Journal of Plant Sciences
2020; 8(5): 123-133
http://www.sciencepublishinggroup.com/j/jps
doi: 10.11648/j.jps.20200805.14
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

In-vitro Screening of Selected Accessions of Wheat (*Tritium Aestivum L.*) Variety for Drought Tolerance in Ethiopia

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To cite this article:

Yirgashewa Asfere, Dejene Zenabu, Eneyew Kassahu, Abdulkerim Adam, Abi Kebede, Alemi Gasha, Aleminesh Dida, Alemshet Atlaw, Biyensh Tefera, Tigist Wolore. *In-vitro* Screening of Selected Accessions of Wheat (*Tritium Aestivum L.*) Variety for Drought Tolerance in Ethiopia. *Journal of Plant Sciences*. Vol. 8, No. 5, 2020, pp. 123-133. doi: 10.11648/j.jps.20200805.14

Received: September 27, 2019; **Accepted**: November 4, 2019; **Published**: September 24, 2020

Abstract: Wheat (Tritium aestivum L.) is among the major staple crops, with about 720 million tons being produced globally. It is also an important cereal crop in Ethiopia that is widely cultivated in a wide range of altitude. But its production is limited highly by drought, which affects the growth and yield of wheat grain. The present investigation was carried out to study the drought tolerance of wheat accessions (5011, 5435, 7145 and 7284) for drought tolerance. The four wheat genotypes were screened in in-vitro method by using Polyethylene glycol (PEG 6000 MW) as a drought simulator to assess their seedling response under drought stress. Four PEG concentrations, 0 (0bar ψw), 50gm/l (-1.5bar ψw), 100gm/l (-2.6bar ψw), and 150gm/l (-3.91bar ψw) of PEG was used to simulate a drought stress for the screening experiment. The experiment was laid in completely randomized design with three replications. Data were recorded on shoot length, root length, total fresh weight, total dry weight, leaf number and root number at four different levels of treatments. Except fresh weight, the parameter of all accessions was decreased with the increasing concentration of PEG concentration. Screening of the four wheat accessions for tolerance to drought at growth stage using various concentrations of PEG 6000 resulted in the identification of two accession namely, 5435 and 7284 compared to the rest accessions with tolerant to drought and accession 5435 was identified as a drought tolerant compared to 7284. The in-vitro screening of wheat growth using PEG 6000 can be considered as a simple, rapid and preliminary bioassay that can be used in mass screening for evaluating seedling of wheat genotypes under drought.

Keywords: Drought Tolerance, *in-vitro* Screening, PEG (Polyethylene Glycol), Wheat Accessions

1. Introduction

1.1. Background of the Study

Food security is a great challenge for the world at least another 40 years due to the continuous increase in the population and high consumption growth rate [1]. Wheat (Triticum aestivum L.), is one of the most important cereal crops though out the world, according to an estimate [2]. It is among the major staple crops, with about 720 million tons being produced globally. Wheat food calories consumption is almost 20 percent. In sub-Saharan Africa (SSA), the crop is grown by millions of resource poor small holder farmers predominantly under rain-fed conditions. Wheat consumption SSA is increasing by approximately 650,000,000 tons per year [3]. Unavailability of the adequate water supply is a very crucial factor affecting the plant growth and development. Ultimately it seriously decreases food production. Drought is one of the major abiotic stress which causes low yield especially in arid and semi-arid regions of the world [4, 5]. Due to the climate change, severe droughts are expected in future. Thus, the wheat yields need to be increased in order to meet the food demands of growing populations [6]. Agriculture is the largest sector of employment and main source of livelihood in Ethiopia. Nearly 85% of the population depends directly on farming

