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IN-VITRO SCREENING OF DIFFERENT TOMATO GENOTYPES AGAINST PEG INDUCED WATER STRESS

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ABSTRACT

Tomato (*Lycopersicum esculentum* L.) is one of the most economically important plants in the *Solanaceae* family. Water scarcity is one of the major climatic constraints which is affecting all crops including tomato in the world. Selection of water stress tolerant cultivars is an important strategy to overcome this problem. Therefore the present research was conducted to determine the effect PEG induced water stress on germination percentage and early growth stages of tomato seedlings. Fifteen different tomato genotypes were screened under *in-vitro* conditions using two different concentrations of PEG (2% and 4%). The experiment was arranged in a completely randomized design (CRD) with three replications. Parameters like germination percentage (%), root length (cm), shoot length (cm) and seedling biomass (g) were recorded during the experiment. Results indicated that PEG induced water stress significantly reduced germination percentage (%) and other growth parameters in drought susceptible tomato genotypes (RUS-1, Rustam, R-2017, Pakit, 17904, Kashmiri, Kiara, Avinash, and K.K.2). Whereas in other genotypes "R. Wonder, Naqeeb, Rio grande, T-4, Patfeeder and Nagina" all parameters increase with an increase in PEG concentration. However among these genotypes, "Patfeeder" exhibited highest germination percentage (100 %) with maximum root and shoot length and seedling biomass at highest PEG concentration 4%. Based on experimental results, "Patfeeder" was considered a drought tolerant genotype due to its better performance in different levels of water stress.

Key word: Drought, *in-vitro*, PEG, screening, seedling, tomato.
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INTRODUCTION

Drought and elevated temperature are the main abiotic stresses that affect crop productivity (Kumar *et al.*, 2017). But with an increase in global climate change and a growing human population, it makes water scarcity a significant threat. According to the World Water Council (WWC), about 66% of consumed water is used for irrigation purposes and in some regions; it reaches up to 90% (Piwowarczyk *et al.*, 2014). Such a huge amount of water shortage poses a great problem for crop production and reduces yield up to 70% (Shamim *et al.*, 2016).

Prolonged dry weather conditions significantly affect seed germination rates and seedling growth. Water stress causes a reduction in leaf relative water content and chlorophyll content which in turn related to a reduction in photosynthesis rate (Sivakumar *et al.*, 2018). Moreover, low water potential in cells causes induction of oxidative bursts that results in increased activity of antioxidant enzymes, protein accumulation and elevate levels of solutes. Drought disturbs the water balance in the plant bodies and adversely affects the plant's ability to uptake water. Damaging effects of water stress had been reported in many crops such as corn, tomato, citrus and soybean (George *et al.*, 2013). Plants respond to drought stress by initiating different defense mechanisms ranges from whole plant level (extended root system, and change the time of life cycle-maturity) to the cellular level (osmoregulation) (Aazami *et al.*, 2010). Plants tolerate drought by maintaining metabolic homeostasis with minimum injury. While plants susceptible to water stress fail to balance their metabolic activities thus causes a growth reduction, ultimately leads to plant death (Shamim *et al.*, 2016).

Tomato (*Lycopersicum esculentum* L.) is the widely grown vegetable throughout the world with an area of 484.8 thousand hectare and production up to 18230.1 thousand tons. In Pakistan, the total area under tomato cultivation is 63 thousand hectares with production of 601 thousand hectares respectively (FAO, 2017). Tomato is eaten in a fresh form such as a salad or consumed in different processed forms like sauces, ketchup, soup, puree and paste (Kumar *et al.*, 2017). Calcium, phosphorus, vitamin C and carbohydrates are present abundantly in tomato. Tomato also has medicinal value such as antitoxicity for kidneys and washes off poisons that infect the body system (Wamache, 2005). It reduces the risk of cancer, mainly head and neck cancer and also provides protection against neurodegenerative disorders (Rao and Balachandran, 2002). Tomato is a water-demanding crop, especially new varieties. It is very sensitive to drought stress, firstly during vegetative development and, later on, when the tomato is in the reproductive stage (Wudiri and Henderson, 1985). Although tomato requires a high quantity of water throughout their growth cycle, drought tolerance of each genotype may change with change in the developmental stage. A genotype can be considered as an ideal if it showed a tolerance at many growth stages with minimum injury to plant and thus these genotypes will be used for the breeding of drought tolerant cultivars and for the development of high yielding varieties (Brdar-Jokanović and Zdravković, 2015). The need to identify tomato genotypes that can tolerate water stress is vital for increasing crop production and this can only be achieved by exploring tomato germplasm (Basha *et al.*, 2015).

