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Tef (*Eragrostis tef*) Recombinant Inbred Line Variety Development for High Potential Areas of Ethiopia

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Abstract: The national average yield of Tef is low at 1.75 t ha^{-1}. This is partially due to lack of high yielding Tef genotypes for different Tef growing areas. Therefore, the present study was designed to develop high yielding, and desirable quality of improved Tef varieties suitable for high and optimum potential farming systems. Eight recombinant inbred lines (RILs) developed from a cross of DZ-01-353 x kaymurri plus two checks were laid out in a randomized complete block design using four replications in multi-environments for two years (2013 and 2014) to see the effect of genotypes, environments and GEI. ANOVA from additive main effect and multiplicative interaction (AMMI) for grain yield revealed highly significant ($p<0.01$) effect for genotypes, environments, and genotype by environment interaction (GEI. The effect of environment, genotypes and genotype by environment interaction accounted for 81.49, 3.98 and 14.15% of the total sum squares, respectively. A large sum of squares for environments indicated that the test environments were diverse with large differences among environmental means which causing most of the variation in grain yield. Therefore, results of combined data analysis across locations and over the years showed that variety DZ- Cr- 429 (RIL 125)/Negus/ performed better and stable across five locations over two years among tested genotypes. Thus, variety Negus was identified and released as best promising Tef variety for production in high and optimum potential tef growing areas in the country. This variety should be used in similar agro ecologies to increase grain yield productivity and ensure food security in the country.

Keywords: RIL, Tef Genotypes, High Potential Area, AMMI, Variety Negus, GEI

1. Introduction

Tef is the major Ethiopian cereal grown on 3.02 million hectares annually [4], and serving as staple food grain for over 73 million people in the country. It constitutes 30% of the total area allocated to cereals and contributes more than 20% of the total cereals production [4].

Tef hybridization began following the discovery of Tef flower opening time and consequent to that the artificial surgical binocular-aided hand emasculation and pollination technique by [24]

The average annual genetic gain in tef grain yield was estimated as 0.8% from 1970 until 1995 [27] and o.58% from 1970 until 2012 [7] under lodging controlled and uncontrolled conditions respectively. Tef varieties developed through hybridization showed a yield advantage of 9.5% over those developed through direct selection from farmers' variety. In Tef improvement effort grain yield constituted the highest priority [15]

Yield is a complex quantitative trait often affected by genotype, environment and genotype by environment interaction (GEI). The differential responses of genotypes across environments occur because of differences in expression of different sets or the same set of genes in different environments [18, 8 & 10]. GEI complicates selection of any superior genotype across environments because it reduces the association between phenotypic and genotypic values [6].

Yield stability usually refers to a genotype's ability to produce high or low yield consistently across a wide range of environments [2]. For grain yield stability analysis, a genotype having minimum cultivar superiority value is considered the most stable genotypes [17].

Nevertheless, the national yield per unit area (at 1.75 tha^{-1}) still remains low, some of the factors contributing to low yield of Tef are; lack of outstanding cultivars and lodging, both biotic and abiotic pressures. Despite low average national yield, the national Tef research program developed 49 improved Tef varieties [20] yet, from the released tef varieties achieved the potential yield of the crop. It shows there is higher difference between the potential of Tef and the actual yield called yield gap which is less than half. This is due to the limitation of well performing and stable tef genotypes in multi environments of Tef growing areas in the Country. In order to alleviate these problems, currently the breeding program is focused on the development of varieties with high yield and good kernel quality. The segregating generations are handled by modified bulk method, i.e., raising bulk families in F3-F4 generations that are derived from individual F2 plants, keeping in view the need to generate more variability through hybridization. Thus, the objective of the present study was to develop high yielding and stable white seeded recombinants inbreed Tef variety (ies) to the farming community.

