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RESEARCH PAPER

Evaluation of drought tolerance in mutant Kenyan bread wheat (*Triticum aestivum* L.) using in vitro techniques

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Abstract

Wheat (*Triticum aestivum* L) is widely cultivated as a small-grain cereal. In Kenya, it is ranked second after maize in its contribution towards food security. Biotic stress conditions such as drought cause extensive losses to agricultural production worldwide. In Kenya, arid and semiarid lands represent 83% of total land area, which experience frequent crop failure due to drought stress. Developing drought-tolerant wheat genotypes has been the focus of many wheat improvement programs. Few drought tolerant varieties are available for commercial production in Kenya. Hence, there is need to develop more drought tolerant wheat varieties. The objective of this study was to screen for drought resistance in two mutant wheat lines *in vitro* using Polyethylene Glycol (PEG). Four wheat germplasm were tested for drought tolerance using -3.0, -9.0 and -15.0 PEG-6000 concentrations and the data was recorded on various seedling parameters including root length, shoot length and root length /shoot length ratio. The experiment was carried out in three replicates using completely randomized design. Data was subjected to analysis of variance (ANOVA) using GENSTAT 12th edition. Correlation was done by Pearson Correlation Coefficients to determine significant associations among the different variables. Results indicated that there was a significant difference (p=0.05) between Mutant 1 and Mutant 2 having longer roots, shoots and a root to shoot ratio compared to Chozi and Duma in the different PEG concentrations used. Hence, the two mutant lines are possible candidates for varieties that can be grown in ASALs regions in Kenya.

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Introduction

Bread wheat is the major food crop in the world and sustains the majority of the world population (USDA, 2011). It is grown on about 225 million hectares worldwide from the equator to latitudes of 60°N and 44°S and at altitudes ranging from sea level to more than 3000 m. Approximately 600 million tons of wheat is produced annually, roughly half of which is in developing countries (Goyal and Manoharachary, 2014). Global wheat production must continue to increase 2% annually until 2020 to meet future demands of imposed population and prosperity growth (Singh *et al.*, 2007; Geleta *et al.,* 2015).

Wheat crops growing in both irrigated and rainfed environments commonly experience environmental stresses .Drought is one of the environmental stresses seriously limiting crop production in the majority of agricultural fields of the world (Abedi and Pakniyat, 2010) and recent global climate change has made this situation more adverse (Anand *et al.,* 2003). Approximately 32% of the wheat-growing regions in developing countries experience some type of drought stress during the growing season. The frequency and severity of soil water deficit is generally greater for rain-fed wheat crops. However, changing weather patterns and worldwide water shortages will likely result in irrigated wheat being grown with the loss of applied water, increasing the likelihood of a soil water deficit (Al-Ghamdi, 2009).

One possible way to ensure future food needs of an increasing world population involves the better use of water through the development of crop varieties which need less water and are more tolerant to drought (Mafakheri *et al.,* 2010). About one fifth of the developing world's wheat (*Triticum aestivum* L.) is grown in the arid and semi-arid lands (ASALs) (Torkamani, 2005; Ndiema *et al.,* 2011). Despite these limitations the world's ASALs and cropping environment are increasingly crucial for food security in developing world. Worldwide, land with inherent characteristics for arable crop production continues to decline, while population growth and demand for wheat are rising (USDA 2013). Therefore, gains in wheat production in ASAL environments are important because it is unlikely that increased production in the favourable environments will be sufficient to meet the projected growth demand for wheat from the present to 2020 (Geleta *et al.,* 2015).

In Kenya, wheat has been grown since the turn of the 20th century at first by large-scale farmers and later by small-scale producers (Kinyua, 1997). It was traditionally cultivated in the high attitudes ranging from 1, 800 meters above sea level to 3,000 meters above sea level. Recently wheat has been introduced into lower dry lands areas of Machakos, Naivasha, Koibatek and Lower Narok among others (Ndiema *et al.,* 2011).

Biotechnology and mutation techniques are being used to improve local varieties of basic food crops for yield and quality, early maturity and tolerance to biotic and abiotic stresses (IAEA, 1998). This is essential especially in Kenya where only three varieties, Chozi, Duma and Ngamia, that had been recommended and released for commercial production in the marginal rainfall areas of Kenya (Kinyua *et al.*, 1998; Ndiema, 2010), therefore there is need to screen more drought tolerant wheat varieties for the ASALS.

