ASSESSMENT OF GENETIC DIVERSITY, GENOTYPE BY ENVIRONMENT INTERACTION, BLAST (*MAGNAPORTHE ORYZAE*) DISEASE RESISTANCE, AND MARKER DEVELOPMENT FOR FINGER MILLET GERMPLASM FROM ETHIOPIA AND INTRODUCED

A THESIS SUBMITTED TO

THE SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF MICROBIAL CELLULAR AND MOLECULAR BIOLOGY

COLLEGE OF NATURAL SCIENCES

ADDIS ABABA UNIVERSITY

BY

DAGNACHEW LULE

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY (PhD) IN BIOLOGY

(APPLIED GENETICS)

MARCH 2015

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DEDICATION

This thesis is dedicated to my lovely wife, Sr. Zufan Hailu, in recognition of her support, love, and encouragement, which she so generously gives to me. I also dedicate it to my son Henok Dagnachew and my daughter Hawi Dagnachew, who endured the pain of separation and did not get much attention and love from me, not only during this study period but also during the last seven years for government duty that forced us to live in different districts.

DECLARATION

I declare that this thesis is submitted to the School of Graduate Studies of Addis Ababa University for the Degree Doctor of Philosophy (PhD) in Biology (Applied Genetics). I would like to attest through my signature affixed below that it is my own independent work and has not previously been submitted elsewhere by me or anybody else. All authors of the references cited in the present study were duly acknowledged.

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thank my father and mother, brother and sisters, for their input. My family is my foundation and the source of my inspiration.

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ACRONYMS AND ABBREVIATIONS

AAU	Addis Ababa University
AEA	Average Environment Axis
AFLP	Amplified fragment length polymorphism
AMMI	Additive Main effect and Multiplicative Interaction
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
APS	Ammonium Per Sulphate
ATA	Average Tester Axis
AUDPC	Area Under Disease Progress Curve
Bis	bisacrylamide
BWPPL	Biomass weight per plant
CB	culm branch
CD	culm diameter
CGIAR	Consultative Group for International Agricultural Research
CLC	Genomics workbench software
cpDNA	Chloroplast DNA
CSA	Central Statistics Agency
CTAB	Cetyl tri-ethyl ammonium bromide
CV	Coefficient of variation
EDTA	Ethylene diamine tetra acetic acid
DAP	Diammonium phosphate
DAP	Days After Planting
ddH_2O	Double distilled water

df	degrees of freedom
DH	days to 50% heading
DM	days to maturity
dNTPs	Deoxynucleoside triphosphates
EIAR	Ethiopian Institute of Agricultural Research
EBI	Ethiopian Biodiversity Institute
FAO	Food and Agriculture Organization (of the United Nations)
FL	finger length
FN	finger number
FW	finger weight
FW	finger width
GA	Genetic advance
GCV	Genotypic Coefficient of Variation
GEI	genotype by environment interaction
GGE	genotype and genotype by environment interaction
GYPPL	grain yield per plant
H’	Shannon diversity index
HBAUDPC	Head Blast Area under Disease Progress Curve
HBRAUDPC	Head Blast Relative Area under Disease Progress Curve
HI	Harvest index
IBPGR	International Board for Plant Genetic Resources
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IPCA	Interaction Principal Component Axes
ISSR	Inter Simple Sequence Repeat

ITS	Internal transcribed spacer
LBAUDPC	Leaf Blast Area under Disease Progress Curve
LBRAUDPC	Leaf Blast Relative Area under Disease Progress Curve
LL	Lesion length
Log	lodging index
m.a.s.l	meter above sea level
MNV	Multiple Nucleotide Variant
MSE	Mean square of error
NGPS	number of grains per spikelet
NBDINC	neck blast disease incidence
PAGE	Polyacrylamide gel electrophoresis
PCA	Principal Component Axis
PCR	Polymerase chain reaction
PCV	Phenotypic Coefficient of variation
PDA	potato dextrose agar
PIC	Polymorphic Information Content
PH	plant height
PTN	productive tiller number
QTL	Quantitative trait locus
RAPD	Random amplified polymorphic DNA
RCBD	randomized complete block design
RFLP	Restriction fragment length polymorphism
RILs	Recombinant inbred lines
rpm	revolutions per minute

SAS	Statistical Analysis System
SE	standard error of mean
SNNP	Southern Nations Nationalities and Peoples Region
SNP	Single Nucleotide Polymorphism
SNV	Single Nucleotide Variant
SSCP	Single Strand Conformation Polymorphism
SSR	Simple sequence repeat
TBE	Tris/Borate/EDTA
TE	Tris EDTA
TEMED	Tetramethylenediamine
TGW	Thousand Grain Weight
TTN	Total Tiller Number

ABSTRACT

Finger millet (*Eleusine coracana* subsp. *coracana)* is extensively cultivated in the tropical and sub-tropical regions of Africa and India. It is the major staple food for millions of resource poor people and plays an important role in the dietary habits and economy of subsistence farmers inhabiting arid and semi-arid regions of Africa and India. The crop is adapted to adverse agro-ecological conditions with minimal inputs, tolerant to moisture stress, produced on marginal land where other crops cannot perform and tolerant to acidic soils. Breeding efforts in finger millet has been limited and farmers are growing unimproved and low yielding cultivars. Improvement in any crop usually involves exploiting the genetic variability in specific traits of interest. Moreover, the use of multiple data sets, such as morphological, biochemical and molecular in combination with appropriate statistical methods are essential to identify inter and intra-species variation to develop improved cultivars.

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)), supplemented by 39 introduced accessions from Kenya, Zambia, Zimbabwe and Eritrea and six improved varieties, were evaluated in the field at Gute and Arsi Negele during 2011 to assess the genetic diversity and eco-geographical patterning. The analysis of variance indicated that the mean square due to environment and genotype were highly significant ($P \leq 0.01$) for all quantitative traits except ear weight for the latter case. Erect type growth habit, open ear type, light green ear (glumes) color, enclosed grains by glumes, lower spikelet density and purple black seed color were the predominant phenotypic classes for the traits recorded in the present study. Cluster analysis for both qualitative and quantitative phenotypic traits indicated that finger millet accessions from neighboring regions of Ethiopia, neighboring African countries and proximity in altitude classes shared strong similarity. Principal component analysis at population level, geographical locations and agro-ecologies of origin indicated that grain yield per plant, thousand grain weight, days to heading, days to maturity, lodging index and biomass weight per plant were the most important traits contributing to the overall variability, implying that breeding efforts on those traits can meet the objectives in improving the trait. The higher heritability coupled with higher genetic advance noted for ear weight (71.14%), lodging index (53.49), finger length (41.94%), thousand grain weights (28.88) and grain yield per plant (26.34) indicated the ease of phenotypic selection for the improvement of those traits. About 68.4% of the total traits association showed positive correlation.

The 150 finger millet test accessions were also evaluated using a set of 20 SSR markers. Results showed variable genetic polymorphism with an average polymorphic information content of 0.57. A total of 188

alleles were recorded with an average of 9.89 alleles per locus. Analysis of molecular variance revealed 69.52% variation within populations and 30.48% among populations. Relatively higher within accession polymorphism were recorded for accessions from Oromia (21.10%), Amhara (16.93%) and Tigray (13.43%) regional states of Ethiopia. Weighted neighbor joining-based clustering grouped the 138 accessions for which the SSR markers worked well, into three major clusters apart from the main node, and accessions collected from the same region or the same altitude classes did not often group entirely together within a given major cluster or sub cluster. Likewise, analysis of population structure distinguished the 138 finger millet accessions in to three subpopulation (k = 3) with the highest ΔK of around 300. The first, second and the third group comprised of accessions from the different geographical locations and the improved varieties with membership proportion 22.9%, 38.5% and 38.6%, respectively.

Magnaporthe oryzea, a fungal pathogen that attacks more than 50 *gramineous* species, is well known to cause immense yield loss, and is particularly severe on rice and finger millet. A total of 225 finger millet materials were evaluated for blast resistance/tolerance on a sick plot supplemented with artificial inoculation at Bako Agricultural Research Center in 2011. The analyses of variance revealed the mean square due to genotypes were highly significant ($P \leq 0.01$) for leaf, sheath, neck and head blast. Based on the head blast disease severity index, the 225 test materials were classified into five groups: only one accession (Acc. BKFM0031) was resistant (head blast severity score of < 3 at maturity with both head blast severity index and relative area under disease progress curve (RAUDPC) values of less than 21%), 10 accessions were moderately resistant with blast severity index of 21-40%, the remaining 214 was in a range of moderately susceptible to highly susceptible. About 66% of the most susceptible accessions gave grain yield below the average (11.29 g/plant), indicating that grain yield reduction in most accessions were due to blast infection. Finger millet accessions collected from the same region showed different levels of disease reaction, implying that diversified materials are produced within a country or region. Both correlation and regression analysis revealed highly significant ($P \leq 0.01$) negative association of head blast to grain yield. An average of 63.2% yield loss was recorded due to head blast disease during the severe infection periods of 2011 as estimated using different yield loss assessment models.

Identification of adaptable, stable and high yielding genotypes under varying environmental conditions are the first steps in plant breeding prior to release of a cultivar and this has direct bearing on the adoption of the variety, its productivity and total production of the crop. To this end, a total of 30 advanced finger millet genotypes were evaluated against two standard checks (Gute and Taddesse) across four locations (Arsi Negele, Assosa, Bako and Gute) in the 2012 and 2013. Additive Main effect and Multiplicative Interaction (AMMI), Genotype and Genotype by Environment Interaction (GGI)

biplot analysis and, Eberhart and Russell model 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 recommended for possible release with wider environmental adaptability. Moreover, Acc. 242111 (3.08 ton ha^{-1}) and BKFM0051 (3.07 ton ha^{-1}) were high yielding, but showed narrow stability and thus recommended for possible release for specific environments.

Single strand conformation polymorphism (SSCP) analysis detected that only one marker was polymorphic among the thirteen primers designed from Rice Orthologous Genes with finger millet for blast resistant. Sequence analysis of this polymorphic marker (Pi9F2) for eight representative finger millet accessions and 15 clone samples revealed the average contigs measurement of 891bp and nucleotide distribution of 30.9% Thymine (T), 28.1% Adenine (A), 24.2% Cytosine (C) and 16.8% Guanine (G). Multiple sequence alignment of those sequence revealed two possible SNPs at consensus positions 460 (G/A) and 780 (G/C) in a window of 66 bp, where the consensus residue is G at both positions. Overall, this study showed the presence of divers finger millet accessions that deserve sustainable conservation and use in breeding program and generated information on the extent of genetic variation among finger millet accessions and eco-geographical regions using multiple markers. Besides, the study has of the preliminary contribution in the application of molecular tools for diversity analysis and thus variety development and identified accessions with variable level of blast tolerance for further utilization in breeding and other genomic research activities.

Keywords: Finger millet (*Eleusine coracana* subsp. *coracana),* Additive main effect and multiplicative interaction (AMMI), Area Under Disease Development Curve (AUDPC), Heritability, *Magnaporthe oryzae*, polymorphism, Simple Sequence Repeat (SSR), Single strand confirmation polymorphism (SSCP).

1. BACKGROUND AND JUSTIFICATION/INTRODUCTION

The predominantly African grass genus *Eleusine* comprise of eight species, among which cultivable finger millet (*E. coracana* subsp. *coracana*, 2n=4x=36), is the most important subsistence crop of Africa and India (Babu *et al.,* 2007). Finger millet represents one of the critical plant genetic resources for the agriculture and food security of farmers inhabiting arid, infertile and marginal lands (Barbeau and Hilu, 1993). It is largely produced in India, Uganda, Tanzania, Kenya, Ethiopia, Rwanda, Zaire, Zambia, Zimbabwe, Eritrea, Somalia, China, and Myanmar (Ekwamu, 1991). In the semiarid tropics of Eastern Africa, it is the major staple food for millions of resource poor people and plays an important role in the dietary habits and economy of subsistence farmers (Ekwamu, 1991).

The major attributes of finger millet are its adaptability to adverse agro-ecological conditions with minimal inputs, tolerance to moisture stress, productivity on marginal land where other crops cannot perform and tolerance to acidic soil (Upadhyaya *et al*., 2007). Since it is rich in calcium, iron and other important nutrients, finger millet serves as a main staple food for rural populations in developing tropical countries where calcium deficiency and anemia are widespread (Babu *et al.,* 2007). The whole grain of finger millet contains per 100 g edible portion: water 10.9 g, energy 1,377 kJ (329 kcal), protein 7.4 g, fat 1.3 g, carbohydrate 77.7 g, fiber 4.3 g, Ca 397 mg, P 190 mg, Fe 17.1 mg, β-carotene traces, thiamin 0.18 mg, riboflavin 0.11 mg and niacin 0.8 mg (Leung *et al*., 1968). The essential amino-acid composition per 100g food is: tryptophan 107 mg, lysine 213 mg, methionine 229 mg, phenylalanine 383 mg, threonine 310 mg, valine 487 mg, leucine 701 mg and isoleucine 324 mg (Leung *et al*., 1968).

In Ethiopia, finger millet occupies diverse agro-ecologies with a vast range of genetic variability and is intensively cultivated in mid and lower altitude regions of Tigray, Gojam, Gonder and Wollega where it constitutes 10 to 20 % of the total cereal production (Kebede and Menkir, 1986). The Ethiopian Central Statistical Authority data from 1995-2013 reveals that the area devoted to finger millet cultivation and its productivity is generally increasing (Fig 1 and 2). The increment could be attributed partly due to the release and dissemination of improved varieties developed through selection breeding that were adaptable to the pocket environment and also due to the awareness created on production and management of the crop.

Although the yield potential reported is more than 3.0 tons ha^{-1} (Mulatu *et al*., 1995; Mulatu and Kebede 1993), the current national average is only limited to 1.7 tons ha^{-1} (CSA, 2013). This could be mainly due to the lack of widely adaptable improved varieties, blast disease infection, limited management recommendations and other biotic and abiotic factors (Kebede and Menkir, 1986; Mulatu *et al.,* 1995; Bezawuletaw *et al*., 2006). Moreover, breeding efforts in finger millet has been limited, in general, and majority of farmers are generally growing unimproved and low yielding cultivars (Dida *et al*., 2007; Neves, 2011; personal observation). This necessitates development of stable high yielding cultivars with other desirable traits.

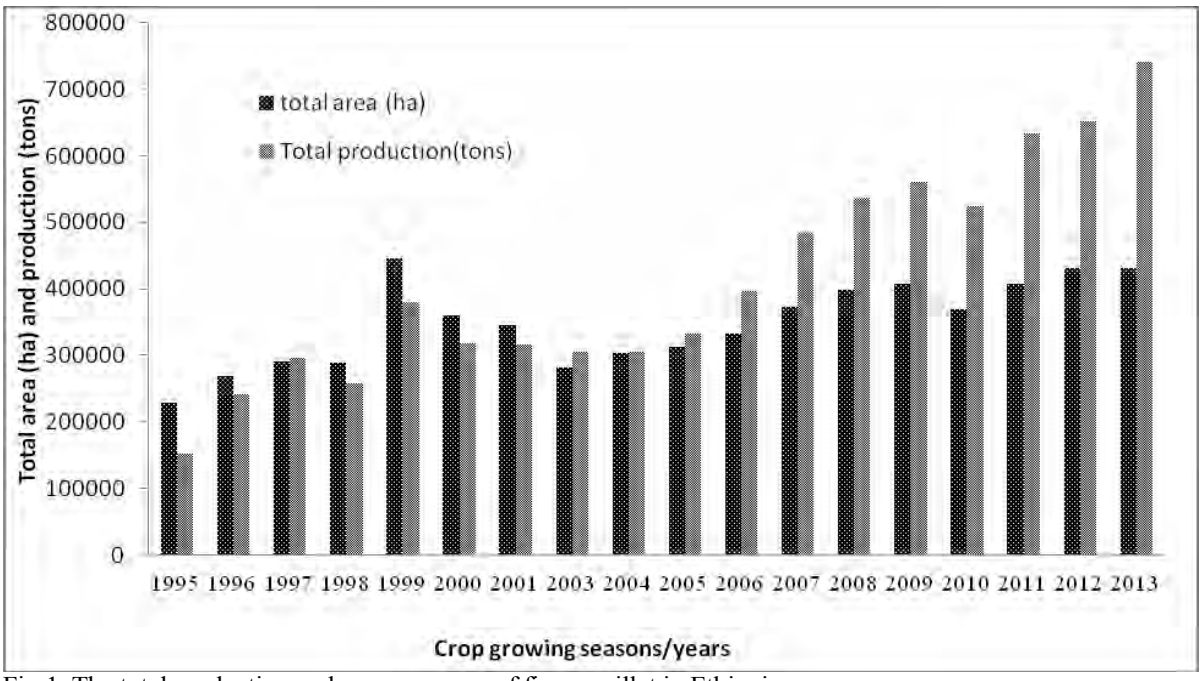


Fig 1. The total production and area coverage of finger millet in Ethiopia
Source: CSA, 1995-2013).

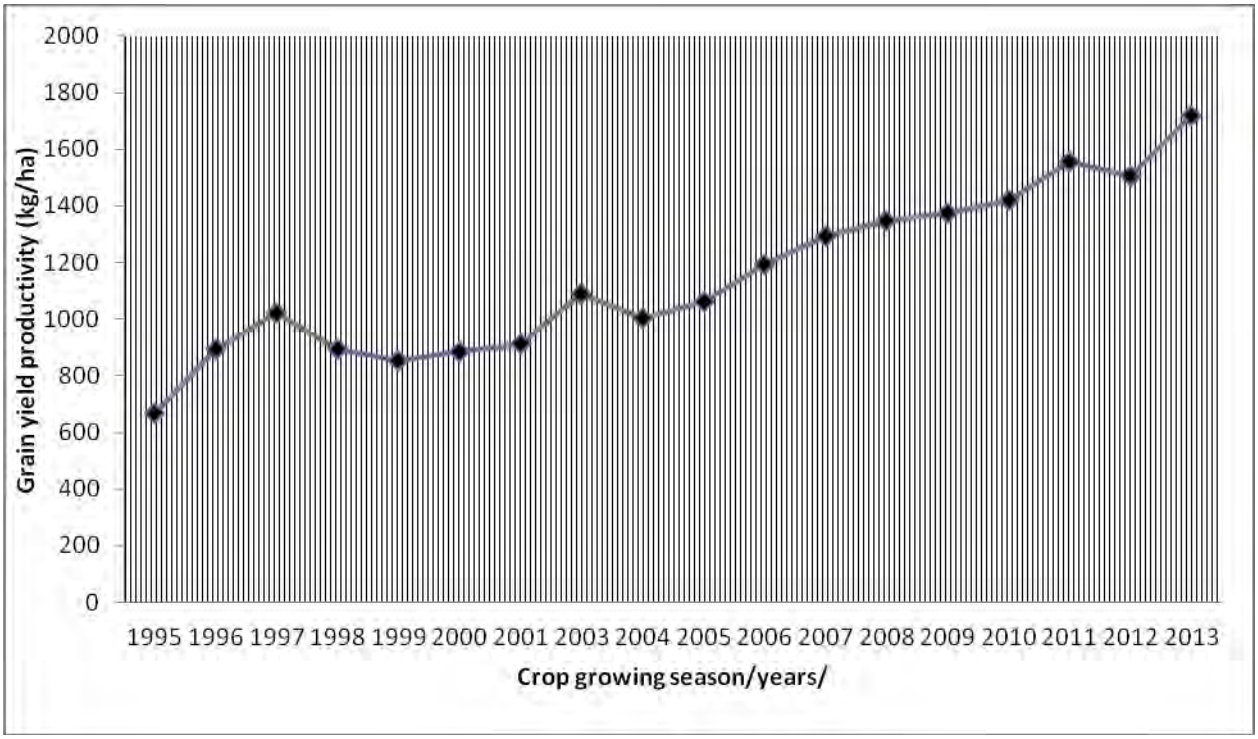


Fig 2. Finger millet productivity per unit area in Ethiopia (1995-2013)
[Source: CSA, 1995-2013].

Blast disease, caused by *Magnaporthe grisea* (anamorph *Pyricularia grisea*), has been identified as the highest priority constraint to finger millet production in East Africa (Takan *et al.*, 2011;

Mgonja *et al*., 2007). For the genetic improvement of any crop plant, it is essential to know the nature and magnitude of variability present in the base germplasm population. Studying the genetic variation of a crop species is essential for effective utilization of germplasm in plant breeding programs, devising appropriate sampling procedures for germplasm collection and conservation, obtaining some collections for efficient germplasm management and explaining the taxonomy, evolution and origin of crop species (Bekele, 1983; Bekele, 1985; Demissie and Bjonstrand, 1996; Keneni *et al*., 2007; Varshney *et al*., 2007).

Genetic diversity assessment, therefore, aids understanding intra-specie crop varietal performance that can be exploited in crop improvement (Aremu, 2011), provides information about the extent of genetic divergence and serves as a platform for specific breeding objectives (Thompson *et al*., 1998) as well as identifies parental combinations to create segregating progenies with maximum genetic potential for further selection (Dje *et al*., 2000). The use of multiple data sets, such as morphological, biochemical and molecular (Aremu, 2011) in combination with appropriate statistical methods such as genetic distance measurements (Rogers, 1972; Singh and Chaudhary, 1985) and phylogenetic relationships among the test genotypes using individual and combined analysis of data sets (Wrigley *et al.,* 1982) are essential to identify inter and intra-species variation to develop improved cultivars.

Identifying adaptable and stable high yielding genotypes with other desirable traits under varying environmental conditions to recommend new varieties for release as cultivar(s) is fundamental and this has direct bearing on the adoption of a variety, productivity and total production of the crop (Flores *et al*., 1998; Showemimo *et al*., 2000; Mustapha *et al*., 2001). Multi- environment yield trials are important to identify adaptable high yielding cultivars and

discover sites that best represent the target environment (Yan *et al*., 2000). Crop improvement through conventional breeding is slow, especially for traits controlled by quantitative gene action. It also takes large amounts of time, resources and energy, and it is often difficult to detect the tolerance/resistance to biological or environmental stresses (Kanchan and Anita, 2010). Hence, the use of molecular markers that can improve this process is of great interest beside morphological markers. There are also modern crop improvement tools such as comparative genomics to transfer genetic information from model species to species of interest that can provide a more timely & robust response to crop production threats (Praba *et al*., 2009).

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 (Bezaweletaw *et al.*, 2006), very few accessions have been characterized at morphological level (Daba *et al*., 2008, Wolie *et al*., 2011, Bezawuletaw *et al*., 2006, Tsehaye and Kebebew, 2002) and only one study has been conducted at the molecular level using RAPD markers (Bezawuletaw, 2011). Yield loss assessment and blast isolate pathogenicity was also conducted in greenhouse conditions two released varieties and a local check (Gashaw *et al*., 2013). This study is therefore initiated for the systematic and intensive evaluation and characterization of finger millet genetic diversity using multiple markers and other genomic tools to minimize the existing research gaps, and provide a clue for germplasm conservation, cultivar development and other phenomics and genomic research.

2. OBJECTIVES

2.1 Major objective

To evaluate the extent and patterns of genetic variation among finger millet accessions collected from different regions of Ethiopia and some introduced using morphological, molecular and other genomic tools to enrich finger millet germplasm data bases and identify stable high yielding and blast disease tolerant genotypes for possible release.

2.2 Specific objectives:

† To assess the extent and pattern of genetic diversity of finger millet germplasms on the basis of phenotypic traits and molecular markers and identify potential germplasm collection and conservations agro-ecologies;

† To screen genetically diverse finger millet accessions for blast disease tolerance for further utilization in breeding programs;

† To estimate the genetic parameters, genotypic and phenotypic coefficient of variations, heritability and genetic advance for quantitative traits;

† To estimate genotype by environment interactions and identify stable high yielding and disease tolerant genotypes for possible release and/or candidate genotype for crossing;

† To identify potential molecular markers for blast disease resistance using rice blast resistant orthologous genes through multiple DNA alignment.

2. LITERATURE REVIEW

3.1 Origin and distribution of finger millet (E. coracana subsp. coracana)

There has been controversy in reports of the exact center of origin for finger millet. Vavilov (1951) considered finger millet an indigenous crop to Ethiopia and the high plateaus of Abyssinia as a possible center of origin. Purseglove (1972) considered Uganda as a center of origin because of the long tradition of religious and other ceremonies connected with its cultivation. Kenneth and LeRoy (1977) reported the eastern Sudan zone and the highlands stretching from Ethiopia to Uganda as a possible area of domestication of this crop. Biosystematics, ethno-botanical, genetic and linguistic evidence confirmed that the East African highlands, particularly Uganda and Ethiopia, are a possible center of origin for finger millet (Hilu and De Wet, 1976).

The oldest domesticated finger millet was found in the archaeological record of a prehistoric site at Axum, Ethiopia, dating back some 5000 years and it resembles race plana (a highly evolved race) which is the principal finger millet still grown in Ethiopia (Hilu *et al*., 1979). Therefore, molecular markers such as Amplified Fragment Length Polymorphism (AFLP), that provide a useful insight into the genetic organization and relationships among the cultivated species and their wild relatives were deemed necessary in finger millet so as to confirm the occurrence of a single domestication event and investigate a more precise origin of domestication of the crop.

Finger millet cultivation extends in Africa from Nigeria eastwards to Eritrea and south towards South- West Africa and Natal in South Africa (Purseglove, 1972). It was introduced to India at a very early date, probably over 3000 years ago (Hilu *et al*., 1979; National Research Council,

1996) and now it is an important staple food in some places, particularly in the hilly country in the north and the south of India. It is said to have reached Europe only around the beginning of the Christian era.

3.2 Taxonomy and Cytology

The genus *Eleusine* Gaertn. is a member of the tribe Chlorideae and comprises eight species, among which the cultivated *E. coracana* subsp. *coracana* (finger millet) is the most important subsistence crop of Africa and India in contrast to *E. indica* (goose grass), which is categorized as one of the most problematic weeds in the world (Holm *et al.*, 1977; Phillips, 1995; Devarumath *et al*., 2005). The genus is characterized by three different basic chromosome numbers of x = 8, 9 or 10; *E. intermedia, E. indica, E. floccifolia* and *E. tristachya* are diploids with 2n = 2x = 18; *E. multiflora* is a diploid with 2n = 2x = 16 and *E. jaegeri* is also diploid with 2x = 2n = 20 (Devarumath *et al*., 2005). The tetraploid *Eleusine spp.* comprise *E. coracana* subsp *coracana* and subsp. *africana* with 2n = 4x = 36 and *E. kigeziensis* with 2n = 38, which all probably have allopolyploid origins involving two diploid species with x = 9 or 10 (Bisht and Mukai, 2002; Neves, 2011). The most common vernacular names for finger millet include: finger, birdsfoot, African millet or ragi millet (in English); ragi, nagli, mandua or kurukkan (India); Dagussa (Ethiopia); Wimbi (in East Africa such as Kenya, Uganda and Tanzania); and *coracan* (French).

3.3 Floral biology of finger millet

Ayyangar (1932) found that the complete emergence of the inflorescence requires about 10 days and flowering extends over at least 7 or 8 days. The plant flowers under a photoperiod of 11 hr and 30 minutes to 11 hrs and 50 minutes (Kenneth and LeRoy, 1977). Finger millet with

compact and semi compact ear type undergo anthesis between 2 and 5 am, while panicles with open spikes tend to flower between 1 and 3 am (Kenneth and LeRoy, 1977). The general tendency of the flowers is to open and progress from the top to the bottom in a finger. In a spikelet, however, the order is reversed and proceeds from the bottom to the top and from the bigger to the smaller flowers (Kenneth and LeRoy, 1977). The stigma remains receptive for a very short period after its emergence from glumes (Kenneth and LeRoy, 1977). The period of anthesis in the flower is very short and is conductive to self pollination, but occasionally cross-pollination occurs (Kenneth and LeRoy, 1977).

3.4 Evolution and phylogenetic of Eleusine coracana *subsp.* coracana

The species of the genus *Eleusine* were categorized into an A genome group comprising *E. indica* and *E. tristachya* and a B genome group comprising three species namely *E. floccifolia*, *E. intermedia* and *E. multiflora* (Dida and Devos, 2006). Since the evolution of the tetraploid species involved both A and B genome groups; *E. coracana* subspecies *coracana* (finger millet), *E. coracana* subsp. *africana* and *E. kigeziensis* belong to both genome groups (Dida and Devos, 2006). All of the species are from African origin except *E. tristachya,* which is native to South America (Devarumath *et al*., 2005; Neves *et al*., 2005). Based on cytotaxonomic and morphological analysis, Mehra (1963) suggested that *E. indica* (2n=18) was crossed with unknown *Eleusine* species (2n=18) and resulted in a diploid wild hybrid that was further developed in to wild tetraploid (2n=36) through chromosome doubling. The wild tetraploid was evolved in to *E.coracana* subspp. *africana* (2n=4x=36) and the cultivated *E.coracana* subspp. *coracana was* developed through natural and artificial selection of *E.coracana* subspp. *africana.* The frequent and naturally occurring hybridization between cultivated *E. coracana* subsp. *coracana* and its wild relative *E. coracana* subsp. *africana* gave rise to many morphological

intermediates (De Wet *et al*., 1985; Phillips, 1995; Neves *et al*., 2005) and evident gene flow between subspecies (Dida *et al*., 2008). This is mainly caused by the co-occurrence of *E. coracana* subsp. *coracana* and *E. coracana* subsp. *africana* in the same crop fields where they grow as cultivated and weeds, respectively (Tsehay, 2012). Several authors also confirmed that the cultivated subsp. *coracana* (finger millet) was domesticated through natural and artificial selection from the wild type finger millet, subsp. *africana* (Hilu and De Wet, 1976; Hilu and Johnson, 1992; Dida *et al*., 2008).

Neves *et al*. (2005) confirmed the close relationship of *E. coracana* and *E. indica*, and of these taxa to *E. kigeziensis* and further noted that all the three species show considerable morphological similarities (Fig 3). According to their closeness to the cultivated *E. coracana* subsp. *coracana*, Maxted and Kell (2009) classified the genus *Eleusine* into: primary wild relatives which include *E. africana, E. indica* and *E. kigeziensis; s*econdary wild relatives (*E. tristachya*, *E. floccifolia* and *E. intermedia*) and tertiary wild relatives (*E. jaegeri* and *E. multiflora*).

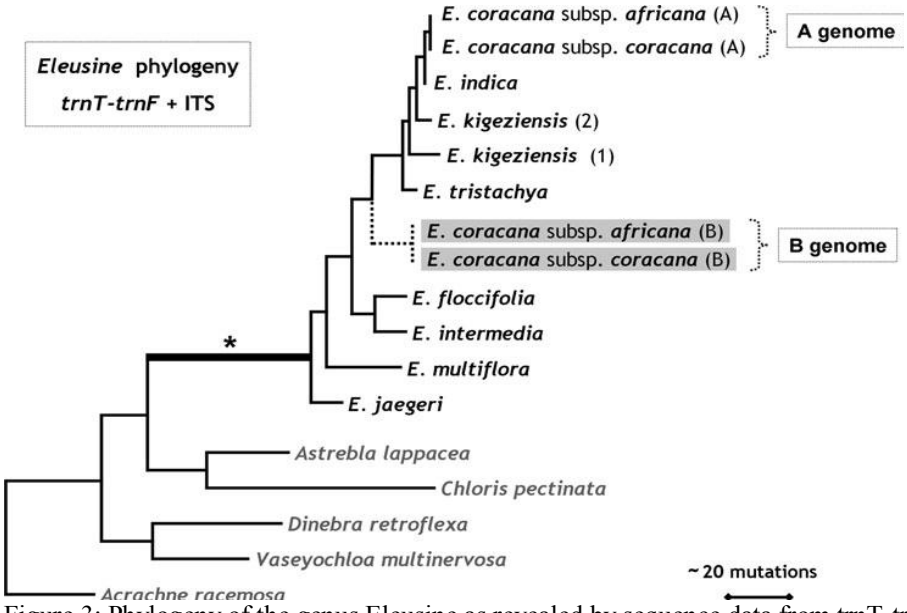


Figure 3: Phylogeny of the genus Eleusine as revealed by sequence data from trnT-trnF and ITS.
(Source: Neves *et al*., 2005)

3.5 Global distribution, cultivation and significance of finger millet

Finger millet is widely cultivated in the tropical and sub-tropical regions of Africa and India and ranked third in importance among millets after pearl millet (*Pennisetum glaucum*) and foxtail millet (*Setaria italica*) (Reddy *et al*., 2009). The precise global area under finger millet is not known because this crop has often been grouped with other millets such as Proso millet (*Panicum miliaceum*), pearl millet (*Pennisetum glaucum*), Foxtail millet (*Setaria italica*), Barnyard (Sawa) millet (*Echinochloa colona*), Kodo millet (*Paspalum scrobiculatum*), Teff (*Eragrostis tef*), Fonio millet (*Digitaria exilis* and *D. iburua*) (Hugh, 1986). Food and Agricultural Organization (FAO) estimated that the total millet production was 29,295,000 and 9,557,000 metric ton and finger millet took only 12.8% and 9% share on the planet and in Africa, respectively (FAOSTAT, 2004). About 36.29 million hectare land was covered by millet and 10% was finger millet (Upadhyaya *et al*., 2006).

Among the merits of finger millet are its ability to adapt to adverse agro-ecological conditions such as soil acidity, moisture stress, minimal inputs and marginal land where other crops cannot perform (Upadhyaya *et al*., 2007). The high content in nutrients such as calcium, iron and other important nutrients made finger millet a main staple food for rural populations of developing tropical countries inhabiting arid and infertile lands where calcium deficiency and anemia are widespread (Babu *et al.,* 2007). The yield potential reported is up to 4.5 tons ha^{-1} in India (Hugh, 1986) and more than 3.0 tons ha^{-1} in Ethiopia (Mulatu *et al*., 1995; Mulatu and Kebede 1993).

3.6 Finger millet research in Ethiopia and achievements

Research on finger millet started in the early nineteenth century at Debrezeit Agricultural Research Center. It was merged with sorghum research at Melkassa Agricultural Research

Center in 1986. About 13 improved varieties were released from the different research centers until 2014 (Table 1). However there are several gaps in terms of addressing peoples' preference on the type of varieties developed so far. For instance, in Metekel area, the two released varieties, Baruda and Dibatsi are red colored which are preferred for making local drinks termed *borde* by the Gumuz people. Productivity of white and black seeded finger millet varieties studied so far in the area had lower yield and were susceptible to lodging.

Purple–black seed color is highly preferred in Northern Ethiopia due to the fact that farmers linked this type of finger millet to high yield, tolerant to moisture stress and poor soil fertility. But no variety was released in the area so far. White seeded finger millet is highly preferred for injera making in the several finger millet growing regions. But there are several yield and agronomic drawbacks associated with such varieties such as low yield, susceptibility to lodging and susceptibility to blast disease. Necho is the only white seeded released variety, which is adapted to higher altitude areas around Yilmana Densa district and fails to adapt in Western Ethiopia where the blast disease is a priority constraints.

There has been very limited research on crop management and protection practices for finger millet. The agronomic recommendations used were variable across regions depending on the soil history and other environmental factors. Fertilizer rate of 105kg/ha DAP and 65kg/ha urea, 40cm row spacing and 15kg/ha seed rate were recommended for western Oromia.

Table 1: Characteristic features of finger millet varieties developed in Ethiopia

Varietal name	Year of release	Pedigree name	Area of potential production/altitude range, (m.a.s.l)	Average grain yield (kg/ha)		Seed color	Maintainer/ released
				on farm	On-station		
Tessema	2014	Acc.229469	1600-1900	1600	2000	Brown	Melkasa ARC
Gudetu	2014	Acc.215990	1400-1900	2050	2200	Brown	Bako ARC
Mecha	2014	Acc. 229371	1900-2500	1950	2450	Brown	Adet ARC
Necho	2011	PGRC/E203572	1900-2500	1750	2250	White	Adet ARC
Debatsi	2010	Debatsi	1100-1600	1750	2500	Brown	Pawe ARC
Bereda	2010	BRC-356-1	1600-2300	2150	2400	Light brown	Bako ARC
Gute	2009	Acc. 229373	1400-1900	2600	2750	Brown	Bako ARC
Wama	2007	KNE#392	1400-1900	2300	2600	Dark brown	Bako ARC
Baruda	2007	PW-001-75	1100-1600	1775	2000	Light brown	Pawe ARC
Degu	2005	PGRC/E215874	1900-2500	1900	2650	Black	Adet ARC
Boneya	2002	KNE#411	1400-1900	2200	2200	Brown	Bako ARC
Padet	1999	KNE#409	1400-1900	2250	2400	Brown	Melkasa ARC
Tadesse	1999	KNE#1098	1400-1900	2500	2500	Brown	Melkasa ARC

Source: Variety release registration volume 1-17 (1999-2014)

Key: ARC= Agricultural Research Center

3.7 Nutritional composition

Finger millet is rich in dietary fiber and minerals such as calcium and iron (Table 2) as compared to major cereal crops (Babu *et al*., 1987; Wondimu and Tekabe, 2001; Mgonja *et al.,* 2006). Its straw makes good fodder and contains up to 61% total digestible nutrients (National Research Council, 1996).

Table 2: Nutrient composition of finger millet as compared to major cereals grown in Ethiopia

Nutrient	Nutrient component per 100g of staple cereals					
	Finger Millet	Wheat	Maize	Rice	Sorghum	Tef
Energy (Kcal)	328.00	346.00	342.00	345.00	349.00	336
Protein (g)	7.30	11.80	11.10	6.80	10.40	8.3
Carbohydrate (g)	72.00	71.20	66.20	78.20	72.60	75.2
Fat (g)	1.30	1.50	3.60	0.50	1.90	2.9
Fibre (g)	3.60	1.20	2.70	0.20	1.60	3.6
Iron (mg)	12.60	5.30	2.30	0.70	4.10	59.0
Calcium (mg)	410.00	41.00	10.00	10.00	10.00	140

Source: Nutritional composition of finger millet compared to major cereals such as wheat, maize, rice and sorghum (Mgonja *et al*., 2006) and tef (Wondimu and Tekabe, 2001).

3.8 Genetic diversity and traits association in finger millet

3.8.1 Phenotypic diversity

Improvement in any crop usually involves exploiting the genetic variability in specific traits, which is expressed as the genetic differences between species, subspecies, varieties, populations or individuals (Jarvis *et al.,* 2000). Species with greater genetic diversity are more likely to be able to evolve in response to a changing environment than those with low genetic diversity. Populations that lack genetic diversity may experience low fertility and high mortality among offspring even in environments that are fairly stable (Hunter, 1996). Well-refined investigation on the genetic variation of a crop species is essential for genetic resource conservation and effective utilization of germplasm in plant breeding programs (Bekele, 1983 and 1985; Demissie and Bjonstrand, 1996; Keneni *et al*., 2007; Varshney *et al.*, 2007; Lule *et al.,* 2011).

Quantitative traits diversity studies conducted on finger millet in different countries by different authors indicated genetic variability among accessions within country and between countries.

Bezawuletaw *et al.* (2006) and Tsehaye and Kebebew (2002) reported substantial genetic variability among finger millet accessions collected from Ethiopia. Reddy *et al.* (2009) reported that finger millet accessions collected from Ethiopia and Burundi each grouped into separate clusters, based on major quantitative traits, but accessions from Kenya, Tanzania and Uganda grouped together.

With respect to qualitative traits diversity, Upadhyaya *et al.* (2007) noted that the erect type growth habit was dominant for 909 finger millet accessions collected from different East African countries. However, Bezawuletaw *et al.* (2006) reported that decumbent types were dominant followed by prostrate types in accessions collected from some of the former Ethiopian administrative regions (Wollega, Gojam, Gamu Gofa, Gonder, Tigray and Eritrea). De Wet *et al.* (1985) reported that open and droopy shaped fingers are a typical characteristic of race coracana. Purple-black colored seed is dominant in Northern Ethiopia due to the fact that farmers linked this type of finger millet with high yield and tolerance to abiotic stresses such as moisture stress and poor soil fertility (Bezawuletaw *et al*., 2006; Tsehaye and Kebebew, 2002).

3.8.2 Variability, heritability, genetic advance and association of major quantitative traits

Heritability interests plant breeders primarily as a measure of the value of selection for a particular character in various types of progenies and as an index of transmissibility. If the percentage is high, the character is heritable but if it is small, the environment is correspondingly prominent in the character expression (Hayes *et al*., 1955). Allard (1960) indicated that the heritability values for quantitative traits are low mainly due to their sensitivity to environmental factors. Moreover, heritability should be used along with genetic advance in

predicting the efficiency of selection. High heritability values could be obtained with genotypes having small or large genetic variance but genetic progress would be larger with larger genotypic variance (Allard, 1960).

High heritability associated with high genetic advance is chiefly due to the additive gene effect but if heritability is mainly due to dominance and epistasis, the genetic gain would be low (Panes, 1957). In general, genetic variability, heritability and genetic advance are prerequisites for a breeding program and provide opportunities to breeder to select high yielding genotypes or to combine or transfer genes having desirable traits (Khorgade *et al.*, 1985). Pandey and Tiwari (1983) indicated the importance of estimating heritability to know the inheritance of quantitative traits as it indicates the genetic gains that may be achieved through selection.

Seed yield is an important and priority trait for plant breeders and other crop researchers. However, seed yield is a complex character and is considered the ultimate product of its components. Hence, selection of superior genotypes based on grain yield is difficult due to the integrated structure of a plant in which most of the characters are interrelated and being governed by a larger number of genes. This necessitates a thorough knowledge on the nature of the relationship prevalent between the contributory characters and grain yield and the extent of genetic variability.

Genetic variability, inheritance and association of quantitative traits in finger millet were studied by several authors. Sharathbabu *et al*. (2008) evaluated 19 white seeded finger millet genotypes and three standard checks and reported very low phenotypic coefficient of variation (PCV) values (6.46 and 8.34%) for days to maturity and days to 50% heading, respectively. The author

also reported that finger number per main ear, number of productive tillers and ear weight per plant had a strong positive association with grain yield per plant and suggested that simultaneous selection for these traits could be more reliable to develop high yielding genotypes in finger millet. Low PCV and genotypic coefficient of variation (GCV) were observed for days to maturity for 230 finger millet accessions (Ganapath *et al*., 2011).

Lower GCV values for days to heading and days to maturity were reported in different crops by several authors (Purseglove, 1972; Misra *et al*., 2009; Sharathbabu *et al*., 2008; Ganapathy e*t al*., 2011). Ganapathy *et al.* (2011) reported high heritability coupled with high genetic advance observed in finger length, days to 50% heading, productive tillers per plant and seed yield per plant. John (2006) also noted that high heritability coupled with high genetic advance as percentage of mean for number of fingers per main ear and ear weight. Wolie and Dessalegn (2011) found that plant height, days to heading and days to maturity had negative direct effects on grain yield, but they had positive indirect effects on grain yield mainly through their high and positive indirect effect on biomass yield.

3.8.3 Molecular diversity

Phenotypic characterization is an important first step in the assessment of genetic diversity, but has certain limitations due to morphological plasticity and parallel evolution. These characters are strongly influenced by environmental conditions and show little variation at the intra-specific level (Sangwan *et al*., 2001). Besides, evaluation of phenotypic traits requires growing the plants to maturity prior to identification. Isozymes are differently charged protein molecules that can be separated using electrophoretic procedures (usually starch gel) (Markert and Moller, 1959). Since enzymes catalyze specific biochemical reactions, it is possible to visualize the

location of a particular enzyme on a gel by supplying the appropriate substrate and cofactors and involving the product of the enzymatic reaction in a color producing reaction. Bands visualized from specific enzymes represent protein products, have a genetic basis and can provide genetic information as co-dominant markers. However, the scarcity of isozyme loci and the fact that they are subjected to post-translational modifications often restricts their utility (Staub and Crubaugh, 1995). The rapid developments in biotechnology allow easy analysis of a large numbers of loci distributed throughout the genome of plants. The most commonly used molecular marker systems in diversity analysis are random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), inter simple sequence repeats (ISSRs), microsatellites or simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs). However, each marker has its own advantages and disadvantages.

Some of the most commonly used molecular markers in finger millet molecular research includes RAPD (Salimath *et al*., 1995; Babu *et al*., 2007, Das and Misra 2010; Panwar *et al*., 2010; Gupta *et al*., 2010; Bezawuletaw, 2011), RFLP (Parani *et al*., 2001), ISSRs (Gupta *et al*., 2010) and SSRs (Dida *et al*., 2007, 2008; Panwar *et al*., 2010; Sinha and Pande, 2010) as well as some efforts to investigate blast resistant R-genes (Panwar *et al*., 2011; Reddy *et al*., 2010).
Previous findings using isozyme and DNA marker analyses have indicated that cultivated finger millet has a narrow genetic base and most likely went through a bottleneck during domestication (Hilu and Johnson 1992; Salimath *et al*., 1995). Variations in the wild subsp. *africana* were relatively higher than the cultivated subsp. *coracana* (Hilu and Johnson 1992; Werth *et al*., 1994; Dida *et al*., 2008; Lule *et al*., 2014).

3.8.4 Major features of SSRs and its advantages over the other molecular markers

Simple sequence repeat (SSR) markers are short tandem DNA repeats, randomly spread in eukaryotic genomes, with repeat units usually equal to or less than six bases in length such as $(GA)_{10}$, $(CAC)_{15}$, $(TTGG)_8$, etc, and the number of repeats highly variable between individuals or genotypes (Reddy *et al*., 2002). SSRs are important molecular markers in both animals and plants. They are very polymorphic due to the high mutation rate affecting the number of repeat units. Such length-polymorphisms can be easily detected on high resolution gels (e.g. sequencing gels). It is suggested that the variation or polymorphism of SSRs are a result of polymerase slippage during DNA replication or unequal crossing over (Levinson and Gutman, 1987). SSRs are not only very common, but also are hyper-variable for numbers of repetitive DNA motifs in the genomes of eukaryotes (Rallo *et al*., 2000; Van der Schoot *et al*., 2000). Polymorphism in the repeat region can be detected by performing a PCR with primers designed from the DNA flanking region.

SSR markers have features that make them particularly suitable for assessing genetic diversity in many plants including that they are co-dominant, abundant, highly reproducible, highly polymorphic, multi-allelic, locus specific and easy to assay (Marcel *et al*., 2007; Pushpendra *et al*., 2007), need a small amount of medium quality DNA, so cheap and easy to run, the analysis can be semi-automated and performed without the need of radioactivity (Guilford *et al*., 1997). With the advances in DNA isolation technologies, it has been possible to identify loci in highly degraded ancient DNA where traditional enrichment procedures have been unsuccessful (Allentoft *et al*., 2009). SSRs have become fast and efficient with the development of high-throughput sequencing platforms (Allentoft *et al*., 2009; Santana *et al*., 2009). Furthermore, SSRs can be used to identify parental lines for new variety development with maximum hybrid

vigor and genetic diversity due to their higher polymorphism compared to other markers (Kubik *et al*., 2009) and hence it is a marker of choice for practical breeding applications, particularly in developing countries (Sharma *et al*., 2010).

3.9 Finger millet blast (Magnaporthe oryzae) *disease and its significance*

Early investigators and observers of *Eleusine coracana* had a common belief that disease of finger millet is relatively unimportant (Coleman, 1920; Patel, 1955). Moreover, these early workers considered *Helminthosporium* species to be more important than other pathogens. More recently, blast has become considered extremely important owing to the high losses that can occur from certain types of infection particularly when the neck and ear/head are affected (Kenneth and LeRoy, 1977).Blast disease, caused by *Magnaporthe oryzae* (anamorph. *Pyricularia oryzae*) is a pathogen of many *gramineous* species (Bheema *et al*., 2010; Ou, 1985) and the most important constraint for rice (Chauhan *et al*., 2002, Rossman *et al*., 1990, Ou, 1985) and finger millet production (Mgonja *et al*., 2007; Kato *et al*., 1977; Pall, 1977; Rath and Mishra, 1975; Anon, 1959; Venkatarayan, 1947). The pathogen also attacks barley (Yaegashi and Nishihara, 1976), wheat (Urashima *et al*., 1993) and maize (Notteghem, 1990). Sources of the inoculum are known to be seeds, crop residues and several weed species, including wild *Eleusine* spp. and *Setaria verticillata* (Pande *et al*., 1995; Borromeo *et al*., 1993). Even though the host range of *M. oryzae* is extensive, individual isolates of the fungus are specific to only a small number of grass species (Kang *et al*., 1995).

3.9.1 The Causal organism

The causal organism for finger millet blast was postulated differently by several authors in different countries. Hansford (1943) in Uganda and Wallace and Wallace (1948) in Tanzania, identified the pathogen as *Pyricularia oryzae*. In India, Ramakrishnan (1948) identified the fungus to be a race of *P. oryzae*, since he could find no obvious difference between the two organisms. Thirumalachar and Mishra (1954) identified the causal organism as *P. grisea.* Later, Couch and Kohn (2002) reported that *Magnaporthe oryzae* is new species that segregated from *M. grisea* and hence grouped blast pathogen isolates from *Digitaria* spp. as *M. grisea* and those from a number of other hosts, including rice and finger millet, as *M. oryzae*. More recently, Takan *et al*. (2011) confirmed that *M. grisea* isolates from *Digitaria* spp. formed a distinct genetic cluster from isolates from cultivated finger millet, its wild types and other weedy species based on analyses using AFLP and internal transcribed spacer (ITS) sequences.

3.9.2 Cross infectivity of the pathogen

Cross infectivity experiments have been carried out by several authors. The finger millet *Pyricularia* strain fails to infect rice and ginger but does infect wheat, barley and oats. The strain from rice and *Panicum repens* would infect only its own host (Kenneth and LeRoy, 1977). Ramakrishnan (1948) reported that the finger millet pathogen readily infects bulrush millet, maize and *Dactyloctenium aegyptium* but would not infect rice and *Digitaria marginata*. Hence, the finger millet strain has alternative host in other crops and wild relative as well.

3.9.3 Varietal reaction to blast pathogen

Environmental conditions have been shown to have considerable influence on the intensity of blast infection. The incidence and severity of the disease is particularly acute during heavy rainy

seasons and highly humid months, but low infection can also occur in hot dry seasons (Kenneth and LeRoy, 1977). Varietal resistance to finger millet blast has apparently not been studied thoroughly since very few lines have been identified as carrying even moderate levels of resistance. For instance, line E.C. 155 possessed medium resistance in the early years of testing, but in later seasons was found to become susceptible in Coimbatore, India (Kenneth and LeRoy, 1977). Another cultivar, R-6, showed field resistance at Kovilpatti, India, but proved susceptible at Coimbatore in the same state. The finger millet line, Mozambique 359, was mentioned as a source of resistance and hence a possible parent for resistance breeding in to local Ugandan lines (Anon, 1959). Furthermore, a general observation stated that purple pigmented types of finger millet are more resistant to blast than non-pigmented ones. In this regard, inheritance of purple pigment is dominant over green (Kenneth and LeRoy, 1977).

3.9.4 Yield losses due to the pathogen

Severe yield losses have regularly been recorded due to the blast fungus that infects the different above ground tissues of finger millet; 80-90% yield loss in Mysore, India (Venkataryan, 1947), up to 46% in Japan in 1973 (Kato *et al*., 1977) and up to 90% in Eastern Africa (Mgonja *et al*., 2007).