In Ethiopia, it is the third major cereal crop in terms of area and production next to teff (Eragrostisteff) and barley (Hordeumvulgare) and followed by maize (Zea mays) and Sorghum (Sorghum bicolor (L) Moench) [7]. Among those grains wheat is an important cereal crop in Ethiopia that is widely cultivated in a wide range of altitude [8]. Ethiopia is the largest wheat producing country in Sub-Saharan Africa, with annual production of more than 4 million tons of grain on 1.6 million hectares of land which accounted for 13% of total land allotted to cereals [9, 10]. It is the main staple food for about 36% of the Ethiopian population [11, 12, 13]. The national average yield of wheat in Ethiopia is about 1.83 t/ha [11]. This is far by bellow the world's average which is about 2.5 t/ha [13]. Multifaceted biotic and abiotic factors are responsible for this low yield. Abiotic stress is the most serious threat to agriculture in many parts of Ethiopia resulting in increased desertification. Abiotic stress limits crop productivity [14], and plays a major role in determining the distribution of plant species across different types of environments. Drought or water stress is a major abiotic factor that limits plant growth and productivity. It is one of the major causes of crop loss Worldwide commonly reduces average yield for many crop plants by more than 50% [15, 16]. The Ethiopian agriculture is mainly rain-fed in that its performance is highly dependent on the timing, amount and distribution of rainfall [17]. This makes the sector vulnerable to drought and other natural calamities. Due to the changing global climate, the rain fall trend is also changing [18, 19, 20]. Decreased ψw (decreased free energy of the water) makes it more difficult for the plant to take up water, and this in turn elicits a range of responses that allow the plant to avoid water loss, allow water uptake to continue at reduced ψw or allow the plant to tolerate a reduced tissue water content. Apparently, under drought stress conditions, an urgent need for plants would be to increase the uptake of water, which is usually more available deep down in the soil [21]. 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 [22, 23] salt [24] flooding [25] low temperature [26] and high temperature [27]. In vitro selection technique has been used to improve abiotic environmental stresses such as cold hardiness, salt tolerance and drought tolerance [28, 29, 30]. One of the screening techniques based on physiological traits is the use of various osmotica to induce stress in plant tissues. Germination in mannitol and polyethylene glycol (PEG) has been suggested for drought screening [31, 32]. Polyethylene glycol (PEG) compounds used to induce osmotic stress in petridish (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 [33, 34, 35, 36, 37]. PEG induced osmotic stress is induct to decrease cell water potential [38]. The upsurge inconcentration of PEG caused a decrease in germination percentage, seedling vigour in certain crop plants [39]. 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 [40, 41]. The Ethiopian wheat germplasm was extensively studied for its variability in agro-morphological and molecular traits [42, 43]. However, most of the previous studies were focussed on the final crop growth stage such as yield and yield related traits, which had overlooked the importance of seedling evaluation for water stress resistance. Therefore, the present study was conducted to evaluate the phenotypic variability among four Ethiopian bread wheat genotypes and to identify the most tolerant genotypes for early-stage water stress. Artificial drought stress conditions was created and Polyethlylene glycol (PEG-6000) was used as drought stimulator as it is considered as non-penetrable, harmless and best way to create the drought stress condition [40, 41]. Parametrs, fresh weight (g), shoot and root length (cm), dry weight (gm), number of leaf and number of leaf was assessed to determine drought resistant wheat accessions from the selected four varieties.

1.2. Statement of the Problem

In some parts of our country which have efficient rain are able to cultivate wheat, but in most part of the country farmers can not able to produce the grain throughout the year, because of shortage of rain that is consequently related to shortage of water or drought. Farmers around the research area (WolkiteUniversity, ChehaWoreda in Gurhage Zone, SNNPR) also have difficulties to cultivate wheat due to drought. The use of sophisticated technologies and modern irrigation system is difficult to apply, costy, and in appropriate in our country (Ethiopia) to solve this problem. Also complex biotechnological methods such as, genetic engineering, to create drought resistant wheat for solving drought problem is costy, require sophisticated equipment and skilled man power for their operation. To overcome this snag, screening and selection of good drought tolerant wheat accessions by using *In vitro* screening method instead is a great importance with less effort, cost effectiveness, accurately and the growth pattern differences are due to genotypes with least environmental influences. In this study selected accessions of wheat from different parts of the country were screened in vitro for their drought resistant ability to solve the problem of farmers of ChehaWoreda and the whole parts of our country (Ethiopia). Therefore, the aim of this study was to screen out drought resistant wheat varieties by using PEG as drought enhancer.

1.3. Significance of the Study

The finding of this study suggests the possible solution of wheat cultivation problems due to drought that leads the community not to produce wheat. Help the researchers to elaborate the knowledge and how to screen out drought resistant wheat and other grain varieties from different

accessions. And it serve as a reference material for other researchers who are interested to do such type of researchers in other areas.

1.4. Scope of the Study

The study was completed at the tissue culture level after germinated seeds were incubated for 18 days and the study's conclusion was made based on tissue culture result

2. Methodology

2.1. Study Area

This study was conducted at Wolkite University, Biotechnology Department of tissue culture laboratory. The university is found in Wolkite, the administrative center of the Gurhagie Zone of the Southern Nations, Nationalities and People's Region (SNNPR), this town has latitude and longitude of 8°17′N 37°47′E and an elevation between 1910 and 1935 meters above sea level (Wikipedia: wolkite). The town is about 155 km far from Addis Ababa. In winter, there is much less rainfall in Wolkite than in summer. The average annual temperature is 18.6°C. The rainfall averages 1244 mm (climate: wolkite, from: climate-data.org).

2.2. Plant Material

Wheat varieties were collected from Ethiopian Biodiversity Institute. As whole five wheat varieties were collected. Out of five varieties, one accession was used for protocol optimization (to see the effect of NaOCl on the germination and media contamination by using different concentration of it). And the rest four varieties were used for the screening experiment.