Screening techniques based on physiological criteria should be rapid, simple and inexpensive, especially for the evaluation of large populations (Meeta *et al.*, 2013). One of the screening techniques based on physiological traits is the use of various osmotic to induce stress in plant tissues. Germination in mannitol and polyethylene glycol (PEG) has been suggested for drought screening (Geravandi *et al.*, 2011; Sayar *et al.,* 2011). Because of its high molecular weight, PEG cannot cross membranes and cannot get into the cell to change its osmotic potential (Geravandi *et al.*, 2011). It stimulates water deficit conditions in cultured cells in a manner similar to that observed in the cells of intact plants subjected to true drought conditions. The objective of this study was therefore to evaluate drought resistance in the mutant wheat lines *in vitro* using Polyethylene Glycol (PEG).

Materials and methods

Study site

The experiment was conducted at Mimea International, Kitengela, under laboratory conditions.

Plant germplasm

Two mutant wheat lines that showed resistance to stem rust disease in the field were used in the present study and were obtained from University of Eldoret. The other seeds were two known drought resistant commercial varieties of wheat (Duma and Chozi) which were obtained from Kenya Agriculture and Livestock Research Organization (KALRO-Njoro). Both Duma and Chozi wheat varieties have been developed for the dry areas of Kenya. In total four wheat varieties were tested.

Procedure

Seeds were initially surface sterilized by dipping them in 70% ethanol for 15 min. Residual ethanol was removed by thorough washing with sterilized distilled water. Twenty-five randomly selected seeds of each wheat variety were placed in Petri dishes on moistened filter paper to provide appropriate moisture stress for seed germination as suggested by Bayoumi *et al.* (2008). Water stress was exerted by preparing different water potential values, -3.0, -9.0 and -15.0 bars, produced by dissolving 138, 222 and 270 grams of PEG in 1000 ml of distilled water, respectively following the method of Hadas (1976). A control set was also included using distilled water (zero bars). Each different water potential had three replicates. All the Petri dishes were placed at random in a growth chamber for 10 days, at average temperature of day and night of 22±2^{o} C and at 50% relative humidity. Five ml of distilled water was added to each Petri dish every 2 days to compensate for losses through evaporation. At the same time, 5 ml of PEG solution was added to each Petri dish under osmotic stress conditions of -3.0, -9.0 and -15.0 bars.

Data collection and analysis

When seedlings were at the stage of first true leaf initiation (10 days after treatment) data was taken at different treatments. These included root length, shoot length and root length to shoot length ratio all measured in centimeters.

The experiment was laid out in a completely randomized design with two factors: wheat variety types and water stress. Data on root length, shoot length and root length/shoot length ratio was subjected to Analysis of Variance. Correlation analysis using Pearson correlation was done on both root length and shoot length in relation to PEG concentration. All the analyses were done using Genstat Statistical software version 12.

Results

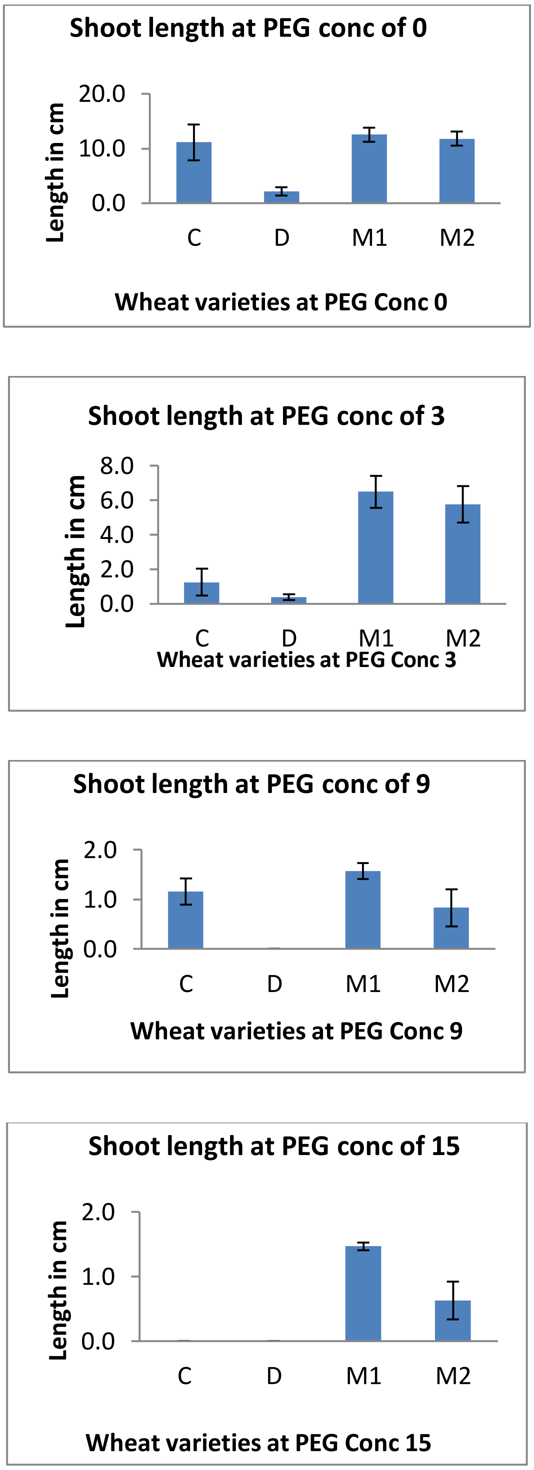
Effects of different concentrations of PEG on shoot length

