and Shi, 2013),flooding (Bailey-Serres *et al*, 2012), low temperature (Theocharis *et al*, 2012) and high temperature (Hasanuzzaman *et al*, 2013). In *Solanum lycopersicum* the fruit is the edible part and it isa source of many essential nutrients such as vitamins, minerals, fibers and bioactive compounds which are significant for human health. According to FAO STAT 2012 (http://faostat.fao.org/) China ranked first in production of tomato followed by India.

Various methods have been employed from time to time to identify drought tolerant genotypes and efforts have been made in the past to screen tomato varieties which differed in drought tolerance (Georgeet *al*, 2013). Polyethylene glycol (PEG) compounds used to induce osmotic stress in petri dish (in vitro) for plants to maintain uniform water potential during the experimental period. Poly ethylene glycol (PEG) has been used often as abiotic stress inducer in many studies to screen drought tolerant germplasm (Turkan *et al*, 2005; Landjeva *et al*, 2008; Almaghrabi, 2012; Ahmad *et al*, 2013; Jatoi *et al*, 2014). PEG is a polymer and considered as better chemical than others to induce water stress artificially (Larher *et al*,1993; Kaur *et al*, 1998). PEG induced osmotic stress is inductee to decrease cell water potential (Govindaraj *et al*, 2010). The upsurge inconcentration of PEG caused a decrease ingermination percentage, seedling vigour incertain crop plants (Khodarahmpour, 2011).

Several reports have shown that in vitro screening technique using PEG is one of the dependable approaches for the selection of desirable genotypes to study in detail on water scarcity on plant germination indices (Kocheva *et al*, 2003;

**Corresponding author:* **P. Osman Basha**
Department of Genetics and Genomics, Yogi Vemana University, Kadapa, A.P., India

Sakthivelu *et al*, 2008). Identification of tomato genotypes that can withstand inadequate water condition is vital to increase the crop production and this can be accomplished only by exploring the drought tolerant germplasm of tomato. Current study was planned to find out appropriate criteria for simple and quick screening of tomato genotypes that have higher tolerance to drought. To achieve this we intend to understand the effect of different PEG-6000 concentrations on germination, root length and shoot length of selective tomato genotypes. This helpsto find out the optimised concentration for quick screening of large number of mutagenized tomato germplasm.

## MATERIALS AND METHODS

### *Plant materials and screening experiments*

The screening experiments were planned with completely randomized design (CRD) with three replications. The germplasm used in the present study were obtained from different sources. *Solanum lycopersicumcv*. Arka Vikas (AV) and Arka Rakshak (AR) seeds were obtained from Indian Institute of Horticulture Research (IIHR), Bangalore, India. The seeds of YVU-1 and YVU-2 were collected from local farmers of Kadapa district, Andhra Pradesh, India and their germplasm is being maintained at Yogi Vemana University, Kadapa. PKM-OP (PO) is a hybrid variety and it is collected from local traders.

Seeds were disinfected with 4% Sodium hypochlorite solution for 15 minutes with gentle shaking at room temperature. After the surface sterilization the seeds were washed four times with autoclaved distilled water or until traces of sodium hypochlorite got removed. Thirty seeds from each germplasm were germinated on two layers of filter paper in 9-cm Petri dishes with respective PEG treatment. Seeds of fivevarieties were incubated at 25±2 ºC at different stress level of PEG-6000 i.e. 0% (control), 2%, 4%, 6%, 8%, 10%, 12%, 14% and 16%. The Petri plates were covered to avoid the loss of moisture by evaporation under laboratory condition (25±2 ◦C) for 12 days. Seeds were considered as germinated if they exhibited radicle extension by more than 3 mm. The experiment was terminated by harvesting seedlings after 12 days and germination rate, shoot length and root length data was noted. The germination percentage, shoot length and root length of three replications of 30 seeds i.e total 90 seeds was recorded. Statistical analysis was carried out using “Graph Pad Prism” software to identify significant difference among the tomato varietiesat different treatments. ANOVA was carried out to test the variation at 0.05 significance and Newman-Keuls Multiple Comparison-Post-HOC test was carried out for Pair wise comparison among all groups of varieties.

