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RESEARCH ARTICLE

Genetic divergence and association of traits in recombinant inbred lines of tef (Eragrostis tef [Zucc.] Trotter)

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Abstract

Forty-nine tef RILs with checks was conducted in Gondar Zuria district, Ethiopia to assess multivariate variability and association among traits using 7×7 simple lattice design. Analysis of variance revealed a highly significant and significant variation among the RILs except for thousand seed weight. Grain yield exhibited highly significant positive genotypic correlation with plant height, biomass yield and harvest index and highly significant positive phenotypic correlation with plant height, main shoot panicle length, number of primary branches per main shoot panicle, main shoot panicle grain yield, biomass yield and harvest index. The path coefficient analyses both at genotypic and phenotypic levels revealed harvest index and above ground biomass yield exert strong direct effect on grain yield; indicating that these traits could be used as selection criteria for yield improvement. Cluster analysis, grouped the tested genotypes into five clusters with an inter- and intra-cluster distance ranging from 12.79 to 85.14 and 7.82 to 15.29, respectively. Also cluster III had larger distance from Cluster II and Cluster V; the divergence of cluster III from these two clusters indicate existence of RILs to be crossed and selected. The first five principal components (PCs) accounted for 70.8% of the total genetic variation. Generally, this considerable genetic variability among the RILs confirmed the possibility of improving tef productivity.

Keywords: Cluster analysis, correlation, path analysis, RILs, tef

Introduction

Tef [*Eragrostis tef* (Zucc.) Trotter] is a small grain cereal crop which is indigenous and mainly grown in Ethiopia. It is adaptable and can be cultivated under diverse agro-ecological conditions and excellently performs better than other cereals under adverse and marginal growing conditions and fits in to various cropping systems. It is useful as a catch and low-risk reliable crop (Kebebew *et al.,* 2011), it specifically performs in areas with temperature ranges of 10-27°C, altitudes of 1800-2100 m.a.s.l and annual rainfall of 750-850 mm (Seyfu, 1997). Tef have high economic value, mainly grown for its grain as a major staple food and market cereal crop (Hailu *et al.,* 2003). Its grain is useful for preparing injera, kitta (unleavened bread), anebaberro (double layered injera), porridge, gruel, and local alcoholic beverages such as tella and katikala. Currently, tef has been gaining global popularity as health food because of its gluten-free, which makes it suitable for peoples suffering from gluten protein allergy known as celiac disease [(Spaenij-Dekking *et al.,* 2005). Its commercial production and experimentation is

currently being conducted in the United States, South Africa, Netherland, Spain, Israel (Kebebew *et al.,* 2011) and known as the"most up-to-date marvelous food of the 21st century" such that its international popularity is rapidly growing. Tef have been cultivated in Ethiopia for centuries. Moreover, a total production of 5283401.2 tons with area coverage of 3023283.5 ha has been recorded in 2018 (CSA, 2018). The Amhara Region is one of the major tef growing regions in the country with productivity of 1.7 t ha^{-1}, which is slightly more than the national average. North Gondar zone is one of the major tef growing area in the region with 132046 ha of land cultivated with it. It is the most important cereals grown in Gondar zuria district with nearly 21032 ha of land grown (GZAO, 2019). However, productivity of tef at national level is still lower due to the low yield potential of landrace varieties used by farmers, as these varieties are more susceptible to lodging, drought and other biotic and abiotic stresses (Kebebew *et al.,* 2011). Therefore, these varieties should be replaced by high yielding and stress resistant tef varieties through exploiting the broad spectrum of diversity through direct selection or hybridization of parental lines with desirable traits (*et al.,* 2015). Screening of tef genotypes using both phenotypic and genotypic data is important to identify high yielder and productive breeding lines (Mizan *et al.,* 2015). The breeding progress is dependent on the magnitude of genetic variation for traits of importance in a given environment (Singh, 2002). Information on the extent and nature of interrelationships among traits helps in planning efficient scheme of multiple trait selection, (Jalal et al., 2019). Above and beyond, multivariate methods such as cluster and principal component analyses using morphological traits are important to identify promising genotypes based on their phenotypic variation (Fisseha, 2017). In Ethiopia, different studies on tef have reported the existence of substantial genetic variability and broader association of traits (Habtamu *et al.,* 2011; Dagnachew and Girma, 2014; Fisseha, 2017). But these are mostly based on the naturally occurring diversity. Though tef cross breeding have resulted in 9% yield advantage as compared to those developed through direct selection from germplasm materials, not much studies have been conducted on genetic divergence through multivariate techniques and trait association in tef recombinant inbred lines. Therefore, the objectives of the study were to determine the correlation among traits and to evaluate the direct and indirect effects of traits on grain yield of recombinant inbred lines; to identify traits having major contribution to the observed variability, to group and to identify diverse promising tef recombinant inbred lines and parents for future breeding programs.

Materials and methods

The field experiment was conducted on farmer's field, at Gondar zuria district, which is located at 15 km and 680 km from the city of Gondar and Addis Ababa respectively and it is found in Central Gondar zone of the Amhara Region, Ethiopia. The site is located at a longitude of 12°36′N 37°28′E and has an altitude of 2023 meters above sea level (GPS reading). The soil of the district is clayish in texture with particle size distribution of 27.28% sand, 31.28% silt and 41.44% clay (GZAO, 2019). Forty-two F8 advanced recombinant inbred lines (RILs), two standard check varieties (Negus and Ebba), four parents of the RILs; namely, Magna (DZ-01-196), HO-TFS-1486, Boset and GA-10-3 along with one local check variety were used for the study (Table 1). The 42 F8 RIL populations were progenies from the cross of four parental varieties; DZ-01-196 × HO-TFS-1486 and Boset × GA-10-3. The local check variety was included from the study area whereas the rest of the experimental materials were collected from Debre Zeit agricultural research centre.

