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Estimation of Agro-Morphological and Yield Related Traits Variability in Tef [*Eragrostis tef* (Zucc.) Trotter] Genotypes

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Tef is an important crop to curb malnutrition and ensure food security in this era of climate change. 36 tef genotypes arranged in a triplicated randomized complete block design were evaluated for agro-morphological traits, variability and diversity of genotypes in the 2019 cropping season. The result of the analysis of variance showed highly significant (P≤0.01) differences for several characters studied, indicating the range of improvement through selection for high mean values of these traits. All of the traits were not found to be higher at the Genotypic Coefficient of Variance level, whereas a moderate genotypic coefficient of variation was recorded for panicle yield, biomass yield and grain yield indicating improvement is possible through simple selection of these traits. Ahigh heritability estimate was computed for days to physiological maturity, days to heading,

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lodging index, grain yield, biomass yield and harvest index. This result showed that advancement is possible through the selection of these traits. On the basis of hierarchical Euclidean cluster analysis, all 36 genotypes were grouped into four non-overlapping clusters. The inter-cluster distance varied from 50.76 between clusters II and III to 16.50 between clusters II and IV. Genetic diversity was observed among the materials studied, indicating that the possibility of better parental selection for future breeding programs.

Keywords: Tef; genetic variability; genetic advance; cluster analysis; Ethiopia.

1. INTRODUCTION

Tef [*Eragrostis tef* (Zucc.) Trotter] is an important crop to curb malnutrition and for food security in this era of climate change. It is a self-pollinated small annual cereal crop. However, physiologically, it is a C_4 plant, along with large cereals like sorghum and maize. It has a chromosome number of 2n = 4X = 40 [1]. Tef is endemic to Ethiopia and its major diversity is found only in that country, which deduces sufficient opportunities for genetic advancement through selection and intra-specific hybridization [2, 3].

In Ethiopia, tef is the main food crop where it is extensively cultivated with an annual coverage of more than three million hectares of land [4]. and yearly accounts for about 30 percent of the gross area and 20 percent of the total grain production of cereals grown in the country [5]. Compared to other cereal crops, tef is known for its plasticity to withstand extreme ecological and edaphic factors mainly water-logging. The crop harbors several useful traits for farmers and consumers and has the ability to yield on a wide range of soil types, from light to poorly drained Vertosols and variety of ecological conditions, from below sea level, to 3000 meters above sea level which most cereals cannot tolerate.

Currently, outside Ethiopia, there is a growing interest in using tef for commercial production due to its high nutritional content and gluten-free nature, hence it is considered a healthy food [6,7]. This result is in agreement with the report by [8] from the genome sequence initiative. Tef is known for its high iron content and plays an essential role in Ethiopia, as there is an absence of anemia related to pregnancy in areas of tef consumption [9]. The grain is also used to make local alcoholic drinks and in mixtures with soyabean, chickpea, and other grains in the baby food industry.

Some of the beneficial traits of the crop are that it is tolerant of extreme environmental conditions, that the seeds are not attacked by storage pests, and that it isgluten-free. Despite all these merits, scientific improvement on the crop has lagged far behind the level made for major cereals such as wheat and rice. Consequently, tef is considered an orphan crop, and its yield is the lowest compared to other major world cereals, which is 1.85 tons ha^{-1}. However, according to [10] its potential yield level is estimated at 6 ton/ha.

The availability of diverse genetic resources is a prerequisite for the genetic improvement of any crop including tef. Besides the availability of genetic resources, their characterization is essential for effective utilization in crop improvement programs. The success of the hybridization program depends to a large extent on the choice of suitable parents of diverse origin, with the possibility of obtaining a large frequency of transgressive segregants. Suitable statistical tools used such as SAS and D2 statistics, were used, to assess the relative contribution of different component traits to the total diversity. Knowledge of genetic diversity among genotypes on the basis of divergence analysis usually helps a breeder choose diverse parents for the breeding program. Hence, the purpose of this research was to look into the genetic diversity and variability in tef. Therefore, attempt has made in this study to investigate genetic variability and diversity in tef.

