Tef (Eragrostis tef) variety development for moisture stress areas of Ethiopia

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Tef is widely cultivated grain crop in Ethiopia by concerning 6.5 million smallholder farmers on about 30% of the full-scale region distributed to cereal crops. The goal of this experiment was to identify and release stable and high yielding tef genotype for moisture stress areas of country and to determine and understand the effect of genotype, environment, and their interaction on grain yield of tef. Fourteen selected tef genotypes obtained from two autonomous crosses and progressed through alternative for a minimum of eight generations, and a local and standard check varieties, were tested over a two years at seven tef growing sites in moisture stress areas of Ethiopia namely Debre Zeit, Alemetena, Dhera, Axum, Sirinka, Minjar and Mehoni, using randomized complete block design with four replications. Among the genotype tested, DZ-Cr-387 x 3774-13(RIL120B) was found predominant in terms of yield at tested moisture stress environments. This genotype was obtained through targeted cross between DZ-Cr-387 (Quncho) selected as a maternal parent for its high yielding capability and wide adaptability and, 3774-13 as a pollen parent for its extraordinarily white seed quality and earliness. The genotype DZ-Cr-387 x 3774-13(RIL120B) gave the average grain yield 2740 kg/ha pooled across all environments. This genotype “DZ-Cr-387 x 3774-13(RIL120B)” was later released as DZ-Cr-453(RIL 120B) or Bora by the National Variety Release Committee in 2013, and with a yield advantage of 5.7% and 24.46% over the standard (Boset) and local check, respectively.

Key words: Eragrostis tef, bora, early maturity, variety release

INTRODUCTION

Tef (Eragrostis tef (Zucc.) Trotter) is an allotetraploid (2n=4x=40) small cereal grain crop that belongs to the family Poaceae, sub-family Eragrostidoae, tribe Eragrostidace and genus Eragrostis. Tef genome has a size of 672 Mb and it is larger than the rice genome (430 Mb) (Cannarozzo et al., 2014). It is a self-pollinated crop with exceptionally low level of out-crossing from 0.2% to 1.0% (Seyfu, 1997). Tef is a major cereal crop in Ethiopia, where it was originated and domesticated (Vavilov, 1951). At present, the crop is highly accepted worldwide thought for its wholesome pivotal facts since it is affluent in supplements and is gluten-free. Consumers prefer tef not only because it makes good quality “injera”, a pancake-like soft bread, but also it is nutritious due to its high protein and mineral content (Geremew et al., 2002), and the absence of gluten (Spaenj-Dekking et al., 2005) which makes it an alternative food for people suffering from celiac disease. Due to this “life-style” nature of the crop, it has been heralded as a super food or super grain (Jeffrey, 2015; Provost and Jobson, 2014). Tef is additionally acknowledged to be lenient to outrageous environmental condition and soil conditions; consequently, it’s a most valued crop in the semi-dry zones (Zenihu and Kebebew, 2012). Despite its numerous relative advantages and economic importance, the productivity of tef in Ethiopia is low amounting to 1.85 tons ha⁻¹ (CSA, 2020). The numerous yield prescribing variables in tef are absence of cultivars tolerant to lodging and drought (Kebebew et al., 2011), even as very little seed size. Yield misfortunes are assessed to achieve up to forty percent throughout extreme moistness stress (Mulu, 1993). Further, yield reduction of up to 77% has been reported as a result of drought at the anthesis stage of tef (Abuhay, 2001). The level of yield reduction due to moisture stress warrants targeted breeding of tef for low moisture stress environments in Ethiopia (Mizan et al., 2017). Accordingly, one of the primary goals of the national tef breeding program in Ethiopia is to develop high yielding, drought tolerant tef varieties (Kebebew et al., 2011). Tef breeders need to continuously search for new sources of drought resistance or tolerance and introgress the genes into the susceptible cultivars. Screening of tef genotypes using both phenotypic and genotypic data are important to identify drought