Table 1: List of the experimental tef recombinant inbred lines and checks used in the study

Entry	Genotype	Source	Entry	Genotype	Source
1	RIL146	DZ-01-196×HO-TF-1486	26	RIL52	DZ-01-196×HO-TF-1486
2	RIL303	Boset×GA-10-3	27	RIL296	Boset×GA-10-3
3	RIL32	DZ-01-196×HO-TF-1486	28	RIL191	DZ-01-196×HO-TF-1486
4	RIL293	Boset×GA-10-3	29	RIL225	DZ-01-196×HO-TF-1486
5	RIL280	DZ-01-196×HO-TF-1486	30	RIL42	Boset×GA-10-3
6	RIL213	Boset×GA-10-3	31	RIL162	DZ-01-196×HO-TF-1486
7	RIL58	DZ-01-196×HO-TF-1486	32	RIL163	Boset×GA-10-3
8	RIL259	DZ-01-196×HO-TF1486	33	RIL152	DZ-01-196×HO-TF-1486
9	RIL134	Boset×GA-10-3	34	RIL174	Boset×GA-10-3
10	RIL122	Boset×GA-10-3	35	Ebba	Kay Muri x 3774-13
11	RIL114	Boset×GA-10-3	36	RIL178	DZ-01-196×HO-TF-1486
12	RIL55	DZ-01-196×HO-TF-1486	37	RIL128	Boset×GA-10-3
13	RIL90	Boset×GA-10-3	38	RIL123	DZ-01-196×HO-TF-1486
14	RIL281	DZ-01-196×HO-TF-1486	39	RIL170	Boset×GA-10-3
15	RIL176	DZ-01-196×HO-TF-1486	40	RIL92	DZ-01-196×HO-TF-1486
16	RIL104	Boset×GA-10-3	41	RIL313	Boset×GA-10-3
17	RIL285	Boset×GA-10-3	42	Local	Farmers' cultivar
18	RIL89	Boset×GA-10-3	43	RIL143	DZ-01-196×HO-TF-1486
19	RIL108	DZ-01-196×HO-TF-1486	44	RIL127	Boset×GA-10-3
20	RIL240	Boset×GA-10-3	45	Boset	Parent
21	RIL105	DZ-01-196×HO-TF-1486	46	GA-10-3	Parent
22	RIL26	DZ-01-196×HO-TF-1486	47	DZ-01-196	Parent
23	RIL281	DZ-01-196×HO-TF-1486	48	HO-TF1486	Parent
24	RIL69	DZ-01-196×HO-TF-1486	49	Negus	DZ-01-353 × Kay Murri
25	RIL242	Boset×GA-10-3			

Experimental design and management

The 49 genotypes were laid out with 7×7 simple lattice design. Each experimental plots had 1 m × 2 m dimensions (2 m^2 area) and five rows spaced 20 cm apart. Plots and incomplete blocks were spaced with 1 m distance while replications were spaced 1.5 m. Each genotype was assigned on plots with randomized allocations within each replication. Seeds were hand drilled along the surface of each row at the rate of 15 kg ha^{-1} (3 g. per plot). The experimental materials were planted on the first week of July, 2021. All recommended packages of practices were followed to raise the healthier crop. Data were collected on the basis of plot and individual plants. For plant basis, five plants were randomly selected from the central two rows of each plot. The mean value of the sample plants from each plot was used for analysis. The following traits were measured on plant basis: plant height (cm), main shoot panicle length (cm), peduncle length (cm), numbers of primary branches per main shoot panicle, main shoot panicle weight (g), main shoot panicle grain yield (g), number of total tillers and number of fertile tillers. Number of total tillers and number of fertile tillers were assessed on randomly taken plants from central rows of each half meter part of the plot as the total number of tillers and the number of panicle bearing tillers, respectively. Traits measured on plot basis were the following: days to heading (days), days to physiological maturity (days), grain-filling period (days), lodging index, grain yield per plot (kg), above ground biomass yield per plot (kg), harvest index (%), thousand-seed weight (g).

Lodging index was recorded following the method of Caldicott and Nuttall (1979) as a product sum of each scale or degree of lodging (0-5) and their respective percentage divided by five; i.e., Lodging index = Sum (Lodging scores or degree × the respective percentage area lodged)/5. The calculated value for lodging index is between zero (no lodging or erect) and 100 (complete lodging). The relevant values generated on plot and plant basis were subjected to analysis of variance (ANOVA) using general linear model and lattice procedures by SAS 9.2 software (SAS Institute, 2007) to estimate the variation among the genotypes. The lattice procedure was used to accurately estimate treatment effects, while general linear model procedure was used to calculate unadjusted sum squares for block, intra block error and treatment. Comparison of trait means of genotypes was done following the significance of mean squares using Duncan's Multiple Range Test. Phenotypic and genotypic correlations with their respective significance level were obtained from the CANDISC procedure using SAS 9.2 software (SAS institute, 2007). based on the variance components of each trait and the covariance components for each pair of traits using the formulae of Robinson et al. (1949). Path-coefficient analysis is a standardized partial regression coefficient, which splits the correlation coefficient into measures of direct and indirect effects. Both phenotypic and genotypic correlation coefficients were partitioned into direct and indirect effects on grain yield as per the procedure of Dewey and Lu (1959). The magnitude of Pr. indicates how best the causal traits account for the variability of the dependent trait (Singh and Chaudhary, 1985). If Pr. value is small (nearly zero) the dependent character considered (yield) is fully explained by the variability in the independent characters, whereas higher Pr value indicates that some other factors which have not been considered, need to be included in the analysis to fully account the variation in the dependent character. Pair wise genetic distance of 49 tef genotypes were estimated using Mahalanobis (1936). D^2 statistics from quantitative traits after standardization as established by Mohammadi and Prasanna (2003) by using JMP 5 software. Average intra distance values were estimated. Significance of the squared distances for each cluster was tested against the tabulated $\chi 2$ values at p-1 degree of freedom at 1% and 5% probability level using D^2 values as calculated chi-square value. Where p= number of traits used for clustering the genotypes (Singh and Chaudhary 1985). The cluster analysis was performed based on D^2 distance by SAS statistical software (SAS Institute, 2007). Number of clusters was determined based on the pseudo F and t2 values (cut points were determined from local peaks of the pseudo F value joined with small values of the pseudo t2 value and then a larger pseudo t2 for the next cluster union) from the analysis of treatment means. Clustering and distance pattern of genotypes was plotted based on average linkage and squared Euclidean distance by using dendrogram with JMP software, from which cluster means were calculated for genotypes within their respective cluster. Principal components based on correlation matrix were calculated using the SAS statistical software. The principal component variables were linear combinations of the original variables X1… Xk… Xm. The eigenvectors table provided coefficients for equations. Yk = Ck1X1 + Ck2X2 + …+ CkmXm. Where Yk is the kth principal component k; C coefficients in the table. Relative significance of traits constituting the PCs was weighed by dividing eigenvectors greater than half the standard deviation (square root) to the eigenvalue of the respective PC (Johnson and Wichern 1988).

