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Efficiency of *In Vitro* Regeneration is Dependent on the Genotype and Size of Explant in Tef [*Eragrostis tef* (Zucc.) Trotter]

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Abstract

Tef [*Eragrostis tef* (Zucc.) Trotter] is the major cereal crop in the Horn of Africa particularly in Ethiopia where it is staple food for about 50 million people. Its resilience to extreme environmental conditions and high in nutrition makes tef the preferred crop among both farmers and consumers. The efficiency of *in vitro* regeneration plays significant role in the improvement of crops. We investigated the efficiency of regeneration in 18 tef genotypes (15 landraces and three improved varieties) using three sizes of immature embryos (small, intermediate and large) as an explant. *In vitro* regeneration was significantly affected by the genotype and the size of the immature embryo used as a donor. Intermediate-size immature embryos which were 101-350 µm long led to the highest percentage of regeneration. Interestingly, the three improved varieties presented very low regeneration efficiencies whereas the landrace Manyi resulted in consistently superior percentage of *in vitro* regeneration from all three sizes of explants. The findings of this work provide useful insight into the tef germplasm amenable for the regeneration technique which has direct application in techniques such as transformation. It also signifies the importance of using tef landraces instead of improved varieties for *in vitro* regeneration.

Keywords: *Eragrostis tef*; Immature embryo; Plantlet; Regeneration; Embryogenesis; tef

Introduction

Tef [*Eragrostis tef* (Zucc.) Trotter] is a cereal crop extensively cultivated in the Horn of Africa where it is annually cultivated on about 3 million hectares of land in Ethiopia alone [1]. This extensive cultivation of the crop is related to some traits beneficial for farmers and consumers including, i) its tolerance to extreme environmental biotic and abiotic conditions, ii) its gluten-free seeds, hence considered as a healthy food, and iii) high palatability of its straw by livestock. Despite all these useful traits, tef is considered as an orphan crop due to the little scientific research done on the crop. As a result, the crop remains largely unimproved which is associated with poor productivity lodging or displacement of the plant from its upright position is the major cause for inferior yield in tef [2]. Tef has a very tall and weak stem which falls on the ground due to wind and rain. The majority of research on tef improvement has been done at the Ethiopian Institute of Agricultural Research where conventional techniques of selection and hybridization are widely implemented to release 35 improved varieties which are suited to diverse agro-ecological regions [3]. The widely cultivated and popular variety called Quncho was developed by the intra-specific crossing between two improved cultivars [4]. The recently published tef genome [5] will accelerate the breeding program if integrated with improvement methods such as tissue culture and genetic transformation.

Tissue culture or also commonly known as *in vitro* regeneration plays a key role in crop improvement. In addition to its significant contribution in genetic transformation of plants, tissue culture is also useful in developing large-scale clonal propagation of genotypes of interest and producing and propagating disease-free plants [6]. The somaclonal variations induced in the tissue culture are also source of variability in plant breeding [7,8]. The percentage of initial explants converted to plantlets or whole plants referred to the culture efficiency of regeneration. This efficiency is mainly affected by the genotypes and explants. The presence of a strong genetic effect was reported for *Arabidopsis* [9], wheat [10-12] and rice [13]. Considerable differences in regeneration ability were observed among four *Arabidopsis* ecotypes, namely Columbia, Landsberg erecta, Cape Verde Island and Wassilewskija based on the source of explant and composition of the culture medium [9]. In wheat, embryogenic capacity or number of somatic embryos formed from cultured immature embryos was mainly altered by the genotype whereby the best performing cultivars scored 1.4-1.8 plants/explant [10].

The source and size of explant affects the efficiency of regeneration. The study in malting barley called Morex showed that smaller embryos (0.5-1.5 mm) had higher regeneration efficiency than larger embryos (1.6-3.0 mm) [14]. Similarly, in Sudan grass (*Sorghum sudanense* Piper)smaller immature embryos (0.7-1.5 mm) were better than larger ones (1.6-2.5 mm) in the speed and frequency of callus and shoot formation [15].

Diverse *in vitro* regeneration techniques were studied for tef. The explants used for these investigations were seedlings, roots, and leaves [16,17], seeds [18], immature spikelets or panicles [19,20], and immature embryos [21] in which the latter resulted in substantially high percentage of regeneration. However, since earlier study using immature embryo was made on only two tef genotypes, it did not represent the existing tef germplasm with huge variations. Hence, the present study was made to investigate the efficiencies of *in vitro* regeneration in 18 tef genotypes with diverse morphological and agronomic properties [22].

