# Genetic Variation, Genotype by Environment Interactions and Grain Yield Stability Analysis in Finger Millet Accessions Resulted in the Release of an Improved Variety

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## Abstract

*The use of multiple data sets, such as morphological, biochemical and molecular in combination with appropriate statistical analysis tools are essential in identifying inter and intra-species variation to develop improved cultivars. To this end, a total of 150 finger millet accessions, of which 105 were collected from Ethiopia, 39 introduced from eastern and south eastern Africa and six commercially released Ethiopian varieties were evaluated at Arsi Negele and Gute research sites in 2011. Among those, 138 accessions were genotyped using 20 Simple Sequence Repeat (SSR) markers at International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Nairobi, in 2012. Highly significant ($P \leq 0.01$) variations were observed among the 150 accessions for grain yield and other agronomic traits. A total of 199 alleles were recorded with an average of 9.95 alleles per microsatellite locus and polymorphism information content (PIC) of 0.57 was observed. Hierarchical clustering based on major phenotypic traits revealed that the majority of accessions from the same region and adjoining geographical region shared strong phenotypic similarity and thus grouped together. Weighted Neighbor Joining based on SSR data grouped the test accessions into three major clusters that were not entirely based on geographical origin. Based on the magnitude of phenotypic and genotypic diversity and blast disease tolerance, 30 finger millet genotypes were selected for further evaluation at multi-location (Arsi Negele, Assosa, Bako and Gute) in the 2012 and 2013. Additive Main effect and Multiplicative Interaction (AMMI), and Genotype and Genotype by Environment Interaction (GGI) biplot analysis revealed that Acc. 203544 was found to be the most stable and highest yielding (3.16 ton ha-1), with yield advantage of 13.7% over the best standard check, Gute (2.78 ton ha-1). Thus, this accession was officially released with the name "Addis-01" and recommended for production in the test environments and similar agro-ecologies in the country.*

**Keywords**: Additive main effect and multiplicative interaction (AMMI), Genotype by Environment Interaction (GGI), Simple Sequence Repeat (SSR).

# Introduction

Finger millet (*Eleusine coracana* subsp. *coracana)* is extensively cultivated in the tropical and sub-tropical regions of Africa and India, and is known to save the lives of poor farmers from starvation during times of extreme drought (Upadhyaya *et al*. 2006). The crop is adapted to adverse agro-ecological conditions, requires minimal inputs, tolerant to moisture stress and acidic soils, and generally thrives on marginal land where other crops cannot perform (Barbeau and Hilu, 1993). Finger millet also plays an important role in food security due to its high nutritional value and good storage quality (Dida *et al*. 2007).

Archaeological records reveal that the primary center of origin for finger millet is East Africa, particularly Ethiopia (Purseglove, 1972). Understanding the extent of genetic diversity of finger millet genotypes cultivated in Ethiopia, therefore, represents an important resource for the study of finger millet genetics and genome evolution. Genetic diversity assessment aids in understanding intra-species crop performance that can be exploited in crop improvement (Aremu, 2011), and provides information on the extent of genetic divergence. Diversity assessment also serves as a platform for specific breeding objectives (Thompson *et al*. 1998) and identifies parental combinations exploitable to create segregating progenies with maximum genetic potential for further selection (Dje *et al*. 2000). Successful genetic conservation and utilization of any crop is largely dependent on understanding the genetic diversity and its distribution in a given region (Varshney *et al*., 2007).

Although Ethiopia is the center of origin and domestication for finger millet, comprehensive studies on finger millet diversity using morphological or molecular markers are generally limited (Kebere *et al*., 2006), few accessions have been characterized at morphological level (Dagnachew *et al*., 2012a,b; Chemeda *et al*., 2008, Andualem *et al*., 2011, Kebere *et al*., 2006, Yemane and Fasil, 2002) and few study has been conducted at the molecular level using RAPD markers (Kebere, 2011) and SSR marker (Dagnachew *et al*., 2014 a,b). Identifying adaptable and stable high yielding genotypes with other desirable traits under varying environmental conditions to recommend a new variety (ies) for release as cultivars is fundamental and this has direct bearing on the adoption of a variety, productivity and total production of the crop (Showemimo *et al*., 2000; Mustapha *et al*., 2001). Therefore, this study aimed to assess the genetic variations in finger millet accessions collected from various regions of Ethiopia and introduced accessions using morphological and molecular markers and further

evaluate selected diverse genotypes at multilocation in order to identify and release stable high yielding genotypes for wider adaptability.

# Materials and Methods

## Phenotypic diversity analysis

One hundred and five finger millet accessions collected from the major finger millet producing regional states of Ethiopia (Amhara, Benishangul Gumuz, Oromia, Tigray and Southern Nations Nationalities and Peoples Region (SNNP)), 39 introduced accessions from Kenya, Zambia, Zimbabwe and Eritrea, and six improved varieties were used. The experiment was conducted at Arsi Negele Research Sub-site (altitude 1947 m a.s.l., $07^0$19" N, $38^0$39" E) and Gute Research Sub-site (1906 m.a.s.l., $09^0$00" N, $36^0$38" E) in 2011 main cropping season to assess the genetic diversity and eco-geographical patterning. Field data were recorded for the major traits such as days to 50% heading, days to maturity, productive tiller number, plant height (cm), finger length (cm), ear weight (g), finger number per main ear, number of grains per spikelet at the center of the finger, culm diameter, finger width, lodging index, harvest index, thousand grain weight (g) and grain yield per plant (g) following the finger millet descriptor (IBPGR, 1985). Phenotypic traits data were analyzed using SAS (SAS, 2008) for ANOVA and principal component analysis and MINITAB14 (MINITAB, 2003) software for cluster analysis.

## DNA Extraction and Polymerase Chain Reaction (PCR)

DNA was extracted from young leaf samples according to the modified CTAB protocol of Mace *et al.* (2003). Extracted DNA was visualized on a 0.8% (w/v) agarose gel and quantified using a Nanodrop® 1000 spectrophotometer (Thermo Scientific, USA). DNA samples were subjected to genotyping using 20 published SSR markers for finger millet (Dida *et al*., 2007) (Table1). All forward primers contained an M13-tag (5'-CACGACGTTGTAAAACGAC - 3') on the 5' end that was fluorescently labeled to allow detection of amplification products (Schuelke, 2000). PCR amplification was performed in 10 µl reaction volume comprising of 1 x PCR buffer (20 mM Tris-HCl, pH 7.6; 100 mM KCl; 0.1 mM EDTA; 1 mM DTT; 0.5% (w/v) Triton X-100; 50% (v/v) glycerol), 2 mM $MgCl_2$, 0.16 mM dNTPs, 0.16 µM fluorescent labeled M13-forward primer, 0.04 µM forward primer, 0.2 µM reverse primer, 0.2 units of Taq DNA polymerase (SibEnzyme Ltd, Russia) and 30ng of template DNA. PCR reactions were performed in 384 well microtiter plates on a GeneAmp 9700 thermocycler (Applied Biosystems) with initial denaturation of 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 59°C for 1 minute, extension at 72°C for 2 minutes and the final elongation at 72°C for 20 minutes.

## SSR fragment detection and data analysis

Amplification was confirmed by running the PCR products on a 2% (w/v) agarose gel stained with GelRed® (Biotium, USA) and visualized under UV light. Amplification products (1.5 µl – 3.5 µl of each) were co-loaded in sets of 3 to 4 markers together with the size standard GeneScan™ –500 LIZ® (Applied Biosystems) and Hi-Di™ Formamide (Applied Biosystems), and separated by capillary electrophoresis using an ABI Prism® 3730 Genetic analyzer (Applied Biosystems). Allele calling was performed with GeneMapper 4.0 (Applied Biosystems). PowerMarker ver. 3.25 software (Liu and Muse, 2005) was used to calculate polymorphism information content (PIC) and other summary statistics. The weighted Neighbor Joining based clustering was computed using DARwin v.5 (Perrier and Jacquemoud 2006).