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resilient breeding lines (Mizan et al., 2015). In Ethiopia, about 42 improved varieties are released by national and regional agricultural research of the country mainly by Debre Zeit Agricultural Research Center. Amongst these, recently released varieties namely Quncho (Kebebew et al., 2011), Kora (Kebebew et al., 2017), Dagim (Solomon et al., 2017) and Tesfa (Worku et al., 2019) showed significant yield benefits. Most of them are targeted to favourable and wide environments, however, they’re not fitted to various and difficult environments. In a plant breeding programs many new genotypes are usually evaluated in different environments (location and periods) to identify and advance desirable ones towards release. A genotype or cultivar is considered as stable if it has adaptability for a trait of economic importance across diverse environments. The environmental part typically represents the biggest component in analyses of variance however it is not relevant to variety choice exclusively; only genotype and genotype by environment interaction are relevant to meaningful cultivar evaluation and must be considered simultaneously for making selection decisions (Yan and Kang 2003). Genotype by environment interaction affects the potency of crop improvement programs which will cause complications recommendation of types across diverse environments. Information on the structure and nature of genotype by environment interactions are particularly useful for breeders (Yayis et al., 2014). Therefore, the present studies are carried out to identify and release stable and high yielding tef genotype for moisture stress areas of country and, to determine and understand the effect of genotype, environment, and their interactions on grain yield of tef.

MATERIALS AND METHODS

Experimental sites

The field experiment was conducted for two years at seven tef growing sites namely Debre Zeit, Alemetena, Dhera, Axum, Sirinka, Minjar and Mehoni in moisture stress regions of the Ethiopia.

Plant materials

The two independent crosses made in 2010 were DZ-Cr-387 x 3774-13 and DZ-01-196 x 3774-13. The purpose was to develop stable, high yielding, early maturing, white seeded and farmers and consumers preferred tef varieties for the moisture stress areas. The maternal parent line as Quncho (DZ-Cr-387), and DZ-01-196 (Magna) is the well-known tef variety (Kebebew et al., 2011) selected for its high yielding capability and wide adaptability. The line 3774-13 was chosen as the pollen parent for its white seed colour and early mature type, and it had been obtained through 7ILLING (Target Induced local lesion IN genomes) technique from the University Bern, Switzerland. From each of the simple crosses, 500 F2 seeds were taken and advanced up to F6 using the single seed descent method (SSD). Eventually, the recombinant inbred lines were considerably reduced to few lines through modified bulk selection. Ultimately, tough selection focusing on standing ability and grain yield was carried out and the best performing lines at the eight filial generations were used for the study. Crossing and early generation testing were carried out for all breeding populations at Debre Zeit Agricultural Research Center from where the National Tef Breeding Program is coordinated.

Genotypes, Testing Sites, and Experimental Design

The performance of fourteen tef genotypes were selected as early maturing inbred lines from the crosses and two controls (farmers’ variety or local and improved standard check variety; Boset). Evaluations were carried out at Debre Zeit/Bishoftu, Alemetena, Dhera, Axum, Sirinka, Minjar and Mehoni using randomized complete block design with four replications during 2015 and 2016 which are main cropping seasons of Ethiopia. The trial was conducted on the plot size of 2m x 2m with ten rows per plot throughout all trial sites and 1.5m between replications, 1m between plot and 20cm between rows. The genotypes were allotted to plots at random within each replication. As per the research recommendations 15 kg ha⁻¹, 6 g plot⁻¹ of seeds was hand broadcasted along the surface of each row. 40 kg N and 60 kg P₂O₅ per hectare for light soil, and 60 kg N and 60 kg P₂O₅ per hectare for black soil were applied. NPS was applied all at planting, while urea was applied two weeks after sowing and top dressed at tillering stage. Agronomical and yield data were recorded and subjected to statistical analysis in order to identify the best performing genotypes.