In the Ethiopian context, although the crop has a potential yield of more than 3.0 tons per hectare (Mulatu *et al*., 1995; Mulatu and Kebede 1993), production and productivity is adversely affected by various biotic and abiotic constraints among which blast is the most severe and the national average yield never exceeded 1.5 tons ha^{-1} during the 1995 – 2012 cropping seasons (Fig 2) and was rose to 1.7 tons ha^{-1} for the 2013 cropping season (CSA, 2013). The severity of the disease was at its climax or high, to such an extent that released and

candidate varieties could not tolerate infection during very humid and high rainfall years (BARC, 2010). All aerial parts of the plant can be affected in wet and humid environments. Leaf surfaces become speckled with oval lesions and plants become prone to lodging when stems are infected. Rath and Mishra (1975) and Pall (1977) described significant losses in grain number and grain weight and increased spikelet sterility due to finger millet neck infection. However, the most severe yield loses occur from panicle infection (Mgonja *et al*., 2007; Ekwamu, 1991).

3.9.4.1 Yield losses assessment methods

The two main approaches used to relate disease intensity to crop losses are experimental and statistical (James, 1974).

The experimental approach

The experimental approach to crop loss assessment usually involves experiments in which the level of disease in a crop is controlled using different levels of inoculation or treatments with biocides (fungicides, nematicides, bactericides). Often, an intensive spraying schedule is used to control disease at a level close to zero, which allows the potential yield of the crop in the absence of disease to be determined. The value of this method depends on the assumption that the biocide treatment itself has no direct effect on yield and that susceptible and resistant cultivars should have similar yield potentials in the absence of disease (James, 1974). Near isogenic lines of a crop, which are genetically similar except for the presence of a specific gene for resistance, are sometimes used to investigate the effects of disease on growth and yield (James, 1974). Therefore, allowances should be made for this when applying data to commercial situations.

Under experimental approaches, various yield loss assessment models have been reported based on disease epidemics, crop types and other host-pathogen-environment interaction effects (Mousanejad *et al*., 2010; Shtienberg *et al*., 1990; Tsai, 1988; Teng and Gaunt, 1980; Katsube and Koshinizu, 1970; James *et al*., 1968). However, the three most common assessment methods are: (1) critical-point models that provide estimates of loss for any given level of disease at a specific time (Mousanejad *et al*., 2010; Katsube and Koshinizu, 1970; James *et al*., 1968) or any specified time when a particular severity of disease is reached (Large, 1952), (2) multiple-point models estimate loss over time as a disease-progress curve consisting of multiple disease assessment time intervals (James *et al*., 1972) and (3) the model that relates the area under the disease-progress curve to yield loss, which can be described as the midway between the critical- and multiple-point models (James, 1974; Van der Plank, 1963). The duration of the epidemic relative to the life cycle of the crop is a primary consideration in applying the appropriate model (James, 1974).

The statistical approach

This approach to disease assessment involves statistical analysis of yields under different levels of disease that occur naturally in the field (James, 1974) and includes: (i) comparisons of yields obtained from crops in seasons with different disease intensities, (ii) comparisons of expected yield under disease free conditions on experimental stations and actual yields, (iii) comparisons of the yields of a particular crop with the average yield for the region and (iv) comparisons of epidemic progress and yield parameters between crops or portions of a crop showing different levels of infection or damage. These comparisons can be very useful because they are based on the real cropping situation in the field rather than on experimental plots, which often give artificially high yields (James, 1974). However, account has to be taken of the fact that in the

field many other factors besides disease will affect the yield (James, 1974). Using statistical analysis of natural disease epidemics, critical point, multiple point and AUDPC models relating crop loss to epidemic development can also be determined (James, 1974).

3.10 Genotype by Environment interaction and grain yield stability

The consequences of phenotypic variation depend largely on the environment. This variation is further complicated by the fact that not all genotypes react in a similar way to changes in the environment and no two environments are exactly the same. Mean yield across environments is an adequate indicator of genotypic performance only in the absence of Genotype by Environment (G × E) interaction. Moreover, G × E interaction results in genotype rank changes from one environment to another, a difference in scale among environments, or a combination of these two situations. If the relative performance of genotypes grown in different environments is different, then G × E interaction becomes a major challenging factor to crop breeding.

It is important for plant breeders to identify specific genotypes adapted to or stable in environment(s), thereby achieving quick genetic gain through screening of genotypes for high adaptation and stability under varying environmental conditions prior to release as a variety (Ario, 1989; Flores *et al.,* 1998; Showemimo *et al*., 2000; Mustapha *et al*., 2001). However, most genotypes exhibit fluctuating yields when grown in different environments or agro-climatic zones. This complicates demonstrating the superiority of a particular variety. To address this challenge, multi- environment yield trials are crucial to identify adaptable high yielding cultivars and discover sites that best represent the target environment (Yan *et al*., 2000). Adaptability is the result of genotype, environment and genotype by environment

interaction and generally falls into two classes: (1) the ability to perform at an acceptable level in a range of environments, referred to as general adaptability, and (2) the ability to perform well only in desirable environments, known as specific adaptability (Farshadfar and Sutka, 2006).

Combined analysis of variance can quantify G x E interactions and describe the main effects but does not explain the interaction effect (Yuksel *et al*., 2002; Worku *et al*., 2013). The fundamental reason Additive Main effects and Multiplicative Interactions (AMMI) is appropriate for agricultural research is that the ANOVA part of AMMI can separate the G and E main effects and the G × E interaction effects (Gauch *et al*., 2008). Besides, its greatest advantage is its ability to extract interaction Principal Component Axis (PCA) along which there is a maximum variation, thereby indicating the number of components necessary to explain the pattern in the interaction residual (Girma, 1999). Additive Main Effect and Multiplicative Interaction model and genotype and genotype by environment interaction (GGE) biplot analysis are the most commonly used analytical and statistical tools to determine the pattern of genotypic responses across environments (Gauch and Zobel, 1996; Yan *et al*., 2000; Yuksel *et al*., 2002).

AMMI and GGE biplot (Gauch and Zobbel, 1996; Yan *et al*., 2000; Yuksel *et al*., 2002) for graphical display of data and Eberhart and Russell (1966) model are the most commonly used analytical and statistical tools to identify stable, high yielding and adaptable genotype(s) for wider and/or specific environments.

3.11 Comparative genomics

The use of modern crop improvement tools such as genomics to transfer information about genes from model species to the species of interest and genetic mapping in order to identify genes controlling traits of interest, can provide a more timely and robust response to crop production threats (Praba *et al*., 2009). It also provides added opportunities to develop crop varieties with multiple stress tolerance.

Comparative genomics is the study of the relationship of genome structure and function across different biological species or strains. It is an attempt to take advantage of the information provided by the signatures of selection to understand the function and evolutionary processes that act on genomes (Soh, 2011). Comparative genetic analyses have shown that different plant species share orthologous genes for similar function and have highly conserved gene content and gene order (Devos *et al*., 2000). Pinpointing a genetic trait on a linkage map is crucial to physically isolating it; and when it is isolated from one species, it may be obtained from any other by homologous cloning (Jennifer, 1998). This enables us to identify genes that control genetic diseases, and even makes it realistic to consider cloning genes involved in quantitative traits. Comparative gene mapping can, therefore, deliver information for the benefit of research into plant and animal health and molecular breeding (Jennifer, 1998).

Comparative genomics provides a tool to utilize the exponentially increasing sequence information from model plants to clone agronomically important genes of less studied crop species (Sanwen *et al*., 2005). The isolation of genes underlying orthologous traits is the first step in conducting comparative functional studies (Devos, 2005). The most popular applications in cereals, especially since the complete rice genomic sequence has been available, are the use

of comparative data in the generation of new markers to tag traits in other species and to identify candidate genes for these traits (Devos, 2005). By comparing unigene sets from soybean to non-legume unigene sets and to the genomic sequences of rice and *Arabidopsis*, Graham *et al*. (2004) were able to identify more than 2,500 legume-specific EST contigs, accounting for approximately 6% of genes in legume unigene sets and identified three major protein related gene families from this putative legume-specific gene set. Comparative analysis based on DNA sequences has also revealed that disease resistance (R) loci may be evolving faster than the rest of the grass genomes (Leister *et al*., 1998).

Finger millet–rice comparative maps demonstrate that, other than the rearrangements that are necessary to account for the difference in chromosome number between finger millet and rice, the finger millet genome has remained highly conserved since its divergence from a common ancestor with rice some 60 million years ago (Dida *et al.,* 2007). The high conservation of colinearity between finger millet and rice will facilitate the exploitation of the information and resources available from rice and other grasses. Traits that are of immediate interest to finger millet breeders are blast and drought resistance, both of which have been the subject of much research in rice (Dida *et al.,* 2007).

The genome size of finger millet was estimated by flow cytometry to be in a range of 3.36–3.87 pg (2C value) and an average of 1.8 pg (1C value) (Mysore and Baird, 1997), where 1 pg =980 Mbp. Although the genome size of the *Eleusine* species in general and finger millet in particular is small when compared with that of many other plants, it is nonetheless considered relatively large from a genetics or genomics point of view (Mysore and Baird, 1997; Dida et al., 2007).

3.12 Single strand conformation polymorphism (SSCP) and its use to detect SNPs

Apart from DNA the sequencing, SSCP technique is a simple and efficient way to detect any small alteration in PCR-amplified product and discover new DNA polymorphisms (Orita *et al.,* 1989). It is based on the assumption that nucleic acid changes affect the migration of single stranded DNA fragments, and therefore, result in visible mobility shifts across a non-denaturing polyacrylamide gel (Orita *et al.,* 1989). SSCP uses amplicons of a specific locus that have been produced in a preceding PCR reaction. SSCP analysis is a useful application for the detection of mutations based on the ability of a single nucleotide change in a given sequence to alter the electrophoretic mobility of that DNA single strand under non-denaturing conditions (Orita *et al.,* 1989). Other than for double stranded DNA, the mobility of single strands (ssDNA) in electrophoretic gels is affected by changes in sequence, leading to instable intra-strand base pairing, resulting in loops and folds that give the single strand a unique secondary structure. It can be used in genotyping to detect homozygous individuals of different allelic states, as well as heterozygous individuals that should each demonstrate distinct patterns in an electrophoresis experiment (Michiei *et al.,* 1993).

During SSCP analysis, dsDNA undergoes denaturation to form ssDNA followed by subsequent folding, generating a unique conformational state based on its sequence. A single nucleotide change therefore dramatically affects the mobility of the ssDNA strand through a gel by altering the conformation. Because of the conformational changes, SSCP can identify heterozygosity of DNA fragments of the same molecular weight and even detect changes of a few nucleotide bases as the mobility of the single-stranded DNA changes with a change in its GC content. This is a rapid technique for allele analysis particularly for the detection of point mutations as well as for the detection of DNA polymorphisms and mutations at multiple sites in DNA fragments.

Apart from regular mutation detection in genes of interest, SSCP has been applied in genotyping (Sheffield *et al.,* 1993) as well as in molecular ecology (Sunnucks *et al.,* 2000) or mutation detection (Martins-Lopes *et al*., 2001). In general, denaturing polyacrylamide gel electrophoresis doesn't allow detection of polymorphism due to a difference of one base pair length/type. However, the SSCP methodology allows detection of polymorphism due to a difference of one or more base pairs in PCR products and is suitable for SNP genotyping (Varshney, 2010).

4. MATERIALS AND METHODS

A total of five subthemes were covered in the present study. Those are; 1) genetic diversity analysis for phenotypic traits, 2) genetic diversity analysis using SSR markers, 3) screening for blast disease resistance/tolerance, 4) genotype by environment interaction and 5) marker development for blast resistant gene/comparative genomics

4.1. Genetic diversity analysis for phenotypic traits

4.1.1 Description of the study area

Field experiments were conducted at Arsi Negele research sub site (1947 m.a.s.l., $07^0$19' 29.9"N; $38^0$39' 27.2"E), 20 km north west of Shashamane town and 230 km south of Addis Ababa and Gute sub site (1906 m.a.s.l, $36^0$38' 24.3" E; $09^0$00' 53.6"N), 320 km west of Addis Ababa in Western Ethiopia and 11 km east of Nekemte town. According to the classification of Agro ecological zonation of Ethiopia, both experimental sites are characterized as sub-humid midlands (Weina Dega) located between 1500-2300 m.a.s.l. and receive an average annual rainfall in a range of 800-1200 mm (Hans, 1998; USDA, 2003; Gorfu and Ahimed, 2008).

4.1. 2 Planting materials and experimental procedures

One hundred and five finger millet accessions collected from major finger millet growing regions of Ethiopia (Oromia, Amhara, Tigray, Benshangule Gumuz and Southern Nation Nationalities and Peoples Regional states), supplemented by 39 introduced accessions from Kenya, Zambia, Zimbabwe and Eritrea were evaluated along with six released varieties, namely Tadesse, Boneya, Gute, Bereda, Padet and Wama (Table 3). Those accessions were primarily

collected by the Ethiopian Institute of Biodiversity Conservation (EIBC), Bako Agricultural Research Center (BARC) and Addis Ababa University (AAU). The experimental design was a Randomized Complete Block Design (RCBD) with two replications and the plot size was single rows of 2m long and 50cm between row spacing. Each block was divided into two. Spacing between plants within each row was adjusted to 10cm. Eight altitude classes were used to group finger millet accessions with relative resemblance of agro-climatic origin following the Agrawal (1996) formula:-

$K = 1+3.32\log_{10}n$ and $W= (L-S)/K$ where K = number of class intervals, W = width of class interval, L = the largest value, S = the smallest value and n = sample size (in this case the number of accessions). Regional and altitudinal distribution of finger millet accessions along with collection passport data are presented in Table 3 and 4.

Table 3. Finger millet accessions evaluated for phenotypic and molecular diversity

SND	Acc.name	Region	SNJ	Alt.	SND	Acc.name	Region	SNJ	Alt.
1	242133	Amhara	1	1825	47	203354	Zimbabwe	128	1420
2	BKFM0034	Oromia	53	1454	48	230102	Eritrea	40	1850
3	230104	Eritria	44	1800	49	229730	Amhara	10	1850
4	Padet*	Released			50	215982	Amhara	11	1850
5	AAUFM-42	Tigray	95	2058	51	Tadesse	Released	85	
6	AAUFM-22	Tigray	96	2142	52	242617*	Tigray		1700
7	BKFM0026*	Oromia		1479	53	238300	Tigray	106	1980
8	215989	Amhara	2	2000	54	203356	Zimbabwe	129	1420
9	230106	Eritrea	38	1800	55	244798*	SNNP		2169
10	BKFM0042	Oromia	54	1867	56	Wama	Released	86	
11	BKFM0028	Oromia	55	1608	57	242616	Tigray	107	1400
12	215985	Amhara	3	1940	58	BKFM0048	Oromia	66	1337
13	203350	Zimbabwe	126	1400	59	BKFM0024	Oromia	67	1913
14	BKFM0032	Oromia	56	1390	60	237584	SNNP	91	1990
15	BKFM0006	Oromia	57	1479	61	229723	B/Gumuz	32	1300
16	AAUFM-4	Tigray	97	1896	62	214991	Zambia	118	1330
17	235835	Amhara	4	1930	63	Bereda	Released	87	
18	BKFM0022	Oromia	58	1926	64	215976	Amhara	12	1860
19	AAUFM-34	Tigray	98	1568	65	242111	Amhara	13	2100
20	AAUFM-32	Tigray	99	1630	66	242624	Tigray	108	1400
21	AAUFM-33	Tigray	100	1620	67	229728	B/Gumuz	33	1440
22	216039	Oromia	59	1950	68	216036	Tigray	109	1900
23	241768	SNNP	90	1500	69	216033	B/Gumuz	34	1930
24	AAUFM-8	Tigray	101	1812	70	214994	Zambia	119	1160
25	BKFM0047	Oromia	60	1334	71	241769	SNNP	92	1500
26	229731	Amhara	5	1950	72	237472	Tigray	110	1800
27	BKFM0029	Oromia	61	1251	73	203358	Zimbabwe	130	1420
28	AAUFM-19	Tigray	102	1811	74	BKFM0005	Oromia	68	1449
29	214995	Zambia	117	1130	75	Gute	Released	88	
30	203353	Zimbabwe	127	1420	76	214996	Zambia	120	1130
31	AAUFM-2	Tigray	103	1896	77	238327	Tigray	111	1900
32	235782	Amhara	6	1860	78	BKFM0018	Oromia	69	1667
33	242117	Amhara	7	1915	79	216046*	Tigray		1910
34	237475	Tigray	104	1750	80	214988	Zambia	121	1300
35	203545	Kenya	46	1590	81	243639	Amhara	14	2070
36	AAUFM-35*	Tigray		1568	82	225892	Amhara	15	1710
37	215802	Amhara	8	1950	83	214987	Zambia	122	1310
38	BKFM0039	Oromia	62	2144	84	230110*	Eritrea		1700
39	230103	Eritria	45	1700	85	215990	Amhara	16	1910
40	BKFM0010	Oromia	63	1484	86	203360	Zimbabwe	131	1420
41	245087	Tigray	105	1923	87	208726	Oromia	70	1880
42	230105	Eritrea	39	1600	88	BKFM0055	Oromia	71	1723
43	215887	Amhara	9	1880	89	237443	Amhara	17	2100
44	BKFM0062	Oromia	64	1923	90	BKFM0011	Oromia	72	1428
45	BKFM0002*	Oromia		1550	91	Boneya	Released	89	
46	BKFM0052	Oromia	65	2200	92	AAUFM-15*	Tigray		1568

Table 3. continued…

SND	Acc.name	Region	SNJ	Alt.	SND	Acc.name	Region	SNJ	Alt.
93	203355	Zimbabwe	132	1420	122	203363	Zimbabwe	136	1420
94	229722	B/Gumuz	35	1750	123	214990*	Zambia		1360
95	BKFM0057	Oromia	73	1707	124	236446	Oromia	79	1930
96	BKFM0060	Oromia	74	1852	125	203357	Zimbabwe	137	1420
97	230109	Eritrea	41	1800	126	235140	Amhara	25	1950
98	100038*	Amhara		1980	127	214997	Zambia	125	1100
99	238344	Amhara	18	2000	128	242112	Amhara	26	2125
100	245091	Oromia	75	1991	129	203547	Kenya	50	1510
101	216056	Oromia	76	1600	130	BKFM0051	Oromia	80	2227
102	AAUFM-23	Tigray	112	2100	131	203544	Kenya	51	1485
103	203542	Kenya	47	1540	132	242109	Amhara	27	2060
104	238341	Amhara	19	1780	133	203351	Kenya	52	1490
105	203362	Zimbabwe	133	1420	134	229738	B/gumuz	37	1830
106	230107	Eritrea	42	1800	135	203359	Zimbabwe	138	1420
107	203352	Zimbabwe	134	1490	136	242132	Amhara	28	1910
108	242135	Amhara	20	1910	137	243636*	Amhara		2100
109	AAUFM-20	Amhara	21	2142	138	BKFM0004	Oromia	81	1445
110	214993	Zambia	123	1340	139	BKFM0008	Oromia	82	1459
111	229724	B/Gumuz	36	1520	140	AAUFM-21	Tigray	114	1722
112	203546	Kenya	48	1620	141	216040	Oromia	83	1940
113	242105	Amhara	22	1860	142	BKFM0058	Oromia	84	1725
114	216057	Oromia	77	1800	143	235700	SNNP	93	1530
115	BKFM0001	Oromia	78	1580	144	235699	SNNP	94	1440
116	238346	Amhara	23	1940	145	AAUFM-12	Tigray	115	1502
117	214989	Zambia	124	1210	146	230101	Eritrea	43	1740
118	203361	Zimbabwe	135	1420	147	215981	Amhara	29	1850
119	AAUFM-14	Tigray	113	1568	148	229725	Amhara	30	1650
120	203543	Kenya	49	1514	149	242120	Amhara	31	1850
121	235783	Amhara	24	2000	150	AAUFM-11	Tigray	116	1502

Key: *= accessions not amplified by ≥ 4 markers and thus reduced from AMOVA, SSR neighbor joining and population structure analysis; SND= serial number as used sequentially for dendrogram generated for 17 quantitative traits of the whole population; SNJP= serial number showing germplasm arrangement on neighbor joining tree and population structure of 138 well amplified accessions.

Table 4. Regional and altitudinal distribution of finger millet accessions used for genetic diversity study.

No.	Region /Country	Altitude classes								
		≤1241	1242-1382	1383-1523	1524-1664	1665-1805	1806-1946	1947-2087	≥2088	Sub total
1	Amhara/Eth	0	0	0	1	2	16	9	4	32
2	B/Gumuz/ Eth	0	1	2	0	1	2	0	0	6
3	Eritrea	0	0	0	1	7	1	0	0	9
4	Kenya	0	0	4	3	0	0	0	0	7
5	Oromia/ Eth	0	3	9	4	5	8	2	3	34
6	SNNP/ Eth	0	0	3	1	0	0	1	1	6
7	Tigray/ Eth	0	0	4	6	4	8	2	3	27
8	Zambia	5	5	0	0	0	0	0	0	10
9	Zimbabwe	0	0	13	0	0	0	0	0	13
Sub total		5	9	35	16	19	35	14	11	144
Released Varieties										6
Grand total										150

4.1.3 Diversity data collection and analysis for morphological traits

Ten individual plants were selected randomly per plot, marked before heading and used as samples for the measurable quantitative traits.

4.1.3.1 Qualitative traits data collection and analysis

Data were recorded on the qualitative traits such as growth habit, ear shape, ear (glumes) color, grain coverage by glumes, spikelet density and grain color following the finger millet descriptor (IBPGR, 1985). The number of each phenotypic class was counted per plot. The percentage frequency of distribution of qualitative traits across regions and altitude classes were manipulated using Microsoft Excel. Hierarchal clustering of accessions were performed regionally and altitudinally using MINITAB14 software (MINITAB, 2003) for the standardized data to mean zero and unity variance. The amount of genetic variation was determined using the Shannon-Weaver diversity index as suggested by Jain *et al*. (1975) using the formula:

$$H' = -\sum_{i=1}^{n} Pi \log ePi$$

where n = the number of phenotypic classes for a character and P_i = the genotypic frequency as the percentage proportion of the total entries in the i^{th} class. The diversity index was estimated at specific accession level, regional level and altitudinal level.

4.1.3.2 Quantitative traits data collection and analysis

Data were recorded for days to 50% heading, days to 50% maturity, plant height (cm), culm length (cm), culm diameter (cm), finger length (cm), number of fingers per main ear, total number of tillers, productive tiller number, number of grains per spikelet, number of culm branches per main plant, lodging index, ear weight (g), biomass weight per plant (g), harvest index (%), thousand grain weight and grain yield per plant (g) following the finger millet descriptor (IBPGR, 1985).

4.1.3.2.1 Analysis of variance

Data collected for all quantitative characters were subjected to analysis of variance (ANOVA) using SAS software (SAS, 2008). The major descriptive statistics such as mean, range and standard deviation of each trait for the whole study accessions, countries/regions of collection and altitude zones were computed by using Microsoft Excel.

4.1.3.2.2 Cluster analysis

Hierarchal clustering of the average linkage method with squared Euclidian distance was performed using MINITAB14 (MINITAB, 2003). Data for all quantitative traits were standardized to mean of zero and variance of one before clustering to avoid any bias that may

have arisen due to differences in measurement scales. The distances between clusters were calculated using the average linkage method of squared Euclidian distance.

4.1.3.2.3 Principal component analysis (PCA)

Principal component analysis for 17 standardized quantitative traits was computed by using Agrobase (2000) software at population level, region of collection and altitude classes to identify the most important traits contributing to the total variations observed among the accessions, countries/regions of origin and altitude classes. As suggested by Johnson and Wichern (1988), principal components with eigen values greater than one were considered.

4.1.3.2.4 Estimation of correlation coefficient

The Pearson's correlation coefficients between all possible pairs of quantitative traits were tested for their significance using SAS software (SAS, 2008).

4.1.3.2.5 Path coefficient analysis

The direct and indirect effects of yield related quantitative traits on grain yield per plant were calculated following the formula suggested by Dewey and Lu (1959) as:

$$rij = Pij + \sum rikPkj$$

where, r_{ij} is mutual association between the independent character (i) and dependent character (j) as measured by the correlation coefficient; Pij is the component of direct effects of the independent character (i) and dependent (j) as measured by the path coefficient and; $\sum rikPkj$ is the summation of indirect effect of a given independent character (i) on the given dependent character (j) via all other independent characters (k).

Residual effect, which determines how best the causal factor accounts for the variability of the dependent factor (grain yield), was estimated by the formula:

$\sqrt{1-R^2}$ where $R^2 = \sum Pijrij$, Pij = Component of direct effects of the independent character (i) and dependent character (j) as measured by the path coefficient; rij, mutual association between the independent character (i) and dependent character (j) as measured by the correlation coefficient.

4.1.3.2.6 Analysis of phenotypic and genotypic coefficient of variation

The variability of each quantitative trait was estimated by simple statistical measures such as mean, range, phenotypic and genotypic variances and coefficient of variation. The phenotypic and genotypic variation and coefficient of variations were calculated following the formula suggested by Singh and Chaundhary (1985) and Allard (1960) as follows;

Genotypic variance (δ^2_g)

$\delta^2_g = (MS_g - MSgl)/rl$

where MS_g = mean square of genotype, MSgl = mean square due to genotype by environment interaction, l = number of locations and r = number of replications.

Genotype by environment interaction variance (δ^2_{gl})

$\delta^2_{gl} = (MSgl\text{-}MSe)/r$

where MSgl = mean square due to genotype by environment interaction, and MS_e = combined error mean square.

Phenotypic variance (δ^2_p)

$\delta^2_p = \delta^2_g + (\delta^2_{gl}/l) + (\delta^2_e/rl)$

Estimates of coefficient of variation were obtained as follows.

Phenotypic coefficient of variation (PCV)

$PCV = \frac{\sqrt{\delta^2_p}}{\mu}$ x 100 where PCV = phenotypic coefficient of variation, δ^2_p = phenotypic variance and μ = population mean for the trait considered

Genotypic coefficient of variation (GCV)

$GCV = \frac{\sqrt{\delta^2 g}}{\mu}$ x 100 where GCV = genotypic coefficient of variation, δ^2_g = genotypic variance and μ = population mean for the trait considered.

Environmental coefficient of variations (ECV)

$ECV = \frac{\sqrt{\delta^2 e}}{\mu}$ x 100

Genotype by environment interaction coefficient of variation (GECV)

$GECV = \frac{\sqrt{\delta^2 gl}}{\mu}$ x 100 where, δ^2_{gl} = genotypic by environment interaction variance and

μ = population mean for the trait considered

4.1.3.2.7 Broad sense heritability (H^2) and genetic advance

Broad sense heritability was estimated according to Allard (1960). Heritability per location is calculated by dividing genotypic variance by phenotypic variance: $H^2 = (\delta^2_g/\delta^2_p)$ x 100, where δ^2_g = genotypic variance and δ^2_p = phenotypic variance. When heritability is calculated for combined analysis of two or more locations, the phenotypic variances combined over location were used. Hence, $H^2 = (\delta^2_g/\delta^2_p)$ x 100, where $\delta^2_p = \delta^2_g + (\delta^2_{gl}/l) + (\delta^2_e/rl)$

Expected genetic advance under selection, assuming a selection intensity of 5%, were computed following the formula developed by Allard (1960) as: GA = (K) (δ_p) (H^2), where GA = expected

genetic advance, K = selection differential that varies depending on the selection intensity and stands at 2.056 for selecting 5% of the genotypes, δ_p = phenotypic standard deviation and H^2 = heritability (in a broad sense). Genetic advance as percentage of the mean was calculated as GA (% of mean) = ($\frac{GA}{\mu}$) x 100%, where GA = genetic advance and μ = population mean for the trait considered.

4.1.3.3. Comparison of selected genotypes with the original study materials

Ten percent of the best performing accessions were sorted for each 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 accessions/populations and sampled accessions trait mean was tested using the Z table in such a way that when the calculated value of the Z-test was more than the tabulated Z value, the difference was considered significant (Singh, 2001).

4.2 Genetic diversity analysis using SSR molecular markers

4.2.1 Experimental accessions

A total of one hundred and fifty finger millet accession and varieties listed in Table 3, were grown in the field (See section 4.1.2) and used for this study. The study materials were assembled from the Institute of Biodiversity Conservation, Bako Agricultural Research Center and Bioinnovate Africa collection mission of Addis Ababa University.

4.2.2 DNA extraction

DNA was extracted from field-grown young leaf samples of all 144 accessions 6 released varieties used in this study according to the modified CTAB protocol of Mace *et al*. (2003), omitting the phenol: chloroform step. 2-3 leaf samples from different plants were used. Extracted DNA was visualized on a 0.8% (w/v) agarose gel and quantified spectrophotometrically using a Nanodrop® 1000 (Thermo Scientific, USA), followed by dilution to 10 ng/µl in TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0).

4.2.3 SSR marker Polymerase Chain Reaction (PCR)

DNA samples were subjected to genotyping using 20 published SSR markers (Table 5) for finger millet (Dida *et al*., 2007). All forward primers contained an M13-tag (5'-CACGACGTTGTAAAACGAC - 3') on the 5' end that was fluorescently labeled to allow detection of amplification products (Shuelke, 2000). PCR amplification was performed in 10 µl in 384 well microtitre plates and each reaction was comprised 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 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 final elongation at 72°C for 20 minutes.

4.2.4 Fragment detection

Amplification was confirmed by running 4 µl of the PCR products on a 2% (w/v) agarose gel stained with GelRed® (Biotium, USA) and visualized under UV light (UV-tech, Cambridge®) on a gel documentation system. Amplification products (1.5 µl – 3.5 µl of each) labeled with different fluorochromes 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).

Table 5: List of SSR markers used in this study with repeat motifs and primer sequences

Primer	Forward primer sequence	Reverse primer sequence	Repeat motif	Map-ped
UGEP05	TGTACACAACACCACACTGAT	TTGTTTGGACGTTGGATGTG	$(TC)_{12}AC(TC)_4$	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)

4.2.5 Genetic polymorphism and neighbor joining based cluster analysis

Allelic data such as polymorphic information content (PIC), observed heterozygosity, major allele frequency, genetic distance between test accessions (Liu and Muse, 2005), analysis of molecular variance (AMOVA), classical F-statistics (Wright, 1965) and allelic frequency based Rogers genetic distance for regions/countries of origin (Rogers, 1972) were calculated using Power Marker V3.25 software (Liu and Muse, 2005). The significance of allelic frequency for

the study accessions at locus level (population differentiation test) was estimated by Mantel test (Mantel, 1967). The weighted neighbor joining based clustering was computed using DARwin v.5 software (Perrier and Jacquemoud, 2006). Only the accessions for which the SSR markers worked well were considered for weighted neighbor joining.

4.2.6 Population structure analysis

The software Structure 2.3.3 (Pritchard *et al.* 2000) was used to establish the number of hypothetical distinct populations. Optimum number of subpopulations were inferred by running an admixture ancestry model with correlated allele frequencies starting from $K = 2$ to $K = 8$, with 10 independent runs at each K. For each run, 5000 burn-ins followed by 50,000 Markov chain Monte Carlo (MCMC) simulations were performed. The ideal number of k according to Pritchard *et al.* (2000) was used as the criterion for defining the number of groups (k). The most trustworthy value was estimated based on the lowest negative number of Ln (the log-likelihood of the data) and the lowest standard deviation found during statistical analysis and the highest peak of the curve at $k = 3$, suggesting that three subpopulations could contain all individuals with greatest probability (Fig 14). Hence, a k value of 3 groups (subpopulations) was selected to describe the genetic structure of the 138 finger millet accessions.

4.3. Screening of finger millet accessions for blast (*Magnaporthe oryzea*) disease

4.3.1. Description of the study area

Field experiments were conducted at Bako Agricultural Research Center (1650 m.a.s.l, 7°17'12.5"N; 38°25'47.5"E), 259 km west of Addis Ababa. During the growing season of 2011, annual rainfall was 1425.30 mm, relative humidity 60.50% and mean temperature 21.20°C. The

last ten years weather condition of this test environment was as indicated in Figure 4a and b below. The dominant soil type of the area was Nitosol.

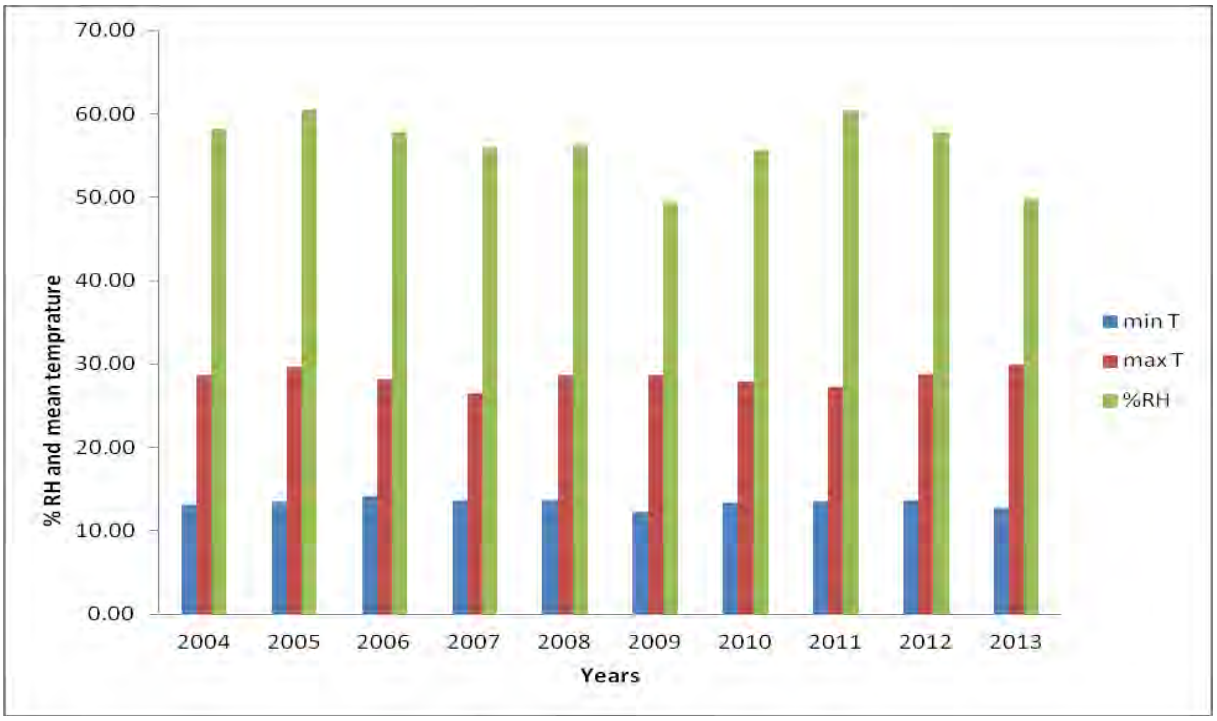


Fig 4a. Mean minimum and maximum temperature (°C) and relative humidity (%) for the period 2004-2013 [Source: Bako Agricultural Research Center Agro-meteorology Research Team]

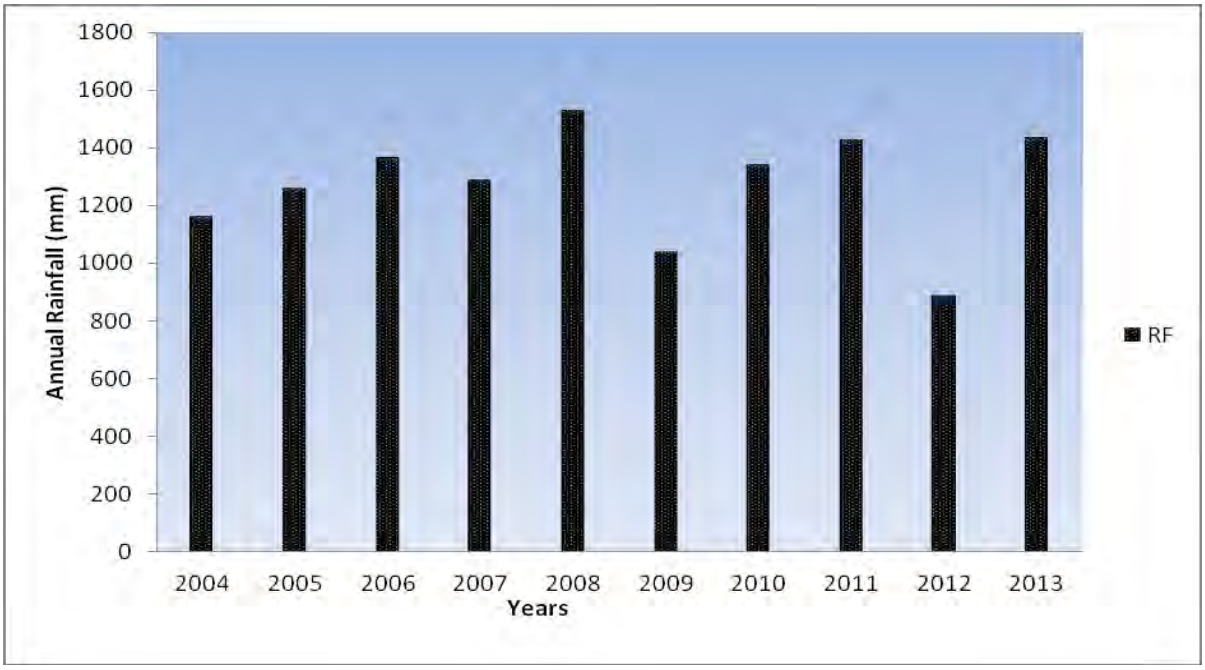


Fig 4b. Annual rainfall for the period 2004-2013
[Source: Bako Agricultural Research Center Agro-meteorology Research Team]

4.3.2 Planting material and experimental procedures

One hundred and eighty-six finger millet accessions collected from major finger millet growing regions of Ethiopia (Tigray, Amhara, Oromia, Benishangul Gumuz and Southern Nation-Nationalities and Peoples Regional States) supplemented by 31 accessions introduced from Eritrea, Kenya, Zambia and Zimbabwe were planted with seven improved varieties and one advanced line in simple lattice design (Table 6). The improved finger millet varieties with their pedigree names in bracket were; Boneya (KNE #411), Wama (KNE #392), Gute (Acc. 229373), Bereda (BRC-356-1), Tadesse (KNE#1098, Padet (KNE#409) and Degu (PGRC/E215874) and the advanced line was PW-001-022. The plot size was single rows of 1.5m long and 60cm between row spacing. Spacing between plants within rows was adjusted to 10cm. Fertilizer rates of 105 kg/ha DAP and 65 kg/ha Urea were used. Urea was applied in split form.

4.3.3 Isolation and inoculation of the pathogen

Artificial inoculation was employed by developing the inoculums on Potato Dexterous Agar (PDA) following protocols described in Tredway *et al*. (2003). The trial was also conducted on a sick plot where finger millet was grown as precursor crop in the previous season. The isolate was collected from naturally highly infected leaf, neck and seeds (heads) of finger millets genotype Acc. 229402 grown in lath house for pathogen development at Bako Agricultural Research Center. Infected leaves were surface disinfected by 10% chlorox and thoroughly washed using distilled and sterile water.

PDA was prepared from 39g of commercial potato dextrose agar (manufacturer) suspended in 1000 ml distilled water and heated to dissolve the agar completely. The media was sterilized by autoclaving at 1.1 kg/cm^2 pressure and 121ºC for 15 minutes and transferred to the plate by

sterile needle in a laminar flow hood. Inoculated plates were incubated for seven days at 27±1^{0}C under 12 hours light/dark in an incubator (Tredway *et al.*, 2003).

Pure cultures were identified as per characteristic mycelia and spores after seven days of incubation, by microscopy. Spore concentrations were adjusted to 2.5 x 10^6 spores/ml in water with a haemocytometer. A 20-liter capacity portable sprayer was adjusted to full coverage for inoculation. Infection was done during humid and cloudy conditions (Han *et al*., 2003). Five drops of surfactant (80% *Cita Wat)* was added to 20 lit spore suspensions to enhance adhesion of spores to the host tissue. Plants were inoculated at early heading and peak vegetative stage.

4.3.4 Data collection and analysis

Ten plants per row were randomly selected and tagged prior to heading for plant-based disease assessment. Leaf blast and neck blast severity were recorded on a scale of 1 to 9 where 1 = no disease to 9 = more than 75% disease (Chen *et al*., 2013; Thakur *et al*., 2009; Mgonja *et al*., 2007; Taken *et al*., 2004). Disease incidence was recorded as percentages. Head blast scores were calculated as the number of diseased plants divided by the total number of plants sampled in a plot and multiplied by 100 (IBPGR, 1985). The length of infected ear/lesion length (cm) and grain yield per plant (g) were also recorded.

Table 6. List of 217 finger millet accessions and 8 improved varieties evaluated for blast disease resistance at Bako Agricultural Research Center, western Ethiopia.

No	Accession	Region	Altitude	No	Accession	Region	Altitude
1	BKFM0014	Oromia/Eth	1409	43	BKFM0052	Oromia/Eth	2200
2	215984	Amhara/Eth	1870	44	208726	Oromia/Eth	1880
3	208725	Oromia/Eth	1920	45	215867	Amhara/Eth	1990
4	245088	Oromia/Eth	2060	46	BKFM0057	Oromia/Eth	1707
5	203358	Zimbabwe	1420	47	216045	Oromia/Eth	1880
6	BKFM0011	B/Gumuz/Eth	1428	48	Gute	Released	
7	BKFM 0031	Oromia/Eth	1368	49	203360	Zimbabwe	1420
8	BKFM0038	Oromia/Eth	2144	50	216057	Oromia/Eth	1800
9	238299	Tigray/Eth	1940	51	216033	Oromia/Eth	1930
10	242623	Tigray/Eth	1590	52	BKFM 0030	Oromia/Eth	1379
11	BKFM0007	B/Gumuz/Eth	1456	53	237472	Tigray/Eth	1800
12	208446	Amhara/Eth	1920	54	BKFM0043	Oromia/Eth	1601
13	BKFM0005	B/Gumuz/Eth	1449	55	229722	B/Gumuz/Eth	1750
14	208440	Amhara/Eth	1850	56	BKFM0054	Oromia/Eth	1511
15	BKFM0062	Oromia/Eth	1923	57	203547	Kenya	1510
16	238344	Amhara/Eth	2000	58	BKFM0009	B/Gumuz/Eth	1498
17	BKFM0046	Oromia/Eth	1367	59	BKFM0003	B/Gumuz/Eth	1455
18	215981	Amhara/Eth	1850	60	215886	Amhara/Eth	1850
19	216051	Oromia/Eth	1910	61	BKFM0047	Oromia/Eth	1334
20	214988	Zambia	1300	62	Wama	Released	
21	216050	Oromia/Eth	1700	63	BKFM0015	Oromia/Eth	1449
22	BKFM0018	Oromia/Eth	1667	64	AAUFM-049	Tigray/Eth	
23	216052	Oromia/Eth	1660	65	242109	Amhara/Eth	2060
24	BKFM0034	Oromia/Eth	1454	66	AAUFM-3	Tigray/Eth	
25	229727	B/Gumuz/Eth	1450	67	242107	Amhara/Eth	2020
26	BKFM0036	Oromia/Eth	2144	68	Tadesse	Released	
27	Bereda	Released		69	BKFM0045	Oromia/Eth	1615
28	208447	Amhara/Eth	1710	70	216035	Oromia/Eth	1900
29	214997	Zambia	1100	71	BKFM0006	B/Gumuz/Eth	1479
30	237445	Tigray/Eth	1940	72	AAUFM-8	Tigray/Eth	1812
31	100045	Amhara/Eth	1650	73	BKFM0024	Oromia/Eth	1913
32	BKFM0039	Oromia/Eth	2144	74	AAUFM-20	Tigray/Eth	2142
33	242619	Tigray/Eth	1810	75	242105	Amhara/Eth	1860
34	216048	Oromia/Eth	1640	76	BKFM0020	Oromia/Eth	1644
35	BKFM0029	Oromia/Eth	1251	77	AAUFM-15	Tigray/Eth	1568
36	PW-001-022	Released		78	229731	Amhara/Eth	1950
37	Boneya	Released		79	BKFM0002	B/Gumuz/Eth	1550
38	214989	Zambia	1210	80	AAUFM-42	Tigray/Eth	2058
39	230101	Eritrea	1740	81	237446	Tigray/Eth	1850
40	238304	Tigray/Eth	1950	82	AAUFM-4	Tigray/Eth	1896
41	203352	Zimbabwe	1490	83	BKFM0059	Oromia/Eth	1730
42	216049	Oromia/Eth	1600	84	238305	Tigray/Eth	1990

Table 6. Continued….

No	Accession	Region	Altitude	No	Accession	Region	Altitude
85	BKFM0063	Oromia/Eth	1923	129	100070	Amhara/Eth	1900
86	244798	SNNP/Eth	2169	130	AAUFM-11	Tigray/Eth	
87	BKFM0019	Oromia/Eth	1596	131	243636	Amhara/Eth	2100
88	Padet	Released		132	242624	Tigray/Eth	1400
89	243640	Amhara/Eth	1890	133	AAUFM-17	Tigray/Eth	
90	BKFM0044	Oromia/Eth	1601	134	AAUFM-19	Tigray/Eth	1811
91	BKFM0012	Oromia/Eth	1420	135	100038	Amhara/Eth	2030
92	BKFM0023	Oromia/Eth	1891	136	216040	Oromia/Eth	1940
93	AAUFM -34	Tigray/Eth	1568	137	230104	Eritrea	1800
94	AAUFM-12	Tigray/Eth	1502	138	214994	Zambia	1160
95	237443	Amhara/Eth	2100	139	214990	Zambia	1360
96	AAUFM-050	Tigray/Eth		140	208730	Oromia/Eth	1900
97	AAUFM-6	Tigray/Eth		141	236445	Oromia/Eth	1870
98	203355	Zimbabwe	1420	142	234199	Tigray/Eth	1920
99	215976	Amhara/Eth	1860	143	203546	Kenya	1620
100	235145	Tigray/Eth	1730	144	AAUFM-14	Tigray/Eth	1568
101	BKFM0010	B/Gumuz/Eth	1484	145	214993	Zambia	1340
102	214995	Zambia	1130	146	245085	Oromia/Eth	1987
103	AAUFM-10	Tigray/Eth		147	242117	Amhara/Eth	1915
104	BKFM0058	Oromia/Eth	1725	148	238341	Amhara/Eth	1780
105	AAUFM-33	Tigray/Eth	1620	149	228202	Amhara/Eth	
106	BKFM0060	Oromia/Eth	1852	150	235700	SNNP/Eth	1530
107	AAUFM-35	Tigray/Eth	1568	151	241768	SNNP/Eth	1500
108	208439	Amhara/Eth	1950	152	100084	SNNP/Eth	1820
109	AAUFM-21	Tigray/Eth	1722	153	216044	Oromia/Eth	1910
110	230105	Eritrea	1600	154	241769	SNNP/Eth	1500
111	AAUFM-2	Tigray/Eth	1896	155	245087	Oromia/Eth	1923
112	229726	B/Gumuz/Eth	1600	156	215887	Amhara/Eth	1880
113	AAUFM-22	Tigray/Eth	2142	157	203362	Zimbabwe	1420
114	BKFM0055	Oromia/Eth	1723	158	242625	Tigray/Eth	1880
115	AAUFM-23	Tigray/Eth	2100	159	238346	Amhara/Eth	1940
116	229725	B/Gumuz/Eth	1650	160	242110	Amhara/Eth	1990
117	AAUFM-32	Tigray/Eth	1630	161	238460	Tigray/Eth	1650
118	AAUFM-41	Tigray/Eth		162	238308	Tigray/Eth	2000
119	BKFM0048	Oromia/Eth	1337	163	235699	SNNP/Eth	1440
120	229730	Amhara/Eth	1850	164	242108	Amhara/Eth	2050
121	AAUFM-44	Tigray/Eth		165	215802	Oromia/Eth	1950
122	215865	Amhara/Eth	1880	166	215959	Amhara/Eth	1960
123	BKFM0028	Oromia/Eth	1608	167	203351	Zimbabwe	1490
124	203359	Zimbabwe	1420	168	216036	Oromia/Eth	1900
125	BKFM0042	Oromia/Eth	1867	169	216039	Oromia/Eth	1950
126	AAUFM-29	Tigray/Eth		170	238343	Amhara/Eth	1970
127	243635	Amhara/Eth	2070	171	229724	B/Gumuz/Eth	1520
128	AAUFM-28	Tigray/Eth		172	242617	Tigray/Eth	1700

Table 6. Continued ……

No	Accession	Region/ Country	Altitude	No	Accession	Country/ Region	Altitude
173	236446	Oromia/Eth	1930	200	Degu	Released	
174	237584	SNNP/Eth	1990	201	BKFM0051	Oromia/Eth	2227
175	203363	Zimbabwe	1420	202	203361	Zimbabwe	1420
176	207964	Oromia/Eth		203	203357	Zimbabwe	1420
177	215853	Amhara/Eth	1780	204	238300	Tigray/Eth	1980
178	237969	Oromia/Eth	1930	205	203356	Zimbabwe	1420
179	203350	Zimbabwe	1400	206	203353	Zimbabwe	1420
180	234189	Tigray/Eth	1900	207	229738	Amhara/Eth	1830
181	242644	Amhara/Eth	1815	208	237970	Oromia/Eth	1620
182	203545	Kenya	1590	209	242106	Amhara/Eth	1955
183	242116	Amhara/Eth	1860	210	BKFM0001	B/Gumuz/Eth	1580
184	203354	Zimbabwe	1420	211	238342	Amhara/Eth	1750
185	100032	Amhara/Eth	1980	212	245092	Oromia/Eth	1954
186	215851	Amhara/Eth	1800	213	229728	B/Gumuz/Eth	1440
187	100002	Amhara/Eth	2004	214	234825	Oromia/Eth	
188	203544	Kenya	1485	215	245084	Oromia/Eth	1915
189	216056	Oromia/Eth	1600	216	215856	Amhara/Eth	1720
190	214991	Zambia	1330	217	242112	Amhara/Eth	2125
191	215854	Amhara/Eth	1750	218	216046	Oromia/Eth	1680
192	234827	Oromia/Eth		219	214987	Zambia	1310
193	242643	Amhara/Eth	1750	220	234826	Oromia/Eth	
194	215863	Amhara/Eth	1850	221	242114	Amhara/Eth	1870
195	242121	Amhara/Eth	1820	222	242115	Amhara/Eth	1890
196	237475	Tigray/Eth	1750	223	215849	Amhara/Eth	1820
197	242618	Tigray/Eth	1950	224	235834	Amhara/Eth	1920
198	203542	Kenya	1540	225	215960	Amhara/Eth	1980
199	215852	Amhara/Eth	1790				

Disease assessment was made every two weeks commencing from 88 days after planting (soon after disease appearance on the leaves) to physiological maturity. Accordingly, both disease severity and incidence for leaf blast, head blast and sheath blast was recorded five times, four times and two times, respectively. Severity score for leaf, head and sheath blast from ten selected plants were converted into a severity index or disease index as:

$$DIj = \frac{[\sum_{i=1}^{n} S_i] \times 100}{n \times 9}$$

Where DIj = disease index at j^{th} round score, Si = the severity recorded from i^{th} sample plant, n = total number of sample plants considered per plot and 9 = maximum value of scoring scale and was constant. The disease indexes obtained for the different assessment periods were used to calculate the area under disease progress curve (AUDPC) of the recording period. AUDPC used to quantify and summarize the severity of disease over time was estimated as per the equation suggested by Shaner and Finnay (1977) as:

$$AUDPC = \sum_{i=1}^{ni-1} \{ \frac{(Y_{xi+1} + Y_{xi})}{2} (t_{i-1} - t_i) \}$$

where AUDPC = area under disease progress curve, Y_{xi} = disease severity index recorded for x-accession at i^{th} assessment period; t_i = time interval of disease recording for the assessment period. The sum total of disease progress during the different assessment periods were used as the final AUDPC at the end of the epidemiological period. The estimates of AUDPC was normalized by dividing with the total area of the graph (*i.e.* the number of days from first appearance of the disease till end of the observation period) in order to facilitate a better visual comparison among host genotypes over seasons of testing (Shtienberg *et al.*, 1990; Fry 1968). In the case of this study, the number of days from first appearance/recording of the disease to the end of the observation period was 60 days for leaf blast and 45 for head blast. The normalized AUDPC was referred to as the Relative Area Under Disease Progress Curve (RAUDPC) and this proportion was employed for different analysis packages used in the study.

