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In vitro Evaluation of Tef [*Eragrostis tef* (Zucc) Trotter] Genotypes for Drought Tolerance

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ኣብዛኛው የኢትዮጵያ ክፋል ዝናብ ኣጠር እና ያልተስተካከለ ስርጭት የሚያገኝ በመሆኑ ድርቅ የጤፍ ምርት ከሚቀንሱ ማነቆዎች በዋናነት ይጠቀሳል። የዚህ ጥናት ዋና ኣላማ ድርቅን ሊቋቋሙ የሚችሉ ዝርያዎችን በቤተ ሙከራ ምርምር ኢንቪትሮ እንዲሁም ከቤተ ሙከራ በሃላም በእፅዋት ማሳደግያ የመስትዋት ቤት መገምገም ነው። በጥናቱ የተካተቱ ሶስት የጤፍ ዝርያዎች ማለትም መልኮ፣ ገመቺስ እና Pop12S2 ሲሆኑ እነዚህ ዝርያዎች በኣምስት የተለያየ መጠን 0፣ 0.5፣ 1፣ 1.5 እና 2 መቶኛ የተዘጋጀ የፖሊ ኢታይሊን ግላይኮል (ሰው ሰራሽ ድርቅ) ተገምግመዋል። ጥናቱ በምልኣ ብሎክ ንድፍ ያረፈ ሲሆን ለእያንዳንዱ ዝርያ ሶስት ድግግሞሽ ነበረው። ውጤቱ እንደሚያመለክትው በሶስቱ ዝርያዎች እንዲሁም በተለያዩ መጠን የተዘጋጀ ፖሊ ኢታይሊን ግላይኮል ከፍተኛ የሆነ ልዩነት ኣሳይታል። በጤፍ ዝርያዎች እና በፖሊ ኢታይሊን ግላይኮል ያለውን ግንኙነት በተወሰነ ደረጃ ላቅ ያለ ነበር። ከዚህም በተጨማሪ የፖሊ ኢታይሊን ግላይኮል መጠኑ ከ0 ወደ 2መቶኛ ከፍ ሲል የካለስ፣ የስር እና የቅጠል እድገቶችን በከፍተኛ ደረጃ እንደሚቀንስ ወጤቱን ያሳያል። በምርምሩ ወጤት መሰረት መልኮ ከተባለው የጤፍ ዝርያ የተገኙ ሪጀነራንቶች በቀጣይ ድርቅን የሚቋቋም የጤፍ ዝርያን ለማሻሻል፣ ለማዳቀል ለሚደረገው ምርምር ልንጠቀምባቸው እንችላለን።

Abstract

Drought is a serious tef production constraint as most of Ethiopia's agriculture is rain dependent with limited and erratic distribution. The present study was conducted to evaluate tef genotypes for drought tolerance under in vitro condition, and to assess the performance of the in vitro developed regenerants under greenhouse condition. The in vitro experiment was arranged in a factorial experiment using completely randomized design with three replications. Three tef genotypes including Melko (drought tolerant), Gemechis (moderately tolerant) and Pop12S2 (susceptible) and five polyethylene glycol levels (0, 0.5, 1, 1.5 and 2%) were used. Analysis of variance indicated significant differences (P≤0.05) among the genotypes as well as the different polyethylene glycol concentrations for all the measured parameters, but total shoot/culture and survival percentage was not genotype dependent. There was significant genotype by polyethylene glycol interactions on total shoot number/culture, total root number/culture, shoot length, root length and survival percentage, while no significant interaction effect was noted on callus induction efficiency, percent plant regeneration, rooting percentage and number of roots/shoot. The results showed that increasing polyethylene glycol concentration from 0% to 2% in the medium causes a gradual decrease in callus induction and plant regeneration efficiency. In most regenerants, fluctuation behavior was observed for the measured parameters. Hence, to determine the most desirable drought tolerant regenerants, ranking method was taken. Accordingly, regenerant Melko (0.5%), Melko (1.5%), and Melko (1%) gave the most desirable regenerants; thus, they could be used for crossing and further improvement of drought tolerance.

Introduction

Tef [*Eragrostis tef* (Zucc.) Trotter] is an important cereal crop in Ethiopia accounting for about 28% of the total acreage and 21% of the gross grain production of all cereals. Among the food crops grown in Ethiopia, tef is cultivated on about 3.01 million hectare producing 5.02 million tons. It is grown by over 6.99 million farmers' households (CSA 2017). Tef is a staple food for about 50 million Ethiopian people. It is versatile to adverse climatic conditions and high in nutritional value makes suitable for both farmers and consumers (Assefa *et al*. 2013).

Ethiopia is prone to drought, which has serious implications on food security, as most of Ethiopia's agriculture is rain dependent. A study by the Ethiopia Central Statistical Agency and the World Food Program found that lack of rainfall is one of the main determinants of food production in Ethiopia. Across most of Ethiopia, households reported that erratic rainfall as the main risk contributing to their food insecurity and overall vulnerability. Overall, there have been declines in amount of rainfall between March and September from 1980 to 2015 (Annette 2015).

Although tef grows in a wide agro-ecological conditions ranging from semi-arid areas with low rainfall to areas with high rainfall, the rainfall pattern in most tef growing regions is not consistent enough to support the normal growth of the crop during the crop cycle (Tadele 2016). In most tef growing regions, greater rainfall variability exists over the growing period than over the year-cycle, and these results in poor agricultural outputs. A recent study confirmed that climate would have a negative impact on the acreage and productivity of tef unless urgent interventions are implemented which favor mitigation and adaptation strategies (ABCIC 2011).

While drought is a major barrier to increase productivity in tef and selection under actual field conditions is tedious due to low heritability and time required, other alternative strategies are needed. Plant tissue culture studies play tremendous role by providing efficient way of understanding plant genetic processes in short period in a controlled environment. Plant tissue culture also plays an important role in the production of agricultural crops and in the manipulation of plants for improved agronomic performance. *In vitro* culture of plant cells and tissues has attracted considerable interest over recent years because it provides the means to study plant physiological and genetic processes in addition to offering the potential to assist in the breeding of improved cultivars by increasing genetic variability (Wani *et al*. 2010).