In the past, various efforts have been made to identify drought tolerant tomato genotypes, thus with the change in time, various innovative techniques have been employed to screen tomato genotypes that have a tolerance to drought (Basha *et al.*, 2015). Normally, under natural conditions, different factors i.e., environmental variation, soil heterogeneity and the use of a large amount of plants with a huge usage of labor and time make screening difficult in field trails. Whereas laboratory experiments are easy to conduct and produce reliable results (Razzaq *et al.*, 2016). Through *in-vitro* screening, a large number of genotypes were evaluated as *in-vitro* propagated plants have the ability to withstand stress even at different developmental stages (Aazami *et al.*, 2010). Also through *in-vitro* cultures, a large number of uniform plants are achieved. Furthermore, through this technique, a large plant population will be screened within a short time space and less space will be utilized. Tissue culture is an effective method to study the effect of drought stress on cell metabolism (Said *et al.*, 2015).

Polyethylene glycol (PEG) a high molecular non-penetrating osmotic compound, is a series of polymers that range from viscous liquids to waxy solids (Kumar *et al.*, 2017). It has been widely used to artificially induce water stress in plants. PEG has no toxic or detrimental effect on plants but it inhibits the plant growth by lowering the water potential of a growing medium (cultural media). Due to a reduction in water potential, explants are unable to uptake water and nutrients from the culture media thus inducing water stress (Said *et al.*, 2015). PEG lowers the water potential in the cultural medium due to its non-toxic nature, chemical inertness and difficulty to absorb by the plants (Baccari *et al.*, 2016).

OBJECTIVES

Keeping in view of the damaging effects caused by drought, the current study was conducted on artificially induced water stress with an aim to screen and identify tolerant tomato accessions.

MATERIALS AND METHODOLOGY

Experimental site: The research was conducted at Plant Tissue Culture Laboratory, Barani Agricultural Research Institute (BARI), Chakwal in 2019. During the experiment, the relative humidity and temperature were maintained up to 60-70% and 23-25 °C, respectively.

Plant material: Fifteen genotypes of tomato (*Lycopersicum esculentum* L.) were investigated against PEG-induced drought stress. The list of the genotypes is presented in Table 1. These tomato genotypes were collected from Plant breeding and Genetics (PBG) department, PMAS Arid Agriculture University Rawalpindi.

Rus-1	17904	Avinash
Rustam	Kashmiri	T-4
R-2017	Naqeeb	K.K.2
Pakit	Kiara	Patfeeder
R. Wonder	Rio grande	Nagina

Table 1: List of tomato germplasm selected for *in-vitro* screening against water stress.

PEG Assay: The screening of tomato germplasm was done under laboratory conditions using PEG-6000. Different concentrations of PEG (2% and 4%) were used for inducing stress conditions. Five seeds of each genotype was grown on a Whatman filter paper No. 1 placed in a petri dish. Seeds were spread on filter paper moistened with distilled water (control) and respective PEG treatments. Afterwards, seeds were covered with another layer of filter paper and petri dish lid to avoid moisture loss through evaporation. All petri dishes were transferred in an incubator for optimal germination. Distilled water and PEG solutions were regularly added to petri dishes when needed. An experiment was arranged in a completely randomized design (CRD) with three replications of each treatment were performed.