## Comparison of selected genotypes with the original study materials

Ten percent of the best performing accessions were sorted for each phenotypic trait independently and compared with the total accessions. The absolute value of Student's Z test was calculated following the formula suggested by Singh (2001) to compare the values of the 10% best selected genotypes with the base population as:

$$Z = \frac{(\bar{X} - \mu)}{\delta/\sqrt{n}}$$

Where $\bar{X}$ = mean of selected genotypes, µ = mean of the base populations, δ = standard deviation for the base populations and n = number of genotypes selected from the base population for better performance. The significance of the difference between the total and sampled accessions trait mean tested using the Z table. When the calculated value of the Z-test is more than the tabulated Z value, the difference is considered significant (Singh, 2001).

## Genotype by Environment interactions and stability analysis

Based on phenotypic and molecular diversity, the SSR genetic distances and their potential resistance/tolerance to blast disease, a total of 30 genotypes were selected and evaluated along with two standard checks (Gute and Tadesse) across four locations (Arsi Negele, Assosa, Bako and Gute) in 2012 and 2013 main cropping seasons. The design used was randomized complete block design (RCBD) replicated three times. Data for grain yield and yield related traits were collected. Additive Main effect and Multiplicative Interaction (AMMI), Eberhart and Russell regression model, Genotype and Genotype by Environment Interaction (GGE) biplot analysis were employed to identify stable and high yielding genotypes for possible release.

Table 1: List of SSR markers used for study, repeat motifs and primer sequences

| Primer | Forward primer sequence | Reverse primer sequence | Repeat motif | Mapped |
|---|---|---|---|---|
| UGEP05 | TGTACACAACACCACACTGAT | TTGTTTGGACGTTGGATGTG | (TC)12AC(TC)4 | 9B |
| UGEP20 | GGGGAAGGCAATGATATGTG | TTGGGGAGTGCCAACAATAC | $(GA)_{20}$ | ND |
| UGEP27 | TTGCTCTGAGGTTGTGTGTTGC | TCAAGCATAGTGCCCTCCTC | $(GA)_{19}$ | ND |
| UGEP24 | GCCTTTTGATTGTTCAACTCT | CGTGATCCTCTCCTCTCTG | (GA)26 | 3B |
| UGEP12 | ATCCCCACCTACGAGATGC | TCAAAGTGATGCGTCAGGTC | (CT)22 | 8B |
| UGEP84 | GGAACTTCCGTCAGTCCTT | TGGGGAAGGTGTTGAATC | $(CT)_{24}$ | ND |
| UGEP96 | TAATGGGCCTAATGGCAATG | CAAAATCCGAGCCAAGATTC | $(CT)_{10}$ | ND |
| UGEP98 | GTCTTCCATTTGCAGCAACC | ACGCGTACTGACGTGCTTG | $(GCC)_8$ | ND |
| UGEP67 | CTCCTGATGCAAGCAAGGAC | AGGTGCCGTAGTTTGTGCTC | $(TC)_{22}TT(GT)_5$ | ND |
| UGEP79 | CCACTTTGCCGCTTGATTAG | TGACATGAGAAGTGCCTTGC | $(CT)_{12}$ | ND |
| UGEP33 | TAGCCGTTTGCTTGTTGTTTTG | AAGGCCCTAGAACGTCAAGC | $(TC)_{18}$ | ND |
| UGEP46 | CAAGTCAAAACATTCAGATGG | CCACTCCATTGTAGCGAAAC | $(GA)_{14}$ | ND |
| UGEP53 | TGCCACAACTGTCAACAAAAG | CCTCGATGGCCATTATCAAG | (AG)26 | 2A |
| UGEP57 | CCATGGGTTCATCAAACACC | ACATGAGCTCGCGTATTGC | $(AG)_{16}$ | ND |
| UGEP64 | GTCACGTCGATTGGAGTGTG | TCTCACGTGCATTTAGTCAT | $(CT)_{23}$ | ND |
| UGEP66 | CAGATCTGGGTAGGGCTGTC | GATGGTGGTTCATGCCAAC | $(AG)_{29}$ | ND |
| UGEP95 | AGGGGACGCTTGGAGTTTG | GCCTCTACCTGTCTCCGTTG | $(TC)_{14}$ | ND |
| UGEP73 | GGTCAAAGAGCTGGCTATCG | ACCAGAACCGAATCATGAGG | $(CT)_4CC(CT)_{10}$ | ND |
| UGEP106 | AATTCCATTCTCTCGCATCG | TGCTGTGCTCCTCTGTTGAC | (AC)12 | 9B |
| UGEP110 | AAATTCGCATCCTTGCTGAC | TGACAAGAGCACACCGACTC | (CT)12 | 7AB |

Key: ND=not done, B=B genome, A= A genome, AB=both A and B genome of *Eleusine coracana* subsp *coracana* (Dida *et al*., 2007)

# Result and discussion

## Analysis of variance and mean performances

The combined analysis of variance (ANOVA) over the two locations showed significant location effects for all quantitative traits considered in the present study (Table 2). Genotype mean squares were also significant ($P \leq 0.01$) for all quantitative traits except ear weight, implying the possibility for selection. Similarly, highly significant variations among finger millet genotypes were reported in previous studies (Kebere *et al.,* 2006; Yemane and Fasil, 2002).

## Patterns of quantitative traits variation and its intrinsic value for breeding

Wider ranges of variations were observed among finger millet accessions for all quantitative traits (Table 3). Such variation is crucial for effective collection, conservation and sustainable improvement of finger millet by combining the desirable traits. Days to maturity ranged from 143 days for accession 230103 of Eritrea collected from altitude of 1700 m.a.s.l. to 167 days for Acc. BKFM0018 of Oromia collected from altitude of 1667 m.a.s.l. This offers great flexibility for developing improved varieties suitable for various agro-ecologies of the countries or

regions with variable length of growing period and also can be recommended for various cropping systems. It also guides breeders to develop a variety which can escape late season drought by improving traits which correlate to days to maturity in the required direction.

Likewise, plant height ranged from 41.13cm for Acc. 214991 of Zambia collected from 1330 m.a.s.l to 103.35 cm for Acc. 215802 of Amhara collected from 1950 m.a.s.l. For finger length, Acc. 229730 collected from Amhara attained the highest score (11.4cm) and Acc. 203357 of Zimbabwe were the shortest (3.53cm). Thousand grain weights ranged from 3.5g for Acc. 203546 (Kenya) to 1.4g for Acc. 229724 (Benishangul Gumuz). Grain yield per plant was highest (41.6g) for Acc. 242132 of Amhara collected from an altitude of 1910 m.a.s.l, and lowest (6.12g) for Acc. 214991 of Zambia collected from 1330 m.a.s.l. The variation in plant height, culm diameter, culm branch and tillering capacity indicated the possibility to combat lodging. Variation in number of fingers per main ear, finger length, number of grains per spikelet, harvest index, thousand grain weight and grain yield per plant implied that it is possible to create a variety with higher grain yield and/or other biological yields.