The *in vitro* drought tolerance screening approach consists of growing cells or tissues of plants or plantlets on a defined drought stressing culture media under an aseptic and controlled environment. The *in vitro* technique provides precise results but the working environment differs from the natural environment of crops. Therefore, the combination of *in vitro* screening with selection under the natural condition could improve the quality of results (Ahloowalia *et al*. 2004). According to Perez and Gomez (2012) report, the *in vitro* culture technique has been successfully applied with increased tolerance to drought stress for plant species such as rape seed (*Brassica napus)*, sour orange (*Citrus aurantium)*,

tomato (*Lycopersicon esculentum)*, bamboo (*Dendrocalamus strictus)*, sweet potato (*Ipomoea batatas)*, sugarcane (*Saccharum* sp.), potato (*Solanum tuberosum) and* wheat (*Triticum aestivum).*

To date, little investment in biotechnology has been applied to tef. *In vitro* plant regeneration from different explants of tef has been demonstrated previously. The explants used for these investigations were seedlings, roots, and leaves (Mekbib *et al*. 1997), seeds (Assefa *et al*. 1998), and immature embryos (Gugsa and Kumlehn 2011). But no improved variety was obtained as a means of increasing yield, drought tolerance, disease resistance and other agronomic traits so far from this technique. Therefore, further work is needed for a successful tef improvement through tissue culture system.

Polyethylene glycol (PEG) of high molecular weights has been used as osmotic stress agents for *in vitro* selection for many years to stimulate water stress in plants including cereals. 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 (Farshadfar *et al*. 2012). This compound is a non-penetrating inert osmoticum that reduces water potential of nutrient solutions without being taken up by the plant or being phytotoxic (Perez and Gomez 2012).

There was no report so far on drought tolerance evaluation using *in vitro* culture technique in tef. Thus, the objective of the current study was to evaluate tef genotypes for drought tolerance under *in vitro* condition, and to assess the performance of the *in vitro* developed tef regenerants for morpho-phenologic, yield and yield related traits.

Materials and Methods

The study was conducted at the Tissue Culture Research Laboratory of Plant the Biotechnology Division in Mekelle Agricultural Research Center, Northern Ethiopia.

Plant material, treatments and experimental design

Three tef genotypes with contrasting drought tolerance including Melko (tolerant), Gemechis (moderate) and Pop12S2 (sensitive) were used for this experiment. Of these, the two varieties (Melko and Gemechis) were released by Debre Zeit and Melkassa Agricultural Research Centers, respectively, and one genotype (Pop12S2) was a landrace collected from central Tigray. The base for selecting these genotypes was based on the moisture stress response in drought screening field experiments. The treatments comprised factorial combinations of three tef genotypes (Melko, Gemechis, and Pop12S2) and five polyethylene glycol (PEG 6000) levels of 0, 0.5, 1, 1.5 and 2% (w/v). The experiment was arranged in completely randomized design with three replications.

Culture media and growth conditions

Murashige and Skoog (1962) medium (MS) was used as basal medium with 3% sucrose and 0.75% agar added by melting on a microwave oven. All media were adjusted to pH

5.8 using drops of 1 N HCl and 1 N NaOH. When the agar became clear solution, 50 ml medium were dispensed in to culture tubes and autoclaved at 121°C for 20 minutes.

Seed sterilization and germination

For sterilization, the seeds were first treated with 70% ethanol for 5 min and then washed in 8% sodium hypochlorite for 30 min, followed by six washes in sterile double distilled water in a laminar airflow cabinet. The sterilized seeds were cultured for two weeks under aseptic conditions containing semisolid MS medium at 27°C. After two weeks, young seedling leaves (Figure 1 A) were excised (Figure 1 B) and used for callus induction.

Callus induction medium

Leaf explants (2 cm) were placed on MS medium containing 0.75% agar and 3% sucrose for each treatment. Callus induction was initiated from the leaf explants placed on MS medium containing 2.4-D (2 mg/l), kinetin (0.2 mg/l) and 1-naphthalene acetic acid (1 mg/l). Different concentrations of PEG (0, 0.5, 1, 1.5, and 2%) were added in to the callus induction medium. The culture tubes were sealed with parafilm and placed in a growth room at 27ºC. In all experiments, three replicates were made, 10 explants of leaf segments were placed with one replication represented by two culture tubes.

Plant regeneration

After four weeks of incubation, the induced calli (Figure 1 C) were transferred to culture tubes, sub-cultured under the same growth conditions and in the same MS medium with various concentrations of PEG (6000) (0, 0.5, 1, 1.5 and 2%). The resulting calli were excised, transferred, into culture tubes containing MS medium supplemented with 1.5 mg/l kinetin + 0.2 mg/l NAA + 3% sucrose + 0.75% agar for shoot initiation (Figure 1 D). This way we were able to check the efficiency of embryogenic calli for further regeneration (shooting and rooting) in the presence of drought stress, calli were exposed to PEG (6000) (0, 0.5, 1, 1.5 and 2%) in the plant regeneration medium. Rooting was initiated on half strength fresh MS medium supplemented with 1.5 mg/l NAA (Figure 1 E). The incubation period was two weeks for shooting and two weeks for rooting.

Acclimatization of regenerated plants

Healthy and well rooted plantlets (Figure 1 F) were washed to remove the medium adhered and subjected to acclimatization, transplanted to plastic tray (Figure 1 G) under high humidity by covering the plant with plastic containing sterilized soils, coco peat and compost, and placed under polythene shed with high humidity (>90% RH) for 3 weeks to harden. After acclimatization, plantlets were transplanted to pot experiment under greenhouse conditions, and the survival percentages were taken four weeks later (Figure 1 H). Finally, the survived plants were assessed for their morpho-phenologic, yield and yield related traits.