2.3. Experimental Design

The experiment was conducted with protocol optimization, in which the result of it was used in the screening experiment. First, one type of wheat accession explants were treated with different concentration (1%, 1.5% and 2%) of sodium hypochlorite (in the form of Chlorox bleach) to determine the best sterilization concentration of sodium hypochlorite and the control without NaOCl sterilization. Then, the best sterilization concentration of sodium hypochlorite was determined. After, four different varieties of wheat seed was sterilized by the determined NaOCl concentration and was allowed to germinate on Petridis containing filter paper, and it was transferred to media containing different concentration of PEG 6000. Four PEG 6000 concentrations, 0 (0bar ψw), 50gm/l (-1.5bar ψw), 100gm/l (-2.6bar ψw), and 150gm/l (-3.91bar ψw) of PEG was used to simulate a drought stress for the screening experiment. Allowed to germinate on Petridis containing filter paper, and it was transferred to media containing different concentration of PEG 6000. Four PEG 6000 concentrations, 0 (0bar ψw), 50gm/l (1.5bar ψw), 100gm/l (-2.6bar ψw), and 150gm/l (-3.91bar ψw) of PEG was used to simulate a drought stress for the screening experiment.

2.4. Stock Solutions and Growth Regulators Preparation

In this study, (Murashige and Skoog, 1962) MS media was used along with the proper type and concentration of plant growth regulator. A stock solution for each of the MS components (Appendix Table 1) was weighed in recommended amounts and completely dissolved in double distilled water by grouping it in five group based on their solubility nature (Appendix Table 1) in order to reduce bottles consumption and stored at +4°C. The stock solutions of MS nutrients, vitamins and amino acid were prepared fresh every two week. Iron and Na2. EDTA mix stock solution was protected from light by storing the solution in bottles wrapped with aluminium foil to protect Iron from degradation.

2.5. Medium Preparation

After MS and plant growth regulators (pgrs) stock solutions were prepared and mixed appropriately, liquid media enriched with 30 g/l sucrose was prepared. The P^H of the medium was adjusted at 5.8 and different concentration of PEG 6000 (0, 50gm/l, 100gm/l and 150gm/l) solution was added. And it was autoclaved at a temperature of 121°C with a pressure of 103 kpa for 20 minutes [44]. But for protocol optimization, solid media containing 8gm/l of agar without PEG solution was prepared.

2.6. Culture Establishment Procedure

2.6.1. Protocol Optimization: Determination of Best Sterilization Concentration of Naocl

One accession wheat seed was washed using tap water. Then the explants were washed thoroughly under running tap water with Largo and Tween 20 with slight shaking and washed tile removed Largo and Tween 20. The explant was then dipped in 70% (v/v) ethanol for 5 min and then rinsed three times with sterile distilled water in aseptic condition in the laminar air flow hood. Followed by treatment with different concentration of NaOCl (0, 0.5%, 1% and 1.5%) of sodium hypochlorite (in the form of Chlorox bleach) with 3 drops of Tween 20 for 15 minutes for disinfection. Explants was then rinse with sterile distilled water 3 times. Sterilized seed explants were inoculated for on a jar containing MS medium to collect explants contamination data under different concentration of NaOCl. After 15 days of incubation in growth chamber at a temperature of 25 ± 2°C and at light conditions of 16 hours photoperiod of white fluorescent light of (20 μ mol/ m^2/s) intensity in growth chamber

2.6.2. Sterilization of Seed Explants

Explants were sterilized by the same method that was used for optimization with the determined best sterilization concentration of NaOCl (1.5%).

2.7. Seed Germination

After Seed explants of different varieties was sterilized with 1.5% of NaOCl for 15 min in laminar air flow cabinet,

seeds of each accessions were placed onto the seed germination and shoot establishment Petridis containing filter paper (whatman paper 1) by using sterilized forceps. 1ml of sterilised distilled water was added and two replicates of 15 seeds per Petridis were used.

The seeds were spread to prevent malt formation and it was allowed to stay up to 36 hours in a dark room at a temperature of 25 ± 2°C. Seeds were considered to be germinated when radicle was emerged approximately ≥ 0.75 mm. According to these studies [44, 45] initiation of seedling in PEG unstressed media and transfer to stressed media facilitate dehydration of the seedlings, which is useful to study plants response to drought rapidly.

2.8. Inoculation of Germinated Seed underPEG Stress Condition

Seeds emerged approximately ≥0.75mm was inoculated to a Petridis containing a liquid medium enriched with 30 g/l Sucrose and supplemented with 0, 50gm/l, 100gm/l, 150gm/l of PEG6000. The petridishes were sealed and incubated at a temperature of 25 ± 2 °C and at light conditions of 16 hours photoperiod of white fluorescent light of (20 µ mol/ m^2/s) intensity in growth chamber. Three replicates of three seeds per petridish were used and it was kept in incubation for 12 days. Both the medium with PEG and control was re-supplemented after seven Days of growth.

