RESEARCH ARTICLE

In vitro selection of sorghum (*Sorghum bicolor* (L) Moench) for polyethylene glycol (PEG) induced drought stress

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Abstract

Drought is one of the main environmental factors affecting growth and yield of sorghum in arid and semi-arid areas of the world. *In vitro* selection of *Sorghum bicolor* for drought tolerance was undertaken by the use of somaclonal variation. The experiment was carried out with a collection of sixteen sorghum genotypes and tested in a completely randomized design. Data were recorded at five different PEG 6000 (polyethylene glycol) levels (0, 0.5, 1.0, 1.5, 2.0% (w/v) treatments)) on coleoptile length (CL), root length (RL), shoot dry weight (SDW), root dry weight (RDW), root number (RN) and statistically analyzed for significant differences. Significant differences were observed among the genotypes, treatments and their interactions for the evaluated plant traits suggesting a great amount of variability for drought tolerance in sorghum. In general, embryogenic callus induction and plantlet regeneration was found to be indirectly proportional to increased PEG concentrations. By taking into consideration all the measured traits, Mann Whitney rank sum test revealed that 76T1#23 and Teshale followed by Gambella-1107 and Melkam showed better drought stress tolerance while Chelenko appeared to be drought sensitive.

Keywords: Callus induction; Moisture stress; Plant regeneration; Polyethylene glycol; *Sorghum bicolor*

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Introduction

Sorghum (*Sorghum bicolor* (L.) Moench, 2n = 2x = 20) is a major staple and multipurpose crop grown in semiarid areas of the world for food, animal feed/forage and industrial purposes. It is also the fifth most important grain and the second highly produced cereal in Africa after maize (Gerda and Christopher, 2007). Although sorghum is generally considered tolerant for heat, drought, salinity and flooding as compared to other cereal crops but still it is sensitive at different critical plant growth stages to many stresses (Ejeta and Knoll, 2007; Ali *et al*., 2011), in addition the crop grown in rainfed areas is highly affected by moisture stress at both pre- and post-flowering stages of development and has the most adverse effect on yield during and after anthesis (Tuinstra *et al*., 1997; Kebede *et al*., 2001; Blum, 2004).

Drought is a serious threat for crop production and food security and it also decreases water and turgor potential of plants (Hsiao, 1973; Jaleel *et al*., 2009). Drought is a major abiotic stress that adversely affects plant production in many parts of the world and results in reduction of yield and plant growth. Plant responses to drought is a complex physical-chemical process, in which many biological macromolecules and small molecules are involved, such as nucleic acids (DNA, RNA, microRNA), proteins, carbohydrates, lipids, hormones, ions, free radicals and mineral elements (Ingram and Bartels, 1996). Identification and understanding the mechanisms of drought tolerance in sorghum have been major goals of plant physiologists and breeders which includes prolific root system, ability to maintain stomatal opening at low levels of leaf water potential and high osmotic adjustment (Turner, 1974; Fekade and Daniel, 1992; Cabelguenne and Debaeke, 1998; Rajendran *et al*., 2011). Water stress affects almost every developmental stage of the plant. However, damaging effects of this stress was more noted when it coincided with various growth stages such as germination, seedling shoot length, root length and flowering (Rauf, 2008; Khayatnezhad *et al*., 2010). Among these critical stages, water stress induced during seedling stage has been exploited in various crop species to screen germplasm i.e. Wheat (Dhanda *et al*. 2004), sorghum (Gill *et al*. 2002; Bibi *et al*., 2010), maize (Mohammadkhani and Heidari, 2008; Farsiani and Ghobadi, 2009; Khayatnezhad, 2010) and sunflower (Rauf *et al*., 2008). Screening genotypes at seedling stages have several benefits, such as low cost, ease of handling, less laborious and getting rid of susceptible genotypes at earliest. Furthermore seedling traits have also shown moderate to high heritability with additive type of genetic variance within and over environments (Rauf *et al*. 2008).

An alternative approach to field experiments related to moisture stress is to induce stress using polyethylene glycol (PEG) in an *in vitro* condition. Polyethylene glycol with molecular mass of 6000 and above are non-penetrable and non-toxic osmotic substance which can be used to lower the water potential of the culture medium and it has been used to simulate drought stress in cultured plant tissues (Muhammad *et al*., 2010; Khodarahmpour, 2011). Hence, experiment was carried out to assess the effect of PEG induced stress on genotypic capacity of coleoptile growth, root growth, dry weight accumulation and root branching of sorghum genotypes; to categorize sorghum germplasm against drought stress and to select suitable accessions for drought tolerance.