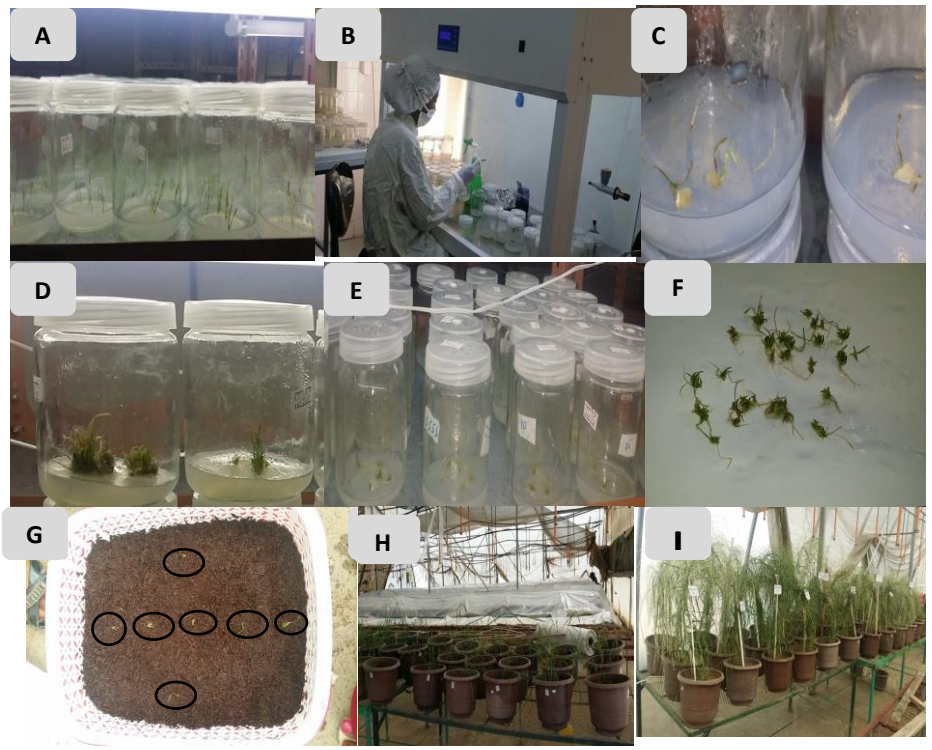

Figure 1. Developmental steps of tef, *in vitro* regenerants. (A) 15-day old seedling explants, (B) Inoculation of the explants, (C) Callus initiation four weeks after inoculation, (D) Shoot regeneration medium, (E) Root regeneration medium, (F) Selected plantlets showing shoots and roots on the shooting and rooting regeneration medium, (G) Plantlets transplanted to plastic tray containing sterilized soils, coco peat and compost for acclimatization (H), Survival of the regenerated plantlets in pot and (I) Regenerant plants during maturity.

Data collection and measurement

Callusing and plant regeneration were measured as follows. Callus induction efficiency (CIE) was assessed as the number of explants induced callus/ total number of cultured explants used for each treatment x 100. Plant regeneration percent (PRP) was recorded as (number of plantlets/total number of calli) × 100 after PEG treatment. Total number of shoots per culture (TSPC) was counted at stage of the shoot multiplication when treated by PEG. Similarly, shoot length (SL) and root length (RL) were measured using an autoclaved square paper and a well-sterilized measuring tape after two weeks of plantlet incubation. Total number of roots per culture (TRPC) and number of roots per shoot (NRPS) were counted at stage of the root regeneration medium. Data was also recorded for rooting percentage as the percent of rooted shoots (RP) per culture. The incubation period for shooting and rooting medium was two weeks for shooting and two weeks for rooting medium. Survival percentage (SP) was calculated as the percent of surviving plants after four weeks of transfer to pots.

The selected regenerants which were transferred to the pots were labeled based on the genotype name from which the regenerants were regenerated and PEG level at which the regenerants were selected. Accordingly, regenerants from genotype Melko were labeled as Melko0, Melko0.5, Melko1, and Melko1.5. Regenerants from genotype Gemechis were labeled as Gemechis0, Gemechis0.5, Gemechis1, and Gemechis1.5. Similarly, regenerants from genotype Pop12S2 were labeled as Pop12S20, Pop12S20.5, Pop12S21 and Pop12S21.5. The numbers included at the end of each genotype, 0, 0.5, 1 and 1.5 indicated the level (percent) of PEG, which were given to each treatment.

Data on days to heading, days to maturity, plant height (cm), panicle length (cm) spikelet length (cm), number of spikelet/panicle, total number of tillers/plant, number of fertile tillers/plant, plant weight (g), plant seed weight (g), panicle weight (g), panicle seed weight (g), 100 seed weight (g), grain yield/pot (g) and harvest index (%) was recorded from five regenerant plants grown in pots.

Data analyses

Data were subjected to analysis of variance (ANOVA) and the interaction means were separated using Tukey's multiple mean comparison while means of main effects were separated using Least Significance Difference (LSD) test at 5% level of probability using the SAS software package (SAS 2009).

Results and Discussion

Analysis of variance

In vitro culture responses of three tef genotypes were assessed with respect to callus induction efficiency, plant regeneration percentage, total shoot per culture, rooting percentage, total roots per culture, number of roots per shoot, shoot length, root length and survival percentage at 0, 0.5, 1, 1.5 and 2% of PEG concentration. Analysis of variance indicated a significant difference ($P \leq 0.05$) among the genotypes as well as the different PEG concentrations (Table 1). This shows the presence of genotype variability, and differential responses of genotypes to different levels of PEG. But total shoot/culture and survival percentage were not genotype dependent. The results also showed significant genotype x PEG interaction for total shoot/culture, total root/culture, shoot length, root length, and survival percentage indicating that genotypes showed differential performances across the different PEG concentrations. On the contrary, callus induction efficiency, plant regeneration percent, rooting percentage and number of roots/shoot were highly significantly ($P \leq 0.01$) affected by genotype as well as PEG main effects, while the interactions of genotype, and PEG were not significant, suggesting that the response of the genotypes in terms of these parameters was consistent across different PEG concentrations.