The relative efficiency of a 15 x 15 simple lattice design over a Randomized Complete Block Design (RCBD) was tested using Agrobase (2000) software and found to be less efficient for all traits recorded in the present study. Therefore, data collected for all characters were subjected to analysis of variance (ANOVA) using SAS software (SAS, 2008) as suggested by Cochran and

Cox (1957). Head blast progress during the epidemic periods at regional/country and varietal level was indicated on monographs generated using Microsoft Excel and pattern file. Regression and correlation coefficient analysis between the major traits were tested using SAS software (SAS, 2008).

4.3.5. Yield loss assessment

Head blast is generally the most severe aspect of blast infection as it has the most significant effect on yield quantity and quality. Therefore, in this study, head blast infection was used as the principal parameter to estimate yield loss. Multiple point models were employed to relate the yield loss (YL) assessments to the percentage disease severity scores of several time points during the growth season. Besides, it helped to predict the trend of yield loss at different recording intervals and thus to recommend the best time of applying disease management options using a multiple regression equation as suggested by James (1974):

$$\%YL = b_1x_1+b_2x_2+b_3x_3+\ldots+b_nx_n$$

Where $b_1 \ldots b_n$ = partial regression coefficients from the first up to n^{th} round assessment periods and $x_1 \ldots x_n$ = corresponding percentage disease severity scores (severity index) for the 1^{st} up to the n^{th} round assessment period.

The blast infection rate in 2011 progressed consistently throughout crop development (Lule *et al.*, 2014). Therefore, the normalized AUDPC model was applied. This approach followed a similar logic to the multiple point models except that it involved integration of the entire disease progress curve (Zadoks, 1985) and assumed that yield loss was proportional to the amount and duration of the disease and that the infection rate progressed consistently (James, 1974; Mousanejad *et al*., 2010).

Furthermore, statistical approaches such as comparisons of yield of infected fingers with yield of normal fingers of the same size on the same ear were employed following the formula;

$$\% \text{ YL} = \frac{\text{Yield of normal finger} - \text{Yield of infected finger}}{\text{Yield of normal finger}} \text{ x } 100$$

4.3.6. Comparison of selected genotypes with the original study materials

Ten percent of the top resistant/tolerant accessions to head blast infection were compared with the total 225 test materials. The absolute value of Student's Z test was calculated to compare genotypic values of the selected 10% best 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 calculated for the base populations and n = number of genotypes selected from the base population for better performance. The significance of the difference between the population parameter (μ) and sample mean ($\bar{X}$) was tested using the Z table as suggested by Singh (2001).

4.4. Genotype by Environment interactions and grain yield stability analysis

4.4.1 Planting material and experimental procedure

Thirty selected genotypes from diversity and blast trials (Table 35) were evaluated against the standard checks (Gute and Tadesse) at Bako, Gute, Assosa and Arsi Negele in 2012 and 2013. The trial was arranged in a RCBD, replicated three times. All the experimental locations are characterized as midlands (Weina Dega) that are located between 1500-2300 m.a.s.l. and receive an average rainfall in a range of 800-1200 mm annually (Hans, 1998; USDA, 2003; Gorfu and Ahimed, 2008).

4.4.2 Data recording and analysis

Important agronomic traits, such as days to 50% heading, days to 50% maturity, plant height (cm), productive tiller number, ear length (cm), finger number per main ear and grain yield per plot (g) were recorded following the finger millet descriptor (IBPGR, 1985).

4.4.2.1 Analysis of variance

All agronomic traits were subjected to ANOVA using SAS (SAS, 2008) software.

4.4.2.2 Additive mean effect and multiplicative interaction model (AMMI)

The AMMI model equation used was: $Y_{ger} = \mu + \alpha_g + \beta_e + \sum_n \lambda_n \gamma_{gn} \delta_{en} + \varepsilon_{ger} + \rho_{ge}$; where Y_{ger} = the observed yield of genotype (g) in environment (e) for replication (r);

***Additive parameters*:** μ = the grand mean; α_g = the deviation of genotype g from the grand mean, β_e = the deviation of the environment e;

Multiplicative parameters: λ_n = the singular value for interaction principal component axis (IPCA) n, γ_{gn} = the genotype eigenvector for axis n, and δ_{en} = the environment eigenvector; ε_{ger} = error term and ρ_{ge} = PCA residuals. Accordingly, genotypes with low magnitude (regardless of the sign) of interaction principal component (IPCA) scores possessed general or wider adaptability while those with high magnitude of IPCA scores had specific adaptability (Gauch and Zobell, 1996).

4.4.2.3 AMMI Stability Value (ASV)

The ASV is the distance from the coordinate point to the origin in a two-dimensional plot of IPCA1 scores against IPCA2 scores in the AMMI model (Purchase, 1997). Because the IPCA1 score contributes more to the G x E interaction sum of square, a weighted value is needed. This

weighted value was calculated for each genotype and each environment according to the relative contribution of IPCA1 to IPCA2 to the interaction sum square as follows:

$$ASV = \sqrt{[(SS_{IPCA1} \div SS_{IPCA2})(IPCA1score)]^2 + (IPCA2score)^2}$$

where SS_{IPCA1} / SS_{IPCA2} = the weight given to the IPCA1-value by dividing the IPCA1 sum of squares by the IPCA2 sum of squares.

The larger the ASV value, either negative or positive, the more specifically adapted a genotype was to certain environments. Smaller ASV value indicated a more stable genotype across environments (Purchase, 1997).

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

GGE Interaction biplot analysis was conducted using GenStat Release 15.1 computer software.

4.4.2.5 Analysis using Eberhart and Russell model

The stability of yield performance for each genotype was calculated by regressing the mean grain yield of individual genotypes on environmental index and calculating the deviation from regression as suggested by Eberhart and Russell (1966) as:

$$Y_{ij}=\mu_i + b_iI_j + s^2d_{ij};$$

where Yij = the mean performance of i^{th} variety in j^{th} environment, μ_i = the mean of i^{th} variety over all environments; b_i = the regression coefficient which measures the response of i^{th} variety to varying environment; s^2d_{ij} = deviation from regression of i^{th} variety in the j^{th} environment, and I_j = the environmental index of j^{th} environment. Regression coefficient (b_i) was considered as an indication of the response of the genotype to varying environment. If the regression coefficient was not significantly different from one (b_i = 1.0), the genotype was adapted to all environments, genotypes with b_i > 1.0 were more responsive or adapted to high yielding

environments, whereas any genotype with b_i significantly lower than 1.0 was adapted to low yielding environments (Eberhart and Russell, 1966). Both AMMI and Eberhart and Russell models were computed using Agrobase software (Agrobase, 2000).

4.5 Marker development for blast resistant gene

4.5.1. Plant materials and field evaluation

A total of 96 finger millet accessions were included in this part of the study (Table 7) and were selected based on the results of an advanced screening of the 225 accessions evaluated for blast resistance in 2011 (See section 4.3.2). Selection were made for the top 17% blast disease tolerant/resistant and 25% of the most susceptible accessions and re-evaluated on field condition at Bako Agricultural Research Center in 2012. Inoculum production and inoculation of the isolates, data collection and analysis were done as indicated in section 4.3.3.

4.5.2 DNA extraction

DNA was extracted from four weeks young leaves grown in greenhouse following the CTAB protocol as described in Mace *et al*., (2003). Extracted DNA was visualized on a 1% (w/v) agarose gel and quantified spectrophotometrically using a Nanodrop® 1000 (Thermo Scientific, USA), followed by dilution to 40 ng/µl in distilled water.

4.5.3 Primer design

A total of 107 candidate genes (85 rice blast resistance (R) genes, 8 genes linked to flowering date and 14 Aluminum tolerant genes) were identified from the literature. Among those, 51 have GenBank but only 21 of them fulfilling the requirement for primer designing were selected

(Table 8). Finger millet orthologs of each of these gene sequences were identified through blastn queries against the available 454 sequences read of finger millet accession KNE796 which was available at UGA. The finger millet sequences were aligned with the sequence of the orthologous rice *Pi* gene using CLUSTALW 2.0 (http://www.ebi.ac.uk/Tools/clustalw2/index.html) and a java multiple alignment editor (Jalview) (http://www.ebi.ac.uk/~michele/jalview/contents.html). Specific primers for each gene were designed using Primer 3 (Rozen and Skaletsky, 2000).

4.5.4 Polymerase Chain Reaction (PCR)

PCR amplification was performed in 20 µl in 96 well microtitre plates and each reaction comprised 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), 1.5mM $MgCl_2$, 0.2mM dNTPs, 0.5 mM of forward primer, 0.5 mM reverse primer, 1unit of Taq DNA polymerase (SibEnzyme Ltd, Russia) and 40ng of template DNA. PCR reactions were performed on a GeneAmp 9700 thermocycler (Applied Biosystems) with initial denaturation of 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 1 minute and 15 seconds and, a final elongation at 72°C for 5 minutes. Amplification was confirmed by running 4 µl of the PCR products on a 2% (w/v) agarose gel stained with Ethidium bromide and visualized under UV light.

Table 7. List of 96 finger millet accessions included in comparative genomic analysis.

SN	Acc	Region	SN	Acc	Region
1	BKFM 0031	Eth/Oromia	49	215865	Eth/Amhara
2	214988	Zambia	50	AAUFM-6	Eth/Tigray
3	214987	Zambia	51	245085	Eth/Oromia
4	BKFM0010	Eth/B/Gumuz	52	242623	Eth/Tigray
5	PW-001-022	Pipeline	53	AAUFM-4	Eth/Tigray
6	203356	Zimbabwe	54	AAUFM-28	Eth/Tigray
7	BKFM0020	Eth/Oromia	55	242116	Eth/Amhara
8	BKFM0029	Eth/Oromia	56	215851	Eth/Amhara
9	214995	Zambia	57	203542	Kenya
10	214997	Zambia	58	242619	Eth/Tigray
11	216035	Eth/Oromia	59	AAUFM-12	Eth/Tigray
12	BKFM0024	Eth/Oromia	60	AAUFM-11	Eth/Tigray
13	BKFM0018	Eth/Oromia	61	235700	Eth/SNNP
14	BKFM0063	Eth/Oromia	62	230101	Eritrea
15	216051	Eth/Oromia	63	AAUFM-10	Eth/Tigray
16	BKFM0042	Eth/Oromia	64	208439	Eth/Amhara
17	BKFM0023	Eth/Oromia	65	AAUFM-17	Eth/Tigray
18	BKFM0001	Eth/B/Gumuz	66	215853	Eth/Amhara
19	216039	Eth/Oromia	67	215852	Eth/Amhara
20	BKFM0007	Eth/B/Gumuz	68	237443	Eth/Amhara
21	BKFM0009	Eth/B/Gumuz	69	AAUFM-050	Eth/Tigray
22	BKFM0038	Eth/Oromia	70	AAUFM-14	Eth/Tigray
23	215984	Eth/Amhara	71	100002	Eth/Amhara
24	BKFM0006	Eth/B/Gumuz	72	203357	Zimbabwe
25	236446	Eth/Oromia	73	242114	Eth/Amhara
26	203353	Zimbabwe	74	AAUFM-21	Eth/Tigray
27	214989	Zambia	75	237475	Eth/Tigray
28	BKFM0028	Eth/Oromia	76	242115	Eth/Amhara
29	216036	Eth/Oromia	77	238299	Eth/Tigray
30	216045	Eth/Oromia	78	AAUFM-15	Eth/Tigray
31	BKFM0014	Eth/Oromia	79	AAUFM-2	Eth/Tigray
32	BKFM0034	Eth/Oromia	80	AAUFM-32	Eth/Tigray
33	243640	Eth/Amhara	81	230105	Eritrea
34	216049	Eth/Oromia	82	AAUFM-22	Eth/Tigray
35	BKFM0047	Eth/Oromia	83	230104	Eritrea
36	208725	Eth/Oromia	84	AAUFM-35	Eth/Tigray
37	BKFM0005	Eth/B/Gumuz	85	AAUFM-23	Eth/Tigray
38	AAUFM-42	Eth/Tigray	86	AAUFM-44	Eth/Tigray
39	238305	Eth/Tigray	87	228202	Eth/Amhara
40	235699	Eth/SNNP	88	238460	Eth/Tigray
41	203350	Zimbabwe	89	238308	Eth/Tigray
42	215863	Eth/Amhara	90	242618	Eth/Tigray
43	238300	Eth/Tigray	91	203362	Zimbabwe
44	AAUFM-3	Eth/Tigray	92	BKFM0043	Eth/Oromia
45	203355	Zimbabwe	93	BKFM0011	Eth/B/Gumuz
46	AAUFM-19	Eth/Tigray	94	Tadesse	Imp. Variety
47	229731	Eth/Amhara	95	242617	Eth/Tigray
48	242617	Eth/Tigray	96	214990	Zambia

Key: Acc= accession, SN=serial number

Table 8: List of Oligonucleotide primers designed, primer sequences and source GenBank ID

Primer name	Forward primer sequence	Reverse primer sequence	GenBank ID
AL4_F1	CCAAAGTCTGTATCTACCCCC	CACCTACATGTTTCACACCC	AB302223
*AL13_F1	CTCCCATTTGGCAAAAGTGCAC	GTATTGCAGCATCGGCAG	EU966615
AL15_F1	AGAGATCAGCCACCTTGTGC	GTTGATGCCGTCGAGATGAG	DQ072260
Pi36_F2	GCTATCATTGCGATAGCTAG	CTTCATGTGACAAGTCACG	DQ900896
HvFT2_F1	ATCATCGGCGACGTGATC	GTGTAGAAGGTGCGCATGTC	DQ297407
PpdH_F1	CTGTTGGTTGTGCCTGGTAG	AGGTCTGGAATATTGTGGATTC	AY970701
VRNH3_F1	CACGCTGGCAGTTGAAGTAG	TGCCGTTTTATTTACGTCGTC	DQ898515
*HvGI_F1	TGGCAACGGTTGAAGCTATAC	TATCCCATTGGCTTTCAGG	AY740524
HvCO1_F1	GCTCTGCTGTTGCTCAGTTG	CTACCTCTGCGCGTCCTG	AF490467
Pi5_F1	TCTTGGATGACCTTTCTAGCAC	ATGGAGCCATGTTTTGATCC	*EU869185*
*Pi5_F2	TTGATCAGGGAGAAGGTTGG	ACATCTCCCCATTCACATAAG	*EU869185*
Pib_F1	TCGTTCCGAGCATGGATAAG	CCGACATATCAGCCCAAAAG	*AB013448*
*Pib_F2	ATGGATGCTGATGAGAAGAAG	CAAGAGTCAATTGATTTCCTGC	*AB013448*
*Pi9_F1	GGAAATGGGCTTTATCAGGC	GATGACGTATGGTCCAGGGA	*DQ285630*
Pi9_F2	GACATTCGCAACCAATCAGC	TAACAAACCCTTCTGCTATCC	*DQ285630*
Pikm2_F1	TCTTTCACATGCTTGGCATC	GGCCATAGTTACCCTTGCTG	*AB498876*
*Pikm2_F2	GATGAAGGACTGGATGAAGC	TCAAGAACCATGTCATGAAC	*AB498876*
*Pb1_F1	CAAGAGCAGGACATTTGCTG	GAGTAGCACCCAGCTAAACCA	*AB498876*
Pb1_F2	CCTTCCAATGCAGAGTTTCC	GCCTGTTGCAGCTGTTATTG	*AB570371*
*Pi37_F1	GTTAAGCATTTGCCCGAAAA	CTGCAACCCAAAGGTGAACT	*DQ923494*
Pi37_F2	TCATTGCTTCACTGCCCTTC	CATTTATCGGCAGGGACTTC	*DQ923494*

*= primers gave no PCR amplicons and were not used for further analysis

4.5.5 Single Stranded Conformation Polymorphism (SSCP) procedures

In non-denaturing polyacrylamide gel electrophoresis (PAGE), the components used to synthesize matrix are acrylamide monomers, N, N-methylene bisacrylamide (Bis), ammonium per sulphate (APS) and N,N,N',N'-tetramethylenediamine (TEMED). Ammonium persulphate (APS) when dissolved in water generates free radicals, which activate acrylamide monomers inducing them to react with other acrylamide molecules forming long chains. These chains cross-linked with Bis. TEMED act as catalyst for gel formation because of its ability to exist in free radical form. The detailed SSCP procedures and required reagents are indicated in Appendix 2. A total of eight representative finger millet accessions were used in the test SSCP electrophoresis analysis. Those accessions were also representative for moderately resistant group (#5), susceptible (#60) and highly susceptible groups (#63, #65, #66, #77, #83 and #85). Based on test SSCP, PCR product of all the 96 accessions were subjected to SSCP analysis.

4.5.6. Cloning and sequencing of PCR products

Cloning PCR products into a stable vector is often desirable for subsequent analysis such as high quality DNA sequencing, hybridization studies, expression, modification or further sub cloning. A wide variety of strategies has been employed and is available in commercially supplied kits (Jiang and Smith, 2002). In the present study, pGEM®-T and pGEM®-T Easy Vector Systems was used with the objective to get high quality DNA sequencing. The detail protocol is found at web site: www.promega.com/protocols/ (accessed on Sep 2013). The pGEMR-T and pGEMR-T Easy Vectors are linearized vectors with a single 3′-terminal thymidine at both ends. The T-overhangs at the insertion site greatly improve the efficiency of ligation of PCR products by preventing re-circularization of the vector and providing a compatible overhang for PCR products generated by certain thermostable polymerases (Robles and Doers, 1994).

Eight finger millet accessions such as PW-001-022 (#5), AAUFM-11 (#60), AAUFM-10 (# 63), Acc 215853 (#66), Acc 238299 (#77), Acc 230104, (#83) and AAUFM-23 (#85) were selected for cloning because they showed different SSCP band patterns. Following PCR amplification, 8 µl of the PCR products were run on a 2% (w/v) agarose gel stained with Ethidium bromide and visualized under UV light. The PCR band for each accession was excised from the gel for cloning and sequencing. Two colonies of the clones with an insert (white colony) per accessions were sequenced. The CLC Genomics Workbench 7.0 software (http://www.clcbio.com/download) was used to analyze single nucleotide variants, nucleotide distributions, contigs measurements and other sequence information.

5. RESULTS AND DISCUSSION

5.1 Phenotypic diversity analysis

5.1.1. Qualitative traits diversity

5.1.1.1 Percentage frequency distribution of qualitative traits at regional and altitudinal level

The percentage frequency of qualitative characters distributed across the regions of origin and altitude classes has been summarized in Table 9 and 10 below. Three phenotypic classes of growth habit (erect, prostrate and decumbent), four types of ear shapes (droop, open, semi compact and compact), four types of ear/glumes color (light green, purple, yellow green and white), three classes of grain covered by glumes (enclosed, intermediate and exposed), three classes of spikelet density (high, intermediate and low) and five classes of grain color (white, light- brown, dark- brown, purple-black and cream) were observed in finger millet accessions included in the present study (Fig 5).

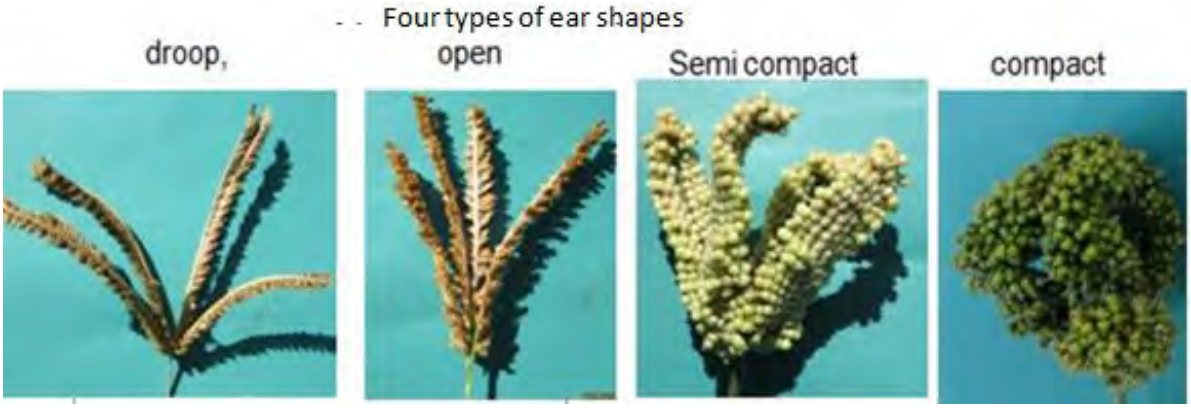
The frequency distribution of growth habit showed that the erect type was dominant across all collection regions and altitude classes followed by decumbent type (Table 9). The possible reason could be due to preferential selection by farmers for ease of spotting weeds under the crop, one of the major bottlenecks for finger millet production (personal observation and communication with farmers). Similarly, the erect growth habit was also dominant for the 909 accessions collected from different East African countries (Upadhyaya *et al.*, 2007). In contrast, decumbent types followed by prostrate type were dominant in 64 accessions collected from some of the former Ethiopian administrative regions (Wollega, Gojam, Gamu Gofa, Gonder, Tigray and Eritrea) (Bezawuletaw *et al.*, 2006). The difference observed across locations and

among genotypes collected from different regions could be due to the genetic or environmental factors that influence the adaptive role of the traits as well as several years of selection by local farmers.

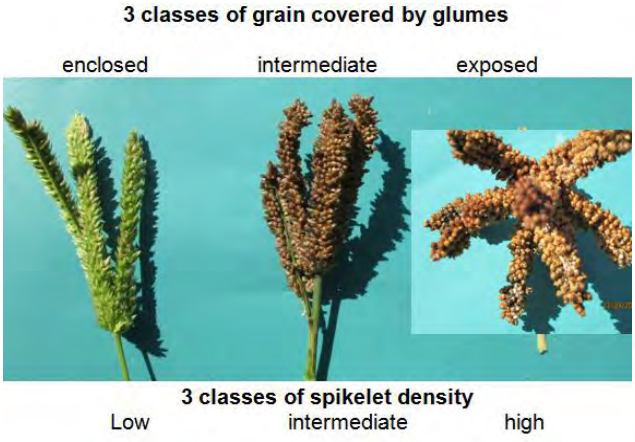
Compact ear shape was dominant in accessions from Zimbabwe (59.7%), Zambia (52.3%) and Kenya (47.9%) (Table 9). Open ear type was popular in Eritrea (58.6%) and across all regional states of Ethiopia except in SNNP, where semi compact dominated (57.7%). Light green ear (glumes) color was abundant across all the study countries and regions whereas purple color was relatively abundant in Kenya, Zimbabwe and Oromia region of Ethiopia (Table 9). Purple-black colored seed was abundant in Eritrea (71.2%) and Ethiopia (42.2%); white was dominant in Zambia (38.3%) and light brown in Kenya (41.1%) and Zimbabwe (43.9%) (Table 9). In Ethiopia, purple-black colored seed dominated in Tigray (49.4%), SNNP region (46.7%), Amhara (43.2%) and Oromia (35.4%) whilst light-brown seeds were dominant in the Benishangul Gumuz region (45.0%) (Table 9).

Open type ear shape, lower spikelet density and grains enclosed by glumes were abundant in Ethiopian and Eritrean accessions, but compact ear shape, exposed grain and higher spikelet density were popular for Kenyan, Zambian and Zimbabwe accessions. This indicated that either selection was in favor of the traits due to their adaptation to the agro-ecologies or they are farmer's priority criteria for selection for several reasons. Therefore, the wide range of agro-climatic conditions of these African countries could favor the development of different races of finger millet. De Wet *et al*. (1985) reported that open and droopy shaped fingers were a typical characteristic of race *coracana*. This also provides support that *E. coracana* was primarily domesticated in East Africa, particularly Ethiopia (Reddy *et al*., 2009; Hilu *et al*., 1979).

Accessions with the grains enclosed in glumes are more resistant to bird damage, minimize grain spoilage due to mold, particularly in areas with high humidity or rain fall, and does not shatter. In contrast, accessions with exposed grains thrash and clean easily, particularly when removing the husk from the grain. Drooping and open ear shaped accessions are mainly characterized by lower numbers of spikelets per panicle. Inflorescence morphology was associated with grain yield and is used by farmers to distinguish complexes of cultivars (De Wet *et al*., 1985).



a) Ear shapes



b).Grain covered by glumes and spikelet density

c) Grain color

Fig 5. Major phenotypic class of qualitative traits recorded in the present study

Table 9. Percentage frequency distribution of six major qualitative traits of 144 finger millet accessions

Country	Region	Growth habit			Ear shape				Ear (glumes) color			
		ER	PR	DC	DP	OP	SC	CM	LG	PP	YG	WT
Ethiopia	-	53.1	15.6	31.4	20.8	51.7	21.9	5.5	91.2	7.3	0.5	1.0
Eritrea	-	37.8	25.6	36.7	38.2	58.6	0.7	2.5	95.6	1.4	3.1	0.0
Kenya	-	70.5	9.3	20.2	1.1	7.0	44.1	47.9	49.6	48.9	1.4	0.0
Zambia	-	64.6	9.4	26.0	2.3	17.4	28.1	52.3	83.0	9.1	7.9	0.0
Zimbabwe	-	71.7	5.9	22.4	1.3	2.6	36.4	59.7	48.3	42.1	9.6	0.0
Mean		**59.5**	**13.2**	**27.3**	**12.7**	**27.5**	**26.2**	**33.6**	**73.5**	**21.8**	**4.5**	**0.2**
Ethiopia	Amhara	39.8	20.4	39.7	28.3	67.4	3.6	0.7	97.1	2.1	0.1	0.8
	B/Gumuz	44.0	22.5	33.5	22.1	63.3	10.0	4.6	99.6	0.4	0.0	0.0
	Oromia	65.6	11.6	22.8	8.1	46.0	35.6	10.3	71.3	28.3	0.4	0.0
	SNNP	71.7	4.8	23.5	7.3	23.1	57.7	11.9	89.6	4.2	2.1	4.2
	Tigray	44.3	18.5	37.3	38.4	58.8	2.8	0.0	98.6	1.4	0.0	0.0
	Mean	**53.1**	**15.6**	**31.4**	**20.8**	**51.7**	**21.9**	**5.5**	**91.2**	**7.3**	**0.5**	**1.0**

Table 9. Continued

Country	Region	Grain covering by glumes			Spikelet density			Grain color				
		EX	IM	EC	H	IM	L	WT	LB	DB	PB	OR
Ethiopia	-	7.1	18.1	74.8	11.0	24.3	64.6	12.5	28.6	7.2	42.2	9.4
Eritrea	-	2.2	2.8	95.0	4.4	8.3	87.2	2.8	18.3	3.3	71.7	3.9
Kenya	-	51.1	40.2	8.8	52.7	38.6	8.8	15.4	41.1	0.0	9.3	34.3
Zambia	-	35.5	35.4	29.1	41.6	36.6	21.8	38.3	11.8	0.0	10.0	39.0
Zimbabwe	-	63.0	23.4	13.7	55.6	34.6	9.8	17.7	23.9	0.4	13.5	44.6
Mean	**-**	**31.8**	**24.0**	**44.3**	**33.1**	**28.5**	**38.4**	**17.3**	**24.7**	**2.2**	**29.3**	**26.2**
Ethiopia	Amhara	2.8	8.8	88.4	3.1	12.0	84.9	19.0	24.8	8.5	43.2	4.5
	B/Gumuz	3.8	8.3	87.9	5.0	13.3	81.7	6.7	45.0	5.4	36.3	6.7
	Oromia	20.7	27.0	52.3	19.2	34.3	46.5	10.3	21.8	1.8	35.4	30.7
	SNNP	2.9	30.8	66.3	23.5	41.5	35.0	15.0	20.0	15	46.7	3.3
	Tigray	5.2	15.7	79.1	4.4	20.5	75.1	11.7	31.6	5.3	49.4	2.0
	Mean	**7.1**	**18.1**	**74.8**	**11.0**	**24.3**	**64.6**	**12.5**	**28.6**	**7.2**	**42.2**	**9.4**

Key: Growth habit: ER=Erect, PR=Prostrate, DC=Decumbent; **Ear shape**: DP=Droop, OP=Open, SC=Semi-compact, CM=Compact; **Ear (glumes) color:** LG=light green, PP=purple, YG=yellow green, WT=white; **Grain covered by Glumes**: EX=exposed, IM=Intermediate, EC=Enclosed; **Spikelet density** = high, IM=Intermediate, L=low; **Grain color**: WT= white, LB=light brown, DB=Dark brown, PB=purple black, OR=Orange-red, SNNP=Southern Nation, Nationalities and Peoples Region

Farmer's preference for grain color depends on the adaptability of local cultivars to the climate and other environmental factors of the growing environment, yielding ability, major use of the grain or cultural roles and ease of production. Accordingly, purple-black colored seed was dominant in the Northern part of Ethiopia due to the fact that farmers linked this type of finger millet to high yield and tolerant such as moisture stress and poor soil fertility. Supporting results were reported by Bezawuletaw *et al*. (2006) and Tsehaye and Kebebew (2002). Countries or regions with better distributions of the different phenotypic classes of a given trait is said to have better diversity for the trait. Consequently, finger millet accessions sampled from Ethiopia showed better diversity for ear shape, ear (glumes) color and seed color, accessions from Zambia showed better diversity for grain coverage by glumes and spikelet density and Eritrean collections were diverse in growth habit.

Although all altitude classes and regions of collections were not represented by the same number of accessions, the different types of ear shapes, grain covering by glumes, ear color and spikelet density were abundant in altitude classes between 1383–1523 m.a.s.l., indicating that this altitude class might be the zone possessing the highest diversity of finger millet accessions. Diversified accessions for grain color were observed at ≥ 2088 m.a.s.l. and 1947– 2087 m.a.s.l altitude classes (Table 10). All released varieties were monomorphic for the traits considered in the study. However, there were differences in the phenotypic classes among these varieties (Table 10).

Table 10. Percentage proportion of six major qualitative traits in 144 finger millet accessions collected from eight altitude classes and 6 released varieties

Altitude	Growth habit			Ear shape				Ear (glumes) color			
	ER	PR	DC	DP	OP	SC	CM	LG	PP	YG	WT
≤1241	59.3	12.0	28.8	3.3	12.0	31.3	53.5	79.0	7.3	13.8	0.0
1242-1382	69.9	5.7	24.4	2.2	29.9	32.6	35.3	81.3	17.6	1.1	0.0
1383-1523	64.9	10.3	24.9	9.9	25.1	33.1	32.0	62.5	32.6	4.2	0.7
1524-1664	60.6	11.9	27.6	20.2	42.1	28.9	8.8	82.5	17.3	0.2	0.0
1665-1805	46.6	20.5	32.9	26.5	53.1	15.9	4.6	89.3	9.2	1.5	0.0
1806-1946	46.7	16.5	36.7	26.1	64.9	5.6	3.4	94.2	4.8	0.4	0.7
1947-2087	39.0	23.5	37.5	31.9	60.2	6.3	1.6	98.0	2.0	0.0	0.0
≥2088	52.2	18.9	28.9	28.0	52.2	15.7	4.1	91.3	8.8	0.0	0.0
sub mean	54.9	14.9	30.2	18.3	42.5	21.3	17.9	84.8	12.4	2.6	0.2
Padet	100.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	100
Taddesse	100.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	100
Wama	100.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	0.0	0.0	0.0
Bereda	100.0	0.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	0.0
Gute	100.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	0.0	0.0	0.0
Boneya	100.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	0.0	0.0

Table 10. Continued

Altitude	Grain covering by glumes			Spikelet density				Grain color			
	EX	IM	EC	H	IM	L	WT	LB	DB	PB	OR
≤1241	38.3	38.3	23.5	39.0	45.5	15.5	38.0	14.0	0.0	2.0	46.0
1242-1382	26.3	31.9	41.8	35.7	31.8	32.5	24.7	13.1	0.8	15.8	45.6
1383-1523	36.2	25.3	38.5	33.8	34.9	31.3	13.6	27.1	3.2	21.0	35.1
1524-1664	17.9	26.8	55.3	20.2	29.8	50.1	10.2	33.6	4.4	33.6	18.3
1665-1805	8.4	14.0	77.6	7.8	19.9	72.4	12.5	27.1	3.2	45.3	12.0
1806-1946	6.1	9.4	84.5	6.1	14.7	79.2	12.9	24.7	4.7	52.6	5.1
1947-2087	4.3	15.4	80.4	5.7	16.3	78.0	6.3	27.5	9.8	51.3	5.2
≥2088	9.3	18.7	72.1	10.8	21.7	67.5	26.7	19.2	6.7	35.8	11.7
Sub mean	18.3	22.5	59.2	19.9	26.8	53.3	18.1	23.3	4.1	32.2	22.4
Padet	100.0	0.0	0.0	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
Taddesse	100.0	0.0	0.0	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
Wama	100.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
Bereda	0.0	0.0	100.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	0.0
Gute	0.0	100.0	0.0	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
Boneya	0.0	100.0	0.0	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0

Key: **Growth habit:** ER=Erect, PR=Prostrate, DC=Decumbent; **Ear shape:** DP=Droop, OP=Open, SC=Semi-compact, CM=Compact; **Ear (glumes) color**: LG=light green, PP=purple, YG=yellow green, WT=white; **Grain covered by Glumes**: EX=exposed, IM=Intermediate, EC=Enclosed; **Spikelet density** H= high, IM=Intermediate, L=low; Grain color: WT= white, LB=light brown, DB=Dark brown, PB=purple black, OR=Orange-red

5.1.1.2 Cluster analysis

A dendrogram generated based on the regional/country mean of the six qualitative traits grouped the regions of origin and the improved varieties into three clusters at 80% cluster-cutting point (Fig 6). All five administrative regions of Ethiopia and Eritrea aggregated in the first cluster and accessions from Kenya, Zambia and Zimbabwe grouped in the second cluster. The improved varieties Taddesse and Padet, both released from Melkasa Agricultural Research Center, were grouped in the third cluster. Cluster analysis partly showed that neighboring regions, countries and proximity in altitude classes shared strong similarity. The similarity could be due to farmer's selection criteria for a given trait that was uniform particularly based on their ecological performance, the original source of seeds that might be the same or seed exchange among neighboring farmers. Other possibilities could be inter- and intra-country/regional migration of farmers along with their seeds and long distance gene flow as facilitated by pollinators and birds/animals via seed dispersal.

Similarly, finger millet accessions collected from Kenya, Tanzania and Uganda were grouped together, but accessions from Ethiopia and Burundi were in separate clusters each (Reddy *et al*., 2009). Due to the monomorphic nature for the different qualitative characters (Table 10), all the released varieties shared minimal similarity with finger millet accessions of all countries and regions (Fig 6). For altitude, accessions from all eight classes were grouped into two classes, but accessions from altitude range of 1383 – 1523 m.a.s.l and 1524 – 1664 m.a.s.l remained ungrouped (Fig. 7).

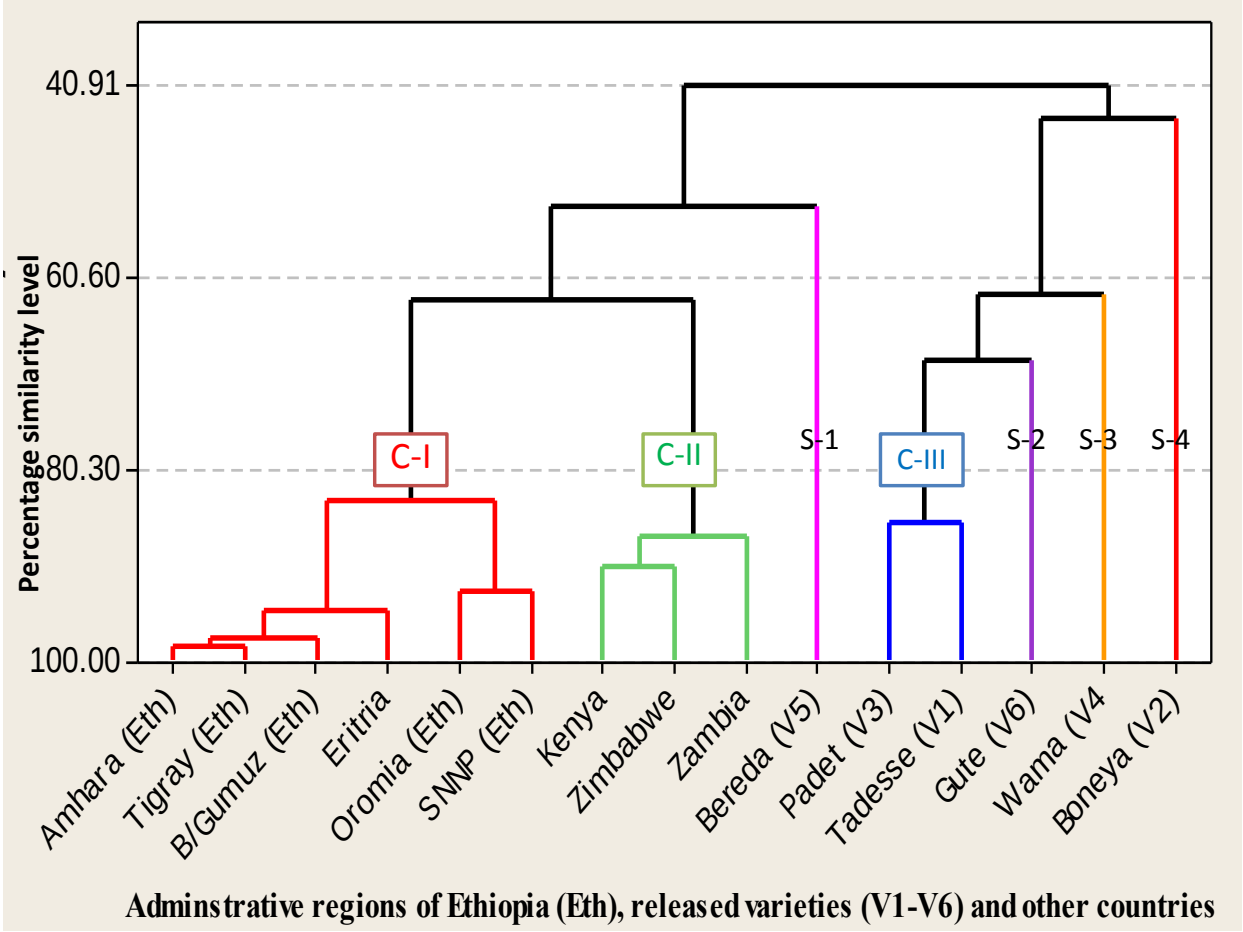


Fig 6. Dendrogram showing the similarities of 144 finger millet accessions for 6 qualitative traits among regions and countries of origin and six released varieties.

Key: C (cluster) followed by the number indicated cluster number and S= solitary or ungrouped

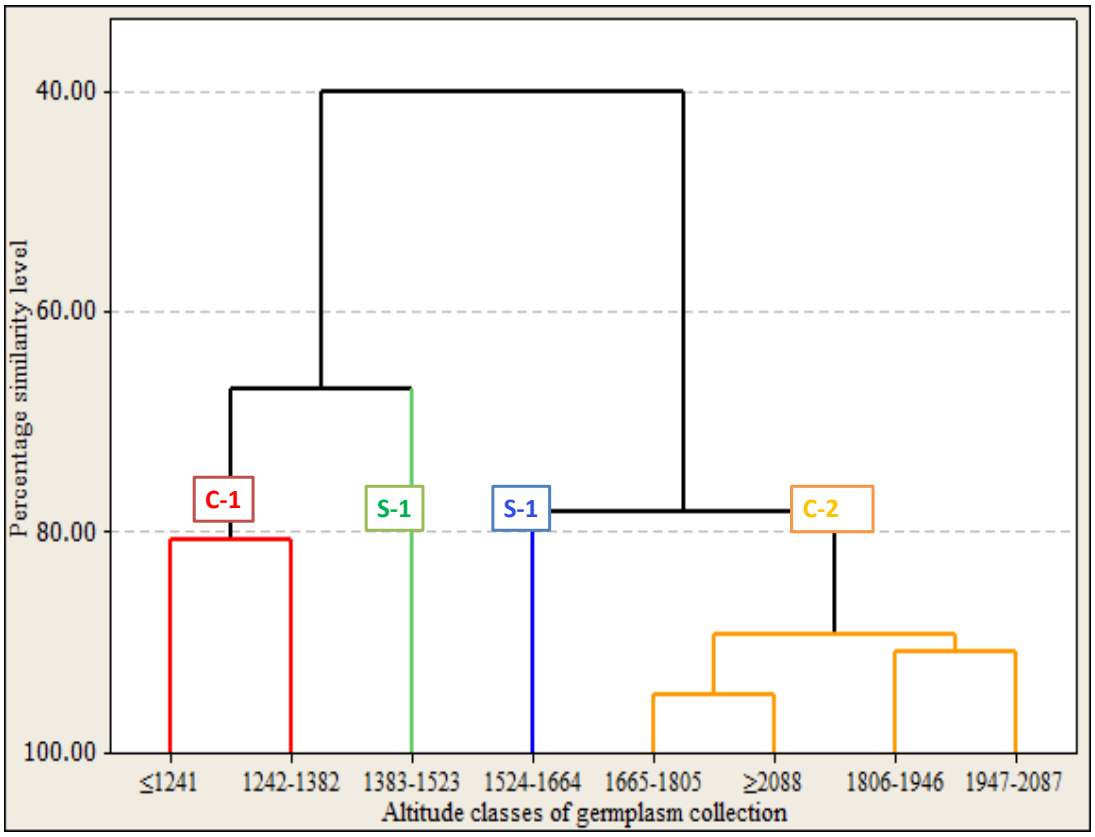


Fig 7. Dendrogram showing the similarities among agro-ecologies of collection for 144 finger millet accessions for six qualitative traits.

Key: C (cluster) followed by the number indicated cluster number and S= solitary or ungrouped

5.1.1.3 Shannon-Weaver diversity index (H') analysis

The mean Shannon weaver diversity index for individual accessions for the six qualitative traits ranged from 0.151 for Acc. 214993 of Zambia collected from altitude of 1340 m.a.s.l. to 0.387 for Acc. 229731 m.a.s.l of Ethiopia (Amhara region) collected from 1950 m.a.s.l. Besides, Acc. 230102 (0.172) and 230110 (0.173) both from Eritrea, Acc. AAUFM-20 (0.176) from Ethiopia (Tigray) and Acc. BKFM0048 (0.182) from Ethiopia (Oromia) were some of the accessions with minimum values. However, relatively higher diversity was observed for Acc. 203543 (0.365) of Kenya, Acc. AAUFM-32 (0.369) of Ethiopia (Tigray region), Acc. BKFM0051 (0.372) and Acc. 216057 (0.385), both from Ethiopia (West Wollega zone of Oromia region) and Acc. 229731 (0.387) of Ethiopia (Amhara region) (Appendix 1.)

Region/country wise, better diversity in growth habit were recorded for Eritrean accessions (0.485) followed by Benishangul Gumuz (0.427), Amhara (0.426) and Tigray regional states of Ethiopia (0.423) but least for Zimbabwe (0.302). Similarly, Kenya (0.345), Eritrea (0.305) and Zambia (0.297) were the major regions possessing accessions with comparatively high diversity of ear shapes. Grain covering by glumes and spikelet density were abundant in SNNP (0.338) and Oromia (0.335). Grain color showed greater abundance in accessions collected from Kenya (0.403), while the least was from Eritrea (0.243). On trait basis, minimum mean diversity within regions was observed for ear/glumes color (0.199) and maximum for growth habit (0.377) followed by grain color (0.353).

The pooled mean diversity indices for the six traits were comparatively higher for the Kenyan collection (0.320) followed by Benishangul Gumuz (0.302), Oromia (0.294), Zambia (0.289) and Zimbabwe (0.287) but least for Eritrea (0.236) and the overall mean was 0.287 with standard deviation of 0.045 (Table 11). Shannon-Weaver diversity index analysis for six qualitative traits indicated that diversity was higher at the lower level (between accessions) than the subsequent level and relatively lower at the higher level (between countries or altitude classes). Similar results were reported for bread wheat (Bekele, 1984), finger millet (Barbeau and Hilu, 1993) and tef (Lule *et al*., 2011). Contradicting results were also reported for bread wheat, where diversity was higher among districts than among populations within a district (Mulatu *et al*., 1991). Therefore, the pattern of germplasm biodiversity is crop-dependent. In general, pooled mean data at geographical or ecological level could provide a mean value for a group of accessions to illustrate eco-geographical patterning, gene flow, migration and trends of seed movement within and between adjoining regions and countries.

Table 11. Regions of origin-based Shannon-Weaver diversity indices (H') and standard error of mean (± SE) of finger millet accessions for 6 qualitative traits.

Country/region	Qualitative characters						Mean ± SE
	GH	ESH	EC	GCG	SPD	SC	
Amhara/Ethiopia	0.426	0.245	0.212	0.239	0.238	0.312	0.279 ± 0.033
B/Gumuz/Ethiopia	0.427	0.253	0.159	0.260	0.338	0.377	0.302 ± 0.040
Oromia/Ethiopia	0.329	0.246	0.255	0.335	0.299	0.296	0.294 ± 0.015
SNNP/Ethiopia	0.391	0.279	0.194	0.236	0.289	0.326	0.286 ± 0.043
Tigray/Ethiopia	0.423	0.277	0.157	0.238	0.288	0.325	0.284 ± 0.033
Eritrea	0.458	0.305	0.055	0.111	0.243	0.243	0.236 ± 0.060
Kenya	0.317	0.345	0.291	0.234	0.330	0.403	0.320 ± 0.024
Zambia	0.325	0.297	0.182	0.284	0.312	0.337	0.289+ 0.240
Zimbabwe	0.302	0.293	0.282	0.264	0.283	0.353	0.288 ± 0.012
Mean	0.377	0.282	0.199	0.244	0.291	0.330	0.287±0.045

Key: B/Gumuz =Benishangul Gumuz, SNNP=Southern Nation, Nationalities and Peoples region, GH=growth habit, ESH= ear shape, EC=Ear/glumes color, GCG=Grain covering by glumes, SPD=Spikelet density, SC=seed color

Table 12. Shannon-Weaver diversity indices (H') and standard error of mean (± SE) of finger millet accessions collected from eight altitude classes (agro-ecologies) for six qualitative traits

Altitude	Qualitative characters						Mean ± SE
	GH	ESH	EC	GCG	SPD	SC	
≤1241	0.329	0.348	0.234	0.242	0.342	0.305	0.300 ± 0.058
1242-1382	0.316	0.296	0.170	0.289	0.284	0.305	0.277 ± 0.035
1383-1523	0.367	0.288	0.239	0.294	0.326	0.388	0.317 ± 0.038
1524-1664	0.371	0.239	0.184	0.200	0.279	0.339	0.269 ± 0.043
1665-1805	0.396	0.282	0.190	0.259	0.281	0.294	0.284 ± 0.042
1806-1946	0.416	0.231	0.186	0.242	0.266	0.264	0.268 ± 0.038
1947-2087	0.433	0.260	0.189	0.267	0.247	0.377	0.295 ± 0.051
≥2088	0.376	0.277	0.194	0.198	0.290	0.304	0.273 ± 0.041
Mean	0.377	0.282	0.199	0.244	0.291	0.330	0.287 ± 0.041

Key: GH=growth habit, ESH= ear shape, EC=Ear/glumes color, GCG=Grain covering by glumes, SPD=Spikelet density, SC=seed color, SE= standard error of mean

For altitude, relatively high mean H' values were noted for accessions collected from altitude classes between 1383 – 1523 m.a.s.l for ear/glumes color (0.239), grain covering by glumes (0.294) and grain color (0.388). Overall, lower mean diversity was observed for ear color (0.199), but higher diversity for growth habit (0.377) and seed color (0.330). The pooled mean diversity for those six traits was lower for the altitude region between 1524-1664 m.a.s.l and higher for 1383 – 1523 m.a.s.l. and the overall mean was 0.287 with deviation from the mean value of 0.041 (Table 12).

5.1.2. Quantitative traits analysis

5.1.2.1 Analysis of variance

The combined analysis of variance (ANOVA) for the two locations showed significant location effects for all quantitative traits considered in the present study (Table 13). The genotypes mean squares were also significant ($P \leq 0.01$) for all quantitative traits except ear weight, implying the possibility to continue further breeding activities. Similarly, highly significant variations between finger millet genotypes were reported in previous studies (Bezawuletaw *et al.,* 2006; Tsehaye and Kebebew, 2002; Prasad *et al*., 1994). Genotype by environment interaction mean square was highly significant ($P \leq 0.01$) for days to 50% maturity, total tiller number, productive tiller number, plant height, finger length, finger number, ear weight and lodging (Table 13). This indicated the test accessions responded differently across environments for those traits. However, no significant differences were observed for days to 50% heading, number of grains per spikelet, culm diameter, finger width, thousand seed weight & grain yield per plant (Table 13).

Table 13. Mean squares for 15 quantitative traits of 144 finger millet accessions and six released varieties as obtained from combined ANOVA of the two locations, Gute and Arsi Negele.

Source of variation	df	DH	DM	TTN	PTN	PLHT	FL	FN
Environment	1	4066.4**	11102.6**	3199.8**	3087.2**	47638.2**	28.1**	36.66**
Genotype	149	315.40**	89.26**	12.02**	11.48**	491.75**	15.1**	4.85**
G x E	149	51.24	44.13**	8.31**	8.20**	122.75**	2.45**	1.21**
Error	298	46.83	13.01	1.10	1.19	35.58	0.94	0.65
CV (%)		7.05	2.29	18.72	19.55	8.68	12.12	11.09
LSD (5%)		7.98	4.21	1.23	1.27	6.95	1.13	0.94
Mean		97.01	157.73	5.61	5.55	68.75	7.98	7.23

Table 13 Continued

Source of variation	df	EW	NGPS	CD	FW	TGW	LOG	HI	GYPLN
Environment	1	72.5**	135**	2129**	13.2**	0.02	228150**	27859.1**	28912**
Genotype	149	5.32*	1.07**	0.389**	0.08**	0.8**	1546.3**	673.3**	182.8**
G x E	149	1.09**	0.37	0.32	0.05	0.20	642.79**	239.7**	111.90
Error	298	0.74	0.34	0.27	0.05	0.17	82.50	199.96	53.61
CV (%)		32.44	12.21	22.01	28.72	18.52	20.57	31.30	35.85
LSD (5%)		1.00	0.71	0.61	0.26	0.49	10.59	16.48	8.54
Mean		2.65	4.39	2.37	0.79	2.26	44.15	23.10	20.42

KEY: df= degree of freedom, DH=days to50% heading, DM= days to maturity, TTN=Total tiller number, PTN= productive tiller number, PLHT= plant height, FL= finger length, FN= finger number, EW= finger weight, NGPS=number of grain per spikelet, CD=culm diameter, FW=Finger width, TGW=thousand grain weight, LOG= lodging index, HI=Harvest index, GYPLN=grain yield per plant., GxE, CV(%), LSD (5%)

5.1.2.2 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 14). 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). Finger number per ear ranged from 5.1 for Acc. BKFM0028 of Oromia to 11.68 for Acc. 215802 of Amhara. For number of grains per spikelet, the highest (6.35) and the lowest (2.95) was recorded for Acc. BKFM0042 and Acc. 216056, respectively, both from Oromia. 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.

