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Multivariate analyses of phenotypic diversity in Northeast Ethiopian tef [*Eragrostis tef* (Zucc.) Trotter] landrace collections

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ABSTRACT

The knowledge of genetic diversity is valuable for germplasm conservation and for crop improvement. An investigation was undertaken at Sirinka and Kobo Research Stations in 2006 to assess phenotypic diversity of 160 landraces and 6 varieties of tef and to identify the most important traits in differentiating the landraces. The result showed appreciable ranges of values for traits like days to maturity, plant height, biomass- and grain-yield suggesting the presence of sizable variability in the tef landraces. Grain yield was positively and significantly correlated with plant height (0.245**), panicle length (0.22**), biomass (0.907**), harvest index (0.474**) and days to heading (0.284**). The stepwise regression analysis suggests linear increment in plant height, harvest index and biomass yield could lead to significant increase in tef grain yield. In the principal component (PC) analysis, 74% of the total variability was explained by three PCs. Biomass yield from the first PC; and day to heading, grain-filling duration, plant height, panicle length and culm length from the second PC axis were important in discriminating the landraces. Two-step cluster analysis divided the landraces into three groups. Landraces with late flowering, short grain-filling period, high biomass, high grain yield and high harvest index were grouped in cluster II. Landraces in cluster II could therefore be used as parent lines for future breeding programs. Biomass yield, harvest index and plant height could be set as indirect selection criteria to improve yield of tef.

Keywords: Cluster analysis, Correlation, *Eragrostis tef*, Phenotypic diversity, Principal component axis, Stepwise regression.

INTRODUCTION

Tef, [*Eragrostis tef* (Zucc.)Trotter], is one of the four important cereal crops grown in Ethiopia. The crop is originated and diversified in Ethiopia (Vavilov, 1951). Tef is adapted to a range of altitudes, and can be grown from sea level up to 2800m above-sea-level (masl). However, it performs best at an altitude of 1800-2100 m, with annual rainfall of 750-850 mm and a temperature range of 10°C-27°C (Ketema, 1997). It has numerous importance for people and for livestock. The flour is used to prepare fermented local flat-bread called *injera*, which is a common food in most of the places in the country. Tef straw is the most palatable and preferred livestock feed. Besides, the straw is also used for plastering mud when constructing walls of houses and traditional granaries in the rural areas.

The existence of genetic diversity within species is essential for its survival and adaptation to changing environments (Gao, 2003; Rao & Hodgkin, 2002). This knowledge is valuable for germplasm conservation, variety identification, and for crop improvement (Duran et al., 2009). Plant breeding involves the continuous development of improved cultivars of crops and the success of this endeavor depends on presence and access to diverse genetic material (Hoisington et al., 1999; Maxted et al., 2002).

About 5169 accessions of tef have been collected and conserved by the Ethiopian Biodiversity Institute (Tesema, 2013). Most of the collections came from locations easily accessible by vehicles and from areas ranging in altitudes from 1800 to 2000 masl. Due partly to relatively low genetic replacement and varietal influx, tef has been less extensively collected as compared to other major cereals. Up to the year 2000, only 356 accessions were collected from Wollo, northeastern part of Ethiopia (Demisie, 2000). Thus, there is a need to

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collect, characterize and evaluate more landraces from this area.

Although some works have been documented on multivariate diversity of tef landraces from various agro-ecological zones of Ethiopia (Assefa et al., 2000, 2003; Adnew et al., 2005; Ayalew et al., 2011), the collection made so far was not exhaustive and the information generated was inadequate. Therefore, this investigation was undertaken with the objective to assess phenotypic diversity of tef landraces for morpho-agronomic traits and to identify the important traits in differentiating the landraces by using multivariate analyses.

MATERIALS AND METHODS

Experimental materials and place of study:

The field experiment was conducted at Sirinka Agricultural Research Center and Kobo Research Sub-Center in 2006 main cropping season. Sirinka and Kobo are located at an altitude of 1850 and 1450 msal, respectively; they are geographically placed at $11^0$45'N and $39^0$36' E and $12^0$9' N and $39^0$38' E, respectively. The two locations represent the deficit moisture-stressed tef growing areas of northeast Ethiopia. The materials used were 166 tef genotypes, out of which six were improved varieties and 160 were landraces collected from seven districts of three administrative zones (Table 1).