Material and Methods

Plant material

Fifteen selected landraces and three improved varieties of tef were used. The 15 landraces were obtained from the National Plant

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Germplasm System (NPGS) of the United States Department of Agriculture [23]. They are: *Ada* (NPGS accession number: 524433), *Addisie* (524434), *Alba* (524435), *Balami* (524436), *Beten* (524437), *Dabbi* (524438), *Enatite* (524439), *Gea Lamie* (524440), *Gommadie* (524441), *Karadebi* (524442), *Manyi* (524443), *Red dabi* (524457), *Rosea* (524444), *Tullu Nasy* (524445) and *Variegata* (524446) while the three improved varieties were *Dukem* (DZ-01-974), *Magna* (DZ-01-196), and *Tsedey* (DZ-Cr-37). Donor plants were grown for three weeks under long-day conditions (16 h light, 8 h dark at 21 ± 1°C) before plantlets were transferred to short day conditions (8 h light, 16 h dark at 20 ± 2°C). The soil used consisted of 5/11 parts of topsoil, 4/11 parts of turf and 2/11 parts of quartz sand. Plants were fertilized once a week with Hauert Plantaktiv 16-6-26 N-P-K (Hauert HBG Dünger Schweiz, Grossaffoltern, Switzerland).

Embryo isolation and *in vitro* regeneration

The procedure for isolating immature embryos from panicles was based on earlier work [21]. Panicles were surface sterilized for 10 minutes with 1% HCl followed by three washings with sterile water. Immature embryos were separated from the sterilized caryopses by squeezing them out through an incision made at its base. Three different sizes of embryos were selected: small and globular (50-100 µm), intermediate (101-350 µm) and large embryos (351-750 µm) (Figure 1). Another distinction between the small and intermediate embryos was the loss of the globular shape in the intermediate ones. Large embryos were extracted from solid endosperm. Thirty immature embryos were plated on 3.5 cm diameter petri dishes containing K99 medium [24] placing the scutellum facing up and incubating in the dark at 25°C ± 2°C. The K99 media contains 90 g/l maltose, 1 g/l glutamine and 2 mg/l of 2,4-D. After two to three weeks in the dark, somatic embryos were transferred to K4NB medium [25] containing 36 g/l maltose, 0.15 g/l glutamine and 0.22 mg/l BAP. The pH of the medium was adjusted to 5.8 where 0.4% phytagel was used as a gelling agent. Plantlets were regenerated under 14-hour photoperiodic conditions for 4 weeks with a sub-culture to fresh medium after 2 weeks. The growth conditions consist of a relative humidity of 50% all day-long and a temperature of 21 ± 2°C during the dark and 25 ± 2°C during the light. The photon luminosity was set to 70 µmol/m^2 s during the light period. Plantlets with well-developed root systems were transferred to soil and grown under the same conditions than for the donor plants (see above). After three weeks of hardening in long-day conditions, plants were transferred to short-day room for the production of seeds. Five replicates each containing 30 immature embryos were tested for each tef cultivar and size of the explant (Figure 1).

The efficiencies in somatic embryogenesis, regeneration and culture were enumerated as follows:

Embryogenesis efficiency, direct (%)=number of embryos/number of explants × 100

Embryogenesis efficiency, indirect (%)=number of callus/number of explants × 100

Regeneration efficiency, direct (%)=number of plantlets/number of embryos × 100

Regeneration efficiency, indirect (%)=number of plantlets/number of callus × 100

Culture efficiency=number of plantlets/number of explants × 100 [26].

Determination of morphological, phenotypic and yield related traits

Morphological traits including numbers of tillers, panicles and internodes, and lengths of culm, panicle and the second culm internode (starting from the base of the plant) were determined at the flowering time. The length of second culm internode was earlier reported to determine the lodging tolerance [27]. Days to heading or flowering and days to maturity and the grain filling period were also determined. Days to heading was defined as the number of days from sowing until the first flower appeared and days to maturity was the number of days for all the grains of a panicle to mature. The time between flower emergence and grain maturity was defined as grain filling period. At harvesting time, shoot biomass was separated into culms and panicles

