
IN VIVO EVALUATION OF WHEAT (*Triticum aestivum* L.) CULTIVARS FOR MOISTURE STRESS

S. Tamiru* and H. Ashagre

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Abstract

In Ethiopia, wheat productivity is constrained by water stress especially at germination and seedling stage. The objective of this research was to determine the effect of moisture stress on bread wheat (*Triticum aestivum*) cultivars. Four wheat cultivars (Danda'a, Kubsaa, Huluka and Local) were treated with six levels of water stress (0, 50, 100, 150, 200 and 250 g L⁻¹) using PEG-6000. The experiment was arranged factorially in completely randomized design with three replications. Data on germination and growth indices were collected and analyzed using one way ANOVA. The result revealed that highest germination percentage (99.7%), germination rate (14.6 plants day⁻¹) and seedling vigor index (9.67) were obtained from the local cultivar. Progressive increase in water stress had also significantly reduced germination indices. There was no significant difference among the cultivars in producing taller root and shoot length. The local cultivar had significantly produced highest root number (4.3 plant⁻¹), shoot fresh weight (0.214 g), seedling fresh (0.314 g) and dry weight (0.097 g). Even though, all the growth parameters of wheat cultivars showed a diminishing trend with increasing the level of PEG-6000 induced water stress, a highly significant decrease in the parameters were observed starting from 150 g L⁻¹ PEG concentration. Higher shoot length inhibition was observed for Danda'a cultivar followed by Huluka. Huluka's root growth was more inhibited than Danda'a. According to the growth and germination indices results, the local cultivar is the drought tolerant wheat cultivar.

Keywords: Bread Wheat, Moisture Stress, Cultivars, PEG-6000

College of Agriculture and Veterinary Sciences, Department of Plant Sciences, Ambo University, Ambo, Ethiopia

*Corresponding author's email: solmill2000@gmail.com (S. Tamiru)

Introduction

Wheat (*Triticum aestivum* L.) is a staple food for more than 35% of the world population and is also the leading grain crops in most of the developing countries (Metwali *et al.*, 2011). In Ethiopia, the total area devoted for wheat production was estimated to be 1.68 million ha with an average yield of 1827 kg ha⁻¹ (CSA, 2011). Wheat productivity in Ethiopia is influenced by biotic and abiotic factors. Moisture stress is among the major limiting abiotic factors hinder the productivity of *Triticum aestivum*. Abiotic stress, especially drought stress is a worldwide problem, seriously constraining global crop production (Pan *et al.*, 2002). It is one of the major causes of crop loss worldwide, which commonly reduces average yield for many crop plants by more than 50% (Bayoumi *et al.*, 2008). Plants may be affected by drought at any time of life, but certain stages such as germination and seedling growth are critical (Dhanda *et al.*, 2004). One of the most important abiotic factors limiting plant germination and early seedling stages is water stress brought about by drought and salinity (Almansouri *et al.*, 2001), which are widespread problems around the world (Soltani *et al.*, 2006)

Germination and seedling growth stages are the most critical periods in the life cycle of plants. Under water stress, low water potential is a determining factor inhibiting seed germination (Xu *et al.*, 2006). It is also important to determine the potential for seed germination in osmotic stress conditions, because, in this phase resistance against osmotic stress is a genetic quality and it is a good criterion for selection of stress resistant populations (Gharoobi *et al.*, 2012). According to Boureima *et al.* (2011), good germination capacity and seedling growth in water deficit conditions are drought tolerance indices, which allow better prediction on the crop establishment. In addition, field experiments related to water stress has been difficult to handle due to significant environmental or drought interactions with other abiotic stresses (Rauf *et al.*, 2008). An alternative approach is to induce water stress through polyethylene glycol 6000 (PEG 6000) solutions for screening of the germplasm (Khodarahmpour, 2011). Therefore, the objective of the current experiment was to determine moisture stress effect on bread wheat cultivars at germination and seedling growth stage.

Materials and Methods

In vivo experiment was conducted in November 2013 at the Department of Plant Sciences, Ambo University, to investigate the effect of moisture stress on germination and seedling growth of wheat. The experiment was arranged factorially in completely randomized design with three replications. Four wheat cultivars (Danda'a, Kubsa, Hulluka, and Local) were treated with six levels of water stress (0, 50, 100, 150, 200 and 250 g L⁻¹ PEG) for the experiment; de-ionized water was used for the control treatment. Polyethylene Glycol Solution (PEG-6000) used as a source of moisture stress was prepared by dissolving the respective treatment amount at 25 °C with deionized water. Seeds were surface sterilized with 0.01 % HgCl₂ solution for 1 min, and rinsed with deionized water.