## RESULTS

### *Germination rate*

The effect of osmotic stress induced by the Poly Ethylene Glycol (PEG) on the germination rate of Arka Rakshak (AR), Arka Vikas (AV), YVU-1, PKM-OP (PO), and YVU-2 varieties is presented in Fig1. Seed germination and seedling development in lab conditions have been recognized as testing procedure in tomato and it is well known that with the increase in PEG concentrations, the germination rate and growth of seedlings got decreased (Fig 1). The highest germination rate was recorded at control as well as at lower PEG treatments and all the varieties was unable to germinate at 22% PEG (Fig 1).The germination rate of each tomato genotype in control and PEG induced water stress remained statistically non-significant up to 12% PEG (Fig 1). At 12% PEG concentration, a drastic reduction in germination rate was noted.

### *Root length data analysis*

The response of tomato varieties against different PEG concentration generated informative results. A strong negative correlation coefficient was noted in between PEG concentration and root length (i.e.root length was decreased with the increasing concentration of PEG). The correlation coefficient values calculated for AR (r=-0.89031), AV (r=-0.92295), YVU-1 (r=-0.90121), PO (r=-0.84434) and YVU-2 (r=-0.81693). Three different types of phenomenon observed among the root length of 12 days old seedlings i.e. enhanced, declined and remained same as compared to control. In control the mean root length of AR, AV, YVU1, PO and YVU2 were 10.27 cm, 7.87 cm, 9.5 cm, 7.73 cm and 8.07 cm respectively. Significant difference was observed in root length in between AR& AV, AR& PO, AR & YVU-2 but not between other varieties (Fig 2). Among all the tomato varieties examined at 2% PEG osmotic stress, significant difference has been observed in between YVU-2 & AV, YVU-2 & AR and YVU-1 & YVU-1 (Fig 2). No significant difference was observed between all varieties at 4%, 6%, 8% and 10% PEG except between PO & YVU-2 at 6% PEG, PO & YVU-2 at 6% PEG, and AV & YVU-2 at 8% PEG (Fig2). Drastic decrease in root length was identified at 12% PEG in AV (3.3 cm) whereas the other varieties represented more or equal to 4.8 cm. significant differences observed between AV & AR, AV & PO and AV & YVU-1(Fig 2). Similarly root length reduction has been observed in YVU-1 variety with average length 1.4 cm and significant root length difference was noted between AV & AR, AV & PO, AR & YVU-1 and PO & YVU-1 but not in between other varieties. The mean root length of all varieties was almost rudiment and differences were not found at 16%. Above this concentration the germination of the all verities was completely inhibited (Fig 2).

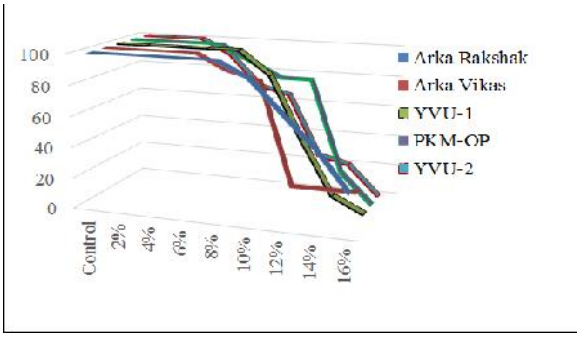


**Figure 1** Effect of PEG induced water stress on germination rate of different germplasm

***Shoot length data analysis***

All the germplasm showed strong negative correlation between shoot length and PEG concentration (i.e. shoot length was decreased with the increasing concentration of PEG). The correlation coefficient values calculated for AR (r=-0.95689), AV (r=-0.88643), YVU-1 (r=-0.95413), PO (r =-0.96421) and YVU-2 (r=-0.94041). A positive correlation between shoot length and root length has been identified and it clearly indicated that increase in root length helps to increase in shoot length. All the varieties showed common trend reduction rate in shoot length with increasing concentration of PEG with one exception at 2% PEG. All the tomato varieties have shown superior growth in shoot length at 2% PEG in comparison with control (Fig 2). The longest shoot length was identified in AR (6.5 cm), AV (5.5 cm), YVU-1 (3.6 cm), PO (5.5 cm) and YVU-2 (3.4 cm) at 2% PEG but not in control. The genotype AR and YVU-1 had better shoot growth when compared to others. At the concentration of 22% PEG all the germplasm shoot growth was completely inhibited. Better shoot growth was observed in AR and PO at 16% PEG (Fig 2).

