

Evaluation of Tef (*Eragrostis tef* (Zucc.) Trotter) Somaclones for Drought Tolerance

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Abstract

Breeding for moisture stress tolerance by conventional methods is not satisfactory in view of current demand to increase crop productivity. Inducing somaclonal variation is a potential technique to obtain stress tolerant variants. The present study was conducted to evaluate the performance of tef somaclones (R1 generation) and their respective parental genotypes for drought tolerance. The materials used in this experiment were obtained from the R0 generation of previous *in vitro* culture experiment. Twelve seed derived somaclones; Melko 0, Melko 0.5, Melko 1, Melko 1.5, Gemechis 0, Gemechis 0.5, Gemechis 1, Gemechis 1.5, Pop12S20, Pop12S20.5, Pop12S21 and Pop12S21.5 and their respective parental genotypes (Melko, Gemechis and Pop12S2) were used under two set of experiments (i.e., under moisture stress and non-stress conditions) in pots under green house. The experiments were laid out in completely randomized design with three replications. Analysis of variance revealed that somaclones (R1 generation) and their parental genotypes showed highly significant ($P \leq 0.01$) difference in all the measured traits under both moisture regimes. Comparisons were made between the somaclones and their parents under moisture stress and non-stress conditions for the measured traits. Under moisture stress condition superiority of the somaclones was observed except for number of spikelet/panicle and total number of tiller/plant. On the other hand, under non-stress condition better performance was observed from parental genotypes for number of spikelet/panicle, panicle weight, panicle seed weight, plant weight, plant seed weight and grain yield. The results showed that all the somaclones and their parental genotypes were negatively affected by moisture stress for all studied parameters. However, somaclones were less affected by moisture stress as compared to the parental genotypes. Hence, *in vitro* culture induced somaclonal variation can effectively be used for selection and improvement of drought tolerant tef genotypes.

Keywords: Generation; *In vitro* culture; Moisture stress; Parental genotypes; Somaclones

Introduction

Tef (*Eragrostis tef* (Zucc.) Trotter) belongs to family Poaceae and genus *Eragrostis* and is believed to have been first domesticated by pre-semitic inhabitants in Ethiopia between 1000 and 500 B.C [1]. Tef is not only staple food crop for more than 50 million Ethiopians, but also the most sources of animal feed, generate household income and fulfilling the nutritional needs [2]. Tef contains high nutritional values; 11% protein, 80% complex carbohydrate and 3% fat [3]. 100 grams of tef grain contains 180 mg calcium, 0.8 mg copper, 7.6 mg iron, 184 mg magnesium, 9.2 mg manganese, 429 mg phosphorus, 427 mg potassium, 12 mg sodium, 3.6 mg zinc and 4.4 mg selenium. Grains of tef are reported to contain vitamins; 0.39, 0.27 and 3.4 mg of Thiamin, Riboflavin and Niacin, respectively [4]. It also contains eight essential amino acids (isoleucine, leucine, methionine, lysine, phenylalanine, threonine, tryptophan and valine) [5].

Even though its importance is indispensable in the Ethiopian agriculture and the national food security, the productivity of tef is low

particularly due to drought and water lodging [6]. Improved varieties of tef under well managed farms produced an average grain yield of 1.95-ton ha⁻¹ on farmers field and 2.5-ton ha⁻¹ on research fields [7,8]. However, the current tef yield is about 1.66-ton ha⁻¹ [9]. Moisture stress has been one of the major causes for its low yield [10].

Ayele [11] described that yield losses of tef due to low moisture stress commenced up to 40% during severe stress on vegetative stages. Admas and Belay [12] and Shiferaw et al. [13] have been reported that about 26% to 51% grain yield reductions for tef due to moisture stress. Furthermore, yield reduction of 7.3% to 85% has been reported to have occurred as a result of drought at the anthesis stage under green house and 69% to 77% yield loss under field conditions occurred at pre-flowering stage of tef [14,15].

Breeding for water stress tolerance by traditional methods is a time consuming and inefficient procedure [16]. Saadalla [17] reported plant breeders should have an effective and reliable screening method; relatively simple, accurate, inexpensive and well correlated with crop performance under actual stressful conditions.