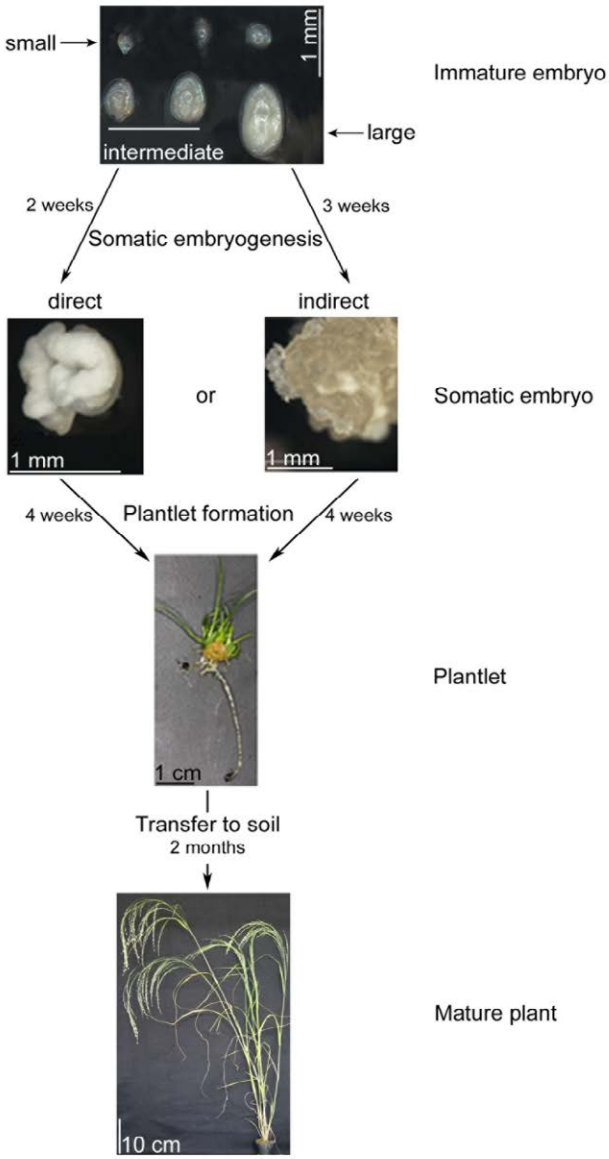

Figure 1: *In vitro* regeneration of tef. Three sizes of immature embryos were used as an explant. Somatic embryogenesis was formed either directly or indirectly through the formation of a callus. Plantlets were established from somatic embryos exposed for four weeks to light. Two months after transferring the plantlet to soil, a plant with mature seeds was obtained.

and dried for 24 hours at 60°C in order to determine the dry weight of all culms and panicles. Harvest index was calculated as the ratio of the grain yield to the shoot biomass.

Statistical analysis

Statistical analysis was performed using SPSS Statistical 17.0 (IBM, Chicago, IL). Non-parametric tests were chosen as it is appropriate for the number of replicates used and the non-homogeneity of the variance in order to compare differences between the treatments ($p \leq 0.05$). For K independent samples, Kruskal-Wallis tests were used, whereas for two independent samples, Mann-Whitney U tests were employed. For correlation analysis, the Pearson correlation test was used on the mean values of selected traits among efficiencies of embryogenesis, regeneration, and culture.

Results

In vitro regeneration

Steps and the timeline from excising the three sizes of immature embryos to the two paths of somatic embryogenesis, plantlet formation and to finally grown on soil to full maturity are shown in Figure 1. The efficiency of somatic embryogenesis was determined for the three sizes of immature embryos used as an explant as well as for the two types of embryos formed. While the three groups of immature embryos were small (50-100 µm), intermediate (101-350 µm) and large (351-750 µm). Somatic embryos made from immature embryos can be either directly without passing an intermediate callus forming step or indirectly through callus. While direct embryogenesis took two weeks once the embryos were placed on the appropriate media, the indirect embryogenesis took an additional week. The whole procedure starting from embryo isolation to fully mature plants on soil takes 12-13 weeks or about 3 months.

A high diversity was found in the somatic embryogenesis depending on the size of the explant and the type of the tef ecotype. While intermediate-size immature embryos resulted in high percentage of somatic embryogenesis, only low proportion of small explants formed somatic embryos (Figure 2). Regarding small immature embryos, the efficiency of somatic embryos formed varied from less than 10% in Ada, Enatite, Rosea, and the two improved varieties (Magna and Dukem) to more than 70% in Manyi (Figure 2A). Surprisingly, small-size explants from the landrace Rosea did not produce any somatic embryos. The proportions of embryos formed through direct and indirect somatic embryogenesis were similar except in Gommadie where significantly higher percentage was formed through direct somatic embryogenesis. In the case of intermediate immature embryos, the percentage of somatic embryogenesis ranged from less than 20% for Ada to around 90% for Gommadie and Manyi (Figure 2B). Unlike small-size embryos which formed the direct or indirect somatic embryogenesis in a similar proportion, in the intermediate-size embryos, the indirect embryogenesis was dominant over the direct one. This favor to the indirect embryogenesis was significant in six landraces, namely Alba, Enatite, Gea Lamie, Karadebi, Rosea and Variegataas well as all the three improved varieties. For large immature embryos used as explants, the percentage of somatic embryogenesis varied from less than 20% in Balami, Rosea and Magna to more than 65% in Addisie, Gommadie and Manyi (Figure 2C). Similar to the intermediate-size embryos, the proportion of indirect embryogenesis was significantly higher than the direct ones for large-size explants except in Gea Lamie land race where the difference between direct and indirect embryogenesis was negligible.

The efficiency of regeneration which refers to the proportion of somatic embryos that result in plantlet formation were quantified for the three types of explants (small, intermediate and large) and the two forms of somatic embryos (direct and indirect) (Figure 3). Substantial variability in regeneration capacity was observed among the ecotypes, sources of explants and forms of embryos. Using small explants, four genotypes, namely Ada, Dabi, Rosea and Magna, did not form any plantlet while Alba and Balami did not regenerate from indirectly formed embryos (Figure 3A). However, over 70% of regeneration was obtained for Karadebi and Manyi. Regeneration efficiencies were variable between direct and indirect embryogenesis in each genotype although significantly higher values were obtained for directly formed embryos. The proportion of plantlets formed from intermediate-size