It also helps to identify traits that load more in explaining the observed variation. In the Principal component analysis, variables that most strongly correlated with each component (i.e. with large eigenvectors, which are furthest from zero in either positive or negative direction) were considered in the correlation matrix.

Results and discussion

The analysis showed the presence of highly significant (P <0.01) differences among tef genotypes for days to heading, days to physiological maturity, grain-filling period, plant height, panicle length, tiller number, fertile tiller number, panicle weight, grain yield per panicle, grain yield, above ground biomass yield and harvest index; while peduncle length, number of primary branches per main shoot panicle and logging index showed significant (P<0.05) difference indicating the existence of adequate variability among the 49 tef genotypes for phenomorphic, yield and yield related traits. The reduced value of coefficient of variation for most of the traits indicate the existence of good precision in the experiment. The ANOVA result generally showed existence of genetic variability in the tef RILs and this variation for those traits indicate the possibility to exploit it in future breeding programs and the higher chance of improving the crop through selection. These findings are in agreement with Haftamu (2018), Habte *et al.,* (2017), Chekole *et al.,* (2016) and Mizan *et al.,* (2016) who found highly significant variation for those traits in study of tef genotypes. However, some disparity were found with Habtamu *et al.,* (2011) for fertile tillers, panicle length, biomass yield, grain yield and harvest index that might have been resulted due to variation of genetic factors carried by the genotypes and difference in the experimental area under which the lines were evaluated. Targeted trait improvement can be achieved by indirect selection via other traits that are more heritable and relevant to select. This requires understanding the interrelationship of the traits among themselves and with the target trait (like grain yield). Yield is the most complex trait and it is subjective to many component traits that determine its productivity. These relationships observed from this experiment among yield and yield related traits are discussed. Estimates of genotypic (rg) and phenotypic (rp) correlation coefficients between each pair of the traits are presented in Table 2. Grain yield showed highly significant (P<0.01) positive genotypic correlation with plant height (rg = 0.46), above ground biomass yield (rg = 0.42) and harvest index (rg=0.77). This suggests that prolific high yielder lines are likely to have good plant height, harvest index and high biomass yield. Therefore, any improvement of these traits would result in a substantial improvement on grain yield. The finding is in agreement with results of Alemayehu *et al.,* (2012) and Habtie *et al.,* (2015) who found a significant positive correlation of grain yield with biomass yield and harvest index. Moreover, positive significant correlation of grain yield with plant height was also reported by Asaye (2017). Besides, grain yield had significant positive genotypic correlation with main shoot panicle length (rg=0.36), peduncle length (rg=0.32) and number of primary branches per main shoot panicle (rg=0.36). This shows the presence of inherent relationship between these traits with grain yield. Solomon *et al.,* (2009) also reported significant positive genotypic correlation of grain yield with panicle length. Therefore, the strong correlation observed between the traits indicates those traits are conditioned by the same set of genes (Falconer, 1989). Consequently, selection for one trait can indirectly change positively in the other trait due to either presence of pleiotropic gene effect or genetic linkage or both.

Table 2: Estimates of genotypic-rg (above diagonal) and phenotypic-rp (below diagonal) correlation coefficients for 15 traits of tef genotypes studied

Traits	Days to heading	Days to maturity	Grain-filling period	Plant height	Main shoot panicle length	Peduncle length	Total tiller number	Fertile tiller number	PBPMS	Main shoot panicle weight	main shoot panicle grain yield	Lodging index	Grain yield	Above ground biomass yield	Harvest index
Days to heading	1	0.25	-0.72**	0.08	0.07	-0.06	-0.06	-0.003	0.14	-0.01	0.03	-0.074	-0.14	-0.11	-0.06
Days to maturity	0.25*	1	0.49**	-0.34*	-0.2	-0.17	0.07	0.27	-0.36*	-0.15	-0.07	-0.02	-0.38**	-0.15	-0.33*
Grain-filling period	-0.71**	0.51**	1	-0.31*	-0.21	-0.07	0.1	0.16	-0.39**	-0.103	-0.08	0.06	-0.15	-0.003	-0.18
Plant height	0.03	-0.29**	-0.24*	1	0.87**	0.49**	-0.03	0.33*	0.82**	0.34*	0.36*	0.57**	0.46**	0.25	0.31*
Main shoot panicle length	0.05	-0.15	-0.15	0.76**	1	0.30*	-0.08	0.30*	0.75**	0.45**	0.29*	0.50**	0.37*	0.11	0.30*
Peduncle length	-0.08	-0.17	-0.05	0.49**	0.25*	1	0.01	0.33*	0.32*	-0.15	0.04	0.27	0.32*	0.28	0.14
Total tiller number	-0.06	0.05	0.09	0.03	-0.05	0.05	1	0.35*	-0.05	0.011	-0.07	-0.12	-0.09	-0.24	0.09
Fertile tiller number	-0.03	0.17	0.15	0.25*	0.25*	0.27**	0.37**	1	0.114	0.12	-0.05	0.10	-0.09	-0.06	-0.06
primary branches per main shoot panicle	0.09	-0.26*	-0.27**	0.7**	0.67**	0.24*	0.02	0.07	1	0.28	0.30*	0.44**	0.36*	0.24	0.24
Main shoot panicle weight	0.01	-0.11	-0.09	0.23*	0.36**	-0.14	0.004	0.16	0.27**	1	0.43**	0.27	0.29	0.07	0.26
Main shoot panicle grain yield	0.02	-0.07	-0.07	0.25*	0.22*	0.04	-0.03	-0.001	0.22*	0.42**	1	0.43**	0.07	0.05	0.05
Lodging index	-0.05	0.02	0.05	0.39**	0.47**	0.16	-0.14	0.04	0.33**	0.15	0.29**	1	0.19	0.21	0.05
Grain yield	-0.13	-0.33**	-0.13	0.36**	0.36**	0.25*	-0.08	-0.09	0.34**	0.25*	0.06	0.22*	1	0.42**	0.77**
Above ground biomass yield	-0.12	-0.12	0.02	0.22*	0.08	0.23*	-0.22*	-0.06	0.19	0.09	0.04	0.16	0.37**	1	-0.25
Harvest index	-0.04	-0.27**	-0.16	0.22*	0.31**	0.10	0.09	-0.06	0.24*	0.20*	0.04	0.12	0.78**	-0.29**	1