Data Collection

Data on grain yield and yield-related traits were recorded on plot. Date of heading was taken once each plot accomplished 50% of heading (panicle emergence), days to maturity and lodging index were taken when the plant 90% physiological maturity stage, and days were determined begin from the date of planting. Data for plant height (cm), panicle length (cm) were recorded from five sample plants that were randomly taken from each plot and therefore the average of five sample plants were utilised for analysis.

Data Analysis and Analysis of Variance

Data from individual environments and combined over seven environments were analyzed by using SAS (2009) software. The analysis of variance for grain yield and yield-related traits for each environment and over seven environments were analyzed by using randomized complete block design. The combined analysis of variance across the environment was carried out in order to determine the differences between genotypes across environments, among environments and their interaction. Bartlett’s test was used to assess the homogeneity of error variances prior to doing combine analysis over environments. After getting significant differences for traits, pair-wise mean comparison was done using Least Significant Difference (LSD) at 0.05 significance level. R software (3.5 version) was used to visualize genotype by environment interaction patterns. Based on principles of GGE biplot, for the yield characters; Environmental evaluation (the power of environments to discriminate among genotypes), Genotype evaluation (the mean performance and stability) and Mega-environment analysis (which-where pattern), whereby specific genotypes are often recommended for specific mega environments (Yan and Tinker, 2006).

Table 1. Environmental conditions of experimental sites during growing period

|             | Alemetena | Minjar | Delze zeit | Dhera | Mehoni | Sirinka | Axum |
|-------------|-----------|--------|------------|-------|--------|---------|------|
| Rain fall (mm) | 500       | 419    | 527        | 172   | 387    | 361     | 431  |
| Max. mean daily temperature (°C) | 29.8      | 31     | 23         | 25    | 30     | 24      | 20   |
| Min. mean daily temperature (°C) | 12.9      | 15     | 12         | 16    | 14     | 13      | 12   |
RESULTS AND DISCUSSION

Performance variations

According to the results of the combined analysis of variance over the seven environments (Table 2), grain yield was highly significantly (P<0.0001) affected by genotypes and environments, which accounted for about 2.69% and 73.05% of the total variance, respectively. The genotype by environment (GxE) interaction effects on grain yield also highly significant by about 8.09% this indicating that the genotypes tested performed different across the test environments. This implies that the genotypes tested exhibit different adaptation to specific environments. The significant variability of genotypes traits showed in the present study for different traits of tef genotypes are in agreement with the previous report by different authors for genotype variability (Habile et al., 2019 and Tiruneh, 2001). Highly significant variations among the genotypes were recorded in grain yield performance of pooled across all environments. The genotype DZ-Cr-387 x 3774-13(RIL120) selected for its high grain yield performance and stay green at visual observation at all tested environments and these desirable traits indicated that it is most adaptable at moisture stress regions. Hence, DZ-Cr-387 x 3774-13(RIL120) was given the vernacular name DZ-Cr-387 x 3774-13(RIL120) and put under variety verification trial for release as a new improved tef variety. Based on the application, the National Variety Release Committee in Ethiopia evaluated the two year performance of DZ-Cr-387 x 3774-13(RIL120) and visited several locations where the new variety was grown for evaluation. Consequently, the committee approved the release of DZ-Cr-387 x 3774-13(RIL120) as a new variety with the name ‘DZ-Cr-453(RIL 120B)’ as Bora.

GGE biplot analysis of tef genotypes

Genotypes code 7 (DZ-Cr-387 x 3774-13(RIL120)), code 1 (Boset), code 11 (DZ-Cr-387 x 3774-13(RIL56B)), code 4 (DZ-Cr-387 x 3774-13(RIL74C)) and code 6 (DZ-Cr-387 x 3774-13(RIL10A)) showed positive interaction with most of the environments (Bishoftu/Debeze Zeit, Alemtena, Dhera, Axum and Mehin), as shown in Fig.1. But the remaining genotypes were not showed positive interaction to most of the environments. This indicates that the genotype DZ-Cr-387 x 3774-13(RIL120) was best performed than all tested genotypes at Bishoftu, Alemtena, Dhera, Axum and Mehin.