2. MATERIALS AND METHODS

2.1 Study Area Description

Field trial was carried out at Naeder Adet district of Axum Agricultural Research Center (AxARC) research site (Fig. 1). it is located at (38°38’ 19” E longitude and 13 °53’ 16” N latitude) in Central zone of Tigray Northern Ethiopia. Naeder is 1064 km and 300 km North of Addis Ababa and Mekelle, respectively at an altitude of 2122 meters above sea level. The site receives an annual average rainfall of 850.5mm during summer. The average annual temperature is

25°C with mean minimum and maximum temperature of 12.5 and 28.9°C, respectively. Specific soil type of the trial site is clay loam. The experimental materials were planted from mid July, 2019 to early October, 2019.

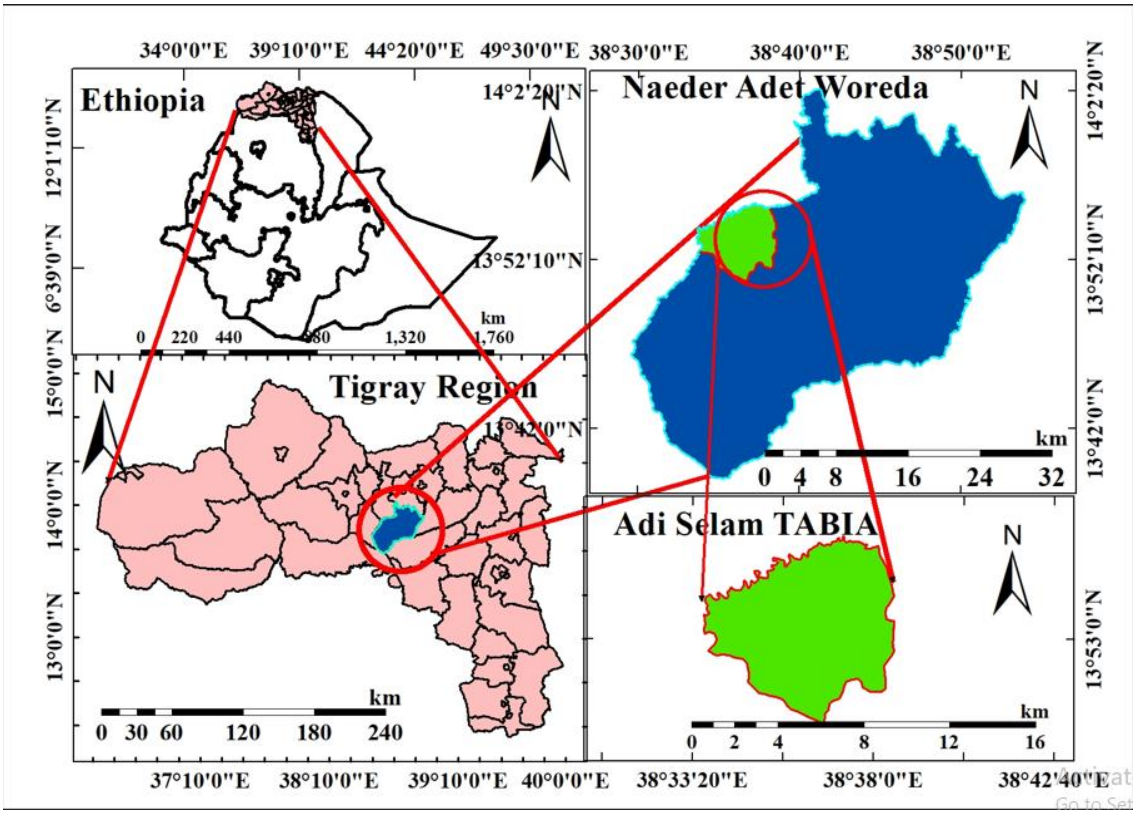

Fig. 1. Location Map of the study area

Table 1. List and description tef genotypes tasted in the study

Entry	Line code	Pedigree	Entry	line code	Pedigree
1	RIL10A	Quncho x Dukem	19	RIL11B	Quncho x Dukem
2	RIL5B	Quncho x Dukem	20	RIL13A	Quncho x Dukem
3	RIL8B	Quncho x Dukem	21	RIL3A	Quncho x Dukem
4	RIL44B	Quncho x Dukem	22	RIL65A	Quncho x Dukem
5	RIL124B	Quncho x Dukem	23	RIL68A	Quncho x Dukem
6	RIL113B	Quncho x Dukem	24	RIL17A	Quncho x Dukem
7	RIL28B	Quncho x Dukem	25	RIL48A	Quncho x Dukem
8	RIL19B	Quncho x Dukem	26	RIL19A	Quncho x Dukem
9	RIL17B	Quncho x Dukem	27	RIL124A	Quncho x Dukem
10	RIL45B	Quncho x Dukem	28	RIL70A	Quncho x Dukem
11	RIL11C	Quncho x Dukem	29	RIL110A	Quncho x Dukem
12	RIL46C	Quncho x Dukem	30	RIL121A	Quncho x Dukem
13	RIL74C	Quncho x Dukem	31	RIL63A	Quncho x Dukem
14	RIL3C	Quncho x Dukem	32	RIL16A	Quncho x Dukem
15	RIL11D	Quncho x Dukem	33	RIL44A	Quncho x Dukem
16	RIL11E	Quncho x Dukem	34	RIL50B	Quncho x Dukem
17	RIL75B	Quncho x Dukem	35	Standard check	Dagm
18	RIL57B	Quncho x Dukem	36	Local chick	Zagrev

Fig. 2. Photo of the plant materials in the field during the experimentation

