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Characterization of Tef {*Eragrostis tef* (Zucc.) Trotter} Genetic Resources Based on Quantitative and Qualitative Traits Under Field Conditions, Northern Ethiopia

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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

Ethiopia possesses extensive genetic diversity in tef, a vital staple crop, yet current breeding efforts have not fully capitalized on this diversity. The aim of the study was to generate information for enhancing Tef germplasm and pre-breeding materials using over 500 Tef accessions, focusing on 12 quantitative and 3 qualitative traits. The trial was conducted at Laleay Machiew disctric, Hastebo sub district in two years 2018/19 and 2019/2020 cropping season by single observation plot. Phenotypic evaluation encompassed days to heading (ranging from 38 to 82 days), days to maturity (70 to 115 days), panicle length (20.80 to 53.66 cm), plant height (62.49 to 127.40 cm), peduncle

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length (10.2 to 28 cm), number of florets per spikelet (3.1 to 28.64), and grain yield (275 to 6491 kg/ha). Additionally, variations in seed and lemma color were documented, with 77% of accessions showing brown and white seeds and 83.28% exhibiting yellow, gray, and variegated lemma colors. The yield variation is the most selected one from this study. Cluster analysis identified five distinct groups among tef accessions, highlighting differences in agronomic performance and adaptation traits. Understanding intra-cluster distances facilitated targeted trait selection and hybridization. The study revealed significant diversity in both quantitative and qualitative traits among tef accessions. To enhance tef accession characterization further, future studies should aim to include all descriptors and utilize genetic markers across various agro-ecological zones.

Keywords: Tef; clustering of tef; tef accession; tef breeding tef traits; and diversity.

1. INTRODUCTION

Tef is the most important cereal crop in Ethiopia, serving as a primary source of food, feed, and income. Its value as a cash crop is significant in the recent years Chanyalew et al., [1], with current prices more than $124,00 USA Ethiopian Birr per quintal, triple than maize and wheat cereal crops in Tiger [2]. Tef is well-adapted to a variety of climatic conditions and soil types [3], particularly in regions with unpredictable and unreliable rainfall [4]. The crop is known for its resilience to drought and waterlogging, making it a low-risk alternative to long-maturing crops such as maize and sorghum. Appart from the above-mentioned attributes the crop is less prone to threats from pests or diseases [5].

Tef cultivation covers 24.11% of Ethiopia's total grain crop area, contributing to 81.46% of the country's grain production, with an average yield of 19 quintals per hectare and a total production of 57,357,101.87 quintals [6]. In the Tigray region, Tef is grown on 188,391.88 hectares, producing 3,117,538.77 quintals with an average yield of 16.55 quintals per hectare. Research station studies have shown potential yields ranging from 20 to 28 quintals per hectare, with productivity averaging 23 quintals per hectare. However, the crop's potential yield can reach 45 to 73 quintals per hectare (Seyfu, 1993; Yifru, [7]; Habte et al., [8].

Characterizing and evaluating Tef germplasm is crucial for enhancing its genetic base and developing new varieties [5]. This involves assessing genetic diversity for different traits to efficiently utilize germplasm [9]. The primary descriptors for Tef include 23 quantitative and 5 qualitative traits, which are detailed in the Tef breeding manual [1]. Previous studies have highlighted the variability in Tef germplasm, essential for improving yield and resilience [10-16], Yifru, 1989; Habte et al., [8].

Tef originated and diversified in Ethiopia Vavilov, [17], through germplasm collected from various sources including farmers, research centers, and biodiversity institutions. Currently, 6797 Tef germplasm accessions are stored in gene banks worldwide, with 6000 accessions held by the Ethiopian Biodiversity Institute (EBI) and 797 in other institutions (Chanyalew et al., 2022). This extensive collection supports the development of improved varieties and hybridization programs. The first step in Tef breeding is characterizing the variability of accessions to identify morphological, physiological, and pathological traits (Poehlman and Sleper, 1995). Identified accessions are then used for hybridization and other breeding methods like mutation [18].