Twenty seeds were uniformly placed on Watman filter paper covered the Petri dish (9.5 cm diameter) using a forceps per treatment, and well soaked by adding 8 ml with the respective solutions. All the Petri dishes were covered with lids and kept at room temperature (22 ± 2°C). Germination continued for 10 days, and germinated seeds were counted daily. Germination was considered to have occurred when radicles attained a length of 2 mm. After 10 days, parameters such as per-cent germination and rate of germination were calculated according to ISTA (1999); and root and shoot lengths of seedling were measured using a scale. Root and shoot dry weights were recorded after oven drying for 72 h at 60°C. The seedling vigor index (SVI) was determined as Hosseini and Kasra (2011). Statistical analysis of the data was performed using one-way ANOVA using SAS statistical software (Version 9). Based on the ANOVA results, mean separations were performed by LSD test at 5% level.

Results and Discussion

Germination percentage

Among all test varieties, the local variety produced the highest germination percentage (99.7%). This indicates the superiority of the local variety over the improved varieties in terms of germination capacity under water stress condition (Table 1). Water stress level induced by 50 g L⁻¹ PEG resulted the highest germination percentage (100%) and it showed a decreasing trend with increase in PEG concentration where 250 g L⁻¹ PEG produced the lowest percentage (92.5%). Progressive increase in moisture stress had adversely affected germination capacity of wheat cultivars (Table 2). Similar results were found for linseed (*Linum usitatissimum* L) and cotton cultivars seeds as a consequence of increasing PEG concentration, because of diminished movement and accessibility of water

for seed imbibitions (Guo *et al.*, 2012; Meneses *et al.*, 2011).

Germination rate

The highest numbers of seeds germinated were recorded for local variety with 14.6 plants day⁻¹, which showed its high genetic capacity for fast velocity of germination. Variety Kubsa showed the lowest number of seeds germinated thus slow velocity of germination followed by Danda'a (Table 1). Kubsa and Danda'a were highly susceptible to moisture stress induced by PEG as compared to the local cultivar. Regarding different PEG induced water stress, the highest numbers of seeds were germinated for non-stress (control) treatment with 17.5 plants day⁻¹, and it followed diminishing trend when PEG concentration was increased from 50 to 250 g L⁻¹ (Table 2). This delay in seed germination result obtained at more water stress levels were also found in cotton cultivars (Meneses *et al.*, 2011).

Seedlings vigor index

The analysis result revealed that the seedling vigor index of the local variety was 23.2, 14.6 and 6% higher than the vigor index of Huluka, Danda'a and Kubsa cultivars, respectively (Table 1). The vigor index of wheat cultivar seeds produced by inducing water stress at 50 and 100 g L⁻¹ PEG were not significantly different from non-stress (0 g L⁻¹ PEG) treatment. Seedling vigor index showed significant difference and decrease starting from seeds treated with water stress at 150 g L⁻¹ to 250 g L⁻¹ PEG where the lowest vigor index (2.793) was produced at the maximum water stress level (Table 2). Cokkizgin (2013) observed that seedling vigor index of pea (*Pisum sativum* L.) was decreased with increasing PEG concentration. Moraes *et al.* (2005) observed that the bean (*Phaseolus vulgaris* L.) seeds also presented a progressive decrease of vigor when PEG-6000 concentrations increase. Decreasing seed vigor index of wheat at high moisture levels was probably due to decreasing trend in shoot and root lengths (Table 2).

Shoot and root length

Both shoot and root lengths were affected by moisture stress induced by PEG but there was no significant difference among the wheat cultivars in producing taller shoot and root under moisture stress. Increasing PEG concentration has significantly reduced root and shoot lengths except at control and 50 g L⁻¹ PEG where maximum shoot and root length were obtained. The reductions in shoot lengths were more drastic than root growth for each increase in PEG concentration. Drought stress suppressed shoot growth more than root growth and in certain cases root growth increased (Bibi *et al.*, 2010; Younis *et al.*, 2000).