Materials and methods

Plant material

The seeds of the genotypes were provided by the National sorghum improvement project of Melkassa agricultural research center, Ethiopia. Sixteen sorghum varieties from more than 19 released cultivars were selected based on the basis of their morphological and agronomic diversity to formulate the representative core sample of the species. Five PEG concentrations (0-2%) were used. The experiment of the study was laid out in a completely randomized design in factorial arrangement with three replications (Gomez and Gomez, 1976).

Callus induction under PEG stress

The study was conducted in the plant tissue culture laboratory of Melkassa Agricultural Research Center Melkassa, Ethiopia. The sorghum seeds were treated with bavistin 0.1% for three hours and washed three times in sterile distilled water. Then surface sterilized in 0.2% HgCl2 for 15-20 minutes followed by three rinses in sterile distilled water in a laminar flow cabinet. Sterilized seeds were cultured in Petri dishes containing semisolid Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 2.5 mg l-1 2,4-D + 0.5 mg l-1 kinetin + 560 mg l-1 proline + 30 g l-1 sucrose + 8 g l-1 agar. The Petri dishes were sealed with parafilm and placed in a growth chamber in the dark at 25 ± 2ºC. After four weeks incubation, the induced calli were excised and sub-cultured, under the same growth conditions, and in the same MS medium to which various concentrations of polyethylene glycol (PEG) (6000) (0, 0.5, 1.0, 1.5, 2.0%) were added. The incubation period was two cycles of two weeks each. The pH of all media was adjusted to 5.8 with 0.1 N NaOH prior to autoclaving. The culture medium was autoclaved at 121ºC for 30 min.

Plant regeneration from induced callus in PEG stress condition

Resulting calli were excised, transferred, to jars containing 25ml MS basal salts medium supplemented with 2.0 mg l-1 benzylaminopurine + 0.5 mg l kinetin + 0.5 mg l naphthalene acetic acid + 30 g l-1 sucrose + 8 g l-1 agar 30 g l-1 for shoot initiation where PEG (6000) (0, 0.5, 1.0, 1.5, 2.0%) has been added for four weeks. The jars were placed in a growth chamber under fluorescent light and at an ambient temperature of 25 ± 2ºC. The medium was changed in 15 days and after four weeks, calli with clearly differentiated shoots were transferred to rooting media. Rooting was initiated on half strength MS medium supplemented with 3 mg l-indole-3-butyric acid (IBA) under the same conditions. Regenerating calli, showing shoot and root formations, were transferred to MS basal medium with no phytohormones, but PEG stress to sustain growth of plantlets for four weeks.

Data recorded and statistical analysis

After four weeks of incubation in MS basal medium with PEG stress, plant parts (shoot and roots) were harvested separately. After washing free of culture media, coleoptile length and root length were measured. Root branching for the lateral roots was measured by visual counting. Finally,

Table 1. Mean value of various treatments of sorghum genotypes under different stress levels

PEG Level (%)	CL (cm)	RL (cm)	RN	SDW (mg)	RDW (mg)
0	15.49a	5.91a	19.94e	1.79a	0.49a
0.5	14.87b	5.73b	21.23d	1.74b	0.48b
1.0	12.98c	5.29c	23.00c	1.69c	0.45c
1.5	11.41d	5.11d	24.56b	1.66d	0.44d
2	11.41d	4.82e	26.69a	1.62e	0.42e