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 14).

Among altitude classes, the least mean value for plant height, finger length, finger number per ear and grain yield per plant was observed below 1241 m.a.s.l. and higher for altitude class above 2088 m.a.s.l (Table 14). In other words, the two extreme altitude classes were described for an extreme mean value of grain yield and yield related traits. Reddy *et al*. (2009) evaluated about 2000 finger millet accessions collected from five east African countries, namely Burundi, Ethiopia, Kenya, Tanzania and Uganda and reported that flowering date ranged from 50 – 120 days, where accessions from Burundi was early but those from Ethiopia were late. The authors also reported Kenyan accessions were dwarf type but the tallest accession was from Uganda. The existence of a vast range of genetic variability in finger millet germplasm were also reported by Hussaini *et al*. (1977) and Tsehaye and Kebebew (2002).

Table 14. Patterns of genetic variability for 17 quantitative traits among finger millet accessions, regions and altitudes of origin

Traits	Accession/population level			Regional/country level			Altitude level			Mean ±SE
	Minimum	Maximum	Range	minimum	maximum	Range	minimum	maximum	Range	
Days to 50% heading	82.25	117.00	34.75	86.69	107.59	20.90	94.17	103.92	9.75	97.01±0.73
Days to 50% maturity	143.0	167.25	24.25	150.72	161.75	11.03	155.84	160.95	5.11	157.73±0.39
Total No. of tillers	2.60	11.35	8.75	4.09	6.96	2.87	4.70	6.71	2.01	5.61±0.14
No. of productive tillers	2.58	10.50	7.92	4.08	6.93	2.85	4.69	6.63	1.94	5.55±0.14
Plant height (cm)	41.13	103.35	62.22	56.03	77.17	21.14	54.89	76.00	21.11	68.75±0.91
Finger length (cm)	3.53	11.40	7.87	5.07	9.83	4.76	5.58	9.56	3.98	7.98±0.16
Finger number	5.10	11.68	6.58	6.71	8.16	1.45	6.87	8.23	1.36	7.23±0.09
Culm branch	1.15	6.08	4.93	2.42	3.32	0.90	2.26	3.51	1.25	3.03±0.08
Ear weight(g)	1.10	5.53	4.43	1.58	4.54	2.96	2.01	3.55	1.54	2.65±0.09
No. of grains/spikelet	2.95	6.35	3.40	3.83	4.65	0.82	4.15	4.50	0.35	4.39±0.06
Culm diameter (cm)	1.57	3.29	1.72	1.99	2.61	0.62	2.24	2.43	0.19	2.38±0.03
Finger width (cm)	0.60	1.77	1.17	0.69	0.89	0.20	0.74	0.84	0.10	0.79±0.01
Lodging (%)	7.50	80.00	72.50	30.18	59.91	29.73	27.88	57.00	29.12	44.15±1.61
Biomass wt/plant (g)	45.45	173.47	128.02	58.16	116.5	58.34	58.16	116.5	58.34	95.03±2.21
Harvest index (%)	9.39	51.96	42.57	15.7	30.47	14.77	15.01	24.9	9.98	22.71±0.65
1000 grain weight (g)	1.40	3.50	2.10	1.92	2.91	0.99	1.91	2.44	0.53	2.26±0.04
Grain yield/plant (g)	6.12	41.60	35.48	15.27	25.85	10.58	14.95	25.18	10.23	20.42±0.49

5.1.2.2 Cluster analysis

5.1.2.2.1 Hierarchical clustering based on major phenotypic traits

At 85% similarity level, all 150 accessions were grouped into eight clusters based on 17 standardized quantitative traits, but three accessions (Acc.216057, 241768 and 238344) and one improved variety (Wama) remained apart (Fig 8). The first cluster (C-I) consist of only two accessions, Acc. 242133 collected from Amhara and AAUFM-22 from Tigray regional states. This cluster recorded the highest total and productive tiller number as well as in grain yield (28.34g/plant) and least in culm diameter (2.09 cm) (Table 15). All 13 introduced accessions from Zimbabwe and six from Kenya were grouped in the second cluster (C-II) along with 15 accessions from Oromia and three accessions from SNNP. The second cluster showed the highest culm diameter (4.85cm) and thousand grain weights (4.62 g).

The third cluster (C-III) was comprised of only two accessions, Acc. 214991 and Acc. 214996, both introduced from Zambia and distinguished by the shortest average plant height (43.61cm) and the lowest grain yield per plant (7.32g) (Table 15). All accessions from Eritrea and Tigray, 26 from Amhara, two from Zambia and one each from Oromia, SNNP and Kenya were grouped in the fourth cluster (C-IV). The fifth cluster (C-V) comprised of 25 accessions collected from different regions and countries, and this group exhibited lower percentage harvest index and was late maturing. The improved variety Boneya and Acc. 229730 grouped together in the sixth cluster (C-VI), which showed the highest mean culm branching and finger length. The seventh cluster (C-VII) comprised of four accessions (Acc. 215802- from Amhara, Acc.244798-from SNNP and Acc. 208726 and BKFM0055 both from Oromia) and characterized by taller plant height, higher finger number and late maturity. Three improved varieties (Padet, Taddesse and Gute) and Acc. 203546 introduced from Kenya were grouped in the eighth cluster (C-VIII).

This cluster was mainly distinguished by the highest mean finger width, 1000 grain weight, culm diameter and number of grains per spike, but the least for finger number and tiller number.

Among the outliers, Acc. 216057 from Oromia (S-1) was characterized by the highest biomass weight, number of total and productive tillers per plant and good yielding ability, but prone to lodging (Fig 8, Table 15). Acc. 241768 (S2, from SNNP) was characterized by earliness in days to maturity (144 days) and lowest biomass weight (52.28g). Improved variety Wama (S3) scored the highest ear weight, number of grains per spikelet, 1000 grains weight and tolerance to lodging, but least in number of fingers per main ear. Acc. 238344 was the other outlier (S4) collected from Amhara region. It had the highest finger width and culm branching (Table 15).

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, the primary seed source could have been the same, or there might have been a high tendency of seed exchange. Supportive results were also reported by Reddy *et al*. (2009) for 2000 finger millet accessions collected from east Africa and evaluated at ICRISAT (India), Bezawuletaw *et al.* (2006) for 64 populations and Tsehaye and Kebebew (2002) for 42 finger millet populations collected from different regions of Ethiopia. On the other hand, collections from Oromia, SNNP, Benishangul Gumuz and Zambia were distributed across several of the clusters to variable degrees (Fig 8), which indicated variation among accessions of the same region or country.

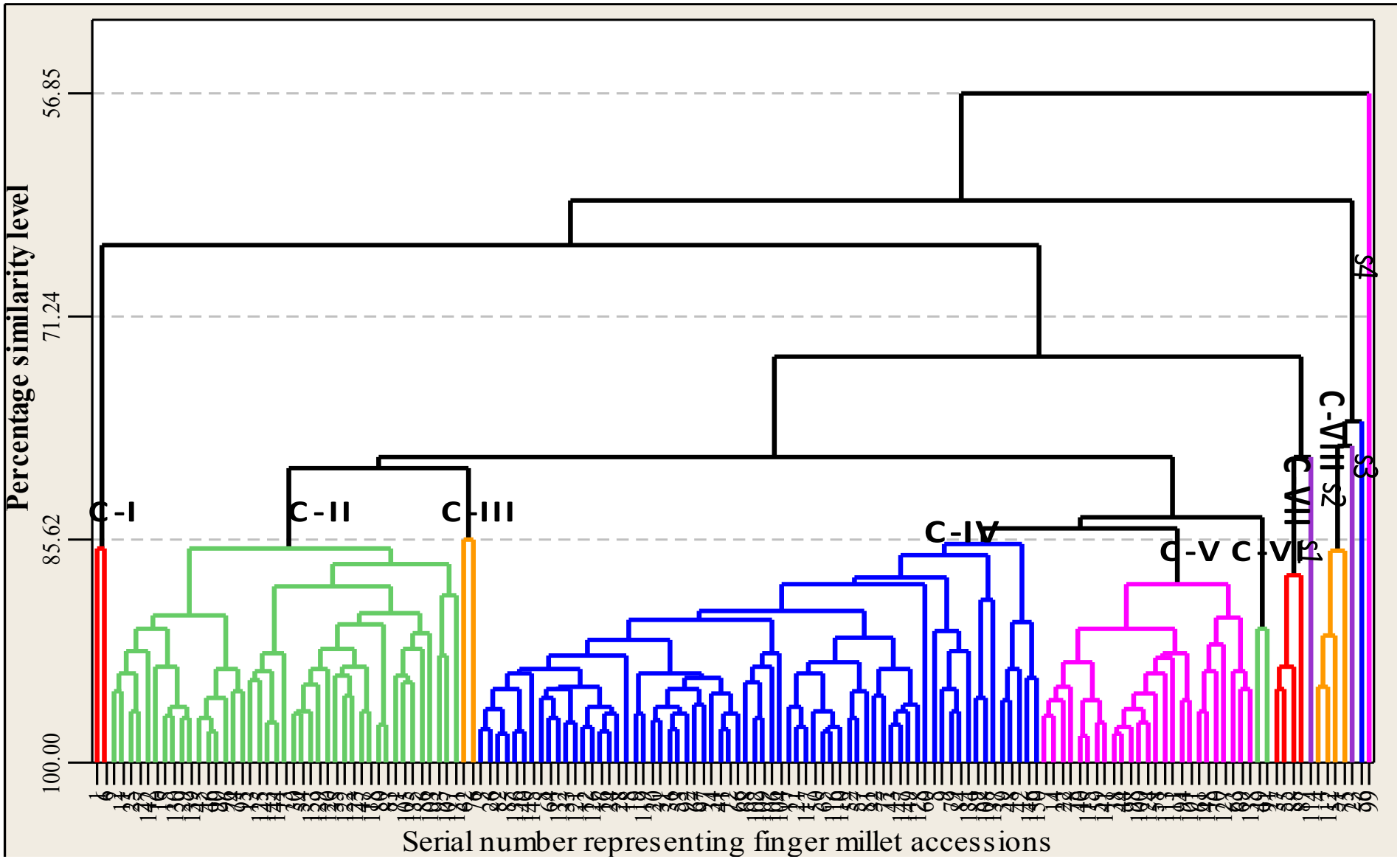


Fig 8. Dendogram showing the similarities based on 17major quantitative traits at 85% similarity level for standardized data among the 150 finger millet accessions evaluated in this study. The serial number above each cluster indicates the cluster number and the numbers below the X-axis indicates each finger millet accession in the same order as listed in Table 3 under column SND (serial number used for dendrogram).

Table 15. Agronomic trait means for clustering of 144 accessions and six improved varieties for 17 quantitative traits

Cluster	DH	DM	TTN	PTN	PLHT	FL	FN	CB	EW	NGPS	CD	FW	TGW	GYPPL	LOG	HI	BWPPL
C1	87.63	156.13	8.83	8.31	67.51	9.81	7.01	3.36	1.61	4.90	2.09	0.72	2.25	28.34	66.88	74.84	68.51
C2	98.64	158.48	4.64	4.58	64.72	5.80	6.72	2.58	3.69	5.42	2.41	0.87	2.43	19.25	25.52	21.17	97.09
C3	105.75	159.25	2.93	2.89	43.61	4.81	5.80	3.11	3.10	5.53	2.25	0.64	1.90	7.37	19.38	31.03	67.81
C4	91.56	155.35	6.18	6.11	67.08	9.21	7.41	3.18	2.02	4.86	2.31	0.74	2.26	23.21	56.47	25.17	85.5
C5	106.42	162.61	6.05	5.99	74.00	8.48	7.26	3.12	2.03	4.44	2.31	0.70	1.90	15.40	48.25	13.87	92.62
C6	95.75	154.63	5.35	5.35	81.23	10.11	7.68	4.20	2.75	5.05	2.99	0.90	2.70	18.87	31.88	17.30	109.37
C7	109.31	162.25	4.85	4.85	100.13	7.01	10.72	3.68	3.58	5.23	2.65	0.79	1.85	16.37	32.19	15.60	92.93
C8	102.69	159.25	3.29	3.26	75.18	7.05	6.22	2.63	5.24	5.76	3.31	1.08	3.45	17.57	11.56	16.07	97.94
S1	102.25	160.75	10.15	9.63	93.43	7.70	10.25	3.20	3.43	4.75	2.52	0.72	2.10	26.53	77.50	23.32	158.33
S2	98.00	144.25	4.38	4.33	73.55	5.15	6.93	2.38	4.25	4.60	2.78	1.06	2.40	17.31	30.00	20.21	52.28
S3	99.25	160.00	3.58	3.58	67.30	7.83	5.55	3.38	5.28	5.80	2.03	0.93	3.50	15.22	6.25	24.02	63.33
S4	90.00	154.25	5.30	5.30	72.25	10.00	7.00	5.28	1.63	4.60	2.30	1.77	1.90	18.06	70.00	31.71	68.33

KEY: DH=days to50% heading, DM= days to maturity, TTN=Total tiller number, PTN= productive tiller number, PLHT= plant height, FL= finger length, FN= finger number, CB=culm branch, EW= ear weight, NGPS=number of grain per spikelet, CD=culm diameter, FW=Finger width, TGW=thousand grain weight, LOG= lodging index, HI=Harvest index, GYPPL=grain yield per plant, BWPPL=Biomass weight per plant, C=cluster, S=solitary or ungrouped

5.1.2.2.2 Hierarchical clustering at regional/country level

At 85% similarity level, all the accessions were grouped into four clusters with three varieties that remained as outliers (Fig 9). Accessions collected from Tigray and Amhara showed strong relatedness with those from Eritrea and grouped in the first cluster (C-I). This cluster was characterized by the highest mean finger length (9.07cm), productive tiller number (6.3), grain yield per plant (22.56g) and harvest index (26.91%), but lowest ear weight (1.86 g) (Table 16). The second cluster (C-II) comprised of accessions from Oromia and Benishangul Gumuz and this group was distinguished for late heading (104 days) and maturity (161). In both the first and second clusters, neighboring regions grouped together for the same likely reasons mentioned in section 5.1.2.2.1 above. The finding of Ayana (2001) also indicated that sorghum accessions collected from neighboring regions were grouped together. The report on finger millet by Bezaweletaw *et al*. (2006) and Tsehaye and Kebebew (2002) were also in agreement to the present study.

Introduced accessions from Kenya, Zambia and Zimbabwe were grouped in the third cluster and this cluster was known for its short mean plant height (63cm) and finger length (5.87cm) (Table 16). Even if the geographical location of Kenya is distant from Zambia and Zimbabwe, accessions from those countries showed strong similarity. The most probable reasons could be, migration, the primary seed source for these countries could have been the same, eco-geographic similarity for the adaptation of similar populations, or combinations of those and other factors. In support of the present study, finger millet accessions collected from Kenya, Tanzania and Uganda were grouped together, but accessions from Ethiopia and Burundi were in separate clusters each (Reddy *et al*., 2009).

The improved varieties such as Gute and Padet were grouped in the fourth cluster, which showed the highest mean plant height (75.45cm), culm diameter (3.14cm) and finger width (1.1cm), but the lowest total and productive tiller numbers (3.09 and 3.08, respectively) and culm branching (1.65). Bereda, Boneya, accessions from SNNP, Taddesse and Wama remained outliers. Bereda gave the least grain yield (9.231) of the groups, Wama showed the lowest finger number (5.5), the SNNP accessions had the highest harvest index and Taddesse showed the highest finger width (1.17cm) but the lowest tiller number (3.13).

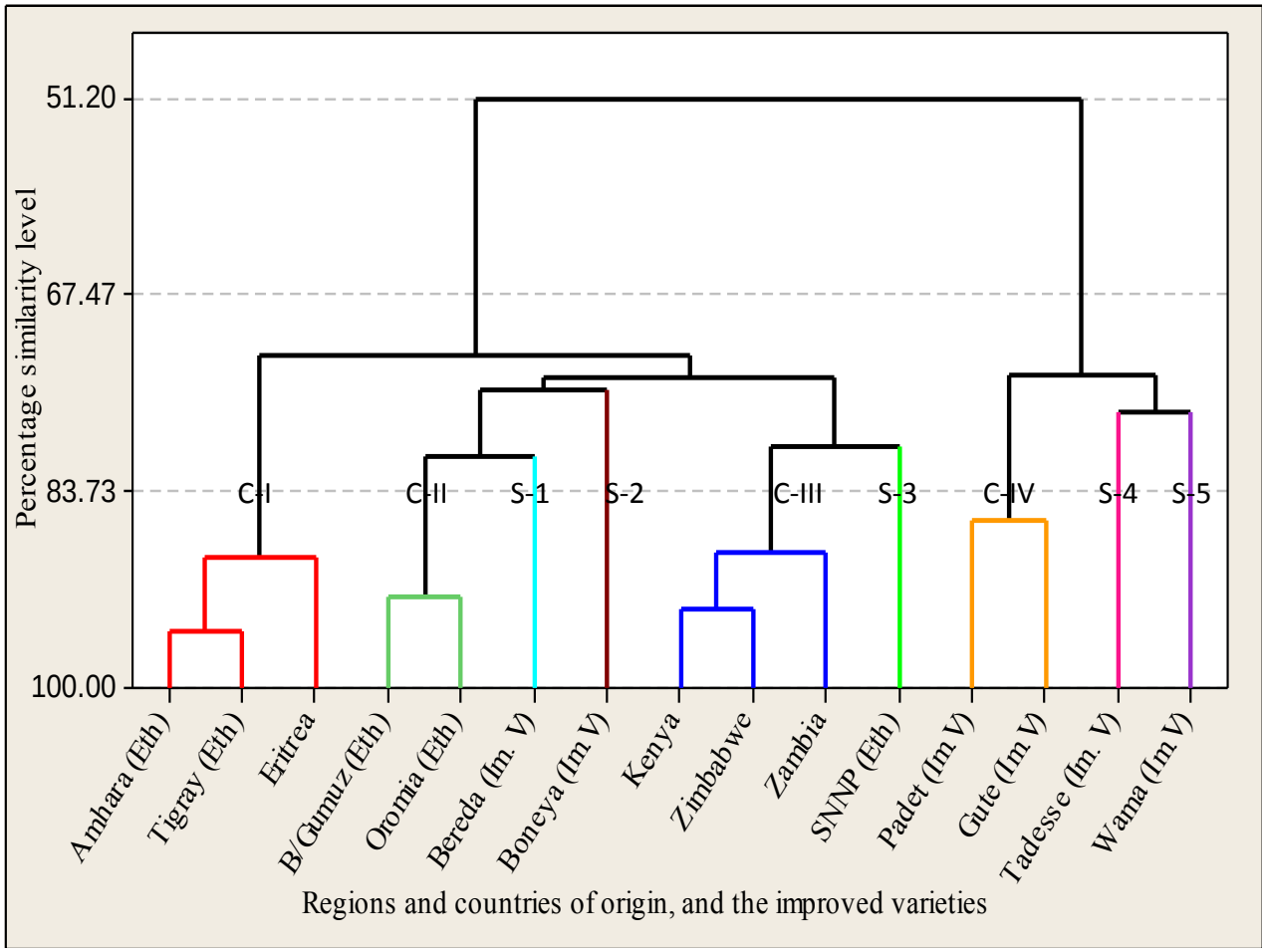


Fig 9. The genetic relatedness of 144 finger millet accessions for 17 quantitative traits among regions and countries of origin and six released varieties.
Key: C= cluster, S= solitary or ungrouped

Table 16. Mean of 17 quantitative traits per cluster (C) and ungrouped accessions (S) at regional level

Clusters	DH	DM	TTN	PTN	PLHT	FL	FN	CB	EW	NGPS	CD	FW	TGW	GYPPL	LOG	HI	BWPPL
C1	90.32	154.33	6.37	6.30	64.41	9.07	7.27	3.18	1.86	4.85	2.25	0.73	2.27	22.56	57.02	26.91	87.86
C2	103.75	161.06	6.10	6.03	72.07	8.30	7.13	3.02	2.43	4.76	2.23	0.73	1.96	17.34	42.12	17.10	97.09
C3	96.34	159.36	4.17	4.14	62.98	5.87	6.96	2.72	4.05	5.33	2.50	0.87	2.62	20.19	30.16	22.05	100.40
C4	104.13	159.88	3.09	3.08	75.45	6.64	5.58	1.65	4.30	5.63	3.14	1.10	3.35	15.51	16.25	16.55	85.50
S1	101.00	163.25	3.23	3.23	65.85	8.93	6.68	2.45	2.08	5.10	2.20	0.79	1.40	9.23	36.25	11.82	77.92
S2	96.75	155.75	5.13	5.13	83.60	8.83	7.63	4.33	2.98	5.05	2.70	0.89	3.10	12.12	26.25	17.92	60.20
S3	97.75	153.33	4.78	4.75	72.26	6.05	8.16	3.13	2.89	4.86	2.32	0.82	2.18	17.83	32.92	39.79	91.68
S4	102.00	159.50	3.13	3.13	71.65	8.05	6.55	3.83	6.85	6.25	3.66	1.17	3.60	20.92	5.00	16.99	116.47
S5	99.25	160.00	3.58	3.58	67.30	7.83	5.55	3.38	5.28	5.80	2.03	0.93	3.50	15.22	6.25	24.02	63.33

Key: DH=days to50% heading, DM= days to maturity, TTN=Total tiller number, PTN= productive tiller number, PLHT= plant height, FL= finger length, FN= finger number, CB=culm branch, EW= ear weight, NGPS=number of grain per spikelet, CD=culm diameter, FW=Finger width, TGW=thousand grain weight, LOG= lodging index, HI=Harvest index, GYPPL=grain yield per plant, BWPPL=Biomass weight per plant

5.1.2.2.1 Hierarchical clustering at clinal level

For altitude, three clusters and one solitary group were formed at 80% similarity level (Fig 10). Proximity in altitude classes was clearly manifested in all three clusters. Accessions collected from altitudes above 2088 m remained ungrouped mainly due to their distinguishing characters such as tallest plant height, longest finger length, highest finger number, longest culm branch and highest grain yield and harvest index.

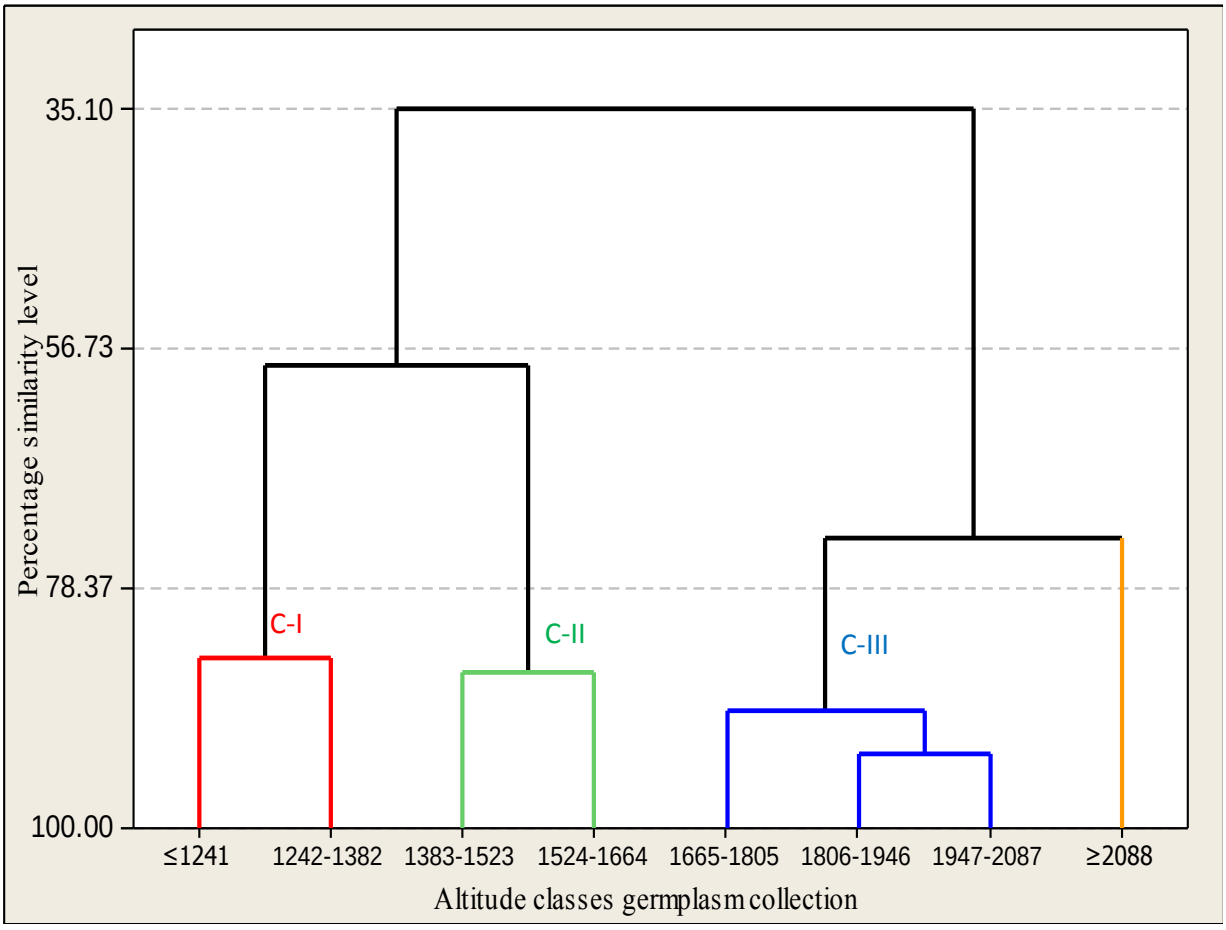


Fig 10. The genetic relatedness of 144 finger millet accessions for 17 quantitative traits among agro-ecological zones of origin

Key: C= cluster, S= solitary or ungrouped

5.1.2.3. Inter-cluster Euclidean distance

Inter-cluster Euclidian distance measured based on the standardized means for the seventeen quantitative traits revealed that relatively wider genetic variation (10.78) was recorded between cluster C-I (mainly comprised of Acc. 242133 from Amhara and AAUFM-22 from Tigray) and C-VIII (comprised of the improved varieties Padet, Taddesse and Gute as well as Acc. 203546 from Kenya). Interestingly, the first cluster recorded the highest in the total and productive tiller numbers, highest in grain yield and least in culm diameter, whereas, C-VIII was mainly distinguished for the least in total and productive tiller numbers and finger number, contrary to the first group. The accessions in the first cluster also showed wider genetic distance or dissimilarity to the seventh and the third clusters, respectively (Table 17).

Table 17. Inter cluster Euclidean distance for the 144 finger millet accessions and six improved varieties in this study

Clusters	C-I	C-II	C-III	C-IV	C-V	C-VI	C-VII	C-VIII	S-I	S2	S3	S4
C-I	0.00	7.67	9.05	5.45	7.52	7.76	9.41	10.78	7.20	9.51	10.65	9.51
C-II		0.00	4.23	3.95	4.29	4.20	5.65	4.98	7.22	5.06	6.39	8.42
C-III			0.00	6.29	5.86	7.05	7.79	7.44	9.97	7.17	7.50	10.46
C-IV				0.00	3.29	3.84	6.02	7.79	5.41	6.71	8.73	7.89
C-V					0.00	4.54	4.91	7.97	5.55	7.37	9.12	8.65
C-VI						0.00	5.05	5.52	6.38	5.95	8.11	7.56
C-VII							0.00	7.67	5.48	7.74	9.65	9.59
C-VIII								0.00	9.98	5.50	5.73	9.88
S1									0.00	9.20	11.44	9.95
S2										0.00	6.46	8.86
S3											0.00	11.21
S4												0.00

5.1.2.4 Principal component analysis

5.1.2.4.1 Accession/Population based

Principal component analysis (PCA) indicated that the first vectors were more important than the second and other vectors (Table 18). The first PC accounted for 26.2% of the total variation, whereas the corresponding values for the second to the fifth PCs were 15.3%, 11.9%, 9.0% and 6.2%, respectively (Table 18). The first five principal components with Eigen values greater than one combined explained about 68.6% of the gross variation, and 83.5% variation was extracted from the first eight PCs. Agronomic and phenotypic characters such as thousand grain weight, ear weight, finger length, productive tiller number, total tiller number and lodging index were the major contributors for the variation observed in the first principal component (Table 18).

The variation in the second PC was mainly due to days to heading, days to maturity, biomass weight per plant, plant height and grain yield per plant. Likewise, finger number per main ear, culm diameter, harvest index, plant height and culm branch were among the major contributors to the variation in the third component. Total tiller number, productive tiller number and lodging index were the major contributors to the variation observed in the fourth component. The variability in the fifth component was attributed mainly to number of grains per spikelet, biomass weight per plant and finger width (Table 18). Several authors indicated that different phenotypic traits for different crops contributed to the overall variability observed between study germplasm for different crops such as tef (Lule *et al*., 2011 and Assefa *et al.*, 2003); sorghum (Ayana and Bekele, 1998) and finger millet (Quendeba *et al*., 1995).

Table 18. Principal component analysis for 17 quantitative traits of 144 finger millet accessions

Variable	PC1	PC2	PC3	PC4	PC5
Days to 50% heading	0.150	-0.492	-0.150	-0.168	-0.101
Days to 50% maturity	0.125	-0.437	0.084	0.002	0.018
Total No. of tillers	-0.328	-0.056	-0.171	-0.506	-0.032
No. of productive tillers	-0.331	-0.060	-0.168	-0.502	-0.027
Plant height	-0.092	-0.379	0.368	-0.215	-0.015
Finger length	-0.373	-0.030	0.216	0.042	0.064
Finger number	-0.090	-0.206	0.440	0.070	-0.043
Culm branch	-0.122	0.011	0.319	0.211	-0.175
Ear weight	0.392	-0.045	0.137	-0.142	0.082
No. of grains per spikelet	0.105	0.044	-0.076	-0.156	0.724
Culm diameter	0.202	-0.092	0.408	-0.160	0.095
Finger width	0.254	0.082	0.153	-0.163	-0.327
Thousand grain weight	-0.393	0.094	0.144	0.174	0.031
Biomass weight per plant	-0.019	0.390	0.105	-0.204	-0.380
Grain yield per plant	0.173	0.338	0.215	-0.070	0.273
Harvest index	-0.158	0.212	0.376	-0.287	0.202
Lodging	0.308	0.185	0.023	-0.332	-0.207
Eigen value	4.4528	2.6094	2.0190	1.5338	1.049
Proportion	0.262	0.153	0.119	0.090	0.062
Cumulative	0.262	0.415	0.534	0.624	0.686

PC: Principle components

5.1.2.4.2 Geographical location (country/region) based PCA

Variances of 45.6%, 18.5%, 16.1% and 10.8% were extracted from the first four PCs with Eigen values greater than one, and 91.0% of the total variance was explained by these components (Table 19). Ear weight, finger width, lodging index, total tiller number, productive tiller number and finger length were the major contributors for the variation in the first principal component. Days to heading, harvest index, grain yield per plant and days to maturity for the second; finger number per main ear, number of grain per spikelet, plant height and days to heading for the

third; plant height, culm diameter, grain yield per plant, harvest index and days to maturity for the fourth were the major traits contributing for the variation observed in the respective principal components (Table 19). Reddy et al. (2009) reported that the basal tiller number, finger length, finger width and finger number per ear are some of the most important traits in the evaluation of East African finger millet germplasm and thus they were the major traits contributing to the 95.1% variation recorded in the first three PCA.

Table 19. Principal component analysis for 17 quantitative traits of finger millet based on geographical location and agro-ecologies of germplasm source

Variables	Country/regional based				Altitude based			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
Days to 50% heading	0.097	0.435	-0.331	0.006	0.268	0.192	0.298	0.068
Days to 50% maturity	0.135	0.386	-0.062	-0.365	0.251	0.214	0.175	0.304
Total No. of tillers	-0.340	0.069	0.081	-0.028	-0.268	0.174	-0.297	-0.097
No. of productive tillers	-0.340	0.068	0.087	-0.017	-0.267	0.173	-0.292	-0.121
Plant height	0.008	0.019	-0.391	-0.442	-0.285	-0.166	0.098	0.192
Finger length	-0.320	0.003	0.006	-0.294	-0.303	0.012	0.171	0.076
Finger number	-0.071	-0.140	-0.523	0.066	-0.231	-0.027	0.487	0.132
Culm branch	-0.249	-0.244	-0.275	-0.204	-0.308	-0.101	0.107	0.023
Ear weight	0.350	-0.005	0.014	-0.103	0.308	-0.104	-0.000	-0.071
No. of grains per spikelet	0.172	0.091	0.396	-0.041	0.097	-0.124	0.257	-0.799
Culm diameter	0.265	-0.101	-0.086	-0.397	0.029	-0.535	0.025	0.303
Finger width	0.344	-0.125	-0.017	-0.062	0.231	-0.194	-0.179	0.248
Lodging	-0.331	-0.123	0.114	-0.119	-0.287	0.169	0.023	0.040
Harvest index	-0.019	-0.418	-0.253	0.361	-0.181	-0.287	0.255	-0.066
Thousand grain weight	0.197	-0.369	0.249	-0.116	-0.026	-0.402	-0.482	-0.077
Grain yield per plant	-0.039	-0.420	0.151	-0.435	-0.274	-0.278	0.100	-0.048
Biomass weight per plant	0.284	-0.191	-0.212	0.129	0.228	-0.344	0.115	-0.088
Eigen value	7.750	3.142	2.744	1.831	9.904	2.877	1.793	1.154
Proportion (%)	45.6	18.5	16.10	10.8	58.3	16.9	10.5	6.8
Cumulative (%)	45.6	64.10	80.20	91.0	58.3	75.2	85.7	92.5

PC: Principle components

The relative positions of the regions of origin and the improved varieties on the first two principal axes (Fig. 11) clearly reflected the hierarchical clustering affinities and the different geographical locations of the germplasm source and released varieties have been scattered across all four quadrants (Fig 11).

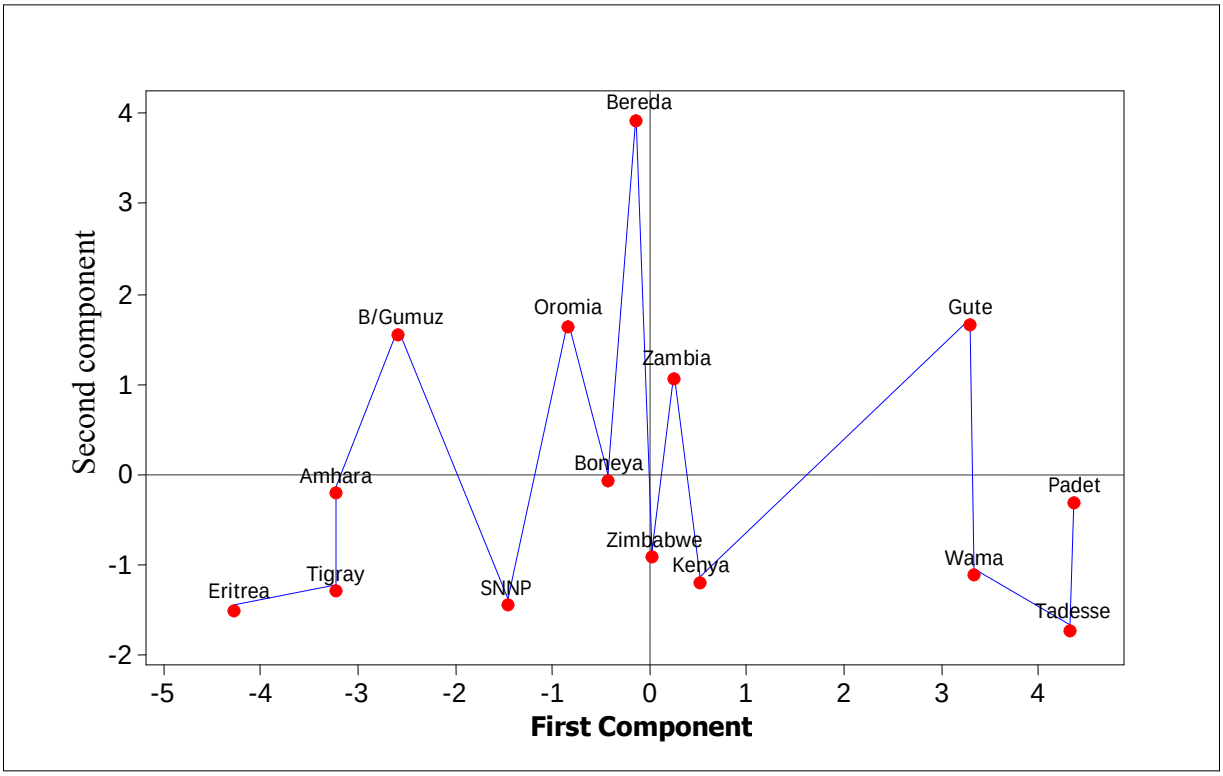


Fig 11. The relative position of countries/regions of origin and released varieties on the first two Principal Component

5.1.2.4.3 Clinal/altitudinal based PCA

With regard to altitude classes, about 92.5% of the total observed variance was recorded by the first four PCs with Eigen values greater than one (Table 19). The first PC accounted for 58.3% of the total variation and this was mainly due to ear weight, culm branch per plant and finger length as the major input. Culm diameter, thousand grain weight and biomass weight per plant was the major contributor for the variation observed in the second PC. Finger number per main ear and thousand grain weights were the major inputs for the variation in the third component.

Likewise, number of grains per spikelet, culm diameter and days to maturity for the fourth component. The eight altitude classes were distributed among the four quadrants and their relative positions on the principal axis partly confirmed the clustering result of altitude zones (Fig 12).

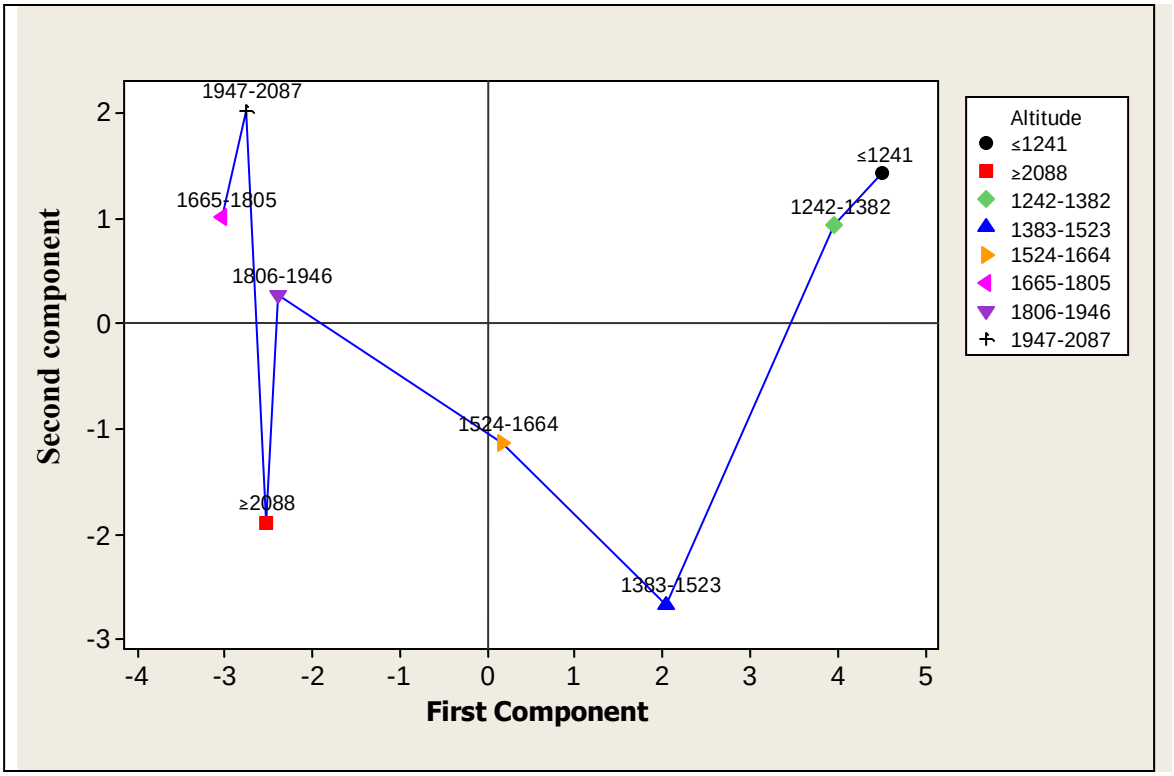


Fig 12. The relative position of eight altitude classes of origin on the first two Principal Component coordinates

In general, the PCA indicated that all polygenic traits considered in the present study contributed to a varying degree to the overall morphological variability observed among the tested finger millet accessions, and among regions and altitude classes of origin.

Based on the mean of the first two PCs of the three Eigen vectors (accession level, country/regional and altitudinal level), grain yield per plant, thousand grain weight, days to

heading, days to maturity, lodging index and biomass weight per plant were the most important traits contributing to the overall variability observed among accessions, geographical locations and agro-ecologies. This is an important backup option for breeder to investigate high yielding (both in food and feed aspect), agro-ecology and climate condition (rainfall) based maturity groups and lodging resistant varieties through conventional breeding. Several authors indicated that different phenotypic traits for different crops contributed to the overall variability observed between study germplasm and eco-geographical zones of origin (Lule *et al*., 2011 and Assefa *et al.*, 2003-for tef; Negash *et al*., 2005-for linseed; Ayana and Bekele, 1998-for sorghum and Quendeba *et al*., 1995-for finger millet).

5.1.2.5 Phenotypic and Genotypic Coefficient of Variation (GCV)

PCV and GCV values below 10%, 10%-20% and above 20% were considered to be low, intermediate and high, respectively (Khorgade *et al.,* 1985). The highest PCV values were noted for lodging index (44.5%), finger length (43.5%), grain yield per plant (33.85%), total number of tillers per plant (30.9%), productive tillers per plant (30.5%) and finger number (24.35%) (Table 20). Intermediate PCV values were observed for plant height (16.13), finger number per main ear (15.23), number of grains per spikelet (11.78), culm diameter (13.18), finger width (17.9) and thousand grain weights (19.16). However, days to 50% heading and days to 50% maturity exhibited very low PCV values, 3.003 and 9.153%, respectively. In line with the present study, Sharathbabu *et al.* (2008) evaluated 19 white seeded finger millet genotypes and three controls and reported very low PCV (6.46 and 8.34%) for days to maturity and days to 50% heading, respectively. Ganapathy *et al. (*2011) evaluated 230 finger millet varieties and reported low PCV and GCV value for the days to maturity. Assefa *et al*. (2001) also reported that PCV was lowest for days to maturity for 120 tef lines.

Estimates of GCV were lowest for days to heading, days to maturity, number of grains per spikelet, culm diameter and finger width, indicating that selection is not effective for those traits. Intermediate GCV values were observed for plant height, total number of tillers per plant, productive tillers number per plant, finger number per main ear and thousand grain weights. Comparatively high GCV values were recorded for finger length, ear weight, grain yield per plant and lodging index, indicating that the genetic component contributed the lion's share to the variability observed between accessions. Similarly, Prabhakar and Prasad (1984) observed high GCVs for number of fingers per ear, finger length and grain yield per plant. Most of the quantitative traits considered in this study had medium to high GCV values, additional evidence of the presence of genetic variability to be exploited for crop improvement as discussed in section 5.1.2.1.

Lower GCV values for days to heading and days to maturity were reported for finger millet (Sharathbabu *et al.,* 2008 and Bezawuletaw *et al*., 2006) and for tef (Lule *et al.,* 2008; Assefa *et al*., 2001 and Hundera *et al.*, 1999). This might have been ascribed to the phenotypic plasticity of these traits as the main source of variation, rather than genetic variance (Guillen *et al*., 1999).

The highest genotype × environment interaction coefficient of variation (G × ECV) was obtained for lodging index (37.9%), total number of tillers per plant (33.8%), productive tiller number per plant (33.7%) and grain yield per plant (26.44%). Those phenotypic traits also showed higher GCV values, indicating the possibility of improving crop varieties for such traits through conventional breeding. The G x ECV values for most of the traits considered in this study was found to be less than the GCV values.

5.1.2.6 Broad sense heritability (H^2) and Genetic advance

Heritability and genetic advance are important factors that determine the success of selection in breeding programs. Pandey and Tawari (1983) documented the importance of estimating heritability to predict the inheritance of quantitative traits as it indicates the genetic gains that is possible through selection. Genetic advance provides a prior quantitative estimate of the magnitude of the progress that can be achieved through selection (Panes, 1957).

Estimates of heritability (H^2) ranged from 17.95% for culm diameter to 83.78% for finger length (Table 20). Hence, the highest heritability estimates were observed for finger length (83.78%), days to heading (83.75%) and ear weight (79.51%). Similarly, in 19 white-seeded finger millet accessions evaluated by Sharathbabu *et al.* (2008) recorded maximum heritability for finger length (98.00%). About 64.3% of the traits considered in the present study showed greater than 50% heritability. Genetic advance was lowest for days to maturity (3.12%) and highest for ear weight (71.14%) (Table 20).

Higher heritability and genetic advance were recorded for ear weight, lodging index, finger length, thousand-grain weight and grain yield per plant. This indicated the predominance of additive gene effects controlling these traits and therefore early and simple selection strategies can be used to exploit them. Grain yield is largely dependent on those major traits and thus the result indicated that it may be possible to improve finger millet for both yielding capacity and lodging resistance. High heritability coupled with high expected genetic gain could result from the high additive gene effect and therefore targeted selection for such traits will lead to yield improvement (Panes, 1957). Similarly, Ganapathy *et al.* (2011) reported high heritability

coupled with high genetic advance observed in finger length and seed yield per plant. John (2006) also reported high heritability coupled with high genetic advance for number of fingers per ear and ear weight.

Days to maturity, culm diameter, finger weight, number of grains per spikelet, days to heading, total tiller number and productive tiller number showed lower percentages of genetic advance. This implied that the variations in these traits were mostly environmental, leading to low expected genetic gains from selection. Contrary to this finding, Ganapathy *et al*. (2011) reported higher heritability and genetic advance for traits such as finger number per ear, days to 50% heading, plant height and productive tillers per plant. Overall, the results for heritability and genetic advance in the current study support the available genetic diversity within the finger millet accessions evaluated in this study and thus several traits can be improved through conventional breeding.

Table 20. Estimation of the different variance parameters, heritability and genetic advance for 14 major quantitative traits of 144 finger millet accessions and six released varieties

Traits	Mean	δ^2_g	δ^2_p	δ^2_e	δ^2_{gl}	GCV	ECV	GECV	PCV	H^2 (%)	GA	GA (%)
Days to 50% heading	97.01	66.04	78.85	46.83	2.21	8.38	7.05	1.53	9.15	83.75	15.29	15.76
Days to 50% maturity	157.30	11.28	22.32	13.01	15.56	2.14	2.29	2.51	3.00	50.56	4.91	3.12
Total tiller number	5.61	0.93	3.01	1.10	3.60	17.17	18.72	33.84	30.90	30.87	1.10	19.61
Productive tiller number	5.55	0.82	2.87	1.19	3.51	16.32	19.63	33.74	30.52	28.57	1.00	17.93
Plant height (cm)	68.75	92.25	122.9	35.58	43.59	13.97	8.68	9.60	16.13	75.04	17.11	24.88
Finger length	7.98	3.16	3.78	0.94	0.75	22.29	12.16	10.88	24.35	83.78	3.35	41.94
Finger number per ear	7.23	0.91	1.21	0.65	0.28	13.19	11.13	7.34	15.23	75.05	1.70	23.50
Ear weight (g)	2.65	1.06	1.33	0.74	0.18	38.81	32.40	15.85	43.52	79.51	1.89	71.14
Number of grains per spike	4.39	0.17	0.27	0.37	0.01	9.39	13.88	2.22	11.78	63.55	0.68	15.39
Culm diameter (cm)	2.37	0.02	0.10	0.27	0.02	5.58	22.05	6.47	13.18	17.95	0.12	4.86
Finger width (cm)	0.79	0.01	0.02	0.05	0.00	9.81	28.59	6.33	17.90	30.00	0.09	11.04
Thousand grain weight (g)	2.26	0.14	0.19	0.18	0.01	16.41	18.56	4.85	19.16	73.33	0.65	28.89
Grain yield per plant (g)	20.42	17.67	45.65	53.60	34.15	20.59	35.85	26.44	33.09	38.72	5.38	26.34
Lodging percentage	44.15	225.86	386.6	82.50	280.15	34.04	20.57	37.91	44.53	58.43	23.62	53.50

Key: $\delta^2_{g=}$ genotypic variation, δ^2_p=phenotypic variation, δ^2_e=environmental variance, $\delta^2_{gl=}$ genotype by location variance, GCV=genotypic coefficient of variation, ECV= environmental coefficient of variation, G *x* ECV=genotype by environment coefficient of variation, PCV=phenotypic coefficient of variation, H^2= heritability in broader sense, GA=genetic advance and GA (%) =genetic advance as percentage of mean

5.1.2.7 Pearson correlation coefficient

The result of analysis of phenotypic correlation coefficients based on the mean of the accessions and improved varieties evaluated in the present study showed positive association for 68.4% of the traits (Table 21). This might be due to the presence of common genetic elements that controlled the characters in the same direction. The observed significant positive correlation could be either due to the strong coupling linkage between the genes or was the result of pleiotropic genes that controlled these characters in the same direction (Kearsey and Pooni, 1996). Similarly, Amsalu (2001) found positive, highly significant correlations for 15 quantitative characters of 415 sorghum accessions collected from different regions of Ethiopia.

Finger length (0.33), tiller number (0.28), thousand grain weight (0.23) and finger number (0.21) exhibited significant ($P \leq 0.01$) positive correlation with grain yield, indicating that low yield could be improved through conventional crosses, a finding that was confirmed by Sharathbabu *et al,* (2008). Days to heading and days to maturity exhibited negative correlation with grain yield. Field observations showed that most of the late maturing genotypes had an open ear type, narrow finger width, few spikelets per centimeter of finger in the centre and fewer grains per spikelet. As tillering capacity increased, number of fertile florets per spikelet, finger width, ear weight and thousand grain weights decreased (Table 21). This was probably due to the depletion of available resources during profuse vegetative growth that resulted in biomass production at the expense of energy that should have been stored in the seeds. In addition, different dominant or pleiotrophic genes may control the character in different directions (Kearsey and Pooni, 1996). Negative association between days to maturity and grain yield were also reported for tef (Assefa *et al.,* 2001) and for linseed (Negash *et al*., 2005).

