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Variability and trait association in culm and grain yield characteristics of recombinant inbred lines of *Eragrostis tef* **×** *Eragrostis pilosa*

Demeke Mewa¹ , Getachew Belay² and Endashaw Bekele³ *

¹Bahir Dar University, P. O. Box 79, Bahir Dar, Ethiopia. ²Common Market for Eastern and Central Africa (COMESA), Zambia. ³ Faculty of Life Science, Addis Ababa University, P. O. Box1176, Addis Ababa, Ethiopia.

Accepted 7 May, 2013

A study was conducted to assess variability and association in some culm and grain yield characteristics of recombinant inbred lines of a cross between *Eragrostis tef* **and** *Eragrostis pilosa***. A total of 81 lines were evaluated using randomized complete block design at Akaki and Debre Zeit, Ethiopia in 2007. Analysis of variance indicated that there exists difference among the recombinant inbred lines of similar and different parental genotypes. Grain yield revealed high (>30%) genetic coefficient of variation while lodging index revealed moderate (>15%). Moderate (>10%) genetic coefficient of variation was also recorded for plant height, panicle length and culm characters. Genetic advance was high for grain yield (>50%) and lodging index (>20%). Moderate (>10%) genetic advance was recorded for culm related characters. Most associations of the characters considered in the study were positive. Lodging index revealed consistently strong association with second culm strength that would be a point of further investigation. The variability among the recombinant inbred lines considered in this study has verified importance of** *E. pilosa* **in diversifying germplasm base for tef crop improvement. Existence of considerably high genotypic variability between recombinant inbred lines of different parental genotypes has also put a promise to further tef crop improvement, through crossing with many more genotypes of** *E. pilosa***. Difference revealed between the sites implicating the need to work on more environments to represent major tef growing conditions.**

Key words: Tef improvement, variation, lodging, genetic advance.

INTRODUCTION

Tef belongs to the family Poaceae and the genus *Eragrostis* with specific nomenclature *Eragrostis tef* (zucc.) Trottor (Ketema, 1997). It has numerous desirable agronomic- and storage- features and high cultural and socio-economic values in its origin (Tefera and Ketema, 2000). The plant bears heavy panicles on relatively thin

culms and tends to lodging particularly under growth and yield promoting conditions (Tefera et al., 2003b).

The species originated and diversified in Ethiopia (Vavilov, 1951). The existing diversity does not impart substantial variation to develop cultivars that are stiff or dwarf enough. Studies indicated that none of the existing

*Corresponding author. E-mail: endashawbw@yahoo.com

germplasm accessions resist lodging satisfactorily under good growth (Tefera et al., 2000); lodging persisted as the major bottleneck constraining tef husbandry (Assefa et al., 2000). Grain yield of tef is not competitive with the most popular cereals; partly due to the low basic productivity of available cultivars, together with susceptibility to lodging. The national average grain yield of tef is low (0.9 ton/ha) but has a potential of yielding four to five tons of grain per hectare if the lodging problem is resolved (Tefera and Peat, 1997).

There is compelling interest to use wild relatives to create genetic variability and improve some production limitations of tef (Tefera et al., 2003a). Tef has closely related species, which might be useful in improvement of some traits through conventional breeding (Bekele and Lester, 1981; Tavassoli, 1986). Difference is found among accessions of *E. tef* and wild relatives for many agronomic and grain quality traits (Ayele et al., 1999; Bekele et al., 1995). *Eragrostis pilosa* is the only wild relative that crosses relatively easily with tef (Tefera et al., 2003a); the species are closely related (Ingram and Doyel, 2003). *E. pilosa* was collected as food in many parts of Africa during famine periods (Ketema, 1997).

A previous breeding attempt has demonstrated importance of *E. pilosa* in diversifying the germplasm pool for tef breeding. Highly significant quantitative trait loci were identified for several agronomic traits including plant height and culm characters. The attempt was the first evaluation of tef germplasm derived from interspecific crosses for breeding purposes (Tefera et al., 2003a). More polymorphism is expected from interspecific recombinant inbred lines (RILs) pertinent to this study, obtained from the second interspecific crossing in the history of tef breeding.