Data collection and design: Germination was tested as per ISTA rules (ISTA, 1985) and data of seed germination was recorded on the 6th day of seed sowing. On the basis of a number of seedlings germination percentage was noted (ISTA, 1985). Root length, shoot length and seedling biomass of randomly selected seedlings were calculated.

The data collected were subjected to the analysis of variance (ANOVA) using M-STATISTIX software and obtained means were compared by LSD at 5% level of significance (Steel *et al.*, 1997).

RESULTS

Germination percentage (%): Impact of PEG induced drought stress on different tomato genotypes germination percentage presented in Figure 1. Data analysis showed that significant differences were studied among different genotypes and PEG treatment. However, germination percent was insensitive of PEG concentrations and no significant difference was observed in between two concentrations. Approximately in all genotypes, germination percentage decrease with PEG treatment but in genotypes 'Patfeeder' 100% germination was examined in both PEG concentrations (2% and 4%). Whereas genotypes "R. Wonder, T-4 and Nagina" showed a slight increase in the germination percentage.

Root length (cm): Statistical analysis revealed that PEG induced water stress significantly reduced root length in different tomato genotypes, except for "R. Wonder, Rio grande, T-4, Patfeeder and Nagina" which exhibited longer roots as compared to control. However, among these genotypes, Patfeeder had a maximum root length (9.95 cm) at 4% PEG concentration whereas in control its root length is 5.05 cm (Table 2)

Shoot length (cm): In genotypes "R. Wonder, Naqeeb, Rio grande, Patfeeder and Nagina" shoot length significantly increase with an increase in PEG concentration, whereas "Patfeeder" had maximum shoot length (10.05 cm) at 4% PEG concentration (Table 2). Moreover, in genotypes "T-4 and Avinash" slight increase in shoot length was examined at the highest concentration of PEG. But in all other genotypes, PEG treatment significantly reduces shoot length.

Seedling biomass (gm): Among the given genotypes, maximum seedling biomass was observed in "RUS-1 and Patfeeder" (0.65 and 0.64 cm) at 4% PEG concentration followed by "Naqeeb (8.10 cm), Riogrande (8.10 cm) and Nagina (4.45 cm) respectively (Table 2). Whereas in other remaining genotypes, seedling biomass slightly decreases at the highest PEG concentration.

DISCUSSION

Seed germination rate and early seedling growth are important plant growth stages and are more prone to drought stress than other stages (Li *et al.*, 2013). The present study showed that PEG treatment affected the germination of susceptible genotype and results in significantly low germination percentage. Water stress at germination stage can delayed germination or completely inhibit germination (Chachar *et al.*, 2014). Moisture is one of the basic