Table 2. Mean squares for 14 quantitative traits of 150 finger millet genotypes evaluated two locations, Gute and Arsi Negele, in Ethiopia 2011

| Source of variation | df | DH | DM | PTN | PLHT | FL | FN | EW |
|---|---|---|---|---|---|---|---|---|
| Environment | 1 | 4066.4** | 11102.6** | 3087.2** | 47638.2** | 28.1** | 36.66** | 72.5** |
| Genotype | 149 | 315.40** | 89.26** | 11.48** | 491.75** | 15.1** | 4.85** | 5.32* |
| G x E | 149 | 51.24 | 44.13** | 8.20** | 122.75** | 2.45** | 1.21** | 1.09** |
| Error | 298 | 46.83 | 13.01 | 1.19 | 35.58 | 0.94 | 0.65 | 0.74 |
| CV (%) | | 7.05 | 2.29 | 19.55 | 8.68 | 12.12 | 11.09 | 32.44 |
| LSD (5%) | | 7.98 | 4.21 | 1.27 | 6.95 | 1.13 | 0.94 | 1.00 |
| Mean | | 97.01 | 157.73 | 5.55 | 68.75 | 7.98 | 7.23 | 2.65 |
| Environment | 1 | 135** | 2129** | 13.2** | 0.02 | 228150** | 27859.1** | 28912** |
| Genotype | 149 | 1.07** | 0.389** | 0.08** | 0.8** | 1546.3** | 673.3** | 182.8** |
| G x E | 149 | 0.37 | 0.32 | 0.05 | 0.20 | 642.79** | 239.7** | 111.90 |
| Error | 298 | 0.34 | 0.27 | 0.05 | 0.17 | 82.50 | 199.96 | 53.61 |
| CV (%) | | 12.21 | 22.01 | 28.72 | 18.52 | 20.57 | 31.30 | 35.85 |
| LSD (5%) | | 0.71 | 0.61 | 0.26 | 0.49 | 10.59 | 16.48 | 8.54 |
| Mean | | 4.39 | 2.37 | 0.79 | 2.26 | 44.15 | 23.10 | 20.42 |

**KEY**: CD=culm diameter, CV=coefficient of variation, df= degree of freedom, DH=days to50% heading, DM= days to maturity, EW= finger weight, FL= finger length, FN= finger number, FW=Finger width, G x E = genotype by environment interaction, GYPLN=grain yield per plant, HI=Harvest index, LSD = least significant difference, LOG= lodging index, NGPS=number of grain per spikelet, PLHT= plant height, PTN= productive tiller number, TGW=thousand grain weight

Among regions and countries of origin, the mean days to maturity, plant height, finger length and finger number were higher for accessions from Ethiopia (B/Gumuz, Oromia, Amhara and SNNP). Accessions from Eritrea were characterized as early maturing with fewer fingers per ear. Accessions from Zambia had shorter plants and least grain yield per plant, but higher number of grains per spikelet. Lowest number of grains per spikelet and thousand grain weight were recorded for B/Gumuz region. The highest mean grain yield per plant and thousand grain weights were recorded for Kenya (Table 3). The existence of a vast range of genetic variability in finger millet germplasm were also reported by Yemane and Fasil (2002).

Table 3. Patterns of genetic variability for 14 major quantitative traits among finger millet accessions and regions of origin

| Traits | Accession/population level | | | Regional/country level | | | Mean ±SE |
|---|---|---|---|---|---|---|---|
| | Minimum | Maximum | Range | mini | max | Range | |
| Days to 50% heading | 82.25 | 117.00 | 34.75 | 86.69 | 107.59 | 20.90 | 97.01±0.73 |
| Days to 50% maturity | 143.0 | 167.25 | 24.25 | 150.72 | 161.75 | 11.03 | 157.73±0.39 |
| No. of productive tillers | 2.58 | 10.50 | 7.92 | 4.08 | 6.93 | 2.85 | 5.55±0.14 |
| Plant height (cm) | 41.13 | 103.35 | 62.22 | 56.03 | 77.17 | 21.14 | 68.75±0.91 |
| Finger length (cm) | 3.53 | 11.40 | 7.87 | 5.07 | 9.83 | 4.76 | 7.98±0.16 |
| Finger number | 5.10 | 11.68 | 6.58 | 6.71 | 8.16 | 1.45 | 7.23±0.09 |
| Ear weight(g) | 1.10 | 5.53 | 4.43 | 1.58 | 4.54 | 2.96 | 2.65±0.09 |
| No. of grains/spikelet | 2.95 | 6.35 | 3.40 | 3.83 | 4.65 | 0.82 | 4.39±0.06 |
| Culm diameter (cm) | 1.57 | 3.29 | 1.72 | 1.99 | 2.61 | 0.62 | 2.38±0.03 |
| Finger width (cm) | 0.60 | 1.77 | 1.17 | 0.69 | 0.89 | 0.20 | 0.79±0.01 |
| Lodging (%) | 7.50 | 80.00 | 72.50 | 30.18 | 59.91 | 29.73 | 44.15±1.61 |
| Harvest index (%) | 9.39 | 51.96 | 42.57 | 15.7 | 30.47 | 14.77 | 22.71±0.65 |
| 1000 grain weight (g) | 1.40 | 3.50 | 2.10 | 1.92 | 2.91 | 0.99 | 2.26±0.04 |
| Grain yield/plant (g) | 6.12 | 41.60 | 35.48 | 15.27 | 25.85 | 10.58 | 20.42±0.49 |

## Marker characterization

Analysis of polymorphism revealed that polymorphic information content (PIC) ranged from 0.12 (UGEP 96) to 0.94 (UGEP 24) with an average of 0.57 PIC per marker (Table 3). Some microsatellite markers such as UGEP024, UGEP067, UGEP064 and UGEP066 revealed the highest PIC of 0.94, 0.90, 0.88 and 0.87, respectively, and amplified higher numbers of alleles but had low major allele frequencies. Markers UGEP110 and UGEP005 amplified sets of alleles in two clearly different size ranges and hence were split and scored as two separate markers each (Table 3). Markers UGEP98 and UGEP20 were excluded from analysis and interpretation due to monomorphisms and poor amplification, respectively. About 60% of the markers depicted PIC values above the average (0.57) indicating that the majority of markers were able to distinguish the differences among the examined finger millet accessions (Table 3). Relatively lower average PIC values were reported in other studies conducted on finger millet; using SSRs (Panwar *et al*., 2010); using RAPD (Kebere 2011;

Das and Misra, 2010); and using Cyt P450 gene (Panwar *et al*., 2010).

The number of alleles per locus varied from 2 (UGEP084) to 24 (UGEP024), and a total of 199 alleles were observed with an average of 9.95 alleles per locus. Similar results were reported by Das and Misra (2010) and Kebere (2011) using different molecular markers in finger millet. Besides, highly significant ($P \leq 0.01$) and significant ($P \leq 0.05$) allelic differences were detected by 15 and 2 SSR markers, respectively, but three markers (UGEP96, UGEP110_1 and UGEP005_1) showed non-significant allelic differences (Table 3). The high number of alleles per locus and total genetic diversity found in this study demonstrated the presence of genetic variation among finger millet accessions studied.

Table 3. Summary of 20 SSR markers used in the present study

| Marker | Maj.Allele Freq. | Allele No | Heterozygosity | PIC | P-Value |
|---|---|---|---|---|---|
| UGEP53 | 0.36 | 13 | 0.11 | 0.78 | 0.001 |
| UGEP84 | 0.53 | 2 | 0.00 | 0.37 | 0.001 |
| UGEP27 | 0.24 | 15 | 0.10 | 0.84 | 0.000 |
| UGEP95 | 0.25 | 7 | 0.07 | 0.77 | 0.001 |
| UGEP64 | 0.16 | 16 | 0.15 | 0.88 | 0.000 |
| UGEP67 | 0.20 | 20 | 0.16 | 0.90 | 0.000 |
| UGEP106 | 0.60 | 9 | 0.17 | 0.57 | 0.018 |
| UGEP110_1 | 0.79 | 5 | 0.00 | 0.32 | 0.383 |
| UGEP110_2 | 0.77 | 3 | 0.00 | 0.30 | 0.000 |
| UGEP57 | 0.44 | 8 | 0.12 | 0.68 | 0.000 |
| UGEP96 | 0.94 | 5 | 0.07 | 0.12 | 0.813 |
| UGEP66 | 0.20 | 20 | 0.11 | 0.87 | 0.000 |
| UGEP79 | 0.64 | 5 | 0.15 | 0.45 | 0.004 |
| UGEP12 | 0.34 | 12 | 0.16 | 0.73 | 0.000 |
| UGEP73 | 0.90 | 6 | 0.03 | 0.18 | 0.014 |
| UGEP005_1 | 0.93 | 6 | 0.00 | 0.13 | 0.141 |
| UGEP005_2 | 0.90 | 5 | 0.02 | 0.18 | 0.001 |
| UGEP24 | 0.10 | 24 | 0.26 | 0.94 | 0.000 |
| UGEP46 | 0.34 | 7 | 0.23 | 0.70 | 0.003 |
| UGEP33 | 0.46 | 11 | 0.30 | 0.62 | 0.001 |
| Mean | 0.50 | 9.95 | 0.11 | 0.57 | |

PIC = Polymorphic information content

## Hierarchical clustering using phenotypic traits

### Population level

At 85% similarity level, all 150 accessions were grouped into eight clusters but three accessions (Acc-216057, Acc-241768 and Acc-238344) and one improved cultivar (Wama) found to be outlier (Fig not shown). The majority of accessions from the same region and adjoining geographical regions, for instance,

Amhara, Tigray and Eritrea shared strong phenotypic similarity and grouped together. The similarity could be either due to the fact that farmer's selection criteria for a given trait might have been similar particularly based on the adaptive role of traits for the environment, or the primary seed source could have been the same, or there might be a high tendency of seed exchange. Supportive results were also reported by Reddy *et al.* (2009); Kebere *et al.* (2006) and; Yemane and Fassil (2002). On the other hand, collections from Oromia, SNNP, Benishangul Gumuz and Zambia were distributed across several of the clusters to variable degrees, which indicated variation among accessions of the same region or country.