Objective

The study is aimed at investigating extent of variability and trait relationship in RILs *of E. tef* × *E. Pilosa* genotypes for some culm and grain yield related morphological characteristics

MATERIALS AND METHODS

Experimental materials

The study materials, made kindly available from the National Tef Research Project, were three parental genotypes; 76 RILs derived from two crosses between the parental *genotypes;* and two tef cultivars. The parental genotypes were DZ-01-974(*E.tef*) × PI-2232259 (*E. pilosa*) and DZ-01-974 (*E.tef*) × PI-222988 (*E. pilosa*). In both crosses, DZ-01-974 is a common mother genotype and the *E. pilosa* genotypes were used as pollen source. The 76 RILs were randomly taken from hundreds of RILs (38 from each cross) at seventh filial generation. The remaining two tef cultivars (Quncho and DZ-cr-37) that are widely grown in Ethiopia were included in the study for comparison.

RILs were generated through single-seed-decent method. The *E. tef* parental genotype (DZ-01-974) has taller stature, thick culm, loose panicle and pale-white seed color (Berhe, 1981). The *E. Pilosa* parental *genotypes* have quite different characteristics: Shorter stature and thin culm; and PI-223259 has red seed color while PI-222988 has deep brown.

Experimental conditions

The experiment was conducted at Debre Zeit Agricultural Research Center and at Akaki research site, Ethiopia, in 2007 main rainy season. Planting was done on typical black soil (vertisol). Debre Zeit has mean annual rainfall of 801.3 mm and altitude of about 1850 m above sea level and Akaki has 2120 m above sea level and is cooler than Debre Zeit (Tefera et al., 2003a).

The experiment was laid out in completely randomized block design with three replications. Sowing was done on August 04 at Debre Zeit and on August 11 at Akaki. Each genotype was planted in two-rows with 1.5 m row length and 0.2 m spacing between the rows. Double-row spacing (0.4 m) was used to separate each double-row representing each of the genotypes in a block. Seed was drilled in rows and thinned to about 0.1 m spacing when grown; to retain 15 plants per row at Debre Zeit but left as such at Akaki in response to lower emergence and seedling vigor. Crop management practices were done as recommended for tef production in the area.

Data collected

Data was collected for: (i) first and second culm diameters (mm); (ii) first and second culm strength (pound); (iii) plant height (cm) and panicle length (cm); (iv) lodging index and (v) grain yield (g). First culm refers to the middle of the first internode above the soil surface and second culm refers to the next (upper). Measurements of variables were average from five randomly sample plants for each genotype (in each block); except for lodging index and grain yield for which general observation and total weighing was done, respectively.

Statistical procedures

Homogeneity and normality of error variance was tested by plotting predicted means versus residuals for all the variables. Except for grain yield and lodging index, the test has indicated no relationship between predicted means and residuals for all other variables. The pattern of relationship for grain yield tends to be semi-circular for which arcsine transformation was done while linear patterns for lodging index was log (x+1) transformed.

The two check genotypes included in the study were taken out in all analyses to reveal variability and correlation parameters within families of RILs. Analysis of variance (ANOVA) was done for single site and sites data (combined analyses) using SAS software. However, ANOVA was repeated with inclusion of the check genotypes to compare performance of the RILs in relation to the check genotypes. Range, coefficients of genotypic and phenotypic variance, and genetic advance were computed from mean square and grand mean values. Estimation of genotypic correlation coefficient was done using SPAR software while phenotypic correlation coefficient was done by SPSS software. Principal component analysis was made using Minitab software.

The model of the experimental analysis used is as follows:

1. For single site:

 $Y_{ij} = \mu + r_j + g_i + e_{ij}$

2. For combined data over sites: $Y_{iik} = \mu + q_i + r_i + I_k + (I)_{ii} + e_{iik}$

Where Yij = observation of the ith genotype in the jth replication, μ = grand mean of trait Y; gi = effect of the ith genotype, e_{ij} = effect of experimental error, r_{j} the effect of j^{th} replication, Y_{ijk} = observation of the ith genotype in the jth location and Kth replication; l_k = effect of jth location, e_{ii} = effect of experimental error.