Table 1. Mean squares from the analysis of variances for genotypes (G), PEG levels and their interaction effects on callus induction and plant regeneration

SoV	DF	Mean square								
		CIE	PRP	TSPC	RP	TRPC	NRPS	SL (cm)	RL (cm)	SP
Genotype	2	215.6**	303.7*	6.1^{ns}	284.4*	296.1**	1.9**	1.2**	0.4**	94.1^{ns}
PEG	4	985.5**	4677.5**	332.7**	12276.3**	4935.9**	43.2**	13.2**	6.3**	14425.1**
G x PEG	8	15.5^{ns}	80.7^{ns}	6.8*	52.3^{ns}	151.7**	0.46^{ns}	0.09**	0.2**	516.6**
Error	28	33.6	101.5	2.6	62.1	40.5	0.21	0.02	0.01	123.2
CV		21.9	20.5	21.5	12.2	21.7	12.0	6.9	8.0	15.7
Mean		26.4	38.8	7.5	64.2	29.2	3.9	2.1	1.5	70.4

** and ** Significant at $P \leq 0.05$ and $P \leq 0.01$ respectively. ns=non-significant, CIE=callus induction efficiency percent, PRP=plant regeneration percent, TSPC=total shoot per culture, RP=rooting percentage, TRPC=total roots per culture, NRPS=number of roots per shoot, SL=shoot length, RL=root length, SP=survival percentage*

Effect of genotypes on callus induction and plant regeneration

Mean comparison of the genotypes revealed that Pop12S2 was significantly better than the other two genotypes in inducing callus (30.7%) while Melko and Gemechis was significantly lower with 25.3 and 23.3%, respectively. The highest CIE from Pop12S2 might be due to good callus induction ability of the genotype as compared to the other two genotypes. Joshi *et al*. (2011) and Gouranga *et al*. (2015) also found differences in callus responses of rice cultivars. In contrast, Mekbib *et al*. (1997) reported similar callus induction efficiency for four tef genotypes. Percent plant regeneration from Melko was significantly higher (43.3%) than the regeneration from callus cultures of the other two genotypes, indicating good plant regeneration potential of the genotype and the induced calli were normal, while significantly lower regeneration potential (34.3%) occurred from Pop12S2 (Table 2).

Table 2 . Main effects of genotypes and different levels of PEG concentration on callus induction and plant regeneration

Genotypes	CIE	PRP	TSPC	RP	TRPC	NRPS	SL (cm)	RL (cm)	SP
Melko	25.3^{b}	43.3^{a}	7.6^{ab}	68.5^{a}	34.0^{a}	4.2^{a}	2.4^{a}	1.6^{a}	70.5^{a}
Gemechis	23.3^{b}	38.9^{ab}	6.8^{b}	64.3^{ab}	25.2^{b}	3.7^{b}	1.9^{b}	1.3^{c}	67.8^{a}
Pop12S2	30.7^{a}	34.3^{b}	8.1^{a}	59.8^{b}	28.5^{b}	3.6^{b}	2.0^{b}	1.5^{b}	72.7^{a}
LSD (5%)	4.3	7.5	1.2	5.9	4.7	0.34	0.1	0.09	8.3
PEG levels (%)									
0	38.9^{a}	60.0^{a}	16.3^{a}	93.1^{a}	62.7^{a}	4.2^{b}	3.0^{a}	1.7^{c}	86.1^{b}
0.5	30.0^{b}	48.1^{b}	9.5^{b}	83.2^{b}	38.6^{b}	4.8^{a}	2.8^{b}	1.8^{b}	100^{a}
1	26.7^{b}	42.6^{b}	7.3^{c}	73.3^{c}	27.0^{c}	5.2^{a}	2.5^{c}	1.9^{a}	79.6^{b}
1.5	26.5^{b}	43.5^{b}	4.4^{d}	71.6^{c}	17.9^{d}	5.2^{a}	2.3^{d}	2.0^{a}	86.0^{b}
2	10.0^{c}	0.0^{c}	0.0^{e}	0.0^{d}	0.0^{d}	0.0^{c}	0.0^{e}	0.0^{d}	0.0^{c}
LSD (5%)	5.6	9.7	1.5	7.6	6.1	0.44	0.14	0.11	10.7

Means within the same column followed by the same letter are not significantly different as judged LSD at $P \leq 0.05$. CIE=callus induction efficiency percent, PRP=plant regeneration percent, TSPC=total shoot per culture, RP=rooting percentage, TRPC=total roots per culture, NRPS=number of roots per shoot, SL=shoot length; RL=root length, SP=survival percentage

The highest rooting percent (68.5%) and number of roots/shoot (4.2) were attributed to genotype Melko. In contrast, Pop12S2 showed the least rooting percent and number of roots/shoot with 59.8% and 3.6, respectively. This could be because the quality of calli

from Pop12S2, might not as good as those from the other two genotypes (Figure 2), thus, the induced calli might be more sensitive to moisture stress during the regeneration and further growth. Helaly *et al*. (2013) and Amaranatha *et al*. (2015) reported that callus induction was a critical phase where the regeneration of plants is highly dependent on the quality of callus.

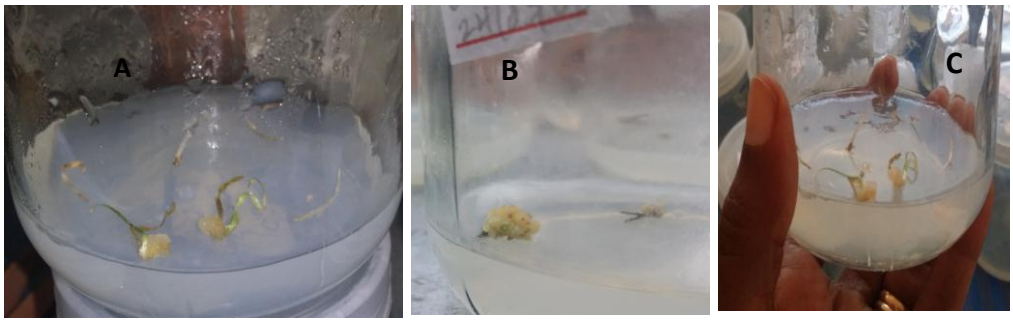

Figure 2. Callus culture from genotypes (A) Melko, (B) Gemechis and (C) Pop12S2 with MS medium at 1.5% PEG Concentration, 4 weeks after

Effect of PEG stress on callus induction and plant regeneration

Mean values of the different PEG concentration (0, 0.5, 1, 1.5 and 2%) were highly significant ($P \leq 0.01$) different for all the studied parameters. Increasing level of PEG from 0 to 2% had significant effect on mean values of the parameters measured. Callus initiation was observed in all the PEG concentrations, but the induction efficiency varied from concentration to concentration (Table 2). The callus growth was rapidly reduced with the relative increase of PEG concentration. Callus induction percentage on callus induction medium containing 0.5, 1 and 1.5% PEG was 30, 26.7and 26.5% respectively, against 38.9% in the control treatment. This showed that increasing PEG concentration had an inhibitory effect on the growth of callus. Gradual decrease in callus induction efficiency with a progressive increase of PEG in the culture medium was also reported by Joshi *et al*. (2011) in rice and Farshadfar *et al*. (2012) in wheat. Tsago *et al*. (2013) in sorghum also reported that the mean callus induction efficiency decreased drastically under higher PEG concentration.