The response of wheat varieties against different PEG concentration generated informative results. There was a negative correlation (-0.139) between increased PEG and the different wheat tested. A negative correlation coefficient (p=0.01; -0.608) was noted between PEG concentration and shoot length, that is, in all the wheat varieties there was a decrease in shoot length as the PEG concentration increased. There was a negative correlation (- 0.148) between the wheat tested and shoot length (Table 3).

In all the wheat tested there was a decrease in shoot length with increase of PEG concentration. At -3 PEG concentration Duma and Chozi had a percentage decrease of 88.1% and 83.3% respectively. Mutant 2 had a 40.3% decrease while mutant 1 had a 43.5% decrease at the same concentration. At -9 PEG concentration there was no growth in Duma, 93.6% decrease in Mutant 2, 86.3% decrease in mutant 1 and 84.5% decrease in Chozi, At -15 PEG concentration, germination in both Chozi and Nduma varieties was completely inhibited. In mutant line 1 there was a 87.2% decrease in shoot length while in mutant line 2 there was a 95.8% decrease in shoot length at similar PEG concentration (Table 2).

Effects of different concentrations of PEG on root length

There was a negative correlation (p=0.01; -0.256) between the root length and the different wheat varieties. There was a negative correlation (p=0.01; -0.649) between the root length with increased PEG concentration. There was a positive correlation (p=0.01; 0.913) between increased root length and shoot length (Table 3).

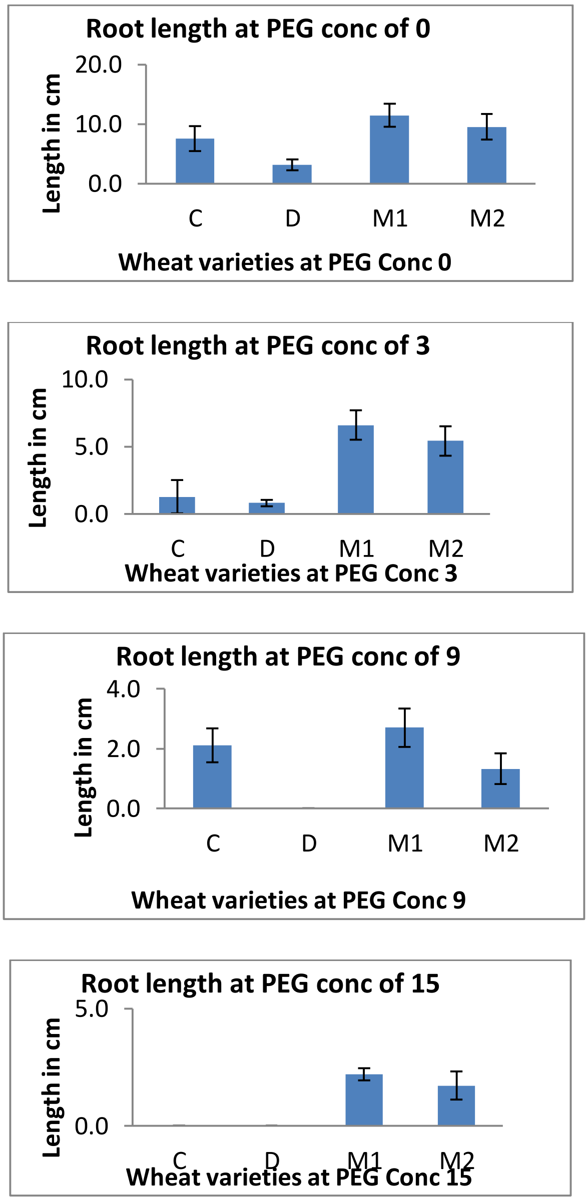


Key: C = Chozi, D = Duma, M1 = Mutant 1 M2 = Mutant 2

Fig. 1. Shoot length at different PEG concentration against for the different wheat varieties.