Germplasm characterization aims to distinguish accessions, detect genetic diversity, and identify desirable agronomic traits. This process involves morphological characterization, phenotypic evaluation, and agronomic performance assessment. Despite the genetic improvement of Tef, yield remains affected by factors such as seed shattering, lodging susceptibility, poor agronomic practices, low-yielding cultivars, drought stress, Tef shoot fly, rust, and soil compaction [19]. In Tigray, yield is particularly impacted by drought, Tef shoot fly, and poor agronomic practices. Although genetic diversity in Tef is substantial, the breeding program has not yet fully exploited this potential [20].

Dissimilarity among individuals in a population is inevitable, and understanding the origin and extent of this variability is crucial for the success of crop improvement programs [21,22]. Genetic distance measures the average number of changes per site that have occurred since two sequences diverged from their common ancestor. The Euclidean distance method, developed by Sneath and Sokal [23], is commonly used to classify divergent genotypes into distinct groups. For instance, Habte et al. [24] clustered Tef genotypes into seven groups. However, the diversity of Tef genotypes is too extensive to be reduced to just a few groups (Mengesha et al., 1965). Genetic improvement through hybridization and selection relies on the genetic diversity between parents. Successful crossing for desirable traits can be achieved by selecting parents from clusters with the greatest and least genetic divergence [25].

Genetic improvement through hybridization and selection relies on understanding genetic diversity. Clustering Tef genotypes based on their genetic distance allows for the selection of parents with desirable traits [21,22]. This research aims to generate information for enhancing Tef germplasm and pre-breeding materials using over 500 Tef accessions, focusing on 12 quantitative and three qualitative traits.

To address these challenges, this study aims to evaluate and characterize Tef accessions in Axum, Tigray, focusing on identifying traits such as drought tolerance, lodging resistance and higher yield. Understanding the variability of these traits under different growing conditions can significantly enhance the development of improved Tef varieties. The study employs phenotypic evaluation, morphological characterization, and agronomic performance assessments to generate comprehensive breeding information and identify desirable traits for immediate use.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The field trial was conducted at the Axum Agricultural Research Center, specifically at the Hatsebo sub-station, which is situated at an altitude of 2100 meters above sea level. The coordinates for this location are 14°6′N latitude and 38°48′E longitude. The site experiences an annual rainfall of approximately 700 mm and has a temperature range of 12.2°C to 26.8°C. The soil type in this area is predominantly Vertisol. This region often faces soil moisture stress during planting, which can cause the Tef seedlings to dry out. However, this issue is mitigated through soil compaction techniques.The rainfall is distributed from end of June to September.

2.2 Planting Material Selection and Crop Management

In 2018, 312 Tef accessions were obtained from the Ethiopian Biodiversity Institute (EBI), along with 45 landraces from the Tigray region. These were planted at the Axum Agricultural Research Center in 2019 and 2020. During the first year, the accessions were purified, resulting in single-panicle plants. By the second year, the number of heads per plant increased to 467, and the 45 Tef landraces had already been purified. These purified single heads were then used for characterization.

Recommended agronomic practices for Tef were followed, including the application of 100 kg/ha of both Urea and Blended fertilizers. Blended fertilizer(NPSB) was applied once at planting, while Urea was applied in two doses: half at planting and the remaining half four weeks later. In the first year, seeds were planted based on availability, while in the second year, a specific number of seeds were used per panicle. Seeds were drilled in two rows with a spacing of 0.20 meters between rows and 0.75 centimeters between accessions.

2.3 Data Collections

Observations were made on 15 different phenological, morphological, and agronomic traits. These included days to heading, days to maturity, plant height, panicle length, peduncle length, number of tillers, number of internodes, number of primary panicle branches, number of florets per spikelet, number of spikelets per primary branch, panicle form, lemma color, seed color, biomass (kg/ha), and grain yield (kg/ha). Since molecular breeding in Tef has not yet been implemented, these traits are crucial for variety development Tef.

- **Days to Heading and Maturity**: Recorded as the number of days from planting to 50% heading and 75% physiological maturity, respectively.
- **Plant Height and Panicle Length**: Plant height was measured from the ground to the tip of the plant, and panicle length from the base to the tip of the panicle. Peduncle length was measured as the length of the topmost culm internode. Five sample plants were taken, and the average was used for characterization.