Table 2. Sum of squares, mean squares and percent of variance explained by different sources of variation from the analyses of variance of grain yield of 14 tef genotypes tested at seven moisture stress environments

| Source                        | Degrees of freedom | Sum of squares | Mean squares | Explained variance (%) |
|-------------------------------|--------------------|----------------|--------------|------------------------|
| Genotype                      | 13                 | 7321039.2      | 563156.9**   | 2.69                   |
| Environment                   | 6                  | 199005996.8    | 3316766.1**  | 73.05                  |
| Rep/Environment               | 21                 | 12201463.1     | 581022.1**   | 4.48                   |
| Genotype x Environment        | 78                 | 22036145.5     | 282514.7**   | 8.09                   |
| Error                         | 273                | 31841100.0     | 116634.1     | 11.69                  |
| Corrected Total               | 391                | 272405744.7    |              |                        |

** denote significance at P≤0.01

Table 3. Mean grain yield performance of fourteen tef genotypes evaluated in the national variety trial over two years main cropping season.

| No. | Genotypes | Environments (Year x Location) | Mean |
|-----|-----------|-------------------------------|------|
|     |           | 1    | 2    | 3    | 4    | 5    | 6    | 7    |
| 1   | DZ-Cr-409 (Boset) | 3252 | 3657 | 3000 | 2260 | 2090 | 2615 | 1267 | 2592 |
| 2   | DZ-Cr-387 x 3774-13 (RIL 49B) | 3006 | 3122 | 2993 | 1645 | 2120 | 1899 | 1226 | 2287 |
| 3   | DZ-Cr-387 x 3774-13 (RIL 8C) | 3037 | 3005 | 3293 | 1768 | 1973 | 2190 | 1802 | 2438 |
| 4   | DZ-Cr-387 x 3774-13 (RIL 74C) | 3455 | 3019 | 3530 | 1833 | 1714 | 2034 | 1567 | 2450 |
| 5   | DZ-Cr-387 x 3774-13 (RIL 93) | 2901 | 2779 | 2888 | 1538 | 2010 | 2529 | 1386 | 2290 |
| 6   | DZ-Cr-387 x 3774-13 (RIL10A) | 3057 | 3342 | 3941 | 1805 | 1720 | 2275 | 1619 | 2537 |
| 7   | DZ-Cr-387 x 3774-13 (RIL 120B) | 4249 | 3787 | 3239 | 1599 | 2283 | 2402 | 1259 | 2740 |
| 8   | DZ-Cr-387 x 3774-13 (RIL 55A) | 3173 | 3317 | 3474 | 1294 | 1750 | 2041 | 1221 | 2324 |
| 9   | DZ-Cr-387 x 3774-13 (RIL 17B) | 2971 | 2979 | 3179 | 1491 | 2258 | 1987 | 1521 | 2341 |
| 10  | DZ-Cr-387 x 3774-13 (RIL 4A) | 2766 | 2759 | 3406 | 1388 | 2050 | 2099 | 1554 | 2289 |
| 11  | DZ-Cr-387 x 3774-13 (RIL 56B) | 3345 | 3084 | 3298 | 1638 | 1485 | 2696 | 1212 | 2394 |
| 12  | DZ-01-196 X 3774-13 (RIL 77) | 3134 | 2894 | 3249 | 1761 | 1761 | 2107 | 1702 | 2373 |
| 13  | DZ-01-196 x 3774-13 (RIL 118) | 3071 | 2752 | 3667 | 1954 | 1751 | 2276 | 1301 | 2396 |
| 14  | Local check | 2653 | 2869 | 3113 | 1709 | 1382 | 2207 | 1472 | 2201 |