Experimental design and data collection:

The experiment was laid out in a randomized complete block design with two replications. Sowing was done on the second week of July 2006. About 0.5gm of seed of each landrace was broadcasted on a plot size of 1m x 0.2m. Inter plot

Table 1. Number of tef landraces and districts where they were collected

Sr. No.	Administrative zone	District	Number landraces	Identification
1	Oromo special zone	Dawa Cheffa	15	FTS0039, FTS0040, FTS0041, FTS0042, FTS0043, FTS0044, FTS0045, FTS0046, FTS0047, FTS0048, FTS0049, FTS0050, FTS0051, FTS0052, FTS0053
2	South Wollo	Tehuledere	16	FTS0206, FTS0207, FTS0208, FTS0209, FTS0211, FTS0213, FTS0216, FTS0217, FTS0219, FTS0221, FTS0222, FTS0224, FTS0225, FTS0226, FTS0228, FTS0229,
3	South Wollo	Tenta	10	FTS0068, FTS0069, FTS0070, FTS0071, FTS0072, FTS0073, FTS0075, FTS0076, FTS0236, FTS0237
4	South Wollo	Dessie Zuria	11	FTS0193, FTS0195, FTS0196, FTS0198, FTS0200, FTS0201, FTS0202, FTS0203, FTS0205, FTS0232, FTS0233
5	South Wollo	Kalu	53	FTS0114, FTS0116, FTS0118, FTS0119, FTS0120, FTS0121, FTS0122, FTS0123, FTS0125, FTS0126, FTS0128, FTS0129, FTS0130, FTS0133, FTS0134, FTS0135, FTS0136, FTS0137, FTS0139, FTS0140, FTS0141, FTS0142, FTS0144, FTS0146, FTS0147, FTS0148, FTS0149, FTS0151, FTS0152, FTS0155, FTS0156, FTS0157, FTS0158, FTS0159, FTS0164, FTS0165, FTS0166, FTS0168, FTS0170, FTS0171, FTS0172, FTS0173, FTS0176, FTS0177, FTS0178, FTS0179, FTS0180, FTS0181, FTS0182, FTS0183, FTS0184, FTS0185, FTS0186
6	North Wollo	Habru	44	FTS0013, FTS0014, FTS0016, FTS0018, FTS0019, FTS0020, FTS0026, FTS0027, FTS0029, FTS0031, FTS0032, FTS0033, FTS0035, FTS0036, FTS0037, FTS0077, FTS0078, FTS0079, FTS0080, FTS0082, FTS0083, FTS0085, FTS0086, FTS0087, FTS0088, FTS0089, FTS0090, FTS0091, FTS0092, FTS0093, FTS0095, FTS0096, FTS0098, FTS0101, FTS0102, FTS0103, FTS0104, FTS0105, FTS0106, FTS0107, FTS0108, FTS0109, FTS0110, FTS0111,
7	North Wollo	Kobo	11	FTS0001, FTS0002, FTS0004, FTS0005, FTS0006, FTS0007, FTS0008, FTS0009, FTS0010, FTS0011, FTS0012
8	-	Improved varieties	6	Tseday, Dukem, Gerado, Koye, Holette-Key, Gola

FTS= Initials of the collectors

and between block distances of 1m and 1.5m were left. Fertilizer was applied at the rate of 41-46 kg ha^{-1} N-P on each plot in the form of Urea and Diammonium Phosphate (DAP). Urea was applied in two splits, while the whole dose of DAP was applied at planting. Weeding and other cultural practices were applied uniformly on each plot.

Data were collected for nine morphological and agronomic traits. The data for traits like days to heading and maturity, grain-filling duration, biomass yield (gm), grain yield (gm) and harvest index (%) were collected on plot basis, while data for plant height (cm), culm length (cm) and panicle length (cm) were collected from five randomly selected plants per plot.

Data Analysis:

Univariate ANOVA was done by using CropStat 7.2 and the mean values of significantly differing traits were used for further analysis. PAST 1.93 (Hammer *et al*., 2001) computer software was used for principal component analysis. Phenotypic correlation, stepwise regression and two-step cluster analyses were worked out using SPSS 19. The mean values were standardized to a mean of zero and variance of unity to remove the biases due to differences in the scale of measurements before the two-step cluster analysis. While running the analysis, log-likelihood was selected as a distance measure and Akaike's information criterion (AIC) was used to determine the number of clusters automatically.

RESULTS

Mean values and range of traits:

The mean, minimum and maximum values of the nine traits of the 166 tef genotypes are presented in Table 2. Appreciable ranges of values were obtained for the various traits analyzed. Days to maturity ranged from 92 for the line FTS0045 collected from Dawa Cheffa to 106 for FTS0213 collected form Tehuledere. Plant height ranged from 56.2cm for FTS0135 collected from Kalu to 83.9cm for FTS0088 collected from Habru. Biomass ranged from 73.87gm for FTS0172 to 410.27gm for FTS0116, both collected from Kalu. In addition, grain yield ranged from 17.97gm for FTS0172 collected from Kalu to 117gm for FTS0047 which was collected from Dawa Cheffa.