* and ** significant ($p \leq 0.05$) and highly significant ($p \leq 0.01$) respectively

On the other hand, days to physiological maturity (rg = -0.38) showed a highly significant negative genotypic correlation with grain yield. These are in line with the results of Abel *et al.,* (2014) and Plaza-Wuthrich *et al.*, (2013) who obtained negative correlation of grain yield with days to maturity. The strong correlation observed between the traits indicates those traits are conditioned by the same set of genes in the negative direction (Falconer, 1989). Grain yield showed positive and highly significant (P<0.01) phenotypic correlation with plant height (rp=0.36), main shoot panicle length (rp=0.36), number of primary branches per main shoot panicle (rp=0.34), main shoot panicle grain yield (rp=0.30), above ground biomass yield (rp=0.37) and harvest index (rp=0.78) (Table 2). This Suggests that vigorous and tall plants were high yielding and serve as indicators of high yielding ability. In agreement to this result, Solomon (2010) also found positive and significant phenotypic correlation of grain yield with plant height, panicle length and biomass yield. The result found by Tsion et al. (2018) shows similar result to this study for correlation of yield with number of primary branches per main shoot panicle, biomass yield and harvest index. Similarly, grain yield had significant (p<0.05) positive phenotypic correlation with peduncle length (rp = 0.25), main shoot panicle weight (rp=0.25) and lodging index (rp=0.22) (Table 2). These are in line with Hailu *et al.,* (2003) who found positive correlation of grain yield with panicle weight, main shoot panicle grain yield and lodging index. Days to physiological maturity (rp=-0.33) showed a highly significant negative relationship with grain yield. Likewise, traits like days to heading (rp= -0.13), grain-filling period (rp= -0.13), total tiller number (rp=-0.08) and fertile tiller number (rp = -0.09) correlated negatively with grain yield (Table 2). This suggested that selection for these traits would not improve grain yield, Plaza-Wuthrich *et al.,* (2013) also reported negative phenotypic correlation of grain yield for days to maturity and tiller number. Tsion *et al.,* (2018) also found negative phenotypic correlation of grain yield with days to heading, days to physiological maturity and tiller number. This demonstrated that plant height, above ground biomass yield and harvest index showed highly significant positive genotypic and phenotypic correlation (P<0.01) with grain yield while, peduncle length and main panicle weight showed positive and significant (P<0.05) association at phenotypic and genotypic levels. This indicates that selection for tall plants, heavier above ground biomass, higher harvest index, long peduncle and heavier main shoot panicle would improve tef grain yield. For genotypic path analysis, seven traits that had significant genotypic correlation with grain yield were selected as casual (independent) traits and partitioned into direct and indirect effects using grain yield as dependent variable. Furthermore, nine traits that provided significant phenotypic correlation with grain yield were selected as independent traits for phenotypic path analysis. The genotypic and phenotypic direct and indirect effects of these casual traits on grain yield are presented in Tables 3 and 4, respectively.

Genotypic direct and indirect effects of traits on grain yield

The direct effects of casual traits on grain yield ranged from -0.046 to 0.935 at genotypic level (Table 3). Genotypic path coefficient analysis showed that harvest index (0.935) followed by above ground biomass yield (0.654) exerted the highest positive genotypic direct effect on grain yield (Table 3). Therefore, above ground biomass yield and harvest index are good contributors to grain yield. As a result, any genetic improvement on those traits can increase grain yield and selection for these traits is helpful to improve grain yield indirectly. This is in line with previous findings of Habte (2019) and Abel *et al.,* (2014) who demonstrated high positive direct effects of biomass yield and harvest index on grain yield.

Plant height, peduncle length and number of primary branches per main shoot panicle also exerted appreciable indirect effects on grain yield via above ground biomass yield and harvest index. Besides, main shoot panicle length also had sizable indirect effect of on yield through Harvest index. Though days to physiological maturity had positive direct effect on grain yield, all its indirect effects except through number of primary branches per main shoot panicle were negative summing up with negative genotypic correlation with grain yield. The genotypic residual effect of this study was 0.093, indicating that the independent traits had explained about 90.7% of the total variation in grain yield. While the remaining 9.3% was contributed by traits not included in the path analysis and errors encountered in the study. Therefore, the studied independent traits have explained more of the yield.

Phenotypic direct and indirect effects of traits on grain yield

Phenotypic path coefficient analysis revealed that above ground biomass yield (0.652) and harvest index (0.968) exerted positive and highest direct effect on grain yield (Table 4). This effect is an indication that the strong phenotypic correlation of above ground biomass yield and harvest index with grain yield was largely due to the direct effect of these traits. Therefore, any genetic improvement on above ground biomass yield and harvest index can increase grain yield and selection for these traits is helpful to improve grain yield indirectly. A number of previous workers on tef reported similar findings (Abel *et al.*, 2014; Dagnachew and Girma 2014; Chekole *et al.*, 2016; Fisseha 2017 and Habte 2019). Moreover, most of the traits exerted higher indirect phenotypic effects on grain yield through above ground biomass yield and harvest index (Table 4). These results are in agreement with Chekole *et al.*, (2016) for days to maturity and main shoot panicle length.