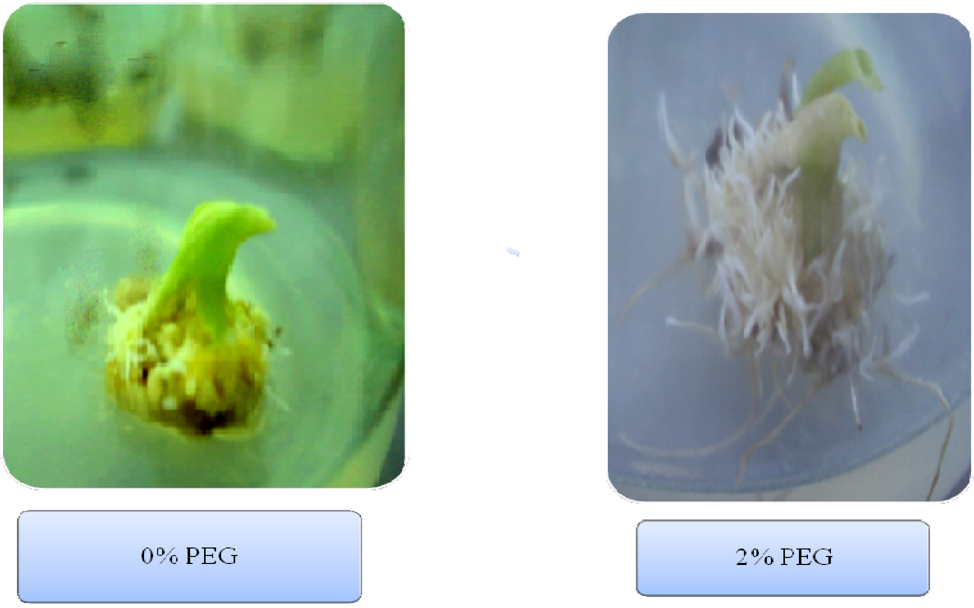

Fig 1. Root Number as differed across PEG treatment levels

each plant part was oven dried at 70°C for 24 h before measurement of shoot dry weight and root dry weight was conducted.

Data for all dependent variables (*i.e.*, coleoptile length (CL), root length (RL), root count (RN), shoot and root dry weight (SDW,RDW) were analysed by one-way ANOVA to test for statistical significance. Fischer's LSD test at 5% probability level using MSTATC software (Freed and Eisensmith, 1986) was used. In addition Pearson correlation analysis was done using Systat software to examine degrees of association between characters.

Results and Discussion

Variation among genotypes

The differences among the water stress treatments for all the traits were highly significant (Table 1). There was decrease in shoot and root related traits with increase in concentration of PEG whereas the mean root number increased with increasing level of PEG treatment in each genotype (Figure 1). The maximum value for coleotile length, root length, shoot and root dry weight was observed in control (distilled water) and minimum value was observed in 2% PEG. The differences among genotypes were also highly significant for all the traits (Table 2).

Melkam showed maximum values of coleoptile length (16.89 cm), root number (32.27), Shoot dry weight (2.18 mg), and root dry weight (0.59 mg). Gubiye showed minimum value of coleoptile length (11.27 cm), Raya showed minimum value of root length (3.68 cm), 76T1#23 showed minimum value of root number (16.20) whereas Hormat and Misikir showed minimum number of shoot and root dry weight (1.54 and 0.39 mg) respectively (Table 2). Reduction in shoot length in cereal crops is mostly linked to drought tolerance (Bibi *et al.*, 2012). The decrease in CL in the studied genotypes may be due to osmotic regulation, which enables them to maintain cell turgor to assist growth under severe stress conditions. 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 coleoptile elongation.

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. Generally in the genotypes scrutinized, reduction in RL across the five PEG stress levels was found. The findings of the present study are in line with earlier studies where severe water stress reduced RL in cereals (Kamran *et al.*, 2009; Ali *et al.*, 2007). In addition, shoot dry weight decreases mainly due to increased partitioning of solutes to the root as a means of osmotic regulation (Ramu *et al.*, 2008; Addisie (2010). Decrease in SDW is a common response of crop plants when subjected to moisture deficit

Table 2. Mean values for various traits of sorghum genotypes under different stress levels

Genotype/variety	Coleoptile length	Root length	Root number	Shoot dry weight	Root dry weight
Abshir	14.05	6.43	28.06	1.79	0.46
Chelenko	12.43	4.71	27.13	1.71	0.43
Raya	12.41	3.68	19.33	1.73	0.43
Hormat	11.67	4.09	21.53	1.54	0.43
Gubiye	11.27	6.97	23.00	1.58	0.43
Gambella-1107	11.35	7.02	24.07	1.60	0.42
Birmash	11.39	4.03	21.26	1.57	0.43
Meko	11.66	6.15	21.80	1.60	0.44
Macia	13.85	4.65	17.60	1.63	0.45
Seredo	15.74	6.66	27.93	2.07	0.55
Misikir	11.71	4.97	21.60	1.57	0.39
Melkam	16.89	6.82	32.27	2.18	0.59
76T1#23	12.25	4.26	16.20	1.56	0.45
ESH-2	11.63	4.78	20.86	1.57	0.42
Girana-1	13.36	3.95	21.93	1.58	0.43
Teshale	14.69	6.77	24.53	1.91	0.54

stress (Sharp and Davies, 1979; Creelman *et al*., 1990).

A means of increasing drought tolerance is by decreasing osmotic potential by accumulation of solutes (Rhodes and Samara, 1994). 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.