In all the wheat tested there was a decrease in root length with increase of PEG concentration. At- 3 PEG concentration Duma and Chozi had a percentage decrease of 62.7% and 88.6% respectively. Mutant 2 had a 54.1% decrease while mutant 1 had a 47.3% decrease at the same concentration. At -9 PEG concentration there was no root growth in Duma, 81.1% decrease in Chozi, 88.7% decrease in mutant 2 and 78.3% decrease in Mutant 1. At -15 PEG concentration germination in both Chozi and Nduma varieties was completely inhibited. In mutant line 1 there was a 82.2% decrease in root length while in mutant line 2 there was a 85.5% decrease in root length at similar PEG concentration (Table 2).

against for the different wheat varieties.

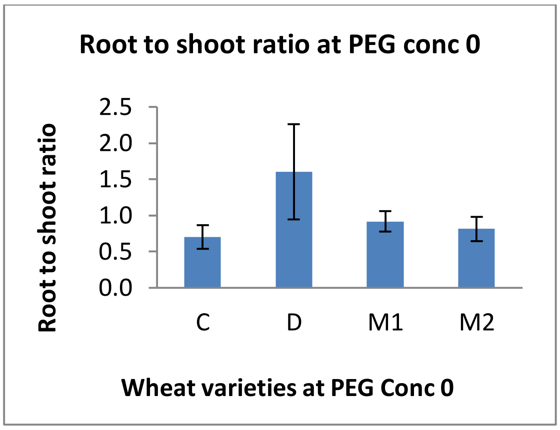


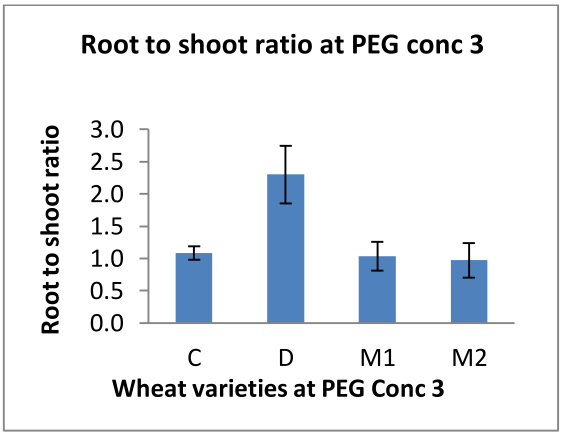
Key: C = Chozi, D = Duma, M1 = Mutant 1 M2 = Mutant 2

Fig. 2. Root length at different PEG concentration for the different wheat varieties.

Effects of different concentrations of PEG on root to shoot ratio

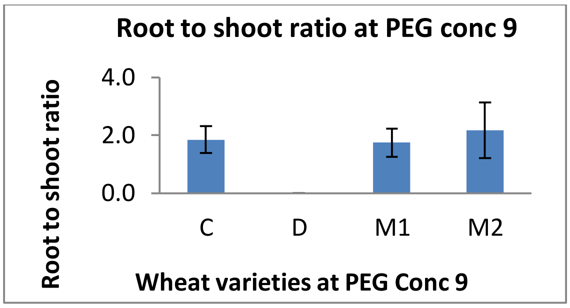
There was a positive correlation at p=0.01 of 0.407 in the shoot to root ratio with increase in PEG concentration. There was however a negative correlation at p=0.01 between the shoot to root ratio and shoot length and root length with -0.53 and -0.364 respectively. There was a negative correlation (-0.06) between the wheat varieties and the shoot to root ratio (Table 3).