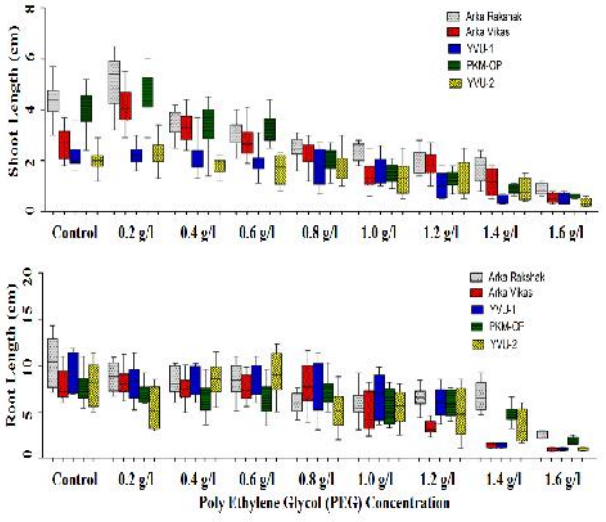


**Figure 2** Comparative response of tomato germplasm under control and PEG induced water stress; (A) Shoot length (cm) and (B) Root length (cm).

Comparison was carried out between varieties at different stress conditions. In control condition, a significant difference has been found among all the tomato varieties, except between AV & YVU-1, AR & PO, YVU-1 & YVU-2 (Fig2). Similar phenomenon was found at 2% PEG except in between YVU-1 & YVU-2. Significant difference was noted among all varieties at 4% and 6% and PEG concentration (Fig2) except between AV & AR, AR & PO, and YVU-1 & YVU-2 at 4% PEG and AV & AR, YVU-1 & YVU-2 at 6% PEG (Fig 2). Significant difference in shoot length at 14% PEG was observed in between AR & YVU-1and all other varieties are not showing such significant difference. Individual varieties at different stress have also been studied. The YVU-1 germplasm showed significant difference in between control and 0.4% PEG and similar results were noted in AR (Fig 2). A noteworthy point is that shoot lengthincreasedsignificantly in AR, AV and PO at 2% PEG in comparison to control, whereas no such significant difference was observed in YVU-1 and YVU-2.

## DISCUSSION

Water stress due to drought is one of the most significant abiotic factors that limitthe seed germination, seeding growth, plants growth and yield (Hartmann *et al*, 2005, Van den Berg and Zeng, 2006). Several methods have been developed to screen drought tolerant germplasm in plant species. Based on the literature available, PEG is considered as a superior chemical to induce water stress (Kaur *et al*, 1998). Polyethylene glycol (PEG) molecules are inert, non-ionic, virtually impermeable chains and have been used frequently to induce water stress in crop plants (Carpita *et al*, 1979; Turkan *et al*, 2005; Landjeva *et al*, 2008; Rauf *et al*,2006). One of the important speculations is that a positive correlation between drought tolerance of the genotypes in the field and in laboratory experiments was noted (Kosturkova *et al*, 2014).

The AR germplasm showed more than 25% germination even at the higher dose of 16% PEG. Among investigated germplasm, the AR genotype showed low germination rate reduction with increasing PEG concentration than the other genotypes AV, YVU-1 and YVU-2 (Fig 1). The PEG inhibited the germination of the susceptible lines and caused them in record low germination percentage. Almaghrabi, (2012) reported that environmentally confined seedlings in laboratory experiments would appear to be suitable for screening large population to improve drought tolerance prior to yield testing. Usually the drought tolerant genotype will have the highest germination rate and better survival. The higher germination rates of the tolerant germplasm may be due to their capability to absorb water even under PEG induced water stress. Hegarty, (1997) and Turk *et al*, (2004) reported that water stress at germination stage delayed or reduced or hinder germination completely. However, once the grain attains a critical level of hydration it will lead to full seed germination. If, the physiological changes happen below the critical level it lead to complete inhibition of seed germination. Dodd and Donavon, (1999) stated that PEG induced reduction in germination percentage was because of reduction in the water potential gradient between seeds and their surroundings. Several reports on wheat varieties suggest that germination rate was affected by various abiotic stresses (Bayoumi *et al*, 2008; Jajarmi, 2009; Alaei *et al*, 2010). A higher level of germination under stress condition was observed in *Vigna aconitifolia*, however this finding may not be applicable to all cases and it depends on the germplasm used in screening (Soni *et al*, 2011). Similar results like reduction in germination rate with the increase PEG were noted in chick pea also (Kaur *et al*, 1998).