The negative effect of moisture stress was stronger in 2% PEG as only 10% of the cultures induced callus and the induced calli lost their regeneration ability and further growth was inhibited. Biswas *et al*. (2002) stated that, this might be due to the interference of PEG in proplastid biosynthesis during morphogenesis. Sakthivelu *et al*. (2008) reported that addition of high PEG-6000 in culture media lowers water potential of the medium that adversely affect cell division leading to reduced further callus growth.

As the PEG concentration in the medium increased, there was a decrease in plant regeneration percentage (Figure 3).The plant regeneration percentage was 60% at 0% PEG and decreased to 48.1% at 0.5%, 42.6% at 1%, 43.5% at 1.5% and reached 0% at 2% PEG concentration. The result also indicated a significant reduction in rooting percentage as the PEG concentration increases. On the contrary, a significant increment of root length

was found at 1% (1.9 cm) and 1.5% (2 cm) PEG concentrations respectively, as compared to the control and the remained concentrations (Table 2). This reflects an adaptive response involving an increase in root length to reach deeper water in the soil. Ahmed (2014) who found an increase in root length associated with increasing PEG concentration observed similar trends in the study.

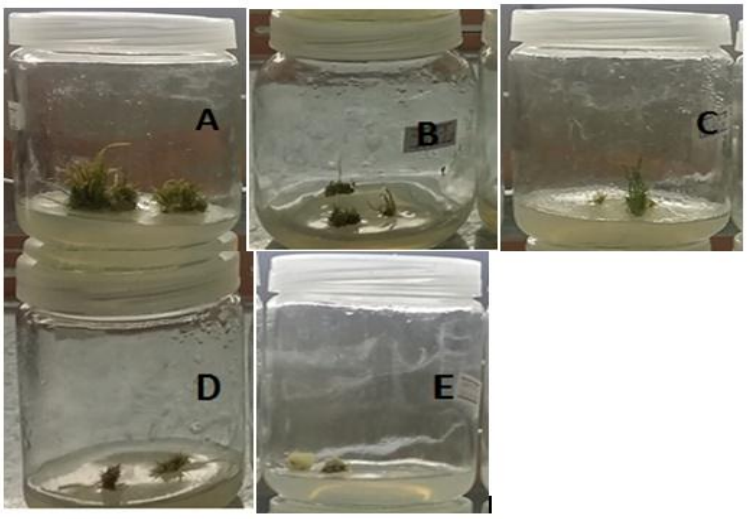

Figure 3. Shoots regeneration medium via indirect somatic embryogenesis (callus) for genotype Melko. (A) 0% PEG 6000, (B) 0.5% PEG 6000, (C) 1% PEG 6000, (D) 1.5% PEG 6000 and (E) 2% PEG 6000.

Effects of genotype x PEG interaction on callus induction and plant regeneration

The genotype × PEG interaction was significant for total shoot/culture, total root/culture, shoot length, root length and survival percentage (Table 3), displaying differential responses of genotypes to different levels of drought inducing PEG. Several reports indicated significant interaction effects between genotypes and PEG concentration for the measured parameters. Leila (2013) in six Pearl millet genotypes subjected to three different PEG 6000 levels, and Tsago *et al.* (2013) using sixteen elite sorghum genotypes at five different PEG 6000 levels (0, 0.5, 1.0, 1.5, 2.0%), found significance differences among genotypes, treatments and their interaction for shoot length, root length, shoot number and root number. On the contrary, the interaction effect for callus induction efficiency, plantlet regeneration percent, rooting percentage and number of roots/shoot was not significantly different indicating that the response of the genotypes was consistent across different PEG concentration. Therefore, focusing only on the main effects would be relevant for these parameters.

Table 3. Interaction effects of genotypes and PEG concentration for drought tolerance in tef regenerants

Regenerants	PEG level	CIE	PRP	TSPC	RP	TRPC	NRPS	SL (cm)	RL (cm)	SP
Melko	0	33.33^{bc}	61.1^{ab}	14.33^{b}	95.39^{a}	59.00^{b}	4.33^{efg}	3.40^{a}	1.53^{ef}	91.67^{ab}
Melko	0.5	30.00^{bcd}	55.6^{abc}	10.67^{c}	92.09^{ab}	52.00^{b}	5.33^{abc}	3.20^{a}	1.90^{c}	100.00^{a}
Melko	1	26.67^{cd}	50.0^{abcd}	7.33^{de}	73.01^{cd}	30.67^{cd}	5.66^{ab}	2.83^{b}	2.30^{a}	61.11^{d}
Melko	1.5	26.66^{cd}	50.0^{abcd}	5.66^{ef}	82.22^{abc}	28.33^{cd}	6.00^{a}	2.77^{bc}	2.40^{a}	100.00^{a}
Melko	2	10.00^{e}	0.00^{e}	0.00^{g}	0.00^{e}	0.00^{g}	0.00^{h}	0.00^{g}	0.00^{g}	0.00^{e}
Gemechis	0	36.66^{b}	55.0^{abc}	15.00^{b}	93.61^{ab}	58.33^{b}	4.17^{fg}	2.70^{bc}	1.37^{f}	66.67^{cd}
Gemechis	0.5	26.66^{cd}	50.0a^{bcd}	8.33^{cde}	82.14^{bc}	27.16^{cd}	4.16^{fg}	2.57^{cd}	1.63^{de}	100.00^{a}
Gemechis	1	23.33^{d}	44.4^{bcd}	7.00^{de}	72.22^{cd}	25.83^{d}	5.16^{bcd}	2.13^{ef}	1.60^{de}	88.90^{ab}
Gemechis	1.5	23.30^{d}	44.0^{bcd}	4.00^{f}	73.89^{cd}	14.67^{cf}	4.83^{cdef}	2.10^{f}	1.90^{c}	83.33^{abc}
Gemechis	2	6.67^{e}	0.00^{e}	0.00^{g}	0.00^{e}	0.00^{g}	0.00^{h}	0.00^{g}	0.00^{g}	0.00^{e}
Pop12S2	0	46.77^{a}	63.3^{a}	19.70^{a}	90.24^{ab}	70.67^{a}	4.00^{g}	2.87^{b}	2.10^{b}	100.00^{a}
Pop12S2	0.5	33.33^{bc}	38.9^{cd}	9.67^{cd}	75.42^{cd}	36.66^{c}	5.00^{bcde}	2.73^{bc}	1.90^{c}	100.00^{a}
Pop12S2	1	30.00^{bcd}	33.3^{d}	7.66^{de}	69.64^{cd}	24.66^{de}	4.66^{c-g}	2.37^{de}	1.90^{c}	88.89^{ab}
Pop12S2	1.5	30.00^{bcd}	36.1^{d}	3.67^{f}	63.89^{d}	10.66^{f}	4.50^{defg}	2.03^{f}	1.73^{cd}	75.00^{bcd}
Pop12S2	2	13.33^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}