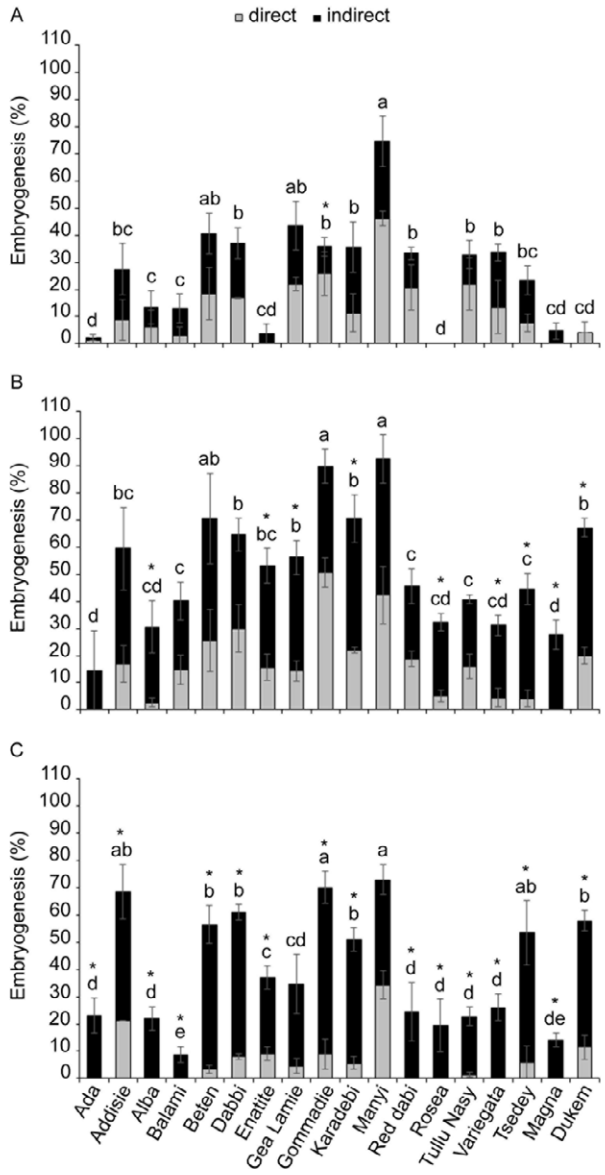

Figure 2: Efficiency of somatic embryogenesis derived from small (A), intermediate (B) and large (C) immature embryos. Values with different letter indicate significant difference ($p<0.05$) among the genotypes for the total embryogenesis (direct + indirect). An asterisk (*) indicates a significant difference ($p<0.05$) between direct and indirect embryogenesis.

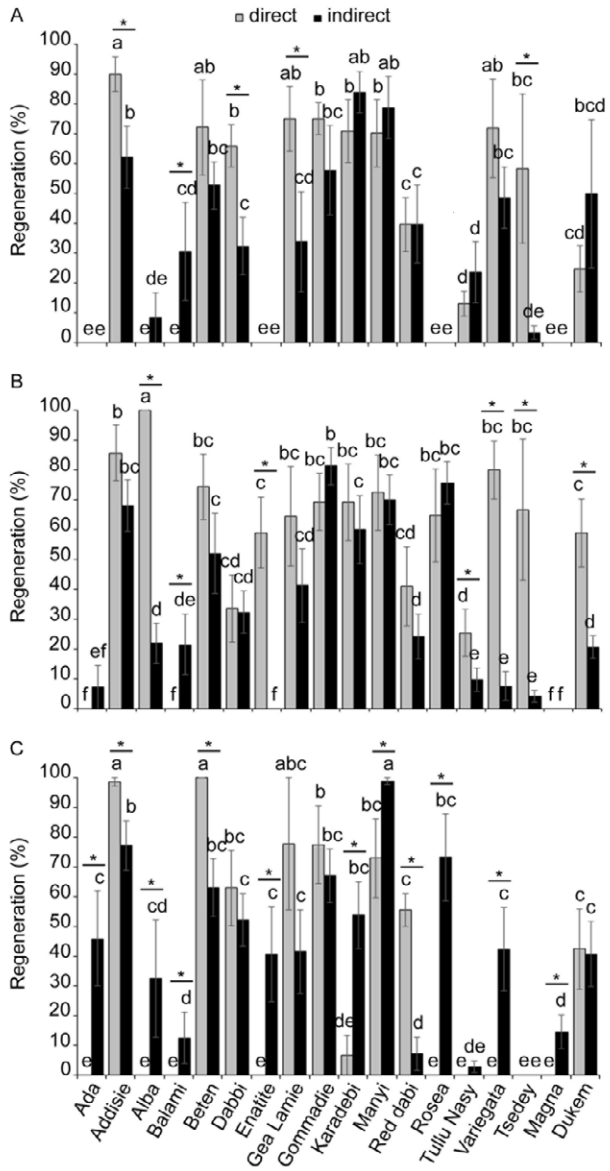

Figure 3: Efficiency of plantlet regeneration derived from somatic embryos which were originally obtained from small (A), intermediate (B) and large (C) immature embryos. Values with different letter represent a significant difference ($p<0.05$) among the genotypes. An asterisk (*) indicates a significant difference ($p<0.05$) between plantlets derived from direct or indirect somatic embryogenesis.

immature embryos ranged from less than 10% in Ada, Balami, Enatite and Magna (direct embryogenesis) as well as in Ada, Variegata, Tsedey and Magna (indirect embryogenesis) to more than 70% in Addisie, Beten, Manyi and Variegata (direct embryogenesis), and Gommadie, Manyi and Rosea (indirect embryogenesis) (Figure 3B). Some inconsistencies were observed in some genotypes between the capacity of plantlet formation and somatic embryogenesis. For instance, in Alba cultivar, although the percentage of direct embryogenesis from the intermediate-size embryo was only 2% (Figure 2), the plantlet formation reached 100% for same size of embryos (Figure 3) indicating high capacity of plantlet formation from extremely low rate of somatic embryo development. Regarding the regeneration capacity from large embryos, variations revealed in the other two embryo sizes were also observed among different genotypes (Figure 3C). Three landraces (Ada and Beten from direct embryogenesis, and Manyi from indirect embryogenesis) gave exceptionally high plantlet formation.