2.2 Experimental Materials

Thirty-four seventh generation recombinant inbred lines (RIL) of tef were developed by independent inter-crossing between the parental lines Dz-cr-387xDz-01-974 for optimum areas were indiscriminately taken from hundreds of RILs from Debre Zeit Agricultural Research Center (DzARC). Standard check Dagm and local check Zagrev were included (Table 1).

2.3 Experimental Design and Procedure

The experiment was set up in a triplicated randomized complete block design. Genotypes were planted on a plot 2.5 m long and 1.2 m wide (3 m^2) that consisted of six rows spaced 20 cm apart with 1m and 1.5m spacing between plots and blocks, respectively. Sowing was done by manual drilling along the rows at a seed rate of 4.5 g per plot on the basis of a15 kg ha^{-1} seed rate. Blended and urea fertilizers were sources for P_2O_5 and N, respectively, applied at a rate of 100 kg ha^{-1} each. Urea was applied in splits, half at tillering and the remaining at early heading and the blended was applied at planting. Sowing was done in mid of July-2019, the main cropping season. Other agronomic management, protection practices and packages needed were applied based on national recommendations for crops.

2.4 Data Collection

Data for quantitative traits such as number of fertile tillers per plant, panicle length (cm), yield per panicle (g), panicle weight (g), and thousand-seed weight (g) were collected on plant bases; ten individual plants were randomly selected from the four middle rows per plot, marked before panicle emergence, and their mean values were used as sample data. On the other hand, data for days to 50% heading, days to 90% physiological maturity, harvest index (%), total dried biomass kg ha^{-1}, grain yield kg ha^{-1}, and lodging index (%) were assessed on a plot basis and subjected for data analysis [11].