Table 1. Mean comparison of wheat cultivars' response to PEG induced water stress

Varieties	Germination (%)	Germination rate (plants day ⁻¹)	Seedling vigor index	Shoot length (cm)	Root length (cm)	Root number
Danda'a	97.2 ^a	11.1(4.2) ^b	8.26 ^{bc}	4.9(3.0) ^a	9.3(3.9) ^a	3.3(2.8) ^c
Kubsa	98.6 ^{ab}	10.7(4.2) ^b	9.09 ^{ab}	5.2(3.1) ^a	9.2(3.9) ^a	3.6(2.9) ^b
Hulluka	97.2 ^b	12.6(4.5) ^{ab}	7.43 ^c	5.7(3.1) ^a	8.9(3.9) ^a	3.6(2.9) ^b
Local	99.7 ^b	14.6(4.8) ^a	9.67 ^a	5.6(3.2) ^a	9.6(4.0) ^a	4.3(3.0) ^a

Means with the same letter are not significantly different; data in parenthesis are square root transformed

Table 2. Effect of PEG induced water stress on germination and growth indices of wheat

PEG (g L ⁻¹)	Germination (%)	Germination rate (plants day ⁻¹)	Seedling vigor index	Shoot length (cm)	Root length (cm)	Root number
0.0	99.6 ^a	17.5 (5.1) ^a	11.389 ^a	10.4(4.2) ^a	13.9(4.7) ^a	4.9(3.2) ^a
50	100 ^a	16.7(5.0) ^{ab}	11.359 ^a	9.5(4.1) ^a	13.8(4.7) ^a	4.8(3.2) ^a
100	99.6 ^a	14.1(4.7) ^b	11.856 ^a	6.8(3.6) ^b	11.4(4.3) ^b	4.6(3.1) ^a
150	98.3 ^a	10.4(4.2) ^c	8.703 ^b	3.3(2.8) ^c	8.8(3.9) ^c	3.5(2.9) ^b
200	99.2 ^a	9.1(4.1) ^c	5.559 ^c	1.6(2.2) ^d	5.8(3.4) ^d	2.8(2.7) ^c
250	92.5 ^b	5.8(3.3) ^d	2.793 ^d	0.6(1.7) ^e	1.8(2.3) ^e	1.6(2.3) ^d

Means with the same letter are not significantly different; data in parenthesis are square root transformed

Root number

Result in table 3 showed that the local cultivar had produced the highest root numbers (4.3) per plant, which indicated its high capacity to tolerate water stress conditions. In contrast, lower root numbers were produced by cultivar Danda'a. There was no significant difference in root number between Kubsa and Huluka cultivars. The reduction in root number became very low when PEG concentration was increased from 0 to 100 g L⁻¹. Wheat cultivars root numbers were reduced by 28.4, 43 and 67.4 % when PEG concentration was increased from control (distilled water) to 150, 200 and 250 g L⁻¹, respectively. Root number become the parameter most affected by water stress i.e. under severe water stress, a preference should be given to wheat cultivars, which could produce more number of roots per plant.

Shoot fresh and dry weight

Shoot fresh and dry weight (Table 3) differed among the four wheat cultivars grown under water stress conditions. The local cultivar produced high shoot fresh weight, which was significantly different from the rest cultivars. Even though, the local cultivar produced high shoot fresh weight, its ability to convert into dry

matter was lower than Huluka and Danda'a, which recorded low shoot fresh weight and relatively high dry weight. Regarding the water stress levels, shoot fresh and dry weight followed a decreasing trend when PEG was increased from 0 to 250 g L⁻¹. Shoot fresh weight decreased significantly at 50 g L⁻¹ and by half when PEG concentrations were increased from 150 to 200 and 200 to 250 g L⁻¹. The reduction in dry weight followed the same trend as fresh weight from 150 to 250 g L⁻¹ PEG concentration. But, the reduction followed slight decrease from 0 to 100 g L⁻¹ PEG concentration.

Root fresh and dry weight

Root fresh and dry weights were less influenced by osmotic concentration of PEG, but both parameters decreased with increase in PEG concentration. Significant difference in root fresh weight was observed between control (0 g L⁻¹ PEG) and water stress greater than 150 g L⁻¹ PEG. Sharp decrease in root fresh weight was noted than root dry weight with increasing the severity of moisture stress to wheat cultivars, suggesting that root dry weight is less affected by increasing moisture stress than fresh weight.