Culture efficiency which refers to proportions of initial immature embryos resulting in plantlets was also investigated. Significant variability was observed among the tef genotypes and the sizes of immature embryos used as an explant (Table 1). The performance of some landraces was high when small starting material was used while in others large explants gave superior results. The large explants from Addise, Gea Lamie and Manyi had extremely high efficiency. Although inconsistencies in performance were observed for the three sizes of embryos, Manyi gave exceptionally high efficiency for all sizes of embryos. Irrespective of the size of immature embryos, all three improved tef varieties performed extremely poor indicating their low application in regeneration and transformation related studies. The main reason for the culture efficiencies above 100% is due to the friable nature of somatic embryos which resulted in the generation of several viable pieces generated from a single somatic embryo.

Correlations among steps of *in vitro* regeneration

Pearson correlation coefficients were used to investigate the relationship among the three parameters of regeneration (embryogenesis-, regeneration- and culture-efficiency) and three sizes of immature embryos used as an explant (small, intermediate and large). Very significant and positive correlations ($p<0.01$) were observed among the three parameters and three sizes of immature embryos as well as their interaction (Table 2). The only non-significant correlation ($p=0.108$) was between the embryogenesis from the small explant and the regeneration from the large explant.

Determination of morphological, phenotypic and yield related traits

The existence of substantial variability in diverse morphological

Genotype	Culture efficiency (%)		
Landrace	small	intermediate	Large
Ada	0.0 *d*	1.7 *e*	13.9 *d**
Addisie	40.0 *bc*	69.3 *a*	106.0 *a**
Alba	12.5 *c*	9.3 *cd*	5.5 *e*
Balami	6.6 *d*	7.4 *d*	2.7 *e**
Beten	69.0 *b*	73.4 *a*	66.8 *b*
Dabbi	36.0 *bc*	27.0 *bc*	66.4 *b**
Enatite	0.0 *d**	14.5 *c*	9.3 *e*
Gea Lamie	29.0 *c*	30.8 *b*	115.8 *a**
Gommadie	54.4 *b*	99.3 *a**	54.1 *c*
Karadebi	72.5 *b*	67.7 *ab*	34.7 *d**
Manyi	95.8 *a*	93.9 *a*	93.1 *b*
Red dabi	21.8 *c*	36.5 *b*	5.9 *d**
Rosea	0.0 *d**	36.4 *b*	24.4 *d*
Tullu Nasy	4.7 *d*+	16.0 *c*+	7.9 *d*+
Variegata	28.5 *c*	16.7 *c*	20.7 *d*
Improved			
Dukem	1.8 *d**	11.8 *c*	21.0 *d*
Magna	0.0 *d*	0.0 *e*	3.7 *e*
Tsedey	11.2 *c*	4.8 *d*	0.9 *e*

Table 1: Culture efficiency of 18 tef genotypes regenerated in vitro from small, intermediate and large immature embryos. Means followed by the same letter are not significantly different ($P<0.05$) for a same column. An asterisk (*) indicates a significant difference ($P<0.05$) between the sizes of a specific tef genotype or landrace. A plus (+) indicates a significant difference ($P<0.05$) among the three sizes of explants.

	EB_I	EB_L	EB_T	RG_S	RG_I	RG_L	RG_T	CEF_S	CEF_I	CEF_L	CEF_T
EB_S	.646**	.640**	.763**	.845**	.474*	.392	.484*	.831**	.672**	.643**	.672**
EB_I		.803**	.928**	.762**	.618**	.508*	.573*	.768**	.820**	.625**	.805**
EB_L			.943**	.719**	.487*	.474*	.509*	.681**	.716**	.661**	.744**
EB_T				.811**	.602**	.553*	.609**	.808**	.831**	.725**	.843**
RG_S					.687**	.582*	.698**	.927**	.830**	.669**	.835**
RG_I						.810**	.954**	.703**	.849**	.610**	.866**
RG_L							.934**	.600**	.797**	.710**	.865**
RG_T								.716**	.868**	.682**	.911**
CEF_S									.874**	.651**	.859**
CEF_I										.661**	.943**
CEF_L											.797**

Table 2: Pearson correlation coefficients among three efficiencies.
EB: Embryogenesis; RG: Regeneration; CEF: Culture efficiency
Three sizes of immature embryos S: Small; I: Intermediate; L: Large; T: Total
The * and ** showed statistical significance at the 0.05 and 0.01 probability levels, respectively.