2.5 Data Analysis

The collected data were subjected to analysis using SAS Computer Statistical Package version 9.1.3 [12] (SAS Institute Inc., 2004). Variance components were derived from a randomized complete block design (RCBD) analysis of variance [13]. Descriptive parameters such as mean, standard error of means, range and mean squares were computed. In addition, genotypic and phenotypic variances, the and coefficient of variability, heritability and expected genetic advance were estimated as formulated by [14].

$$\text{Genotypic variance } (\sigma^2_g) = \frac{MSg - MSe}{r}$$

Environmental variance (σ^2e) = MSe
Phenotypic variance (σ^2ph) = $\sigma^2g + \sigma^2e$

Where, σ^2 g = variance of genotype MSg = genotypic mean square MSe = error mean square r = number of replications σ^2 e = l variance of Environment and σ^2 p = variance of phenotype.

Genotypic Coefficient of Variation (GCV) =

$$\left(\frac{\sigma \mathbf{g}}{\mathbf{grand\ mean}}\right) \times 100$$

Phenotypic Coefficient of Variation (PCV) =

$$\left(\frac{\sigma \mathbf{ph}}{\mathbf{grand\ mean}}\right) \times 100$$

Heritability in broad sense (h^2_b) = (σ^2_g/σ^2_{ph}) x 100

As presented by [15] heritability estimates were classified (0-30%) as low, (30- 60%) moderate and (60% and above) high. Genetic advance under selection (GA) was also calculated as suggested [16].

$$EGA = k * \sigma_{ph} * h^2_b$$

Genetic advance per population means =

$$\left(\frac{\mathbf{EGA}}{\mathbf{grand\ mean}}\right) \times 100$$

Where; k = selection differential (with a value of 2.06 at 5% selection intensity), SDp = phenotypic standard deviation, H^2 = heritability in broad sense, x = Grand mean. Genetic advance as a percentage mean was categorized as low (0-10%), moderate (10- 20% and high (≥20%) as suggested by [16].

In addition to this, using statistical software program based on the square distance (D^2) cluster analysis was estimated based on Ward method as adopted by [17].

3. RESULTS AND DISCUSSION

3.1 Genetic Variability

The analysis of variance result showed a highly significant difference (P≤ 0.01) among genotypes for days to heading, days to physiological maturity, plant height, number of tillers, panicle weight and biomass yield, indicating considerable variability among them (Table 2). Working on these genotypes could capitalize on better genetic variability in the future breeding program. The value of the phenotypic coefficient of variation component differs from the genotypic coefficient of variation component, indicating that the existent variability was due to the interaction of the genotypes' inherent character and the influence of environmental factors. Thus, selection based on phenotypic characteristics could be a good indication of genotypic potential. This is in line with the results reported by [18, 19].

As the result in Table 3 revealed that Genotypic Coefficient of Variation (GCV) varied from 2.7 to 12.7 for plant height and biomass yield, respectively. Whereas moderate GCV was recorded for panicle yield (11.7), biomass yield (12.7) and grain yield (10.4), indicating improvement could be possible through the selection of these traits. Whereas days to heading (4.2), days to 90% physiological maturity (5.3), plant height (2.7), panicle length (5), number of tillers (8.6), panicle weight (8.8), thousand seed weight (7), harvest index (9.5) and lodging index (3.7) showed lower GCV values. [20] also recorded low GCV for plant height, days to heading, days to maturity, thousand seed weight and lodging index.

The PCV ranged from 4.2 (lodging index) to 29.9 (panicle weight) (Table 3). A higher PCV was recorded for panicle yield (20.4) and panicle weight (29.9) showing the influence of the environment on these traits. Whereas moderate PCV was estimated for plant height (10.5), number of tillers (18.4), thousand seed weight (14.1), biomass yield (14) and grain yield (10.4).