- **Number of Tillers and Internodes**: The number of fertile tillers per plant and the number of internodes were counted from five sample plants and averaged.
- **Primary Panicle Branches and Spikelets**: The number of primary branches on the main panicle and the number of spikelets per primary branch were counted from five sample plants.
- **Florets per Spikelet**: Counted from five sample spikelets and averaged.

Qualitative traits were assessed visually:

- **Lemma Color**: Classified at physiological maturity as yellow, variegated, red, purple, gray, or pale.
- **Panicle Form**: Recorded at physiological maturity as very loose, loose, fairly loose, semi-compact, or compact and Gorado.
- **Seed Color**: Classified after threshing as brown, white, pale white, deep brown, yellow-white, or pale white.

Biomass and grain yield were weighed for the entire plot and converted to kilograms per hectare. For both experiments, the techniques used for characterization and evaluation included physiological traits, morphological traits, and agronomic performance. Phenological data were measured in days, agronomic traits in centimeters and numbers, and qualitative traits were expressed by count percentage for the Tef germplasm. Measurements for phenological and agronomic traits involved days, centimeters, and numbers, as well as kilogram per hectare. The qualitative traits were expressed as a count percent for the tef genotypes.

2.4 Data Analysis

Descriptive statistical measures were obtained for each trait based on data from 467 accessions and 45 landraces. Variance analysis was performed on the data for each trait. Additionally, the performance of 382 Tef accessions for 10 phenological and agronomic traits was used for cluster analysis (CA). CA used the Average Linkage-Between Groups-Squared Euclidean Distance method. The number of Tef accessions was reduced from 467 to 382 for clustering due to incomplete data of the rest. The cluster analysis of inter distance matrix was analyzed by SAS statistical package[26].

2.5 Cluster Analysis of Tef Genotypes

A cluster analysis was performed to group the tested Tef genotypes into genetically distinct classes using SAS Statistical Software Version 9.3. The analysis followed the average linkage cluster analysis method. The number of clusters was determined based on the Pseudo-F and Pseudo-t2 criteria provided by the SAS clustering procedure. Genetic distances between clusters, standardized using Mahalanobis's D2 statistics, were calculated with the formula [27].

$$D2ij = (xi - xj)' \text{cov}-1(xi - xj)$$

D^2ij=the distance between cases I and j; xi and xj=vectors of the values of the variables for cases I and j; and to identify its significance level of genetic diversity between and within cluster was used chi-square table based on the degree of freedom of traits at significance level of 0.05 or 0.01. These were compared against the tabulated $\chi 2$ values for 'P' degrees of freedom, where 'P' represents the number of traits considered [28].

3. RESULTS AND DISCUSSION

3.1 Quantitative Traits

3.1.1 Phenology of tef

The descriptive statistics for all measured traits of Tef accessions were summarized, focusing on the mean, confidence interval, standard error, and the range of minimum and maximum values (Table 1). The days to heading of the Tef accessions ranged, with a 95% confidence interval (CI), from 54 to 55 days (average mean of 54.61 days) and a standard error of 0.291. The individual observations ranged from 38 to 76 days.

The variability in days to heading was significant, with more than five weeks' difference among Tef accessions. This variability is crucial for areas with late drought and uneven rainfall distribution, as early heading can be an escape mechanism, leading to early maturity and helping cope with moisture deficits. However, both extremely early and late heading can negatively impact Tef yield. Early heading can cause grain filling during the main rainy season, leading to lodging and rot, damaging both grain and straw, while late heading can expose the crop to late drought. Among the 467 Tef accessions, 5% were categorized as early and intermediate (less than 44 days), and 95% were classified as late heading (more than 44 days) (Table 3).

According to Kefyalew et al. [16], days to heading can be classified into early (less than 39 days), intermediate (39 to 44 days), and late (more than 44 days). This study aligns with previous research by Ebba [10], Ayele et al. [11], Assefa et al. [12], [29], Zeid et al. [13] and Habte et al. [8], Ayele et al. [11] reported a minimum of 18 days to heading, while Assefa et al. (2011) reported a maximum of 81 days. Thus, the variability in days to heading is substantial and significant for breeding objectives.