Phenotypic correlation and stepwise regression analyses:

Grain yield had significant phenotypic correlation with all the traits studied. However, its correlation with maturity and grain-filling duration was negative. Biomass yield was significantly correlated with all the traits except harvest index; its correlation with days to maturity and grain-filling duration was negative. Grain-filling duration was significantly correlated with days to heading and maturity, biomass yield, grain yield and harvest index; except days to maturity, the correlation with the other four traits was negative (Table 3).

Stepwise regression analysis was performed to determine the traits that contributed to yield. The result revealed that three out of the nine traits had significant linear relationship with yield. Plant height, harvest index and biomass yield had linear relationship (Table 4). Based on the regression analysis, the following model was obtained:

$$Y=-22.867+0.282BY+1.394HI-0.251PH$$

Where Y= yield, BY= biomass yield, HI= harvest index and PH= plant height

The traits included in the analysis explained 92.6%

Table 2. Estimates of means, ranges, standard error of mean of the characters investigated in the 166 Tef genotypes combined over locations.

Traits	Mean± SE	Minimum			Maximum			LSD (5%)
		Value	Line	District	Value	Line	District	
DH	51.86±3.520	45	FTS0157	Kalu	61	FTS0164	Kalu	9.78
DM	99.47±2.241	92	FTS0045	Dawa Cheffa	106	FTS0213	Tehuledere	6.23
GFD	47.65±4.217	36	FTS0164	Kalu	55.25	FTS0069	Tenta	11.72
PH (cm)	69.83±4.903	56.2	FTS0135	Kalu	83.9	FTS0088	Habru	13.62
PL (cm)	32.66±2.130	27	FTS0070	Tenta	40.75	FTS0140	Kalu	5.92
CL (cm)	37.17±3.728	28.25	FTS0114	Kalu	46.55	FTS0040	Dawa Cheffa	10.36
BY (gm)	208.58±57.873	73.87	FTS0172	Kalu	410.27	FTS0116	Kalu	160.78
GY (gm)	54.93±19.051	17.97	FTS0172	Kalu	117	FTS0047	Dawa Cheffa	52.93
HI (%)	26.15±4.215	14.95	FTS0176	Kalu	47.13	FTS0078	Habru	11.71

DH= days to heading, DM= days to maturity, GFD= grain-filling duration, PH= plant height, PL= panicle length, CL= culm length, BY= biomass yield, GY= grain yield, HI= harvest index of the total variations relative to grain yield; only 7.4% was attributed to other factors (error and traits not included)

Table 3. Phenotypic correlation coefficients of characters of the 166 tef lines used in the study.

	Days to heading	Days to maturity	Grain-filling duration	Plant height	Panicle length	Culm length	Biomass yield	Grain yield
DM	-.095							
GFD	-.848**	608**						
PH	-.021	.038	.037					
PL	-.027	.001	.022	.740**				
CL	-.013	.051	.038	.928**	.437**			
BY	.233**	-.167*	-.275**	.342**	.327**	.276**		
GY	.284**	-.166*	-.315**	.245**	.220**	.206**	.907**	
HI	.209**	-.118	-.229**	.050	-.023	.079	.181*	.474**

DM= days to maturity, GFD= grain-filling duration, PH= plant height, PL= panicle length, CL= culm length, BY= biomass yield, GY= grain yield, HI= harvest index; **, *= significant at 0.01 and 0.05 probability levels, respectively

Table 4. Stepwise regression analysis of yield related traits on grain yield of the 166 tef lines

Model	Unstandardized Coefficients		Sig.
	B	Std. Error	
Constant	-22.867	5.904	.000
Biomass	.282	.007	.000
Harvest index	1.394	.095	.000
Plant height	-.251	.082	.003

$R^2 = 0.926$

Principal component analysis:

The result of the principal component (PC) analysis is presented in Table 5. In the analysis, nine principal components, equal to the total number of traits, were produced. However, from the total number of principal component axes, only three had eigenvlaues greater than one. The first, second and third principal component axes explained 34.3, 26.4 and 13.4% of the variability contained in the landrace lines of tef, respectively. The three PC axes totally explained 74% of the total variability. As evidenced by the highest value of loading or eigenvector (absolute), biomass yield was the most important trait contributing to the first PC. Similarly, grain-filling duration, plant height, day to heading, culm length and panicle length were important in the second PC axis. In the third PC, however, days to maturity, grain yield and harvest index were important traits.