In vitro culture offers breeders an alternative strategy to conventional methodology for crop improvement. It has been widely

accepted that applying drought stress through *in vitro* culture is an efficient strategy for selecting drought tolerant cell lines and the regeneration of tolerant plants in limited space and short time duration [18]. During plant tissue culture, genetic variability, i.e., somaclonal variation may occur and the variation has an alternative tool for obtaining better performed plants regardless of important morphological and agronomical traits with increased tolerance to moisture stress. Somaclonal variations, for drought tolerance have been reported in many cereal crops, such as durum wheat [19], rice [20], sorghum [21] and peanut [18]. Rahayu [18] in peanut and Verma [20] in rice found drought tolerant somaclonal variants with better yield than the mother plants (non-tissue cultured plants). To date, little investment in biotechnology has been applied to tef. However, there was no report so far on somaclonal variation as a means of evaluating drought tolerance, disease resistance and other agronomic traits in tef. Validation of somaclonal variants and the parental genotypes is an important step to assess the performance of tissue culture derived somaclones relative to non-tissue cultured (mother plant) plants. Therefore, this study was undertaken to evaluate the performance of tef somaclones (R1 generation) and their respective parental genotypes for drought tolerance.

Materials and Methods

Description of the study area

The experiments of this study were conducted in green house at Debre Zeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR) in 2016/17. Debre Zeit is located 47 km away south East of Addis Ababa.

Experimental materials and design

The materials used in this experiment were obtained from the R0 generation of previous *in vitro* culture experiment. Twelve seed derived somaclones (R1 generation); Melko 0, Melko 0.5, Melko 1, Melko 1.5, Gemechis 0, Gemechis 0.5, Gemechis 1, Gemechis 1.5, Pop12S20, Pop12S20.5, Pop12S21 and Pop12S21.5 and their respective parental genotypes (Melko, Gemechis and Pop12S2) were used under two set of experiments (under moisture stress and non-stress conditions). Water stress was induced by withholding irrigation for 20 days at anthesis stage, while the non-stress experiment was regularly watered with optimum condition (field capacity) until plants were physiological matured. In each experiment five plants per pot were planted at a pot

size of 40 cm diameter containing 6 kg black soil under green house. The experiments were laid out in completely randomized design [22] with three replications.

Data collection and measurement

Morpho-phonologic yield and yield related data was recorded from pots on the green house and each pot contains five plants. Days to heading and days to maturity were counted from planting to the date of 50% and 75% of the plants head emerged and matured respectively. When 75% of the stems, leaves, and floral bracts of the crop stand in a pot changed to light yellow (straw) color, a plant assumed to be physiologically matured. Plant height (cm) was measured at physiological maturity from the ground level to the tip of panicle from five plants in each pot and the average value was taken. Panicle length (cm) was taken from the node where the first panicle branch starts to the tip of the panicle as the average number of five plants. Spikelet length (cm) was measured from the base to the tip of the spikelet from five plants in each pot and the average value was taken. Number of spikelet/panicle was taken from the average number of spikelets of five plants in each pot. Total number of tillers/plant and number of fertile tillers/plant were determined from average values of five plants in each pot. Plant weight, plant seed weight, panicle weight and panicle seed weight were determined in grams from average value of five plants and panicles, respectively. 100 seed weight was the weight of hundred seeds on a sensitive balance in gram. Grain yield was determined the weight of grain harvested from the pot in gram. Harvest index (%) is the ratio of grain yield to above ground biomass multiplied by 100.

Data analyses

Collected data was analyzed using the SAS software package [23]. Analysis of variance (ANOVA) was carried out for each parameter while the differences between treatments means were separated using Least Significance Difference (LSD) test at 5% level of probability.

Results

Analysis of variance

Analysis of variance revealed that seed derived somaclones (R1 generation) and their parents showed highly significant ($P \leq 0.01$) difference in all the traits measured under both moisture regimes (Table 1).

Source Variation	of DF	Stress							
		DH	DM	PH	PL	SL	NSPP	TNT	NFT
Treatment	14	11.56**	58.20**	348.29**	21.51**	5.11**	40686.7**	2.31**	0.91**
Error	30	1.38	7	24.68	7.65	1.2	3324	0.12	0.04
CV		3.4	3.49	6.55	9.52	8.92	10.45	9.68	8.43
LSD (5%)		1.96	4.41	8.28	4.61	1.83	96.14	0.55	0.33
		PW	PSW	PTW	PTSW	HSW	GY	BMV	HI %
Treatment	14	0.026**	0.005**	2.69**	0.099**	0.00006**	0.50**	35.73**	70.83**
Error	30	0.0008	0.0003	0.052	0.008	0.00001	0.025	0.427	2.088