2.9. Data Collected and Data Analysis

The experiment of the study was conducted out in a completely randomized design in factorial arrangement with three replications [46]. There were four treatments including control for each wheat varieties which was replicated three times After 18 days of growth period, data was recorded to investigate the effect of PEG at different treatment on shoot length, root length (cm), fresh weight (gm), dry weight (gm), number of leaf and number of root. Shoot length (cm) was measured from the base to the apex of the shoots and root length (cm) was measured from the base of the root to the root tip using a scale. Leaf number and root number of each plantlet per replication was recorded. Fresh weight (gm) of plantlets was measured by using measuring balance. Dry weights (gm.) of plantlets were measured by electric balance after the plantlets was incubated at 120 ^oC for 24 hours. Data was analyzed by SPSS and means were compared by Least Significant Difference (LSD) test at 5% level of probability for results [47] and the mean differences were adjusted by one way ANOVA (Post Hoc Tests) of multiple comparison. Bar charts was used to show the mean differences of accessions at different concentration of PEG.

3. Result and Discussion

3.1. Shoot Length of Wheat Accessions Under Drought Stress

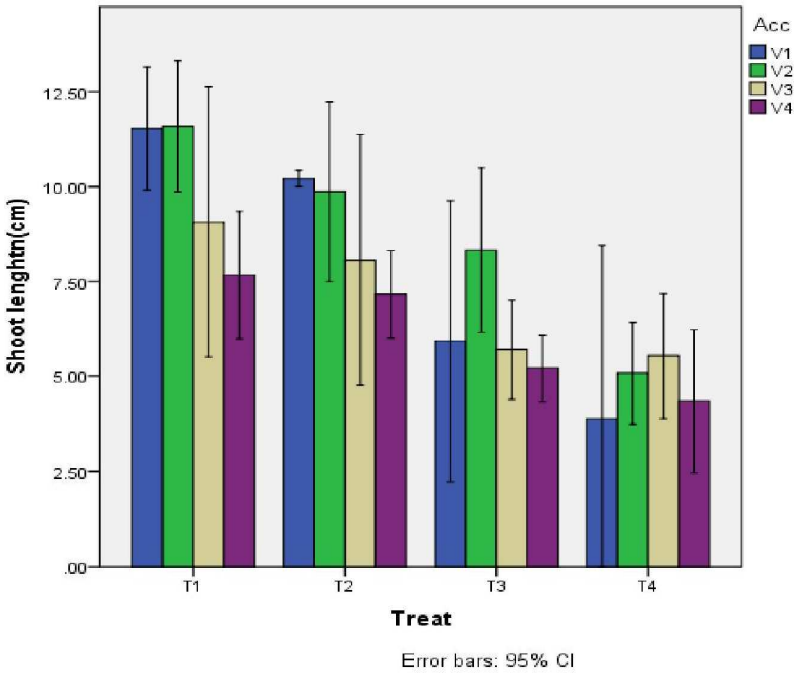

Figure 1. *Mean shoot length of four wheat accessions.*

The comparison of shoot length on the media with and without selective agent revealed that shoot length was adversely affected by PEG. And their was a significant difference (Appendix table 2a and figure 1) at 5% level of probability. Shoot length compared to media without the addition of PEG showed substantial differences between the accession genotypes studied. The highest shoot length was observed on 5011 (12.5) and 5435 (12.2) in control

experiment and the lowest was recorded on 5011 (2.5cm) in highest concentration of PEG (150gm/l). These results indicated that the high level of water stress negatively influences the shoot length of wheat accessions. All shoot length of all accessions decreased from control to highest concentration of PEG (150gm/l), these results corroborates with the findings of these studies [38, 48], in which they had also observed the retardation in growth of shoot and root length in response to increasing moisture stress under field as well as laboratory condition.

3.2. Root Length

Root length showed a significant difference (Appendix table 2b and figure 2) at 5% level of probability. Early and rapid elongation of root is an important indication of drought tolerance. A root system with longer root length at deeper layer is useful in extracting water in upland conditions [49, 48]. In the present investigation, the root length also significantly declined with increased external water potential and consequently, all treatments caused a decrease in root elongation in all genotypes compared to their controls. Out of the four accession genotypes studied, the highest root length (4.6cm) was observed on 5011 in control and the lowest on 7145 (0.5 cm) in 100gm/l of PEGThe mean root length was varied from 4.07cm (5011 in control) to 0.87cm (7145 in 100gm/l of PEG). The accession 5011 showed a significant root length in control and in 50gm/l of PEGsolution alone and it was highly affected by 100 and 150gm/l of PEG. 7145 showed a smalldifference between in control and media with a high concentration of PEG. Besides, 5011 and 7145, accession 5435 and 7284 showed a greater performance in high concentration of PEG with a reduction from control to media with 150 gm/l of PEG. At higher concentration of PEG (150gm/l), accession 5435 recorded highest root length of 3.2 cm when compared to the rest accession.

