

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 kgha⁻¹ 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 2019, and with a yield advantage of 5.7% and 24.48% 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 Eragrostoideae, tribe Eragrostidae and genus *Eragrostis*. Tef genome has a size of 672 Mbp and it is larger than the rice genome (430 Mb) (Cannarozzi 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 (Spaenij-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 (Zerihun 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 proscribing 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 upto 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

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., 2018) 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 complicates 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 parental line as Quncho (DZ-Cr-387), and DZ-01-196 (Magna) is the

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

Table1. Environmental conditions of experimental sites during growing period

Weather data	Alemtena	Minjar	Debe 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

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 *TILLING* (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 F₈ 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

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-won-where pattern), whereby specific genotypes are often recommended for specific mega environments (Yan and Tinker, 2006).

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

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(RIL120B) was given the vernacular name DZ-Cr-387 x 3774-13(RIL120B) 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(RIL120B) and visited several locations where the

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	33167666.1**	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 $P \leq 0.01$

genotype by environment (G×E) 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 differential 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 (Habte et al., 2019 and Tiruneh, 2001). Highly significant variations among the genotypes were recorded in grain yield performance of pooled across all environments.

new variety was grown for evaluation. Consequently, the committee approved the release of DZ-Cr-387 x 3774-13(RIL120B) 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(RIL120B)), 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(RIL110A)) showed

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 (RIL110A)	3057	3342	3941	1805	1720	2275	1619	2537
7	DZ-Cr-387 x 3774-13 (RIL 120B)	4249	3787	3239	1959	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	12.5618.9113	3314.5429.0					15.95
	LSD (5%)	466.5427.4NS	464.5359.1465.9NS						201.68

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

The average grain yield of DZ-Cr-387 x 3774-13(RIL120B) was 2740 kg ha^{-1} (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

positive interaction with most of the environments (Bishoftu/Debre Zeit, Alemtena, Dhera, Axum and Mehoni), 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(RIL120B) was best performed than all tested genotypes at Bishoftu, Alemtena, Dhera, Axum and Mehoni.

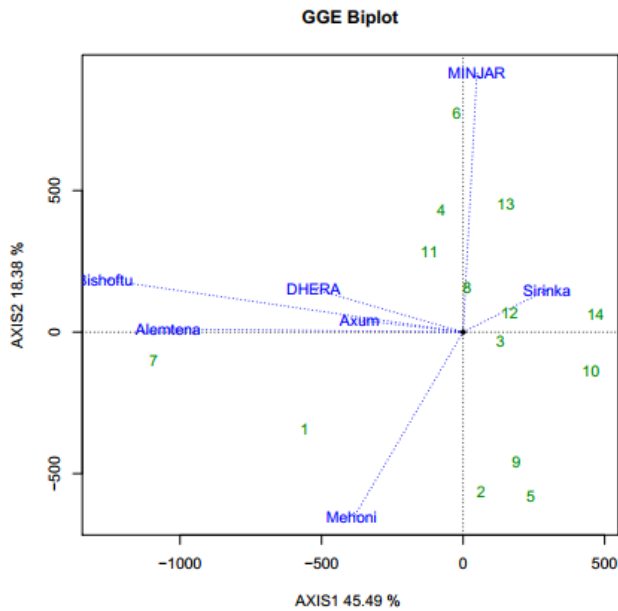


Figure 1. GGE biplot analysis of fourteen tef genotypes tested at seven locations