CV		8.64	12	5.56	10.06	11.96	6.79	4.66	8.21
LSD (5%)		0.047	0.028	0.38	0.148	0.0055	0.264	1.09	2.41
Non-stress									
		DH	DM	PH	PL	SL	NSPP	TNT	NFT
Treatment	14	15.14**	105.89**	150.87**	56.43**	6.46**	49230.9**	1.52**	0.78**
Error	35	1.45	2.93	19.63	5.11	1.41	4022	0.012	0.009
CV		3.52	2	4.97	6.32	8.87	10.45	3	3.27
LSD (5%)		2.03	2.86	7.39	3.77	1.98	105.75	0.18	0.16
		PW	PSW	PTW	PTSW	HSW	GY	BMV	HI %
Treatment	14	0.11**	0.033**	13.14**	1.37**	0.00008**	5.93**	186.58**	50.51**
Error	35	0.0007	0.0002	0.826	0.046	0.0000004	0.098	2.98	2.03
CV		3.41	3.64	9.36	8	1.69	5.59	5.27	8.08
LSD (5%)		0.045	0.024	1.52	0.36	0.001	0.52	2.88	2.37

Table 1: Analysis of variance of 12 seed derived tef somaclones and the 3 parental genotypes for 16 morpho-phenologic, yield and yield related traits evaluated under moisture stress and non-stress conditions. ** Significant at $P \leq 0.01$ 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 spikelet/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, BMV=biomass yield, HI=harvest index.

Evaluation of somaclones (R1 generation) and their respective parental genotypes for drought tolerance

Twelve tef somaclones (Melko 0, Melko 0.5, Melko 1, Melko 1.5, Gemechis 0, Gemechis 0.5, Gemechis 1, Gemechis 1.5, Pop12S20, Pop12S20.5, Pop12S21 and Pop12S21.5) and three parental tef genotypes; Melko, Gemechis and Pop12S2, were evaluated for 16 morpho-phenologic, yield and yield related traits under moisture stress and non-stress conditions. The result showed that all the somaclones and their parental genotypes were negatively affected by moisture stress for all studied parameters. The differences among the means of the somaclones and their respective parental genotypes for the studied parameters were highly significant ($P \leq 0.01$) for both experimental conditions (Table 2). In line with the present results, significant differences through the *in vitro* regenerated plants and the donor parents have been reported previously in many crops [19,24-26].

The mean values of the current results for both moisture stress and non-stress conditions are presented in Table 2. Average number of days to heading and days to maturity across the somaclones and their

parental genotypes was 34.47 and 75.60 days under stress and 34.62 and 85.89 days under non-stress conditions, respectively. The mean difference in days to heading under moisture stress (34.47 days) and non-stress (34.62 days) conditions were very small. This might be due to moisture stress was imposed after heading. Under the stress condition, difference in days to maturity of early and late maturing was 13 days, while under non-stress condition the difference was 17 days which was larger. This might be due to the plasticity of the somaclones/ parental genotypes for maturity in optimum environments. Under the stress condition, somaclone Pop12S21 was an early matured, while under the non-stress conditions Pop12S2 (parent) was an early matured genotype. This result indicates that the somaclones could escape from the late moisture stress as compared to the parental genotype. Arun et al. [27] in bread wheat, Bouiamrine et al. [19] in durum wheat and Rahman et al. [28] in sugar cane reported somaclones with earliness in heading and in maturity as compared to their donor parents. In the present result Melko 0.5 was the late maturing under both moisture regimes.

Somaclones/ Genotypes	DH		DM		PH (cm)		PL (cm)	
	S	NS	S	NS	S	NS	S	NS
Melko0	37.0 ^a	37.3 ^a	79.7 ^{abc}	92.7 ^{ab}	63.7 ^{hi}	90.4 ^{bcd}	25.6 ^{ef}	39.1 ^a
Melko0.5	37.0 ^a	37.3 ^a	81.7 ^a	93.0 ^a	76.3 ^{cde}	88.2 ^{cd}	29.9 ^{a-e}	39.7 ^a
Melko1	36.7 ^{ab}	37.3 ^a	80.0 ^{ab}	92.7 ^{ab}	64.9 ^{ghi}	93.2 ^{abc}	26.7 ^{def}	41.0 ^a
Melko1.5	36.0 ^{abc}	36.3 ^{ab}	80.0 ^{ab}	92.3 ^{ab}	66.9 ^{ghi}	88.5 ^{cd}	28.3 ^{b-f}	37.6 ^{abc}