2. Materials and Methods

2.1. Inbred Line Development

Hybridization/crossing between DZ-01-353 x kaymurri (RIL 125) was made in 2003. The purpose was to develop stable, high yielding; and farmers and consumers preferred Tef varieties for the high rainfall and optimum moisture (high potential) areas of the country. In other words, it was targeted at developing varieties with high yielding potential and better quality than the improved contemporary standard check varieties Quncho [14] and Dagim [23]. DZ-01-355 was selected as maternal parent for its high yielding ability and wide adaptability. Likewise, Kaymurri was selected as a parent for its extra white seed color, relatively large kernel size, thick culm and vigorous growth habit. After a successful crossing rapid generation advancement up to two to three generations per year was made using off-season irrigation facilities. After homogeneity attained at F7 selection was made and transferred to observation nursery trial. Genotypes showed uniform and desirable traits selected for preliminary variety trial and from the preliminary variety trial those eight recombinant inbred lines showed good performance selected for multi-location trial. Among tested genotypes, variety Negus [DZ-01-353 x kaymurri (RIL 125)] was selected by its performance and tested for variety verification trial in 2017 and then the national variety release technical committee approved it. Finally, Variety Negus was developed as a recombinant inbred line through an F2 derived single-seed descent method; and following series of multi-environment yield tests in various major Tef growing areas of the country.

2.2. Genotypes, Testing Site and Design

The field experiment was tested at five locations, Debrezeit light soil, Debrezeit black soil, Minjar, Holota and Adadimariyam

Table 1. *Description of the study areas.*

location	Name	Latitude	Longitude	Altitude (m.a.s.l)	Annual rainfall	Annua Temperature (°c0)	Soil type
1	Minijar	8°45' N	39°45' E	1781	963	19.5	nitosol
2	Debrezeit-1	8°45' N	38°59' E	1860	832	17.05	nitosol
3	Debrezeit-2	8°45'N	38°59' E	1860	832	17.05	vertisol
4	Adadimariam	08°31'N	38°13'E	2383	1105	16.9	Vertisol
5	Holeta	09°03'N	38°30'E	2400	1102	14.5	nitosol

The trial was conducted using a RCB design with four replications throughout the testing sites. The description of testing sites is presented in Table 1. The trial was evaluated on the plot size of 4 m^2 with ten rows of 2 m length throughout all trial sites and 1.5 m between replication, 1 m between plot, 0.2 m between rows distances were maintained. The varieties were assigned to plots at random within each replication based on the randomization table made in computer. As per the research recommendations of 15$kgha^{-1}$, equivalent to 6 g $plot^{-1}$ of seeds was disseminated along the surface of each row by hand drilling. 60 kg P_2O_5 and 40N per hectare for light soil, 60 kg N and 60 kg P_2O_5 per hectare for black soil fertilizer was applied, respectively, all at planting while urea was applied two times, the first application two weeks after sowing and top dressed at tillering stage. Hand weeding was made three times during the crop growth stage. A variety verification trial was conducted at Minjar, Debre Zeit, Holeta and Adet on the trial station and in eight farmers' field during 2016/17 main production season.

2.3. Data Recorded

Data on agronomic yield and yield related traits were collected both on plot and individual plant base. Data on days to heading or panicle emergence using the sowing date as a reference, lodging index, grain and biomass yield were taken on plot bases. Days of heading and maturity were taken when each plot attained 50% heading (panicle emergency) and 90% physiological maturity respectively, and days were calculated beginning from the date of sowing. Lodging index was assessed using the method [3] by considering assessments of both the lodging degree or the angle of leaning on 0 (completely upright) - 5 (completely flat on the ground) and the severity as the percentage of the plot stand manifesting each of the 0-5 degrees of lodging. Then, lodging index for each plot was taken as the product sum of the degree of leaning and the respective per cent severity divided by five. Grain yield of each plot was measured on clean, sun dried seed and the measured grain yield value (g) was converted to kilogram per hectare for data analysis. Plant height (cm), and panicle length (cm) were taken on the five individual samples of plants which were randomly taken from the central rows of each plot, and the averages of five sample plants were as used for analysis.