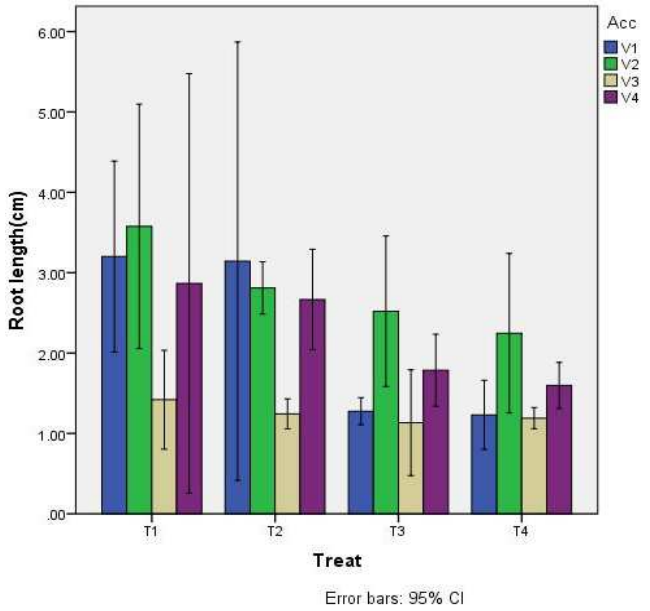

Figure 2. *Mean root length of four wheat varieties.*

The decreasing of root length may due to the drought. So, the high value recorded in 5435 maydue to its resistant to drought. And the result showed agreement with the study made in Iranian almond seedlings [50]. Long roots was reported as a component trait for drought tolerance by these researchers [38] and [51] as they play a direct role with high penetration ability and have large xylem vessel radii and lower axial resistance to water flux aiding in greater water acquition.

3.3. Total Fresh Weight

The highest value of total fresh weight (0.25gm) was recorded in 7284 at 100gm/l of PEG, while the lowest value was recorded in 5011, 5435, and 7145 at 150gm/l of PEG and in 7284 at 50gm/l of PEG. Even though drought has a negative effect in total fresh weight of a plant, significant reduction of total fresh weight was not observed. But 5011 and 5435 showed a reduction of total fresh weight across control to the highest concentration of PEG (150gm/l).

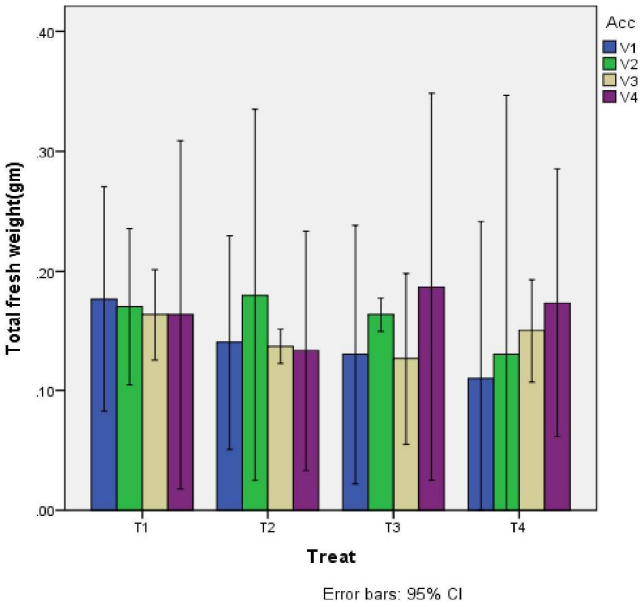

Figure 3. *Mean total fresh weight of the four wheat accession.*

From the result obtained, 5435 showed highest in control, 5435 in 50gm/l and 7284 in 100gm/l and 150gm/l compared to other accessions and 7284 showed highest total fresh weight at high concentration of PEG (150gm/l and 100gm/l). No significant changes in total shoot were observed inresponse to drought stress treatments (Appendix table 2c and figure 3). Therefore, it seems that this trait may not be used as a drought stress marker in wheat varieties.

3.4. Total Dry Weight

Except 5011, which showed a significant reduction in total dry weight with increasing stress conditions, all accessions showedconsiderably constant dry weight with increasing of stress condition (Appendix table 2d and figure 4) And 5435 showed considerably constant total dry weight from constant

to 150gm/l of induce media with a very small difference between them. Content of dry weight in 7145 increased the most and in 5011 the least under the influence of severe osmotic stress generated by the highest dose of PEG (150g/l).