Genotypic, environmental and phenotypic variances were estimated (Falconer, 1981) as follows:

Genotypic variance (σ 2 $_{g}$) for single site = (MS_g - MS_e) / r Genotypic variance for combined data (σ 2 _g) = (MS_g – MS_I) **/** rl Interaction variance (σ ²₁) = (MS_I – Ms_e) / r Environmental variance (σ $_{\rm e}$) = MS_e/r Phenotypic variance (σ $_{\rm p})$ = σ $_{\rm g}$ + σ $_{\rm e}$

Where $r =$ number of replication, $g =$ number of genotypes, $L =$ number of sites, $I =$ interaction, MSe = mean square of error, MSr $=$ mean square of replication, MSg $=$ mean square of genotypes, MS $₁$ </sub> = mean square of interaction.

The model for phenotypic and genotypic coefficient of variation is as follows:

 $PCV = [(\delta^2 P)^{1/2} / \mu] \times 100$

Where, PCV = phenotypic coefficient of variation; μ = Population mean

 $GCV = [(\delta^2_{g})^{1/2}J\mu] \times 100$

Where, GCV = phenotypic coefficient of variation; μ = Population mean

PCV and GCV values >20% is regarded as high, 10 to 20% is considered as medium and <10% is considered as low (Kherdade et al., 1985).

Genetic advance (GA) was estimated (Chanyalew et al., 2006) as follows:

 $GA = (\sigma_{g}^2 / \sigma_{g}^2) x k^* \sigma_{g}^2$

Genetic advance as percent of mean = $(GA / mean) \times 100\%$

 σ_{q} and σ_{p} are defined as aforementioned, k (= 2.056) is the selection differential expressed in phenotypic standard deviation depending on the selection intensity chose (= 5%), $\sigma_{\scriptscriptstyle{\text{p}}}$ is phenotypic standard deviation.

Phenotypic (r_{pxy}) and genotypic (r_{gxy}) Pearson's correlation coefficients between two variables were estimated as follows (Robinson et al., 1954; Johnson et al., 1955; Miller et al., 1958):

$$
\begin{array}{c}\nr_{\text{pxy}} = \sigma_{\text{pxy}} / [(\sigma_{\text{px}}^2) (\sigma_{\text{px}}^2)]^{1/2} \\
r_{\text{gxy}} = \sigma_{\text{gxy}}^2 / [(\sigma_{\text{px}}^2) (\sigma_{\text{my}}^2)]^{1/2}\n\end{array}
$$

Where, r_{pxy} = phenotypic and r_{gxy} = genotypic correlation coefficients *between characters x and y;* σ ²_{ρ *xy* = phenotypic covariance and} σ $_{\textrm{\tiny{guy}}=}$ genotypic covariance between x and y

Principal component analysis was done using MINITAB statistical software. The software was ordered such that data of every variable was pre-standardized into a mean of zero and variance of one. Eigenvalue of one was used as minimum threshold to explain the entire variability (Assefa et al., 2003).

RESULTS AND DISCUSSION

Variability

Analysis of variance

ANOVA for data combined over the sites revealed significant (P<5%) variation for all the characters *(*Table 1*)*. The two sites revealed significant (P<1%) difference for all the characters, which was apparent from the experimental field. Accordingly, emphasis was given to site-wise analyses. RILs revealed significant (P<1%) interaction with the environments for all characters except first culm strength.

Individual site also revealed significant variation among genotypes for all the characters at Akaki *(*Table 2) and for all the characters except first culm strength at Debre Zeit (Table 3). Similar result was obtained among the earlier interspecific RILs (Tefera et al., 2003a).

Throughout the discussion, comparable results for similar characters are inclusively stated (for example: First and second culm diameters are stated together without prefix as culm diameter).

Estimates of variability parameters

At Akaki, high PCV (40%) and GCV (32%) values were obtained for grain yield. Lodging index and panicle length showed PCV in the range of 20 to 30%; GCV value of panicle length also fall in the same range. Culm diameter, lodging index, plant height and first culm strength revealed medium GCV (10 to 20%); only second culm strength revealed GCV below 10% (Tables 4 and 5).

Similar pattern of the variability measures were obtained at Debre Zeit. Except for grain yield, however, GCV and GA were narrower*.* A pattern of similarity was observed in other related study (Tefera et al., 2003b). The potential genetic advance as % of mean was very high (53.71%) for grain yield and moderate (≥20%) for all the other characters except culm strength.