Broad sense heritability values based on the analyses of variance ranged from 29.4% for panicle weight to 95.4% for days to physiological maturity as indicated in Table 3. High heritability values were estimated for days to heading (87.5%), days to 90% physiological maturity (96.4%), biomass yield (91.4%), grain yield (96.4%), harvest index (84.1%), and lodging index (88.1%). [18] also reported high heritability for days to heading (96.98%) and days to physiological maturity (85.59%) which is in line with this study. However moderate heritability was observed for panicle length (47.6%), number of tillers (46.7%), thousand seed weight (49.6), panicle yield (57.4%) and plant height

(46.6%). While lower value was observed for panicle weight (29.4%) signifying selection could be considerably difficult for this trait.

In the current study, a high genetic advance as a percent of the mean was observed for panicle yield (23.5%), number of tillers (53.3%), biomass yield (26.2%) and grain yield (21.4%). According to [16] the estimate of high genetic advance as percentage of mean accompanied by high heritability estimates is more pivotal as a selection tool than heritability alone. Thus, a high heritability together with a high genetic advance as a percentage of the mean for both grain yield and biomass yield suggested the grandness of additive genes for the advancement of these traits, and this could make selection more fruitful. This research is consistent with the findings that [18] obtained.

3.2 Clustering of Genotypes

According to the Euclidean dissimilarity distance, the genotypes were divided in to into four distinct clusters. Cluster-I and II each consisted of five genotypes and cluster-IV comprised of four genotypes, while cluster-III was the largest cluster with twenty-two genotypes (Fig. 3). This study is in concurrent with the result reported by [20] who classified 14 tef populations in to 4 distinct clusters, whereas [21] classified 64 genotypes in to 3 clusters.

3.3 Cluster Mean Analysis

The highest cluster mean value of days to physiological maturity, biomass yield, grain yield and panicle length were noted for cluster-I (Table 4) implying selection for high yielding genotypes could be made more effectively in this cluster. The lowest cluster mean value for lodging index was estimated in cluster-III, which could be due to low biomass yield and relatively lower grain yield. implied that selection of genotypes in cluster-III could be used when a decrease in logging index is desired, which is the most important bottleneck in tef breeding. Minimum days to heading and physiological maturity were observed in cluster-II indicating that selection of genotypes for early maturity could be made more successful in cluster-II, which would be used for environments with erratic rain fall distribution. Whereas maximum plant height, panicle length and panicle yield were computed for cluster-IV.