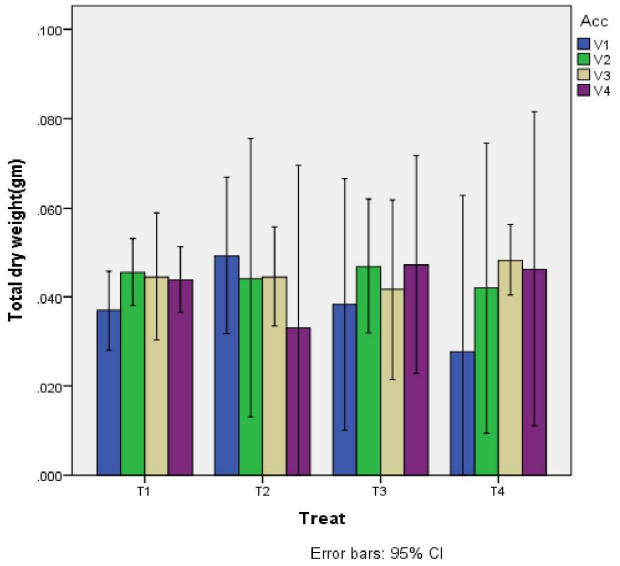

Figure 4. *Mean total dry weight of the four wheat varieties.*

As suggested by [52] decreasing root DW under drought conditions may be caused by a decrease in the accumulation of root carbohydrates. A decrease in dry matter may be due to the considerable reduction of photosynthesis and plant growth [53]. Therefore, plants with high amounts of dry mass under drought stress can be considered as drought tolerant genotypes. But in this study, increasing of stress condition was not showed a decrease in dry weight in all varieties and agrees with the conclusion made by [54].

3.5. Leaf Number

Although leaf number showed significant difference (Appendix table 2e and figure 5) at 5% level of probability, leaf number of wheat varieties showed small differences after 18 days of inoculation. At high stress conditions (100 1nd 150gm/l of PEG), 3 leafs were recorded only in 5435 and only two leafs were recorded in the rest of varieties. However, 3 leafs per explants was recorded in control and 50gm/l of PEG induced media. During water stress, depending on the intensity and duration of the drought, plants tend to minimize transpirational water loss by reducing their number of leaves [55].

3.6. Root Number

The root number of all studied wheat variety accessions was showed a considerable decrease with increasing of stress condition (Appendix table 2f and figure 6). The highest root number (7 per one explants) was observed on 5435 at 50gm/l of PEG and thelowest (2) was recorded on 5011 (in 100gm/l of PEG), 7145 (in 50 and 100gm of PEG) and on 7284 (in 50 and 150gm/l of PEG). Out of the four studied varieties, 5435 showed a high mean root number in low and highly stressed media. The ability to maintain the number of roots in sunflower accession indicates the drought tolerance. Drought stress conditionsfavours the lateral rootsparticularly in seedlings [56].