Table 3. Mean comparison of wheat cultivars' growth response to PEG induced water stress

Varieties	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Seedling fresh weight (g)	Seedling dry weight (g)
Danda'a	0.144(1.339) ^b	0.0246(1.149) ^c	0.113(1.319) ^{ab}	0.0587(1.232) ^a	0.257(1.470) ^{bc}	0.083(1.276) ^{bc}
Kubsa	0.163(1.370) ^b	0.0279 (1.159) ^{bc}	0.123(1.339) ^a	0.0635(1.246) ^a	0.287(1.507) ^{ab}	0.092(1.293) ^{ab}
Hulluka	0.145(1.342) ^b	0.0286(1.161) ^b	0.085(1.280) ^c	0.0466(1.209) ^b	0.230(1.446) ^c	0.075(1.265) ^c
Local	0.214(1.419) ^a	0.0393(1.187) ^a	0.099(1.307) ^{bc}	0.0574(1.235) ^a	0.314(1.526) ^a	0.097(1.303) ^a

Means with the same letter are not significantly different; data in parenthesis are square root transformed

Table 4. Effect of PEG induced water stress on growth indexes of wheat

PEG (g L ⁻¹)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Seedling fresh weight (g)	Seedling dry weight (g)
0.0	0.308 (1.55) ^a	0.047(1.22) ^a	0.15(1.38) ^a	0.067(1.26) ^{ab}	0.454 (1.671) ^a	0.114 (1.338) ^a
50	0.281(1.52) ^a	0.043(1.21) ^{ab}	0.14(1.37) ^a	0.070(1.26) ^a	0.414 (1.638) ^{ab}	0.114 (1.336) ^a
100	0.222(1.47) ^b	0.042(1.20) ^b	0.13(1.36) ^a	0.078 (1.28) ^a	0.361 (1.597) ^b	0.119 (1.344) ^a
150	0.125(1.34) ^c	0.029(1.17) ^c	0.10(1.32) ^b	0.059 (1.24) ^b	0.229 (1.467) ^c	0.088 (1.294) ^b
200	0.048(1.21) ^d	0.013(1.11) ^d	0.08(1.28) ^c	0.042 (1.20) ^c	0.129 (1.349) ^d	0.056 (1.233) ^c
250	0.014(1.12) ^d	0.006(1.08) ^e	0.03(1.16) ^d	0.023 (1.14) ^d	0.042 (1.201) ^e	0.029 (1.163) ^d

Means with the same letter are not significantly different; data in parenthesis are square root transformed

Seedling fresh and dry weight

High seedling fresh and dry weight of 0.314 and 0.097 g, respectively were obtained from local cultivar. Low seedling fresh weight was recorded by Huluka. Inducing progressive moderate water stress (100 and 150 g L⁻¹) caused a significant decrease in seedling fresh weight whereas drastic decreases in seedling fresh and dry weights were observed at progressive severe stress (200 and 250 g L⁻¹) level.

Shoot and root growth inhibition

Higher shoot length inhibition were observed for Danda’a cultivar followed by Huluka. On the other hand, Huluka’s root growth was more inhibited than Danda’a. Moderately, more root and shoot growth (less inhibition) was recorded by Kubsa. Higher difference inhibition percentage

between shoot and root was observed for Danda’a and Kubsa cultivar thus shoot growth was more inhibited than root growth. Huluka and local cultivar shoot and root were less affected by water stress (Fig. 1). Wheat shoot growth was more inhibited than root length under PEG induced water stress. Shoot growth inhibition was more at 150 g L⁻¹ PEG than shoot at 200 g L⁻¹ at PEG. At maximum water stress, the inhibition power of PEG for shoot was slightly higher than root length indicating that PEG induced water stress had inhibited root elongation at lesser rate than shoot growth. Similar results were also reported in pearl millet and chickpea by Kalefetogllu *et al.* (2009) and Govindaraj *et al.* (2010), respectively. Shoot and root growth inhibition increased with increase in water stress levels (Fig. 2).