## Regional/country level

All the accessions were grouped into four clusters at 85% similarity level but three varieties that remained as an outliers (Fig. 1). Accessions collected from Tigray and Amhara strongly related with those from Eritrea and grouped in the first cluster (C-I). Accessions in this cluster are mainly characterized by longer mean finger (9.07cm), larger number of productive tillers (6.3), high grain yield per plant (22.56 g) and harvest index (26.91%), but found to have the lowest ear weight (1.86 g). The second cluster (C-II) comprised of accessions from Oromia and Benishangul Gumuz regions, and the accessions were found to have late heading (104 days) and maturity (161) periods. Accessions introduced from Kenya, Zambia and Zimbabwe were grouped in the third cluster, and this cluster was known for its shorter mean plant height (63cm) and longer finger (5.87cm). Reddy *et al.* (2009) reported finger millet accessions collected from Kenya, Tanzania and Uganda were grouped together, but accessions from Ethiopia and Burundi were in separate clusters each.

The improved varieties such as Gute and Padet were grouped in the fourth cluster, these varieties showed the highest mean plant height (75.45cm), culm diameter (3.14cm) and finger width (1.1cm), but the lowest number of productive tillers (3.08). Bereda, Boneya, Taddesse and Wama as well as accessions from SNNP stood alone as outliers.

## Comparison of selected accessions with the original study materials

The base finger millet accessions were compared against the top 10% best performing accessions for major quantitative traits having significant association with grain yield. Highly significant differences were observed between the means of selected subsets of the top 10% of accessions and the base finger millet accessions for all quantitative characters (Table 4). This revealed the possibilities for different levels of improvement through selection. The highest differences of 90.94% and 73.57% were observed for ear weight and lodging index, respectively (Table 4). The selected 10% best yielding accessions showed

53.77% increment over the mean of the base population. Comparison of mean grain yield of the 10% best accessions showed 30.75% yield advantage over the nationally released variety (Tadesse). Most of those best performing genotypes for grain yield and other traits were promoted to further multilocation evaluation for grain yield stability and other important agronomic traits.

## Weighted Neighbor joining analysis

The weighted Neighbor Joining tree constructed from pairwise genetic distances in the current study revealed that, unlike the phenotypic traits, clustering of African accessions was not entirely based on their geographical origin (Fig. 2). Dida *et al.* (2008), Kebere (2011) and Manyasa *et al.* (2014) reported similar findings to the present study.

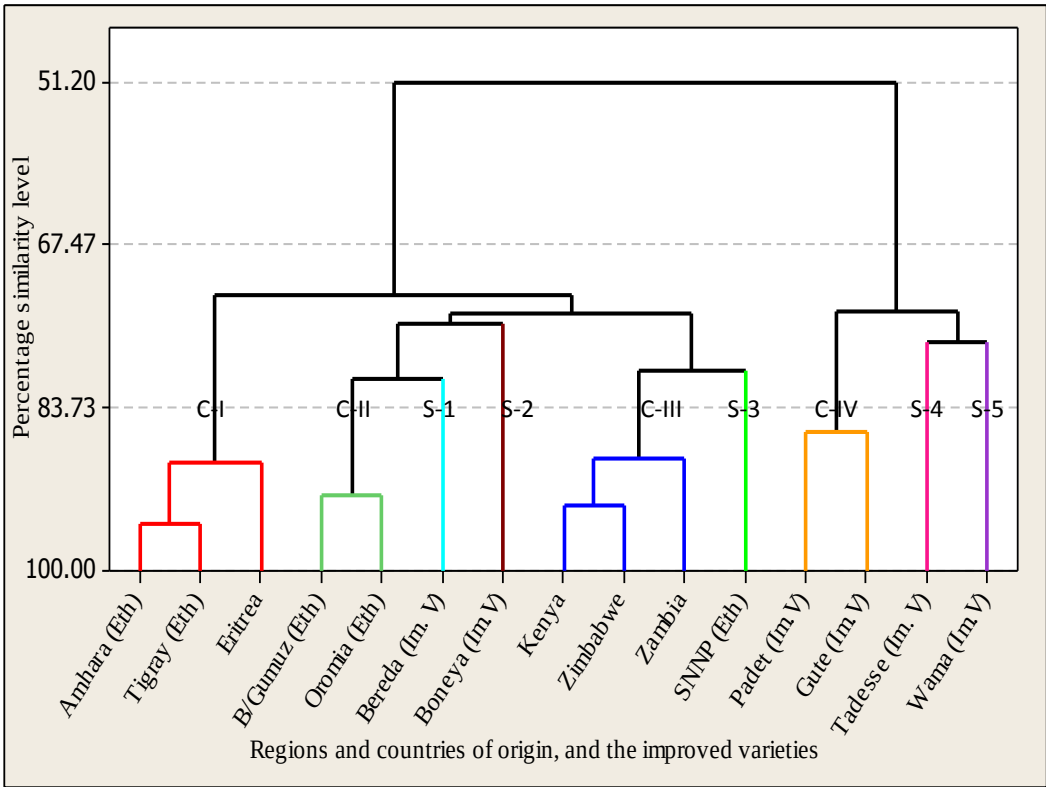



Fig1. The genetic relatedness of finger millet accessions collected from different regions and countries based on 14 quantitative traits
Key: C= cluster, S= solitary or ungrouped

Table 4. Comparison of the mean performances of the top 10% highest yielding accessions with the average performances of all finger millet accessions used in the present study.

| Parameters/ Traits | Selected genotype mean ($\bar{X}$) | Total population mean(μ) | Change due to selection | Change as % of population parameters | Z-calc |
|---|---|---|---|---|---|
| Productive tiller number | 8.60 | 5.50 | 3.10 | 56.36 | 7.18** |
| Finger length | 10.79 | 7.98 | 2.81 | 35.21 | 5.60** |
| Finger number | 9.57 | 7.23 | 2.34 | 32.37 | 8.31** |
| Ear weight | 5.06 | 2.65 | 2.41 | 90.94 | 8.09** |
| Number of grains per spike | 5.72 | 4.39 | 1.33 | 30.30 | 7.08** |
| Finger width | 1.08 | 0.79 | 0.29 | 36.71 | 7.94** |
| Thousand grain weight | 3.20 | 2.26 | 0.94 | 41.59 | 8.35** |
| Grain yield per plant | 31.40 | 20.42 | 10.98 | 53.77 | 7.12** |
| Lodging index | 11.67 | 44.15 | 32.48 | 73.57 | 6.40** |