Melko (parent)	34.3 ^{cd} e	34.7 ^b	80.3 ^{ab}	90.0 ^{bc}	74.5 ^{def}	76.7 ^e	28.9 ^{b-f}	28.5 ^{ef}
Gemechis0	35.7 ^{abc}	35.7 ^{ab}	76.0 ^{bcd}	88.7 ^c	72.9 ^{efg}	90.9 ^{bcd}	28.5 ^{b-f}	37.5 ^{abc}
Gemechis0.5	35.3 ^{abcd}	36.0 ^{ab}	75.3 ^{cd}	87.7 ^c	69.9 ^{e-i}	97.6 ^{ab}	29.4 ^{a-f}	40.9 ^a
Gemechis1	35.7 ^{abc}	35.3 ^{ab}	76.0 ^{bcd}	88.0 ^c	61.8 ⁱ	90.9 ^{bcd}	25.3 ^f	38.4 ^{ab}
Gemechis1.5	35.0 ^{bcd}	36.0 ^{ab}	75.3 ^{cd}	87.3 ^c	71.0 ^{efgh}	92.4 ^{bc}	26.3 ^{def}	39.9 ^a
Gemechis (parent)	33.7 ^{def}	34.3 ^b	78.0 ^{abc}	82.3 ^d	71.7 ^{efgh}	72.5 ^e	26.3 ^{def}	27.6 ^f
Pop12S20	32.3 ^{fg}	32.0 ^c	70.3 ^e	79.3 ^e	83.1 ^{bc}	86.1 ^{cd}	30.8 ^{abcd}	33.3 ^d
Pop12S20.5	32.3 ^{fg}	32.0 ^c	69.3 ^e	79.3 ^e	97.4 ^a	99.8 ^a	31.9 ^{ab}	31.7 ^{de}
Pop12S21	31.0 ^g	32.0 ^c	69.0 ^e	79.3 ^e	82.5 ^{bcd}	84.5 ^d	31.3 ^{abc}	32.8 ^d
Pop12S21.5	33.0 ^{ef}	31.3 ^c	69.3 ^e	79.3 ^e	90.7 ^{ab}	92.3 ^{bc}	33.9 ^a	35.1 ^{bcd}
Pop12S2 (parent)	32.0 ^{fg}	31.7 ^c	73.3 ^{de}	76.3 ^f	90.4 ^{ab}	92.4 ^{bc}	32.5 ^{ab}	34.1 ^{cd}
Mean	34.47	34.62	75.6	85.89	75.83	89.09	29.04	35.81
Somaclones/ Genotypes	SL (cm)		NSPP		TNT		NFT	
	S	NS	S	NS	S	NS	S	NS
Melko0	12.8 ^{ab}	14.4 ^{ab}	367.6 ^{ef}	404.3 ^{ef}	4.53 ^b	4.97 ^a	2.73 ^b	4.00 ^a
Melko0.5	13.2 ^{ab}	14.5 ^{ab}	618.9 ^b	680.7 ^b	3.53 ^{de}	4.27 ^c	2.13 ^{de}	3.27 ^{cd}
Melko1	12.9 ^{ab}	13.9 ^{ab}	618.8 ^{bc}	642.2 ^{bc}	2.93 ^{fg}	3.33 ^f	1.87 ^{ef}	2.67 ^f
Melko1.5	13.2 ^{ab}	13.9 ^{ab}	409.6 ^{ef}	450.5 ^{ef}	4.60 ^b	4.47 ^b	2.73 ^b	3.07 ^e