Table 3: Estimates of direct effect (bold diagonal) and indirect effect (off diagonal) at genotypic level of seven traits on grain yield in tef genotypes

Traits	Days to maturity	Plant height	Main shoot panicle length	Peduncle length	Number of primary branches/ main shoot panicle	Above ground biomass yield	Harvest index	Genotypic correlations with grain yield)
Days to maturity	0.012	-0.007	-0.004	-0.001	0.016	-0.095	-0.304	-0.38**
Plant height	-0.004	0.020	0.019	0.003	-0.037	0.162	0.292	0.45**
Main shoot panicle length	-0.002	0.0176	0.02	0.002	-0.034	0.070	0.281	0.35*
Peduncle length	-0.002	0.0100	0.006	0.00	-0.014	0.182	0.131	0.32*
Number of primary branches per main shoot panicle	-0.004	0.0166	0.016	0.002	-0.04	0.154	0.220	0.36*
Above ground biomass yield	-0.001	0.005	0.002	0.002	-0.010	0.65	-0.233	0.41**
Harvest index	-0.003	0.006	0.006	0.001	-0.010	-0.163	0.93	0.77**

Residual effect=0.093. ** and * indicates highly significant at ($p<0.01$) and significant at ($p<0.05$) probability levels respectively

Although days to physiological maturity had positive direct effect on grain yield, it had negative indirect effects through all the traits except number of primary branches per main shoot panicle leading to a positive phenotypic correlation with grain yield. On the other hand, number of primary branches per main shoot panicle (-0.033) and lodging index (-0.003) exhibited negative direct effect on grain yield. The latter is due to the use of lodging resistant parent GA-10-3 with its progeny RILs that exhibited stronger culm and a relatively reduced height resisted lodging and becomes high yielders. The negative direct and indirect effects of number of primary branches per main shoot panicle on grain yield through desirable traits should be managed during selection as its selection might have direct and indirect reducing effect on grain yield. Dagnachew and Girma (2014) have also reported the negative direct effect of lodging index on grain yield. The residual factor for phenotypic path was 0.094, meaning the explained variation for grain yield by the independent traits was 90.6%, whereas the remaining 9.4% was contributed by traits not included in the study and errors encountered in the experiment.

Table 4: Estimates of phenotypic direct (bold diagonal) and indirect (off diagonal) effects of nine traits on grain yield in tef genotypes studied

Traits	**Days to maturity**	**Plant height**	**Main shoot panicle length**	**Peduncle length**	**Number of primary branches / main shoot panicle**	**Above ground biomass yield**	**Harvest index**	**Days to maturity**	**Plant height**	**Phenotypic correlations with grain yield**
Days to maturity	0.011	-0.002	-0.003	-0.000	0.008	0.000	0.000	-0.078	-0.26	-0.331**
Plant height	-0.003	0.008	0.016	0.002	-0.022	0.000	-0.001	0.145	0.216	0.362**
Main shoot panicle length	-0.001	0.006	0.021	0.001	-0.021	0.000	-0.001	0.054	0.298	0.357*
Peduncle length	-0.001	0.004	0.005	0.004	-0.007	0.000	-0.000	0.150	0.099	0.254**
Number of primary branches / main shoot panicle	-0.002	0.005	0.014	0.00	-0.032	0.000	-0.001	0.125	0.230	0.341**
Above ground biomass yield	-0.001	0.001	0.007	-0.00	-0.008	0.000	-0.000	0.059	0.194	0.25*
Harvest index	0.000	0.003	0.010	0.00	-0.010	0.000	-0.003	0.105	0.116	0.223*
Days to maturity	-0.001	0.001	0.001	0.001	-0.006	0.000	0.000	0.65	-0.27	0.37**
Plant height	-0.003	0.001	0.006	0.000	-0.007	0.000	0.000	-0.187	0.96	0.77**

Genetic divergence analyses

The cluster analysis based on D^2 distance matrix grouped the 49 tef genotypes into five clusters based on their pseudo–F and t2 values at similarity level of 0.46. In line with this study, Habtamu et al. (2011) also reported five clusters. The numbers of genotypes in each cluster varied from five in cluster III to 17 in cluster I. Cluster I comprised 34.7% of the tef genotypes studied including the improved mutagenized parent GA-10-3 along with HO-TFS-1486 and 15 recombinant inbred lines from both crosses. This cluster was more heterogeneous than the others as it contains more lines from both crosses and two of the parents i.e. lines from different genetic back ground (Fig. 1). Similarly, the second cluster was composed of 13 genotypes where 11 of the RILs were progenies of the cross DZ-01-196×HO-TFS-1486, one RIL was progeny of the cross Boset×GA-10-3 and the parent Boset. Likewise, genotypes included in cluster III accounted for 10.2% of the tef genotypes (5 genotypes) where four RILs were progenies of the cross Boset×GA-10-3 and the the other was the local check. On the other hand, cluster IV contained eight genotypes that accounted for 16.3% of the total genotypes; in this cluster, the two recently released improved check varieties (Ebba and Negus) and RILs which were progenies of the cross DZ-01-196×HO-TFS-1486 were included. Finally, cluster V comprised of six (12.2% of the total) genotypes studied; the parent variety Magna (DZ-01-196) and RILs that were progenies of both crosses have been included in this cluster (Table 5). The result showed that genotypes that have been obtained from crosses of the same parents clustered together, this may indicate that the members shared the same genetic materials. The genotypes grouped within a given cluster were assumed to be more closely related in terms of the studied traits than the genotypes that have been grouped into different clusters (Fig. 1). The cluster analysis indicated the existence of variability among the lines resulting from the two crosses.

Cluster mean values

The mean values of the 15 quantitative traits in each cluster are presented in Table 6. Cluster I is composed of genotypes that has lower mean values of harvest index (26.36%), and higher lodging index (46.24%) and heavier above ground biomass (8461.58 Kg ha-1); but the rest of the traits had moderate mean values. Cluster II, exhibited a relatively lower mean values for total tiller number (108.69) and grain yield (2110.65 Kg ha-1), and harvest index. However, it had higher mean value lodging index (46.44%); the other traits considered in the study had medium mean values. Furthermore, the third cluster was characterized by genotypes with moderate mean values for all traits studied.