Even if it was not significant, number of grains per spikelet was negatively associated with tiller number, finger length, finger number and plant height. The wild relative *E coracana* subspp. *africana* is naturally characterized by high tiller number, higher finger number, taller finger length and plant height. The co-occurrence of the two sub species of *E. coracana* (*E. coracana* sub spp. *africana* and *E. cor*acana sub spp. *coracana*) in the same crop fields, for instance in Western and North Western Ethiopia (Tsehay, 2012) could have resulted in intermediate races through natural hybridization and gene flow and such races showed the characteristics of wild relatives such as fewer spikelets, longer but narrow fingers, taller plant heights, late maturity, high tillering capacity, lower seed number per spikelet and lower grain yield. The frequent and naturally occurring hybridization between cultivated *E. coracana* subsp. *coracana* and its wild relative *E. coracana* subsp. *africana* already produced many morphological intermediates races (De Wet *et al*., 1985; Phillips, 1995; Neves *et al*., 2005; Dida *et al*., 2008).

5.1.2.8 Path coefficient analysis

Path coefficient analyses showed direct and indirect effects of some morphological traits on grain yield per plant and are presented in Table 22. Lenka and Mishra (1973) proposed scales for path coefficients in rice with values 0.00 to 0.09 as negligible, 0.10 to 0.19 low, 0.20 to 0.29 moderate, 0.30 to 0.99 high and more than 1.00 as very high. Accordingly, productive tiller number revealed high and positive direct effects (0.356) and thousand grain weight showed a moderate direct effect (0.285) on grain yield (Table 22). This indicated that any improvement in those traits could increase grain yield. Similarly, Sharathbabu *et al.* (2008) reported that the number of productive tillers exhibited a positive direct effect on grain yield.

Days to heading (-0.386), days to maturity (-0.144), lodging index (-0.290) and plant height (-0.104) had a negative direct effect on grain yield. This indicated the likelihood that grain yield could be improved by focusing on medium maturing genotypes with optimum plant height that can resist lodging. In line with this study, Wolie and Dessalegn (2011) found that plant height, days to heading and days to maturity had a negative direct effect on grain yield. None of the traits considered in this study showed any effect on grain yield. Singh and Chaundhary (1985) suggested that an indirect effect seemed to be the cause of correlation and hence, these indirect causal factors (traits) should be considered simultaneously for selection. Even though not significant, finger length, finger number, ear weight, number of grains per spikelet and culm diameter exhibited positive direct effects on grain yield, confirming that an increase in those traits could possibly increase grain yield.

Table 21. Pearson correlation coefficient for major quantitative traits of 144 finger millet accessions and six improved varieties

Traits	DH	DM	TTN	PTN	PLHT	FL	FN	EW	NGPS	CD	FW	TGW	GYPPL
DH	1	0.60**	-0.05	-0.04	0.38**	-0.26**	-0.01	0.28**	0.08	0.16	0.09	-0.28**	-0.42**
DM		1	-0.16	-0.16	0.31**	-0.14	0.13	0.27**	-0.07	0.18*	0.04	-0.07	-0.14
TTN			1	0.99**	0.16	0.38**	0.03	-0.51**	-0.08	-0.31**	-0.33**	-0.33**	0.28**
PTN				1	0.17*	0.38**	0.03	-0.51**	-0.09	-0.31**	-0.34**	-0.33**	0.28**
PLHT					1	0.33**	0.43**	0.02	-0.08	0.31**	0.02	-0.11	0.13
FL						1	0.28**	-0.57**	-0.14	-0.14	-0.34**	-0.24**	0.33**
FN							1	-0.02	-0.13	0.10	-0.16	-0.23**	0.21**
EW								1	0.26**	0.48**	0.54**	0.43**	0.10
NGPS									1	0.14	0.03	0.15	0.04
CD										1	0.39**	0.33**	0.04
FW											1	0.33**	0.05
TGW												1	0.23**
GYPPL													1

KEY: DH=days to heading, DM= days to maturity, TTN=Total tiller number, PTN= productive tiller number, PLHT= plant height (cm), FL= finger length (cm), FN= finger number, EW=ear weight (g), NGPS=number of grain per spikelet, CD=culm diameter (cm), FW=finger width (cm), TGW=thousand grain weight (gram), GYPPL=grain yield per plant (gram), **= highly significant (P≤0.01), *= significant (P≤0.05)

Table 22. Estimates of direct (bold and diagonal) and indirect effect of major finger millet quantitative traits on grain yield per plant on the basis of phenotypic correlation.

Traits	DH	DM	PTN	PLHT	FL	FN	CB	EW	NGPS	CD	TGW	LODG
DH	**-0.386**	0.087	-0.015	-0.040	-0.007	-0.001	-0.012	0.038	0.009	0.006	-0.079	-0.126
DM	-0.232	**-0.144**	-0.056	-0.032	-0.004	0.022	-0.001	0.037	0.004	0.007	-0.021	-0.080
PTN	0.016	-0.023	**0.356**	-0.017	0.010	0.006	0.001	-0.070	-0.038	-0.012	-0.093	0.113
PLHT	-0.147	0.045	0.059	**-0.104**	0.009	0.074	0.009	0.003	-0.014	0.011	-0.031	0.031
FL	0.102	-0.020	0.137	-0.034	**0.027**	0.048	0.015	-0.077	-0.052	-0.005	-0.069	0.199
FN	0.003	0.018	0.012	-0.045	0.008	**0.171**	0.013	-0.003	-0.020	0.004	-0.065	0.074
CB	0.082	-0.002	0.009	-0.016	0.007	0.039	**0.056**	-0.030	-0.027	0.002	-0.003	0.064
EW	-0.109	0.039	-0.182	-0.002	-0.015	-0.004	-0.012	**0.136**	0.079	0.018	0.121	-0.197
NGPS	-0.027	0.005	-0.108	0.012	-0.011	-0.027	-0.012	0.085	**0.125**	0.011	0.094	-0.148
CD	-0.060	0.025	-0.112	-0.032	-0.004	0.018	0.004	0.066	0.036	**0.037**	0.095	-0.096
TGW	0.107	-0.011	-0.116	0.011	-0.007	-0.039	-0.001	0.058	0.041	0.012	**0.285**	-0.085
LODG	0.168	-0.040	0.139	-0.011	0.019	0.044	0.012	-0.093	-0.064	-0.012	-0.083	**-0.290**

KEY: DH=days to heading, DM= days to maturity, PTN= productive tiller number, PLHT= plant height, FL= finger length (cm), FN= finger number, CB=Culm branch, EW=ear weight (g), NGPS=number of grain per spikelet, CD=culm diameter (cm), TGW=thousand grain weight (gram), LODG= lodging index

5.1.2.9. 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 either positive or negative significant association with grain yield. Highly significant differences were observed between the means of the selected subsets of the top 10% of accessions and the base finger millet accessions or populations for all quantitative characters (Table 23). 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 23). The selected 10% best yielding accessions showed 53.77% difference from the base population. Comparison of the

10% best accessions with a nationally released variety (Taddesse) resulted in 30.75% yield advantage. Some among these best performing genotypes for grain yield that were superior to the checks were promoted to the next evaluation phase in multi-location trials.

Table 23. Comparison of the performance of the 10% best selected accessions for grain yield and other agronomic traits to that of the total study accessions/population

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

5.2 Genetic diversity analysis using microsatellite markers

5.2.1 The extent of genetic polymorphism and regional patterning

The 20 microsatellite markers used to study genetic diversity revealed a Polymorphic Information Content (PIC) ranging from 0.12 (UGEP 96) to 0.94 (UGEP 24) with an average of 0.57 per marker (Table 24). Microsatellite markers such as UGEP024, UGEP067, UGEP064 and UGEP066 revealed the highest polymorphism with 0.94, 0.90, 0.88 and 0.87, respectively, and amplified higher numbers of alleles and exhibited 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 24). Markers UGEP98 and UGEP20 were excluded from analysis and interpretation due to monomorphisim 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 differences among the examined finger millet accessions (Table 24). Lower average PIC values were reported in other studies of finger millet using SSRs (Panwar *et al*., 2010); RAPD (Bezawuletaw, 2011; Panwar *et al*., 2010; Das and Misra, 2010) and Cyt P450 gene (Panwar *et al*., 2010).

The number of alleles per locus varied from 2 (UGEP084) to 24 (UGEP024) and a total of 188 alleles were observed with an average of 9.89 alleles per locus. Similar results were reported by Gupta *et al*. (2010), Das and Misra (2010) and Bezawuletaw (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 not for UGEP96, UGEP110_1 and UGEP005_1 (Table 24). The high number of alleles per locus and total genetic diversity found in this study demonstrated the presence of genetic variation within the finger millets accessions.

Geographical regions/countries of collection showed significant disparity in contributing to the variation observed within the finger millet accessions studied. Although the number of accessions represented from the different regions and countries of collection were not proportional, the highest within population polymorphism was recorded for accessions from Oromia (21.10%) followed by Amhara (16.93%), Tigray (13.43%) and Zimbabwe (6.66%) (Table 25). Highly significant ($P \leq 0.01$) intra-region polymorphism was observed among

accessions from all regions/countries of origin except for Benishangul Gumuz (1.27%), SNNP (1.15%) and the improved varieties (0.87%) (Table 25). Previous studies reported that the degree of polymorphism depended on the sample size (Sharma *et al*., 2010), sampling strategy (Kong *et al*., 2011) and types of test material (He *et al*., 2011). Contradicting the postulation of sample size as a factor of polymorphism, Salimath *et al*. (1995) reported higher within species polymorphism exhibited by two accessions of *E. floccifolia* than the 16 accessions of *E. coracana* in a study conducted using ISSR, RAPD and RFLP markers. Dida *et al*. (2008) also reported higher variation within *E. africana* compared to *E. coracana* despite the fact that the *E. africana* sample size was considerably smaller.

5.2.2 Analysis of molecular variance (AMOVA)

Analysis of molecular variance allocated 69.52% of the total allelic variation within populations and 30.48% among populations of the regions (Table 25), in line with the studies of Tsehay (2012), Keneni *et al*. (2011), Sharma *et al.* (2010) and Garris *et al*. (2005) for *Eleusine* species, chick peas, sorghum and rice, respectively. This implies the presence of moderate gene flow among populations in different geographic locations.

Table 24: Summary of genetic parameters as revealed using 20 polymorphic SSR markers for finger millet accessions collected from different countries and regions

Marker	Maj.Allele Freq.	Allele No	Heterozygosity	PIC	P-Value
UGEP53	0.36	13.00	0.11	0.78	0.001
UGEP84	0.53	2.00	0.00	0.37	0.001
UGEP27	0.24	15.00	0.10	0.84	0.000
UGEP95	0.25	7.00	0.07	0.77	0.001
UGEP64	0.16	16.00	0.15	0.88	0.000
UGEP67	0.20	20.00	0.16	0.90	0.000
UGEP106	0.60	9.00	0.17	0.57	0.018
UGEP110_1	0.79	5.00	0.00	0.32	0.383
UGEP110_2	0.77	3.00	0.00	0.30	0.000
UGEP57	0.44	8.00	0.12	0.68	0.000
UGEP96	0.94	5.00	0.07	0.12	0.813
UGEP66	0.20	20.00	0.11	0.87	0.000
UGEP79	0.64	5.00	0.15	0.45	0.004
UGEP12	0.34	12.00	0.16	0.73	0.000
UGEP73	0.90	6.00	0.03	0.18	0.014
UGEP005_1	0.93	6.00	0.00	0.13	0.141
UGEP005_2	0.90	5.00	0.02	0.18	0.001
UGEP24	0.10	24.00	0.26	0.94	0.000
UGEP46	0.34	7.00	0.23	0.70	0.003
UGEP33	0.46	11.00	0.30	0.62	0.001
Mean	0.50	9.95	0.11	0.57	

Key: Maj.Allele Freq= major allele frequency, PIC=Polymorphic information content

Table 25. Analysis of Molecular Variance (AMOVA) showing percentage contribution for the variation observed within accessions of the same region and among accessions of the different regions of origin

Source	DF	Sum of squares	MS	Contribution for variation (%)	Variance	St.dev	F-calc	F-tab.
Amhara	30	97.521	3.251	16.93	3.32	1.822	9.93	1.459**
B/Gumuz	6	7.333	1.222	1.27	5.38	2.318	1.39	2.802
Eritrea	7	13.819	1.974	2.4	2.96	1.719	3.25	2.639**
Kenya	6	13.548	2.258	2.35	3.53	1.878	3.18	2.802**
Oromia	30	121.55	4.052	21.1	6.68	2.584	8.73	1.459**
Imp. varieties	4	5.033	1.258	0.87	9.24	3.040	0.93	2.379
SNNP region	5	6.65	1.330	1.15	6.73	2.593	1.26	2.214
Tigray	23	77.334	3.362	13.43	1.83	1.351	12.19	1.758**
Zambia	8	19.2	2.400	3.33	1.64	1.280	5.63	2.511**
Zimbabwe	13	38.384	2.953	6.66	0.01	0.080	13.78	1.783**
Within Pop. total	137	400.37	2.922	69.5	28.56	5.344	6.42	1.120**
Among Populations	9	175.573	19.508	30.48	61.52	7.844	7.86	2.407**

Key: DF = degrees of freedom, ** = highly significant, MS = mean square, st.dev = standard deviation, F-calc = F-calculated, F-tab = F-tabulated

5.2.3 Microsatellite data based neighbor joining

Weighted neighbor-joining based clustering grouped the 138 accessions into 3 major clusters from the main node (Fig 13) comprising, 16, 71 and 51 accessions in the first, second and third cluster, respectively. Regardless of their sample size, collections from all regional states and agro-ecologies of Ethiopia as well as the introduced accessions were distributed across the 3 clusters to varying degrees except for the absence of accessions from Benishangul Gumuz regional state and the released varieties in the third cluster (Fig 13).

Generally, finger millet accessions collected either from the same region of origin or from the same altitude class (agro-ecology) did not often group entirely together within a given major cluster or sub cluster. Similarly, Babu *et al*. (2007) reported that 32 finger millet accessions fingerprinted using 50 RAPD markers, were grouped into two major clusters and the grouping was independent of ecological similarity. Bezawuletaw (2011) also reported that a total of 64 finger millet accessions grouped into nine clusters with no clear-cut separation among accessions related to their origin.

Very close genetic similarities were observed between some of the accessions collected from countries that were geographically far apart. For example, Acc. 214993 of Zambia (collected from altitude of 1340 m.a.s.l) shared strong similarities with Acc. 216056 from Oromia (1600 m.a.s.l). One likely reason could be that a few materials were originally introduced from the same source (probably from the international research and genetic resource center for the crop such as ICRISAT). Eco-geographical proximity was also manifested in clustering of a few accessions. In this regard, Acc. BKFM0060 from Oromia (1852 m.a.s.l) showed strong similarity with Acc. 235782 from Amhara (1860 m.a.s.l) and Acc. 230104 from Eritrea (1800 m.a.s.l) with AAUFM-97 from Tigray (1896 m.a.s.l). Cross-boundary farmer-to-farmer seed exchange, migration and gene flow could be the possible reasons for this relatedness. Similar findings were reported for other crops such as chickpea (Keneni *et al*., 2011) and sorghum (Ayana and Bekele, 1998).

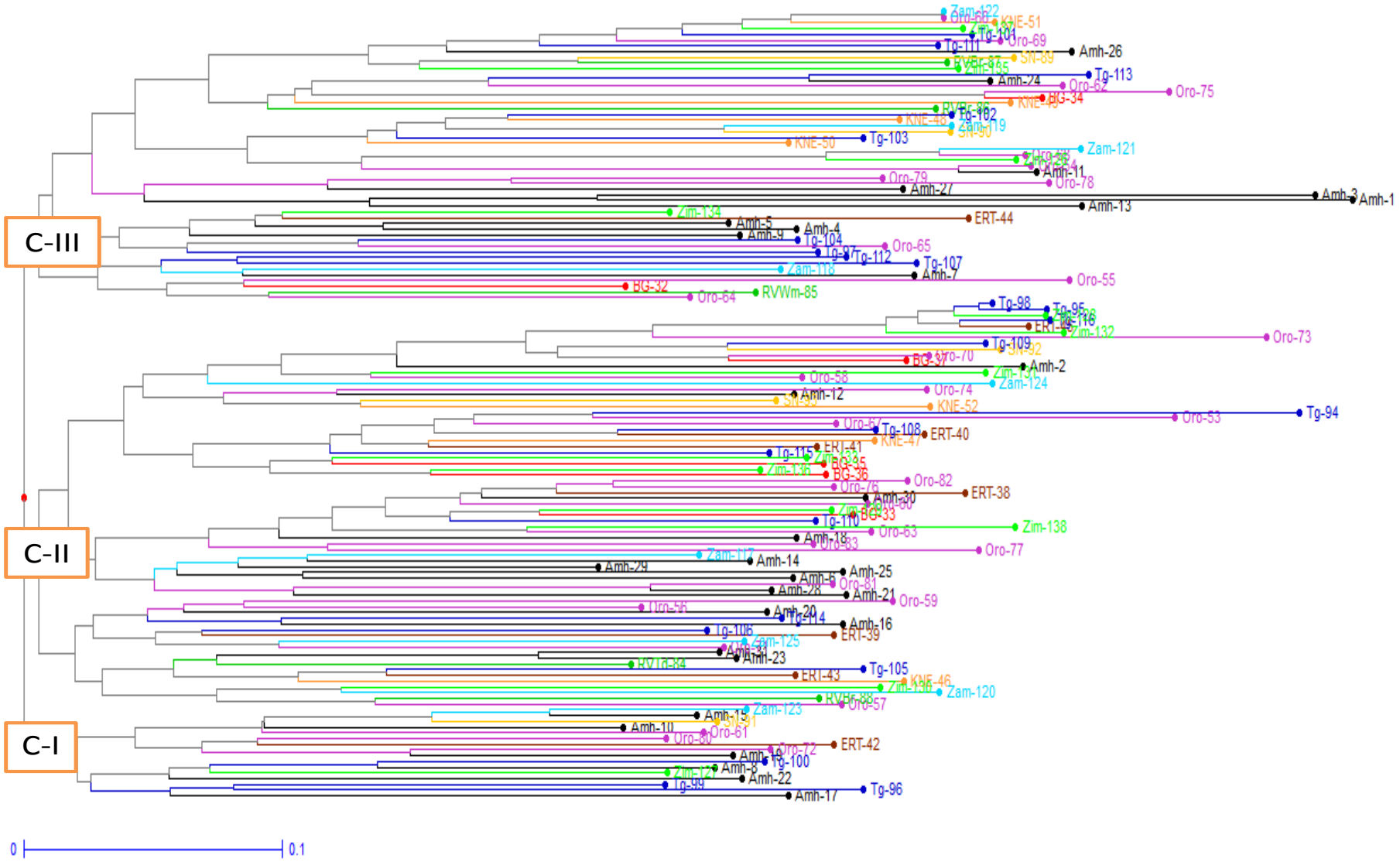


Fig 13. Weighted neighbor joining based clustering of 138 finger millet accessions for 19 polymorphic SSR markers. Key: Amh = Amhara, Oro = Oromia, Tg = Tigray, BG = Benishangul Gumuz, SN = Southern Nation Nationalities and peoples region, KNE = Kenya, Zam = Zambia, ERT = Eritrea, Zim = Zimbabwe. The number following each region/letter on the graph indicates F. millet accessions serial number sequentially as listed Table 3 under column SNJP

5.2.4 Population structure analysis

Analysis of population structure distinguished the 138 finger millet accessions into three subpopulation (groups) with the highest ΔK of around 300 (Fig 14). About 98% of the population displayed partial membership to multiple groups. Similarly, Vanniarajan *et al*. (2012) categorized 31 rice landraces along with 9 *indica* and *japonica* test cultivars into three major subpopulations. Kannan *et al*. (2014) reported four subpopulations (k=4) for 250 Full Sibs progenies of Pearl Millet. The 155 chick pea entries were grouped into five clusters (k=5) following analysis of population structure (Keneni *et al*. 2011).

In agreement to neighbor joining trees, analysis of population structure revealed that accessions collected from the same region of origin did not often grouped entirely together within a given major groups (Fig 15). For instance, among the 27 finger millet accessions having group-I as their largest component, finger millet populations collected from all regions and countries were aligned together in a varying degree except Zambian accessions. This group revealed the membership proportion of 22.9%, an average distances (expected heterozygosity) of 0.489 between individuals in same cluster (Table 26). The second and the third group were also comprised of accessions from the different geographical locations and the improved varieties with membership proportion of 38.5% and 38.6%, respectively. The fixation index (F_{ST}) values of the subpopulations ranged from 0.0723 (group-II) to 0.3322 (group-I), while the pair-wise allele frequency divergence values were maximum (0.1158) between the first subpopulation (group-I) and the third subpopulation (group-III) but minimum (0.053) between group-II and group-III (Table 26).

Table 26. Membership proportion, expected heterozygosity and fixation index of the three finger millet subpopulations

Sub-population or group	membership proportion	expected heterozygosity between individual in the same cluster	mean value of F_{ST}
1	0.229	0.489	0.3322
2	0.385	0.5936	0.0723
3	0.386	0.5397	0.1154

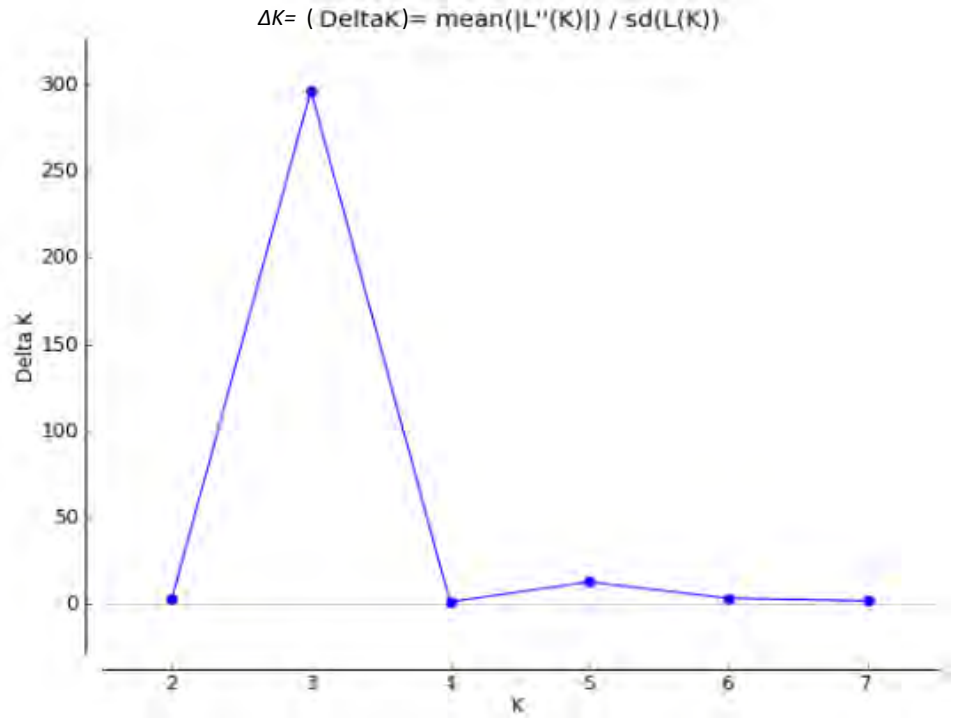
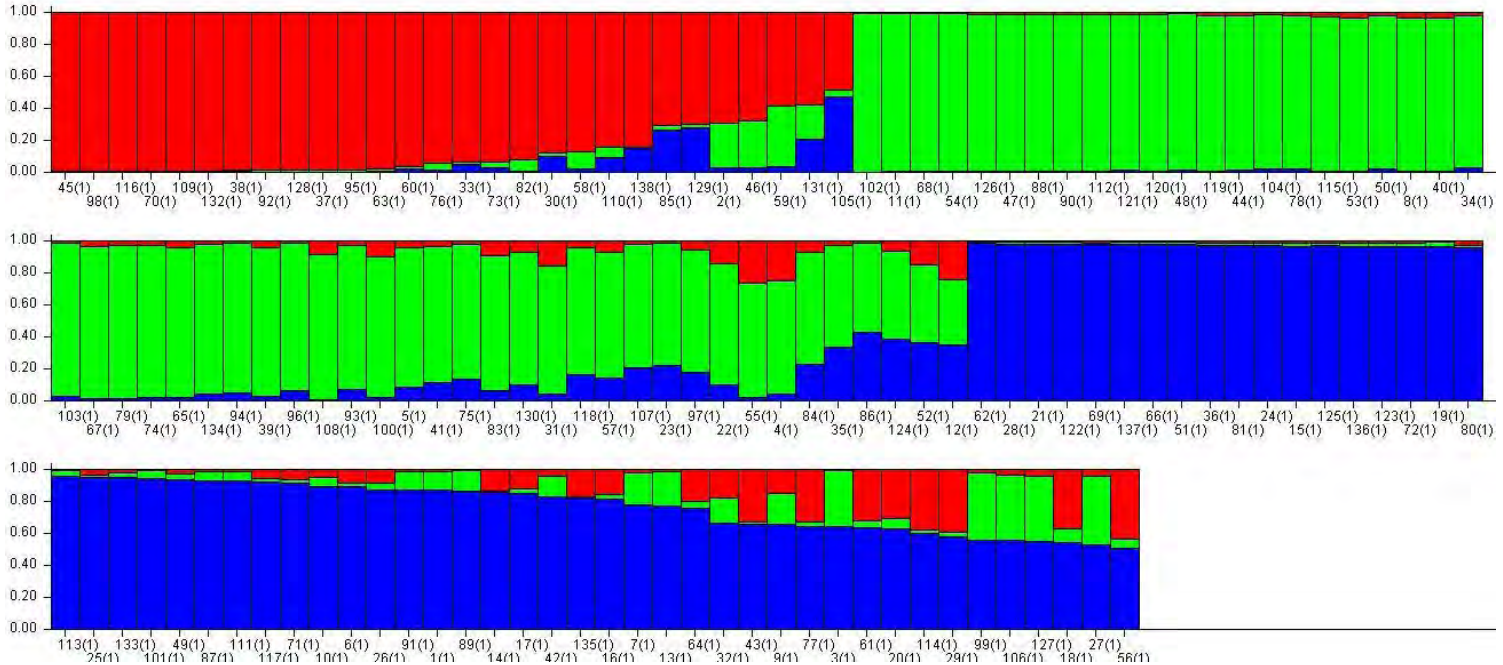


Figure 14: The number of groups (k) from the STRUCTURE runs. The plateau of the graph at *k* = 3 indicates the likelihood number of groups (subpopulations) possible in the panel.

(a)



(b)

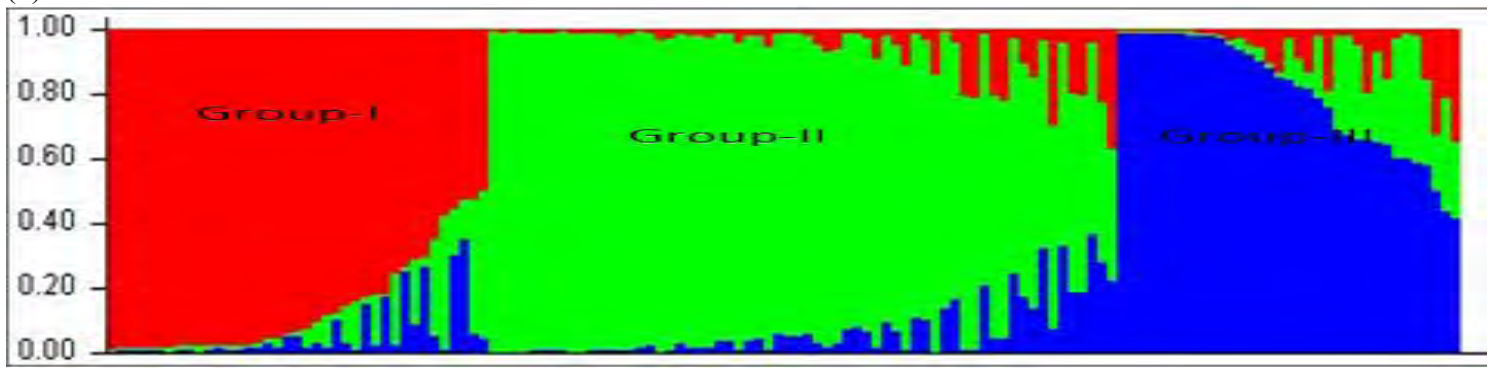


Figure 15: Estimated population structure of 138 finger millet genotypes plotted in a single line with population ID (a) and sorted by ancestor coefficient Q (b) for k =3. The y-axis is the group (subpopulation) membership, and the x-axis is the individual finger millet genotypes. The number under each line indicates finger millet accessions serial number sequentially as listed in Table 3 under column SNJ.

5.2.4 Estimation of Genetic distance

5.2.4.1 At population/accession level

The genetic distances between pairs of accessions were estimated using the shared allele distance method. Due to the unmanageable table size, the 10 most distantly related accessions were selected (genetic dissimilarity value ≥ 0.912 or 91.2%) and the 10 closest related accessions with genetic distances ≤ 0.092 (9.2%) (Table 27). Accordingly, a range of genetic distances was displayed between accessions, from as small as 0.001 between Acc. BKFM0024 (from Oromia) and Acc. 214987 (from Zambia), to complete divergence (1.00 or 100%) recorded between Acc. 242133 (from Amhara) and AAUFM-42 (from Tigray) (Table 27). This implied that, despite the presence of variable genetic diversity among the finger millet accessions studied, the SSR markers could distinguish between lines with minimum divergence (0.001) to complete dissimilarity (1.00). Likewise, high genetic distances were recorded between Acc. BKFM0060 of Oromia and Acc.242133 of Amhara region (0.997), Acc. 215985 of Amhara and AAUFM-42 of Tigray (0.995), BKFM0034 of Oromia and Acc. 242133 of Amhara (0.96) and others (Table 27), implying the possibility to create heterosis with likely substantial variation in resultant segregating progenies. Dida *et al*. (2007) also emphasized the potential for improvement of finger millet through the application of hybridization in breeding.

Some accessions seemed genetically similar (minimum genetic distance), such as BKFM0024 of Oromia and Acc. 214987 of Zambia (0.001 or only 1% dissimilarity), AAUFM-22 and AAUFM-33 (0.033) both from Tigray, Acc. 203354 of Zimbabwe and AAUFM-32 of Tigray (0.050), BKFM0042 of Oromia and Acc. 215982 of Amhara (0.059), AAUFM-11 of Tigray and Acc. 230103 of Eritrea (0.063) and others (Table 27). As discussed previously, there was no clear-cut genetic distance differentiation among finger millet accessions in relation to their

regions of origin. Therefore, some accessions from the same country showed wider genetic distance compared to some other accessions collected from geographically distant countries and vice versa. Furthermore, the accessions with very close genetic distances could be similar; an indication of germplasm exchange that occurred earlier through regional trial networks such as East Africa Regional Sorghum and Millet Network (EARSAN) coordinated by ICRISAT. Accessions form Tigray region of Ethiopia were genetically very close, and indication of farmer preference or same environmental adaptation.

Table 27. Numerical value of genetic distance (GD) for the 10 most distantly related and ten closest related finger millet accessions.

Accession with narrow genetic distance (<0.092)			Accessions with wider genetic distance (≥0.912)		
Accessions	GD value	Accessions	Accessions	GD value	Accessions
214987 (Zam)	0.001	BKFM0024 (Eth/Oro)	242133 (Eth/Amh)	1.00	AAUFM-42 (Eth/TG)
AAUFM-22 (Eth/TG)	0.033	AAUFM-33 (Eth/TG)	»	0.997	BKFM0060 (Eth/Oro)
AAUFM-32 (Eth/TG)	0.050	203354 (Zim)	215985 (Eth/Amh)	0.995	AAUFM-42 (Eth/TG)
215982 (Eth/Amh)	0.059	BKFM0042 (Eth/Oro)	242133 (Eth/Amh)	0.962	BKFM0034 (Eth/Oro)
AAUFM-11 (Eth/TG)	0.063	230103 (ERT)	215985 (Eth/Amh)	0.983	BKFM0060 (Eth/Oro)
AAUFM-22 (Eth/TG)	0.071	203354 (Zim)	»	0.947	BKFM0034 (Eth/Oro)
AAUFM-32 (Eth/TG)	0.072	230103 (ERT)	216056 (Eth/Oro)	0.939	AAUFM-42 (Eth/TG)
AAUFM-32 (Eth/TG)	0.080	AAUFM-11 (Eth/TG)	216056 (Eth/Oro)	0.926	BKFM0060 (Eth/Oro)
214987 (Zam)	0.088	BKFM0018 (Eth/Oro)	203355 (Zim)	0.918	242133 (Eth/Amh)
203354 (Zim)	0.092	230103(ERT)	242133 (Eth/Amh)	0.912	AAUFM-22 (Eth/TG)

Key: Eth/Amh = Amhara regional state of Ethiopia, Eth/Oro = Oromia regional state of Ethiopia, Eth/TG = Tigray regional state of Ethiopia, ERT = Eritrea, KNE = Kenya, Zam = Zambia, Zim = Zimbabwe

5.2.4.2 Geographical location based Rogers's genetic distance

The allelic frequency based genetic distance among regions of origin estimated following the Rogers (1972) approach indicated relatively narrow genetic distance between accessions from Amhara and Oromia regions (0.149) followed by between Oromia and Tigray regions (0.158) (Table 28). Wider genetic distances were recorded between Eritrean accessions and the improved varieties (0.33) followed by between Benishangul Gumuz and SNNP (0.297).

In general, the genetic distance assessment in the current study revealed the presence of wider genetic variability among some of the accessions or groups of accessions and the possibility to identify promising breeding materials through intensive characterization of a population or cultivars using multiple markers followed by multivariate and genetic distance analysis. Several authors reported the use and success of genetic distance analysis in selecting promising parental lines for different crops (African Yam - Adewale *et al*., 2010; wheat - Mostafa *et al*., 2011; bentgrass - Kubik *et al*. 2009; Sesame- Pham *et al*., 2011).

Table 28. Rogers's genetic distance among regions of origin and improved varieties.

Regions	Amhara	B/Gumuz	Eritrea	Kenya	Oromia	Imp. v	SNNP	Tigray	Zambia	Zim
Amhara	0.000	0.204	0.218	0.219	0.149	0.264	0.249	0.184	0.181	0.190
B/Gumuz		0.000	0.255	0.279	0.196	0.282	0.297	0.232	0.247	0.205
Eritrea			0.000	0.261	0.222	0.330	0.261	0.201	0.281	0.211
Kenya				0.000	0.211	0.275	0.234	0.207	0.209	0.226
Oromia					0.000	0.227	0.240	0.158	0.165	0.159
Imp. Var						0.000	0.278	0.266	0.223	0.255
SNNP							0.000	0.221	0.241	0.226
Tigray								0.000	0.220	0.175
Zambia									0.000	0.206
Zimbabwe										0.000

Key: Imp. var = improved varieties, B/Gummuz =Benishangul Gumuz, SNNP = Southern Nations Nationalities and peoples of Ethiopia, Zim=Zimbabwe

5.2.5. Overall Genetic diversity in finger millet and its implication from breeding and germplasms management perspectives

It is generally agreed that genetically diverse parents will exhibit maximum heterosis and offer the best chance of isolating transgressive segregants (Dje *et al*., 2000). As observed from the classic F-statistics, genetic distance and cluster analysis, large phenotypic and genetic variations were observed between some of the tested accessions included in the present study. Interestingly, such accessions existed within and between the different regions and countries (Table 26, Fig 13, Fig 15). Based on allelic frequency, Acc. 242133 was very distantly related to some of the test accessions. Phenotypically, this accession had lower 1000 grain weight, highest number of productive tillers per plant and least ear weight of all. However, improved varieties such as Taddesse and Wama showed higher ear weight and high 1000 grain weight but fewer productive tillers per plant and fingers per main ear. Crossing these two groups could possibly result in several recombinants with collective quality parameters from either parent.

Similarly, estimated genetic distance for Acc. 242133 and Acc. 214988 was 0.91 (Table 26). Acc. 214988 had short plant heights, relatively high ear weight and finger width. Therefore, those two accessions were diverse in both genetic and morphological aspects and hence good parental lines for crossing. Likewise, utilization of accessions for mass or pure line selection, hybridization or pedigree selection from distantly related clusters with diverse functional traits as observed from hierarchical clustering (Figs 8, 9, 10). Accessions with wider allelic dissimilarity as revealed by neighbor joining and structure analysis (Figs 13, 15), and genetic distance analysis (Tables 26, 27) could be potential candidate varieties with broad genetic bases. Similar results were reported by Keneni *et al*. (2011), Mostafa *et al*. (2011) and Kubik *et al*. (2009) for different crops.

Accession 215802 had tall plants (103cm) and Acc. 214991 is the shortest plants (41cm). The former was genetically grouped in the first cluster and the latter in the third cluster (Fig 13). Those accessions could be the best parental lines for producing medium sized cultivars to combat yield loss due to lodging. Moreover, Acc. 214987 was among the top blast disease tolerant (Lule *et al*., 2013) but a low yielder, whereas AAUFM-21 was among high yielding accessions (33.7g/plant) but moderately infected by leaf blast. Acc 214987 was allied in the third major cluster but AAUFM-21 was in the first major cluster (Fig 13). Therefore, those two accessions were genetically and phenotypically diverse and hence could be used as parental lines to produce high yielding and blast disease tolerant progenies. Similarly, Acc. 230101 was early maturing (145 days) and acc BKFM0018 late maturing (167 days). The former was grouped in the first cluster and the latter in the third cluster (Fig 13). Crossing these two genotypes could possibly produce segregants with variable maturity periods.

During efforts to exploit the genetic diversity to attain maximum heterosis or higher heritability and genetic advance in the subsequent generations, care should be taken to increase the number of test accessions or parental lines at the start of screening for desirable traits with a collection from wider eco-geographical regions, possibly among countries or otherwise within country. Even if variation existed within regions or within agro-ecologies for quantitative traits, qualitative traits and or allelic traits; localizing the collection of breeding materials to only a few administrative zones or regions would likely result in a lower probability of capturing such wider inter and intra population variation to successfully continue with a hybridization program or selection of lines with traits of improved heritability and genetic advance.

Path coefficient analysis for the present finger millet accessions showed negative direct effect of plant height and lodging index on grain yield per plant. To this end, crossing of a genetically diverse pair of accessions from C-II and C-VII (Fig 8), that also have better yield and other related traits, could produce heterotic progeny particularly for plant height to combat yield reduction caused by lodging. It should also be noted that, due to the gradual change in rain fall distribution, particularly in the short rainy season, late maturing finger millet accessions included in the current study produced less grain yield. Besides, due to severe blast disease infection, some extremely early maturing accessions also yielded poorly (Lule *et al*., 2013). These two critical issues emphasized the need to generate early maturing but high yielding and blast resistant/tolerant variety(s) or medium maturing, high yielding and blast resistant/tolerant variety (s). In this regard, crosses between selected late maturing accessions in C-II (Fig. 8) with early maturing accessions such as Acc. 230103 and Acc. 241768 could possibly result in a wider range of segregating progenies, fulfilling multiple quality parameters. Finger length, finger number, 1000-grain weight and productive tiller numbers showed positive significant associations with grain yield per plant (Table 21). Therefore, genetic recombination of accessions/varieties in C-VI with accessions in C-VII (Fig. 8) or other accessions with desirable traits may result in segregating progenies with better yield potential.

Phenotypic traits and genotypic (SSR) data based clustering moderately disagreed in how it grouped accessions according to eco-geographical patterning. The discrepancies may be due to the discrete nature of DNA-based SSR data compared to the continuous variable nature of the morphological data (Wrigley *et al*., 1982, Aremu 2012), or the variation that was detected by molecular markers might not be adaptive and therefore not affected by selection and environmental conditions. On the other hand, due to the effect of morphological plasticity,

genetically similar genotypes could show different phenotypic characteristics according to the varying environmental conditions and *vice versa*. Similar results were reported by Karuri *et al*. (2010) for sweet potato, Vieira *et al*. (2007) for bread wheat, Jian-Feng *et al*. (2013) for *Lappula* Moench and Wrigley *et al*. (1982) for Triticale.

5.3 Screening for blast (Magnaporthe oryzea) *disease resistance*

5.3.1 Analysis of variance

Mean squares due to genotypes were highly significant ($P \leq 0.01$) for leaf blast relative area under disease development curve (LBRAUDPC), head blast relative area under disease development curve (HBRAUDPC), neck blast incidence, lesion length and grain yield per plant (Table 29). Besides, significant variations were observed among finger millet accessions for leaf and head blast incidence recorded during the whole assessment period except for head blast at 147 days after planting (DAP). This result implied the available opportunity to generate resistant/tolerant cultivars for further utilization in breeding programs. Similarly, significant variations among finger millet accessions for blast disease, grain yield and other agronomic traits were reported by Prasado *et al*. (1994); Muyonga *et al*. (2000) and, Tsehaye and Kebebew (2002).

Table 29. Mean squares for blast incidence and severity recorded at different assessment periods from different plant tissues and grain yield per plant.

Source of variation	df	Leaf blast incidence - days after planting (DAP)					Head blast incidence (DAP)		
		88	102	117	132	147	102	117	132
Block	1	4795.5**	23995.**	5760.2**	24053.5**	364.5**	624.22	22022.01**	893.24
Genotype	224	277.71**	881.32**	637.46*	440.38**	67.36**	2897.1**	1480.49**	1059.1*
CV (%)		29.03	25.59	24.81	16.83	5.15	28.56	26.61	23.11
Mean		13.22	56.88	63.91	76.86	93.92	45.31	73.33	89.77

Table 29. Continued ….

Source of variation	df	147	SHBDI	NBINC	LBRAUDPC	HBRAUDPC	LL(cm)	GY (g/plant)
BLOCK	1	338.0**	180.3	2572.8**	1298.87**	1765.53**	7.45*	23.76**
Genotype	224	58.93	62.28	800.30**	126.51**	598.27**	2.69**	8.09**
CV (%)		7.07	25.4	25.7	10.52	14.76	21.1	20.03
Mean		98.33	29.54	91.19	54.67	77.93	5.89	11.29

KEY: df = degree of freedom, SHBDI = sheath blast disease index, NBINC = neck blast incidence, LBRAUDPC = leaf blast relative area under disease progress curve, HBRAUDPC = head blast relative area under disease progress curve, LL = Lesion length, Gy = grain yield (gram per plant), ** = highly significant (P≤0.01), * = significant (P≤0.05)

5.3.2 Pathogenicity and epidemiological patterns of disease infection

Blast infectivity showed wider variation among accessions of various regions of collection and the improved varieties at early development stage, but the range gets narrow at later recording periods (Fig 16). The infection rate progressed consistently throughout the growing period for the majority of accessions collected from the different corners of the countries (Fig 16) except that accessions from Oromia, Benishangul Gumuz, Zambia, the improved variety Bereda and the advanced line PW-001-022 showed little infectivity at early heading stage (Fig 16). The

minimum infections observed might be attributed to the late maturing characters of some of the accessions in the group.

The leaf blast AUDPC ranged from 1995.37% - days (LBRAUDPC = 33.26%) for Acc. 242644 of Ethiopia (Amhara at1815 m.a.s.l) to 4309.72%-days (LBRAUDPC = 71.81%) for Acc 235700 of Ethiopia (SNNP at1530 m.a.s.l.). Sheath blast severity index ranged from 17.9% for Acc. 208447 of Amhara (collected from 1710 m.a.s.l.) to 54.94% for Acc. 238299 of Tigray (from 1940 m.a.s.l). Neck blast incidence ranged from 40% for Acc. BKFM0001 of B/Gumuz (1580 m.a.s.l.) to 100% for about 138 finger millet accessions collected from different regions/countries.

All aerial parts of the plant were affected by blast disease in wet and humid environments in 2011. However, several authors reported that the most severe yield loses occurred from panicle infection (Lule *et al*., 2014; Mgonja *et al*., 2007; Ekwamu, 1991; Kenneth and LeRoy, 1977). Genetic variation for disease resistance in natural populations is usually estimated by quantitative variation in visual symptoms (Parker, 1986; Burdon, 1995). Therefore, the total accessions were ranked based on relative head blast AUDPC value and it ranged from 20.33% (AUDPC = 915%-day) for Acc.BKFM0031 of Oromia (1368 m.a.s.l.) to 100% (AUDPC = 4500%-days) for 7 accessions, all collected from Northern Ethiopia (Table 30).

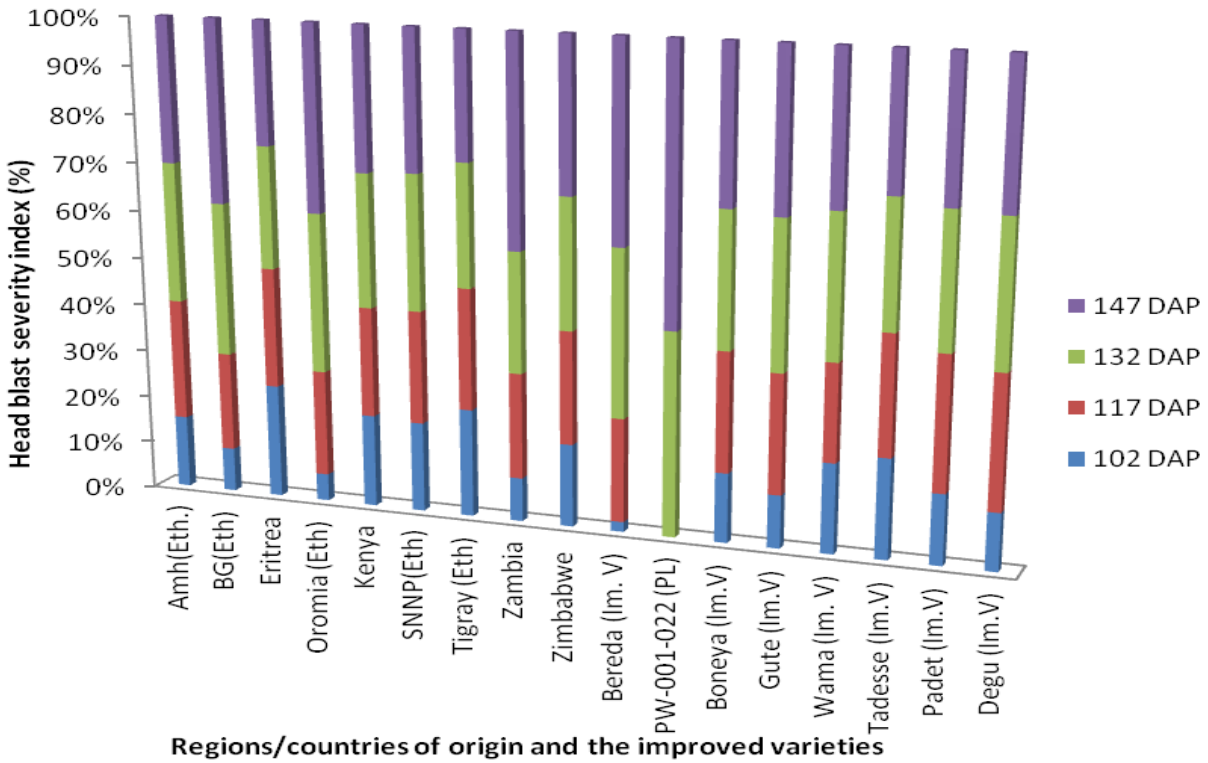


Fig 16. Patterns of head blast severity during the different assessment periods based on regional mean values.

There are different hypotheses for crop disease resistance mechanisms and the host-pathogen relationship. The first is that crop disease resistance could be the ability to reduce pathogen contact or reduce the pathogen growth rate once infection has occurred (Carrillo *et al*., 2012; Clarke, 1986; Burdon, 1995). This refers for quantification of disease traits than the grain yield. In this respect, Acc. BKFM0031 was the most resistant accession with minimum percentage disease infection but low grain yield. The second postulation is that resistance in crop varieties is usually evaluated by their relative yield with and without pathogen infection (Albar *et al*., 1998; Stout *et al*., 2001). In the present study, it was observed that among the 25 accessions least affected by head blast infection, 80% gave optimum grain yields above the average (11.29 g/plant) of the base population and 20% gave grain yield below the average (Table 30). On the contrary, 66% of the 25 most severely infected accessions gave grain yields below the average

(Table 30). This indicated that grain yield reduction in most of the accessions was due to the severe blast infection.

5.3.3. Host reaction to *M. oryzae*

Based on head blast disease severity index recorded in 2011, the total of 225 test materials was classified into five groups. Only one accession (Acc. BKFM0031) was resistant with a head blast severity score of < 3 at physiological maturity and head blast severity index and head blast RAUDPC values of less than 21%; 10 accessions were moderately resistant with blast infection ranging from 21-40%, 24 accessions were moderately susceptible with blast infection of 41-60%, 75 accessions were susceptible with infection of 61-80% and the remaining 115 accessions were highly susceptible with infection percentages of 81-100%.

Similarly, Gashaw *et al*. (2014) evaluated two improved finger millet varieties (Taddesse and Boneya) and one local accession for blast reaction and none of them were resistant. Moreover, Mgonja *et al*. (2007) reported nine out of 95 finger millet germplasms were resistant to blast disease, while Thakur *et al*. (2009) evaluated 432 different pearl millet lines for blast resistance and found only 25 lines consistently resistant to the disease. Jain *et al.* (1991) also reported that out of 18 genotypes of foxtail millet assessed under natural infection conditions, eight showed stable resistance to leaf blast throughout the assessment periods.

Finger millet accessions collected from the same region showed different levels of disease reaction, implying that diversified finger millet accessions are produced within country or region. Accession BKFM0031 was the most resistant of all, but gave low grain yield per plant.

This emphasizes the need to further confirm its consistence in dipping the pathogen and employ it as a parental line in crossing programs. Similarly, Anon (1959) reported that a poor yielding variety MO 359 from Mozambique; remained immune and hence was a possible parent for breeding of disease resistant varieties. On the contrary, Muyonga *et al*. (2000) reported that among the five different varieties evaluated for blast resistance, the highest grain yield was recorded from the most resistant variety and least grain yield was recorded from the most susceptible variety.

Regardless of the infection level, Acc. 238308 and Acc.AAUFM-35 both with relative head blast AUDPC of 100% and both collected from Tigray Regional State of Ethiopia tolerated serious disease infection and yielded well (13.08g/plant and 16.86 g/plant, respectively). This revealed that these accessions were tolerant (they did not limit infection, but reduced or offset their fitness consequences) as described by Roy and Kirchner, (2000). Similarly, Carrillo *et al*. (2012) reported *Ascochyta* blight resistant accessions in *Pisum sativum* subspp. *sativum* showed lower colony establishment and lower lesion sizes than the susceptible accessions but that the reduced lesion sizes were associated with a higher frequency of epidermal cell death and protein cross-linking in resistant accessions.