The mean physiological maturity was 95.32 days, with a range from 70 to 115 days. The standard error for days to maturity was low (0.333) due to the high number of observations, resulting in a narrow confidence interval. However, individual observations showed a wide range of 70 to 115 days, indicating significant variability. This six-week difference is essential for selecting parent plants for hybridization. While the average days to maturity pose a challenge in regions like Tigray, which face late droughts, the variability allows for the selection of suitable varieties. Kefyalew et al. reported maturity classes as early (less than 80 days), intermediate (80 to 95 days), and late (more than 95 days). The findings show 3.67% early, 43.52% intermediate, and 52.81% late-maturing types (Table 3). The near fifty percent of intermediate and late maturity types offer a good selection for variety and hybridization.

Other studies on Tef phenology have reported similar variability. Melak Hail et al. [15], Ebba [10], Yifru (1989), Kbebew et al. (1999), Ayele et al. [11], Assefa et al. [9], Zeid et al. [12] and Habte et al. [8]. reported a minimum of 60 days to heading Ebba, [10], Assefa et al., 2011 and a maximum of 140 days to maturity [10]. This difference might be due to genotype by environment interaction, suggesting that characterizing Tef accessions in various locations and seasons can provide more accurate results for developing Tef cultivars.

The wide range of days to heading and maturity indicates high variability among Tef accessions/germplasms. Early heading accessions are crucial for moisture-stressed areas, as they help escape drought and lead to early maturity, providing optimal yield. Although early heading is beneficial for variety development, intermediate days to heading and maturity are preferable. The test location experienced interrupted rainfall, so early heading and maturity could lead to grain yield loss and reduced straw quality.

3.2 Agronomic Performance of Tef Traits

3.2.1 Plant height

The plant height of the Tef germplasm, as presented in Table 1, ranged from 62.40 cm to 127.4 cm, with an average of 94.21 cm. One of the major challenges in Tef production is lodging, which is often due to weak stems and relatively tall plants compared to semi-dwarf varieties. This wide range of plant heights provides an advantage for developing Tef varieties with lodging tolerance. Additionally, taller plants might be beneficial in producing varieties with higher biomass, leading to more carbon assimilation and grain yield in optimal growing conditions with adequate inputs. However, while there is significant variability in plant height, measuring stem thickness, number and length of nodes, and internode length is crucial for addressing lodging problems effectively. This study's results fall within the range reported by previous works, with a minimum of 20 cm and a maximum of 156 cm (Assefa et al., 2011), while other studies have reported similar findings Ebba, [10] Kebebew et al., 2001; Habte et al., [8]. Plant height is a combination of panicle length, peduncle length, and internode length; thus, future breeding programs should consider their correlation.

3.2.2 Panicle length

The panicle length of the Tef accessions ranged from 20.8 cm to 53.6 cm, with an average of 37 cm. Longer panicles, combined with a higher number of primary panicle branches, spikelets per branch, and florets per spikelet, can increase the number of seeds per plant, thereby enhancing Tef productivity per unit area. This result is consistent with previous reports (Seyfu, 1993a; Assefa et al., 1999 and [29], Solomon et al., [4], Habte et al., [8]. The variability in panicle length aids in selecting long panicle lengths for yield improvement in Tef farming.

3.2.3 Peduncle length

Peduncle length varied from 10.2 cm to 28 cm, with an average of 18.24 cm. Shorter peduncles might help strengthen the panicle, correlating with shorter and thicker internodes, while longer and weaker peduncles can cause the plant to bend and lodge. Previous studies reported peduncle lengths ranging from 5.85 cm to 42.30 cm Fufa et al., 1999; Solomon et al., 2019; Assefa et al., [29].

Table 1. Mean confidence interval at 95% of the mean, standard error, minimum and maximum of the descriptors for phenological traits and agronomic performance of from 45 and 476-tef populations planted at 2020