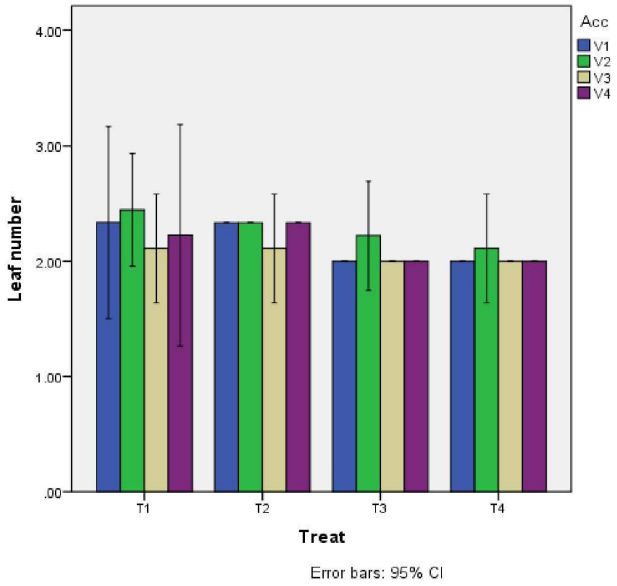

Figure 5. *Mean leaf number of the four wheat varieties.*

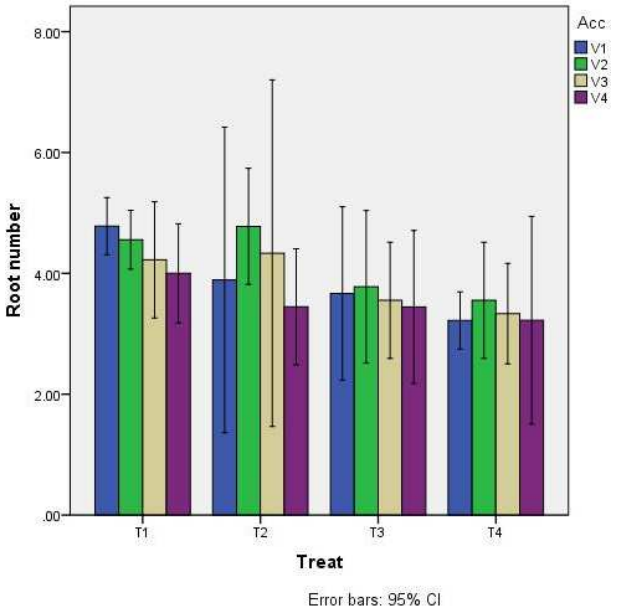

Figure 6. *Mean root number the four wheat accessions.*

In this experiment increase innumber of roots may be due to enhance the water uptake under PEG mediated water deficit conditions. Roots numbers were also increased in sugarcane on culture media supplemented with the PEG concentrations [57].

4. Conclusions and Recommendation

4.1. Conclusions

In the present study, the results proved the use of PEG 6000 for the experimental control of external water potential

as an effective method for studying the effect of water stress on growth characters and also showed it as a simple cost effective method to screen large set of germplasm within very less time period and accuracy, which is in accordance with [38]. In this experiment, most of the parameters were negatively affected by the increase of polyethylene glycol levels. It might be due to the drought stress induced by PEG for plant which reduced the shoot length, root length, leaf number and root number. The results from the present study indicated that accession 5435 and 7284 were drought tolerant and these two accessionsmay be utilized as a selection indicator for breeding program and used as a baseline for improvement of wheat varieties in Ethiopia. Therefore, in vitro method to screen out drought tolerant wheat accessions by using PEG as a drought simulator would be a simple, rapid and cost effective method for screening growth traits of large set of germplasm for drought tolerance.

4.2. Recommendation

Although, the results of this study clearly showed that screening of wheat accessionsunder field conditions provide efficacy in vitro screening method for drought tolerance, the effectiveness of this approach, should be further tested on theses wheat accessionswith known performance for root and shootgrowth characteristics related to drought-tolerance under field conditions. Further study is needed on different locations by including other accessions to account to identify genotypic differences for drought tolerance among varieties. As we observed, using a highly controlled environment and effective sterilization method for the prevention of contamination would be good to know the genotype difference of wheat accessions. (And learning how data's can be analyzed would be important for effective and rapid data analysis).

Appendix

Table 1. *Murashige and Skoog (MS) media composition for many plant species culture.*

(I) Treat	(J) Treat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
T1	T2	1.50250	.76956	.057	-.0484	3.0534
	T3	4.10833*	.76956	.000	2.5574	5.6593
	T4	6.16000*	.76956	.000	4.6091	7.7109
T2	T1	-1.50250	.76956	.057	-3.0534	.0484
	T3	2.60583*	.76956	.002	1.0549	4.1568
	T4	4.65750*	.76956	.000	3.1066	6.2084
T3	T1	-4.10833*	.76956	.000	-5.6593	-2.5574
	T2	-2.60583*	.76956	.002	-4.1568	-1.0549
	T4	2.05167*	.76956	.011	.5007	3.6026
T4	T1	-6.16000*	.76956	.000	-7.7109	-4.6091
	T2	-4.65750*	.76956	.000	-6.2084	-3.1066
	T3	-2.05167*	.76956	.011	-3.6026	-.5007

Note: 1. in preparation of stock solutions each nutrient should completely dissolve in distilled water before adding the next nutrient then the final volume should be adjusted by adding distils water.

2. For the case of Iron and EDTA stock solution preparation both should be dissolved in slightly hot water separately and after dissolving add Iron solution over EDTA: Never Opposite way it may case firing. Then make final volume by distil water.

Table 2. *Post Hoc Tests of Multiple Comparision.*

2a) Multiple Comparisons of shoot length

Dependent Variable: SL

(I) Treat	(J) Treat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
T1	T2	1.50250	.76956	.057	-.0484	3.0534
	T3	4.10833*	.76956	.000	2.5574	5.6593
	T4	6.16000*	.76956	.000	4.6091	7.7109
T2	T1	-1.50250	.76956	.057	-3.0534	.0484
	T3	2.60583*	.76956	.002	1.0549	4.1568
	T4	4.65750*	.76956	.000	3.1066	6.2084
T3	T1	-4.10833*	.76956	.000	-5.6593	-2.5574
	T2	-2.60583*	.76956	.002	-4.1568	-1.0549
	T4	2.05167*	.76956	.011	.5007	3.6026
T4	T1	-6.16000*	.76956	.000	-7.7109	-4.6091
	T2	-4.65750*	.76956	.000	-6.2084	-3.1066
	T3	-2.05167*	.76956	.011	-3.6026	-.5007

*. The mean difference is significant at the 0.05 level.