Table 5: Number and list of tef genotypes (RILs and checks) included in each clusters

Cluster number	Number of genotypes	Name of genotypes clustered
I	17	RIL146, RIL313, RIL213, RIL240, RIL26, RIL303, RIL285, RIL242, RIL280, RIL281, RIL90, RIL104, RIL42, RIL128, RIL163, Ho-TFS-1486, GA-10-3
II	13	RIL32, RIL123, RIL143, RIL122, RIL162, RIL178, RIL92, RIL191, RIL55, RIL225, RIL69, RIL281, Boset
III	5	RIL293, RIL174, RIL89, RIL127, Local
IV	8	RIL58, RIL152, RIL259, RIL296, RIL170, RIL105, Ebba, Negus
V	6	RIL134, RIL114, RIL108, RIL176, RIL52, Dz-01-196 (Magna)

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Fig. 1: Dendrogram grouping of tef genotypes by D^2 generalized distance and paired group clustering method estimated from the means of 15 traits

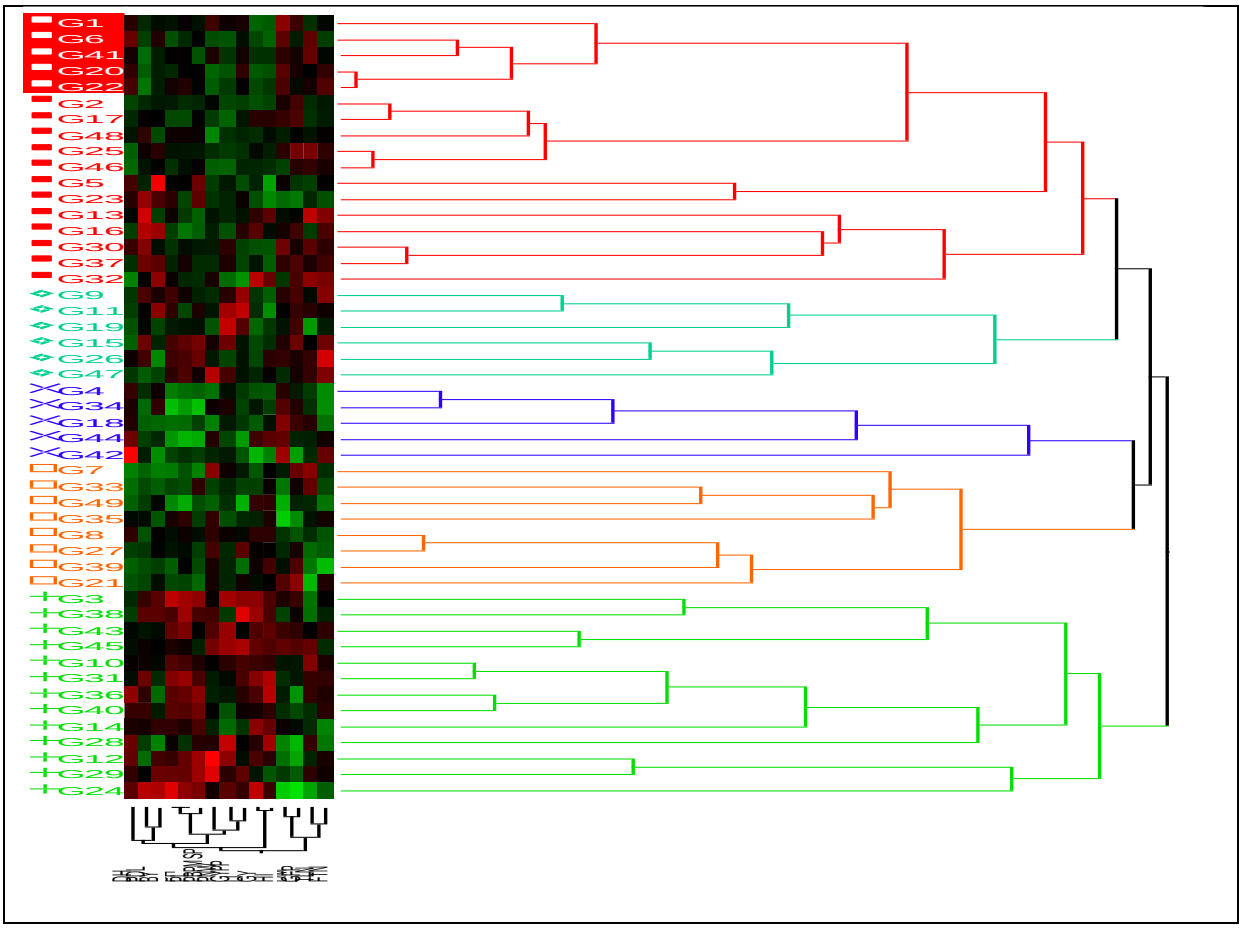


Table 6: Cluster mean values of the 49 tef genotypes for 15 traits studied

Trait	CLI	CLII	CLIII	CLIV	CLV
Days to heading	36.50	38.00	36.78	39.94	34.83
Days to physiological maturity	80.20	79.34	78.21	80.38	78.12
Grain-filling period	43.56	41.31	41.39	40.88	43.25
Plant height	82.88	83.74	82.73	84.99	77.43
Main shoot panicle length	39.14	39.57	39.28	43.56	36.40
Peduncle length	18.23	18.09	17.91	18.23	17.06
Total tiller number	111.79	108.69	111.58	108.75	114.75
Fertile tiller number	69.74	69.19	69.19	70.94	70.25
Number of primary branches per main shoot panicle	25.7	26.01	25.53	25.69	24.05
Main shoot panicle weight	2.36	2.25	2.27	2.4	2.34
Main shoot panicle grain yield	1.86	1.87	1.8	1.76	1.79
Lodging index	46.24	46.44	44.99	45.39	41.79
Grain yield	2213.95	2110.65	2175.93	2249.95	2264.94
Above ground biomass yield	8461.58	8103.37	8111.72	7987.35	7922.29
Harvest index	26.36	26.35	27.08	27.99	28.13

The fourth cluster contained genotypes characterized by lower mean value of total tiller number, above ground biomass and comparatively lower harvest index, but a relatively high mean values for days to heading, plant height, panicle length (Table 6). Finally, the fifth cluster was characterized by genotypes with lower mean value of days to heading, plant height, main shoot panicle length, number of primary branches per main shoot panicle, lodging index, above ground biomass yield and peduncle length; nonetheless, a relatively high mean values for total tiller number, grain yield and harvest index (Table 6). Hence, these genotypes could be helpful for yield and lodging resistance improvement, and their early heading indicates the scope for selecting tef genotypes that escape late coming terminal drought.