Genotype	NTP		NPP		NIC		CL		PL		SCIL	
							cm				mm	
Landrace	*In vitro*	*seed*	*In vitro*	*seed*	*In vitro*	*seed*	*In vitro*	*seed*	*In vitro*	*seed*	*In vitro*	*seed*
Ada	2.8 *c**	1.0*d*	4.8 *b**	1.7*d*	4.5*e*	3.6*cd*	41.0 *a*	34.7*cd*	39.0*ab*	32.1*c*	8.6 *ab*	6.4*e*
Addisie	3.6*c*	2.0*bc*	3.9*c*	2.8*abc*	5.0 *c*	4.6*bc*	37.4 *b**	30.6*d*	37.3 *b**	29.2*d*	6.8*e**	8.6*cde*
Alba	4.0 *bc**	0*e*	5.0*b**	1.3*e*	4.3*de*	4.3*bcd*	46.1 *a**	37.2*cd*	47.5 *a**	33.7*c*	10.9 *ab*	11.2*ab*
Balami	5.2 *b**	0*e*	4.9*bc**	1.0*e*	5.3 *bc**	6.0*a*	37.5 *b**	43.6*b*	44.9 *a*	45.1*a*	9.3*b*	9.9*bc*
Beten	7.5*a**	1.5*bcd*	5.7 *b**	2.0*c*	5.7 *ab**	4.5*bc*	39.4 *ab*	39.5*c*	34.8 *bc*	35.7*c*	7.3*e*	9.2*cd*
Dabbi	4.1*b**	2.0*bc*	6.5*b*	4.0*a*	4.3*d*	4.0*c*	36.3 *b*	40.5*bc*	19.7 *e*	25.3*d*	11.3 *a*	13.2*a*
Enatite	4.7*b**	1.2*bcde*	5.9 *b**	2.3*bc*	4.6*d*	5.2*abc*	38.3 *b*	34.2*cd*	38.9 *ab*	35.4*c*	8.7*c*	8.0*de*
Gea Lamie	6.3 *a**	2.0*bc*	9.0 *a**	3.7*ab*	3.7*e*	3.0*d*	31.9 *c*	35.9*cd*	19.6 *e*	21.3*d*	8.9*c**	6.4*e*
Gommadie	3.5 *c**	1.0*cd*	4.2 *c**	1.5*e*	4.7 *cd*	4.5*bc*	36.7 *b*	36.8*cd*	30.3 *c*	26.3*d*	9.7*b*	8.6*cde*
Karadebi	3.7 *c**	1.2*cd*	4.7*c**	2.0*bc*	4.6 *d**	4.0*cd*	35.5 *b**	46.7*a*	25.0 *de**	29.4*c*	9.5*b*	11.2*ab*
Manyi	5.1 *b**	1.0*d*	5.6 *b**	1.8*d*	5.9*a*	5.6*ab*	37.0 *b*	40.6*bc*	36.4 *bc*	39.8*b*	6.7*e**	9.9*bc*
Red dabi	5.9 *a**	1.6*bcd*	8.8 *a**	3.3*abc*	4.0*e*	4.0*c*	31.7 *c*	32.0*cd*	22.5 *de*	27.4*d*	8.8*c*	9.2*cd*
Rosea	5.5 *b**	0.2*e*	7.9 *b**	2.3*bc*	4.2 *e*	4.5*bc*	30.1 *c*	34.1*cd*	30.0 *cd*	34.1*c*	7.9*d**	13.2*a*
Tullu Nasy	5.0 *b**	0.4*de*	8.0 *b**	1.7*d*	4.2 *e*	3.7*cd*	39.9 *a**	30.0*de*	25.9 *d*	29.7*c*	10.7 *ab**	6.4*e*
Variegata	3.5 *c**	0*e*	3.4*c**	1.5*e*	4.5 *d**	3.7*cd*	31.7 *c*	29.1*de*	24.6 *de*	22.8*d*	9.8*b*	8.6*cde*
Improved												
Dukem	5.9*a*	3.0*ab*	6.7 *b**	2.0*c*	4.2 *e*	4.0*c*	33.6 *b*	33.0*cd*	40.1 *ab*	37.8*b*	8.5*c**	9.3*bc*
Magna	5.7 *ab*	3.4*a*	8.1*b**	3.8*ab*	4.1 *e*	4.6*bc*	29.2 *c**	36.6*cd*	32.7 *c*	32.9*c*	7.8 *d*	8.4*cde*
Tsedey	4.9 *b**	2.0*bc*	8.1*b**	2.8*bc*	3.7 *e**	4.4*bc*	28.9 *c*	29.6*e*	28.3 *d**	32.9*c*	6.8*e*	7.5*de*