Parameters	Number of observation	Mean	Confidence interval at 95%	SE±	Minimum	Maximum
Days to heading(days)	467	54.57	54.04-55.18	0.291	38	76
Days to maturity(days)	462	95.32	94.67-95.98	0333	70	115
Plant height(cm)	465	94.205	93.21-95.19	0.504	62.4	127.4
Panicle length(cm)	465	37.022	36.55-37.48	0.237	20.8	53.6
Peduncle length(cm)	465	18.249	18.02-18.47	0.115	10.2	28
Primary panicle branch(PPB) (number)	383	20.06	19.67-20.45	0.201	8	39
#of spiklites/PPB(number)	382	28.904	28.02-29.78	0.45	8.72	60.2
#florets/#spk	382	6	5.8-6.2	0.101	3.08	28.64
Number of tillers per plant	45	6.57	6.14-7	0.431	4.2	12
Number of internode	45	4.95	4.87-5.03	0.08	4.4	5.6
Biomass(kg/ha)	427	12752.41	12357.22-13147.59	201.62	2500	75000
Grain yield(kg/ha)	439	3369.365	3280.94-3457.78	45.11	275	6491.67

Table 2. Qualitative traits of 476 tef accession and their counts

panicle form	Count	% count	lemma color	Count	%counts	Seed color	Count	%
Fairly loose	33	7.06	Purple	22	4.71	Brown	208	50.12
Gorado	8	1.71	Gray	94	20.12	Deep brown	28	6.746
Loose	283	60.55	Pale	43	9.20	Pale white	28	6.746
Semi compact	8	1.71	Red	11	2.35	White	113	27.228
Very loose	124	26.55	Yellow	141	30.19	Yellow white	10	2.41
Compact	11	2.35	V(r+y)	65	13.91	Pale brown	28	6.746
		100%	V(gr+y)	1	0.21			100%
			V(pale+y)	55	11.77			
			V(purple+y)	35	7.49			
Total	**467**			**467**	**100%**		**467**	

Variegated (yellow plus red), V(pale plus yellow), V(Purple plus yellow) and V (purple plus yellow)

3.3 Number of Internodes and Tillers

The number of internodes ranged from 4.4 to 5.6, with an average of 4.95, while the number of tillers per plant ranged from 4.2 to 12, with an average of 6.57 (Table 1). The number of tillers can have both positive and negative effects under irregular rainfall conditions. With optimal rainfall and distribution, increased tillering can enhance yield. However, in areas with uneven rainfall, increased tillering can divert resources from grain production to vegetative growth, reducing yield. Hence, the number of tillers per plant should be selected based on the rainfall conditions to maximize yield. This finding aligns with reports by Seyfu (1993) and Assefa et al. [29].

The number of florets per spikelet in the accession ranged from 3.1 to 28.64, with an average of 6 florets. Increasing the number of florets can enhance the productivity of tef due to the increased number of seeds per panicle/plant and per unit area. This finding shows a wider range than reported by Assefa et al. [9,29,12], and Demissie [30], who measured a range of 4 to 13 fertile florets per spikelet.

The characterized material exhibited significant diversity in biomass, with a range of 2,500 to 75,000 kg/ha and an average of 12,572.41 kg/ha. However, there was a relatively low range of grain yield compared to the biomass. The grain yield varied from 275 kg/ha to 6,491.67 kg/ha, with an average of 3,369.36 kg/ha. The 95% confidence interval for yield was recorded as 3,280.94 to 3,457.78 kg/ha. The study found that physiological, phenological, and agronomic performance ultimately influenced grain yield, revealing a general diversity in these traits. Consequently, the characterized tef accessions demonstrated high diversity in grain yield performance.

The study had limitations, including the fact that not all tef descriptors were measured (Table 1). Despite these limitations, twelve phenological and agronomic traits of tef were characterized for potential future breeding programs. Additionally, the yield data obtained might have been affected by the small plot size and subsequent conversion to hectares. Nonetheless, the data indicated a high variability in yield. Assefa et al. (2011) cited Tiruneh (1999) and Yifru [7], reporting yields of 1,058 to 4,599 kg/ha and 3.7 to 7.3 t/ha, respectively, while Habte et al. [8] reported yields of 3.7 to 7.3 t/ha. Furthermore, Ketema [31] reported a potential yield of up to 6 t/ha for tef. Therefore, the findings suggest that the potential yield of tef can exceed 6 t/ha.