Strong negative correlation coefficient was noted between root length and PEG concentration with more than -0.81 correlation coefficient values. Roots are the primarily effected plant part under drought conditions than any other parts (Ghafoor, 2013). Root trait of all varieties provided useful information against different levels of PEG and this is very important attribute to study the drought stress. The germplasm which has better growth under stressed environment may have drought tolerance mechanism in it and these plants may have capability of holding a homeostasis under stressed conditions (Saxena and Toole, 2002). With few exceptions, the response of tomato varieties for root length was more or less similar against the

different levels of PEG. A gradual reduction in root length with an increasing concentration of PEG was the common tendency observed among all varieties. The reduction rate in root length is different in the varieties investigated. The root length at control varied in between 9.59 to 12.80 cm in the AR with mean root length 11.0 cm. At the highest concentration of PEG (14%) a drastic reduction in root length in all tomato varieties was noted (Fig 2). Among Tomato varieties AR has shown low reduction in root length. The AV and YVU-1 germplasm were highly affected in comparison to other varieties at higher PEG concentration. Worthy seed germination and seedling vigour may lead to deep and good root system and it permits seedlings to extract moisture in the soil. It is well known fact that root architecture influences the yield and other agronomic traits, particularly under stress conditions (Ludlow and Muchow, 1990; Dorlodot *et al*, 2007). Remarkable decrease in root length has been observed with increasing PEG concentrations was reported by Jajarmi *et al*, (2009) and similar results like reduction in root length with increasing osmotic stress was identified in pea plants (Whalley *et al*, 1998). Kulkarni and Deshpande, (2007) reported that early and rapid elongation of roots is a key trait of drought tolerance. Similarly, one of the important part noted in the present study i.e. AR showed better resistance to stress induced by PEG, it may be because of its hybrid nature.

A strong negative correlation between shoot length and PEG concentration has been observed and a positive correlation between shoot length and root length was identified and it clearly indicated that increase in root length helps in increase of shoot length. All the varieties showed common trend i.e. reduction rate in shoot length with increasing concentration of PEG with a lone exception at 2% PEG. The decline in shoot length traits in response to induced osmatic stress is a commonly observed phenomenon which is depends on the tolerance capacity of the plant. Decreasing in growth rate with increasing osmatic stress was reported in several studies (Waseem *et al*, 2006; Kulkarni and Deshpande, 2007; Abdel-Raheem *et al*, 2007; Aazami *et al*, 2010; Hamayun *et al*, 2010b). Careful analysis of the results revealed that, YVU-2 was the only germplasm in which a decline for all the traits was recorded against the drought stress. YVU-2 germplasm showeda lot of variation in shoot length than the other varieties but it did not showrapid decrease at higher concentration of PEG (Fig 2). A detailed physiological study is required for evaluating their variable response. Comprehensive investigations such as using various plant growth regulators (Hussain *et al*, 2010), proline accumulation under stress (Ali *et al*, 2011), anti-oxidants assays etc., on these varieties could givemore important information for selecting appropriate germplasm.

The germplasm which is showing better performance can be considered as drought tolerant. Hence, germplasm with the capability of early vigour under stress conditions may be beneficial by increasing seedling competitiveness against weeds (Lemerle *et al*, 2001). The early vigour of seedling with good development can be used as a trait of interest for the selection of tolerant germplasm (Richards, 2000; Botwright *et al*, 2002). Root system with the ability of better growth under stress conditions can be considered as tolerant germplasm (Abdel-Raheem *et al*, 2007). Siddique *et al*, (1990) explained that plants with better early vigour can increase the crop water use efficiency. Several reports indicated that better growth under stress conditions as a trait to select germplasm to improve the yield (Richards, 2000). Lemerle *et al*, (2001) reported that early seedling vigour may be useful by increasing seedling effectiveness against weeds.

## CONCLUSION

Water deficit is one of the abiotic stresses caused by less water source, extreme temperatures and low atmospheric humidity and it ultimately affects the plant productivity. Development of new varieties is one of the ultimate methods to overcome the problem associated with the drought stress. The development of new varieties could be assisted by screening of germplasm for higher drought tolerance. The present study was planned to standardize the screening procedure as well as to identify the better genotypes that can be useful to scientific community. All the germplasm showed strong negative correlation between PEG induced water stress and root length and similar results were noted with shoot length also. The germplasm AR showed better growth than others and this resistance may be due to its hybrid nature. Current study was also erudite for the optimization of appropriate concentration of PEG for screening thegenetic resource of tomato for further detailed studies. The results highlight the importance of the PEG as an artificial stress inducer for quick screening in the laboratory conditions for identification of drought tolerant germplasm for breeding programs in tomato.

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