2.4. Data Analysis

Data from individual locations combined over two years and location by years were analyzed by using SAS version 9.0 (2002) software [21]. The analysis of variance for grain yield and yield-related traits for each location and over two years was analyzed by using a randomized complete block design Factorial ANOVA model [9]. The combined analysis of variance across the location was done in order to determine the differences between genotypes across location, over two years and their interaction. Bartlett's test, [13] was used to assess the homogeneity of error variances prior to doing combine analysis over location and years and variance effect were considered as significant and highly significant at P< 0.05 and P< 0.01, respectively. GEA-R (2015) version 2.0 was used for the stability analysis. Mean comparison using Least Significant Difference (LSD) was performed to explain the significant differences among means of genotypes and environments (location x year).

3. Results and Discussion

3.1. Analysis of Variance

ANOVA from additive main effect and multiplicative interaction (AMMI) for grain yield revealed highly significant (p<0.01) effect for genotypes, environments, and genotype by environment interaction (GEI) (Table 2). The effect of environment, genotypes and genotype by environment interaction accounted for 81.49, 3.98 and 14.15% of the total sum squares (Table 2), respectively. A large sum of squares for environments indicated that the test environments were diverse with large differences among environmental means which causing most of the variation in grain yield. This might be due to the presence of variation in temperature, rainfall, soil type, soil fertility, and moisture availability. The AMMI analysis also showed that the first interaction principal component (PC1) and second interaction principal component (PC2) explained 44.14 and 22.42 of the interaction sum squares, respectively. The significant interaction indicated that the genotypes respond differently across the different environments. The significant variability of Tef genotypes in the present study are in line with the previous findings [1, 10, 16, 5, 25].

Table 2. *AMMI analysis of variance for grain yield grown at 10 environments.*

Source	d.f.	s.s.	m.s.	v.r.	F pr	SS%GEI
Treatments	99	3150720	31825	9.60	<0.001	
Genotypes	9	125291	13921	4.20	<0.001	3.98
Environments	9	2567514	285279	27.23	<0.001	81.49
Block (E)	30	314352	10478	3.16	<0.001	
Interactions	81	457915	5653	1.71	<0.001	14.53
IPCA 1	17	202138	11890	3.59	<0.001	44.14
IPCA 2	15	102650	6843	2.06	0.0119	22.42
Error	270	894932	3315			

d.f = Degree of freedom, s.s = Sum square, m.s = Mean square. v.r = F calculated, Fpr = Probability

As indicated in Table 3, the average grain yield across location over two years ranged from 1547±110 kgha^{-1} (RIL 89) at L2 (Debrezeit light soil) to 3208±264 kgha^{-1}(RIL 125) at L1 (Minjar (Table 3). Moreover, performances of genotypes were not consistent across five locations over two years. For instance, at L1 genotype RIL 125, at L2 genotype RIL 29, at L3 genotype RIL 125, at L4 genotype RIL 87 and at L5 genotype RIL 125 were the top ranking genotypes with mean grain yield of 3208±264, 2142±192, 2645±143, 3100±330 and 2795±147, respectively. Thus, such inconsistent in yield ranking from location to location indicated the presence of possible cross over GEI as described by [10, 5].

3.2. Combined Analysis of Variance and Mean Performance of Genotypes Across Locations over Two Years

Combined analysis of variance also showed highly significant difference (<.01) effect of location and genotypes for days to heading, days to maturity, days to grain filling, plant height, panicle length. Alike, year showed significant effect in all traits recorded except days to maturity and plant height. Variability among Tef genotypes for different traits across locations/environment was reported by [10, 12, 26].

As indicated in Table 4 and Figure 1, the interaction of genotype by year by location was highly significant (p<0.01) for grain yield and significant (P <0.05) effect for days to heading and above ground biomass. Likewise, year by location were highly significant for all recorded traits. Similarly genotype by year was significant for plant height and panicle length. In contrast, the interaction of genotype by year was not significant for days to maturity, grain filling period, lodging index, grain yield and above ground biomass. This significant effect due to genotype, location, year and their interaction effect indicated that the genotypes, years and locations were divergent to show considerable variation in Tef traits. Therefore, the significance of GEI indicated that the relative performances of the genotypes were not consistent across the test locations and years that had different effects on the yield potential of the tef genotypes. This result is in agreement with the previous reports [5, 11 & 22] for yield related traits.