Two-step cluster analysis:

The two-step cluster analysis automatically separated the entire genotypes into three groups with 61, 52 and 53 members. Subsequently one-way ANOVA was run to declare whether any of the mean differences of the traits between the three groups were significant. Accordingly, the result showed that the between-group means were significant (p<0.01) for all the traits except for plant height (Table 6).

Cluster I contained three improved varieties (Gerado, Dukem and Tseday) and tef landraces collected from all districts except Kobo. Similarly, cluster II was composed of tef landraces collected from all districts except Kobo. Cluster III, however, contained three improved varieties (Koye, Holetta-Key and Gola) and tef landraces collected from all districts (Table 7)

DISCUSSION

Since the center of both origin and diversity of tef is Ethiopia, systematic and inclusive (of various geographic locations and altitudinal ranges) collection, conservation and evaluation of landraces is vital for gene bank curators and breeders to develop improved tef varieties.

In the present study, the appreciable ranges of values obtained for the various traits like days to maturity, plant height, biomass- and grain-yield indicates the presence of ample variability in the tef landraces included in the study. Generally, most of the landraces had higher mean values for all traits studied than the released varieties. From the 160 tef landraces, 41 out-yielded the six improved tef varieties; this shows the possibility of improvement of tef for yield by phenotypic selection. Some landraces were selected and are under multi-stage and multi-environment (location and year) evaluation to accommodate the environmental variability (Assefa et al., 2014).

The significant phenotypic correlation of grain yield with all the traits studied suggestes the possibility of combining higher yield with higher biomass, higher harvest index, tall plant type and late flowering in a single landrace. Teklu and Tefera (2005) reported that improved plant height, panicle length and kernels per panicle are features of most modern tef varieties. The stepwise regression analysis of grain yield on some yield component traits suggests linear increment in plant height, harvest index and biomass yield could lead to significant increase in tef grain yield. In agreement to the result, Chanyalew et al. (2010) demonstrated that increased shoot biomass and harvest index could serve as selection criterion for increasing tef grain yield. The results of Teklu and Tefera (2005) also revealed that biomass yield was the most important yield attribute accounting for 56.7% of the variation in tef grain yield.

The aim of principal component analysis (PCA) is to reduce the dimensionality of a data set consisting of a large number of interrelated variables. The analysis transforms the data into a new set of variables, the principal components (PCs), which are orthogonal and independent of each other, and ordered so that the first few retain most of the variation present in the original variables (Jolliffe, 2002; Mohammadi and Prasanna, 2003). In this study, 74% of the total variability was explained by three PCs, the first being the most important. PCA, conducted by using different number of tef genotypes, have explained 71% of the phenotypic diversity by five PCs (Assefa et al., 2000), 81% of the total variance

Table 5. Eigenvalues, total variance, cumulative variance and eigenvectors for nine morpho-agronomic traits in the 166 Tef genotypes.

Traits	PC 1	PC 2	PC 3
Days to heading	0.248	**-0.400**	-0.287
Days to maturity	-0.164	0.306	**0.326**
Grain-filling duration	-0.285	**0.482**	0.402
Plant height (cm)	0.388	**0.434**	-0.240
Panicle length (cm)	0.319	**0.344**	-0.194
Culm length (cm)	0.342	**0.390**	-0.213
Biomass (gm)	**0.454**	-0.047	0.355
Grain yield (gm)	0.452	-0.135	**0.483**
Harvest index (%)	0.226	-0.188	**0.387**
Eigenvalue	3.085	2.374	1.204
Variance (%)	34.275	26.380	13.373
Cumulative variance (%)	34.275	60.655	74.028

Table 6. Between-groups mean squares and cluster means of the nine morpho-agronomic traits of the 166 tef genotypes.