3.4 Qualitative Traits of Tef

In addition to quantitative traits, this study measured qualitative traits such as panicle form, lemma color, and seed/caryopsis color (Table 2). The distribution of panicle forms showed that the majority were of the loose type, with 60.55% of the samples, followed by very loose at 26.55%, and fairly loose at 7.06%, accounting for 94% of the total. The remaining 6% consisted of other panicle forms. The dominance of the loose panicle form is significant as it may help the plants escape pests like ants and reduce shattering. Additionally, the loose panicle form is higher yielding, preferred by tef growers, and adaptable to different agro-ecological zones.

The importance of the loose panicle form in breeding programs has been highlighted by several authors [10,32,16,33,19]. This form's structural variation, such as the number of primary panicle branches and spikelets, is crucial for breeding purposes.

Regarding lemma color, 83.28% of the samples fell into five primary categories: yellow (30.19%), gray (20.12%), variegated red and yellow (13.91%), variegated pale and yellow (11.77%), and variegated purple and yellow (7.49%). The remaining percentage was distributed among other colors (Table 2). Lemma color is a valuable trait in tef breeding as it serves as a genetic marker for offspring, indicating the genetic material inherited from both parents. Previous studies (Ebba, 1975; Kefyalew et al., 2000; Assefa et al., 2002) have also reported variations in lemma color percentages, emphasizing its role in qualitative differentiation in breeding programs.

For seed color, the study identified six types, with 77.40% of the samples being brown (50.12%) and white (27.28%). The remaining colors accounted for the rest. Comparatively, Assefa et al. [33] reported that 80% of the samples had deep brown and white seed colors. Dawit and Hirut [34] also found that light-colored seeds were more common than dark ones. The higher frequency of brown seeds suggests better adaptability to diverse growing conditions and higher iron content, as noted by Melak Hail (1966). Although white seeds are highly valued in the market for their income potential, brown seeds are more prevalent among tef accessions, indicating a breeding strategy that prioritizes these preferred traits. Overall, the study highlights the significant variability in qualitative traits among tef accessions, providing valuable information for future breeding programs.

3.5 Cluster Analysis

3.5.1 Intra and inter cluster distance analysis

The determination of cluster number was based on pseudo-F and t2 values, with the pseudo-F reaching its peak and being higher than preceding and succeeding values in the list. Concurrently, pseudo t2 values were minimized followed by higher values, resulting in classification of the test materials into six distinct clusters, with one accession remaining ungrouped at approximately 81% similarity level, which could be further classified.

Out of 467 tef accessions, 382 were utilized for similarity-based grouping, while the remaining accessions were excluded due to incomplete trait data for clustering. Cluster analysis revealed that the first and third clusters contained the largest number of tef accessions, comprising 207 (54.19%) and 122 (31.97%) accessions, respectively (Table 4) [35]. The second cluster comprised 38 accessions (10%), while the remaining clusters contained very few accessions, with one remaining ungrouped accession [36,37]. The highest inter-cluster distances were observed between Cluster I and Cluster II, with a Euclidean squared distance value of 51, and between Cluster I and Cluster III, with a value of 31. The other clusters did not show significant differences in distance from Cluster I [38].

Breeding strategies can focus on these clusters and inter-cluster distances, particularly between Clusters I, II, and III (Table 5). Crossbreeding between tef accessions can be effective when the accessions have diverged sufficiently, whereas selection within clusters may involve a broad range of measured traits to enhance tef yield [39]. Traits such as days to heading, number of spikelets per primary panicle branch, and number of florets per spikelet exhibited higher intra-cluster distances within Cluster I. This suggests that selecting tef accessions for variety development could target traits related to earliness, higher yield, and increased floret numbers per spikelet within this cluster.

Table 3. Out of 467 tef population the phonological classes

Phenology	Classes	Frequency	Percent%
Days to heading(Days)	Early heading (less than 39 days)	4	0.86%
	Intermediate between 39 to 44 days	14	2.99%
	Late heading more than 44 days	450	96.35%
Days to physiological maturity(days)	Early maturing less than 80 days	17	3.67%
	Intermediate between 80 to 95 days	201	43.52%
	Late more than 95 days	244	53.81%

Table 4. Mean values for traits of the six clusters of tef accessions Evaluated at Axum ARC