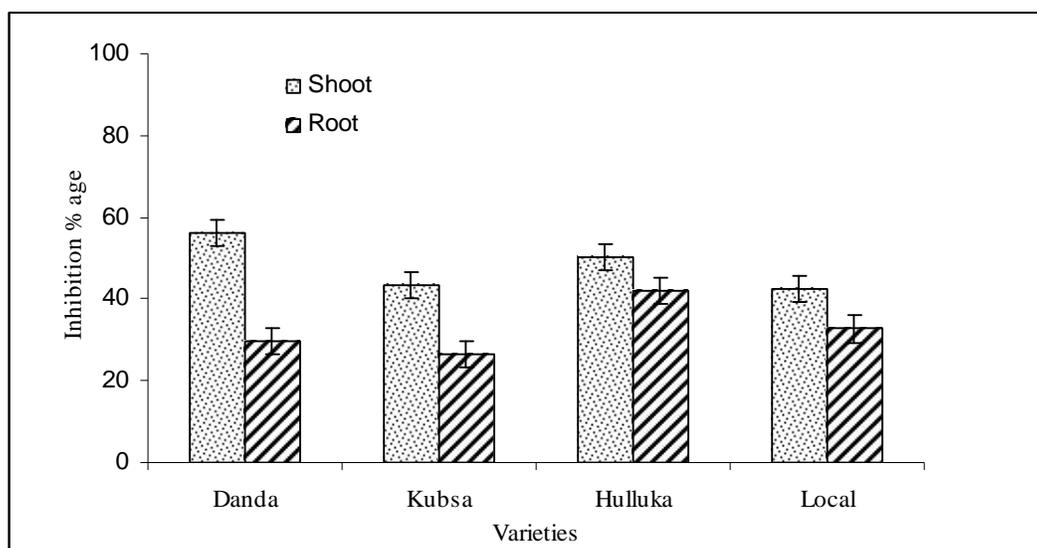


Fig. 1. Shoot and root length inhibition response of wheat cultivars under water stress

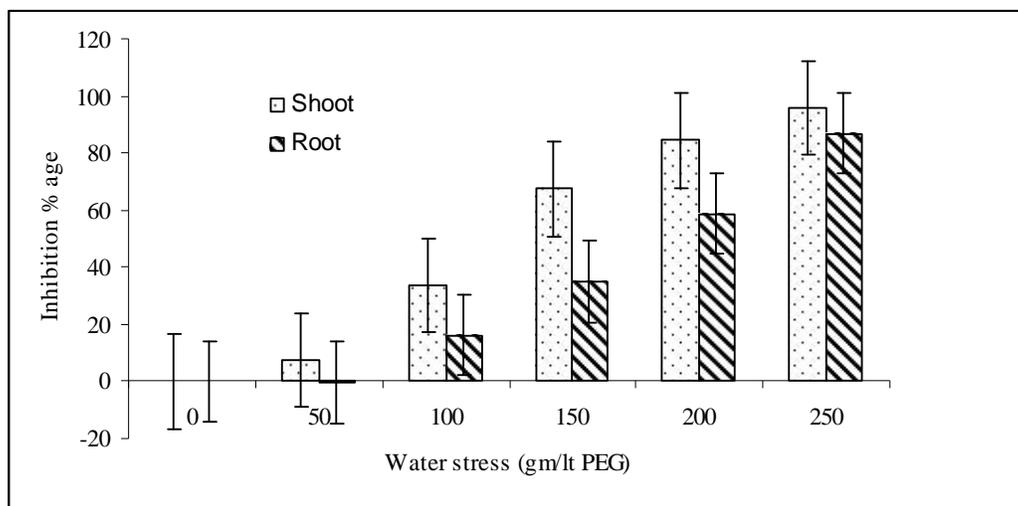


Fig. 2. Inhibition effects of different PEG concentration on wheat shoot and root length

Conclusion

All the wheat cultivars showed significant difference in germination percentage and rate. The local variety was superior over the released varieties in producing highest germination percentage and rapid rate of germination. In addition, the seedling vigor index of the local variety was superior to the rest cultivars. The germination indexes of wheat cultivars showed a diminishing trend with progressive increase in PEG concentration. The result showed that there was no significant difference among the wheat cultivars in producing taller shoot and root length. Increasing water stress levels has significantly reduced both root and shoot lengths. PEG induced water stress inhibited shoot growth more than root growth. The local cultivar was also able to produce more root numbers, shoot fresh and dry weight, seedling fresh and dry weight. The progressive moisture stress significantly reduced the wheat seedling growth parameters. Therefore, the current research suggests that the local cultivar is more tolerant to moisture stress and it could be used for breeding tolerant cultivars. In addition, both germination indices and seedling growth parameters could be used for screening wheat cultivars for drought tolerance.

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