Traits	MS (df=2)	Mean		
		Cluster I (n=61)	Cluster II (n=53)	Cluster III (n=52)
Days to heading	449.628**	50.660	55.175	49.760
Days to maturity	92.920**	100.090	97.934	100.317
Grain-filling duration	950.310**	49.430	42.759	50.558
Plant height (cm)	1434.166	65.109	69.965	75.214
Panicle length (cm)	113.942**	31.269	32.828	34.104
Culm length (cm)	742.106**	33.839	37.137	41.111
Biomass (gm)	123718.786**	163.245	256.366	213.055
Grain yield (gm)	13542.745**	41.058	71.915	53.880
Harvest index (%)	271.645**	24.208	28.574	25.962

**=significant at 0.01 probability level

Table 7. Distribution of tef landraces by district and cluster

Sr. No.	Collection district	Cluster					
		I		II		III	
		Landraces	**Total**	**Landraces**	**Total**	**Landraces**	**Total**
1	Dawa Cheffa	FTS0041, FTS0044, FTS0048, FTS0050, FTS0053	5	FTS0040, FTS0051	2	FTS0039, FTS0042, FTS0043, FTS0045, FTS0046, FTS0047, FTS0049, FTS0052	8
2	Dessie Zuria	FTS0196, FTS0198, FTS0203	3	FTS0193, FTS0195, FTS0201, FTS0202, FTS0233	5	FTS0200, FTS0205, FTS0232	3
3	Habru	FTS0014, FTS0026, FTS0031, FTS0077, FTS0080, FTS0083, FTS0085, FTS0087, FTS0089, FTS0092, FTS0102, FTS0104, FTS0105, FTS0110	14	FTS0016, FTS0019, FTS0029, FTS0032, FTS0036, FTS0078, FTS0082, FTS0086, FTS0088, FTS0090, FTS0093, FTS0095, FTS0101, FTS0103, FTS0106, FTS0107, FTS0108, FTS0109	18	FTS0013, FTS0018, FTS0020, FTS0027, FTS0033, FTS0035, FTS0037, FTS0079, FTS0091, FTS0096, FTS0098, FTS0111	12
4	Kalu	FTS0114, FTS0121, FTS0122, FTS0126, FTS0129, FTS0130, FTS0135, FTS0136, FTS0142, FTS0151, FTS0155, FTS0156, FTS0159, FTS0165, FTS0171, FTS0172, FTS0173, FTS0177, FTS0180, FTS0181, FTS0183	21	FTS0116, FTS0118, FTS0119, FTS0123, FTS0125, FTS0134, FTS0137, FTS0140, FTS0144, FTS0146, FTS0147, FTS0148, FTS0152, FTS0157, FTS0170, FTS0176, FTS0182, FTS0185	18	FTS0120, FTS0128, FTS0133, FTS0139, FTS0141, FTS0149, FTS0158, FTS0164, FTS0166, FTS0168, FTS0178, FTS0179, FTS0184, FTS0186	14
5	Kobo	-	-	-	-	FTS0001, FTS0002, FTS0004, FTS0005, FTS0006, FTS0007, FTS0008, FTS0009, FTS0010, FTS0011, FTS0012	11
6	Tehuledere	FTS0206, FTS0216, FTS0219, FTS0221, FTS0229, FTS0213, FTS0224, FTS0225, FTS0226	9	FTS0208, FTS0209, FTS0217, FTS0222, FTS0228, FTS0211	6	FTS0207	1
7	Tenta	FTS0069, FTS0070, FTS0071, FTS0072, FTS0075, FTS0076	6	FTS0068, FTS0236, FTS0237	3	FTS0073	1
8	Improved varieties	Gerado, Dukem, Tseday	3	-	-	Koye, Holetta-Key, Gola	3
	Total		61		52		53

FTS = Initials of collections

with five PCs (Assefa et al., 2003) and 88.8% of the variance with six PCs (Adenew et al., 2005). However, Habtamu et al. (2011) explained 75% of the variability by the first three PCs. Similarly, by studying of 20 tef genotypes, Bedane et al. (2015) explained 73% of the variability with two PCs. The traits included in the first few PCs with comparatively high loadings are important in differentiating the landraces. Biomass yield was the most important trait contributing to the first PC. While grain-filling duration, plant height, day to heading, culm- and panicle-length were important in the second PC axis. The result is in agreement with the one reported by Assefa et al. (2003).

From the two-step cluster analysis (Table 6), it is evident that cluster I is characterized by short culm, low biomass- and grain-yield. Similarly, late flowering, short grain-filling period, high biomass- and high grain-yield and high harvest index characterized cluster II. This cluster does not contain any of the improved varieties and landraces from Kobo. However, tall plant type (tall panicle and culm) were the distinguishing features of cluster III. The number of clusters identified in this study is smaller than the ones reported by Assefa et al. (2000) and Adenew et al. (2005) who reported six and eight distinct classes, respectively.

Stepwise regression and phenotypic correlation analyses were in agreement in identifying the traits associated with tef grain yield. Biomass yield, harvest index and plant height could be set as indirect selection criteria to improve yield in tef. Landraces in cluster II could be exploited as parents in future breeding programs and could be crossed with improved varieties or other elite lines.

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