Table 30. List of the top 10% least infected accessions (No.1-25) and most susceptible (No.26-50) out of 225 finger millet accessions ranked based on HBRAUDPC with their grain yield and other disease traits

No	Acc	Region	SHBDI	NBINC	LBRAUDPC	HBRAUDPC	GYPPL
1	BKFM 0031	Ethiopia/Oromia	34.72	60.00	45.35	20.33	6.78
2	214988	Zambia	32.63	50.00	49.61	31.67	11.32
3	214987	Zambia	22.22	70.00	41.36	35.50	13.90
4	BKFM0010	B/Gumuz	23.46	80.00	38.71	39.00	16.48
5	PW-001-022	Released	25.93	70.00	57.77	39.11	18.55
6	203356	Zimbabwe	23.46	70.00	51.13	39.56	15.31
7	BKFM0020	Ethiopia/Oromia	25.93	70.00	38.46	39.67	16.11
8	214995	Zambia	24.07	50.00	46.59	39.67	14.74
9	BKFM0029	Ethiopia/Oromia	27.78	70.00	51.15	39.78	9.19
10	214997	Zambia	25.31	70.00	54.59	40.00	15.85
11	216035	Ethiopia/Oromia	22.84	80.00	40.08	40.00	18.02
12	BKFM0024	Ethiopia/Oromia	24.69	90.00	44.27	45.50	12.25
13	BKFM0018	Ethiopia/Oromia	24.69	60.00	39.47	47.58	16.02
14	BKFM0063	Ethiopia/Oromia	28.40	100.00	50.42	49.50	23.05
15	216051	Ethiopia/Oromia	24.69	70.00	37.95	50.00	13.32
16	BKFM0042	Ethiopia/Oromia	29.01	100.00	59.66	50.83	17.05
17	BKFM0023	Ethiopia/Oromia	26.54	60.00	35.92	52.00	13.77
18	BKFM0001	B/Gumuz	20.37	40.00	37.93	54.17	10.26
19	216039	Ethiopia/Oromia	17.90	100.00	34.37	54.33	11.83
20	BKFM0009	B/Gumuz	25.31	80.00	51.66	55.00	18.11
21	BKFM0007	B/Gumuz	38.27	90.00	53.40	55.00	11.82
22	BKFM0038	Ethiopia/Oromia	41.36	80.00	60.86	55.42	9.06
23	215984	Amhara	40.74	100.00	57.31	55.92	11.20
24	BKFM0006	B/Gumuz	29.63	80.00	56.89	56.33	13.96
25	236446	Ethiopia/Oromia	27.16	100.00	49.07	56.67	11.68

Table 30. Continued

No	Acc	Region	SHBDI	NBINC	LBRAUDPC	HBRAUDPC	GYPPL
26	230101	Eritrea	24.07	100.00	60.51	95.83	10.73
27	215852	Amhara	37.04	100.00	61.23	95.83	8.41
28	242114	Amhara	27.78	100.00	53.73	96.67	10.66
29	AAUFM-050	Tigray	25.93	100.00	53.87	96.67	10.55
30	100002	Amhara	29.63	100.00	55.09	96.67	4.66
31	AAUFM-14	Tigray	27.16	100.00	59.17	96.67	11.18
32	237443	Amhara	25.31	100.00	59.61	96.67	2.74
33	203357	Zimbabwe	40.74	100.00	65.39	96.67	15.92
34	AAUFM-21	Tigray	25.93	100.00	51.90	97.50	11.89
35	242115	Amhara	30.25	100.00	57.16	97.50	13.20
36	237475	Tigray	33.95	100.00	61.63	97.50	9.77
37	AAUFM-15	Tigray	33.33	100.00	61.14	98.33	7.93
38	AAUFM-2	Tigray	27.16	100.00	61.54	98.33	8.08
39	AAUFM-32	Tigray	33.95	100.00	63.67	98.33	12.17
40	238299	Tigray	54.94	100.00	70.18	98.33	9.05
41	230105	Eritrea	27.78	100.00	58.36	99.17	4.35
42	230104	Eritrea	26.54	100.00	64.07	99.17	6.30
43	AAUFM-22	Tigray	31.48	100.00	69.38	99.17	6.79
44	AAUFM-23	Tigray	32.10	100.00	53.82	100.00	7.73
45	AAUFM-35	Tigray	30.25	100.00	55.31	100.00	16.86
46	238460	Tigray	28.40	100.00	61.62	100.00	4.08
47	238308	Tigray	35.19	100.00	62.47	100.00	13.08
48	242618	Tigray	33.95	100.00	64.97	100.00	4.12
49	AAUFM-44	Tigray	31.48	100.00	65.11	100.00	5.58
50	228202	Amhara	37.04	100.00	65.41	100.00	6.46

KEY: Acc=accession, LBRAUDPC= leaf blast relative area under disease progress curve, HBRAUDP= head blast relative area under disease progress curve, NBINC=Neck blast incidence, SHBDI= sheath blast severity index, GYPPL =grain yield per plant

5.3.4 Regional patterns of blast infectivity

Eighteen of the 10% least infected accessions were from western Ethiopia (13 from Oromia and five from Benishangul Gumuz), four from Zambia, one from Zimbabwe and one was the advanced line PW-001-022 (Table 30). However, among 25 extremely infected accessions, 21 were from Northern Ethiopia (15 from Tigray and six from Amhara), three from Eritrea and one from Zimbabwe (Table 30).

Generally, lower blast incidence and severity were recorded for some Western Ethiopian collections (particularly western Oromia) and Zambian accessions during the whole assessment period (Table 30). This could be either due to the genetic potential or due to their better adaptive reaction to the test environment and the pathogen or the effect of phenotypic plasticity. On the contrary, accessions from Northern Ethiopia, particularly Tigray and introduced accessions from Eritrea were highly infected by the disease (Table 30).

5.3.5 Regression and correlation analysis

Both correlation (r^2) and regression (r) analysis revealed highly significant ($P \leq 0.01$) negative association of head blast RAUDPC (r^2 =-0.321, r = -0.854) with grain yield. Neck blast showed highly significant ($P \leq 0.01$) negative correlation (r^2= -0.139) and significant ($P \leq 0.05$) negative regression (r = -0.213) to the grain yield (Table 31). Besides, leaf blast showed significant ($P \leq 0.05$) negative correlation (r^2= -0.117) and regression (-0.220) on grain yield per plant (Table 31). Association analysis also showed that infection was progressive throughout the plant growth stages. In other words, an infection on the leaves or neck contributed significantly to development of head infection (Table 32). Overall, the present study revealed that infections on

above ground parts of the plant severely affected grain yield, but most severe yield losses were recorded for head infection. Lule *et al*., 2014; Mgonja *et al*., 2007; Ekwamu, 1991 and, Kenneth and LeRoy (1977) reported similar results on finger millet.

Table 31. Regression and correlation analysis among blast parameter or traits (as independent) and grain yield per plant (as dependent) variable

Traits	NBINC	LBRAUDPC	HBRAUDPC	LL	GYPPL	Regression analysis	
						Estimate for intercept	Estimate for dept. variable
SHBDI	0.13	0.51**	0.24**	-0.14**	-0.07	30.57**	-0.09
NBINC		0.38**	0.41**	-0.16*	-0.139**	93.48**	-0.213*
LBRAUDPC			0.57**	-0.29**	-0.117*	57.02**	-0.220*
HBRAUDPC				0.06	-0.321**	92.59**	-0.854**
Lesion length					-0.19**	6.56**	-0.059

Key: LBRAUDPC = leaf blast relative area under disease progress curve, HBRAUDPC = head blast relative area under disease progress curve, NBINC = Neck blast incidence, SHBDI = sheath blast disease index, LL = lesion length, GYPPL = grain yield per plant, ** = highly significant (P≤0.01), * = significant (P≤0.05)

5.3.6 Yield loss assessment

Percentage yield loss due to the blast fungal pathogen estimated using HBRAUDPC recorded during the severe infection of 2011 cropping season ranged from 17.36% for Acc. BKFM0031 to 85.4% for Acc. AAUFM-23, AAUFM-35, AAUFM-44, Acc.228202, Acc. 238460, Acc.238460, Acc.238308 and Acc.242618 with an average of 66.10%. On the other hand, yield loss estimated using multiple point models during the same season ranged from 17.63% for Acc. BKFM0031 to 99.3% for AAUFM-23, AAUFM-35, AAUFM-44, Acc.228202, Acc. 238460, Acc.238460, Acc.238308, Acc.242618 and AAUFM-22 and Acc.230104 with an average of 82.30%. The trends in yield indicated that maximum loss was recorded at the early stage (102

DAP) followed by the second recording interval (from 102 DAP to 117 DAP) with reduced losses at subsequent recording intervals (Fig 17). This indicated that any control measure should be applied at the early stages of disease occurrence.

Following a statistical approach, an average yield loss of 41.15% was estimated using head blast data recorded in 2011, where yield of infected fingers were compared with yield of normal fingers on the same ear of the same size. In general, based on the mean of the three different yield loss assessment models, an average of 63.2% loss was recorded due to blast [*Magnaporthe oryzea*] disease during the highly humid crop growing season and the severe infection periods such as 2011.

The mean yield loss estimated for 12 cultivated finger millet and four of its wild relatives treated with four different fungal isolates ranged from 40.22% to 99.06% with an average of 73.32% based on HBRAUDPC approaches in 2011, but when estimated using multiple point models, it was much less at 9.8% for the 2012 cropping season (Lule *et al*., 2014). The possible reason for the variation in yield loss between the 2011 and 2012 seasons might be due to the differing weather conditions particularly rainfall shortage at the later growing stage in 2012, relative humidity and generally, variation in host-pathogen-environment interactions reduced the severity of the infection in 2012. Stetina *et al*. (2006) also reported that seasonal variations caused different yield losses in Soybean due to *Rhizoctonia* Foliar Blight. Other studies found average finger millet yield losses of 41.8% in Ethiopia (Gashaw *et al*., 2014); up to 90% in Eastern Africa (Mgonja *et al*., 2007) and up to 46% in Japan (Kato *et al*., 1977).

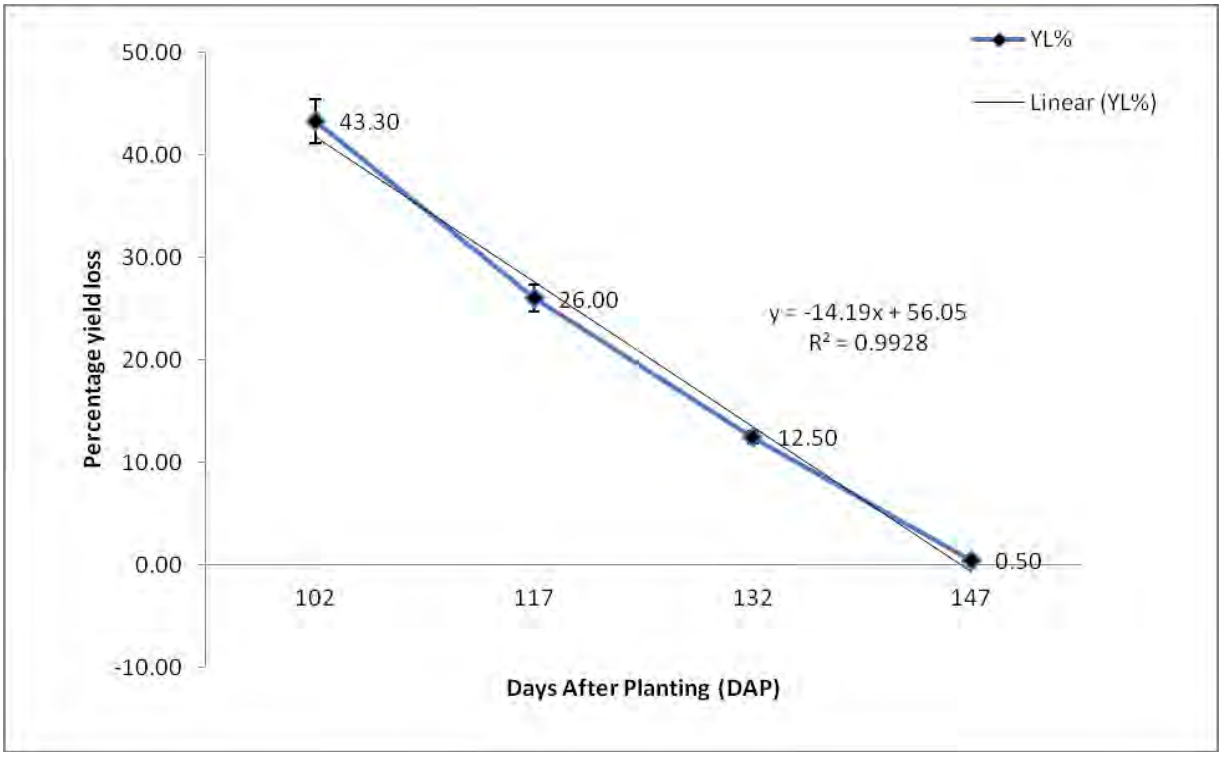


Fig 17. Percentage yield loss estimated at the different disease assessment periods and growth stages

5.3.7. Comparison of selected 10% accessions with the original study materials

The total of 225 study materials were compared to the 10% best performing accessions for blast disease traits and grain yield per plant and the Z-value indicated that there were highly significant differences (Table 32). The highest differences of 74.4% and 39.79% were observed for grain yield and HBRAUDPC, respectively (Table 32). The advanced line PW-001-022 was among the 10% most tolerant to blast disease (head and neck blast) and high yielding accessions. Subsequently, an additional nine of the 25 best performing accessions that were superior to PW-001-022 were promoted to the next evaluation phase at multiple locations.

Table 32. Comparison of the performance of the 10% best selected accessions for grain yield and other agronomic traits from the study population.

Parameters/ Traits	Mean of selected genotype	Population mean (μ)	Change due to selection	Change as % of population parameters	Z-calc
SBDI	22.15	29.55	7.40	25.04	6.64**
NBINC	58.80	92.19	33.39	36.22	8.46**
LBRAUDPC	38.95	54.67	15.72	28.75	9.88**
HBRAUDPC	46.67	77.51	30.84	39.79	9.54**
Grain Yield	19.69	11.29	8.40	74.40	9.23**

Z-calc =Z-calculated, SBDI = Sheath blast disease index, NBINC = neck blast incidence, LBRAUDPC = leaf blast relative area under disease progress curve, HBRAUDPC = head blast relative area under disease progress curve, Z-calc=Z-calculated

5.4 Genotype by Environment interactions and grain yield stability analysis

5.4.1 Analysis of variance

Analysis of variance (ANOVA) for the major quantitative traits of 30 finger millet genotypes and two released varieties showed highly significant ($P \leq 0.01$) variations among genotypes, environments, genotype by environment interaction (GEI) for days to heading, days to maturity, plant height, productive tiller number, ear length, finger number per main ear and grain yield per hectare (Table 33). The significant difference between genotypes for all quantitative traits indicated that they were properly selected from the preliminary trial to advanced multi location evaluations and provided a good basis for further breeding activities. Other authors also reported

that the mean square due to environment, genotypes and GEI were highly significant for major finger millet quantitative traits (Prasado *et al*., 1994; Tsehaye and Kebebew, 2002).

Table 33. Analysis of variance for major quantitative traits of finger millet

Source	DF	DH	DM	PLHT	PTN	EL	FN	GY (kg/ha)
Rep	2	23.61	10.17	83.53	22.44**	2.58*	1.21	130314.00
Loc	3	52870.1**	32545.2**	36085.1**	538.32**	152.21**	75.27**	197792577.6**
Trt	31	538.55**	193.69**	1721.7**	12.52**	114.18**	30.97**	1906605.7**
Loc*Trt	93	55.76**	94.14**	217.49**	6.74**	1.39**	1.83**	3484685.0**
Mean		101.35	158.66	67.40	5.94	7.00	7.10	2558.79
CV		3.61	2.66	11.09	29.38	12.56	13.96	19.01

Key: DF = degree of freedom, DH = days to 50% heading, DM = Days to 50% maturity, PLHT = Plant height, PTN = Productive tiller number, EL = ear length, FN = Finger number, GY = Grain yield, Rep = replication, Loc = location, Trt = treatment, CV = coefficient of variation

5.4.2 Additive Main Effects and Multiple Interaction (AMMI) model

Combined analysis of variance revealed highly significant ($P \leq 0.01$) variations among environments, GEI and IPCAs (Table 34). This result revealed that there was a differential yield performance among finger millet genotypes across testing environments and strong GEI. The result was in agreement with previous reports on wheat (Sial *et al*., 2000), rice (Panwar *et al*., 2008) and finger millet (Misra *et al*., 2009). Substantial percentage of GEI was explained by IPCA-I (66.05%) followed by IPCA-II (12.81%) and IPCA-III (9.46%) (Table 34). The remaining five IPCA were contributed only 11.67% GEI.

Because of their high proportional contribution to the GEI, the first two principal components (IPCA-I and IPCA-II) were used to create a 2-dimensional GGE biplot. Gauch and Zobel (1996)

suggested that the most accurate model for AMMI can be predicted by using the first two PCAs. Moreover, several authors took the first and second IPCA for GGE biplot analysis and, greater percentage of GEI were explained by the first IPCA for maize (Abera and Labuschagne, 2005), bread wheat (Yuksel *et al*., 2002; Farshadfar, 2008; Worku *et al*., 2013), common bean (Temesgen *et al.*, 2008), finger millet (Misra *et al*., 2009) and field pea (Mengistu *et al*., 2011).

Table 34. ANOVA for grain yield using AMMI model

Source	df	SS	MS	Eigenvalue	% G x E interaction	% cumulative interaction
Environments	7	1126.965	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

Acc. 203544 (G6) produced the best average yield (3.16 ton ha^{-1}) and attained an IPCA-I value relatively close to zero (-0.15) indicating that it was a stable and widely adaptable advanced line (Table 35, Fig 18). Genotypic stability was crucial in addition to grain yield (Naroui *et al*., 2013). Acc. 203362 (G30) attained the lowest IPCA-I score (-0.0002) and medium grain yield (2.85 ton ha^{-1}) (Table 35, Fig 18). Genotypes with below average yield, such as Acc. 242617, Taddesse, Acc. 229722 and Acc. BKFM0034 also showed IPCA-I nearing zero, indicating consistence in yield performance across locations. Acc. 242111 (3.08 ton ha^{-1}), BKFM0051 (3.07 ton ha^{-1}) and Acc. 229738 (2.99 ton ha^{-1}) yielded better than Gute (2.82 ton ha^{-1}) but

attained relatively high IPCA-I scores (0.70, 0.52 and 0.85, respectively) (Table 35, Fig 18), indicating inconsistent yield performance across years and location, but site specific adaptability for these accessions. Acc. BKFM0028 (G23) yielded the least grain (1.99 ton ha^{-1}) and attained the highest IPCA-I score (-1.06) implying that it was not adaptable and unstable (Fig 18, Table 35). Besides, Acc. 203546 (G17), Acc. 229722 (G4) and Acc. 230104 (G1) are among the low yielding genotypes (Table 35).

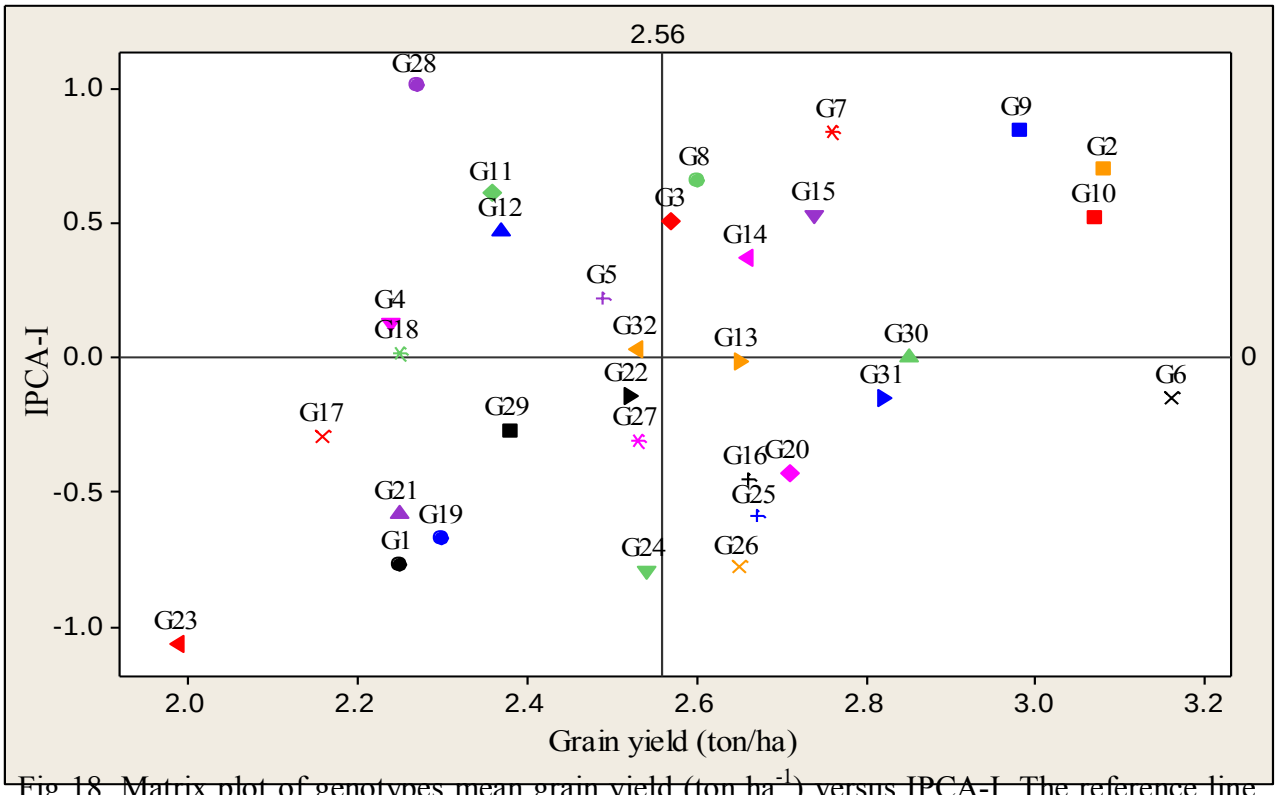


Fig 18. Matrix plot of genotypes mean grain yield (ton ha^{-1}) versus IPCA-I. The reference line on the x-axis is the average grain yield (2.56 ton ha^{-1}) and on the y-axis is the IPCA-I value indicating genotype stability (IPCA-I=0)

Except for Bako, all test environments exhibited fluctuating mean grain yields and IPCA scores during 2012 and 2013 (Fig 19). For instance, the overall mean grain yield at Arsi Negele during the 2012 crop growing season was 2.63 ton ha^{-1}, while the mean grain yield at the same location during the 2013 cropping season was 5.5 ton ha^{-1} (Fig 19, Table 35). The variation in weather conditions, experimental plots and other edaphic factors could be the possible reason.

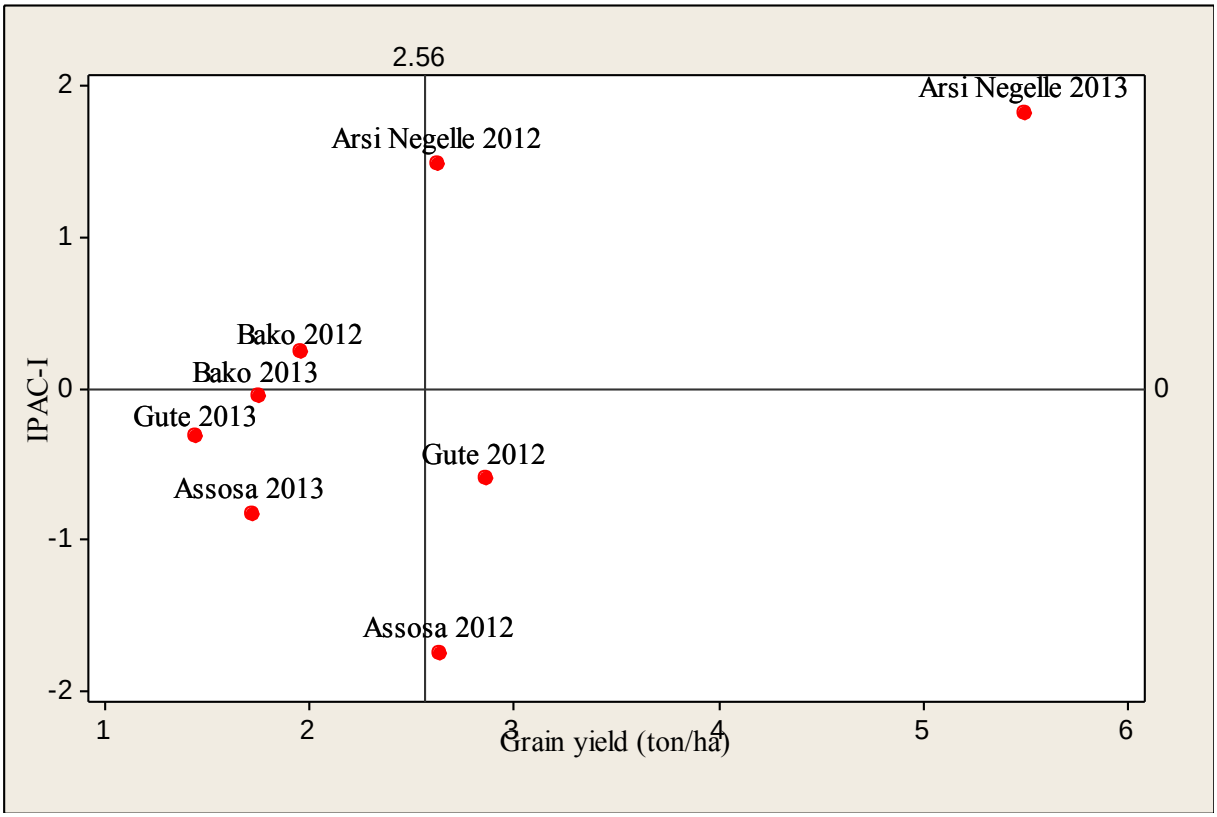


Fig 19. Matrix plot of Environment focused mean grain yield versus Interaction Principal Component Axis (IPCA-I) score. The reference line on the x-axis is at the average grain yield of genotypes across locations (2.56 ton ha^{-1}) and on the y-axis is the IPCA-I value indicating genotype stability (IPCA-I=0)

5.4.1.1 AMMI Stability Value (ASV)

AMMI Stability Value aids selection of relatively stable high yielding genotypes. An ideal genotype should have high mean grain yield and small ASV value. Accordingly, Acc. 203362 (G30), an introduced accession from Zimbabwe, showed the lowest ASV (0.266) and moderate grain yield (2.85 ton ha^{-1}). Furthermore, Acc. 203544 (G6), an introduced accession from Kenya, was the highest yielding genotype (3.16 ton ha^{-1}) with relatively lower ASV (1.18) (Table 35). This revealed that those accessions showed relatively better stability compared to the other accessions.

Nevertheless, stability needed to be considered in combination with yield (Farshadfar, 2008). Thus, Acc. 242111, Acc. BKFM0051 and Acc. 229738 yielded well (3.08 ton ha^{-1}, 3.07 ton $ha^{-1}$and 2.99 ton ha^{-1}, respectively) but high ASV (4.91, 3.66 and 5.97, respectively) and were identified as good genotypes to validate for yield performance and specific adaptability. ASV further confirmed that Acc. BKFM0028 was unstable and not adaptable and that Acc. 242617 and the released variety Taddesse were consistent low yielders across locations and years. Similarly, Odewale *et al*. (2013) evaluated five coconut varieties across nine environments and found two most stable varieties. Farshadfar (2008) evaluated twenty bread wheat genotypes for four years across two location and found that two genotypes were consistently stable as revealed by AMMI stability value and genotype selection index.

5.4.3 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 35, Fig 20). 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.

On the other hand, Acc. 230104 (G1), Acc. BKFM0028 (G23) and Acc. BKFM0042 (G24) gave grain yield below the average and a regression coefficient lower than one (0.58, 0.47 and 0.51, respectively) indicating that they were not stable, and adapted to low yielding environments (Table 35, Fig 20). Farshadfar (2008) reported similar results in bread wheat. The results from the Eberhart and Russell (1966) model for this study also confirmed those obtained from the AMMI model discussed above in section 5.4.2.

Table 35. 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	ASV	IPCA -1	IPCA -2
		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	5.35	-0.77	0.05
2	**242111***	**3.97**	**2.00**	**2.33**	**2.90**	**7.00**	**1.87**	**2.50**	**2.03**	**3.08**	**1.40**	**0.54**	**4.91**	**0.70**	**0.09**
3	203360*	3.43	1.90	1.57	2.10	6.63	1.93	2.23	0.76	2.57	1.30	0.45	3.52	0.51	0.28
4	229722*	2.47	1.57	1.77	2.67	5.20	1.47	0.99	1.77	2.24	0.96	0.18	0.95	0.13	-0.22
5	242120*	2.53	2.20	1.80	3.10	6.23	1.43	1.53	1.13	2.49	1.26	0.01	1.23	0.22	-0.08
6	**203544***	**3.30**	**3.43**	**2.03**	**4.73**	**5.50**	**2.07**	**2.27**	**2.00**	**3.16**	**1.08**	**0.42**	**1.18**	**-0.15**	**-0.53**
7	238346**	4.23	1.70	2.50	1.60	7.00	0.94	2.40	1.53	2.76	1.32	1.17	5.88	0.84	0.41
8	214993*	3.13	1.00	1.93	3.23	7.00	1.20	1.57	1.70	2.60	1.40	0.61	4.62	0.66	-0.28
9	**229738***	**5.40**	**1.40**	**2.43**	**3.37**	**6.67**	**1.70**	**1.67**	**1.20**	**2.99**	**1.32**	**1.40**	**5.97**	**0.85**	**-0.57**
10	**BKFM0051****	**3.23**	**2.43**	**2.63**	**2.70**	**7.60**	**1.83**	**2.30**	**1.80**	**3.07**	**1.41**	**0.27**	**3.66**	**0.52**	**0.37**
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	4.26	0.61	-0.22
12	229730*	3.87	0.90	2.07	3.50	5.03	0.79	1.77	1.05	2.37	0.99	0.97	3.36	0.47	-0.81
13	BKFM0047**	2.37	3.17	2.10	2.00	6.07	2.10	2.00	1.40	2.65	1.07	0.23	0.59	-0.01	0.59
14	203545*	3.07	2.20	2.70	1.63	6.17	1.43	2.33	1.73	2.66	1.05	0.53	2.67	0.37	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	3.69	0.53	0.19
16	230103*	2.43	3.97	2.67	1.97	4.40	2.27	2.27	1.30	2.66	0.61	0.49	3.17	-0.45	0.53
17	203546*	2.10	2.07	1.07	3.30	4.00	1.43	1.50	1.80	2.16	0.68	0.24	2.11	-0.29	-0.51
18	242617*	2.30	2.60	1.73	2.03	5.40	1.57	1.70	0.70	2.25	1.05	0.08	0.34	0.02	0.32
19	214995**	1.37	3.27	1.90	3.50	3.93	2.17	0.83	1.43	2.30	0.65	0.64	4.70	-0.67	-0.29
20	BKFM0005**	2.70	3.50	1.93	3.17	4.37	2.10	1.63	2.30	2.71	0.63	0.18	3.03	-0.43	-0.16
21	214988**	1.40	3.67	2.17	2.13	4.13	2.03	1.60	0.88	2.25	0.67	0.54	4.11	-0.58	0.41
22	BKFM0034**	2.43	3.27	1.70	1.97	5.33	1.73	1.97	1.77	2.52	0.90	0.20	1.08	-0.14	0.45
23	BKFM0028**	0.92	3.83	0.60	3.40	2.87	2.20	0.79	1.30	1.99	0.47	1.41	7.42	-1.06	-0.34
24	BKFM0042**	1.67	4.20	1.77	3.10	3.77	1.90	1.97	1.90	2.54	0.51	0.64	5.58	-0.79	0.04
25	BKFM0043**	1.90	3.83	1.70	3.73	4.60	2.10	2.00	1.50	2.67	0.76	0.49	4.11	-0.59	-0.16
26	BKFM0010**	1.30	3.33	2.77	3.33	3.70	2.87	2.10	1.80	2.65	0.37	0.53	5.46	-0.78	-0.04
27	214990**	1.83	3.90	1.77	2.67	5.63	1.77	1.37	1.30	2.53	1.08	0.38	2.23	-0.31	0.36
28	237443*	4.40	0.75	2.20	2.10	6.63	0.44	1.06	0.61	2.27	1.47	1.34	7.05	1.01	-0.19
29	214989**	1.47	2.50	1.73	3.77	5.20	1.83	1.12	1.43	2.38	0.99	0.39	1.95	-0.27	-0.33
30	203362*	3.10	3.20	1.11	3.93	6.17	1.93	2.23	1.10	2.85	1.23	0.31	0.27	-0.00	-0.26
31	**GUTE**	**2.23**	**3.07**	**2.13**	**3.53**	**5.79**	**1.97**	**2.07**	**1.73**	**2.78**	**1.02**	**0.09**	**1.02**	**-0.15**	**-0.03**
32	Taddesse	2.07	2.77	1.13	2.23	6.37	1.97	1.53	2.13	2.53	1.17	0.38	0.68	0.03	0.43
	MEAN	**2.63**	**2.64**	**1.96**	**2.86**	**5.50**	**1.72**	**1.75**	**1.44**	**2.56**	**0.99**	**0.53**	**3.32**	**0.001**	**0.001**

Key: *= accessions promoted from diversity study, ** = accessions promoted from disease screening trial of 2011, G# = Genotype number, AN = Arsi Negelle, AS = Assosa, BK = Bako, GT = Gute, the number following each location indicates the year (12 = 2012, 13 = 2013), IPCA = Interaction Principal Component Axis, CV = Coefficient of variation, LSD = Least significance difference, GEI = Genotype by Environment Interaction, Cumu.Int = cumulative interaction, b_i = Regression coefficient, s^2di = Squared deviation from regression, ASV = AMMI Stability Value, IPCA = Interaction Principal Component Axis,

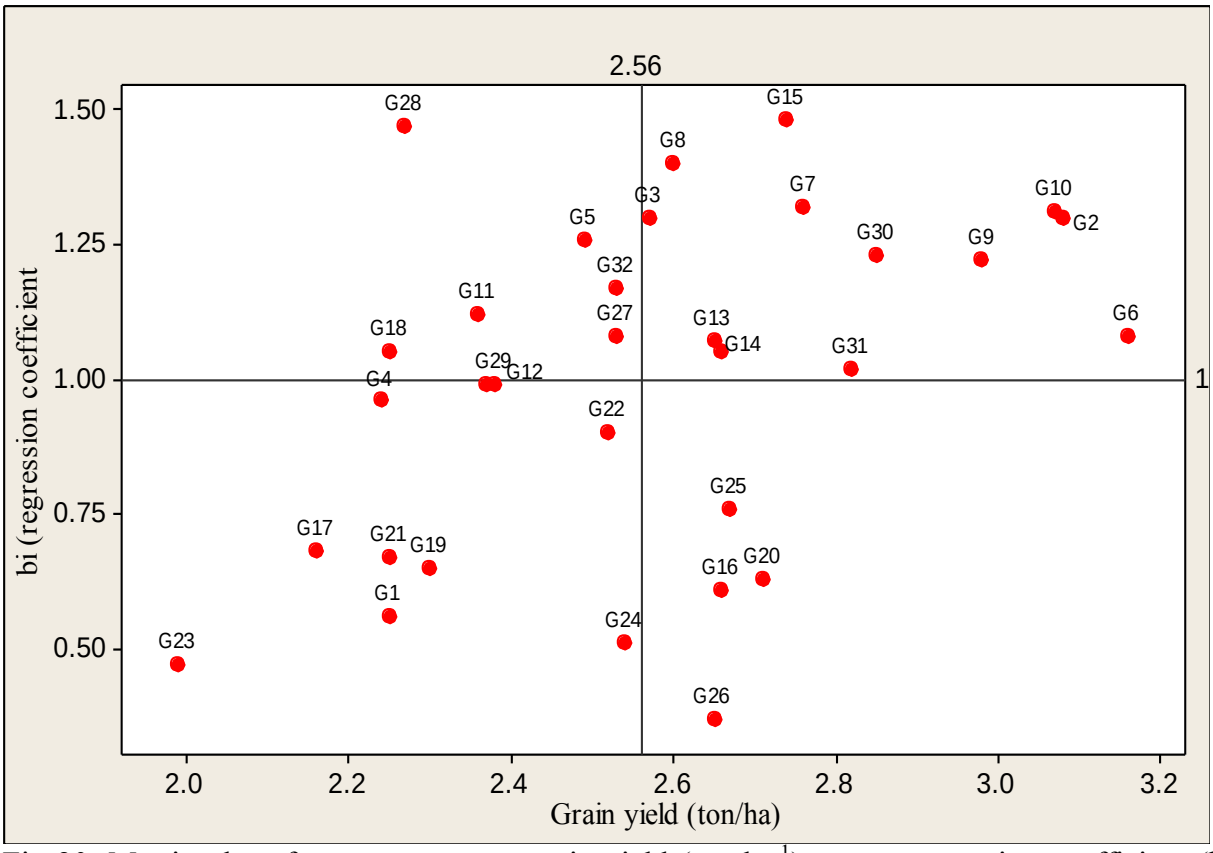


Fig 20. Matrix plot of genotypes mean grain yield (ton ha^{-1}) versus regression coefficient (b_i) indicating stability and yield performance of the test genotypes. The reference line on the x-axis is the average grain yield of genotypes and on the y-axis is the regression coefficient (b_i=1) indicating genotype stability.

5.4.4. Genotype and Genotype by Environment interaction (GGE) biplot analysis

5.4.4.1 Relationship among test environments

Mean grain yield data of both years were used to assess the relationships between the different test environments and was visualized by the line connecting each environment to the biplot origin or environment vectors (Fig 21). The cosine of the angle between two environments was used to calculate the correlation between them (Dehghani *et al*., 2009; Kaya *et al*., 2006). Based on mean grain yield of 2012 and 2013, environments Bako (BK) and Gute (GT) correlated

positively (acute angle), Assosa (AS) and Arsi Negele (AN) correlated negatively (obtuse angle) and AN and GT did not correlate (at about right angle) (Fig 21).

A strong negative correlation indicated high cross-over or GEI (Yan and Tinker, 2006). Tukamuhabwa *et al*. (2012) and Choukan (2010) reported that if two test locations correlated consistently across years, one could be dropped without significant loss of information about the genotypes. In the current study, variable environmental conditions among sites as well as within a given site from year to year resulted in inconsistent performance of genotypes (Fig 21, Table 35) and thus resulted in inconsistent correlations between the test locations. For instance, Gute and Bako showed relatively similar genotypes performance in 2013 and thus positively correlated, but different response to genotypes in 2012 (Fig 19; Table 35).

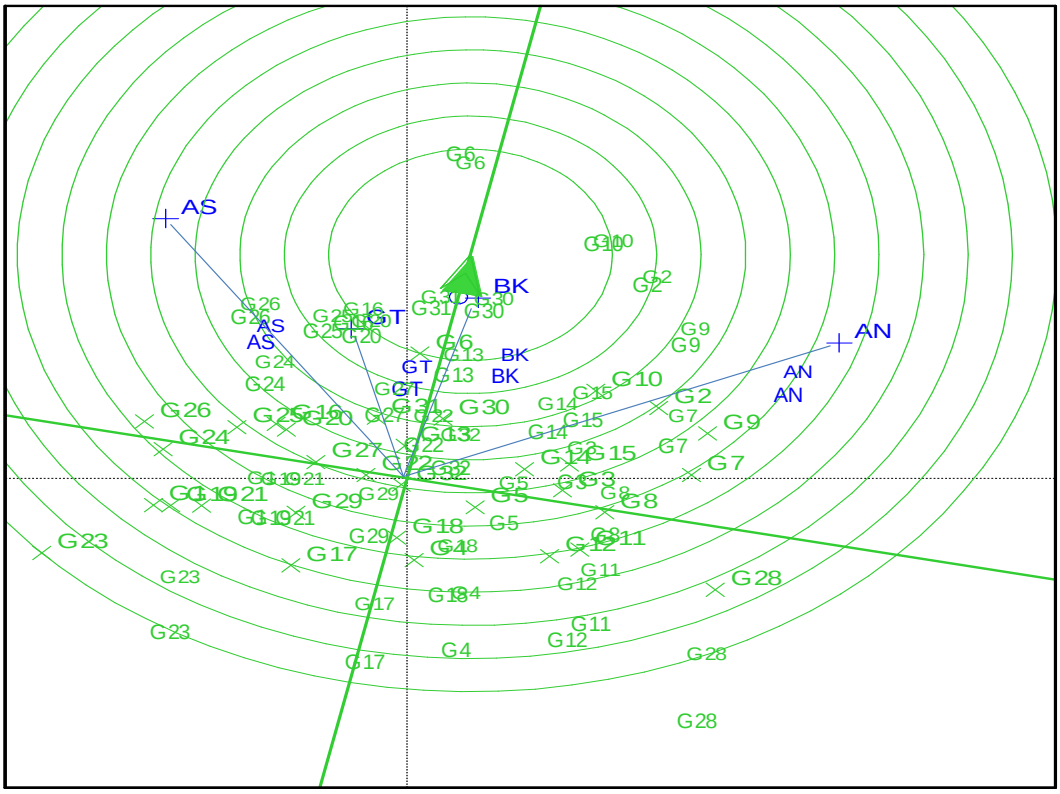


Fig 21. GGE biplot based on test environments-focused comparison for their relationships

5.4.4.2 Discriminating ability of the test environment and genotype stability

The concentric circles on the biplot help to visualize the length of the environment vectors, which are proportional to the standard deviation within the respective environments and are a measure of the discriminating ability of the environments (Worku *et al*., 2013). Environments and genotypes that fall in the central (concentric) circle are considered ideal environments and stable genotypes, respectively (Yan and Rajcan, 2002).

An environment is more desirable and discriminating when located closer to the centre circle or to an ideal environment (Naroui *et al*., 2013). Consequently, in the present study, Bako was the most discriminating environment (Fig. 21, 22). 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; Worku *et al*., 2013). Therefore, Bako followed by Gute were more representative and discriminating environments. Arsi Negele and Assosa were non-discriminating and less representative sites although the first was high yielding and the second a poor yielding environment (Fig. 19 and 21, Table 35). Similarly, Odewale *et al*. (2013) reported that only one environment was stable, representative and discriminating among the nine environments for the performance of 5 coconut genotypes.

Ranking based on the genotype-focused scaling assumed that stability and mean yield were equally important (Yan and Rajcan, 2002). The best candidate genotypes were expected to have high mean grain yield with stable performance across all test locations. In practice, such genotypes are very rare. Therefore, high yielding and relatively stable genotypes can be considered as references for genotype evaluation (Yan and Tinker, 2006). Both environments-

focused biplot and genotype-focused comparison of genotypes revealed that Acc. 203544 (G6) fell in the central circle indicating its high yield potential and relative stability compared to the other genotypes (Fig 21 and 22). Besides, Acc.242111 (G2) and BKFM0051 (G10) fell close to the ideal genotype or around the center of concentric circle indicating that they possessed specific adaptability with better grain yield potential. Among those, Acc. 203544 (G6), Acc.242111 (G2) and BKFM0051 (G10) were the best performing pipeline cultivars with 13.7%, 10.8% and 10.43%, yield advantage over the best standard check Gute (G31), and hence recommended for further verification and possible release for year 2014. The GGE biplot analysis result also supported those obtained using AMMI and the Eberhart and Russell model.

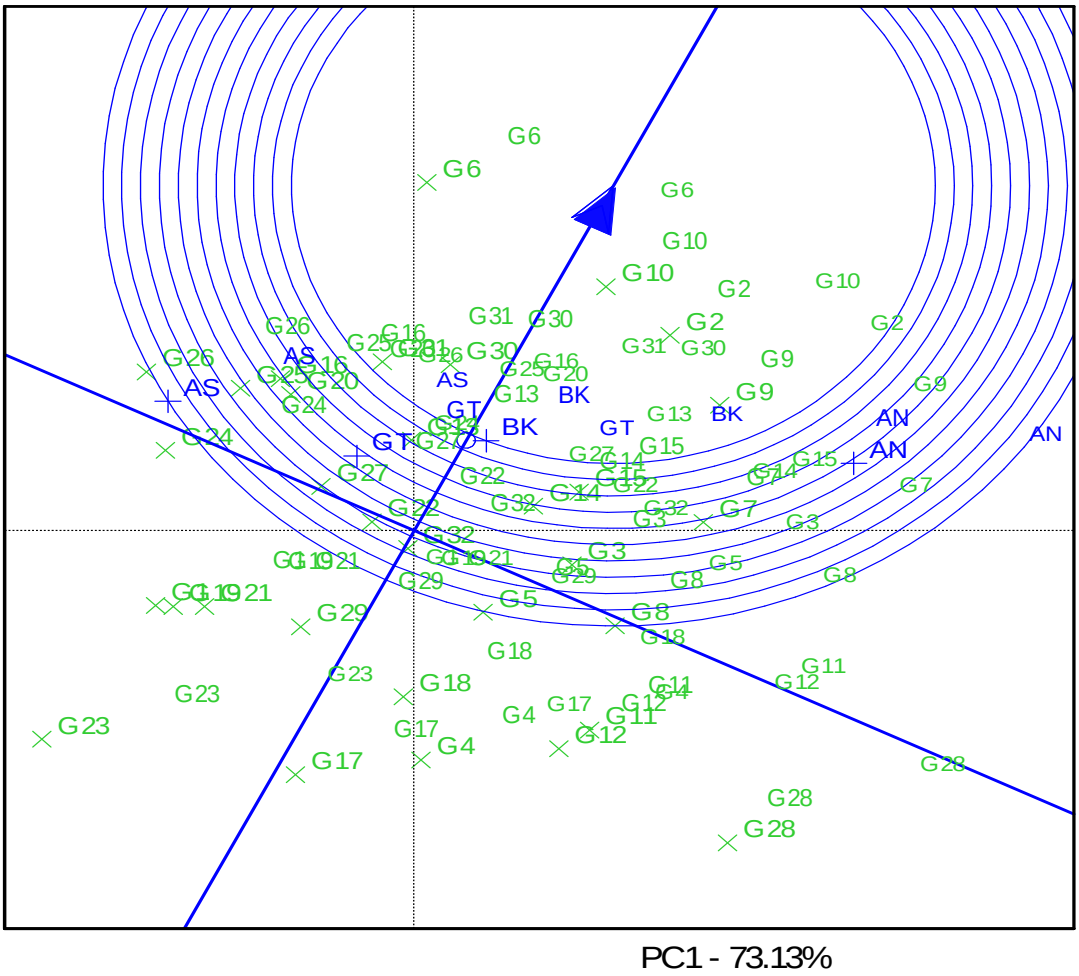


Fig 22. GGE biplot based on genotype-focused scaling for comparison of genotypes for their yield potential and stability.

5.5 Comparative genomic analysis of rice orthologous gene with finger millet for blast resistance

5.5.1 PCR amplification

A total of 21 primers (13 Pi-genes, 5 flowering date and 3 aluminum tolerant genes) were ordered based on the requirements of primer design such as located within a coding gene/region, product size of 400-600, appropriate melting temperature and absence of self-annealing (Table 8). The test PCR was conducted on two finger millet accessions with different levels of blast resistance; where Acc#5 or PW-001-022 was moderately resistant, but Acc#77 or Acc.238299 belonged to the highly susceptible groups (Fig 23). An annealing temperature gradient of 52 - 60°C was used to test the best amplification condition for the newly designed primers. Among these, only 13 primers (7 rice blast resistance genes, 2 aluminum tolerance genes and 4 genes linked to flowering date) gave amplicons (Table 8). Twelve of them were amplified at an annealing temperature of 58°C. One primer designed against aluminum tolerance gene (AL4) was amplified at 54°C. About 86 major rice blast R-genes have been identified until 2013, but only 18 of them have been cloned (Lui *et al*., 2013)

Clear variation was observed in the size of amplicons produced with different primer combinations with amplicon sizes ranging from about 400 bp for Pi36 and AL15 to about 800 bp for Pi9 (Fig 23). The variation was due to the differences in product sizes selected during primer design. As expected, there was no variation detected in amplicon size between the two extreme (blast resistant and susceptible) finger millet accessions based on agarose gel electrophoresis in reference to the standards ladder (Fig 23). PCR amplification and agarose gel

electrophoresis was conducted for all the 96 accessions using the 13 primer sets that amplified well. Similarly, no clear variation in amplicon size was recorded between the resistant and susceptible groups amplified using a single marker as revealed by agarose gel electrophoresis (Fig. 24).

Although the whole genome of finger millet is not yet sequenced, the majority of the mapped rice blast resistant genes gave good hits when used as blastn queries against the 454 sequence reads of the finger millet accession KNE796. Seven out of the 13 Pi blast resistance primers designed against conserved region identified through multiple alignments of the different finger millet hits against the Pi gene and the rice Pi gene itself showed PCR amplicons in finger millets. This implied a high sequence conservation of the Pi genes in finger millet and rice. The fact that both rice and finger millet are attacked by the same fungal pathogen *Pyricularia oryzea* could indicate that the function of Pi genes is also conserved.

Comparative analysis of the finger millet maps with the rice genome sequence suggests that the *Eleusine* and rice genomes are highly collinear, and that the chromosome rearrangements that differentiate the two species are specific either to finger millet or to the Chloridoideae subfamily (Srinvasachary *et al*., 2007; Devos, 2005). Such genetic colinearity could facilitate the exploitation of the information and resources available from rice and other grasses (Dida *et al.,* 2007; Devos, 2005). Genetic polymorphisms of those markers were further analyzed using single strand conformation polymorphism (SSCP) approaches.

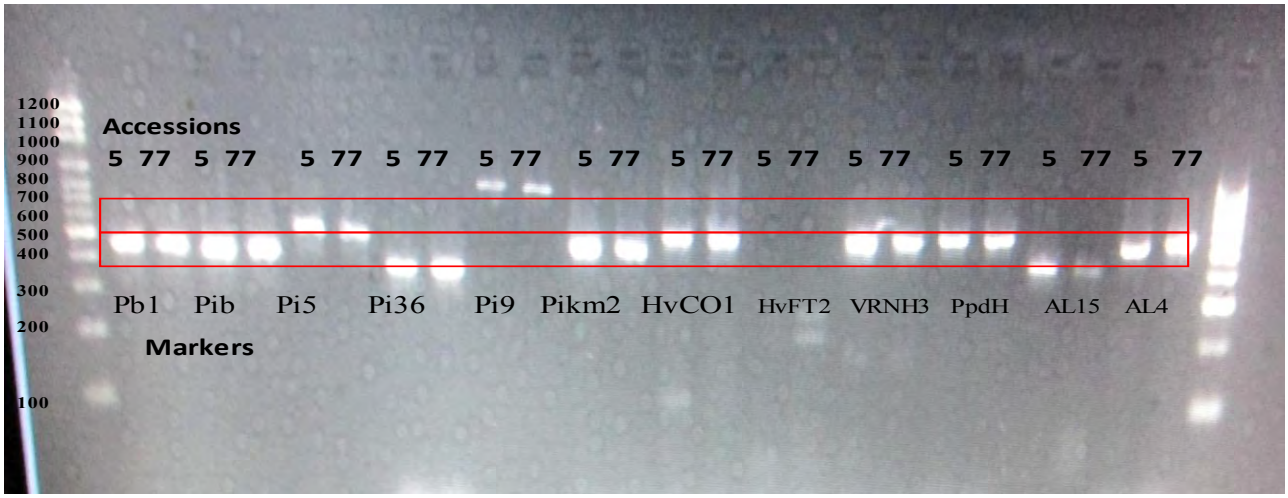


Fig 23. PCR product for 6 Pi genes, 4 flowering date and 2 aluminum tolerant genes.
The band indicated the PCR product tested on agarose gel in reference to 1kb ladder (left) and 100bp ladder (right) using two different finger millet accessions. Accession #5 or PW-001-022 was among moderately resistant and Acc #77 or Acc. 238299 was among the highly susceptible group. Marker Pi37F2 was not included and HvFT2 was not amplified.