Traits	CL-I	CL-II	CL-III	CL-IV	CL-V	VL-VI
DH	55.222	52.974	55.156	57	65	55
DM	96.536	94.395	95.992	97.417	99.5	93
PH	94.969	90.568	92.593	98.233	94.8	82.4
PL	37.149	35.678	36.446	39.86667	38.4	32.8
PDL	18.329	17.847	18.107	19.16667	22.8	18.6
Spk/PPB	28.798	29.898	28.752	29.29	32.66	19.4
Flort/Spk	5.996	5.856	6.058	5.756667	8	5.28
PPB	20.072	19.952	20.269	17.35	27.3	18.2
Biom	13,582.93	17,214.91	10,214.03	4,421.296	22,916.67	19,444.44
GY	3454.928	3738.996	3328.882	3884.12	4756.25	275

DH=Days to Heading, DM=Days Maturity, PH= Plant Height, PL=Panicle Length, PDL=Peduncle Length, Spk/PPB= number of spikelet per primary panicle branch, number of florets per number of spikelet's, PPB=Primary Panicle Branches , biom=Biomass and GY=Grain Yield

Table 5. Pair wise generalized squared distances (D2) values between clusters constituting 382 tef accessions

Clusters	Generalized square distance					
	CL-I	**CL-II**	**CL-III**	**CL-IV**	**CL-V**	**CL-VI**
CL-I	0					
CL-II	51.382**	0				
CL-III	30.944**	4.335ns	0			
CL-IV	16.856ns	11.201ns	6.507ns	0		
CL-V	14.353ns	14.622ns	4.129ns	4.688ns	0	
CL-VI	138.975**	21.676*	42.411**	61.743**	70.743**	0

**Significance at 0.05, for $x^2 = 16.919$, **significance at 0.01 for $x^2 = 21.666$, ns =Non Significance*

Across the six clusters analyzed, three showed significant genotype divergence (Table 5). Cluster I exhibited significant differences with Clusters II and III, while other cluster pairs did not show statistically significant differences in clustering [40]. Days to maturity was identified as a crucial trait in Cluster I, where accessions exhibited a wide range from higher to lower maturity days [41]. Cluster II emphasized earliness and a higher number of primary panicle branches, traits important for selection to enhance tef yield in moisture-deficient regions with late-maturing but high-yielding parents. These materials could be utilized for breeding with existing tef accessions or with those identified as tef parents in previous studies. The sixth cluster, which remained ungrouped, was characterized by significantly lower grain yield, nearly three quintals per hectare, deviating from other tef accessions. Overall, the clustering approach identified groups of tef accessions sharing similar traits, providing opportunities for targeted trait selection.

Understanding the diversity among genotypes informs strategies for improvement through selection and hybridization. Greater genetic divergence between genotypes increases the potential for improvement through these methods [42]. Getahun [43] classified 49 semi-dwarf tef accessions into four clusters, while Assefa et al. [12] clustered 320 tef genotypes into 14 groups, demonstrating the varied approaches and outcomes in tef genotype classification [44,2].

4. CONCLUSION

The tef characterization and evaluation on phenological and agronomic performance of tef accessions is yet not accomplished satisfactorily and systematically from the genetic resources. This study revealed there is a vast genetic potential of tef crop. Assessing a range of key morphological, agronomic, and phenological traits, the study identified valuable sources of variability, which used for future tef improvement effects. The desirable accession identified early maturing, high biomass, and superior grain yield and other qualitative traits provides breeders with a rich groups of tef germplasm to draw upon. This increases targeted and efficient utilization of the tef gene pool, to accelerating the enhancement of varieties tef grower demand on the diverse production environment. The cluster analysis of tef accessions provides a comprehensive understanding of genetic diversity and trait distribution, informing targeted breeding strategies. The significant inter-cluster distances between specific clusters offer promising opportunities for crossbreeding to develop high-yielding and adaptable tef varieties. This study reinforces the importance of utilizing genetic divergence in breeding programs to enhance crop improvement and sustainability.

Overall, traits with higher yielding, early maturing and higher straw are the most important traits in Tigray region. In addition to the indicated problems tef shoot fly and lodging are the other major problems that require]future line of works. Characterization of tef accessions would better if it includes the characterization and evaluation having all descriptors and the method of characterizations through genetic analysis (using markers) in different agro-ecologies for all tef accessions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of this manuscript. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1.I do have used AI for grammer editing and paragraph writing of my work.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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