Identification of stable genotypes with highest performance

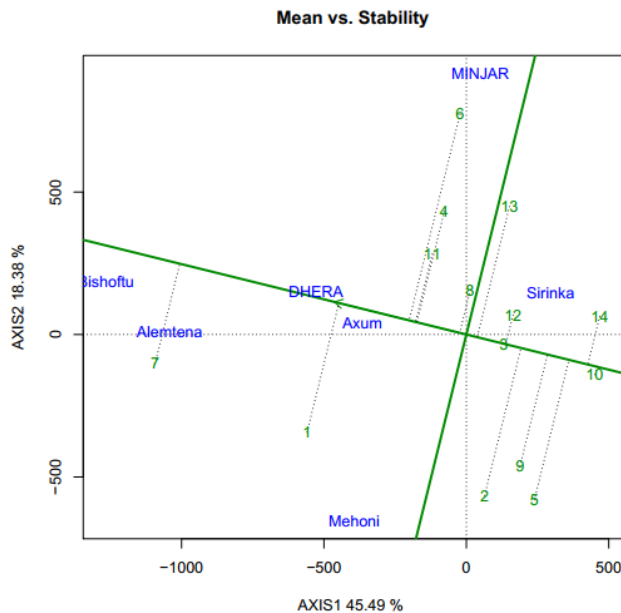


Figure 2. Identification of stable genotypes with highest performance

AEC abscissa (or AEA) is the single-headed 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.

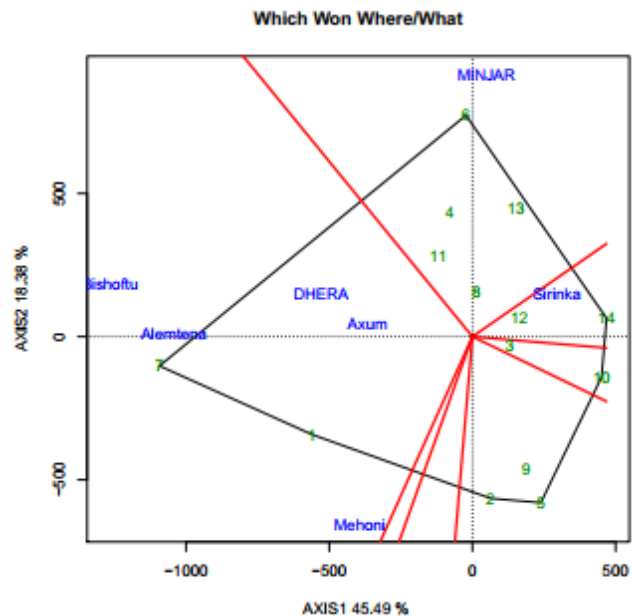


Figure 3. Which won where pattern of the GGE biplot of fourteen tef genotypes tested at seven locations

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.

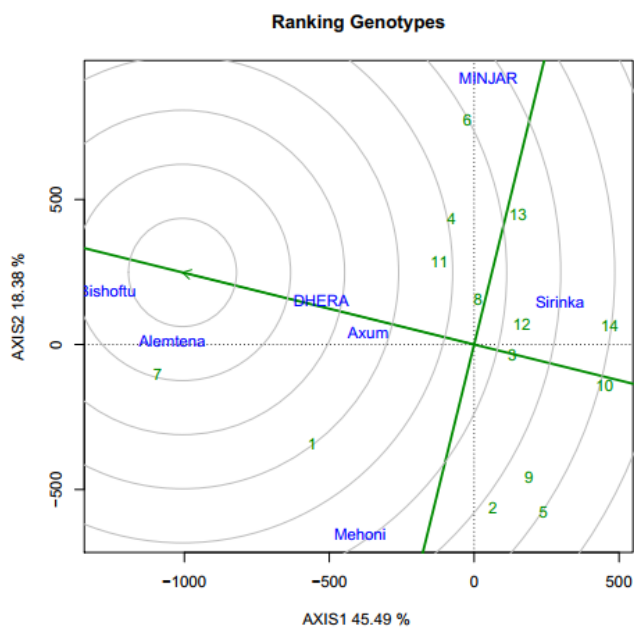


Figure 4. Ranking genotypes

Brief description of the distinguishing features and performance of the new variety

A brief description of the new tef variety DZ-Cr-387 x 3774-13(RIL120B) including its names, distinguishing pheno-morphic features and yield is given on Table 4.

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
4	Plant height (cm)	90-116
5	Panicle length (cm)	38-46
6	Lemma colour	Yellowish
7	Anther colour	Yellowish
8	Caryopsis colour	Very white
9	Grain yield – On-station (kg/ha)	2400-3000
10	Grain yield - On-farm (kg/ha)	1900-2600
11	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 kgha⁻¹ 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 x environments interaction (GEI) for grain yield. The significant genotypes x 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 Zerihun 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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