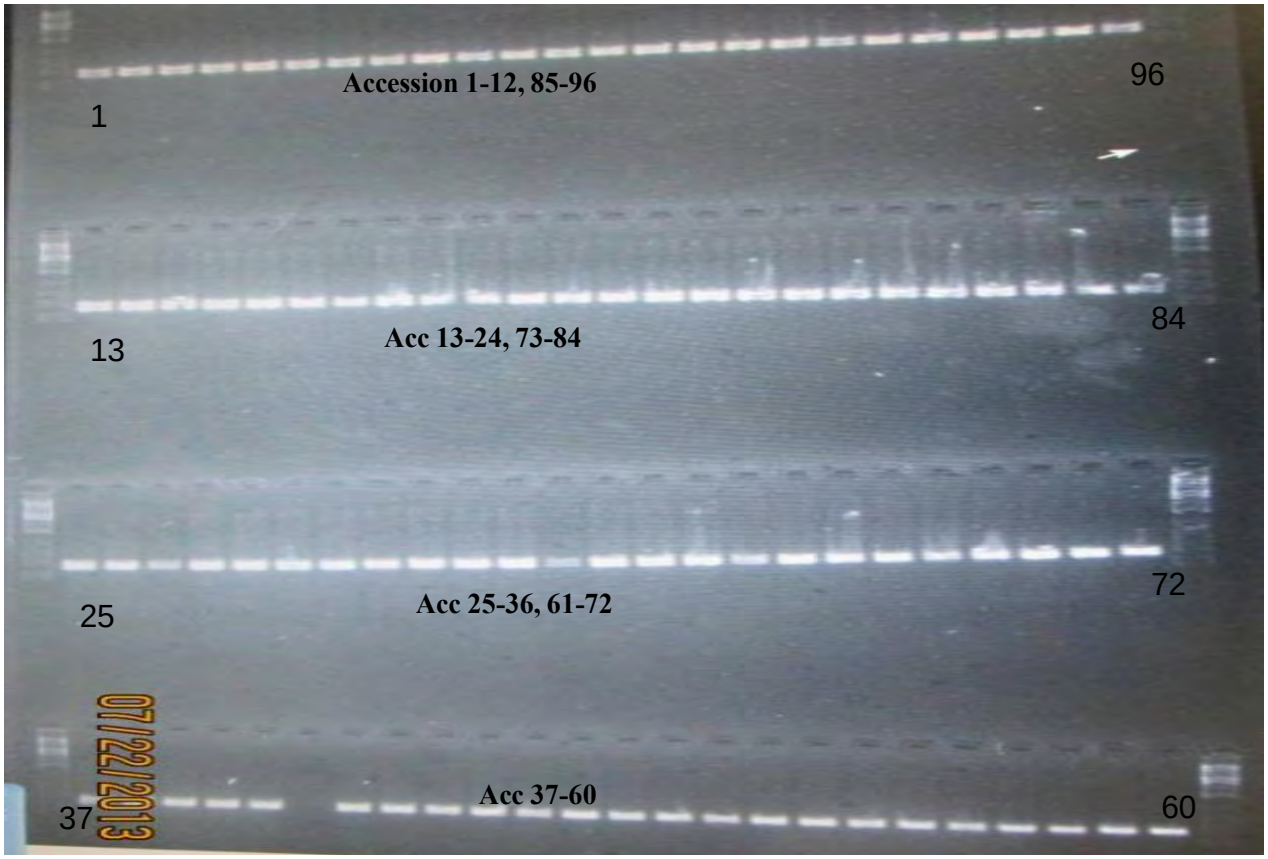


Fig 24. PCR amplification for blast resistant gene marker (PibF1) on 96 finger millet accessions.
Acc # 1=resistant, Acc # 2-5=moderately resistant, Acc # 6-35=moderately susceptible and Acc # 35-96 were susceptible to highly susceptible based on head blast infection recorded in 2011

5.5.2 Single Strand Conformation Polymorphism Analysis (SSCP)

An important step when using this SSCP technique is determining where each product will run within the gel under the chosen conditions. There is no a priori way of predicting the mobility of single strand fragments under non-denaturing conditions. Mobility of PCR products of different markers on the SSCP gel is therefore performed in reference to the standards ladder. In the present study, no clear polymorphism was noticed in the test SSCP gel for all Pi markers except Pi9F2 (Fig 25). Further SSCP analysis using 96 finger millet genotypes and the seven Pi-markers were conducted to confirm whether a marker is polymorphic or monomorphic. However, apparent polymorphism was recorded only for Pi9 F_2.

As the major objective of this study was to develop blast disease resistance markers, cloning, sequencing and other analyses were conducted using the polymorphic Pi genes (Pi9 F2). Four different SSCP band patterns were recorded for this primer set among the entire 96 finger millet samples. Eight accessions with different banding pattern were selected for cloning and sequencing (Fig 26). Band patterning was regardless of the disease history of the accessions. In other words, no distinguishing patterns were observed between blast resistant and susceptible groups (Fig 26).

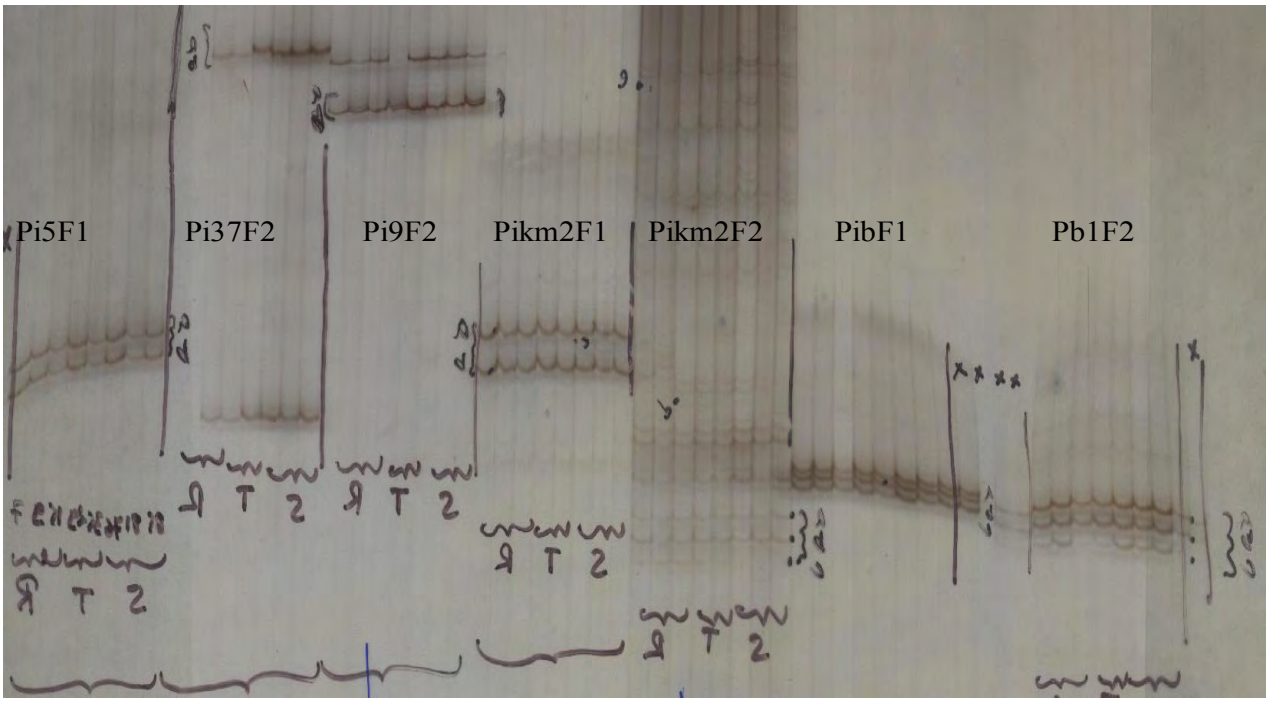


Fig 25. Test SSCP gel image for the seven amplified Pi genes

Key: The tset SSCP was conducted on 8 finger millet accessions and 7 Pi genes. Two accessions were among moderately resistant (R), three of them were among moderately susceptible (T) and three accessions were highly susceptible (s), all sequentially arranged from left to right for all markers.

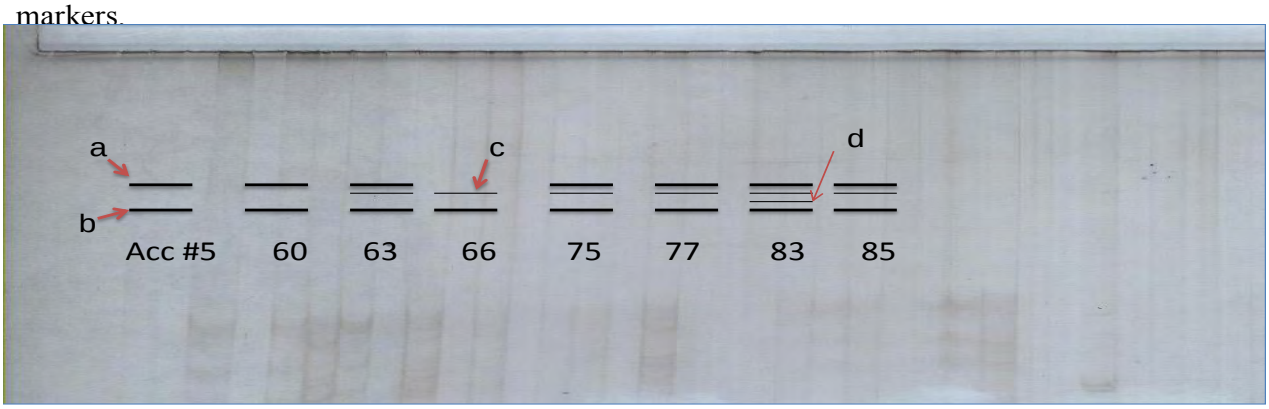


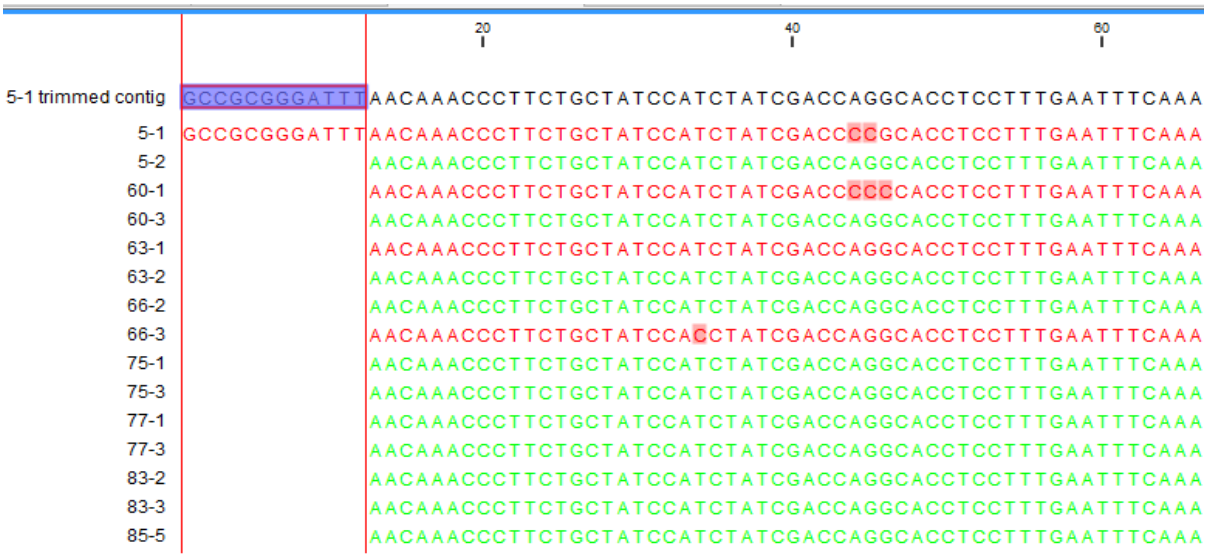
Fig. 26 SSCP gel electrophoresis banding patterns for some of the selected polymorphic finger millet genotypes for marker Pi9F2

Key: Accession name for finger millet Acc#5= PW-001-022, #60=AAUFM-11, #63= AAUFM-10, #66=Acc 215853, #77= Acc 238299, #83= Acc 230104, and #85= AAUFM-23. Four different bands were recorded for the entire accessions and labeled a, b, c, and d. The selected band pattern is therefore, represented all the 96 finger millet accessions used in the study. PW-001-022 (#5) was among moderately resistant, AAUFM-11 (#60) was susceptible, and the remaining accessions (#63, #65, #66, #77, #83 and #85) were among the highly susceptible groups. The band was recorded as absent (0) or present (1) based on the presence and absence of the labeled letters.

5.5.3. Single nucleotide variant detection

The mean length of the obtained sequence for the 15 reads was 859bp and about 13kb in total. Nucleotide distribution revealed that 30.9% Thymine (T), 28.1% Adenine (A), 24.2% Cytosine (C) and 16.8% Guanine (G). Basically, the sequence reads could be trimmed to remove bases with low quality scores, ambiguous sequences (e.g. stretches of Ns), and the adapter sequence. In the present study, the sequence was trimmed to remove low coverage sequence in the either side of the sequence. Therefore, about 12bp of the low coverage from both extremes were trimmed (Fig 27).

A total of 48 nucleotide variants (consensus position) were recorded across all 15 reads derived from 8 samples (Table 36). The variants were single nucleotide variant (SNV) when one base is replaced by one other base or multiple nucleotides variant (MNV) when two or more SNVs in succession compared to the reference and the other experimental populations (Fig 28). In species that lack a complete genome sequence, the consensus of the read clusters across the sequence tagged sites becomes the reference (Poland *et al*., 2012).The variant at consensus position 44, 45 and 46 for Acc #60 (Acc. AAUFM-11) is good example of MNV (Fig 28). SNV is preferred over SNP because the latter includes an extra layer of interpretation about variants in a population. This means that SNV could potentially be a SNP but this cannot be determined at the point where the variant is detected in a single sample (www.bioinformatics.dnalc.org/ptc/pdf accessed on August 2013).



a)

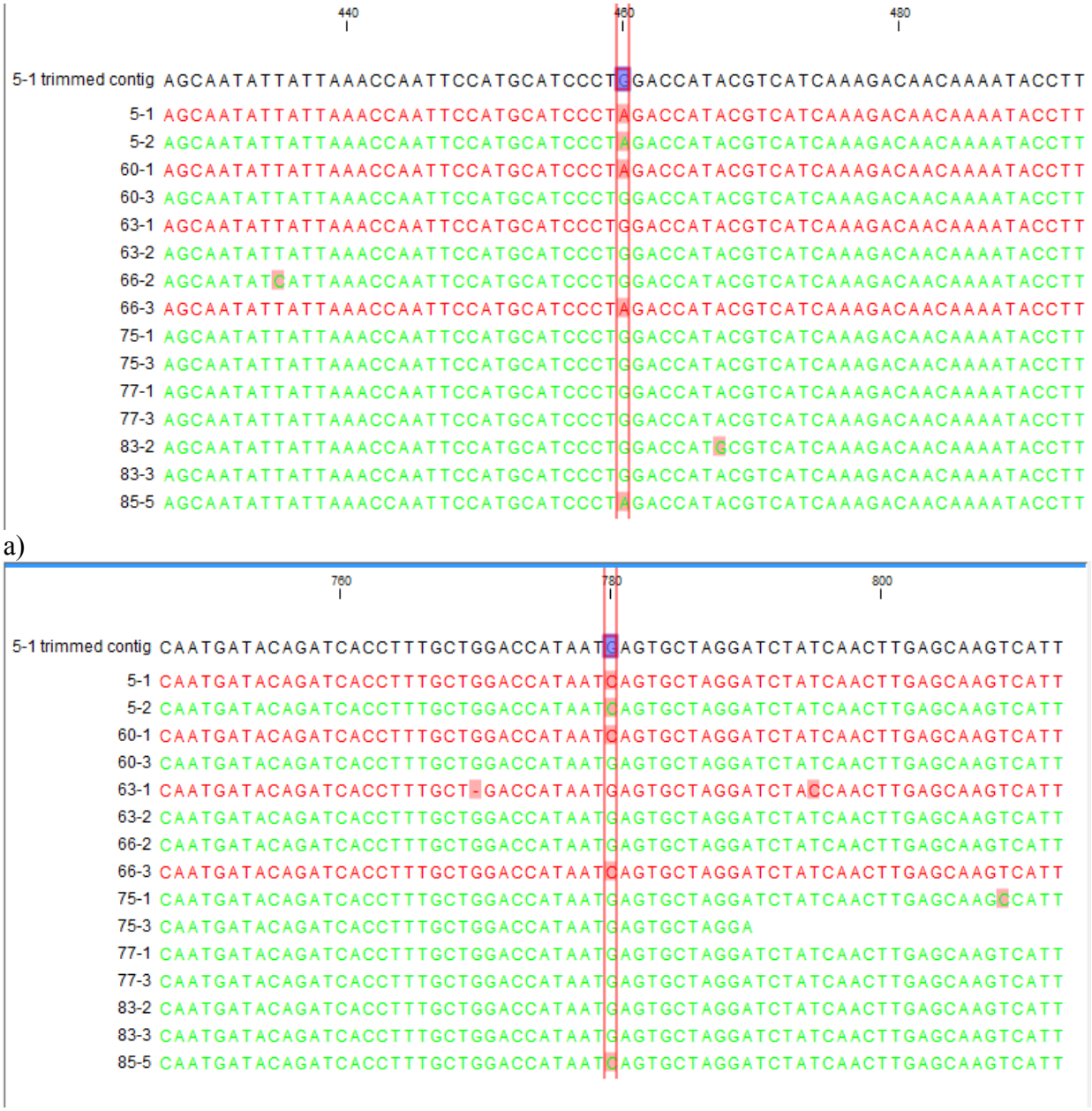
b)

Fig 27. The trimmed nucleotides and reads for its low coverage at start (a) and the end of the sequences (b).

A single nucleotide polymorphism (SNP) is a variation at a single position in a DNA sequence among individuals. If more than 1% of a population does not carry the same nucleotide at a specific position in the DNA sequence, then this variation can be classified as a SNP (www.bioinformatics.dnalc.org/ptc/pdf accessed on August 2013). To identify SNPs in the

populations, all pairs of tags were evaluated for one or two base-pair difference (Varshney, 2010). Multiple sequence alignment of the sequence data generated for 15 DNA fragments from 8 accessions using Pi9F2 reveals two likely SNPs at consensus positions 460 (G/A) and 780 (G/C) in a window of 66 bp, where the consensus residue or the reference sequence was G at positions 460 and 780 but was replaced by A and C, respectively (Fig. 28, Table 36). Because, with the exception of accession #85 (AAUFM-23), we have two sequences per accession, we were able to distinguish sequencing errors from true SNPs. A true SNP will be present in both sequences derived from the same accession, while a sequencing error will be present in only 1 of the two duplicates. Translation of the finger millet sequences into a protein sequence indicated that all sequences contained a stop codon (which was not present in the corresponding rice sequence), suggesting that the finger millet candidate blast resistance gene was not a functional gene.

Because of the lack of a reference genome sequence for finger millet, the present study invested lots of time but had limited success in designing finger millet blast resistance markers to use in a candidate gene approach association mapping. However, the current progress (and near completion) of the whole genome sequencing of finger millet at the Beijing Institute of Genomics (BGI) with financial support of SIDA and the African Orphan Crops Project, will herald a breakthrough in the genetic improvement of finger millet. With the revelation of the genome sequence map and its final annotation and reconciliation, information on the DNA structure and arrangements of structural and regulatory genes underlying economically important traits will be identified, thereby enhancing our understanding of the function of important traits, and allowing detailed comparisons of the genome structure and sequence across related and unrelated crop species and thus describe their evolutionary relationship.



a)

b)

Fig 28. The two probable SNP positions; at consensus position 460 (a) and 780 (b) to the reference sequence and sequences of majority of the populations.

Table 36. Position of variants, variant nucleotides and the highly conserved sequences in the population.

Consensus position	Consensus residue	Other residues	IUPAC
102	G	A (1)	G (14)
140	A	A (14)	G (1)
153	T	C (1)	T (14)
161	A	A (14)	G (1)
178	A	A (14)	G (1)
196	T	C (1)	T (14)
221	A	A (14)	G (1)
256	C	C (14)	G (1)
285	T	C (1)	T (14)
326	T	C (1)	T (14)
34	T	C (1)	T (14)
379	T	C (1)	T (14)
385	T	C (1)	T (14)
388	T	C (1)	T (14)
425	A	A (14)	G (1)
435	T	C (1)	T (14)
44	A	A (13)	C (2)
45	G	C (2)	G (13)
46	G	C (1)	G (14)
460	G	A (5)	G (10)
467	A	A (14)	G (1)
556	T	C (1)	T (14)
579	C	- (1)	C (14)
588	C	C (14)	T (1)
617	T	C (1)	T (14)
637	A	- (2)	A (13)
661	A	A (14)	G (1)
684	G	C (1)	G (14)
696	T	A (1)	T (14)
710	T	C (1)	T (14)
738	C	C (13)	T (2)
770	G	- (1)	G (14)
780	G	C (5)	G (10)
795	T	C (1)	T (13)
809	T	C (1)	T (13)
840	C	C (12)	N (1)
844	C	A (1)	C (11) N (1)
846	C	C (12)	G (1)
847	A	A (12)	G (1)
87	G	G (14)	N (1)
882	T	C (1)	T (3)
92	A	A (14)	N (1)
98	C	C (14)	T (1)

6. CONCLUSION AND RECOMMENDATION

Finger millet (*Eleusine coracana* subsp. *coracana*) is an allotetraploid (AABB, 2n=4x=36) crop widely produced in the tropical and sub-tropical regions of Africa and India and serves as a main staple food for rural populations in those countries. The major feature of finger millet is its adaptability to adverse agro-ecological conditions with minimal inputs, tolerance to moisture stress, its ability to produce on marginal land where other crops cannot perform and tolerance to acidic soil. It constitutes 10 to 20 percent of the total cereal production in major finger millet growing regions of Ethiopia. Despite the fact that Ethiopia is the center of origin and domestication of finger millet, little research attention has been given to improve finger millet compared to other food crops. The current national average productivity is 50% less than the yield potential. This is mainly due to the lack of widely adaptable improved varieties, blast disease infection, lack of improved management technologies and other biotic and abiotic factors. Therefore, this study was aimed to contribute towards such pressing needs and also generate some basic information to improve productivity of finger millet.

Genetic diversity analysis for qualitative traits showed erect growth habit, open ear type, light green ear (glumes) color; enclosed grains by glumes, lower spikelet density and purple black seed color were the predominant phenotypic classes in the studied finger millet accessions. The significant variation between genotypes for most of the quantitative traits as revealed by descriptive analysis, ANOVA, AMOVA and Shannon index indicated the possibility to improve productivity of this stress tolerant crop to cope even better with changes in climate and edaphic factors.

At 85% similarity level, the 150 finger millet accessions evaluated for phenotypic traits were grouped into eight clusters based on 17 major quantitative traits, but three accessions (Acc.216057, 241768 and 238344) and one improved cultivar (Wama) remained apart. 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 have been either due to farmer's selection criteria for a given trait that might be similar particularly based on their adaptability to the environment or yield potential or other quality parameters of common interest, the primary seed source could have been the same, or a high tendency of seed exchange could have ensured that the same varieties spread across the collection areas. Besides, cluster analysis based on regional mean of six qualitative traits and 17 quantitative traits depicted that finger millet accessions from Ethiopia and Eritrea were grouped together and, Kenyan, Zambian and Zimbabwe's accessions clustered together. Altitudinal proximity was also partly manifested in the clustering of accessions for both qualitative and quantitative traits. On the other hand, collections from Oromia, SNNP, Benishangul Gumuz and Zambia were distributed across some of the clusters to variable degrees, which indicated variation among accessions of the same region or country.

PCA indicated that all quantitative traits considered in the present study contributed to the overall genetic variation observed among finger millet accessions and eco-geographical origins. The higher heritability observed, followed by higher genetic advance recorded for grain yield and other major yield related traits such as ear weight, finger length and thousand grain weight as obtained from inheritance analysis as well as the positive association between those traits and grain yield as observed from the correlation and path coefficient analysis could point breeders to the traits they need to focus on in order to change the current low productivity of the crop.

Molecular marker based investments in finger millet has been neglected compared to other cereals, particularly in Ethiopia. The analysis of molecular variance and genetic polymorphism for 150 finger millet accessions genotyped using a set of 20 SSR markers confirmed the presence of genetic variability among accessions studied, within and between regions of origin. Furthermore, neighbor-joining based clustering discriminated the potential variability existing within accessions from the same region. Shared allele genetic distance and Rogers's genetic distance also portrayed a range of genetic divergence among accessions and regions of origin that can guide the selection of potential heterotic breeding lines. Analysis of population structure distinguished the 138 finger millet accessions into three subpopulations (k = 3) with the highest ΔK of around 300. In agreement with the result of neighbor joining trees, finger millet accessions collected from the same region of origin did not often grouped entirely together within a given major groups.

Overall, nil variation was not recorded between pairs of accessions analyzed in the current study either for polygenic traits, monogenic traits or with the SSR markers used. However, minimum variation between some accessions was recorded for both phenotypic and molecular markers apart from eco-geographical origins. Only 144 germplasm accessions and 6 improved cultivars were addressed in the present study. If the thousands of accessions available at international or national gene banks were to be evaluated in a similar way, redundant germplasms or germplasms with narrow genetic base, which may not be sufficient to utilize as breeding stock, could be eliminated. Therefore, the present study reiterates that attention should be given to further strategic collection of finger millet from less addressed or unaddressed areas, use of larger number of accessions with equal regional and altitudinal representations, reduction of

redundant germplasms and replacing such with new and diverse accessions combined with availability and easy-access of inter-country germplasms from international research institutes such as ICRISAT. Moreover, only six accessions collected from SNNP region were included in this study, which is too little to represent this vast region. Those few accessions showed wider variations genetically and phenotypically. This urges further strategic collection mission from such regions and also other unaddressed areas at the national level.

Analysis of variance for blast disease traits portrayed highly significant ($P \leq 0.01$) differences between accessions for LBRAUDPC, HBRAUDPC, neck blast incidence, lesion length and grain yield per plant and thus indicated the available opportunity to obtain resistant/tolerant cultivar for further utilization in breeding programs. Blast infectivity showed wider variation among accessions of various regions of collection and the improved varieties at early development stage, but the range narrowed at later recording periods. Based on head blast disease severity index recorded in 2011, the total 225 finger millet test materials were classified into five groups; only one accession (Acc. BKFM0031) was resistant, 10 accessions were moderately resistant, 24 accessions were moderately susceptible, 75 accessions were susceptible and the remaining 115 accessions were highly susceptible. Even though finger millet blast is an economically important pathogen, information regarding the different races present in the country, their virulence level and genetic characters are not known and thus should be given due attention.

Finger millet accessions collected from the same region showed different levels of disease reaction, implying that diversified finger millet accessions are produced within a country or region. Among the top 25 accessions least infected by head blast, 80% gave optimum grain

yield above the average (11.29 g/plant) of the base population and 20% gave grain yield below the average. On the contrary, 66% of the top 25 highly infected finger millet accessions gave grain yield below the average indicating that grain yield reduction in most of the accessions were due to the severe blast infection.

Both correlation and regression analysis revealed highly significant ($P \leq 0.01$) negative association of head blast and significant ($P \leq 0.05$) negative association of neck blast and leaf blast on grain yield per plant indicating that infections on above ground parts of the plant greatly affected grain yield. An average of 63.2% finger millet yield loss was recorded due to blast (*Magnaporthe oryzea*) disease during the humid crop growing season and the severe infection periods of 2011 as estimated using three different models; statistical approach, AUDPC and multiple point models. This further emphasizes ecologically and economically feasible blast management options should be developed. Nine of the 25 most tolerant and high yielding accessions that were also superior to the advanced line PW-001-022 were promoted to the next evaluation phase at multi-locations.

Combined analysis of variance for 30 advanced finger millet genotypes and two improved varieties evaluated across four locations for two years depicted highly significant variation between genotype, environments and genotype by environment interactions for all characters. GGE biplot analysis, AMMI and Eberhart and Russell model revealed that Acc. 203544 was a stable, high yielding (3.16 ton ha^{-1}) candidate variety with 13.7% yield advantage over the best standard check Gute (2.78 ton ha^{-1}) and thus should be recommended for release with wider environmental adaptability. Acc. 242111 (3.08 ton ha^{-1}), Acc. BKFM0051 (3.07 ton ha^{-1}) and Acc.229738 (2.99 ton ha^{-1}) were also high yielding, but inconsistent and thus should be

recommended for verification and possible release for specific adaptability. About 66.05% of GEI was explained by IPCA-I and a total of 88.33% GGE interaction by the first three IPCAs. GGE biplot based analysis on test environments-focused comparison for their consistence revealed that, except at Bako, the test environments were inconsistent for mean grain yield and IPCA scores during 2012 and 2013. This observed instability might have been due to variation in weather conditions, soil and other uncontrolled edaphic factors in 2012 and 2013.

Single strand conformation polymorphism analysis detected that only one marker was polymorphic among the thirteen primers designed against finger millet sequences with orthology to rice blast resistance genes. Multiple sequence alignment of the sequence data generated for 15 samples from eight representative finger millet accessions using Pi9F2 revealed two possible SNPs at consensus positions 460 (G/A) and 780 (G/C) in a window of 66 bp. Genomic research in the present study was generally a preliminary finding. With the revelation of the finger millet genome sequence map and its final annotation and reconciliation, information on the DNA structure and arrangements of structural and regulatory genes underlying economically important traits should be identified and detailed comparisons of the genome structure and sequence across related and unrelated crop species and thus describe their evolutionary relationship.

In general, the present study has made contributions to the application of molecular tools in discovering the extent of genetic variation among finger millet accessions and eco-geographical regions and thus crucial input for variety development. Furthermore, accessions with variable level of blast tolerance for further utilization in breeding and other genomic research activities were identified. These results can be applied to develop heterotic progenies and to select the

best recombinant inbred lines, particularly for improving yield and resistance to the most important finger millet disease, blast (*Magnaporthe grisea*) in eastern Africa in general and Ethiopia in particular. Besides, the three adaptable and high yielding candidate cultivars (Acc. 203544, BKFM0051 and Acc.242111) were identified through intensive evaluation in multiple sets of trial and evaluated by the National Variety Releasing Committee and we expect a release of at least one candidate variety that will be a potential input to boost finger millet productivity in the study areas and other regions with similar agro-ecology.

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7. REFERENCES

Abera, W. and Labuschagne, M. (2005). Stability analysis of Ethiopian maize varieties using AMMI model. *Ethiopian Journal of Agricultural Science* **18**(2):173-180.

Adewale, B.D., Kehinde, O.B., Aremu, C.O., Popoola, M. and Dumet, J. (2010). Seed metrics for genetic and shape determinations in African yam bean [*Fabaceae*] (*Sphenostylis stenocarpa). African Journal of Plant Science* **4(4)**: 107-115.

Agrawal, B.L. (1996). *Basic Statistics*, 3rded. New Age International (P) Limited, New Delhi, pp.713.

Agrobase (2000).AGROBASE Generation II®. Plant and Crop Breeding Software. Waterloo St. Winnipeg, Manitoba R3NNOS4, Canada.

Albar, L., Lorieux, M., Ahmadi, N., Rimbault, I., Pinel, A., Sy, A.A., Fargette, D. and Ghesquiere, A. (1998). Genetic basis and mapping of the resistance to rice yellow mottle virus: QTLs identification and relationship between resistance and plant morphology. *Theoretical and Applied Genetics* **97**: 1145–1154.

Allard, R.W. (1960). *Principle of Plant Breeding,* 2nd ed. John Wiley and Sons, New York.

Allentoft M.E., Schuster, S., Holdaway, R.N., Hale, M.L., McLay, E. (2009). Identification of microsatellites from an extinct moa species using high throughput (454) sequence data. *Biotechniques* **46**: 195–200.

Anon, B. (1959). Annual Report of the Department of Agriculture, Uganda, for the year ended 31 December 1958.

Aremu, C.O. (2012). Exploring Statistical Tools in Measuring Genetic Diversity for Crop Improvement. In: *Genetic Diversity in Plants.* Prof. Mahmut Caliskan (ed.), ISBN: 978-953-51-0185-7.

Aremu, C.O. (2011). Genetic Diversity: A review for need and measurements for intra-species crop improvement. *Journal of Microbiology and Biotechnology Research* **1(2)**:80-85.

Ariyo, J. (1989). Factor analysis of pod yield in Okra (*Abelmoschus esculentus*).*Theoretical and Applied Genetics* **64**: 82–85.

Assefa, K., Arnulf, M. and Tefera, H. (2003). Multivariate analysis of diversity of tef (*Eragrostis tef* (Zucc.) Trotter) germplasm from western and south western Ethiopia. *Hereditas* **138**: 228-236.

Assefa, K., Tefera, H., Arnulf, M., Kefyalew, T. and Hundera, F. (2001). Variability, heritability and genetic advance in phenomorphic and agronomic traits of tef [*Eragrostis tef* (Zucc.) Trotter] germplasm from eight regions of Ethiopia. *Hereditas* **134**: 103-113.

Ayana, A. and Bekele, E. (1998). Geographical patterns of morphological variation in sorghum [*Sorghum bicolor* (L.) Moench] germplasm from Ethiopia and Eritrea: Qualitative characters. *Hereditas* **129**:195-205.

Ayana, A. (2001). Genetic diversity in sorghum [*Sorghum bicolor* (L.) Moench] germplasm from Ethiopia and Eritrea. PhD thesis, Addis Ababa University.

Ayyangar, G.N.R.(1932). The inheritance of character in ragi, *E. coracana* Gaertn. *Madras Agricultural Journal* **20**:1-9

Babu, B.V., Ramana, T. and Radhakrishnan, T.M. (1987). Chemical composition and protein content in hybrid varieties of finger millet. *Indian Journal of Agricultural Science* **57**:520-552.

Babu, B., Senthil, N., Gomez, S., Biji, K., Rajendraprasad, N., Kumar, S. and Babu, R. (2007). Assessment of genetic diversity among finger millet accessions using molecular markers. *Genetic Resource and Crop Evolution* **54:** 399–404.

Bako Agricultural Research Centre (BARC) (2010). Research Progress Report for the year 2010.

Ballini, E., Morel, J. B., Droc, G., Price, A., Courtois, B. (2008). A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. *Mol. Plant Microbe Interact*. **21**: 859–868

Barbeau, W.E. and Hilu, K. (1993). Protein, calcium, iron and amino acid content of selected wild and domesticated cultivars of finger millet. *Plant Foods & Human Nutrition* **43**:97–104

Bekele, E. (1985). The biology of cereal landrace populations. Problem of gene conservation, plant breeding schemes and sample size requirement. *Hereditas* **103**: 119-134.

Bekele, E. (1984). Analysis of regional patterns of phenotypic diversity in the Ethiopian tetraploid and hexaploid wheat. *Hereditas* **100**: 131-154.

Bekele, E. (1983). Some measures of genetic diversity analysis on landrace population of Ethiopian barley. *Hereditas* **98:** 127-143.

Bezawuletaw, K. (2011). Genetic diversity of finger millet [*Eleusine coracana* (L.) Gaertn] landraces characterized by Random Amplified Polymorphic DNA Analysis. *Innovative Systems Design and Engineering* **2**: 207-218.

Bezaweletaw, K., Sripichit, P., Wongyai, W., Hongtrakul, V. (2006). Genetic variation, heritability and path-analysis in Ethiopian finger millet (*Eleusine coracana* (L.)Gaertn) landraces. *Kasetsart Journal of Natural Science* **40**: 322-334.

Bheema, I.N., Lingeswara Reddy, Srinivas Reddy, Lakshmi Narasu and Sivaramakrishnan, S. (2010). Characterization of disease resistance gene homologues isolated from finger millet (*Eleusine coracana* L. Gaertn). *Molecular Breeding* **27**: 315-328.

Bisht, M.S. and Mukai, Y. (2002). Genome organization and polyploidy evolution in the genus *Eleusine* (Poaceae). *Plant Systematic and Evolution* **233**: 243-258.

Borromeo, E.S., Nelson, R.J., Bonman, J. and Leung, H. (1993). Genetic differentiation among isolates of *Pyricularia* infecting rices and weed hosts. *Phytopathology* **83:**393-399.

Burdon, J. J., Ericson, L. and Muller, W. J. (1995). Temporal and spatial changes in a metapopulation of the rust pathogen *Triphragmium ulmariae* and its host, Filipendula ulmaria, *Journal of Ecology* **83**: 979–989.

Carrillo, E., Rubiales, D., Pérez-de-Luque, A. and Fondevilla, S. (2012). Characterization of mechanisms of resistance against *Didymella pinodes* in *Pisum* spp. *European Journal of Plant Pathology* **135**: 761–769.

Central Statistical Authority (CSA) (2013). Agricultural sample survey. Report on the preliminary results of area, production and yield of temporary crops, (Meher season, private peasant holding), Addis Ababa.

Chauhan, R.S., Farman, M.L., Zhang, H.B. and Leong, S. (2002). Genetic and physical mapping of a rice blast resistance locus, Pi-CO39 (t) that corresponds to the virulence gene AVR1-CO39 of *Magnaporthe grisea*. *Molecular Genetics and Genomics* **267**: 603–612.

Chen, Y.u., Jian Yao, Wen-Xiang Wang, Tong-Chun Gao, Xue Yang and Ai-Fang Zhang (2013). Effect of epoxiconazole on rice blast and rice grain yield in China. *European Journal of Plant Pathology* **135**:675–682.

Choukan, R. (2010). Genotype, environment and genotype by environment interaction effects on the performance of maize (*Zea mays* L.) inbred lines. *Crop Breeding Journal* **1**:97-103.

Chrispus O.A. Oduori (2008). Breeding investigations of finger millet characteristics including blast disease and *Striga* resistance in western Kenya. PhD Dissertation. University of KwaZulu-Natal, Republic of South Africa

Clarke, D.D. (1986). Tolerance of parasites and disease in plants and its significance in host-parasite interactions. *Advanced Plant Pathology Journal* **5**: 161–197.

Cochran, W.G. and Cox, G. M. (1957). *Experimental Designs*. 2nd ed., John Wiley and Sons, Inc., New York.

Coleman, L.C. (1920). The cultivation of ragi in Mysor. Bulletin of Department of Agriculture, Mysore, Genetics. Ser. 11.

Couch B.C. and Kohn, L.M. (2002). A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea. Mycologia* **94(4)**: 683–693.

Daba, C. and Debelo, A. (2008). Correlation and path analysis in finger millet. *Ethiopian Journal of Crop Science* **1(1)**:38-44.

Das, S. and Misra, R.C. (2010). Assessment of genetic diversity among finger millet genotypes using RAPD markers. *Indian Journal of Agricultural Research* **44** (2): 112 – 118

Dehghani, H., Sabaghnia, N., Moghaddam, M. (2009). Interpretation of genotype-by-environment interaction for late maize hybrids grain yield using a biplot method. *Turkish Journal of Agriculture and Forestry* **33**:139–148.

Demissie, A. and Bjonstrand, A. (1996). Phenotypic diversity of Ethiopian barley in relation to geographic regions, altitudinal range and agro ecological zones: As an aid to germplasm collection and conservation strategy. *Hereditas* **124:** 17-29.

De Wet, J.M.J., Prasada Rao, K.E., Brink, D.E. and Mengesha, M.H. (1985). Systematics and evolution of *Eleusine coracana*. *American Journal of Botany* **71**:550–557.

Dewey, D.R. and Lu, K.H. (1959). A correlation and path coefficient analysis of crested wheat grass grain production. *Agronomy Journal* **51**: 515-518.

Devarumath Rachayya Mallikharjun, Subhash C. Hiremath, Satyawada Rama Rao, Arun Kumar and Suman Shivamurti Sheelavanthmath (2005). Genome interrelationship in the genus *Eleusine* (Poaceae) as revealed through heteroploid crosses. *Caryologia* **58**: 300-307.

Devos, K.M. (2005). Updating the 'Crop Circle' *Current Opinion in Plant Biology*. **8**:155–162

Devos, K.M., Pittaway, T.S., Renolds, A. and Gale, M.D. (2000). Comparative mapping reveals a complex relationship between the pearl millet genome and those of foxtail millet and rice. *Theoretical and Applied Genetics* **100:**190-198.

Dida, M. and Devos, K.M. (2006). Finger millet. pp 333–343. In: Kole C (ed) Genome mapping and molecular breeding in plants, vol 1, Cereals and millets. Springer, Heidelberg.

Dida, M., Srinivasachary, M., Ramakrishnan, S., Bennetzen, J. L., Gale, M. D. and Devos, K. M. (2007). The genetic map of finger millet, *Eleusine coracana*. *Theoretical and Applied Genetics* **114**:321–332.

Dida, M., Wanyera, N., Dunn, M., Bennetzen, J. and Devos, K.M. (2008). Population structure and diversity in finger millet germplasms. *Tropical Plant Biology* **1**: 131–141.

Dje, Y., Hevretz, M., Letebure, C. and Vekemans, X. (2000). Assessment of genetic diversity within and among germplasms accessions in cultivated sorghum using microsatellite markers. *Theoretical and Applied Genetics* **100***:918*-925.

Eberhart, S.A. and Russell, W.A. (1966). Stability parameters for comparing varieties. *Crop Science* **6:** 36–40.

Ekwamu, A. (1991). Influence of head blast infection on seed germination and yield components of finger millet (*Eleusine coracana*). *Tropical Pest Management* **37:** 122-123

FAOSTAT (2004). Food and Agriculture Organization of the United Nations, report on agricultural production of 2004

Farshadfar, E. (2008). Incorporation of AMMI Stability Value and Grain Yield in a Single Non-Parametric Index (Genotype Selection Index) in Bread Wheat. *Pakistan Journal of Biological Sciences* **11**: 1791-1796.

Farshadfar, E. and Sutka, J. (2006). Biplot analysis of genotype-environment interaction in durum wheat using the AMMI model. *Acta Agronomy Hung.* **54**: 459-467.

Flores, F., Moreno, M.T., Cubero, J.I. (1998). A comparison of univariate and multivariate methods to analyze G x E interaction. *Field Crops Research* **56**: 271–286.

Fry, W.E. (1968). Quantification of general resistance of potato cultivars and fungicide effect for integrated control of potato late blight. *Phytopathology* **68**: 1650-1655.

Fukuoka, S., Saka, N., Koga, H., Ono, K., Shimizu, T., Ebana, K., Hayashi, N., Takahashi, A., Hirochika, H., Okuno, K., Yano, M. (2009). Loss of function of a proline-containing protein confers durable disease resistance in rice. *Science* **325**:998–1001

Ganapathy, S., Nirmalakumari, A. and Muthiah, A. (2011). Genetic variability and interrelationship analyses for economic traits in finger millet germplasms. *World Journal of Agricultural Sciences* **7:** 185-188

Garris, A.J., Tai, T.H. and Coburn, J. (2005). Genetic structure and diversity in *Oryza sativa* L. *Genetics* **169**:1631–163.

Gashaw, G., Alemu, T. and Tesfaye, K. (2014). Evaluation of disease incidence and severity and yield loss of finger millet varieties and mycelial growth inhibition of *Pyricularia grisea* isolates using biological antagonists and fungicides in vitro condition. *Journal of Applied Bioscience* 73:5883– 5901.

Gauch, H.G., Piepho, H.P. and Annicchiarico, P. (2008). Statistical analysis of yield trials by AMMI and GGE: Further considerations. *Crop Science* **48**:866–889.

Gauch, H. G. and Zobel, R.W. (1996). AMMI analysis of yield trials. In: Genotype by environment interaction. pp. 85-122 (Kang, M. and Gauch, H. eds.). Boca Raton. CRC press, New York.

Girma T, (1999). Modeling Genotype by environment interaction: A Review of procedures. In: Proceeding of the Eight Annual Conference of the Crop Science Society of Ethiopia, pp 147 – 158, Addis Ababa, Ethiopia.

Gorfu Dereje and Ahmed Eshetu (2008). Crops and Agro-ecological Zones of Ethiopia.Ethiopian Institute of Agricultural Research, Review Report.

Graham, M.A., Silverstein, K.A., Cannon, S.B., VandenBosch, K.A. (2004). Computational identification and characterization of novel genes from legumes. *Plant Physiology* **135**: 1179–1197

Guilford, P., Prakash, S., Zhu, J.M., Rikkerink, E., Gardiner, S., Basset, H. and Foster, R. (1997). Microsatellites in Malus × domestica (apple): abundance, polymorphism and cultivar identification. *Theoretical and Applied Genetics*, **94**:249-254.

Guillen, F.R., Baltensperger, D.D., Nelson, L.A. and Mason, D.C. (1999). Variability in Plainsman Grain amaranth. **In**: Janack, J. (ed.) Perspective on New Crops and New Uses. ASHS press, Alexandria, VA.

Gupta, R., Verma, K. and Joshi, D.C. (2010). Assessment of genetic relatedness among three varieties of finger millet with variable seed coat color using RAPD and ISSR Markers. *Genetic Engineering and Biotechnology Journal* **2**:1-9.

Han, Y., Bonos, S., Clarke, B.B. and Meyer, W.A. (2003). Inoculation techniques for selection of gray leaf spot resistance in perennial ryegrass. *USGA Turf grass and Environmental Research Online* 2:1-9.

Hans Hurni (1998). Research Report on Agroecological Belts of Ethiopia. Soil Conservation Research Programme and Centre for Development and Environment, Wittwer Druck AG, Bern, Switzerland (http://library.wur.nl/isric/fulltext/isricu_t4d384c8b_001.pdf)

Hansford, C.G. (1943). Contribution towards the fungus flora of Uganda. V.fungi imperfect. Proc. Linn. Soc., London. 43:34-67.

Hayes, H.K., Immer, F.R. and Smith, D.C. (1955). *Methods of Plant Breeding*. 2nd ed. McGraw-Hill Book company, Inc. Tokyo. 551pp.

He, Q., Li, X.W. and Liang, G.L. (2011). Genetic diversity and identity of Chinese loquat cultivars/accessions (*Eriobotrya japonica*) using apple SSR markers. *Plant Molecular Biology Reporter* **29**:197–208 doi: 10.1007/s11105-010-0218-9.

Hilu, K.W. and De Wet, J.M. (1976). Domestication of *E. coracana*. *Economic Botany* **30**:199–208.

Hilu, K.W., De Wet, J.M. and Harlan, J.R. (1979). Archaeo-botanical studies of *Eleusine coracana* sub spp. *coracana* (finger millet). *American Journal of Botany* **63**: 1311-1318.

Hilu, K.W. and Johnson, J.L. (1992). Ribosomal DNA variation in finger millet and wild species of *Eleusine* (Poaceae). *Theoretical and Applied Genetics* **83**:895–902.

Holm, L.G., Plucknet, D.L., Pancho, J.V. and Herberger, J.P. (1977). *Eleusine indica* (L.) Gaertn. **In**:The World's Worst Weeds: Distribution and Biology. University Press of Hawaii, Honolulu, USA.

Hugh Doggett (1986). Small millets-A selective overview. In: Small millets in global agriculture (Seetharam, A., Riley, K. W. and Harinarayana, G., eds) Proceedings of the first International Small Millets Workshop, Bangalore, India, 29 October – 2 November, 1986. Pp 1-17

Hundara, F., Tefera, H., Assefa, K., Tefera, T. and Kefyalew, T. (1999). Genetic variability and correlation of agronomic characters in tef landraces. *Tropical Science* **39**: 140-146.

Hunter, M. (1996). *Fundamentals of Conservation Biology*. Blackwell Science, Inc., USA.

Hussaini, S.H., M.M. Goodman and D.H. Timothy (1977). Multivariate analysis and geographical distribution of the world collection of finger millet. *Crop Science* **17**: 257- 263.

IBPGR **(**1985). Descriptors for finger millet (*Eleusine coracana* (L.) Gaertn). Rome, Italy: International Board for Plant Genetic Resources (IBPGR). 20 pp.

Jain, A.K., Gupta, J.C. and Yadava, H.S. (1991). Stability of resistance to blast in foxtail millet. *Mysore Journal of Agricultural Science* **25**: 221-223.

Jain, K.S., Quaset, C.O., Bhatt, G.M. and Wu, K.K. (1975). Geographical patterns of phenotypic diversity in a world collection of Durum wheat. *Crop Science* **15:** 700-704.

James, W.C. (1974). Assessment of plant diseases & losses. *Annual Review of Phytopathology* **12**: 27-48

James, W.C., Jenkins, J.E. and Jemmett, J.L. (1968). The relationship between leaf blotch caused by *Rhynchosporium secalis* and losses in grain yield of spring barley. *Annals of Applied Biology*. **62**:273-88.

James, W.C., Shih, C.S., Hodgson, W.A. and Callbeck, L.C. (1972). The quantitative relationship between late blight of potato and loss in tuber yield. *Phytopathology* **62**:92-96.

Jarvis, D.I., Myer, L.K., Klemick, H., Guarino, L., Smale, M., Brown, A., Sadiki, M. and Hodgkin, K. (2000). A training Guide for *insitu* Conservation, Version 1, pp. 75-92. International Plant Genetic Resources Institute, Rome, Italy.

Jennifer, A.M.G. (1998). Background and Overview of Comparative Genomics. *ILAR (Institute of Laboratory Animal Resource) Journal* **39**: 48-65.

Jian-Feng Huang, Ming-Li Zhang and James I. Cohen. (2013). Phylogenetic analysis of *Lappula* Moench (Boraginaceae) based on molecular and morphological data. *Plant Systematic and Evolution* **299** (5): 913-926.

Jiang, W. and Smith, B.F. (2002). Cloning PCR Products. In: Springer Lab Manual. (Bird, R.C. and Smith, B.F, eds.). Genetic Library Construction and Screening © Springer-Verlag Berlin Heidelberg

John, K. (2006). Variability and correlation studies in quantitative traits of finger millet. *Science Digest* **26:** 166 – 169.

Jones, M.P., Dingkuhn, M., Aluko, G.K.and Semon, M. (1997). Interspecific Oryza sativa L. x O. glaberrima Steud. Progenies in upland rice improvement. *Euphytica* **92**: 237-246.

Johnson, R.A. and. Wichern, D.W (1988). *Applied Multivariate Statistical Analysis*. Prentice-Hall.