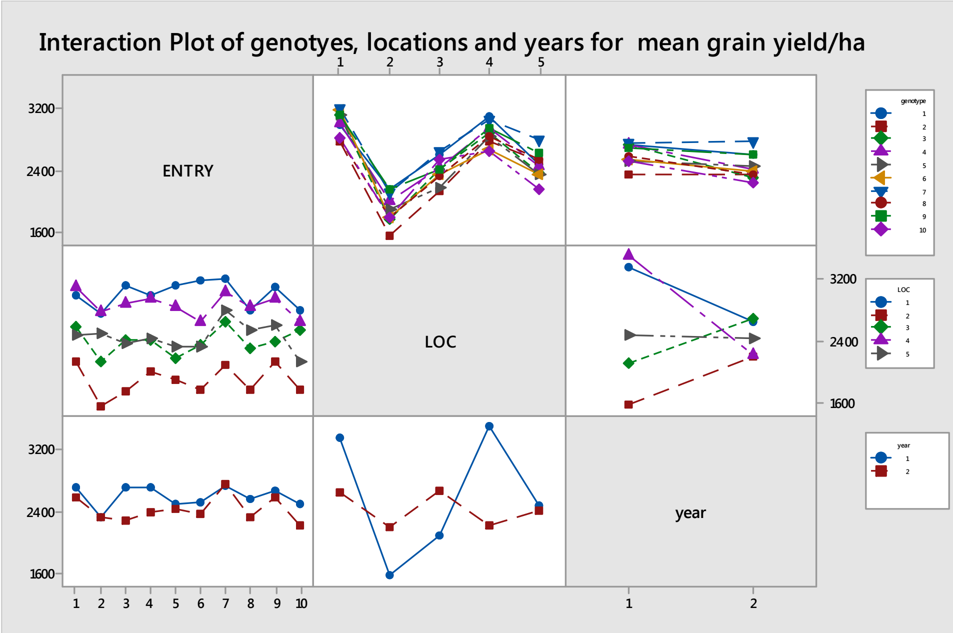

Figure 1. *Interaction of genotype, location and years for grain yield.*

Among the tested genotypes, RIL 125 was the highest yielder genotype with the mean grain yield of 2758 kgha^{-1} followed by genotype RIL 87 (2658 kgha^{-1}) and genotype RIL 29 (2638 kg ha^{-1}) respectively, whereas the lowest mean grain yield (2342 kg ha^{-1}) and 2376 kkgha^{-1} were registered from Genotype RIL 89 and a local check (Table 4). As indicated in the same Table 4, four genotypes scored highest grain yield over the grand mean (i.e., 2522 kg ha^{-1}) and four candidate genotypes scored mean grain yield above the standard check variety Quncho. This finding is in agreement with the previous study with [5, 11, 27] who reported in yield variation among Tef genotype. The candidate genotype, Genotype RIL 125, was statistically high yielder than the other genotypes and showed 11.7% and 16.1% yield advantage over the standard check Quncho (2470 kgha^{-1}) and local check (2376kgha^{-1}), respectively. Therefore, this genotype has been verified in 2017 and visited by the national variety releasing technical committee. Accordingly, genotype RIL 125 has been officially released for its high yielding, medium maturity, very white grain color, and high adaptability in the high potential Tef growing areas of Ethiopia.