In contrast to all other parameters, the mean RN increased with increasing level of PEG treatment in each genotype (Figure 1). The increment in mean RN was genotype dependent. The ability to maintain a variable root number among sorghum accessions indicates their drought tolerance. Water deficit favors the growth of seminal and lateral roots in seedlings (Abd Allah *et al*., 2010). Such an increase in RN 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. Seminal and lateral root growth enhances water uptake which is much needed to sustain growth under severe moisture deficit conditions. The result is in agreement with Begum *et al*. (2011) in which root initiation on culture media supplemented with different level of PEG increased RN in sugarcane after one week.

Correlation studies among different characters

Simple correlation coefficients between studied traits are illustrated in Table 3. Results showed that coleoptile length had positive and significant correlation with root length, shoot dry weight and root dry weight but no correlation with root number. In addition, root length exhibited positive correlation with shoot dry weight, root dry weight and root number. Likewise, shoot dry weight showed positive correlation with root dry weight and root number. Root dry weight also revealed positive correlation with root number. The positive correlation between shoot dry weight and root dry weight is also supported by findings of Ali *et al*. (2009). Dhanda *et al*. (2004) also reported positive association of root length with coleoptile length which is in agreement with the results of this study. Moreover, Matsuura *et al*. (1996) reported a positive correlation between drought tolerance traits and root length in sorghum and millet. Therefore, these characters could be selected simultaneously with their positive effects

Table 3. Correlation coefficients of studied traits on sorghum genotypes under drought stress

	CL	RL	SDW	RDW	RN
CL	1				
RL	0.357*	1			
SDW	0.596*	0.480*	1		
RDW	0.653*	0.546*	0.873*	1	
RN	-0.045	0.326*	0.398*	0.231*	1

* Significant at P=0.05 probability level.

Table 4: Rank sum of measured parameters

Variety	CL	RL	SDW	RDW	RN	Rank Mean	Rank SD	RS
Abshir	15	03	12	16	01	09.4	6.95	16.35
Chelenko	10	04	15	15	13	11.4	6.62	18.02
Raya	06	06	14	08	12	09.2	3.63	12.83
Hormat	16	12	13	06	14	12.2	3.77	15.97
Gubiye	14	13	09	09	03	09.6	4.34	13.94
Gambella-1107	11	10	04	02	04	06.2	4.02	10.22
Birmash	12	07	06	13	05	08.6	3.65	12.25
Meko	03	05	08	07	15	07.6	4.56	12.16
Macia	08	14	07	04	06	07.8	3.77	11.56
Seredo	07	01	16	10	11	09.0	5.52	14.52
Misikir	09	11	03	14	08	09.0	4.06	13.06
Melkam	04	09	11	12	02	07.6	4.39	11.99
76T1#23	02	02	01	01	09	03.0	3.39	06.39
ESH-2	13	15	10	11	07	11.2	3.03	14.23
Girana-1	05	16	02	03	16	08.4	7.02	15.42
Teshale	01	08	05	05	10	05.8	3.42	09.22

on drought tolerance at seedling stages of crop growth in sorghum.

Mann–Whitney–Wilcoxon Rank Sum Test of the Measured Traits

To determine the most desirable drought tolerant genotypes based on all traits measured, mean rank sum method was used according to Ezatollah *et al.* (2012). In this method, all the indices mean rank and standard deviation of ranks of all *in vitro* drought tolerance parameters were calculated and summed (RS) (Table 4).

By taking in to consideration all indices, genotypes: 76T1#23(RS= 6.39), Teshale (RS= 9.22) Gambella-1107 (RS= 10.22) and Melkam (RS= 11.99) respectively exhibited relative tolerance to PEG induced water deficit stress in terms of measured traits. On the other hand, genotype Chelenko (RS =18.02), found to be the most sensitive genotype to PEG induced drought. The result is also supported by the previous report of Tsago *et al.* (2013) where the same genotypes were evaluated based on their callus induction, callus fresh weight and plant regeneration capacities. The susceptible genotypes can be recommended for crossing and genetic analysis of drought tolerance using diallel mating design or generation mean analysis and also for the QTL (quantitative trait loci) mapping and marker assisted selection.

The detection of superior genotypes 76T1#23, Teshale, Gambella-1107 and Melkam for drought tolerance at *in vitro* level together with their high potential for callus induction leads to the conclusion that a hybridization breeding procedure using these superior plant materials supplemented with *in vitro* selection for drought tolerance might be beneficial for improving drought tolerance in sorghum. Hence, study at *in vitro* level can be useful to speed up sorghum improvement.

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