Cross-1 versus cross-2 and transgression

Here, RILs obtained from different pollen source (two accessions of *E. pilosa*) were compared as cross-1 and cross-2; the mother plant was the same genotype of *E. tef*, in both crosses and presents RILs that transgress beyond the better parent. Transgrants were identified as per the objective of the study. RILs that revealed lower index than the parental genotype with better performance against lodging were seen for lodging index; while RILs with higher value for the character grain yield and panicle length. The culm characters and plant height were also seen for the important association they would have with both lodging and grain yield.

Table 1. ANOVA: Mean square values, coefficient of variation (CV), and R² of eight characters for 79 genotypes of *E. tef x E. pilosa* crosses – sites data combined.

SV = source of variation; df = Degree of freedom; Rep = replication; RIL= recombinant inbred lines; G x E = interaction; *, **, *** = significant at 5, 1 and 0.1%, respectively; ns = none significant; R 2 = extent (in %) by which a trait explains variability in RILs; Log(x+1) = log(x+1) transformed; Arcsine = Arcsine transformed

Table 2. ANOVA mean square values, coefficient of variation (CV), and R² of eight characters for 79 genotypes of the *E. tef* x *E. pilosa* crosses at Akaki.

 SV = source of variation; df = Degree of freedom; Rep = replication; RIL= recombinant inbred lines; G x E = interaction; *, **, *** = significant at 5, 1 and 0.1%, respectively; ns = none significant; R^2 = extent (in %) by which a trait explains variability in RILs; Log(x+1) = log(x+1) transformed; Arcsine = Arcsine transformed.

At Akaki, RILs transgressed for lodging, panicle length and grain yield. Seven RILs only from cross-2 for lodging index; two RILs for panicle length and three RILs for grain yield transgressed while no transgression was revealed for the culm characters. At Debre Zeit, 23 RILs transgressed for lodging index (three of the RILs transgressed at both sites) while no transgression was revealed for grain yield. Four RILs transgressed for plant height at Debre Zeit; opposing/alternating output was obtained for plant height and panicle length in the two locations.

No RIL transgressed from cross-1 for lodging index and grain yield. The transgression suggested presence of additive gene action for the transgressed characters; those favorable additive alleles are brought by both parents (Tefera et al., 2003b).

Most of the RILs that revealed good performance compared with the check cultivars were from cross-2 for panicle length and grain yield implying potential of obtaining more variability by further alternating parental accessions in future breeding efforts. RIL2-148 and RIL2-126 at Akaki and together with RIL2-47 and RIL2-118 at Debre Zeit revealed longer panicle length over one of the two check cultivars (DZ-Cr-37). No RIL outsmarted

Table 3. ANOVA mean square values, coefficient of variation (CV), and R² of eight characters for 79 genotypes of the E. tef x E. pilosa crosses at Debre Zeit.

SV = source of variation; df = Degree of freedom; Rep =replication; RIL= recombinant inbred lines; *, **, *** = significant at 5, 1, and 0.1%, respectively; ns = non significant; R² = extent (in %) by which a trait explains variability in RILs; Log $(x+1) = log(x+1)$ transformed: Arcsine = Arcsine transformed.

DZ-Cr-37 while RIL2-188 was higher than quncho (the other check cultivar) for grain yield at Debre Zeit. Four RILs from cross-2 and quncho were tallest at Akaki, while only quncho was tallest but three RILs of cross-2 were taller than DZ-Cr-37 at Debre Zeit.

In culm characters, Relative difference was revealed between the crosses in culm character; cross-2 was better from among highest grain yielding genotypes. No RIL was thicker than quncho while few RILs revealed thicker culm over the other check cultivar (DZ-Cr-37). Culm strength is assumed to contribute more for lodging resistance than culm thickness. Quncho was among the most rigid culms while DZ-Cr-37 revealed lower rigidity than RIL2-126 and RIL1-84 only at Akaki.

Association of characters

Estimates of correlation coefficients

Analysis of all (28) bivariate association indicated that almost all the associations are positive. Pattern of strength between phenotypic and genotypic associations among most pairs of traits was very close and similar which may imply considerably higher effect of inheritance in the phenotypic expression. However, the dual nature of phenotypic correlation makes it clear that the magnitude of genetic correlation cannot be determined from phenotypic correlation (Saleem et al., 2006).

Test of significance was done for phenotypic correlation. Discussion for genotypic association was done by comparing strength of correlations relative to each other. Associations with reference to grain yield and lodging were given due attention as per the objective of the crossing. These characters result from cumulative effects (Izge et al., 2006); may also provide generalized statements-within for associations of other characters; consistent with results of other studies (Chanyalew et al., 2006; Tefera et al., 2003a).