Melko (parent)	13.6 ^a	14.7 ^a	721.0 ^a	793.1 ^a	4.13 ^{bc}	4.20 ^c	2.40 ^{cd}	3.27 ^{cd}
Gemechis0	12.1 ^{abc}	13.5 ^{ab}	517.1 ^{cd}	568.8 ^{cd}	3.86 ^{cd}	3.83 ^{de}	2.53 ^{bc}	3.47 ^b
Gemechis0.5	11.5 ^{bcd}	12.6 ^{bc}	324.1 ^f	356.5 ^f	3.46 ^{def}	3.47 ^{ef}	2.73 ^b	2.73 ^f
Gemechis1	10.1 ^d	10.9 ^{cd}	663.8 ^{ab}	730.2 ^{ab}	2.86 ^g	3.00 ^g	1.47 ^g	2.30 ^h
Gemechis1.5	10.4 ^{cd}	11.0 ^{cd}	429.9 ^{de}	472.9 ^{de}	5.20 ^a	4.47 ^b	3.67 ^a	3.37 ^{bc}
Gemechis (parent)	9.7 ^d	10.4 ^d	629.6 ^{ab}	692.5 ^{ab}	2.8 ^g	3.87 ^d	1.87 ^{ef}	3.00 ^e
Pop12S20	13.1 ^{ab}	14.7 ^a	602.1 ^{bc}	662.3 ^{bc}	3.07 ^{efg}	3.60 ^e	2.53 ^{bc}	3.13 ^{de}
Pop12S20.5	13.1 ^{ab}	14.4 ^{ab}	570.3 ^{bc}	627.3 ^{bc}	2.13 ^h	2.80 ^h	1.60 ^{fg}	2.47 ^g
Pop12S21	13.1 ^{ab}	14.1 ^{ab}	602.0 ^{bc}	662.2 ^{bc}	2.6 ^{gh}	2.93 ^{gh}	1.93 ^e	2.40 ^{gh}
Pop12S21.5	13.7 ^a	14.5 ^{ab}	589.5 ^{bc}	648.4 ^{bc}	2.8 ^g	2.80 ^h	2.33 ^{cd}	2.33 ^{gh}
Pop12S2 (parent)	12.3 ^{ab}	13.2 ^{ab}	642.7 ^{ab}	707.0 ^{ab}	2.73 ^g	2.80 ^h	2.27 ^{cd}	2.33 ^{gh}
Mean	12.31	13.39	551.48	606.63	3.42	3.65	2.32	2.92
Somaclones/Genotypes	PW (g)		PSW (g)		PTW (g)		PTSW (g)	
	S	NS	S	NS	S	NS	S	NS
Melko0	0.327 ^{ef}	0.577 ^h	0.134 ^{def}	0.247 ⁱ	4.29 ^{de}	8.88 ^{fg}	0.823 ^{de}	1.615 ⁱ
Melko0.5	0.402 ^{bc}	0.713 ^e	0.157 ^{cd}	0.448 ^c	4.55 ^{cd}	11.81 ^{bc}	1.013 ^{abc}	3.126 ^c
Melko1	0.298 ^{efg}	0.800 ^d	0.167 ^{cb}	0.416 ^d	4.14 ^e	10.48 ^{cde}	1.013 ^{abc}	2.970 ^{cd}
Melko1.5	0.382 ^{cd}	0.883 ^c	0.170 ^{bc}	0.411 ^d	4.56 ^{cd}	10.65 ^{cd}	1.047 ^{abc}	2.622 ^{ef}