2b) Multiple Comparisons of root length
Dependent Variable: RL

(I) Treat	(J) Treat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
T1	T2	.30000	.32064	.355	-.3462	.9462
	T3	1.09500*	.32064	.001	.4488	1.7412
	T4	1.20000*	.32064	.001	.5538	1.8462
T2	T1	-.30000	.32064	.355	-.9462	.3462
	T3	.79500*	.32064	.017	.1488	1.4412
	T4	.90000*	.32064	.007	.2538	1.5462
T3	T1	-1.09500*	.32064	.001	-1.7412	-.4488
	T2	-.79500*	.32064	.017	-1.4412	-.1488
	T4	.10500	.32064	.745	-.5412	.7512
T4	T1	-1.20000*	.32064	.001	-1.8462	-.5538
	T2	-.90000*	.32064	.007	-1.5462	-.2538
	T3	-.10500	.32064	.745	-.7512	.5412

*. The mean difference is significant at the 0.05 level.

2c) Multiple Comparisons of Total fresh weight
Dependent Variable: FW

(I) Treat	(J) Treat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
T1	T2	.02042	.01765	.254	-.0152	.0560
	T3	.01825	.01765	.307	-.0173	.0538
	T4	.02983	.01765	.098	-.0057	.0654
T2	T1	-.02042	.01765	.254	-.0560	.0152
	T3	-.00217	.01765	.903	-.0377	.0334
	T4	.00942	.01765	.596	-.0262	.0450
T3	T1	-.01825	.01765	.307	-.0538	.0173
	T2	.00217	.01765	.903	-.0334	.0377
	T4	.01158	.01765	.515	-.0240	.0472
T4	T1	-.02983	.01765	.098	-.0654	.0057
	T2	-.00942	.01765	.596	-.0450	.0262
	T3	-.01158	.01765	.515	-.0472	.0240

*. The mean difference is not significant at the 0.05

2d) Multiple Comparisonsof Total dry weight
Dependent Variable: DW

(I) Treat	(J) Treat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
T1	T2	.01053	.02121	.452	-.0223	.0654
	T3	.00826	.02121	.401	-.0212	.0432
	T4	.00984	.02121	.022	-.0090	.0789
T2	T1	-.01046	.02121	.321	-.0432	.0202
	T3	-.01218	.02121	.812	-.0344	.0222
	T4	.00944	.02121	.423	-.0111	.0444
T3	T1	-.02228	.02121	.897	-.0123	.0189
	T2	.00177	.02121	.765	-.0334	.0267
	T4	.00168	.02121	.675	-.0240	.0568
T4	T1	-.03984	.02121	.111	-.0555	.0057
	T2	-.01943	.02121	.221	-.0351	.0344
	T3	-.00151	.02121	.526	-.0567	.0123

*. The mean difference is significant at the 0.05 level

e) Multiple Comparisons of Mean leaf number
Dependent Variable: LN

(I) Treat	(J) Treat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
T1	T2	.02042	.01765	.254	-.0152	.0560
	T3	.01825	.01765	.307	-.0173	.0538
	T4	.02983	.01765	.098	-.0057	.0654
T2	T1	-.02042	.01765	.254	-.0560	.0152
	T3	-.00217	.01765	.903	-.0377	.0334
	T4	.00942	.01765	.596	-.0262	.0450
T3	T1	-.01825	.01765	.307	-.0538	.0173
	T2	.00217	.01765	.903	-.0334	.0377
	T4	.01158	.01765	.515	-.0240	.0472
T4	T1	-.02983	.01765	.098	-.0654	.0057
	T2	-.00942	.01765	.596	-.0450	.0262
	T3	-.01158	.01765	.515	-.0472	.0240

*. The mean difference is significant at the 0.05 level.

f) Multiple Comparisons of Mean root number
Dependent Variable: RN

(I) Treat	(J) Treat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
T1	T2	.0234	.02121	.278	-.0222	.0100
	T3	.0246	.03232	.111	-.0004	.0547
	T4	.0678	.0655	.123	-.0057	.0156
T2	T1	-.01234	.07622	.345	-.0540	.0452
	T3	-.00900	.06517	.309	-.0455	.0634
	T4	.00133	.03451	.632	-.0377	.0850
T3	T1	-.0223	.04567	.703	-.0212	.0073
	T2	.00076	.06789	.309	-.0777	.0977
	T4	.02341	.01876	.155	-.0288	.0572
T4	T1	-.00012	.05671	.008	-.0555	.0157
	T2	-.00666	.01116	.021	-.0543	.0162
	T3	-.02222	.02344	.123	-.0237	.0340

*. The mean difference is significant at the 0.05 level.

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