Table 3: Selected morphological traits for 18 tef genotypes developed by *in vitro* regeneration. Values followed by the same letter were not significantly different (P<0.05) between plants regenerated by *in vitro* method (this study) and those produced from seeds [22].
NTP: Number of Tillers per Plant; NPP: Number of Panicles per Plant; NIC: Number of Internodes per Culm; CL: Culm Length; PL: Panicle Length; SCIL: Second Culm Internode Length

and phenotypic traits were earlier reported for the same 18 tef ecotypes derived from seed [22,28]. In Table 3, comparisons were made for selected traits between those obtained from the seed [22] and those developed through *in vitro* regeneration (the current study). Tef lines generated through *in vitro* method were more robust than those from seeds for key morphological traits especially in the numbers of tillers and numbers of panicles per plant which have positive impact on the productivity of the crop. While most landraces derived from seeds had a maximum of two tillers per plant, those from the *in vitro* had up to seven tillers per plant. Astonishingly, three landraces (namely, Alba, Balami and Variegata) which did not develop a single tiller when generated from seeds were able to form 3-5 tillers when generated *in vitro*. Number of panicles which is also dependent on the form of the panicle is normally low for plants with compact panicles (e.g. Gommadie) and high for those with loose panicles (e.g. Gea Lamie). Compared to those developed from seeds, up to 3-fold increase in the number of panicles was obtained for those from *in vitro* regenerated plants. Although substantial variability was obtained among the tef genotypes for the number of internodes per plant, culm length and plant height, differences between those generated from seeds and those from *in vitro* were not obvious for the three traits.

Significant diversity was also observed for phenotypic and yield related traits among the 18 tef genotypes generated from the seed or from *in vitro* method (Table 4). However, the differences between those from seed and those from immature embryos were inconsistent for most of the traits. Amazingly, all the three improved tef varieties

Genotype	DH		DM		GFP		WAC		WAP		GY		HI	
					Days				mg				%	
Landraces	*Invitro*	*seed*	*Invitro*	*seed*	*Invitro*	*seed*	*Invitro*	*seed*	*Invitro*	*seed*	*Invitro*	*seed*	*Invitro*	*Seed*
Ada	61.0 *e**	90.0*a*	95.2 *d*	110.0*c*	42.8 *a**	20.0*e*	1924.2 *a**	292.4*de*	826.7 *a**	159.8*ab*	251.1 *b**	113.7*ab*	11.9 *bc**	28.9*a*
Addisie	65.7 *e*	65.5*f*	94.8 *d*	90.0*ef*	31.3 *b**	24.5*d*	744.1 *d**	497.8*cd*	417.8 *c**	234.7*ab*	198.6 *bc*	168.8*a*	16.7 *b*	20.6*a*
Alba	75.3 *cd*	78.0*cd*	120.7 *b**	105.0*cd*	45.3 *a**	27.0*cde*	1229.3 *b**	369.5*d*	497.1 *c**	210.3*ab*	147.7 *c*	135.8*ab*	8.5 *d**	23.8*a*
Balami	100.6 *b**	86.2*a*	139.9 *a**	108.2*c*	40.8 *a**	22.0*e*	2137.9 *a**	636.5*bc*	618.2 *b**	342.5 *a*	254.0 *b*	229.9*a*	8.1 *d**	23.6*a*
Beten	85.6 *c*	87.5*a*	116.5 *bc**	108.0*c*	31.3 *b**	20.5*e*	1863.0 *a**	964.6 *a*	619.3 *b**	292.1 *a*	321.2 *b**	131.0*a*	11.3 *c*	10.4*b*
Dabbi	57.1 *f*	58.0*g*	86.2 *e*	87.4*fg*	29.9 *b*	29.4*cd*	538.9 *e*	309.2*de*	187.0 *d*	180.9*ab*	72.0 *e**	38.5*bc*	7.6 *d*	8.1*bc*
Enatite	61.4 *e*	65.0*fg*	97.1 *d**	118.3*a*	35.6 *a**	53.3*a*	1340.4 *b**	499.6*bcd*	340.0 *c*	225.2*bc*	74.7 *e**	147.5*a*	3.7 *e**	20.1*ab*
Gea Lamie	60.3 *e*	58.0*g*	82.4 *e*	87.5*fg*	22.3 *d**	29.5*c*	358.6 *f**	195.2*e*	136.2 *d*	88.3*bc*	34.9 *f*	26.9*c*	4.4 *e**	8.7*bc*
Gommadie	72.3 *d*	69.5*e*	100.6 *d**	92.5*e*	28.1 *c*	23.0*e*	814.2 *c*	776.0*ab*	299.6 *c*	338.8*a*	129.5 *d*	147.7*ab*	9.8 *c**	16.7*ab*
Karadebi	63.9 *e*	60.2*f*	92.7 *d*	85.2*g*	27.8 *c*	25.0*d*	959.5 *b**	550.7*bc*	317.6 *c**	137.3*ab*	153.9 *c**	41.9*b*	10.6 *c**	6.6*cd*
Manyi	81.2 *c*	86.8 *a*	119.4 *b**	107.6*c*	37.5 *a**	20.8*e*	1347.8 *b**	630.8*bc*	308.1 *c*	359.1*a*	128.5 *d**	290.1*a*	6.5 *d**	31.6*a*
Red dabi	56.9 *f*	56.5*h*	84.2 *e*	83.5*g*	28.7 *c*	27.0*d*	818.8 *c**	450.6*cd*	335.0 *c*	228.0*ab*	99.0 *de*	96.4*b*	9.8 *c**	16.9*ab*
Rosea	74.6 *cd*	70.3*e*	113.6 *bc**	103.6*c*	39.0 *a*	33.3*bc*	1196.4 *b**	340.3*d*	499.3 *c**	203.5*ab*	154.6 *c*	95.4*bc*	7.2 *d**	17.7*ab*
Tullu Nasy	47.1 *g**	60.0*f*	70.3 *f**	88.8*ef*	23.6 *d*	28.8*cd*	892.7 *c**	143.7*e*	404.7 *c**	80.7*c*	230.9 *c**	43.1*bc*	16.3 *b*	18.5*ab*
Variegata	64.3 *e**	72.4*de*	91.6 *d*	90.4*e*	27.4 *c**	18.0*e*	850.0 *c**	345.0*d*	192.7 *d**	82.3*c*	79.4 *e**	25.5*c*	6.6 *d*	5.4*d*
Improved														
Dukem	114.0 *a**	80.0*bc*	136.7 *a**	116.3*b*	23.5 *d**	36.3*b*	1195.0 *b**	660.0*ab*	1481.7 *a**	253.6 *a*	734.3 *a**	79.0*b*	35.0 *a**	8.0*c*
Magna	84.1 *c*	83.4*ab*	108.8 *c**	122.8*a*	24.9 *d**	39.4*b*	680.5 *d*	665.7*ab*	423.2 *c**	182.7*ab*	172.6 *c**	33.3*c*	15.4 *b**	3.7*d*
Tsedey	70.1 *d**	*73.6 de*	100.2 *d**	106.6*d*	30.6 *c**	33.0*c*	810.8 *c**	317.9*de*	666.4 *ab**	130.9*ab*	311.2 *b**	34.6*bc*	18.1 *ab**	7.6*c*