Associations with lodging index

Phenotypically, lodging index was found associated with second culm strength, plant height and panicle length at Akaki. However, only

the association with second culm strength was very strong (0.90); all other associations revealed r_p below 0.40. The same trend was also observed at Debre Zeit, with slightly reduced strength of association to second culm strength (0.90 to 0.77) and panicle length (0.30 to 0.25) at this site.

Genotypically, association between lodging index and second culm strength was very high (0.9) at both sites. Association of most other traits with lodging index was also substantially high including plant height and grain yield mainly at Akaki and except culm diameter at Debre Zeit. From this result, one would be confident about the consistently strong association of lodging index with second culm strength; no study is found to be compared with. The obvious association of lodging index with traits like plant height and panicle length is in line with results of many studies including recent ones (Chanyalew et al., 2006).

Lodging index was least associated with other traits. It revealed very little association with first culm strength. This may contradict with the obvious logic if not explained by lack of objectivity in collecting the data and/or the scale of indexing the lodging which might have underestimated

Table 4. Range, grand mean, phenotypic (PCV) and genotypic (GCV) coefficients of variation, heritability (H) and genetic advance as % of mean (GA % mean) for eight characters in 79 genotypes of *E. tef* x *E. pilosa* crosses at Akaki.

Min = minimum, max = maximum, PH = plant height, LI = lodging index, FCD = first culm diameter, SCD = second culm diameter, FCS = first culm strength, SCS = second culm strength, PL = panicle length, GYId = grain yield.

Table 5. Range, grand mean, phenotypic (PCV) and genotypic (GCV) coefficients of variation, heritability (H) and genetic advance as % of mean (GA % mean) for eight characters in 79 genotypes of *E. tef* x *E. pilosa* crosses at Debre Zeit.

Min = Minimum, max = maximum, PH = plant height, LI = lodging index, FCD = first culm diameter, SCD = second culm diameter, FCS = first culm strength, SCS = second culm strength, PL = panicle length, GYld = grain yield.

Table 6. Phenotypic (above the diagonal) and genotypic (below the diagonal) coefficients of correlation among eight characters in 79 lines of the *E .tef* x *E. pilosa* crosses at Akaki.

FCD = first culm diameter, SCD = second culm diameter, FCS = first culm strength, SCS = second culm strength, PH = plant height, GYld = grain yield, LI = lodging index, PL = panicle length.

the index.

Associations with grain yield

Phenotypically, grain yield was found associated with

plant height, panicle length and culm characters except first culm strength at Akaki and not associated with lodging index (Table 6). Genotypically, greater association was found and was considerably higher with plant height and panicle length. Genotypic association of grain yield with lodging index was considerably high $(r_q=0.53)$ at

Table 7. Phenotypic (above the diagonal) and genotypic (below the diagonal) coefficients of correlation among eight characters in 79 lines of the *E. tef* × *E. pilosa* crosses at Debre Zeit.

FCD = first culm diameter, SCD = second culm diameter, FCS = first culm strength, SCS = second culm strength, PH = plant height PL = panicle length, LI = lodging index, GYId = grain yield.

Table 8. Eigenvector, eigenvalue and variability explained by first three principal components for eight characters of the 79 lines of *E. tef* × *E. pilosa.*

Akaki though it was the weakest $(r_q=0.25)$ compared to others at Debre Zeit.

The weak association of grain yield with lodging index may require unique explanation. In small plots, as in experimental fields, yield may easily be secured from lodged plants without loss that would come due to lodging otherwise. In wide acres of tef production, yield loss due to lodging may be high during harvesting for mechanical reasons and due to post maturity climatic hazards like rainfall.

In a casual route of associations with grain yield, high lodging by no means can be a cause for high grain yield rather to reduced grain yield. It is logical to consider grain yield as causal variable always; high lodging index due to high yield. Reducing effect of lodging on grain yield is partly of post physiological mechanism. Low grain yield and shorter plant height was recorded at Akaki that might have contributed to the weak association between grain yield and lodging index there; effects could rather be more

of environmental.