Melko (parent)	0.440 ^{ab}	1.238 ^a	0.174 ^{bc}	0.649 ^a	5.17 ^{ab}	12.46 ^{ab}	0.978 ^{bc}	4.270 ^a
Gemechis0	0.474 ^a	0.707 ^e	0.185 ^{ab}	0.298 ^h	4.82 ^{bc}	10.69 ^{cd}	1.083 ^{ab}	2.193 ^{gh}
Gemechis0.5	0.452 ^a	0.720 ^e	0.207 ^a	0.306 ^{gh}	5.13 ^{ab}	8.31 ^{fgh}	1.157 ^a	2.066 ^h
Gemechis1	0.293 ^{efg}	0.877 ^c	0.156 ^{cd}	0.418 ^d	5.40 ^a	9.82 ^{def}	0.920 ^{cd}	2.768 ^{cde}
Gemechis1.5	0.293 ^{efg}	0.893 ^c	0.085 ^g	0.428 ^{cd}	3.66 ^{fg}	11.32 ^{bcd}	0.643 ^{fg}	2.936 ^{cd}
Gemechis (parent)	0.280 ^{fg}	1.130 ^b	0.117 ^f	0.551 ^b	3.98 ^{ef}	13.38 ^a	0.760 ^{ef}	3.646 ^b
Pop12S20	0.277 ^g	0.655 ^f	0.150 ^{cd}	0.357 ^f	4.03 ^{ef}	5.88 ⁱ	1.043 ^{abc}	2.169 ^h
Pop12S20.5	0.338 ^{de}	0.633 ^f	0.133 ^{def}	0.309 ^{gh}	4.05 ^e	7.23 ^{hi}	0.755 ^{ef}	2.089 ^h
Pop12S21	0.154 ^h	0.813 ^d	0.084 ^g	0.384 ^e	2.02 ⁱ	9.07 ^{efg}	0.587 ^g	2.893 ^{cd}
Pop12S21.5	0.270 ^g	0.627 ^{fg}	0.123 ^{ef}	0.299 ^h	2.52 ^h	7.68 ^{gh}	0.743 ^{ef}	2.299 ^{fgh}
Pop12S2 (parent)	0.180 ^h	0.588 ^{gh}	0.080 ^g	0.325 ^g	3.29 ^g	8.02 ^{gh}	0.657 ^{fg}	2.534 ^{efg}
Mean	0.324	0.79	0.142	0.39	4.11	9.71	0.882	2.68
Somaclones/Genotypes	HSW (g)		GY (g)		BMY (g)		HI (%)	
	S	NS	S	NS	S	NS	S	NS
Melko0	0.036 ^a	0.040 ^c	2.72 ^b	3.14 ^j	15.0 ^c	32.8 ^{ef}	18.1 ^{cd}	9.6 ^g
Melko0.5	0.029 ^{bc}	0.039 ^c	2.16 ^{ef}	6.53 ^{cd}	14.7 ^c	44.7 ^a	14.6 ^{fgh}	14.6 ^{def}
Melko1	0.026 ^{cd}	0.049 ^a	2.51 ^{bc}	6.49 ^{cd}	14.7 ^c	36.4 ^{cd}	17.0 ^{de}	17.9 ^{bc}
Melko1.5	0.030 ^{bc}	0.035 ^d	2.71 ^b	4.90 ^g	15.1 ^c	35.6 ^{de}	17.9 ^{dc}	13.8 ^{ef}
Melko (parent)	0.032 ^{ab}	0.035 ^d	2.11 ^{efg}	8.27 ^a	17.0 ^b	37.5 ^{bcd}	12.4 ^{hi}	22.0 ^a
Gemechis0	0.017 ^e	0.044 ^b	2.47 ^{bcd}	5.01 ^{fg}	14.6 ^c	39.3 ^b	16.9 ^{def}	12.7 ^f
Gemechis0.5	0.027 ^{bcd}	0.035 ^d	2.43 ^{cd}	4.25 ^{hi}	16.6 ^b	27.1 ^{hi}	14.6 ^{fgh}	15.7 ^{cde}
Gemechis1	0.027 ^{bcd}	0.035 ^d	2.11 ^{efg}	5.48 ^{ef}	20.8 ^a	31.5 ^{fg}	10.1 ⁱ	17.4 ^{bc}
Gemechis1.5	0.027 ^{bcd}	0.035 ^d	2.23 ^{de}	6.22 ^d	12.5 ^e	38.7 ^{bc}	17.8 ^{cd}	16.0 ^{bcde}
Gemechis (parent)	0.027 ^{bcd}	0.035 ^d	2.16 ^{ef}	7.25 ^b	14.3 ^{cd}	44.3 ^a	15.2 ^{efg}	16.4 ^{bcd}
Pop12S20	0.027 ^{bcd}	0.030 ^e	3.35 ^a	3.74 ⁱ	14.6 ^c	16.3 ^k	23.1 ^b	23.0 ^a
Pop12S20.5	0.027 ^{bcd}	0.030 ^e	1.74 ^h	4.28 ^h	13.5 ^{de}	23.5 ^j	12.9 ^{gh}	18.3 ^b
Pop12S21	0.032 ^{ab}	0.035 ^d	1.88 ^{gh}	6.81 ^{bc}	7.0 ^f	29.1 ^{gh}	26.9 ^a	23.5 ^a
Pop12S21.5	0.027 ^{bcd}	0.035 ^d	1.90 ^{fgh}	5.56 ^e	7.2 ^f	25.9 ^{ij}	26.6 ^a	21.5 ^a
Pop12S2 (parent)	0.023 ^d	0.035 ^d	2.51 ^{bc}	6.19 ^d	12.8 ^e	28.4 ^{hi}	19.6 ^c	21.9 ^a
Mean	0.028	0.036	2.33	5.61	14.03	32.75	17.59	17.62