Intra- and inter-cluster distances

Highly significant (P<0.01) inter-cluster distances were obtained between clusters I and II (D^2=41.115), clusters II and III (D^2=85.135), clusters II and IV (D^2=37.736), clusters III and IV (D^2=30.027), and between clusters III and V (D^2=59.934). Likewise, significant (P<0.05) inter- cluster distance was found between cluster II and cluster V (D^2=29.077) (Table 7). Moreover, cluster II and III are the most divergent (D^2=85.135) clusters. Similarly, cluster III and V showed the next largest (D^2=59.934) inter-cluster distance. Those higher inter-cluster distances noted may have been resulted from the genetic differences (dissimilarities) of the genotypes included in the study. Hence, crosses involving clusters II and III will exhibit broad-spectrum of variability which is helpful for selecting genetically divergent genotypes. Moreover, cross between genotypes drawn from these two clusters would bring maximum heterosis.

The intra-cluster distance ranged from 7.82 for clusters II to 15.29 for clusters I, while clusters III, IV and V had intra-cluster distances of 9.58, 9.14 and 9.58, respectively. Therefore, cluster I had the highest intra-cluster distance indicating genotypes within this cluster are genetically divergent.

Table 7: Pair-wise generalized squared intra- (bold diagonal) and inter-cluster (below diagonal) distances between five clusters constructed from tef genotypes

Cluster	CLI	CLII	CLIII	CLIV	CLV
CLI	**15.29**				
CLII	41.115**	**7.82**			
CLIII	18.857^{ns}	85.135**	**9.58**		
CLIV	12.797^{ns}	37.736**	30.027**	**9.14**	
CLV	18.863^{ns}	29.077*	59.934**	23.082^{ns}	**9.58**

$\chi 2$ = 23.68 and 29.14 at 5% and 1% probability levels respectively, with 14 degrees of freedom

Principal component analysis

The result of the analysis showed that 15 principal components (PCs), which were equal to the total number of traits studied, were produced. However, only the first five PCs having eigenvalues greater than one were selected as suggested by Holland (2008). These five PCs altogether explained about 70.8% of the total variability among 49 tef genotypes (RILs along with parent and check varieties) using 15 pheno-morphic and yield related traits as depicted in Table 8.

Table 8: Eigenvectors, eigenvalues and variance explained by the first five principal components for 15 traits in study of tef genotypes

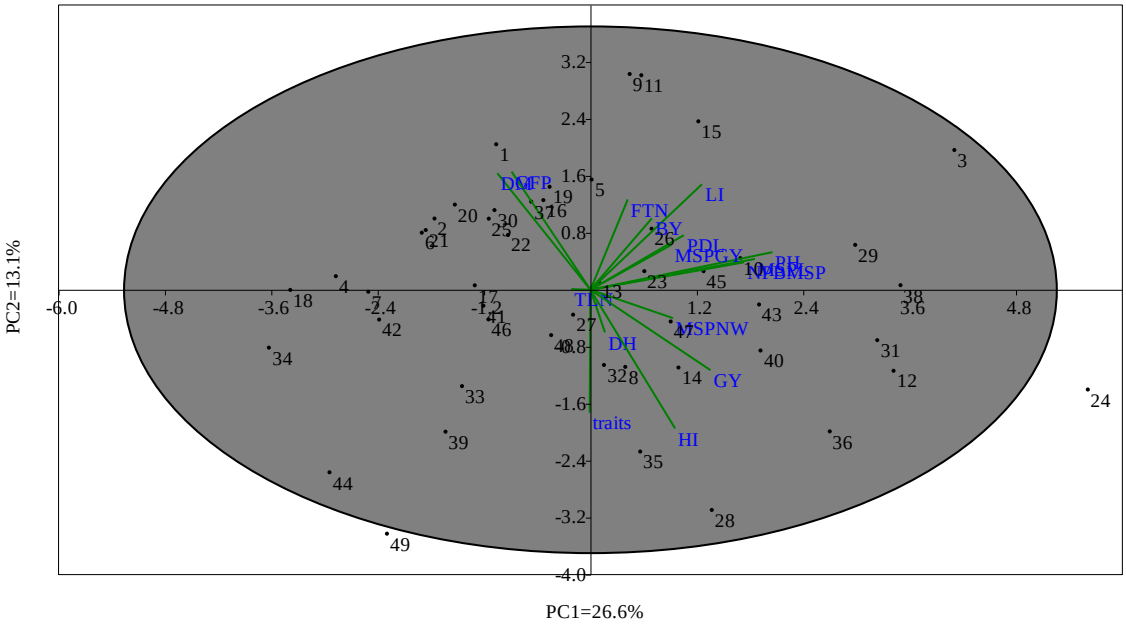
	Eigen vectors				
Traits	**PC_1**	**PC_2**	**PC_3**	**PC_4**	**PC_5**
Days to heading	0.036	-0.132	-0.624	0.19	-0.087
Days to physiological maturity	-0.233	0.362	-0.072	0.196	0.088
Grain-filling period	-0.197	0.367	0.502	-0.022	0.149
Plant height	0.454	0.117	-0.019	0.108	-0.057
Main shoot panicle length	0.411	0.096	-0.023	0.164	0.07
Peduncle length	0.233	0.17	0.149	0.074	-0.49
Total Tiller number	-0.048	0.002	0.177	0.492	0.032
Fertile tiller number	0.093	0.281	0.12	0.541	-0.138
Primary branches/main shoot panicle	0.383	0.086	-0.197	0.049	-0.1
Main shoot panicle weight	0.206	-0.088	0.028	0.013	0.537
Main shoot panicle grain yield	0.202	0.14	-0.105	-0.081	0.513
Lodging index	0.278	0.328	0.018	-0.081	0.212
Grain yield	0.3	-0.25	0.333	-0.202	-0.11
Above ground biomass yield	0.153	0.222	0.038	-0.457	-0.264
Harvest index	0.211	-0.431	0.312	0.105	0.08
Eigen values	4.248	2.088	1.816	1.706	1.433
Variance proportion explained (%)	26.60%	13.10%	11.40%	10.70%	9%
Cumulative variance explained (%)	26.60%	39.70%	51.10%	61.80%	70.80%