Sometimes correlation coefficients greater than one can be found and considered strong (Izge et al., 2006). At Debre Zeit, this phenomenon was reflected by few bivariates; second culm strength with plant height and panicle length. Other very strong association was panicle length with plant height ($r_a > 0.85$) (Tables 7 and 8).

Principal component analysis

In the analysis made to estimate relative contribution of the traits studied towards the overall phenotypic variation among the 79 lines, the first three principal components with eigenvalues of greater than unity explained greater than 75% of the total variation. The first principal component ($PC₁$) alone explained about 44% of the entire variability at Akaki, while the second and third principal components (PC $_2$ and PC $_3$) explained about 19 and 13%, respectively, of the entire variability among the lines.

Similarly, PC_1 , PC_2 and PC_3 each explained 49, 18 and 13%, respectively, of the entire variability at Debre Zeit.

The principal component of gross variability among the recombinant inbred lines, which is greater than 44% of the variation, was mainly accounted by culm diameter, plant height and panicle length at Akaki while comparably distributed among all the variables except lodging index and grain yield at Debre Zeit.

The second PC, about 18%, is predominantly accounted by second culm strength and lodging index at both sites. The third PC, about 13%, is by far originated from by grain yield at both sites. However contribution of most variables to the third PC, except second culm strength and lodging index at both sites, was considerable.

It was interesting to see such consistent result between the two sites. It may also be worth dealing with all the variables considered in this study towards articulating major amount of the gross variation in the subject genotypes. Accordingly, the major gross variation among the recombinant inbred line and between genotypes of their parental species *(E. tef and E. pilosa)* would considerably be accounted by all the variables included in this study; however contribution of plant height, panicle length and culm diameter appears more conspicuous.

CONCLUSION AND RECOMMENDATION

The study revealed presence of considerable genetic variability among the RILs. For most of the characters, the variability did not show huge gap from phenotype to genotype revealing prominence of the genetic components and hence appropriate to draw breeders' attention. In this study, data collected on shattering behavior and plant stand-count revealed presence considerable variability among the RILs which might have accounted for some of the variability observed in grain yield. The genotype x site interaction indicated importance of testing the RILs in more sites to represent major tef growing conditions.

Genetic advance for grain yield was very high; considerably high genetic advance could be attained through working with plant height, panicle length and lodging index. The genetic advance for lodging index would be promising in view of meager variability that has been reported for decades.

The association of grain yield with most other traits may be a common scenario and its improvement would require dealing with many traits. The maximum variability recorded for grain yield would also have reflected immense variability within for vast majority of traits of tef that are not considered in this study. Future studies need to consider more specific traits that would be pivotal for grain yield improvement. Lodging index in association with second culm strength has indicated new dimension for further investigation as it showed consistently strong association in all the conditions considered in this study. Instinctively, lodging is more dependent on grain yield

than the reverse. As a consequence, however, lodging complicates plant conditions around maturity and reduces the total grain harvest especially in wider scale tef cultivation as compared to research plots. In the study, however, effect of grain yield on lodging index was weak. Many characters would interplay between grain yield and lodging. If plant height is considered for example it has positive association with both grain yield and lodging index. Perhaps the character plant height should have been viewed excluding the length containing the grain (the panicle length) or characters like spikelet per unit length of panicle and seed-weight shall receive more attention. Different aspects of looking on characters would open an entry point in an effort to reducing such contradicting desires.

The study demonstrated availability of genetic variability for a number of heritable characters in the RILs for exploitation through selection and presence of promising inbred RILs for further breeding. Plant height, panicle length and second culm strength are front-liner characters with respect to gross variability observed among the RILs. The study also implied potential of obtaining more variability through alternating parental accessions of *E. Pilosa* for future endeavours. In general, it can be said that the study has verified the importance of *E. pilosa* in diversifying the germplasm pool for tef breeding.

ACKNOWLEGMENT

Mcknight Foundation Collaborative Crop Research Program in Tef Genetics and Breeding (Grant No. 02-451) and the host institute, EIAR, deserve thanks for funding and facilitating the work. The authors gratitude also goes to Dr Solomon Chanyalew of Melkassa Agricultural Research Center, Dr Abiy Zegeye of Addis Ababa University and Nigusu Hussien of Debre Zeit Agricultural Research Center for their unreserved assistance. Staff members of EIAR Head Quarter, Debre Zeit and Pawe Agriculture Research Centers also played considerable role for this work to be effective, many thanks.

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