Key: ** = highly significant, Z-calc = Z-calculated

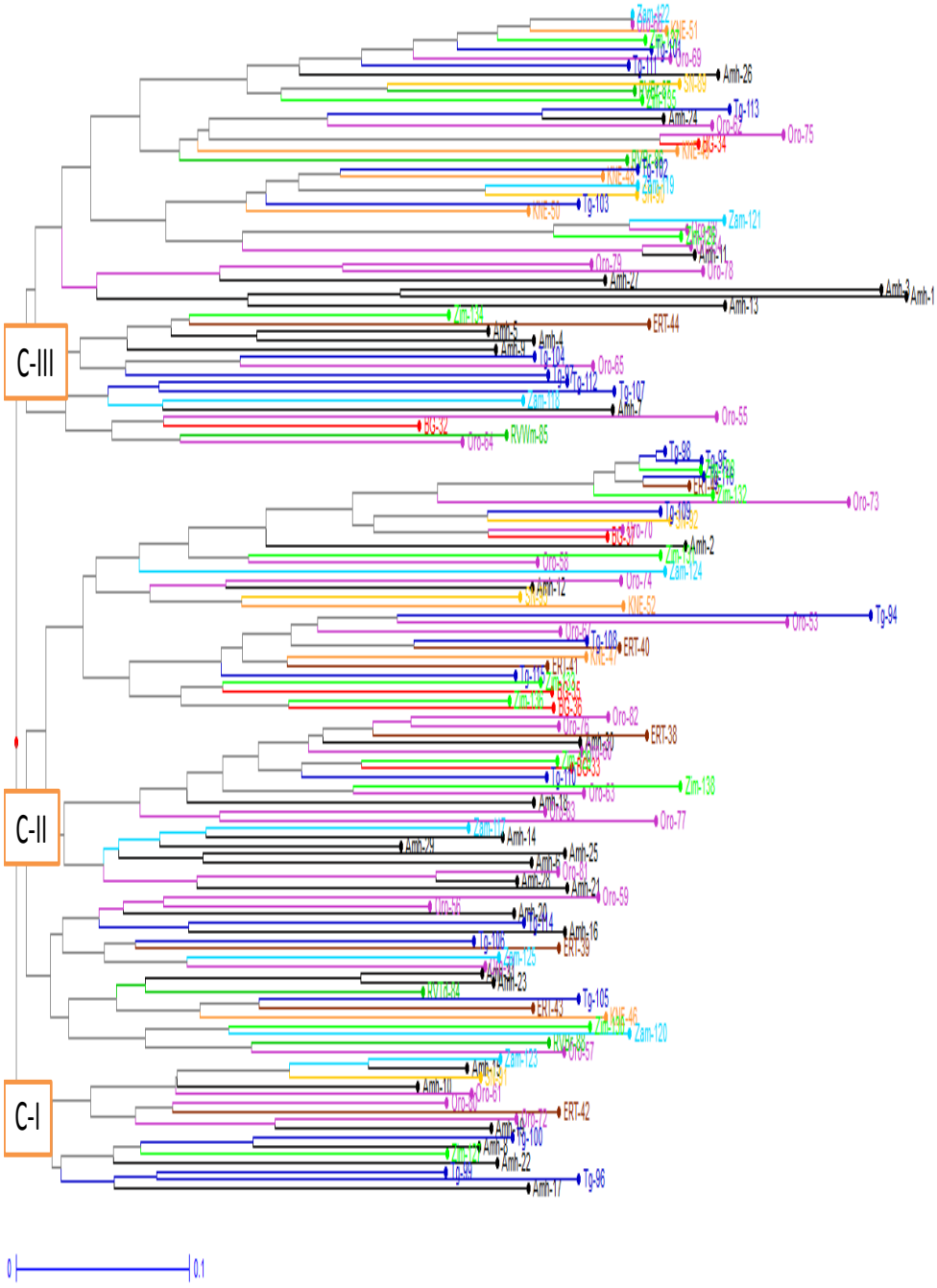
## Genotype by Environment Interaction (GEI) and grain yield stability analysis

### Additive Main Effects and Multiple Interaction (AMMI) model

Combined analysis of variance revealed highly significant (P≤0.01) variations among environments, GEI and Interaction Principal Component Axis (IPCA) (Table 5). This revealed that there was a differential yield performance among the finger millet genotypes across the testing environments and also the existence of strong GEI. About 88.3% of the total GEI was explained by the first three IPCAs: IPCA-I (66.0%), IPCA-II (12.8%) and IPCA-III (9.5%) (Table 5). The remaining five IPCAs explained only 11.7% of the total GEI. Because of their higher proportional contribution to the GEI, the first two principal components (IPCA-I and IPCA-II) were used to create a 2-dimensional GGE biplot (Fig. 4). Gauch and Zobel (1996) suggested that the most accurate model for AMMI can be predicted by using the first two PCAs. Several authors took the first and second IPCA for GGE biplot analysis (Yuksel *et al*., 2002; Farshadfar, 2008; Misra *et al*., 2009).

The GGE biplot indicate that Acc. 203544 (G6) produced the best average grain yield (3.16 ton $ha^{-1}$) and attained an IPCA-1 value relatively close to zero (-0.15) indicating that it was a stable and widely adaptable cultivar (Fig 3). Genotypic stability is crucial in addition to grain yield (Naroui *et al*., 2013) for a given variety to be considered for commercialization. Acc. 203362 (G30) had the lowest IPCA-1 score (-0.0002) and medium grain yield of 2.85 ton $ha^{-1}$ (Fig 3). Genotypes with below average yield, such as Acc. 242617 (G1),

Fig 2.




Weighted neighbor joining based clustering of 138 finger millet accessions using 20 polymorphic SSR markers.
Key: Amh = Amhara, Oro = Oromia, Tg = Tigray, BG = Benishangul Gumuz, SN = Southern Nation Nationalities and peoples region, KNE = Kenya, Zam = Zambia, ERT = Eritrea, Zim = Zimbabwe. The number following each region/letter on the graph indicates finger millet accessions serial number sequentially as listed in Annex 1 under column SNJP

Table 5. ANOVA for grain yield using AMMI model

| Source | df | SS | MS | Eigenvalue | % G x E | % cumulative interaction |
|---|---|---|---|---|---|---|
| Environments | 7 | 1126.96 | 160.99** | | | |
| Genotype | 31 | 59.542 | 1.921* | | | |
| G x E interaction | 217 | 433.914 | 2.00** | | | |
| IPCA 1 | 37 | 286.61 | 7.75** | 95.53552 | 66.05 | 66.05 |
| IPCA II | 35 | 55.5 | 1.6** | 18.52138 | 12.81 | 78.86 |
| IPCA III | 33 | 41.1 | 1.244** | 13.68863 | 9.46 | 88.32 |
| Residual | 496 | 32.523 | 0.065 | | | |

Key: df = degree of freedom, SS = sum of squares, MS = mean squares, IPCA = Interaction Principal Component Axis, ** = highly significant, * = significant

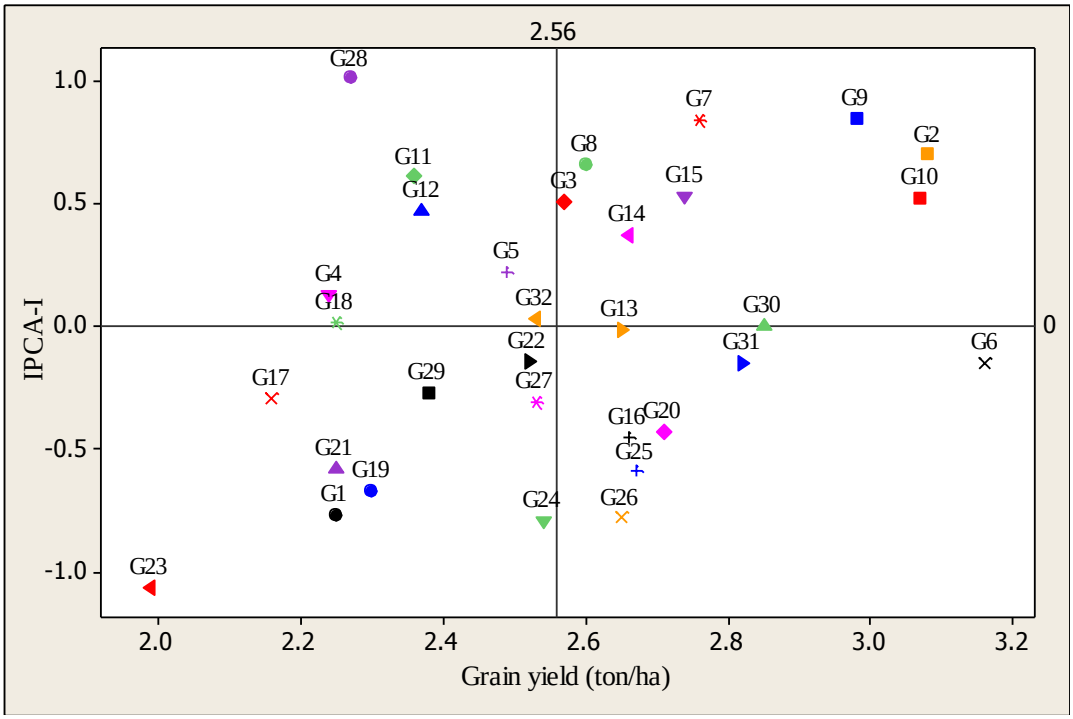



Figure 3. AMMI biplot of genotypic mean grain yield (ton ha$^{-1}$) versus IPCA-1 for 30 accessions evaluated across eight environments.