Mean | 3148 | 3098 | 3305 | 1717 | 1882 | 2240 | 1436 | 2404 |

CV 10.369.44 | 12.5618 | 91.13 | 33.14 | 5429.0 | 15.95 |

LSD (5%) 466.5427.4NS | 464.5359.1465.9NS | 201.68 |

NS = Not significant, 1=Bishoftu, 2=Alemtena, 3=Mekello, 5=Axum, 6=Srinika and 7=Dhera

The average grain yield of DZ-Cr-387 x 3774-13(RIL120B) was 2740 kg/ha (Table 3) which is maximum grain yield recorded among tested genotypes across pooled environments. This result is in close agreement with that of Yazachew et al. (2020). The genotype DZ-Cr-387 x 3774-13(RIL120B) showed grain yield advantage of 5.7% and 24.48% over the standard (Boset) and local checks, respectively. Based on two years of multi-location trial, the genotype DZ-Cr-387 x 3774-13(RIL120B) was selected for commercial cultivation under moisture stress regions in Ethiopia.
Identification of stable genotypes with highest performance

AEC abscissa (or AEA) is the single-arrowed line and points to higher mean yield across environments (Fig.2). Thus, the “7 (DZ-Cr-387 x 3774-13(RIL120B))” genotype code had the highest mean yield, followed by “1 (Boset)” and “6 (DZ-Cr-387 x 3774-13(RIL110A))” genotype code, while “14 (Local)” and “10 (DZ-Cr-387 x 3774-13(RIL4A))” genotype code had the lowest mean yield. Besides, an ideal environment is a point on the AEA in the positive direction of the biplot origin and is equal to the longest vector of all environments (Yan and Tinker 2006). This line was reported to be useful to evaluate mean grain yield and stability of genotypes (Yan and Tinker 2006). The AEC ordinate passes through the origin of the plot and is perpendicular to the AEC abscissa and points to greater uncertainty in either direction (poorer stability). The genotype code “6 (DZ-Cr-387 x 3774-13(RIL110A))” was therefore highly unstable, while the “7 (DZ-Cr-387 x 3774-13(RIL120B))” genotype was highly stable among tested genotype attested environments. Therefore, genotype DZ-Cr-387 x 3774-13(RIL120B) should be recommended for mega environments of moisture stress areas of country.

Which genotypes (s) won where?

In the polygon view, genotypes found extremely away from the origin are the vertex genotypes having the highest yield in their respective sector (Farshadfar et al., 2011 and Yan, 2002). The genotype code “7 (DZ-Cr-387 x 3774-13(RIL120B))”, “6 (DZ-Cr-387 x 3774-13(RIL110A))”, “14 (Local)” , “10 (DZ-Cr-387 x 3774-13(RIL4A))” and “5 DZ-Cr-387 x 3774-13(RIL93)” were as the corner or vertex genotypes (Fig. 3). This infers that, the genotype DZ-Cr-387 x 3774-13 (RIL120B best performed in grain yield at all environments except Minjar and Sirinka. Environments of Dhera, Axum, Minjar and Sirinka fell in the sector in which genotype code “6 (DZ-Cr-387 X 3774-13(RIL110A)” was the vertex genotype. This means that the genotype code “6(DZ-Cr-387 x 3774-13(RIL110A))” was the best genotype for those environments. The five environments fell in the sector in which ‘code 7(DZ-Cr-387 x 3774-13(RIL120B))’ was the vertex cultivar, meaning that ‘code 7 (DZ-Cr-387 x 3774-13(RIL120B))’ was the best cultivar for these five environments. Among the tested genotypes, the genotype code “7(DZ-Cr-387 x 3774-13(RIL120B))” had the highest mean yield, followed by genotype code “1(Boset)” and “6(DZ-Cr-387 x 3774-13(RIL110A))”, whereas genotype code “14(Local)” and “10(DZ-Cr-387 x 3774-13(RIL4A))” had the lowest mean yield.