Variety Negus is white seeded, broad adaptability, medium maturity, high yielding Tef variety with grain yield advantage of 11.7% and 16.1% over the standard check (Quncho) and local check, respectively. Negus takes 50 days to head and 105 days to mature (Table 4). It is 94 cm tall in height with 37 cm panicle length (Tables 4). It has got yellowish lemma color, red anther color, loose panicle form and very white seed color. Negus has gained immense farmers' attention due to its yielding potential and stable performance, very white caryopsis color and good straw yield (straw yield is no less important than grain yield) at participatory variety selected

Table 3. *Grain yield (kgha^{-1}) of ten Tef genotypes across locations over two years (2013-2014).*

No.	Genotypes	Locations				
		L1	L2	L3	L4	L5
1	DZ-01-353 x kaymurri (RIL 87)	2993±218	2135±141	2589±173	3100±330	2475±122
2	DZ-01-353 x kaymurri(RIL 89)	2762±161	1547±110	2130±141	2774±245	2502±146
3	DZ-01-353x kaymurri(RIL 194)	3116±225	1752±135	2411±78	2876±368	2363±166
4	DZ-01-353 x kaymurri(RIL 113)	3001±327	2001±225	2422±101	2949±272	2442±118
5	Variety Quncho (standard (check)	3115±213	1889±217	2168±150	2841±282	2336±118
6	DZ-01-353 x kaymurri(RIL 119)	3184±213	1778±129	2341±167	2651±221	2332±127
7	DZ-01-353 x kaymurri(RIL 125)	3208±264	2100±163	2645±143	3040±319	2795±147
8	DZ-01-353 x kaymurri(RIL 205)	2793±109	1774±112	2315±181	2841±224	2533±176
9	DZ-01-353 x kaymurri(RIL 29)	3103±259	2142±192	2390±218	2947±246	2608±160
10	Local variety	2804±148	1767±140	2535±140	2638±283	2139±154
	Mean	3008±70.4	1888±53	2395±49	2866±86	2452±47
	LSD(0.05)	485.9	292	311	392	353
	CV	16	15	13	14	14
	R^2 (%)	57	72	64	81	50

L1= Minjar, L2= Debrezeit light soil, L3= Debrezeit vertisol, L4= Adadimariam, L5 = Holeta, CV = coefficient of variation, R^2 (%) = the model explain the variability of the response data around its mean

3.3. Stability Analysis

Mean grain yield performance and its stability 10 Tef genotypes across five locations over two years are shown in Table 5 and Figure 2. The mean grain yield value of genotypes averaged over location by year indicated that genotype RIL 125 had the highest (2758 kgha^{-1}), genotype RIL 89 (2343 kgha^{-1}) and local variety (2376 kgha^{-1}) the lowest grain yield, respectively. Genotype superiority with the small measured value indicates the more stable genotypes. Therefore, from the present study, genotype RIL 125 was the most stable and genotype RIL 89 was the most unstable genotypes, respectively. The comparison of variety Negus with the standard check variety Qunco has shown in Figure 3. From this Figure 3 the performance of Variety Negus was superior to standard variety Quncho in tested sites.

3.4. Description of "Negus" Tef Variety

This variety designated as; [DZ-01-353 X Kay Murri (DZ-Cr-429 (RIL No. 125)] can be explained as follows: Genotype DZ-01-353 was the female (ovule) parent and Kaymurri was the male (pollen) parent. And the cross was numbered as "429". RIL No.125 (Recombinant Inbred Line 125) is a designation of the homozygous line among the tested at F7. Finally, DZ-Cr-429 (RIL No. 125) christened as "Negus" was released in 2017 [19] (Table 6).

Table 4. *Mean performances of Tef genotypes for yield and yield related traits at five locations during for two consecutive main cropping season (2013-2014).*