Tadesse (G32), Acc. 229722 (G4) and Acc. BKFM0034 (G22) also showed IPCA-1 near to zero, indicating that these accessions had consistently lower grain yield across locations. Acc. 242111 (G2) (3.08 ton ha$^{-1}$), BKFM0051 (G10) (3.07 ton ha$^{-1}$) and Acc. 229738 (G9) (2.99 ton ha$^{-1}$) yielded better than Gute (2.82 ton ha$^{-1}$) but showed relatively high IPCA-1 scores of 0.70, 0.52 and 0.85, respectively (Fig 3). This shows site specific adaptation of the accessions. Acc. BKFM0028 (G23) had the least grain yield (1.99 ton ha$^{-1}$) and the highest IPCA-1 score (-1.06). Besides, Acc. 203546 (G17), Acc. 229722 (G4) and Acc. 230104 (G1) are among the low yielding genotypes (Fig 3).

## Analysis based on Eberhart and Russell regression model

Eberhart and Russell (1966) model also revealed that the best yielding accession, 203544 (G6) showed regression coefficient ($b_i$) closer to unity (1.08) and was thus a more stable and widely adaptable candidate cultivar than the remaining accessions, although its deviation from regression was quite different from zero (0.42) (Table 6). High yielding genotypes with regression coefficients ($b_i$) closer to one, but squared deviation from regression ($s^2di$) different from zero should also be considered as stable and adaptable cultivars (Eberhart and Russell, 1966). The next three highest yielding accessions, Acc. 242111 (G2), Acc. BKFM0051 (G10) and Acc. 229738 (G9) recorded regression coefficients higher than one (1.40, 1.41 and 1.32, respectively) and squared deviation from regression different from zero (0.54, 0.27 and 1.40, respectively). This implied that these genotypes were highly responsive to the changes in environment and were therefore recommended for favorable environmental conditions with appropriate agronomic practices.

## Genotype and Genotype by Environment interaction (GGE) biplot analysis

Mean grain yield data of both years were used to assess the relationships between the different test environments, which was visualized by the line connecting each environment to the biplot origin or environment vectors (Fig 4). The cosine of the angle between two environments was used to estimate the correlation between the environments (Dehghani *et al*., 2009; Kaya *et al*., 2006). Environments, Bako (BK) and Gute (GT), correlated positively (acute angle), Assosa (AS) and Arsi Negele (AN) correlated negatively (obtuse angle), whereas AN and GT did not correlate at all (right angle). According to Yan and Tinker (2006), a strong negative correlation indicated high crossover or GEI.

Environments and genotypes that fall in the central (concentric) circle are considered ideal environments and stable genotypes, respectively (Yan and Rajcan, 2002). Acc. 203544 (G6) fell in the central circle indicating its high yield potential and relative stability compared to the rest of genotypes evaluated in this study (Fig 4). An environment is more desirable and ideal when located closer to the center circle (Naroui *et al*., 2013). The Average-Environment Axis (AEA) or Average-Tester-Axis (ATA) is the line that passes through the average environment and the biplot origin (Yan and Rajcan, 2002). A test environment with a small angle with the AEA is more representative than other environments (Yan and Rajcan, 2002). In the present study, Bako was the most stable environment due to the

stable performance of genotypes during both years followed by Gute (Fig. 4). Arsi Negele and Assosa showed variable genotype performance over years and thus high crossover, although the former was high yielding and the latter a poor yielding environment. Similarly, Odewale *et al*. (2013) reported that only one environment as stable, representative and discriminating among nine environments for the performance of 5 coconut genotypes evaluated in southern Nigeria.

Table 6. Mean grain yield (ton ha$^{-1}$) per location, AMMI and regression analysis parameters