Means within the same column followed by the same letter are not significantly different as judged by Tukey's multiple comparison at P ≤ 0.05. CIE=callus induction efficiency percent, PRP=plant regeneration percent, TSPC=total shoot per culture, RP=rooting percentage, TRPC=total roots per culture, NRPS==number of roots per shoot, SL=shoot length; RL=root length, SP=survival percentage

Culture media without PEG (0%) showed the highest total shoot/culture (19.7), total root/culture (70.6), root length (2.1 cm), and survival percentage (100%) in Pop12S2 and highest shoot length (3.4 cm) for Melko. Gemechis exhibited the lowest performance for total root/culture (58.3), shoot length (2.7 cm), root length (1.37 cm) and survival percentage (66.7%) in the control treatment. At 0.5% PEG concentration, Melko showed better performance for total shoot/culture (10.6), total root/culture (52), shoot length (3.2 cm), root length (1.9 cm), and survival percentage (100%). Maximum root length (1.9 cm) and survival percentage (100%) was also recorded from Pop12S2.Gemechis was the lowest performing genotype for total shoot/culture (8.3), total root/culture (27.1), shoot length (2.5 cm) and root length (1.6 cm) in the same PEG concentration.

On MS medium supplemented with 1% PEG, Melko produced highest total root/culture (30.6), shoot length (2.8 cm) and root length (2.3 cm). In contrast, Pop12S2 had produced the least total root/culture (24.6). The lowest shoot length (2.1 cm) and root length (1.6 cm) were recorded for the genotype Gemechis. The highest total shoots/culture (7.6) was noted for Pop12S2 and the least was from Gemechis, while the latter two genotypes exhibited no difference in survival percentage (88.9%) (Table 3). At 1.5% PEG concentration the highest callus induction efficiency (30%) was recorded from Pop12S2. When the induced calli were transferred to regeneration media in the same PEG concentration, highest plant regeneration percent (50%), total shoots/culture (5.7), rooting percent (82.2), total roots/culture (28.3), number of roots/shoot (6), shoot length (2.8 cm), root length (2.4 cm) and survival percentage (100%) was recorded from the genotype Melko.

Fluctuation behavior of regenerants was observed for almost of all the parameters. Hence, to determine the most desirable drought tolerant regenerants based on all traits measured and for the overall judgment, ranking method was used. Mean rank, rank sum and standard deviation of ranks were used according to Farshadfar *et al*. (2012). In this

method all indices, rank, standard deviation of rank, rank mean, rank sum of all *in vitro* drought tolerance criteria and final rank were calculated (Table 4). Taking all *in vitro* regeneration characteristics in to consideration, regenerants from Melko (0.5), Melko (1.5) followed by Melko (1) were the most desirable drought tolerant regenerants in that order. While regenerants from Pop12S2 (1.5), Pop12 S2 (1), Gemechis (0.5) and Gemechis (1) were the most sensitive to drought. Farshadfar et al. (2012) have used the same procedures for in vitro screening of drought tolerance in bread wheat and Tsago *et al*. (2013) in sorghum.

Table 4. Ranks (R), ranks mean ($\bar{R}$), rank sum (RS) and standard deviation of ranks (SDR) of tef regenerants for drought tolerance

Regenerant	CIE	PRP	TSPC	RP	TRPC	NRPS	SL	RL	SP				
	R	R	R	R	R	R	R	R	R	SDR	$\bar{R}$	RS	FR
Melko (0)	3	2	3	1	2	1	1	2	2	0.78	1.89	2.67	5
Melko (0.5)	2	1	1	1	1	1	1	1	1	0.33	1.11	1.44	1
Melko (1)	2	1	2	1	1	1	1	1	2	0.5	1.33	1.83	2
Melko (1.5)	2	1	1	1	1	1	1	1	1	0.33	1.11	1.44	1
Melko (2)	2	-	-	-	-	-	-	-	-	-	-	-	-
Gemechis(0)	2	3	3	2	3	2	3	3	3	0.5	2.67	3.17	7
Gemechis (0.5)	3	2	2	2	3	3	3	2	1	0.71	2.33	3.04	6
Gemechis (1)	3	2	3	2	2	2	3	3	1	0.71	2.33	3.04	6
Gemechis(1.5)	3	2	2	2	2	2	2	2	2	0.33	2.11	2.44	4
Gemechis (2)	3	-	-	-	-	-	-	-	-	-	-	-	-
Pop12S2 (0)	1	1	1	3	1	3	2	1	1	0.88	1.56	2.44	3
Pop12S2(0.5)	1	3	2	3	2	2	2	1	1	0.78	1.89	2.67	5
Pop12S2 (1)	1	3	1	3	3	3	2	2	1	0.93	2.11	3.04	6
Pop12S2(1.5)	1	3	3	3	3	3	3	3	3	0.67	2.88	3.44	8
Pop12S2 (2)	1	-	-	-	-	-	-	-	-	-	-	-	-

Values in parenthesis indicated PEG concentration in %, CIE=callus induction efficiency percent, PRP=plant regeneration percent, TSPC=total shoot per culture, RP=rooting percentage, TRPC=total roots per culture, NRPS=number of roots per shoot, SL=shoot length, RL=root length, SP=survival percentage, SDR=standard deviation of rank, $\bar{R}$=rank mean, RS=rank sum, FR=final rank

Evaluation of *in vitro* developed tef regenerants in related morpho-phonologic yield and yield related traits in green house

Twelve tef regenerants developed from *in vitro* culture (R0 regenerants) were assessed for morpho-phenologic, yield and yield related traits. All regenerants were morphologically normal, reached to physiological maturity, and set seeds well. Analysis of variance revealed that the regenerants showed highly significant difference in all the traits measured (Table 5). Gadakh *et al.* (2015) and Rahman *et al*. (2016) reported studies on in vitro developed regenerants validation using agronomical and morphological traits.