Ranking genotypes

The average environment coordination view of the GGE biplot shows the ranking of genotypes based on the performance of an ideal genotypes (Fig. 4). The relative adaptation of the ideal genotype is evaluated by drawing a line passing through the biplot origin and the best genotype
marker. This line is called a genotype axis and is connected to the best genotype (Yan et al., 2000). The bolt is the place where an ideal genotype ought to be. Its projection on the AEA was intended to be equivalent to the longest vector, everything being equal, and its projection on the AEC was clearly zero, implying that it is totally steady. Along these lines, genotypes found nearer to the ideal genotype are more attractive than others. Consequently, genotype code "7 (DZ-Cr-387 x 3774-13(RIL120B))" was more attractive than the genotype code "1(Boset)" and "6 (DZ-Cr-387 x 3774-13(RIL110A))". This indicates that, the genotype DZ-Cr-387 x 3774-13(RIL120B) was ideal genotype for all tested mega environments.

**Table 4. Summary of description of the new tef variety DZ-Cr-387 x 3774-13(RIL120B)**

| No. | Descriptor parameter       | Description          |
|-----|---------------------------|----------------------|
| 1   | Breeder’s name            | DZ-Cr-453 RIL120B    |
| 2   | Pedigree                  | DZ-Cr-387(Quncho) x 3774-13-RIL120B |
| 3   | Vernacular name given     | Bora                 |
| 4   | Days to panicle emergence | 34-42                |
| 5   | Days to maturity          | 70-80                |
| 6   | Plant height (cm)         | 90-116               |
| 7   | Panicle length (cm)       | 38-46                |
| 8   | Lemma colour              | Yellowish            |
| 9   | Anther colour             | Yellowish            |
| 10  | Caryopsis colour          | Very white           |
| 11  | Grain yield – On-station (kg/ha) | 2400-3000          |
| 12  | Grain yield - On-farm (kg/ha) | 1900-2600          |
| 13  | Straw yield (kg/ha)       | 13100-14000          |

**CONCLUSION**

From the study, we can conclude that yield and agronomic performance of the selected tef genotypes showed wide variation for the studied traits. As grain yield is the economic trait in tef yield improvement program in the genotype DZ-Cr-387 x 3774-13(RIL120B) gave the average grain yield 2740 kg/ha\(^2\) across all environments. Therefore, it is recommended to use the selected genotypes for the moisture stress areas of Ethiopia. In addition, analysis of variance for combined over seven environments showed significant differences among genotypes, environments, and genotypes × environments interaction (GEI) for grain yield. The significant genotypes × environments interaction effects indicated the inconsistent performance of genotypes across the tested environments except for DZ-Cr-387 x 3774-13(RIL120B) which is stable genotypes with best performance at all tested environments. Therefore, this genotype recommended for all moisture stress areas of Ethiopia. Considering the seven environments data and field performance evaluation during the variety verification trial, the national variety releasing committee has approved the official release of candidate genotype, genotype code “7 (DZ-Cr-387 x 3774-13(RIL120B))” with the vernacular name of "Bora" for moisture stress areas of the country.

**AUTHOR CONTRIBUTIONS**

Worku Kebede, Yazachew Genet, Tsion Fikre, Kidist Tolosa, Mengistu Demissie, Kidu Gebremeskel, and Atinkut Fentahun were carried out the experiment and collect data for analysis. Worku Kebede, Kebebew Assefa, Solomon Chanyalew were written the manuscript and Zenihun Tadele financial supported through Syngenta Foundation for Sustainable Agriculture and develop TILLING (Target Induced local lesion IN genomes) technique for the material used at University Bern, Switzerland. All authors read and approved the final manuscript.

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**COMPETING INTERESTS**

The authors declare that they have no competing interests.

**ETHICS APPROVAL**

Not applicable.

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