| G# | Acc. name | Mean grain yield over locations (ton ha$^{-1}$) | | | | | | | | | $b_i$ | s2di |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AN12 | AS12 | BK12 | GT12 | AN13 | AS13 | BK13 | GT13 | Mean | | |
| 1 | 230104 | 1.23 | 3.60 | 1.40 | 2.93 | 3.67 | 2.13 | 2.07 | 0.99 | 2.25 | 0.56 | 0.64 |
| **2** | **242111** | **3.97** | **2.00** | **2.33** | **2.90** | **7.00** | **1.87** | **2.50** | **2.03** | **3.08** | **1.40** | **0.54** |
| 3 | 203360 | 3.43 | 1.90 | 1.57 | 2.10 | 6.63 | 1.93 | 2.23 | 0.76 | 2.57 | 1.30 | 0.45 |
| 4 | 229722 | 2.47 | 1.57 | 1.77 | 2.67 | 5.20 | 1.47 | 0.99 | 1.77 | 2.24 | 0.96 | 0.18 |
| 5 | 242120 | 2.53 | 2.20 | 1.80 | 3.10 | 6.23 | 1.43 | 1.53 | 1.13 | 2.49 | 1.26 | 0.01 |
| **6** | **203544** | **3.30** | **3.43** | **2.03** | **4.73** | **5.50** | **2.07** | **2.27** | **2.00** | **3.16** | **1.08** | **0.42** |
| 7 | 238346 | 4.23 | 1.70 | 2.50 | 1.60 | 7.00 | 0.94 | 2.40 | 1.53 | 2.76 | 1.32 | 1.17 |
| 8 | 214993 | 3.13 | 1.00 | 1.93 | 3.23 | 7.00 | 1.20 | 1.57 | 1.70 | 2.60 | 1.40 | 0.61 |
| **9** | **229738** | **5.40** | **1.40** | **2.43** | **3.37** | **6.67** | **1.70** | **1.67** | **1.20** | **2.99** | **1.32** | **1.40** |
| **10** | **BKFM0051** | **3.23** | **2.43** | **2.63** | **2.70** | **7.60** | **1.83** | **2.30** | **1.80** | **3.07** | **1.41** | **0.27** |
| 11 | AAUFM-33 | 3.77 | 1.07 | 3.10 | 2.37 | 5.63 | 1.27 | 1.17 | 0.46 | 2.36 | 1.12 | 1.03 |
| 12 | 229730 | 3.87 | 0.90 | 2.07 | 3.50 | 5.03 | 0.79 | 1.77 | 1.05 | 2.37 | 0.99 | 0.97 |
| 13 | BKFM0047 | 2.37 | 3.17 | 2.10 | 2.00 | 6.07 | 2.10 | 2.00 | 1.40 | 2.65 | 1.07 | 0.23 |
| 14 | 203545 | 3.07 | 2.20 | 2.70 | 1.63 | 6.17 | 1.43 | 2.33 | 1.73 | 2.66 | 1.05 | 0.53 |
| 15 | 243636 | 2.63 | 1.87 | 2.20 | 2.83 | 7.53 | 1.53 | 1.53 | 1.80 | 2.74 | 1.48 | 0.31 |
| 16 | 230103 | 2.43 | 3.97 | 2.67 | 1.97 | 4.40 | 2.27 | 2.27 | 1.30 | 2.66 | 0.61 | 0.49 |
| 17 | 203546 | 2.10 | 2.07 | 1.07 | 3.30 | 4.00 | 1.43 | 1.50 | 1.80 | 2.16 | 0.68 | 0.24 |
| 18 | 242617 | 2.30 | 2.60 | 1.73 | 2.03 | 5.40 | 1.57 | 1.70 | 0.70 | 2.25 | 1.05 | 0.08 |
| 19 | 214995 | 1.37 | 3.27 | 1.90 | 3.50 | 3.93 | 2.17 | 0.83 | 1.43 | 2.30 | 0.65 | 0.64 |
| 20 | BKFM0005 | 2.70 | 3.50 | 1.93 | 3.17 | 4.37 | 2.10 | 1.63 | 2.30 | 2.71 | 0.63 | 0.18 |
| 21 | 214988 | 1.40 | 3.67 | 2.17 | 2.13 | 4.13 | 2.03 | 1.60 | 0.88 | 2.25 | 0.67 | 0.54 |
| 22 | BKFM0034 | 2.43 | 3.27 | 1.70 | 1.97 | 5.33 | 1.73 | 1.97 | 1.77 | 2.52 | 0.90 | 0.20 |
| 23 | BKFM0028 | 0.92 | 3.83 | 0.60 | 3.40 | 2.87 | 2.20 | 0.79 | 1.30 | 1.99 | 0.47 | 1.41 |
| 24 | BKFM0042 | 1.67 | 4.20 | 1.77 | 3.10 | 3.77 | 1.90 | 1.97 | 1.90 | 2.54 | 0.51 | 0.64 |
| 25 | BKFM0043 | 1.90 | 3.83 | 1.70 | 3.73 | 4.60 | 2.10 | 2.00 | 1.50 | 2.67 | 0.76 | 0.49 |
| 26 | BKFM0010 | 1.30 | 3.33 | 2.77 | 3.33 | 3.70 | 2.87 | 2.10 | 1.80 | 2.65 | 0.37 | 0.53 |
| 27 | 214990 | 1.83 | 3.90 | 1.77 | 2.67 | 5.63 | 1.77 | 1.37 | 1.30 | 2.53 | 1.08 | 0.38 |
| 28 | 237443 | 4.40 | 0.75 | 2.20 | 2.10 | 6.63 | 0.44 | 1.06 | 0.61 | 2.27 | 1.47 | 1.34 |
| 29 | 214989 | 1.47 | 2.50 | 1.73 | 3.77 | 5.20 | 1.83 | 1.12 | 1.43 | 2.38 | 0.99 | 0.39 |
| 30 | 203362 | 3.10 | 3.20 | 1.11 | 3.93 | 6.17 | 1.93 | 2.23 | 1.10 | 2.85 | 1.23 | 0.31 |
| **31** | **GUTE** | **2.23** | **3.07** | **2.13** | **3.53** | **5.79** | **1.97** | **2.07** | **1.73** | **2.78** | **1.02** | **0.09** |
| 32 | Taddesse | 2.07 | 2.77 | 1.13 | 2.23 | 6.37 | 1.97 | 1.53 | 2.13 | 2.53 | 1.17 | 0.38 |
| | **MEAN** | **2.63** | **2.64** | **1.96** | **2.86** | **5.50** | **1.72** | **1.75** | **1.44** | **2.56** | **0.99** | **0.53** |

Key: G# = Genotype number, AN = Arsi Negelle, AS = Assosa, BK = Bako, GT = Gute, the number following each location indicates the year (12 = 2012, 13 = 2013), CV = Coefficient of variation, LSD = Least significance difference, GEI = Genotype by Environment Interaction, $b_i$ = Regression coefficient, $s^2di$ = Squared deviation from regression

Generally, GGE biplot analysis and AMMI model revealed that Acc-203544 (G6) was a stable and high yielding (3.16 ton $ha^{-1}$) variety with 13.7% yield advantage over the best standard check Gute (2.78 ton $ha^{-1}$) and therefore, officially released in 2015 and recommended for production under wider environmental conditions. The name Addis-01 was given to the variety with the pedigree of Acc-203544, and it was registered as the first variety released from Addis Ababa University in collaboration with Bako Agricultural Research Center.

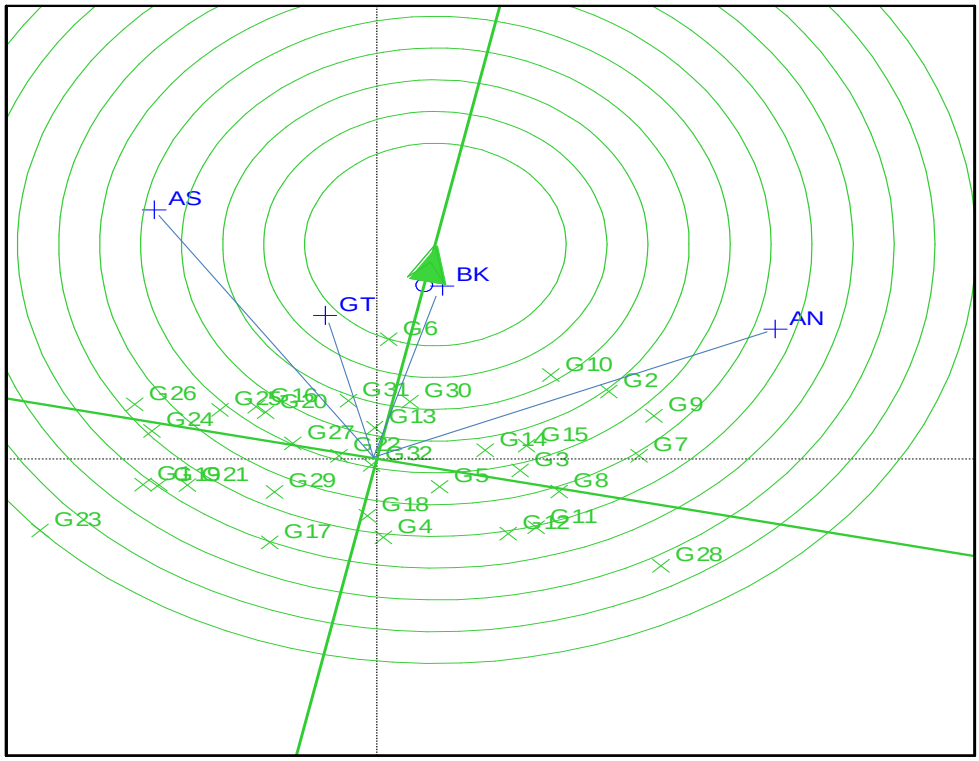



Fig. 4: GGE biplot analysis of 30 Finger millet accessions evaluated across four environment for two years showing ideal environments and stable genotype

# Conclusion and Recommendation

Hierarchical clustering based on phenotypic traits revealed regional grouping of accessions from Amhara, Tigray and Eritrea. Whereas, Weighted Neighbor Joining-based clustering grouped the total test accessions into three major clusters with no particular regional clusters observed. Based on phenotypic, genotypic and blast disease record, widely diverse and best performing genotypes for grain yield, blast disease resistance and other agronomic traits were selected for further multi-location trial. Accordingly, Additive Main effect and Multiplicative Interaction (AMMI) and Genotype and Genotype by Environment Interaction (GGI) biplot analysis revealed that Acc. 203544 was stable and high yielding (3.16 ton $ha^{-1}$) with a yield advantage of 13.7% over the best standard check, Gute (2.78 ton $ha^{-1}$), and thus officially released for production with wider environmental adaptability. In general, the present study portrayed the application of different breeding tools in identifying potential varieties for release

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Annex 1. Passport data of finger millet accessions used for phenotypic and molecular characterization in the present study.