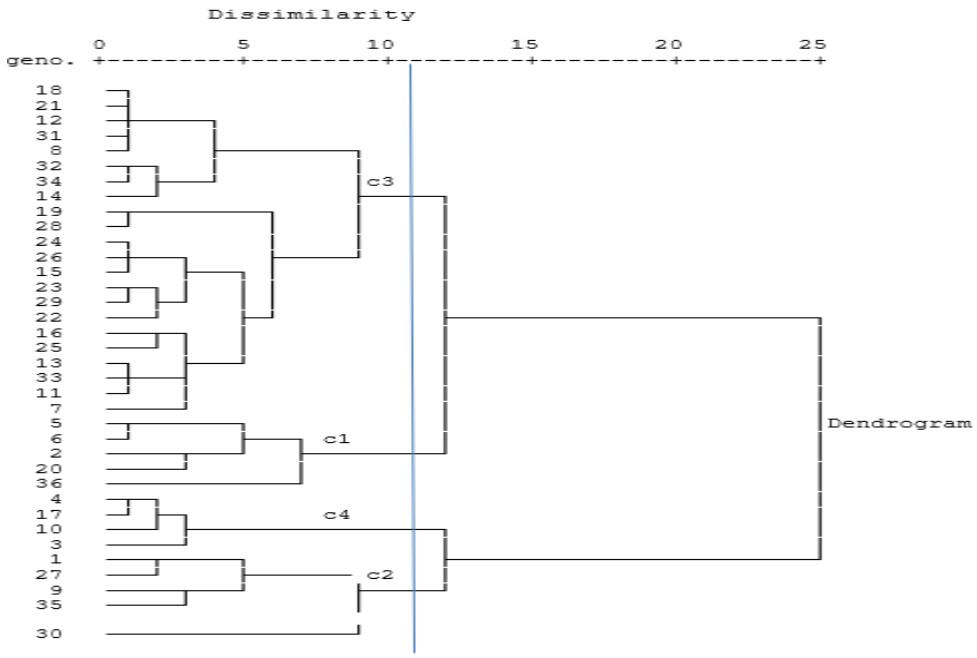

Fig. 3. Tree diagram of 36 tef genotypes for twelve traits studied

Where: 1=RIL10A, 2 =RIL5B, 3=RIL8B, 4=RIL44B, 5=RIL124B, 6=RIL113B, 7=RIL28B, 8=RIL19B, 9=RIL17B, 10=RIL45B, 11=RIL11C, 12=RIL46C, 13=RIL74C, 14=RIL3C, 15=RIL11D, 16=RIL11E, 17=RIL75B, 18=RIL57B, 9=RIL11B, 20=RIL13A, 21=RIL3A, 22=RIL65A, 23=RIL68A, 24=RIL17A, 25=RIL48A, 26=RIL19A, 27=RIL124A, 28=RIL70A, 29=RIL110A, 30=RIL121A, 31=RIL63A, 32=RIL16A, 33=RIL44A, 34=RIL50B, 35=Dagm , 36=Local and Geno.= genotypes

Table 2. Mean squares from Analysis of Variance (ANOVA) for different traits of tef genotypes

	MSr (df=2)	MSg (df=35)	MSe (df=70)	CV	R^2
Days to heading	11.8**	21**	2.15	2.4	84
Days to physiological mature	1.36**	105.9**	2.7	1.4	95
Plant height	229.45**	61.4**	33.2	5.2	53
panicle length	13.8ns	34.7**	17	9.2	51
Number of tillers	0.78**	0.16**	0.087	16.4	54
Panicle weight	0.06*	0.65**	0.02	8.8	64
Panicle yield	0.002ns	0.04**	0.018	16	53
Thousand seed weight	0.006ns	0.005ns	0.004	15	38
Biomass yield	728543.8*	4753549.6**	302744.6	5.7	88
Grain yield	4997.79ns	336679.6**	11248.51	3	94
Harvest index	0.0005ns	0.003**	0.0004	6	79
Lodging index	6ns	33.39**	2.9	2	85

** Significant at p ≤ 0.05 probability level, ** significant p ≤ 0.01 probability level, ns = non-significant, df = degrees of freedom, MSr = mean square of replications, MSg = mean square of genotypes, MSe = mean square of error, R^2 = coefficient of determination and CV = coefficient of variation*

Table 3. Estimated values of variance components, phenotypic and genotypic coefficients variance, broad sense heritability and expected genetic advance for 12 traits of 36 tef genotypes based on analysis of variance

Trait	Range	Mean ±SE	$\sigma^2 g$	$\sigma^2 p$	GCV (%)	PCV (%)	H^2 (%)	GA	GAM (%)
DH	54-66.7	60.35±1.5	6.28	8.43	4.2	4.8	87.5	5.2	8.6
DPM	98.7-121.7	110.8±1.7	34.4	37.1	5.3	5.5	96.4	12.1	10.9
PH	102.9-122	111.3±5.8	9.4	42.6	2.7	5.8	46.6	6.3	5.7
PL	41.4-60.8	44.8±4.1	5.9	22.9	5	10.5	47.6	4.7	10.5
NT	1.5-2.4	1.8±0.3	0.024	0.11	8.6	18.4	46.7	0.96	53.3
PW	1.3-1.9	1.6±0.14	0.21	0.23	8.8	29.9	29.4	0.29	18.1
PY	0.6-1.2	0.85±0.14	0.01	0.03	11.7	20.4	57.4	0.2	23.5
TSW	0.36-0.54	0.45±0.07	0.001	0.004	7	14.1	49.6	0.06	13.3
BY	7972.3-13130.8	9603.6±550.2	1483601.7	1786346.3	12.7	14	91.4	2516.5	26.2
GY	2511.7-3798	3178.2±106.1	108477.1	119725.5	10.4	10.9	95.4	680	21.4
HI	0.24-0.43	0.33±0.02	0.001	0.0014	9.5	11.3	84.1	0.06	18.2
LI	81.33-96.7	86.75±1.7	10.2	13.1	3.7	4.2	88.1	6.5	7.5