NO	Genotype	DTH (days)	DTM (days)	GFP (days)	PH (cm)	PL cm)	LI (%)	ABG (Kgha^{-1})	GY (Kgha^{-1})
1	DZ-01-353 x kaymurri (RIL 87)	48	107	57	94.7	37.5	82.8	12165.6	2658.4
2	DZ-01-353 x kaymurri(RIL 89)	47	106	59	96.8	36.3	82.3	10943.8	2342.8
3	DZ-01-353x kaymurri(RIL 194)	51	107	57	98.4	38.1	82.0	11756.3	2503.7
4	DZ-01-353 x kaymurri(RIL 113)	52	106	54	98.8	38.6	82.6	12175.0	2562.9
5	Quncho	54	108	54	104.2	41.7	81.5	12890.6	2469.7
6	DZ-01-353 x kaymurri(RIL 119)	50	105	55	95.4	36.2	85.4	12131.3	2457.4
7	DZ-01-353 x kaymurri(RIL 125)	50	105	55	94.1	37.5	83.7	12403.1	2757.6
8	DZ-01-353 x kaymurri(RIL 205)	52	107	55	95.7	36.6	83.3	11100.0	2451.2
9	DZ-01-353 x kaymurri(RIL 29)	54	110	56	104.3	41.6	81.3	13578.1	2638.0
10	Local variety	49	104	56	93.1	36.8	86.1	11700.0	2376.4
	MEAN	51	107	56	97.5	38.1	83.1	12084.4	2521.8
	LSD	1	2.00	2	3.26	1.59	NS	774.9	167.12
	CV	5.69	4.28	9.66	7.61	9.50	10.34	14.50	15.05
	R^2	0.93	0.95	0.83	0.71	0.85	0.68	0.73	0.77
	Genotype	**	**	**	**	**	Ns	**	**
	Location	**	**	**	**	**	**	**	**
	Year	**	Ns	**	Ns	**	**	**	**
	Genotype x location	**	**	**	**	**	**	Ns	Ns
	Genotype xyear	**	Ns	Ns	**	**	Ns	Ns	Ns
	Year xlocation	**	**	**	**	**	**	**	**
	Genotype x location x year	*	Ns	Ns	Ns	Ns	Ns	*	**

NB. * = significant at P≤ 0.05), ** = significant at P≤0.0, Ns = noon significant.
DTH = days to heading, DTM= days to maturity, PH= Plant height, PL = panicle length, ABG = aboveground biomass kilogram per hectare, GY = grain yield kilogram per hectare

Table 5. *Stability coefficient analysis of mean grain yield of ten Tef genotypes across environments.*

No.	Genotypes	yield (kgha^{-1})	Standard deviation	Cultivar superiority
1	DZ-01-353 x kaymurri (RIL 87)	2658	393	15194(3)
2	DZ-01-353 x kaymurri(RIL 89)	2343	516	101104(10)
3	DZ-01-353x kaymurri(RIL 194)	2504	526	45177(5)
4	DZ-01-353 x kaymurri(RIL 113)	2563	416	26026(4)
5	Quncho	2470	500	57781(7)
6	DZ-01-353 x kaymurri(RIL 119)	2457	514	64087(8)
7	DZ-01-353 x kaymurri(RIL 125)	2758	427	533(1)
8	DZ-01-353 x kaymurri(RIL 205)	2451	434	55227(6)
9	DZ-01-353 x kaymurri(RIL 29)	2638	394	13439(2)
10	Local variety	2376	419	96020(9)

N.B: Numbers in brackets give the position of each genotype, ranked according to the stability coefficient (running downwards from 1 = best)