Table 2: Mean performance of 12 seed derived somaclones and the 3 parental tef genotypes for 16 traits evaluated under moisture stress (S) and non-stressed (NS) conditions, respectively. Mean values within column followed the same letters are not significantly different ($P \leq 0.01$). S=moisture stress, NS=non-stress, DH=days to heading, DM=days to maturity, PH=plant height, PL=panicle length, SL=spikelet length, NSPP=number of spikelet/panicle, TNT=total number of tiller/plant, NFT=number of fertile tillers/plant, PW=panicle weight, PSW=panicle seed weight, PTW=plant weight, PWSW=plant seed weight, HSW=hundred seed weight, GY=grain yield, BMY=biomass yield, HI=harvest index.

Plant height ranged from 62.0 cm (somaclone Gemechis 1) to 97.4 cm (somaclone Pop12S20.5) in the stress, and 72.5 cm (Gemechis parent) to 99.8 cm (somaclone Pop12S20.5) in the non-stress condition with the mean value of 75.8 and 89.1 cm respectively, reflecting the impact of moisture stress on plant height. The somaclones showed the

highest plant height as compared to the parents under both moisture regimes. The current result agreed with previous studies of Bouiamrine et al. [19] in durum wheat, Zarif et al. [21] in sorghum and Rahman et al. [28] in sugar cane who reported somaclones were superior over their donor parents for plant height. The average panicle length among

the somaclones and the parental genotypes was 29.0 and 35.8 cm in moisture stress and non-stress conditions, respectively. The mean values for panicle length ranged from 25.3 cm (somaclone Gemechis1) to 34 cm (somaclone Pop12S21.5) in stress condition and from 27.6 cm (Gemechis parent) to 41.0 cm (somaclone Melko 1) for non-stress condition indicating the somaclone plants showed highest panicle length. This result indicates that somaclone plants were capable of producing high panicle length under both moisture regimes. Similarly, Zarif et al. [21] in sorghum reported superiority of the somaclones as compared to the parental genotypes for panicle length. Mean values of spikelet length under moisture stress condition varied from 9.7 cm (Gemechis parent) to 13.7 cm (somaclone Pop12S21.5) with the average value of 12.3 cm. Under non-stress condition, the average value for spikelet length was 13.4 cm and the mean values ranged from 10.4 to 14.7 cm (Table 2). Melko and Pop12S2, both parental genotypes showed good performance for spikelet length (14.7 cm), while the least performance was recorded from Gemechis parent (10.4 cm).

The results also showed considerable variation among the somaclones and the parents for number of spikelets/panicle, total number of tillers/plant and number of fertile tillers/plant under both moisture conditions (Table 2). The mean number of spikelets/panicle under moisture stress and non-stress was 551.5 and 606.6 respectively, indicating the significant effect of moisture stress. This could be due to high sensitivity of the trait to the moisture stress. Under moisture stress and non-stress conditions the highest number of spikelets/panicle was obtained from the parent genotype Melko with mean values of 721 and 793, respectively. On the contrary, somaclone Gemechis 0.5 had less number of spikelets/panicle under both moisture regimes. Total number of tillers/plant and number of fertile tillers/plant under moisture stress varied from 2.1 (somaclone Pop12S20.5) to 5.2 (somaclone Gemechis 1.5) and from 1.5 (somaclone Gemechis 1) to 3.7 (somaclone Gemechis 1.5) with the mean values of 3.4 and 2.32, respectively. Under non-stress condition, maximum total number of tillers/plant was recorded from Melko parent (5.0) while, the least performance was recorded from somaclones Pop12S20.5, Pop12S21.5 and Pop12S2 parent (2.8). Maximum number of fertile tillers/plant was recorded from somaclone Melko0 (4) while the least was recorded from somaclone Gemechis 1 (2.3). The mean values for total number of tillers/plant and number of fertile tillers/plant under non-stress condition were 3.7 and 2.9, respectively. The result indicated the superiority of the somaclones over the parental genotypes for total number of tillers/plant and number of fertile tillers/plant under both moisture regimes except Melko parent for total number of tillers/plant under non-stress condition. Our result was similar with Bouiamrine et al. [19] in durum wheat, Zarif et al. [21] in sorghum and Rahman et al. [28] in sugar cane who reported that the regenerant plants showed better performance in the number of fertile tillers/plant and total number of tillers/plant. Danci et al. [29] reported none of the studied somaclones had shown superiority over the parents for number of fertile tillers/plant in bread wheat which was contradictory from the current result.

In terms of panicle weight, somaclones Gemechis 0 (0.474 g) and Gemechis 0.5 (452 g) showed better performance under moisture stress condition. On the other hand, Melko parent (1.238 g) had the highest mean value under non-stress condition. The lowest panicle weight was recorded from somaclones Pop12S21 (0.154 g) and Melko0 (0.577 g) under moisture stress and non-stress conditions with average mean values of 0.324 g and 0.790 g respectively. Under moisture stress condition somaclone Gemechis 0.5 was found to have better panicle seed weight (0.207 g), whereas, Pop12S2 parent produced lowest

panicle seed weight (0.080 g). On the other hand, under non-stress condition better panicle seed weight was recorded from Melko parent (0.649 g) while the least panicle seed weight was recorded from somaclone melko0 (0.247 g).

Somaclone Gemechis 1 (5.40 g) and Gemechis parent (13.38 g) had a high plant weight the former in the stress and the latter in the non-stress condition. The mean plant weight under moisture stress and non-stress conditions was 4.11 g and 9.71 g, respectively. The highest plant seed weight under moisture stress was found from somaclone Gemechis 0.5 (1.157 g) and the lowest from somaclone Pop12S21 (0.587 g) with the mean value of 0.882 g. On the other hand, under the non-stress condition the highest plant seed weight (4.270 g) was recorded from Melko parent and the lowest was from somaclone Melko0 (1.615 g) with mean value of 2.680 g (Table 2). Both the somaclones and the parents were affected by moisture stress. But comparatively the somaclones were better than the parents for these traits. Similar to our result, seed weight/plant was increased significantly in somaclones of sorghum as compared to the parents [30]. On the contrary, Symillides et al. [31] in Chinese spring wheat reported no significance difference between the somaclones and the donor parents for seed weight/plant.