| No | Acc.name | Region | Altitude | No | Acc.name | Region | Altitude |
|---|---|---|---|---|---|---|---|
| 1 | 242133 | Amhara | 1825 | 46 | BKFM0052 | Oromia | 2200 |
| *2 | BKFM0034 | Oromia | 1454 | 47 | 203354 | Zimbabwe | 1420 |
| *3 | 230104 | Eritria | 1800 | 48 | 230102 | Eritrea | 1850 |
| **4 | Padet | Released | | *49 | 229730 | Amhara | 1850 |
| 5 | AAUFM-42 | Tigray | 2058 | 50 | 215982 | Amhara | 1850 |
| 6 | AAUFM-22 | Tigray | 2142 | 51 | Tadesse | Released | |
| **7 | BKFM0026 | Oromia | 1479 | **52 | 242617 | Tigray | 1700 |
| 8 | 215989 | Amhara | 2000 | 53 | 238300 | Tigray | 1980 |
| 9 | 230106 | Eritrea | 1800 | 54 | 203356 | Zimbabwe | 1420 |
| *10 | BKFM0042 | Oromia | 1867 | **55 | 244798 | SNNP | 2169 |
| 11 | BKFM0028 | Oromia | 1608 | 56 | Wama | Released | |
| 12 | 215985 | Amhara | 1940 | 57 | 242616 | Tigray | 1400 |
| 13 | 203350 | Zimbabwe | 1400 | 58 | BKFM0048 | Oromia | 1337 |
| 14 | BKFM0032 | Oromia | 1390 | 59 | BKFM0024 | Oromia | 1913 |
| 15 | BKFM0006 | Oromia | 1479 | 60 | 237584 | SNNP | 1990 |
| 16 | AAUFM-4 | Tigray | 1896 | 61 | 229723 | B/Gumuz | 1300 |
| 17 | 235835 | Amhara | 1930 | 62 | 214991 | Zambia | 1330 |
| 18 | BKFM0022 | Oromia | 1926 | 63 | Bereda | Released | |
| 19 | AAUFM-34 | Tigray | 1568 | 64 | 215976 | Amhara | 1860 |
| 20 | AAUFM-32 | Tigray | 1630 | *65 | 242111 | Amhara | 2100 |
| *21 | AAUFM-33 | Tigray | 1620 | 66 | 242624 | Tigray | 1400 |
| 22 | 216039 | Oromia | 1950 | 67 | 229728 | B/Gumuz | 1440 |
| 23 | 241768 | SNNP | 1500 | 68 | 216036 | Tigray | 1900 |
| 24 | AAUFM-8 | Tigray | 1812 | 69 | 216033 | B/Gumuz | 1930 |
| *25 | BKFM0047 | Oromia | 1334 | 70 | 214994 | Zambia | 1160 |
| 26 | 229731 | Amhara | 1950 | 71 | 241769 | SNNP | 1500 |
| 27 | BKFM0029 | Oromia | 1251 | 72 | 237472 | Tigray | 1800 |
| 28 | AAUFM-19 | Tigray | 1811 | 73 | 203358 | Zimbabwe | 1420 |
| *29 | 214995 | Zambia | 1130 | *74 | BKFM0005 | Oromia | 1449 |
| 30 | 203353 | Zimbabwe | 1420 | 75 | Gute | Released | |
| 31 | AAUFM-2 | Tigray | 1896 | 76 | 214996 | Zambia | 1130 |
| 32 | 235782 | Amhara | 1860 | 77 | 238327 | Tigray | 1900 |
| 33 | 242117 | Amhara | 1915 | 78 | BKFM0018 | Oromia | 1667 |
| 34 | 237475 | Tigray | 1750 | **79 | 216046 | Tigray | 1910 |
| *35 | 203545 | Kenya | 1590 | *80 | 214988 | Zambia | 1300 |
| **36 | AAUFM-35 | Tigray | 1568 | 81 | 243639 | Amhara | 2070 |
| 37 | 215802 | Amhara | 1950 | 82 | 225892 | Amhara | 1710 |
| 38 | BKFM0039 | Oromia | 2144 | 83 | 214987 | Zambia | 1310 |
| *39 | 230103 | Eretria | 1700 | **84 | 230110 | Eritrea | 1700 |
| *40 | BKFM0010 | Oromia | 1484 | 85 | 215990 | Amhara | 1910 |
| 41 | 245087 | Tigray | 1923 | *86 | 203360 | Zimbabwe | 1420 |
| 42 | 230105 | Eritrea | 1600 | 87 | 208726 | Oromia | 1880 |
| 43 | 215887 | Amhara | 1880 | 88 | BKFM0055 | Oromia | 1723 |
| 44 | BKFM0062 | Oromia | 1923 | *89 | 237443 | Amhara | 2100 |
| **45 | BKFM0002 | Oromia | 1550 | 90 | BKFM0011 | Oromia | 1428 |

Annex 1 continued

| No | Acc.name | Region | Altitude |
|---|---|---|---|
| 91 | Boneya | Released | |
| **92 | AAUFM-15 | Tigray | 1568 |
| 93 | 203355 | Zimbabwe | 1420 |
| *94 | 229722 | B. Gumuz | 1750 |
| 95 | BKFM0057 | Oromia | 1707 |
| 96 | BKFM0060 | Oromia | 1852 |
| 97 | 230109 | Eritrea | 1800 |
| **98 | 100038 | Amhara | 1980 |
| 99 | 238344 | Amhara | 2000 |
| 100 | 245091 | Oromia | 1991 |
| 101 | 216056 | Oromia | 1600 |
| 102 | AAUFM-23 | Tigray | 2100 |
| 103 | 203542 | Kenya | 1540 |
| 104 | 238341 | Amhara | 1780 |
| *105 | 203362 | Zimbabwe | 1420 |
| 106 | 230107 | Eritrea | 1800 |
| 107 | 203352 | Zimbabwe | 1490 |
| 108 | 242135 | Amhara | 1910 |
| 109 | AAUFM-20 | Amhara | 2142 |
| *110 | 214993 | Zambia | 1340 |
| 111 | 229724 | B. Gumuz | 1520 |
| *112 | 203546 | Kenya | 1620 |
| 113 | 242105 | Amhara | 1860 |
| 114 | 216057 | Oromia | 1800 |
| 115 | BKFM0001 | Oromia | 1580 |
| *116 | 238346 | Amhara | 1940 |
| *117 | 214989 | Zambia | 1210 |
| 118 | 203361 | Zimbabwe | 1420 |
| 119 | AAUFM-14 | Tigray | 1568 |
| 120 | 203543 | Kenya | 1514 |
| 121 | 235783 | Amhara | 2000 |
| 122 | 203363 | Zimbabwe | 1420 |
| **123 | 214990 | Zambia | 1360 |
| 124 | 236446 | Oromia | 1930 |
| 125 | 203357 | Zimbabwe | 1420 |
| 126 | 235140 | Amhara | 1950 |
| *127 | 214997 | Zambia | 1100 |
| 128 | 242112 | Amhara | 2125 |
| 129 | 203547 | Kenya | 1510 |
| *130 | BKFM0051 | Oromia | 2227 |
| *131 | 203544 | Kenya | 1485 |
| 132 | 242109 | Amhara | 2060 |
| 133 | 203351 | Kenya | 1490 |
| *134 | 229738 | B. Gumuz | 1830 |
| 135 | 203359 | Zimbabwe | 1420 |
| *136 | 242132 | Amhara | 1910 |
| **137 | 243636 | Amhara | 2100 |
| 138 | BKFM0004 | Oromia | 1445 |
| 139 | BKFM0008 | Oromia | 1459 |
| 140 | AAUFM-21 | Tigray | 1722 |
| 141 | 216040 | Oromia | 1940 |
| 142 | BKFM0058 | Oromia | 1725 |
| 143 | 235700 | SNNP | 1530 |
| 144 | 235699 | SNNP | 1440 |
| 145 | AAUFM-12 | Tigray | 1502 |
| 146 | 230101 | Eritrea | 1740 |
| 147 | 215981 | Amhara | 1850 |
| 148 | 229725 | Amhara | 1650 |
| *149 | 242120 | Amhara | 1850 |
| 150 | AAUFM-11 | Tigray | 1502 |

Key: **=accessions excluded from molecular analysis; *=accessions selected for G x E study