Table 5. Analysis of variance of 12 direct regenerants of tef evaluated for 16 traits

Source of variation	DF	Mean squares							
		DH	DM	PH (cm)	PL (cm)	SL(cm)	NSPP	TNT	NFT
Treatment	11	46.3**	60.0**	889.0**	167.2**	27.4**	52296.8**	1.85**	1.014**
Error	24	6.30	0.72	116.40	13.96	1.31	4249.3	0.034	0.030
CV		8.61	1.08	10.31	8.87	6.70	10.53	4.69	5.47
LSD (5%)		4.23	1.43	18.18	6.30	1.93	109.85	0.31	0.29
Source of variation	DF	Mean squares							
		PW (g)	PSW (g)	PTW (g)	PTSW (g)	HSW (g)	GY (g)	BMY (g)	HI %
Treatment	11	0.117**	0.030**	14.01**	2.015**	0.0001**	10.45**	228.7**	27.92**
Error	24	0.003	0.0005	0.663	0.089	0.000	0.432	5.85	2.73
CV		6.78	7.47	7.96	8.61	2.22	10.45	6.75	9.39
LSD (5%)		0.09	0.038	1.31	0.503	0.005	1.11	4.08	2.78

*** Significant at ≤ 0.01 level of probability. DF=degree of freedom, DH=days to heading, DM=days to maturity, PH=plant height, PL=panicle length, SL=spikelet length, NSPP=number of spikelets/panicle, TNT=Total number of tiller/plant, NFT=number of fertile tillers/plant, PW=panicle weight, PSW=panicle seed weight, PTW=plant weight, PTSW=plant seed weight, HSW=hundred seed weight, GY=grain yield, BMY=biomass yield, HI=harvest index*

Regenerants obtained from Melko (Figure 4 A) and Gemechis (Figure 4 B) showed vigorous growth as compared to regenerants obtained from Pop12S2 (Figure 4 C). On the other hand, regenerants obtained from Pop12S2 were better for their earliness.

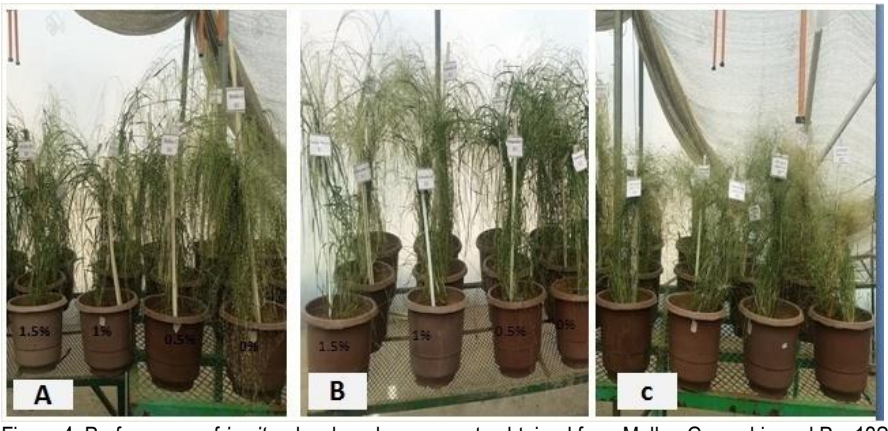

Figure 4. Performance of *in vitro* developed regenerants obtained from Melko, Gemechis and Pop12S2 (A, B and C) genotypes, respectively under greenhouse condition.

Table 6 shows, the mean comparisons of the regenerants based on morpho-phenologic, yield and yield related parameters. Our result showed that Pop12S20 and Pop12S20.5 were an earliest heading regenerants (24 days), while regenerant Melko0.5 (36.3 days) was the late heading. Pop12S21.5, Pop12S20 and Pop1S21 was an early-matured regenerants with mean values of 71.7, 72.0, and 72.7 days. Gemechis0 and Gemechis0.5 was the late matured regenerants. The average value for days to heading and days to maturity was 29.1 and 78.2 of days, respectively indicating the regenerants were earliest for both days to heading and maturity that could be an important opportunity for drought prone areas. Plaza-Wüthrich *et al*. (2013) reported that earliness for days to heading and maturity are important traits on tef for areas with low rainfall to escape terminal drought and, in high rainfall with long growing season areas, can be employed in double cropping systems.

Table 6. Mean performance of 12 direct regenerants of tef evaluated for 16 traits under greenhouse condition