This result is closely related with Kebebew *et al.*, (2000) who explained 71% of the variation by five PCs using 17 quantitative traits in a study of 320 tef lines and 35 landraces. In the current study, the 70.8% of the variation among the RILs has close similarity to the findings of Thomas *et al.*, (2018) who extracted five PCs to explain 79.5% of the variability. Likewise, Fisseha (2017) has found a quite closer variability of 74.03% but with less number of PCs. On the other hand, only four PCs were reported to have explained about 81% of the gross variation by Habtamu et al. (2011) in a study of 37 tef landrace lines. Generally, the higher number of PCs extracted revealed the presence of appreciable variability among the RILs studied. In the study, the first principal component (PC1) explained about 26.6% of the total variation, while the second (PC2), third (PC3), fourth (PC4) and fifth (PC5) principal components in that order accounted for 13.1%, 11.4%, 10.7% and 9% of the total variability contained in the recombinant inbred lines of tef, but this is still lower than Fisseha (2017), whose first three PCs explained about 74% of the total variability. But in this study, the first three PCs together accounted for 51.1% of the total variation indicating that, much of the variability among the genotypes was originated from the traits included in these PCs. From the 15 traits studied, six of them showed positive and larger contribution to the first PC and these traits included plant height, main shoot panicle length, peduncle length, number of primary branches per main shoot panicle, lodging index and grain yield as evidenced by its highest value of loading or eigenvector (absolute value) (Table 8).

This is mainly due to the variation in those traits. Thomas et al. (2018) found substantial variation for plant height, panicle length and number of primary branches per main shoot panicle.

In PC2, 13.1% of the gross variability was contributed by days to physiological maturity, grain-filling period, fertile tiller number, lodging index and harvest index towards the positive direction (Table 8). This result is similar to Tsion *et al.*, (2018) with 13% variation in the second principal component; whereas slightly larger (16%) variability was reported by Thomas *et al.*, (2018). The third PC contributed 11.4% of the variability in which days to heading, grain-filling period, grain yield and harvest index were the most important traits. Similarly, PC4 with 10.7% of the total variability mainly contributed by high variation in total tiller number, fertile tiller number and above ground biomass yield. Finally, the fifth PC accounted for about 9% of the total variability which was contributed by peduncle length, main shoot panicle weight, main shoot panicle grain yield and above ground biomass yield, as illustrated in Table 8 and Fig. 2. Furthermore, the PCA biplot Figure was plotted using PC1 and PC2, which together accounted for most of the variation (39.7%) to show relationships and correspondence between the measured traits of the genotypes (Fig. 2). Accordingly, all the tef genotypes were categorized into four groups along with their respective important traits. Thus, group I contains tef genotypes RIL32, RIL280, RIL114A, RIL134, RIL176, RIL191, RIL52, RIL281B, RIL123, RIL122 and Beset, which had higher values of plant height, peduncle length, main shoot panicle length, fertile tiller number, number of primary branches per main shoot panicle, main shoot panicle grain yield and above ground biomass yield (Fig. 2). Since most of these traits are directly related to yield, selection of genotypes with in this group based on these traits would improve yield. The second group on the other hand, consisted of genotypes like RIL55, RIL281A, RIL259, RIL162, RIL163, RIL92, RIL178, RIL191, RIL143, Ebba and Magna (Dz-01-196), which had higher values of main shoot panicle weight, grain yield and harvest index (Fig. 2). Genotypes with in this group could be utilized for the improvement of those traits.

Fig. 2: Biplot showing the contribution of traits and distribution of the studied tef genotypes



The third group included genotypes such as RIL146, RIL303, RIL293, RIL213, RIL240, RIL105, RIL26, RIL242, RIL42, RIL108, RIL104, RIL285 and RIL128, which were found to have longer days to physiological maturity and grain-filling period. The fourth group, however, included genotypes RIL89, RIL152, RIL174, RIL170, RIL313, RIL127, Ho-TF1486, Ga-10-3 and local check which had relatively lower values of the studied traits except total tiller number (Fig. 2). This result is in line with Habte (2019). In this biplot, some groups of traits that exhibited acute angle between them have strong positive correlations so as to be improved simultaneously. Hence, traits such as plant height and number of primary branches per main shoot panicle with main shoot panicle length, lodging index with above ground biomass yield in group I, grain yield with main shoot panicle weight in group II and days to physiological maturity with grain-filling period in group III provided acute angle (Fig. 2). Hence in conclusion the significant variation among the tef genotypes for most of the traits shows the presence of genetic variability. Most of the lines are earlier maturing than variety Boset. Moreover, 53% and 24.5% of the genotypes had higher grain yield than the high yielder checks and these can be selected for yield improvement. The highly significant positive genotypic and phenotypic correlations of grain yield with plant height, above ground biomass yield and harvest index implies these traits positively affect grain yield and indirect selection of these traits would result in a substantial improvement in grain yield. Genotypic and phenotypic path coefficient analyses revealed harvest index and above ground biomass yield directly determined grain yield indicating genetic improvement of these traits would increase grain yield and selection for these traits is helpful to improve grain yield. Cluster analysis of the studied 49 tef genotypes provided five clusters. Largest inter-cluster distance found between clusters II and III; and between clusters III and V. Therefore, genotypes in these clusters may lead to developing potential varieties by selection. This increased divergence between clusters is helpful for selecting parents for hybridization. The principal component analysis revealed that five PCs explained 70.8% of the total variability based on 15 traits indicating that traits considered have explained substantial portion of the observed variability among the 49 tef genotypes and the potential for further improvement through directional selection and hybridization. To have more representative information, however, the study should be carried out on more locations and seasons.

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