Under moisture stress, somaclone melko0 exhibited maximum hundred seed weight (0.036 g) and the lowest hundred seed weight was recorded for somaclone Gemechis0 (0.017 g) with the average value of 0.028 g. On the other hand, under non-stress condition the average value was 0.036 g with maximum seed weight was from Melko parent (0.049 g) and minimum was from somaclones Pop12S20 and Pop12S20.5 (0.030 g) (Table 2). The result indicated that under the moisture stress condition maximum hundred seed weight was observed from the somaclonal plants whereas under non-stress condition maximum hundred seed weight was obtain from the parental genotype. This was in agreement with the result of Tripathy et al. [26] in grass pea who reported better performance of the somaclones for hundred seed weight. On the contrary, Bouiamrine et al. [19] reported hundred seed weight of somaclonal plants did not show better performance compared to the parental genotypes in durum wheat.

Under moisture stress somaclone Pop12S20 produced maximum grain yield of 3.35 g/pot, indicating the somaclones performed better as compared to the donor parents, whereas under non-stress condition Melko parent produced better grain yield (8.27 g/pot). The somaclones, Pop12S20.5 and Melko0 produced minimum grain yields of 1.74 and 3.14 g/pot under moisture stress and non-stress conditions, respectively (Table 2). Saxena et al. [32] in pigeonpea, Widoretno et al. [33] in soya bean and Zarif et al. [21] in sorghum reported superiority of the somaclones over the donor parents for grain yield. On the contrary, Carver and Johnson [34] and Cheng et al. [35] both in winter wheat reported grain yield in somaclones were low as compared to the parents. The result obtained from comparison of means exhibited that the highest biomass yield was recorded by somaclone Gemechis1 (20.8 g) and Gemechis parent (44.3 g) with mean values of 14.0 and 32.7 g under moisture stress and non-stress conditions, respectively. The two somaclones; Pop12S21 and Pop12S21.5 had highest harvest index under both moisture regimes.

Discussion

As far as we know, this is the first study of somaclonal variants derived from tissue culture regarding to drought tolerance evaluation

on tef. However, several interesting somaclonal variants have been reported in other crops. For instance, rice somaclones with increased drought tolerance have been reported [20] under field conditions. Somaclonal variants of wheat with increased drought tolerance than their donor parents (non-tissue cultured plants) have been identified in pots under greenhouse [25]. The study of Widoretno [33] on soybean and Hemon and Sudarsono [24] on peanut also reported that the increase in drought tolerance of the somaclonal variants than the parents. In the current study twelve tef somaclones (R1 generation) derived from R0 generation and three parental genotypes were evaluated under moisture stress and non-stress conditions. All the somaclones and their parental genotypes were negatively affected by moisture stress for all studied parameters. However, plants from the somaclonal variants were less affected by moisture stress as compared to the parental genotypes. Better performance was recorded from the somaclones for the measured traits under both moisture regimes except for number of spikelets/panicle under moisture stress and for number of spikelet/panicle, panicle weight, panicle seed weight, plant weight, plant seed weight and grain yield under non-stress condition. According to Obute and Aziagba [36] superiority of somaclones under stressed environments might be due to the somaclones may have genes associated with moisture adopted traits through methylation alteration in genes, ploidy change or translocation of genes during the *in vitro* culture process.

Conclusion

This study was conducted to assess the performance of tef somaclones and their respective parents with respect to drought tolerance. Considerable variability existed among the somaclones and their respective parents under moisture stress and non-stress conditions. Compared to parental genotypes significant higher moisture stress tolerance of the somaclones was observed except for number of spikelets/panicle under moisture stress and for number of spikelet/panicle, panicle weight, panicle seed weight, plant weight, plant seed weight and grain yield under non-stress condition. Although inconsistency performance of the somaclones was observed among the screened somaclones, Pop12S20 showed considerable grain yield performance under moisture stress, and could be recommended for moisture deficit areas. Genotype Melko (parent) gave potential grain yield under non-stress condition. In conclusion, somaclones were less affected by water stress comparing to the parental genotypes, though further studies are needed under actual field condition in different climatic conditions.

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