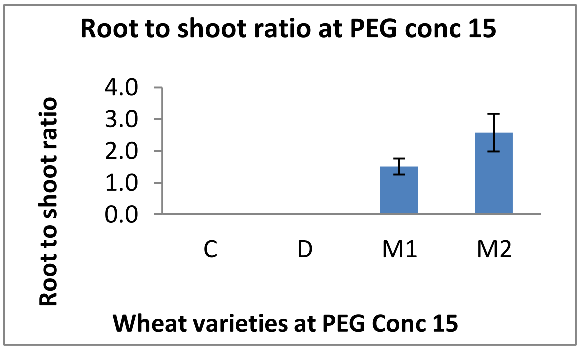




In all the wheat tested there was an increase in the shoot to root ratio with increase of PEG concentration. At -3 PEG concentration Duma had a percentage increase of 28.5%, Chozi had a percentage increase of 11.2%. Mutant 2 and Mutant 1 had a percentage increase of 11.9% and 8.9% respectively at the same concentration. At -9 PEG concentration there was no shoot or root growth in Duma. There was a 60.3% increase in Chozi, 55,9% increase in mutant 2 and 44.5% increase in Mutant 1. At -15 PEG concentration germination in both Chozi and Nduma varieties was completely inhibited.

In mutant line 1 there was a 9.27% increase in the shoot to root ratio while in mutant line 2 there was a 57.4% increase at similar PEG concentration (Table 2).





Key: C = Chozi, D = Duma, M1 = Mutant line 1, M2 = Mutant line 2

Fig. 3. Root to shoot ratio at different PEG concentration for the different wheat varieties.

Table 1. Analysis of variance (ANOVA) of PEG, shoot length, root length and shoot to root ratio.

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
PEG conc.	Between Groups	9.028	3	3.009	3.607	.015
	Within Groups	141.845	170	.834		
	Total	150.874	173			
Shoot	Between Groups	757.438	3	252.479	12.107	.000
	Within Groups	3545.180	170	20.854		
	Total	4302.618	173			
Root	Between Groups	426.564	3	142.188	11.843	.000
	Within Groups	2041.029	170	12.006		
	Total	2467.593	173			
Shoot_root	Between Groups	7.632	3	2.544	4.634	.004
	Within Groups	93.334	170	.549		
	Total	100.966	173			

Table 2. Percentage decrease in Root and shoot length and increase in root to shoot ratio of wheat at different concentrations of PEG (P=0.05).

PEG conc	Wheat variety	Percent shoot length decrease	Percent root length decrease	Percent Root length to Shoot length ratio increase
3	Duma	88.1% a	62.7% b	28.5% a
	Chozi	83.3% a	88.6% c	11.2% a
	Mutant 2	40.3% b	54.1% ab	11.9% a
	Mutant 1	43.5% b	47.3% a	8.9% a
9	Duma	100% b	100% c	0 a
	Chozi	84.5% a	81.1% ab	60.3% b
	Mutant 2	93.6% b	88.7% b	55.9% b
	Mutant 1	86.3% a	78.3% a	44.5% b
15	Chozi	100% a	100% a	0 a
	Duma	100% a	100% a	0 a
	Mutant 2	95.8% ab	85.5 % b	57.4% b
	Mutant 1	87.2% b	82.2% b	9.27% ab

Mean separation using Duncan Multiple range test at α = 0.05: means followed by the same letter are not significantly different from each other.

Table 3. Correlation coefficient between the root, shoot and shoot to root ratio.

	Variety	PEG Conc	Shoot	Root	Shoot Root
Variety	1	-0.139	-0.148	-0.256**	-0.060
PEG conc		1	-0.698**	-0.649**	0.407**
Shoot			1	0.913**	-0.530**
Root				1	-0.364**
Shoot _Root					1

** Correlation is significant at the 0.01 level.

Discussion

Both biotic and abiotic factors limit productivity of any cop worldwide. Among the abiotic factors, water stress due to drought is one of the most significant factors that limit the seed germination, seeding growth, plants growth and yield (Hartmann *et al*, 2005). 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 under laboratory conditions. One of the important reports noted in research is that a positive correlation between drought tolerance of the genotypes in the field and in laboratory experiments exists (Kosturkova *et al*, 2014).

Effects of different concentrations of PEG on bread wheat germplasm shoot length

There was a significant difference on the shoot length in the different PEG concentrations. Reduction in shoot length in cereal crops is mostly linked to drought tolerance (Bibi *et al.,* 2012). The decrease in shoot length in this study in the mutant genotypes may be due to osmotic regulation, which enables them to maintain cell turgor to assist growth under severe stress conditions. Mutant 1 and Mutant 2 had shoot growth even at higher PEG concentration of -15 even when Chozi and Duma varieties were not able to grow. This could be due to alteration in the genetic makeup due to mutation (Acquaah, 2012) enabling the two mutant lines withstand drought simulation situation in the laboratory. The variability in the decreasing trend of osmotic regulation of the genotypes indicates the genotypic variability in response to water deficit stress. Similar findings were reported by Raziuddin *et al.* (2010) in wheat, Takele, (2000), Ambika *et al.* (2011) and Khodarahumpour (2011) in sorghum in relation to the reduction in coleoptiles elongation.