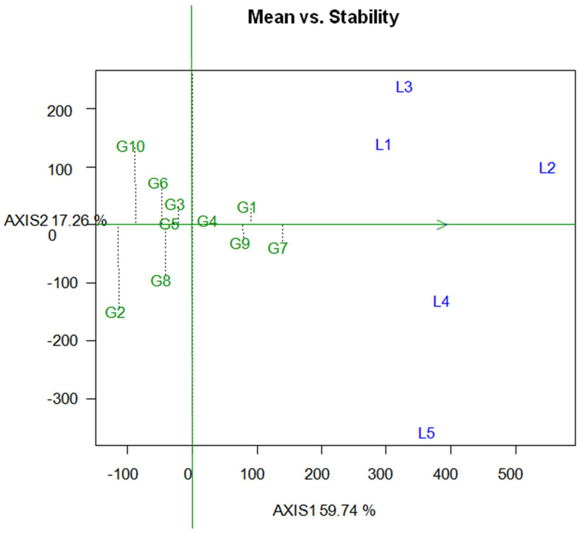

***Figure 2.** Grain yield means VS stability of ten Tef genotype across five locations combined over two years.*

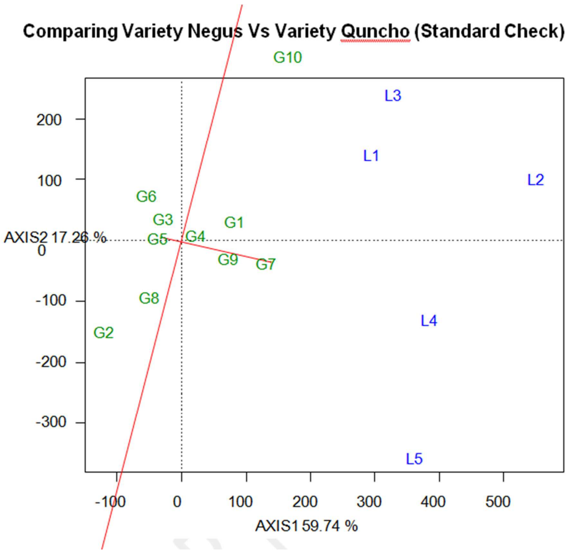

***Figure 3.** performance comparison of variety Negus with the Variety Quncho (standard Check) at five locations combined over two years.*

***Table 6.** Agronomic and morphological characteristics of variety: Negus DZ-Cr-429 (RIL.125).*

No.	Parameters	Description
1	Breeders Name	DZ-.Cr-429
2	Pedigree	DZ-01-353 X kay murri
3	Vernacular name given	Negus
4	Days to heading (days)	50
5	Days to maturity (days)	105
6	Plant Height (cm)	94
7	Panicle length (cm)	37.5
8	Panicle form	Very loss
9	Lemma color	Yellowish
10	Anther color	Yellowish
11	Caryopsis color	Very white
12	Growth habit	Erect
13	1000 seed weight (g)	0.3
14	Grain yield on Station (Kgha^{-1})	2758
15	Mean rain yield on farm (Kgha^{-1})	2200
16	Dried above ground biomass yield (Kgha^{-1})	12403
17	Adaptation Area	high and medium agro ecologies
18	Altitude (m.a.s.l.)	1700-2500
19	Rain fall (mm)	700-1200
20	Seed rate (Kgha^{-1})	10-15
21	Planting method	both broad casting and row sowing
22	Row spacing (cm)	20
23	Planting date	July 10-30
24	Fertilizer Use	recommended rate for tef
25.	Pest reaction	Not significant
26	Year of release	2017

4. Conclusion and Recommendation

Genotype by location by year interaction has a key effect on crop variety development by complicating the release of varieties across challenging environments (location x year). Analysis of variance for every five locations and combined over two years showed significant differences among Tef genotypes, locations, years and year by location interaction (GEI) for grain yield and most of the yield-related traits. Likewise the three way interaction Genotype by location by year reveal significant difference for days to heading and above ground biomass and highly significant difference for grain yield. The significant interaction effects indicated the inconsistent performance of genotypes across the tested locations and seasons. Among the tested genotypes, RIL 125, RIL 87, RIL 29 and RIL 113 had mean grain yield above the overall mean grain yield of evaluated Tef genotypes. However, only the candidate genotype RIL 125 (Negus) had mean grain yield above the standard check variety Quncho.

Considering the 10 environments data (location x years) and field performance evaluation during the variety verification trial, the national variety releasing committee has approved the official release of candidate genotype, DZ-01-353 x kaymurri (RIL 125), with the vernacular name of "Negus" for high, medium and similar agro ecologies.

Competing Interests

All the authors do not have any possible conflicts of interest.

Author Contributions

This work was carried out in collaboration among all authors. Yazachew Genet designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Tsion Fikire and Kebebew Assefa managed the analyses of the study. Solomon Chanyalew and others managed the literature searches. All authors read and approved the final manuscript.

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