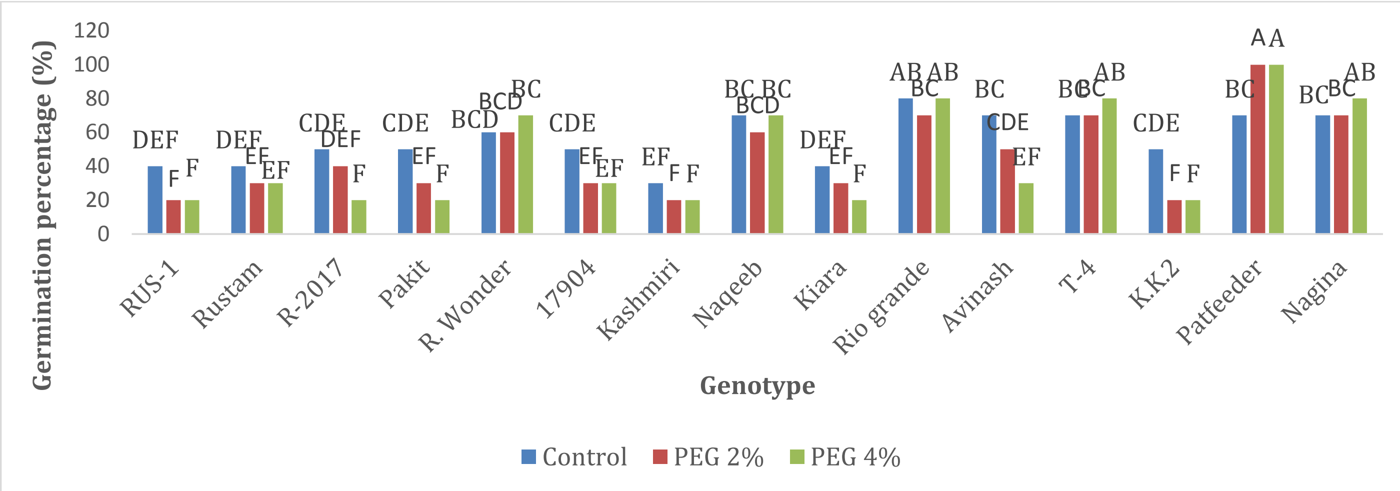

Figure 1: Impact of PEG induced water stress on germination percentage (%) of different tomato genotypes.

Genotype	**Root length (cm)**			**Shoot length (cm)**			**Seedling biomass (gm)**		
	Control	**PEG 2%**	**PEG 4%**	**Control**	**PEG 2%**	**PEG 4%**	**Control**	**PEG 2%**	**PEG 4%**
RUS-1	3.45 T	2.75 U	1.75 V	6.50 E	5.15 H	3.65 NO	0.33 JKL	0.27 NOPQ	0.65 A
Rustam	4.45 NOP	3.65 RST	3.45 T	5.85 FG	4.65 IJ	4.45 JK	0.31 KLM	0.26 PQ	0.26 PQ
R-2017	3.65 RST	2.95 U	1.40 W	5.90 F	4.85 HI	2.15 ST	0.38 FGHI	0.33 JKL	0.20 ST
Pakit	6.40 G	4.95 LM	3.60 RST	6.05 F	4.3 KL	3.55 OP	0.30 LMN	0.27 OPQ	0.25 QR
R.Wonder	4.55 NO	6.40 G	9.15 BC	6.40 E	7.60 D	8.20 C	0.49 D	0.51 CD	0.50 D
17904	7.10 F	6 H	3.90 QR	4.01 LM	3.65 NO	3.41 OP	0.28 NOPQ	0.25 QR	0.22 RS
Kashmiri	6.45 G	4.35 OP	2.95 U	3.90 MN	1.90 T	1.40 U	0.19 T	0.11 U	0.08 U
Naqeeb	8.40 D	7.95 E	8 E	4.05 LM	6.40 E	8.10 C	0.20 ST	0.39 FGH	0.455 E
Kiara	5.40 JK	3.85 QRS	1.45 VW	6.45 E	5.55 G	3.30 PQ	0.41 F	0.36 HIJ	0.19 ST
Rio grande	4.75 MN	5.75 HI	9.40 B	4.10 LM	6.50 E	8.10 C	0.39 FG	0.49 D	0.55 B
Avinash	5.55 IJ	4.25 OP	3.40 T	3.49 OP	3.49 OP	3.65 NO	0.38 FGHI	0.36 GHIJ	0.35 IJ
T-4	4.13 PQ	8.85 C	9.05 C	3.045 QR	3.10 QR	3.52 OP	0.21 ST	0.34 JK	0.54 BC
K.K.2	7.10 F	5.15 KL	3.55 ST	4.74 IJ	2.95 R	1.95 T	0.29 MNOP	0.21 ST	0.21 ST
Patfeeder	5.05 LM	8.15 DE	9.95 A	8.40 C	9.55 B	10.05 A	0.49 D	0.545 B	0.64 A
Nagina	5 LM	6.65 G	7.95 E	2.45 S	2.90 S	4.45 JK	0.30 MNO	0.29 MNOP	0.32 KLM

Table 1: Impact of PEG induced water stress on early growth stage of different tomato genotypes.