Table 4: Phenotypic and yield related traits for 18 tef genotypes regenerated by *in vitro* method. Values followed by the same letter were not significantly different (P<0.05) between plants regenerated by *in vitro* method (this study) and those produced from seeds [22].
DH: Days to Heading; DM: Days to Maturity; GFP: Grain Filling Period; WAC: Weight of all Culms; WAP: Weight of all Panicles; GY: Grain Yield per Plant; HI: Harvest Index.

developed from immature embryos were superior over those from the seed for all traits investigated. This positive effect from tissue cultured plants was revealed on grain yield (up to 8-fold) and harvest index (up to 3-fold) over those from seed. Although improved varieties were mainly selected based on high grain yield and harvest index, the three improved varieties in the current study were inferior to some land races for these two valuable traits.

Discussion

The diversity in regeneration capacity among tef genotypes indicates the existence of a genetic control of this process which differs among diverse tef lines as earlier reported for *Arabidopsis* [9] and rice [29]. This variation among genotypes might be related to the level of endogenous hormones [30] or the effect of exogenous growth regulators on the level of endogenous hormones through influencing their biosynthesis and distribution [31], which subsequently alters the *in vitro* regeneration responses [32-34]. Interestingly, all three improved tef varieties used in the current study had extremely poor regeneration capacity. This was mainly due to the development of tef varieties in the past focused on selecting genotypes with superior grain yield without considering their *in vitro* regeneration efficiency.

The size and/or age of an explant is also responsible for controlling the frequency and speed of regeneration in plants. Although not a single albino plant or plantlet was observed in the current study, up to 50% of shoots of barley derived from large immature embryos were albinos unlike those from small embryos [14]. The absence of albinos in tef plants developed through *in vitro* regeneration increases the acceptability of the technique by researchers as it has positive impact on the viability and productivity of the crop. Large immature embryos of tef tend to initially form a callus before differentiating into somatic embryo. This indirect somatic embryogenesis requires an additional one week to the direct embryogenesis to form somatic embryos. This makes large embryos less favorable to use as an explant. As earlier reported for Sudan grass and tef, large embryos are more determined to germinate than to produce a callus [15,21]. The strong positive correlation among the three steps of the *in vitro* regeneration suggests the existence of general internal/genetic mechanism which controls the response of a particular genotype to the tissue culture, irrespective of the size of the explant or the step of the *in vitro* regeneration process. This means, a highly-responsive genotype performs better than a low responsive one for all sizes of explant and steps of the tissue culture. In winter wheat, only culture efficiency tended to increase as regeneration capacity was enhanced [35].

The comparison between the 18 tef genotypes developed from the seed [22] and those generated via the tissue culture (the current study) revealed significant differences for major morphological, phenotypic and yield related traits. Plants developed through *in vitro* regeneration were more vigorous and productive than those from seed especially in the number of tillers and panicles.

Since tissue culture or in vitro regeneration could result in useful stable somaclonal variations, thorough investigation of progenies needs to be done as some of the changes could be used in developing tef cultivars with improved traits. Using somaclonal variation, potato cultivar with reduced plant height, and sorghum and rice varieties tolerant to drought were developed [36-38].

In conclusion, based on the regeneration efficiency, the intermediate-size explants are the best. Among the germplasms, the Manyi landrace provides the highest culture efficiency. Since the three improved tef varieties were inferior in the efficiency of regeneration, they are not suggested for use especially with the technique and type of the explant indicated in the current study.

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