DH = Days to heading, DM = days to 90% physiological maturity, PH = plant height (cm), PL = panicle length (cm), NT = number of productive tillers per plant, PW = panicle weight per plant per plant (g),PY = yield panicle-1(g) , TSW = thousand seed weight (g), BY = biomass yield(kg ha-1), GY = grain yield (kg ha-1), HI = harvest index (%), LI = lodging index (%), $\sigma^2 g$=genotypic variance, $\sigma^2 p$=phenotypic variance PCV=phenotypic coeficient of varience, GCV=genotypic coeficent of varience, H^2=broad sense heritability, GA=Genetic advance, GAM=genetic advance as as percent of mean and SE=Standard error of mean

Table 4. Estimates of mean values of four clusters for twelve traits of the 36 tef genotypes studied 2019

Trait	Cluster-I	Cluster-II	Cluster-III	Cluster-IV
Days to heading	59.668	59	61	59.1
Days to physiological mature	118.3	105.9	109	117.9
Plant height	113.04	110	109.5	120
panicle length	47.786	43.6	43.9	47.6
Number of tillers	1.754	2.13	1.71	1.96
Panicle weight	1.48	1.65	1.54	1.83
Panicle yield	0.75	0.89	0.83	1.03
Thousand seed weight	0.42	0.45	0.45	0.44
Biomass yield	11456.04	9682.8	8965.9	10696
Grain yield	3442.068	2995.5	3086.2	3582.4
Harvest index	0.34	0.31	0.34	0.34
Lodging index	87.3	90.8	85.7	86.6

Table 5. Generalized Squared Distance between clusters based on twelve variables of 36 genotypes of Tef

cluster	II	III	IV
I	42.50**	50.26**	36.12**
II		50.76**	16.50ns
III			32.22**
V			

*ns= non-significant, * and **, significant ($\chi 2$= 19.68) and highly significant ($\chi 2$= 24.72) at 5 and 1% probability levels, respectively*

3.4 Distance Analysis

The generalized pair-wise intra- and inter-square distance (D^2) among the four clusters (Table 5) revealed a highly significant ($P<0.01$) difference for all cluster distances except between cluster II and IV. Distance analysis is usually performed to identify the diverse genotypes for hybridization purposes. The maximum inter-cluster distance was observed between Cluster-II and Cluster-III (50.76), followed by between Cluster-I and III (50.26) and cluster-I and cluster-II (42.50). The large inter-cluster distance between members of any two clusters indicates that genotypes falling in to such clusters would be more genetically divergent. Therefore, the crosses between genotypes selected from those clusters, may produce transgressive segregants [22].A low-inter cluster distance was recorded between cluster-II and cluster-IV (16.50), indicating that the genotypes of these two clusters were relatively less genetically divergent. Therefore, crossing genotypes from these two clusters may not result in offspring with a greater amount of heterotic effect.

4. CONCLUSION

Though tef is the most important indigenous cereal crop with various usesis to curb malnutrition and for food security in this era of climate change, its productivity is still far below its expected genetic potential and as compared to other major cereal crops grown in Ethiopia. Evaluation of genetic, phenological and agro-morphological variation of recombinant inbred lines of tef is very crucial towards the development of new tef varieties with traits of interest. In the present investigation, agro-morphological trait diversity in tef revealed the existence of wide range of trait variations for yield and yield related traits, phenological traits and morphological traits. Such variation could, therefore, be used in future tef breeding program to develop varieties useful to combat the effect of climate change and to increase tef productivity.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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