A strong negative correlation between shoot length and PEG concentration was 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. 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.

The current findings are in agreement with previous studies. Abdel-Raheem *et al.* (2007) reported a decrease in shoot length of tomato varieties under polyethylene glycol (PEG) and mannitol treatments. Hamayun *et al.* (2010) also recorded a decrease in shoot length soybean with increasing concentration of PEG.

Effects of different concentrations of PEG on bread wheat germplasm root length

There was a significant difference on the root length in the different PEG concentrations. The response of root growth to drought can be variable; under moderate moisture stress, root growth is favored whereas, severe drought often limits root growth (Prasad *et al.*, 2008). The extent of root development is closely related to the ability of the plant to absorb water and the tolerant genotypes have higher capacity of these character. M1 and M2 had longer root lengths in all the different PEG concentrations tested compared to Chozi and Duma varieties and could be as a result of gene alteration during mutation.

Generally in the genotypes scrutinized, reduction in root length across the four PEG stress levels was found. The findings of this study are in line with earlier studies where severe water stress reduced root length in cereals (Kamran *et al.,* 2009). Generally plants accumulate some kinds of organic and inorganic solutes in the cytosol to raise osmotic pressure and thereby maintain both turgor and the driving gradient for water uptake. Under mild drought stress, pattern of resource allocation shifts to the roots rather than to the shoot. Water deficit favors the growth of seminal and lateral roots in seedlings (Abdi *et al.,* 2010). Such an increase in root length in response to PEG induced water stress might be due to limited water up take by the amount of roots in a particular volume of growth media. In this study, Mutant 1 and Mutant 2 lines had longer roots at higher PEG concentrations of 9 and 15 as compared to Chozi and Duma varieties.

Matsuura *et al.* (1996) reported a positive correlation between drought tolerance traits and root length in sorghum and millet. Similarly, a better root development under drought stress enables plant to reach deeper available water in the soil and hence survive to maturity (Radhouane, 2007).

The ability to develop extensive root systems contributes to differences among cultivars for drought tolerance and root length is considered an important trait in selection of drought resistant cultivars (Abdi *et al.,* 2010).

The results of this study indicate that the M1 variety which had longer roots than other wheat varieties in all the PEG concentration (Fig. 2). M1 plant line could therefore be used as a drought tolerant variety. Many plants successful in dry habitats have no specific adaptation for controlling water loss but rely on the development of very extensive and deep root systems that can obtain water from a large volume of soil deep in the water table (Ridge, 1991).

Effect of osmotic stress on root/shoot ratio of different bread wheat germplasm

Apart from the root and shoot lengths, root/shoot ratio also plays a major role in selecting the line for drought tolerance as balanced root and shoot growth was observed in drought resistant genotypes (Gesimba, 2000). The results of this study revealed significant variations for the root/shoot ratio among the cultivars (fig. 3). Dhanda *et al.*, (2004) also reported positive association of root length with coleoptile length in wheat which is in agreement with the results of this study.

Root and shoot lengths are envisaged as prominent characters for screening for drought resistant in wheat (Bayoumi *et al.*, 2008). According to Frazer *et al.*, (1990), reduction in root and shoot lengths may be due to an impediment of cell division and elongation leading to a kind of tuberization. This tuberization and lignification of the root system allow the stressed plant to enter a slowing growth state, while waiting for the conditions to become favorable. The results reported in this study are similar to earlier studies of Dhanda *et al.* (2004) in wheat; Kulkarni and Desphpande (2007) in tomato (*Lycopersicon esculentum* L.) and Govindaraj *et al.* (2010) in pearl millet (*Pennisetum glaucum* L.). The different authors have reported the effect of drought stress induced by PEG on the plants roots and shoots.

Conclusion and recommendations

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 identify the better varieties that can be useful to ASAL regions of Kenya. All the varieties showed strong negative correlation between PEG induced water stress and root length and similar results were noted with shoot length also. Based on these findings we can recommend mutant lines M1 and M2 to be considered as drought tolerant varieties that can be grown in Kenyan arid and semiarid lands.

The results of this study also emphasise the importance of the PEG as an artificial stress inducer for quick screening in the laboratory conditions for identification of drought tolerant wheat varieties

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