Regenerant	DH	DM	PH (cm)	PL (cm)	SL (cm)	NSPP	TNT	NFT
Melko0	34.3^{ab}	80.3^{c}	123.6^{a}	49.4^{a}	18.7^{ab}	434.7^{ef}	5.29^{a}	4.30^{a}
Melko0.5	36.3^{a}	82.0^{b}	125.0^{a}	46.2^{a}	18.9^{ab}	731.8^{ab}	4.61^{b}	3.51^{bcd}
Melko1	25.7^{e}	79.7^{c}	119.2^{ab}	50.1^{a}	18.8^{ab}	690.3^{abc}	3.60^{de}	2.86^{e}
Melko1.5	32.3^{ab}	79.0^{c}	112.2^{ab}	48.3^{a}	18.0^{b}	484.3^{ef}	4.80^{b}	3.31^{d}
Gemechis0	30.6^{bc}	84.3^{a}	117.2^{ab}	46.5^{a}	20.3^{a}	611.5^{cd}	4.13^{c}	3.73^{b}
Gemechis0.5	30.6^{bc}	84.0^{a}	112.0^{ab}	44.9^{a}	18.9^{ab}	383.3^{f}	3.73^{d}	2.93^{e}
Gemechis1	27.7^{cde}	79.0^{c}	110.3^{ab}	45.5^{a}	19.7^{ab}	784.9^{a}	3.30^{ef}	2.47^{f}
Gemechis1.5	30.3^{bcd}	79.0^{c}	101.7^{bc}	44.5^{a}	19.1^{ab}	508.4^{de}	4.83^{b}	3.62^{bc}
Pop12S20	24.0^{e}	72.0^{e}	77.3^{d}	31.0^{b}	12.3^{cd}	712.0^{abc}	3.86^{cd}	3.38^{cd}
Pop12S20.5	24.7^{e}	75.0^{d}	88.0^{cd}	34.0^{b}	14.2^{c}	674.4^{bc}	3.00^{f}	2.67^{ef}
Pop12S21	26.6^{cde}	72.7^{e}	77.0^{d}	29.5^{b}	11.9^{d}	711.9^{abc}	3.18^{f}	2.53^{f}
Pop12S21.5	30.3^{bcd}	71.7^{e}	91.3^{cd}	35.2^{b}	14.1^{c}	697.1^{abc}	3.00^{f}	2.50^{f}
Mean	29.14	78.20	104.57	42.12	17.07	618.73	3.94	3.15
Regenerant	PW (g)	PSW (g)	PTW (g)	PTSW (g)	HSW (g)	GY (g)	BMY (g)	HI (%)
Melko0	1.003^{a}	0.402^{bc}	14.67^{a}	5.164^{a}	0.040^{c}	9.84^{a}	53.30^{a}	18.51^{bcd}
Melko0.5	0.737^{d}	0.270^{de}	9.77^{c}	2.433^{h}	0.040^{c}	3.58^{f}	34.12^{cd}	10.56^{f}
Melko1	0.892^{bc}	0.293^{d}	10.10^{c}	2.790^{fgh}	0.050^{a}	4.49^{ef}	32.69^{cdef}	13.77^{e}
Melko1.5	0.850^{c}	0.440^{a}	9.13^{cd}	3.957^{bc}	0.035^{d}	6.13^{c}	28.65^{fg}	21.46^{a}
Gemechis0	0.923^{abc}	0.417^{ab}	10.44^{c}	3.783^{cd}	0.045^{b}	5.88^{cd}	33.74^{cde}	17.41^{cd}
Gemechis0.5	0.953^{ab}	0.377^{c}	9.83^{c}	3.263^{ef}	0.035^{d}	4.94^{de}	30.21^{def}	16.36^{de}
Gemechis1	0.855^{c}	0.282^{de}	12.16^{b}	4.017^{bc}	0.035^{d}	8.05^{b}	43.75^{b}	18.50^{cd}
Gemechis1.5	0.870^{bc}	0.287^{de}	10.40^{c}	3.369^{de}	0.035^{d}	6.17^{c}	34.63^{c}	17.83^{cd}
Pop12S20	0.733^{d}	0.302^{d}	12.98^{b}	4.400^{b}	0.030^{e}	8.83^{ab}	50.26^{a}	17.56^{cd}
Pop12S20.5	0.543^{e}	0.253^{e}	7.15^{e}	2.596^{gh}	0.030^{e}	4.47^{ef}	24.86^{g}	17.93^{cd}
Pop12S21	0.497^{e}	0.128^{f}	7.94^{de}	2.806^{fgh}	0.035^{d}	6.36^{c}	29.83^{ef}	21.28^{ab}
Pop12S21.5	0.380^{f}	0.137^{f}	8.25^{de}	2.994^{efg}	0.035^{d}	6.76^{c}	33.66^{cde}	20.14^{abc}
Mean	0.78	0.299	10.23	3.46	0.037	6.29	35.80	17.60

Mean values within column followed the same letters are not significantly different ($P \leq 0.01$). DH=days to heading, DM=days to maturity, PH=plant height, PL=panicle length, SL=spikelet length, NSPP=number of spikelets/panicle, TNT=Total number of tiller/plant, NFT=number of fertile tillers/plant, PW=panicle weight, PSW=panicle seed weight, PTW=plant weight, PTSW=plant seed weight, HSW=hundred seed weight, GY=grain yield, BMY=biomass yield, HI=harvest index

Maximum plant height was recorded from regenerants Melko0.5 (125 cm) and Melko0 (124 cm). Highest mean values for panicle length and spikelet length was recorded from Melko1 (50.1 cm) and Gemechis0 (20.3 cm), respectively. On the other hand, poor

performance for plant height (77.0 cm), panicle length (29.5 cm) and spikelet length (11.9 cm) were recorded from regenerant Pop12S21.

Regenerant Melko0 showed good performance in total number of tillers/plant (5.3), number of fertile tillers/plant (4.3), panicle weight (1.00 g), plant weight (14.67 g), plant seed weight (5.16 g), grain yield (9.84 g) and biomass yield (53.3 g). On the other hand, regenerants obtained from Pop12S2 showed poor performance for total number of tillers/plant (3), panicle weight (0.38 g), panicle seed weight (0.128 g), plant weight (7.15 g), plant seed weight (2.53 g), hundred seed weight (0.03 g) and biomass yield (24.9 g).

In general, most of the regenerants obtained from Melko showed best performance under greenhouse were drought tolerant under the *in vitro* condition (Table 2 and 6). This suggests the accrued performance of the tested regenerants under *in vitro* condition was realized under greenhouse condition. It also indicated that, *in vitro* culture is an important tool to develop drought tolerant genotypes and to improve desirable agronomical traits.

Conclusion

The results of this study showed differential responses of callusing and regeneration efficiency under different concentrations of PEG. High concentration of PEG significantly reduced callus growth characteristics such as callus induction efficiency, plantlet regeneration percentage, total shoots/culture, rooting percentage, total roots/culture, and shoot length. However, number of roots/culture and root length increased under high PEG concentration. This may be an adaptive mechanism to moisture stress in the culture medium.

In vitro screening showed that regenerants of Melko (0.5%), Melko (1.5%) and Melko (1%) were drought tolerant, while those of Pop12S2 (1.5%) were the most sensitive regenerant to moisture stress. The performance of regenerants obtained from Melko genotype under *in vitro* condition was also realized under greenhouse condition.

Regenerants obtained from Melko genotype appeared to be more tolerant to moisture stress as compared to Gemechis and Pop12S2 and can be selected for crossing and further improvement of drought tolerance. This genotype could be cultivated in environments where water scarcity is a frequent constraint.

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