requirements for germination thus lowest germination percentage in different tomato genotypes was may be due to the reduction in imbibition of water by seeds because of decrease in water potential of growing medium. This water deficiency in seeds leads to certain metabolic changes such as degradation or inactivation of enzymes that slows the hydrolysis of reserved food in endosperm and transportation of that hydrolysis food product to the developing embryo (Toosi *et al.*, 2014; Channaoui *et al.*, 2019). Partap and Sharma (2010) suggested that decrease germination rate under PEG treatment was due to the osmotically enforced "dormancy", an adaptive response of seeds to overcome stress conditions thus ensuring healthy seedling establishment. But the highest germination percentage of "Patfeeder" depicted that it is drought tolerant genotype and have a better chance of survival under field conditions. Maximum germination percentage (100%) of "Patffeder" was due to its ability to absorb water from medium even under PEG induced water stress conditions (Basha *et al.*, 2015). It is reported that tolerance of treated seeds towards induced water stress at germination stage may have a better chance to the produced stronger root system and can develop healthy seedlings which can subsequently lead to the production of high green matter and the other traits like yield (Channaoui *et al.*, 2019).

During drought stress conditions, root plays an important role in plant survival as roots are in direct contact with soil and absorb water directly from it. It has been reported that roots suffer first from stress followed by other plant parts. Root length is an important trait against drought and genotypes with a longer and extensive root system are considered to be tolerant to drought (Kumar *et al.*, 2017) and these plants have the ability to maintain homeostasis under stressed conditions (Basha *et al.*, 2015). Production of longer roots in "R. Wonder, Rio grande, T-4, Patfeeder and Nagina" in response to water-deficient stress was due to certain gene expression, which activates under drought stress. Also these genotypes required a high amount of photosynthetic products for their growth, thus elongating roots to absorb water from deeper surface (Megha *et al.*, 2017). However, reduction in root length in different tomato genotypes were linked to the reduction in cell division or elongation at the root level (Ashagre *et al.*, 2014) that leads to a kind of tuberization. The tuberization and lignification cause plants to slow down their metabolic activities while waiting for the environmental conditions to become favorable for growth and development (Khakwani *et al.*, 2011).

Like root length, shoot length could also be used as an early and rapid selection criteria for screening of drought tolerant genotypes. Shoot length under PEG treatments significantly decreases in different tomato genotypes. The development of shorter shoots may be due to the fact that plants increases root length and root volume to extract water from the deep at the expense of shoot biomass. Also the reduction in shoot length was a defensive response of plants towards drought stress to slow down transpiration from the shoot surface (Megha *et al.*, 2017). Increase in root and shoot length under stress conditions in tomato genotypes (R. Wonder, Rio grande, Patfeeder and Nagina) were also reported by Basha *et al.* (2015) in tomato under PEG induced osmotic stress.

According to results, an increase in seedling biomass in genotypes "R. Wonder, Rio grande, Patfeeder and Nagina" was due to formation of longer roots and shoots. Moreover, during stress conditions different inorganic and organic solutes are started accumulating in the cytosol in order to maintain the turgor pressure and the potential gradient for effective uptake of water from the medium (Razzaq *et al.*, 2016). It was observed that although PEG induced water stress reduces both root and shoot length in genotype "RUS-1" but it has maximum seedling biomass which may be due to the production of shorter but stronger and thicker roots and shoot which increase the fresh weight of seedling. Whereas other genotypes produce longer roots and shoots but their roots and shoots are thin and fragile.

CONCLUSION

In-vitro screening is an effective technique for screening of a large number of tomato germplasm against PEG induced water stress. Different tomato genotypes respond differently to water stress in terms of germination and seedling early growth parameters. At highest PEG concentration 4%, genotypes "R. Wonder, Naqeeb, Rio grande, T-4, and Nagina Patfeeeder" were considered as tolerant to water stress whereas "Patfeeder were evaluated as relatively more tolerant to drought due to its better performance against induced stress. These genotypes will be further evaluated under field conditions for better evaluation of drought tolerance.

CONFLICT OF INTEREST

Author has no conflict of interest

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