Kannan Baskaran, Senthilvel Senapathy, Arcot Gajaraj, Bhasker Raj, Subhash Chandra, Arunachalam Muthiah, Arun Prabhu Dhanapal and Charles Thomas Hash (2014). Association Analysis of SSR Markers with Phenology, Grain, and Stover-Yield Related Traits in Pearl Millet (*Pennisetum glaucum* (L.). *The Scientific World Journal* **1**: 1-14

Kanchan kumara and Anita Pande (2010). Study of the genetic diversity in finger millet *Eleusine coracana* L. Gaertn) using RAPD markers. *African Journal of Biotechnology* **9**(29): 4542 – 4549.

Kang, S., James, A., Sweigard, S., and Barbara Valent (1995). The PWL Host specificity gene family in the blast fungus, *Magnaporthe grisea. American Phytopathological Society* **8**: 939-948.

Karuri, H., Ateka, E., Amata, R., Nyenda, A., Muigai, A., Mwasame, E. and Gichuki, S.T. (2010). Evaluating diversity among kenyan sweet potato genotypes using morphological & SSR markers. *International Journal of Agricultural Biology* **12**:33–38

Kato, H., Yamaguchi, T. and Nishihara, N. (1977). Seed transmission, pathogenicity and control of ragi blast fungus and susceptibility of ragi to *Pyricularia* spp. from grasses, cereals and mioga. *Annals of the Phytopathological Society of Japan* **43(4)**:392-401

Katsube, T. and Koshimizu, Y. (1970). Influence of blast disease on harvests in rice plant. I: Effect of panicle infection on yield components and quality. *Bulletin of Tohoku National Agricultural Experiment Station* 39:55-96.

Kaya, Y., Aksura, M. and Taner, S. (2006). GGE-Biplot analysis of multi-environment yield trials in bread wheat. Bahari Dagdas International Agricultural Research Institute. *Turkish Journal of Agriculture and Forestry* **30**: 325-337.

Kearsey, M.J. and Pooni, H.S. (1996). *The Genetical Analysis Of Quantitative Traits*. Chapman and Hall, London, Weinhein, New York, pp. 381.

Kebede, Y. and Menkir A. (1986). Improvement of finger millet in Ethiopia, pp. 173-176. In: Seetheram, A., Rilley, K.W. and Upadhyaya, G. (eds.). Small Millet in Global Agriculture: Proceeding of the First International Small Millet Workshop. October 29-November 2, 1986. Bangalore, India.

Keneni, G., Bekele, E., Muhammad, I., Dagne, K., Getu, E. and Assefa, F. (2011) Genetic Diversity and Population Structure of Ethiopian Chickpea (*Cicer arietinum* L.) Germplasm Accessions from Different Geographical Origins as Revealed by Microsatellite Markers. *Plant Molecular Biology Reporter* **30**:654-666. dio: 10.1007/s11105-011-0374-6.

Keneni, G., Jarso, M. and Wolabu, T (2007). Eco-geographical and microcenters of genetic diversity in faba bean (*Vicia Faba* (L) and field pea (*Pisum sativum* (L.)germplasm collection from Ethiopia. *East African Journal of Science* **1**(1):10-24.

Kenneth O. Rachie and LeRoy V. Peters (1977). THE *ELEUSINES*. A review of the World Literature. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

Khorgade, P., Narkhede, W. and Raut, S. (1985). Genetic variability in chickpea. International Chickpea Newsletter, No. 5.pp 3-4.

Kong, Q., Li, X., Xiang, C., Wang, H., Song, J. and Zhi, H. (2011). Genetic diversity of radish (*Raphanus sativus* L.) germplasm resources revealed by AFLP and RAPD markers. *Plant Molecular Biology Reporter*. **29**:217–223. doi: 10.1007/s11105-010-0228-7.

Kubik, C., Honig, J., Meyer, A. and Bonos, S.A. (2009). Genetic diversity of creeping bentgrass cultivars using SSR Markers. *International Turfgrass Society Research Journal* **11**: 533-547.

Large, E.C. (1952). The interpretation of progress curves for potato blight and other plant diseases. *Plant Pathology* **1**:109-117.

Leister, D., Kurth, J., Laurie, D.A., Yano, M., Sasaki, T., Devos, K.,Graner, A. and SchulzeLefert, P. (1998) Rapid reorganization of resistance gene homologues in cereal genomes. *Proceeding of National Academy of Science*, USA, **95**: 370–375.

Lenka, D. and Mishra, B. (1973). Path coefficient analysis of yield in rice varieties. *Indian Journal of Agricultural Science* **43**: 376-379.

Leung, W., Busson, F. and Jardin, C. (1968). Food composition table for use in Africa.FAO, Rome, Italy. pp 306.

Levinson, G. and Gutman, G.A. (1987). Slipped-strand mispairing: A major mechanism for DNA sequence evolution. *Molecular Biology and Evolution* **4**:203−221.

Liu, K. and Muse, S.V. (2005) Power Marker: Integrated analysis environment for genetic molecular data. *Bioinformatics* **21**: 2128-2129

Lule, D., Bekele, E. and Ayana, A. (2008). Variability, heritability & genetic advance in quantitative traits of tef: Implication for genetic conservation and improvement strategies. *Ethiopian Journal of Biological Science* **7(2)**:159-177.

Lule, D., Bekele, E. and Ayana, A. (2011). The extent and pattern of genetic diversity for quantitative traits in tef [*Eragrostis tef* (Zucc.) Trotter] landrace populations. In: Proceeding of 13th Conference of Crop Science Society of Ethiopia **13**: 183-195.

Lule, D., De Villiers, S., Tsehay, S., Dida, M., Fetene, M., Kimani, W. and Tesfaye, K. (2014). Genetic diversity and eco-geographical distribution of *Eleusine* species collected from Ethiopia. *African Crop Science Journal* **22** (1):45 – 57.

Lule, D., Tesfaye, K., Fetene, M. and De Villiers, S. (2013). Evaluation of Ethiopian finger millet (*Eleusine coracana*) germplasm against *Magnaporthe oryzea*, the cause of finger millet blast disease. Paper presented on the first Bio-innovate Regional Scientific Conference, United Nation Conference Center (UNCC-ECA), Addis Ababa, 25-27 Feb. 2013.

Lule, D., De Villiers, S., Fetene, M., Bogale, T., Alem, T., Geremew, G., Gashaw, G. and Tesfaye, K. (2014). Pathogenicity and yield loss assessment caused by *magnaporthe oryzea* isolates in cultivated and wild relatives of finger millet (*Eleusine coracana*). *Indian Journal of Agricultural Research* **48** (4) 258 – 268.

Mace, E.S., Buhariwalla, H.K. and Crouch, J.H. (2003). A high-throughput DNA extraction protocol for tropical molecular breeding programs. - *Plant Molecular Biology Report* 21:459a-459h.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**:209-220.

Marcel, T.C., Varshney, R.K., Barbieri, M., Jafary, H., de Kock, M., Graner, A. and Niks, R. (2007). A high-density consensus map of barley to compare the distribution of QTLs for partial resistance to *Pucciniahordei* and of defence gene homologues. *Theoretical and Applied Genetics* **114**(3):487–500.

Markert. C.L and F. Moller (1959). Multiple forms of enzymes: Tissues. Ontogenetic and species specific patterns. *Proceeding of Natural Academy of Science* USA. **45**:753-763.

Martins-Lopes, P., Zhang, H. and Koebner, R. (2001).Detection of single nucleotide mutations in wheat using single strand conformation polymorphism gels. *Plant Molecular Biology Reporter* **19:** 159–162.

Maxted, N. and Kell, S.P. (2009). *Establishment of Global Network for the in Situ Conservation of Crop Wild Relatives: Status and Needs*.FAO commission on Genetic Resources for Food and Agriculture, Rome, Italy.

Mengistu, G., Daba, C., Temesgen, A., Lule, D. and Geleta, N. (2011). Genotype x Environment interaction for yield in field pea (*Pisum sativum* L.). *East African Journal of Science* **5**(1): 6-11.

Mehra, K.L. (1963). Consideration on the African origin of *E. coracana* (L.) Gaertn. *Current science* **32**:300-301

Mgonja, M., Manyasa, E., Kibuka, J., Kaloki, P., Nyaboke, S. and Wandera, G. (2007). Finger Millet in East Africa: Importance, Blast Disease Management and Promotion of

Identified Blast Resistant Varieties in Western and Nyanza Provinces of Kenya. PP 23 -33. In: Mgonja, M.A., Lenné, J.M., Manyasa, E. and Sreenivasaprasad, S. (eds.). Finger Millet Blast Management in East Africa. Creating opportunities for improving production and utilization of finger millet. Proceedings of the First International Finger Millet Stakeholder Workshop, Projects R8030 & R8445 UK Department for International Development – Crop Protection Programme held 13-14 September 2005 at Nairobi. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 196 pp. ISBN: 978-92-9066-505-2.

Michiei Oto, Satoshi Miyake and Yasuhito Yuasa (1993). Optimization of nonradioisotopic single strand conformation polymorphism analysis with a conventional minislab gel electrophoresis apparatus. *Analytical Biochemistry* **213**: 19-22.

MINITAB (2003).MINITAB statistical software, version 14.13.0.0, Minitab Inc.

Misra, R., Das, S. and Patnaik, M. (2009). AMMI model analysis of stability and adaptability of late duration finger millet (*Eleusine coracana*) Genotypes. *World Applied Science journal* **6**: 1650-1654.

Mostafa, K., Mohammad, H. and Mohammad, M. (2011). Genetic diversity of wheat genotype baspdon cluster and principal component analyses for breeding strategies. *Australian Journal of Crop Science* **5** (1): 17-24

Mousanejad, S., Alizadeh, A. and Safaie, N. (2010). Assessment of yield loss due to rice blast disease in Iran. *Journal of Agricultural Science and Technology* **12**: 357-364.

Mulatu, T., Debelo, A., Gutema, Z. and Degu, E.(1995). Finger millet: A potential crop in Ethiopia. In: Proceedings of Workshop Organized to Re-establish Sorghum and Millet in Eastern & Central Africa. Kampala, Uganda 6-9 Nov. 1995.

Mulatu T, Getachew B, Werede M (1991). Morphological diversity in wheat landrace populations from central highlands of Ethiopia. *Hereditas* 114: 172-176.

Mulatu, T. and Kebede, Y. (1993). Finger millet importance and improvement in Ethiopia. In: Riley K.W., Gupta S.C., Seetharam A. and Mushonga J.N. (eds), *Advances in Small Millets*. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi pp. 51–59.

Mustapha, A.A., Showemimo, F.A. and Aminu-kano, A. (2001). Yield stability analysis of promising Triticale cultivars in Nigeria. *Journal of Arid Agriculture* **11**: 1–4.

Muyonga, C. K., Nzabi, A. W., Kidula, N. L., Okoko, N. and Mwasi, G.(2000). Screening of finger millet varieties for tolerance to Blast disease in Nyatieko location of South West Kenya. Research Progress Report of Kenya Agricultural Research Institute, Regional Research Center, Kissi, Kenya.

Mysore, K.S. and Baird, V. (1997). Nuclear DNA content in species of *Eleusine* (Gramineae): a critical re-evaluation using laser flow cytometery. *Plant Systematis and Evolution* **207**:1-11.

Naroui, R.M.R., Abdul Kadir M., Rafii Hawa, M.Y., Jaafar, Z.E., Naghavi, M.R.and Farzaneh Ahmadi. (2013). Genotype by environment interaction by AMMI and GGE biplot analysis in three consecutive generations of wheat (*Triticum aestivum*) under normal and drought stress conditions. *Australian Journal of Crop Science* **7(7):**956-961.

National Research Council (1996). *Lost Crops of Africa, Volume I, Grains*. National Academy Press, Washington, DC.

Negash, W., Asfaw, Z. and Yibra, H. (2005). Variation and association analysis on morphological characters of linseed (*Linum usitatissimum* (L.) in Ethiopia. *SINET: Ethiopian Journal of Science* **28 (2):**129-140.

Neves, S.S. (2011). *Eleusine.* In: Kole C. (ed.), *Wild Crop Relatives: Genomic and Breeding Resources, Millets and Grasses*, DOI 10.1007/978-3-642-14255-0_7, # Springer-Verlag Berlin Heidelberg.

Neves, S.S., Swire-Clark, G., Hilu, K.W. and Baird, W.V. (2005). Phylogeny of *Eleusine* (*Poaceae*: *Chloridoideae*) based on nuclear ITS and plastid trnT-trnF sequences. *Molecular Phylogenetic and Evolution* **35**:395–419.

Notteghem, J.L. (1990). Results and orientation of EC projects on rice resistance to blast. *Summa Phytopathology* **16**:57-72.

Odewale, J.O., Ataga, C.D., Agho, C., Odiowaya, G., Okoye, M.N. and Okolo, E.C. (2013). Genotype evaluation of coconut (*Cocos nucifera L.*) and mega environment investigation based on additive main effects and multiplicative interaction (AMMI) analysis. *Research Journal of Agricultural and Environmental Management* **2(1)**: 1-10

Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K., and Sekiya, T. (1989). Detection of polymorphism of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proceeding of National Academy of Science*. USA 86, 2766-2770.

Ou, S.H. (1985). *Rice Diseases*. 2nd ed. Common Wealth Mycological Institute, Surrey, England.

Pall, B.S. (1977). Assessment of losses due to neck blast of ragi (*Eleusine coracana*). *Food Farming and Agriculture* **9:** 55.

Pande, S., Makuru, S. K., King, S. B. and Karunakar, R. I. (1995). Biology of, and resistance to finger millet blast in Kenya and Uganda, Proceedings of the Eighth Regional Workshop on Sorghum and Millets, 30 Oct –5 Nov, 1992, WAD Medani, Sudan

Pandey, R.L. and Tiwari, A.S (1983). Heritability and genetic gain in chickpea. *International Chickpea Newsletter*, No. 9: 5-6.

Panes, V.G. (1957).Genetics of quantitative characters in relation to plant breeding. *Indian Journal of Genetics* **17**:318-328.

Panwar, L.L., Joshi, V.N. and Mashiat Ali (2008). Genotype x environment interaction in scented rice. *Oryza* **45**(1): 103-109.

Panwar, P., Jha, A.K., Pandey, P.K., Gupta, A.K. and Kumar, A. (2011). Functional markers based molecular characterization and cloning of resistance gene analogs encoding NBS-LRR disease resistance proteins in finger millet (Eleusine coracana). *Molecular Biology Reporter* **38**(5):3427-3436 doi: 10.1007/s11033-010-0452-0.

Panwar, P., Manoj Nath, Vijay Kumar and Anil Kumar (2010). Comparative evaluation of genetic diversity using RAPD, SSR and cytochrome P450 gene based markers with respect to calcium content in finger millet (*Eleusine coracana* L. Gaertn). *Journal of Genetics* **89**: 121-133

Parani, M., Rajesh, K., Lakshmi, M., Parducci, L., Szmidt, A. and Parida, A. (2001). Species identification in seven small millet species using polymerase chain reaction -restriction fragment length polymorphism of trnS-psbC gene region. *Genome* **44**: 495–499.

Parker, M. A. (1986). Individual variation in pathogen attack and differential reproductive success in the annual legume (*Amphicarpaea bracteata). Oecologia* **69**: 253-259.

Patel, M.K. (1955). A short note on diseases of millets. *Poona Agricultural College Magazine.* 46(2):188

Perrier, X. and Jacquemoud, J. (2006). DARwin software. Available at http:// Darwin.cirad.fr/Darwin

Pham, T. D., Geleta, M., Bui, T. M., Arnulf, M. and Anders. S. C (2011). Comparative analysis of genetic diversity of sesame (*Sesamum indicum* L.) from Vietnam and Cambodia using agro-morphological and molecular markers. *Hereditas* **148**:28–35.

Phillips, S. (1995). Poaceae (Gramineae) *Flora of Ethiopia and Eritrea*, vol 7. In: Hedberg I. and Edwards, S. (Eds.). The National Herbarium, Addis Ababa University/Department of Systematic Botany, Uppsala University, Addis Ababa/Uppsala, Sweden.

Poland J.A., Patrick J. Brown, Mark E. Sorrells, Jean-Luc Jannink (2012). Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. *PLoS ONE* **7**:1-8.

Praba, M.L., Cairns, J.E., Babu, R.C. and Lafitte, H.R. (2009). Identification of physiological traits underlying cultivar differences in drought tolerance in rice and wheat. *Journal of Agronomy and Crop Science* **195**: 30-46.

Prabhakar, M. and Prasad, N (1984). Variability, heritability, genetic advance and gene action studies in segregating population of ragi (*Eleusine coracana* Gaertn). *Madras Agricultural Journal,* **71**: 285-288

Prasado Rao, De Wet, V., Gopal Reddy and Mengesha, M.H. (1994). Diversity in the small millets collection at ICRISAT, pp.331- 345. **In:** K.E. Riley, S.C. Gupta, A. Seetharam and J.N. Mushonga (eds.). *Advances in Small Millets*. International Science. *Kasetsart Journal of Natural Science* **40**: 331-345.

Pritchard, K. J, Matthew Stephens, Noah, A.R. and Peter Donnelly (2000). Association Mapping in Structured Populations. *American Journal of Human Genetics*. **67**:170–181

Purchase, J.L. (1997).Parametric analysis to describe G X E interaction and stability in winter wheat. PhD Dissertation. Department of Agronomy, Faculty of Agriculture, University of the Orange Free State, Bloemfontein, South Africa.

Purseglove, J.W. (1972). *Tropical Crops*: Monocotyledons. Longman Group Limited, London.

Pushpendra, K.G., Upadhyayandra, S.B., Pawan, L.K., Neeraj, K., Ajay, K., Reyazul, R.M., Amita, M., Jitendra, K. (2007). QTL analysis for some quantitative traits in bread wheat. *Journal of Zhejiang University Science* **8**(11):807–814.

Quendeba, B., Ejeta, G., Hannan, W.W. and Kumar, A.K. (1995). Diversity among African pearl millet landrace populations. *Crop Science* **35**: 919-924.

Rallo, P., Dorado, G. and Martin, A. (2000). Development of simple sequence repeats (SSRs) in olive tree (*Olea europaea* L.). *Theoretical and Applied Genetics* **101**: 984−989.

Ramakrishnan, K. V. (1948). Studies on the morphology, physiology and parasitism in the genus *Pyricularia* in Madras. *Proceeding of Indian Academy of Science Bulletin* **27**:174-193.

Rath, G.C. and Mishra, D. (1975). Nature of losses due to neck blast infection in ragi. *Science and Culture.* **41**(7):322-323.

Reddy INBL, Reddy, D.S., Narasu, M.L. and Sivaramakrishnan, S. (2010) Characterization of disease resistance gene homologues isolated from finger millet *(Eleusine coracana L. Gaertn)*. *Molecular Breeding* **27**: 315-328.

Reddy, P.M., Sarla, N. and Siddiq, E.A. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* **128**-2: 9-17

Reddy G, Upadhaya, H.D., Gowda, C.L.L., Sube Singh (2009). Characterization of East African finger millet germplasm for qualitative and quantitative characters at ICRISAT. *Journal of SAT Agricultural Research*, pp 7-9. (An open access journal published by ICRISAT).

Robles, J. and Doers, M. (1994). pGEMR-T Vector Systems troubleshooting guide. *Promega Notes* **45:**19–20.

Rogers, J.S. (1972). Measures of genetic similarity and genetic distance. In: *Studies in Genetics* VII, pp. 145-153. University of Texas Publication 7213, Austin, TX.

Rossman, A.Y., Howard, R.J. and Valent, B. (1990). *Pyricularia grisea*, the correct name of the rice blast disease fungus. *Mycologia* **82**:509–512.

Roy, B.A. and Kirchner, J.W. (2000). Evolutionary dynamics of pathogen resistance and tolerance. *Evolution* **54**: 51–63.

Rozen, S., Skaletsky, H.J. (2000). Primer-3 on the WWW for general users and for biologist programmers. In: Krawetz , S. and Misener, S. (eds) Bioinformatics Methods and Protocols: *Methods in Molecular Biology*. Humana Press, p.365-386.

Salimath, S.S, de Oliveira, A.C. Godwin, I.D. and Bennetzen, J.L. (1995). Assessment of genome origins and genetic diversity in the genus *Eleusine* with DNA markers. *Genome* **38**: 757–763.

Sangwan, N., Yadav, U. and Sangwan, R. (2001). Molecular analysis of genetic diversity in elite Indian cultivars of essential oil trade types of aromatic grasses (*Cymbopogon*species). *Plant Cell Report* **20**: 437-444.

Santana, Q.C., Coetzee, M.P.A., Steenkamp, E.T., Mlonyeni, O.X. and Hammond, G.N.A. (2009). Microsatellite discovery by deep sequencing of enriched genomic libraries. *Biotechniques* **46**:217–223

Sanwen Huang, Edwin A.G. van der Vossen, Hanhui Kuang, Vivianne G.A.A. Vleeshouwers, Ningwen Zhang, Theo J.A. Borm, Herman J. van Eck, Barbara Baker, Evert Jacobsen and Richard G.F. Visser (2005). Comparative genomics enabled the isolation of the R3a late blight resistance gene in potato. *The Plant Journal* **42**: 251–261

SAS Institute Inc. (2008). SAS/STATA Guide for personal computers version 9.2 edition. SAS Institute Carry NC, USA.

Shaner, G. and Finney, R. (1977). The effect of nitrogen fertilization on the expression of slow-mildewing in knox wheat. *Phytopathology* **36**: 1307-1311.

Shang, J., Tao, Y., Chen, X., Zou, Y., Lei, C., Wang, J., Li, X., Zhao, X., Zhang, M., Lu, Z., Xu, J., Cheng, Z., Wan, J., Zhu, L. (2009). Identification of a new rice blast resistance gene, Pid3, by genome-wide comparison of paired nucleotide-binding site-leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. *Genetics* 182:1303–1311

Sharathbabu, S., K. Sonnad, G. Shanthakumar and P. M. Salimath (2008). Genetic variability and character association studies in white ragi (*Eleusine coracana* Gaertn). *Karnataka Journal of Agricultural Science* **21(4):** 572-575.

Sharma R, Deshpande, S.P., Senthilvel, S., Rao, V.P., Rajaram, V., Hash, C,T. and Thakur, R.P. (2010). SSR allelic diversity in relation to morphological traits and resistance to grain mould in sorghum. *Crop and Pasture Science* **61**: 230-240

Sheffield, V.C., Beck, J.S., Kwitek, A.E., Sandstrom, D.W. and Stone, E.W. (1993). The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. *Genomics* **16**: 325-332.

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* **18**:233-234.

Showemimo, F.A., Echekwu, C.A. and Yeye, M.Y. (2000). Genotype x environment interaction in Sorghum trials and their implication for future variety evaluation in Sorghum growing areas of northern Nigeria. *The Plant Scientist* **1**: 24–31.

Shtienberg, S.N., Bergeron, A.G., Nicholson, S., Fry, W.E. and Ewing, E.E. (1990). Development and Evaluation of a general model for yield loss assessment in potatoes. *Phytopathology* **80(5)**:466-472.

Sial, M.A., Arain, M.A. and Ahmad, M. (2000). Genotype by environment interaction on bread wheat grown over multiple sites and years in Pakistan. *Pakistan Journal of Botany* **32**(1): 85-91

Singh, P. (2001). *Numerical Problems in Plant Breeding and Genetics*. Kalyani Publishers, New Delhi, India.

Singh, R.K and Chaundhary, B. D. (1985). *Biometrical Methods in Quantitative Genetic Analysis*. Kalyani publishers, New Delhi-Ludhiana

Sinha, A. and Pande, A. (2010) Finger-printing of *Eleusine coracana* L. (Gaertn) using microsatellite markers. *Bioresearch Bulletin* **2**: 51-58 51.

SOH, A. C. (2011). Genomics and plant breeding. *Journal of Oil Palm Research* **23**:1019-1028

Srinivasachary, Mathews, M. Dida, Mike, D. Gale and Katrien, M. Devos (2007). Comparative analyses reveal high levels of conserved colinearity between the finger millet and rice genomes. *Theory and Applied Genetics* **115**:489–499

Staub. J.E. and Crubaugh, L. (1995). Selection for multiple lateral determinate cucumber genotypes. *Cucurbit Gen. Coop. Rpt.* **18**:5-6.

Stetina, K,C., Stetina, S. and Russin, J. (2006). Comparison of severity assessment methods for predicting yield loss to *Rhizoctonia* foliar blight in soybean. *Plant Disease* **90**: 39-43

Stout, M.J., Rice, W.C., Linscombe, S.D., Bollich, P.K. (2001). Identification of rice cultivars resistant to *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae), and their use in an integrated management program. *Journal of Economic Entomology* **94:** 963-970.

Sunnucks, P., Wilson, A.C., Beheregaray, L.B., Zenger, K., French, J. and Taylor, A. C. (2000). SSCP is not so difficult: The application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. *Molecular Ecology* 9: 1699 – 1710.

Takan, J.P., Akello, B., Esele, P., Manyasa, E.O., Obilana, A., Audi, P.O., Kibuka, J., Odendo, M., Oduori, C.A., Ajanga, S., Bandyopadhyay, R., Muthumeenakshi, S., Coll, R., Brown, A. E., Talbot, N.J., and Sreenivasaprasad, S. (2004). Finger millet blast pathogen diversity and management in East Africa: a summary of project activities and outputs. *International Sorghum and Millets Newsletter* 45:66-69.

Takan, J.P., Chipili, J., Muthumeenakshi, S., Talbot, N.J., Manyasa, E.O., Bandyopadhyay, R., Sere, Y., Nutsugah, S.K., Talhinhas, P., Hossain, M., Brown, A.E. and Sreenivasaprasad, S. (2011). *Magnaporthe oryzea* populations adapted to finger millet and rice exhibit distinctive patterns of genetic diversity, sexuality and host interaction. *Molecular Biotechnology* **50**(2):145-58.

Temesgen, A., Daba, C., Mengistu, G., Lule, D. and Geleta, N. (2008). Regression and additive main effects and multiple interactions (AMMI) in common bean (*Phaseolus vulgaris* L.) genotypes. *Ethiopian Journal of Biological Science* **7**(1): 45-53.

Teng, P.S. and Gaunt, R.E. (1980). Modeling systems of disease and yield loss in cereals. *Agricultural Systems* **6**: 131-154.

Thakur, R.P., Sharma, R., Rai, K.N., Gupta, S.K. and Rao, V.P. (2009). Screening techniques and resistance sources for foliar blast in pearl millet. An open access journal published by ICRISAT, No.7.

Thirumalachar, M. J. and Mishra, J. N. (1954). Some diseases of economic plants in Bihar, India. *FAO Plant Protection Bullet* **1**(10):145-146.

Thompson, J.A., Nelson, R.L. and Vodkin, L.O. (1998) Identification of diverse soybean germplasm using RAPD markers. *Crop Science* **38**:1348-1355.

Tredway, L.P., Stevenson, K.L. and Burpee, L.L. (2003). Components of resistance to *Magnaporthe grisea* in 'Coyote' and 'Coronado' tall fescue. *Plant Disease* **87**:906-912.

Tsai, W.H. (1988). Estimation of rice yield losses caused by panicle blast disease. *Journal of Agricultural Research China* **37**: 86-90.

Tsehay, S. (2012). Genetic diversity and relationships among cultivated and wild *eleusine* species collected from Ethiopia as revealed by ISSR marker. MSc thesis, Addis Ababa University, Ethiopia.

Tsehaye, Y. and Kebebew, F. (2002). Morphological diversity and geographical distribution of adaptive traits in finger millet (*Eleusine corracana* (L.) Gaertn.Subsp. *coracana* (poaceae) population from Ethiopia. *Ethiopian Journal of Biological Science* **1:**37-62.

Tukamuhabwa, P., Asiimwe, M., Nabasirye, M., Kabayl, P. and Maphosa, M. (2012). Genotype by environment interaction of advanced generation soybean lines for grain yield in Uganda *African Crop Science Journal* **20**(2):107-115.

Upadhyaya, H.D., Gowda, C.L. and Gopal Reddy (2007). Morphological diversity in finger millet germplasm introduced from Southern and Eastern Africa SAT eJournal | ejournal.icrisat.org Volume 3, Issue 1

Upadhyaya, H.D., Gowda, C.L.L., Pundir, R.P.S., Reddy, V.G. and Singh, S. (2006). Development of core subset of finger millet germplasm using geographical origin and data on 14 quantitative traits. *Genetic Resource and Crop Evolution* **53**: 679–685.

Urashima, A., Igarashi, S. and Kato, H. (1993). Host range, mating type and fertility of *Pyricularia grisea* from wheat in Brazil. *Plant Disease* **77**:1211-1216

USDA. 2003. United States Department of Agriculture (USDA). Production Estimates and Crop Assessment Division (PECAD). Foreign Affairs Service (FAS). Agro-climatic Zones. http://www.fas.usda.gov/pecad/

Van der Plank, J.E. (1963). *Plant Diseases: Epidemics and Control*. *Academic Press*, New York. 349 pp.

Van der Schoot, J., Pospiskova, M. and Vosman, B. (2000). Development and characterization of microsatellite markers in black poplar (*Populus nigra* L.). *Theoretical and Applied Genetics* **101**:317−322.

Vanniarajan, C., Vinod, K. K. and Andy Pereira (2012). Molecular evaluation of genetic diversity and association studies in rice (*Oryza sativa* L.). *Journal of Genetics* **91**:9-19

Varshney, R.K. (2010). Gene-based marker systems in plants: high throughput approaches for marker discovery and genotyping. In: *Molecular Techniques in Crop Improvement,* 2nd edition, (Jain S.M. and D.S. Brar, eds.). Springer Science + Business Media. PP 123-142

Varshney, R.K., Chabane, K., Hendre, P.S., Aggarwal, R.K. and Graner, A. (2007). Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. *Plant Science* **173**: 638-649.

Vavilov, N. (1951). The origin, variation, immunity and breeding of cultivated plants. pp. 37-38. Ronald Press, NewYork, USA. [Translated from the Rusian by K. Starrchester].

Venkatarayan, S.V. (1947). Diseases of ragi. *Mysore Agricultural Journal* **24**: 50-57.

Vieira, E., Carvalho, F., Bertan, I., Kopp, M., Zimmer, P., Benin, G., Da Silva, J., Hartwig, I., Malone, G. and De Oliviera, A. (2007). Association between genetic distances in

wheat (*Triticum aestivum* L.) as estimated by AFLP and morphological markers. *Genetics and Molecular Biology* **30**: 392–399.

Wallace, G.B. and Wallace, M. (1948). Tanganyika Territory fungus list. Recent records XI. Mycol. Cir.Dept.Agric. Tanganyika Ser. 24, pp.5.

Werth, C.R., Hilu, K.W., Langner, C.A. (1994). Isozymes of *Eleusine* (Gramineae) and the origin of finger millet. *American Journal of Botany* **81**:1186–1197.

Wolie, A. and Dessalegn, T. (2011). Correlation and path coefficient analyses of some yield related traits in finger millet (*Eleusine coracana* (L.) Gaertn.) germplasms in northwest Ethiopia. *African Journal of Agricultural Research* **6:** 5099-5105.

Wondimu, A. and Tekabe, F. (2001). Utilization of tef in the Ethiopian diet. **In**: *Proceedings of the International Workshop on Tef Genetics and Improvement*, pp. 239-244, (Tefera, H., Belay, G. and Sorrells, M. eds). Debre Zeit, Ethiopia. 16-19 October 2000.

Worku, A., Henry, M., Zewotir, T. and Taye, G. (2013). Additive main effects and multiplicative interactions model (AMMI) and genotype main effect and genotype by environment interaction (GGE) biplot analysis of multi-environmental wheat variety trials. *African Journal of Agricultural Research* **8**(12):1033-1040.

Wright, S. (1965). The interpretation of population structure by F-statistics with special regard to systems mating. *Evolution* **19**: 395-420.

Wrigley, C.W., Autran, J.C. and Bushuk, W. (1982). Identification of cereal varieties by gel electrophoresis of the grain proteins. *Advanced Cereal Science and Technology* **5**:211-259.

Yaegashi, H. and Nishihara, N. (1976). Production of the perfect stage in *Pyricularia* from cereals and grasses. *Annals of the Phytopatho Society of Japan* **42**:511–515.

Yan Liu, Bin Liu, Xiaoyuan Zhu, Jianyuan Yang, Alicia Bordeos, Guoliang Wang, Jan E. Leach and Hei Leung (2013). Fine-mapping and molecular marker development for Pi56(t), a NBS-LRR gene conferring broad-spectrum resistance to *Magnaporthe oryzae* in rice. Theoretical and Applied Genetics, **126**:985–998

Yan, W., Hunt, L.A., Sheng, Q., Szlavnics, Z. (2000). Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Science* **40**: 597-605.

Yan, W. and Rajcan, I. (2002). Biplots analysis of the test sites and trait relations of soybean in Ontario. *Crop Science* **42**:11-20.

Yan, W. and Tinker, N.A. (2006). Biplot analysis of multi-environment trial data: principles and application. *Candian Journal of Plant Science* **86**:623-645.

Yang, Q.Z., Lin, F., Feng, S.J., Wang, L. and Pan, Q.H. (2009). Recent progress on molecular mapping and cloning of blast resistance genes in rice (oryza sativa L.). *Sci Agric Sin* 42:1601–1615

Yuksel Kaya, Cetin Palta, Seyfi Taner (2002). Additive main effects and multiplicative interactions analysis of yield performances in bread wheat genotypes across environments. *Turkish Journal Agriculture and Forestry* **26**: 275-279.

Zadoks, C. (1985). On the conceptual basis of crop loss assessment: The Threshold Theory. *Annual Review of Phytopathology*. **23**:455-73

APPENDIXES

Appendix 1. The average performance of 150 finger millet accessions for 17 quantitative traits and mean Shannon weaver index 6 qualitative traits

T.No	DH	DM	TTN	PTN	PLHT	FL	FN	CB	EW	NGPS	CD	FW	TGW	BWPPL	GYPPL	HI	Lodg	Mean H'
1	90	157	11.35	10.50	63.98	9.10	6.55	3.73	1.10	3.70	1.81	0.72	2.30	78.00	25.91	33.22	60.00	0.25
2	107	157	7.00	6.35	70.05	6.18	6.10	1.48	3.93	5.40	2.85	0.85	2.00	138.89	18.51	13.33	7.50	0.33
3	97	151	7.93	7.70	60.55	8.95	6.78	2.63	2.85	3.95	1.83	0.81	2.00	108.14	30.15	27.88	58.75	0.36
4	104	160	3.48	3.45	75.05	5.38	6.33	1.45	5.00	5.90	3.34	1.08	3.20	83.91	15.34	18.28	8.75	**
5	88	159	5.30	5.25	59.98	8.48	7.35	2.25	2.48	4.25	1.83	0.71	2.30	49.28	19.19	38.94	46.25	0.32
6	86	155	6.30	6.13	71.05	10.53	7.48	3.00	2.13	5.15	2.37	0.72	2.20	59.24	30.78	51.96	73.75	0.28
7	110	165	8.68	8.55	77.43	8.48	6.65	1.95	1.53	3.20	2.34	0.68	1.90	103.17	16.47	15.96	58.75	0.24
8	95	162	8.73	8.48	75.30	10.85	7.15	2.98	1.28	4.50	2.44	0.64	1.90	89.83	17.58	19.57	72.50	0.27
9	89	152	5.65	5.58	52.40	7.65	6.00	2.20	1.53	3.40	1.74	0.68	2.30	99.44	14.06	14.14	60.00	0.31
10	115	163	4.03	4.03	65.20	5.75	7.25	1.63	4.63	6.35	2.55	1.04	2.30	71.83	15.32	21.33	26.25	0.33
11	113	162	7.80	7.68	61.35	5.53	5.10	1.15	3.40	4.55	2.55	0.92	2.20	114.08	13.23	11.60	8.75	0.29
12	98	157	6.68	6.68	68.80	8.98	7.28	2.55	1.75	5.00	2.14	0.74	1.80	92.59	21.30	23.00	53.75	0.25
13	89	159	5.88	5.43	51.65	3.70	6.23	2.63	2.03	3.65	2.27	0.94	2.60	62.00	15.42	24.87	33.75	0.29
14	114	160	7.68	7.43	74.88	6.78	6.25	2.75	1.40	4.55	2.24	0.73	1.80	123.33	13.94	11.30	43.75	0.26
15	107	156	4.80	4.65	70.70	6.80	6.85	1.50	2.63	4.70	2.02	0.92	1.70	79.49	12.37	15.56	28.75	0.37
16	84	152	7.03	6.53	64.30	9.15	7.58	2.70	1.65	4.50	2.46	0.70	2.10	68.66	19.43	28.30	58.75	0.25
17	103	160	6.38	6.38	66.20	9.35	7.83	3.08	2.85	3.90	2.12	0.84	2.50	107.55	26.64	24.77	51.25	0.27
18	107	154	8.48	8.43	83.35	9.68	6.45	2.73	1.43	4.15	2.12	0.70	2.20	138.71	21.97	15.84	61.25	0.24
19	86	152	5.05	5.05	61.40	7.80	6.85	2.18	1.80	4.30	2.29	0.68	2.30	65.63	21.14	32.21	61.25	0.28
20	85	153	6.15	6.05	61.33	7.85	6.83	3.00	2.38	5.00	2.09	0.85	2.40	106.78	24.89	23.31	47.50	0.37
21	97	162	7.18	7.18	65.35	5.78	7.93	2.85	2.48	4.00	2.44	0.90	2.40	101.30	25.84	25.51	30.00	0.27
22	115	164	7.60	7.45	83.75	9.83	7.83	1.63	1.68	4.15	2.03	0.73	1.90	115.28	12.71	11.03	37.50	0.28
23	101	144	4.38	4.33	73.55	5.15	6.93	2.38	4.25	3.30	2.78	1.06	2.40	51.05	17.31	33.91	30.00	0.30
24	94	151	6.45	6.40	71.70	9.68	7.00	2.15	1.43	4.65	2.34	0.74	2.10	59.76	17.08	28.58	60.00	0.25
25	102	154	7.73	7.73	70.55	6.80	6.23	1.85	2.55	4.35	2.20	0.79	2.00	80.00	18.10	22.63	18.75	0.35
26	89	153	8.78	8.20	69.70	9.38	6.93	2.18	2.20	5.00	2.09	0.73	2.00	126.67	29.24	23.08	70.00	0.39
27	117	158	7.43	7.43	71.80	5.75	6.13	1.33	2.78	4.10	2.21	0.87	1.80	90.91	15.52	17.07	17.50	0.34
28	88	153	6.20	6.20	72.40	9.53	7.68	2.95	1.98	4.90	2.25	0.77	2.20	58.11	22.66	39.00	80.00	0.27
29	103	158	5.05	5.05	62.05	6.55	7.68	1.43	4.70	4.20	2.11	0.95	1.90	103.45	17.50	16.92	23.75	0.37
30	97	158	4.53	4.53	64.00	5.10	7.13	2.25	3.45	5.40	2.73	0.95	2.80	75.41	21.05	27.91	21.25	0.30
31	83	151	7.08	6.58	55.10	8.58	7.88	3.18	1.40	3.90	2.39	0.80	2.10	77.19	25.55	33.10	51.25	0.26
32	89	156	3.68	3.68	73.80	10.38	8.38	3.43	2.73	5.20	2.58	0.74	1.90	101.92	17.69	17.36	65.00	0.33
33	91	155	6.50	6.50	74.40	10.50	7.38	4.13	1.65	3.60	1.95	0.68	2.20	126.53	25.27	19.97	77.50	0.29
34	88	152	4.78	4.78	64.63	7.93	7.23	4.15	1.73	4.40	2.65	0.81	2.20	103.64	24.27	23.42	57.50	0.36
35	95	159	5.30	5.30	79.05	5.63	6.68	3.28	4.25	3.90	2.62	0.94	2.20	95.31	25.52	26.78	35.00	0.35
36	87	156	6.53	6.53	60.90	9.00	7.28	3.88	1.80	5.35	2.53	0.70	2.40	87.69	25.18	28.71	62.50	0.33

Appendix 1. Continued

T.No	DH	DM	TTN	PTN	PLHT	FL	FN	CB	EW	NGPS	CD	FW	TGW	BWPPL	GYYPPL	HI (%)	Lodg	Mean H'
37	113	164	4.05	4.05	103.35	5.80	11.68	3.78	3.58	3.90	2.62	0.73	2.10	118.57	18.53	15.63	31.25	0.24
38	108	161	5.25	5.25	85.25	9.58	7.40	3.68	1.55	4.40	2.54	0.66	2.10	105.77	18.13	17.14	60.00	0.23
39	82	143	9.25	9.25	62.00	8.73	6.58	3.20	1.23	3.25	2.09	0.74	2.10	68.66	19.65	28.62	56.25	0.23
40	111	166	6.78	6.73	89.65	8.80	6.88	3.68	2.33	4.55	2.87	0.85	2.10	120.83	18.21	15.07	33.75	0.24
41	88	148	6.00	6.00	56.85	9.10	6.73	3.85	2.03	3.65	2.05	0.79	1.90	57.35	15.53	27.08	63.75	0.25
42	86	149	7.90	7.90	51.15	6.73	6.30	3.00	1.83	5.10	2.06	0.63	2.90	89.58	23.44	26.17	27.50	0.22
43	96	159	4.53	4.53	71.65	9.23	7.58	3.00	1.75	3.60	2.37	0.80	2.40	98.08	24.73	25.21	62.50	0.24
44	107	162	5.70	5.60	74.15	8.78	7.98	4.05	1.63	4.60	2.30	0.65	1.80	86.44	19.12	22.12	68.75	0.28
45	102	160	7.13	7.13	68.20	5.98	5.98	3.73	2.68	4.20	2.36	0.77	1.80	97.30	15.53	15.96	18.75	0.26
46	105	160	5.88	5.88	69.25	6.70	5.48	3.95	2.75	5.75	2.08	0.81	1.70	86.42	17.90	20.71	22.50	0.31
47	93	156	4.90	4.83	59.25	4.30	8.20	3.20	5.13	4.20	2.51	0.89	2.40	91.53	15.81	17.27	22.50	0.33
48	84	146	7.95	7.95	58.20	9.15	6.93	3.30	1.43	5.25	2.25	0.66	2.50	78.33	23.76	30.33	60.00	0.17
49	95	154	5.58	5.58	78.85	11.40	7.73	4.08	2.53	3.75	3.29	0.90	2.30	158.54	25.62	16.16	37.50	0.20
50	95	159	5.83	5.75	78.10	9.83	8.35	4.18	2.43	5.35	2.60	0.79	2.00	116.36	27.15	23.33	45.00	0.29
51	102	160	3.13	3.13	71.65	8.05	6.55	3.83	6.85	4.75	3.66	1.17	3.60	116.47	20.92	17.96	5.00	**
52	98	162	5.45	5.43	66.55	7.08	8.30	3.95	2.50	5.15	2.77	0.87	2.30	93.33	21.96	23.53	31.25	0.31
53	85	155	5.95	5.45	66.10	9.68	6.85	4.73	1.85	3.80	2.35	0.71	2.20	63.10	22.79	36.12	46.25	0.35
54	90	162	4.65	4.65	63.95	5.35	8.13	2.65	4.05	5.25	2.54	0.79	3.00	97.92	24.88	25.41	16.25	0.30
55	112	163	6.15	6.15	96.85	7.73	11.58	3.58	3.58	4.05	2.40	0.76	1.80	104.00	20.01	19.24	22.50	0.28
56	104	160	3.58	3.58	67.30	7.83	5.55	3.38	5.28	5.80	2.03	0.93	3.50	63.33	15.22	24.03	6.25	**
57	88	161	6.35	6.35	69.75	7.35	7.53	3.68	2.33	3.40	2.61	0.75	2.30	83.78	23.15	27.63	35.00	0.30
58	101	155	5.55	5.55	70.85	8.98	7.88	3.70	1.25	4.15	2.15	0.72	1.60	66.23	12.05	18.19	46.25	0.18
59	108	163	5.78	5.78	76.90	9.00	6.53	2.98	2.33	3.20	2.62	0.76	2.00	132.69	16.27	12.26	47.50	0.23
60	98	150	7.18	7.18	81.00	7.80	8.50	4.35	2.50	4.60	1.94	0.61	1.70	88.14	20.01	22.70	70.00	0.22
61	106	163	4.90	4.90	50.70	6.30	8.18	2.93	2.38	3.40	2.11	0.62	1.70	88.24	15.61	17.69	32.50	0.35
62	100	155	2.68	2.68	41.13	4.93	5.50	3.88	2.18	5.60	2.04	0.67	1.40	56.94	6.12	10.75	21.25	0.31
63	101	163	3.23	3.23	65.85	8.93	6.68	2.45	2.08	3.90	2.20	0.79	1.40	77.92	9.23	11.85	36.25	**
64	95	164	6.53	6.53	72.05	10.63	7.28	3.00	1.68	5.05	2.45	0.66	2.10	98.18	20.23	20.61	67.50	0.24
65	89	160	5.53	5.53	76.80	10.68	8.78	2.88	2.38	4.00	2.44	0.90	2.10	159.57	29.86	18.71	46.25	0.23
66	88	154	3.75	3.75	61.25	9.30	7.25	3.95	1.50	4.75	2.33	0.70	2.00	61.76	14.22	23.02	53.75	0.28
67	88	155	7.83	7.83	58.65	9.33	6.63	3.45	2.48	3.90	2.06	0.74	2.10	113.64	17.98	15.82	31.25	0.31
68	97	159	7.43	7.43	69.25	8.55	6.30	3.98	2.45	4.85	2.38	0.74	2.60	119.51	33.42	27.96	48.75	0.22
69	108	166	4.63	4.63	80.85	8.98	6.68	3.58	2.65	3.50	2.19	0.77	2.30	81.54	10.99	13.48	23.75	0.26
70	97	158	5.25	5.25	48.30	4.58	6.78	2.40	1.65	5.05	2.06	0.65	1.70	83.85	12.15	14.49	56.25	0.32
71	100	160	3.78	3.78	58.85	6.20	7.83	3.83	2.70	3.80	2.10	0.87	2.20	149.09	21.36	14.33	30.00	0.35
72	83	151	5.30	5.30	65.63	9.55	6.80	3.35	1.55	4.85	2.27	0.71	2.30	55.06	17.91	32.53	67.50	0.30

Appendix 1. Continued

T. No	DH	DM	TTN	PTN	PLHT	FL	FN	CB	EW	NGPS	CD	FW	TGW	BWPPL	GYPPL	HI (%)	Lodg	Mean H'
73	88	154	3.88	3.88	47.83	5.35	6.65	2.78	4.40	4.05	2.10	0.88	2.30	78.85	11.44	14.51	28.75	0.30
74	109	158	4.25	3.85	60.40	6.08	5.63	2.60	3.75	5.70	2.46	0.76	2.20	98.25	13.50	13.74	11.25	0.34
75	105	160	2.70	2.70	75.85	7.90	4.83	1.85	3.60	3.95	2.95	1.13	3.50	87.10	15.69	18.01	23.75	**
76	111	164	3.18	3.10	46.10	4.70	6.10	2.35	4.03	5.45	2.46	0.61	2.40	78.70	8.63	10.97	17.50	0.31
77	86	159	3.55	3.30	70.53	11.30	8.00	3.18	2.78	3.85	2.14	0.71	2.60	56.79	13.18	23.21	48.75	0.23
78	117	167	8.30	8.23	74.80	7.93	6.03	3.15	2.38	4.30	2.36	0.61	2.00	124.64	15.54	12.47	26.25	0.29
79	87	154	4.00	4.00	52.95	8.50	6.55	2.95	1.50	3.55	1.95	0.68	2.50	45.45	16.75	36.85	48.75	0.30
80	108	161	3.83	3.83	54.50	5.45	6.45	2.25	4.60	4.95	2.52	1.00	2.20	75.00	14.24	18.99	7.50	0.26
81	95	162	4.73	4.68	60.15	9.13	8.03	3.58	2.48	3.05	2.73	0.78	2.30	88.44	24.43	27.62	37.50	0.24
82	97	154	8.25	8.00	62.43	9.48	7.65	3.45	2.00	4.70	2.13	0.80	1.90	88.89	25.97	29.22	65.00	0.28
83	108	163	4.75	4.70	55.30	4.98	7.10	2.20	5.10	4.45	2.52	0.98	2.00	99.58	17.76	17.83	13.75	0.20
84	87	155	4.10	4.10	51.15	8.55	7.10	3.00	1.18	4.85	1.57	0.63	2.90	57.95	14.06	24.26	75.00	0.17
85	93	156	8.93	8.63	60.95	9.15	7.45	2.83	1.45	3.65	2.30	0.70	2.10	73.08	26.21	35.86	61.25	0.25
86	106	157	3.28	3.28	57.05	5.55	6.63	2.63	3.43	4.55	2.29	0.89	2.90	124.84	20.44	16.37	21.25	0.28
87	102	162	3.30	3.30	101.50	5.88	9.55	3.98	4.60	4.70	3.26	0.86	1.80	73.91	14.69	19.88	42.50	0.33
88	110	161	5.90	5.90	98.80	8.65	10.08	3.40	2.58	4.65	2.31	0.79	1.70	76.67	12.24	15.96	32.50	0.25
89	91	155	4.08	4.08	66.30	10.13	9.65	4.55	2.45	3.90	2.66	0.72	1.90	71.70	19.31	26.93	50.00	0.30
90	102	158	4.58	4.50	67.30	5.53	6.20	3.95	3.20	5.70	2.25	0.78	2.00	102.86	18.56	18.04	20.00	0.30
91	97	156	5.13	5.13	83.60	8.83	7.63	4.33	2.98	4.10	2.70	0.89	3.10	58.16	12.12	20.84	26.25	**
92	93	156	6.15	6.10	65.00	8.68	6.78	3.75	2.43	4.85	2.46	0.81	2.20	63.33	25.61	40.44	32.50	0.26
93	92	160	3.60	3.60	54.10	5.10	7.75	4.75	3.38	3.50	2.25	0.79	3.10	153.66	29.09	18.93	50.00	0.33
94	101	165	7.85	7.60	82.05	10.30	8.30	3.93	2.28	4.50	2.20	0.67	1.90	107.41	27.83	25.91	56.25	0.31
95	101	160	3.05	3.00	66.20	5.58	5.65	3.85	3.95	4.55	2.74	0.95	2.30	84.42	17.44	20.66	13.75	0.30
96	101	160	4.90	4.85	64.95	5.83	5.70	4.25	3.38	5.15	2.33	0.87	2.40	74.24	15.08	20.31	27.50	0.33
97	87	158	6.25	6.25	55.65	7.70	6.83	3.53	1.25	3.55	2.16	0.71	2.30	105.13	19.04	18.11	55.00	0.21
98	109	162	5.00	5.00	67.40	9.95	8.33	4.38	1.58	4.40	2.65	0.61	1.90	115.22	15.18	13.17	53.75	0.32
99	90	154	5.30	5.30	72.25	10.00	7.00	5.28	1.63	3.55	2.30	1.77	1.90	68.33	18.06	26.43	70.00	0.31
100	104	163	4.90	4.90	73.85	8.63	7.70	2.98	2.13	4.45	2.14	0.62	1.80	110.77	16.11	14.54	53.75	0.30
101	104	162	7.80	7.80	89.05	9.38	9.38	4.10	2.05	2.95	2.15	0.64	2.00	124.24	13.20	10.62	56.25	0.22
102	88	156	6.25	6.25	71.35	9.33	7.60	5.33	2.15	4.85	2.24	0.74	2.70	73.24	28.69	39.17	46.25	0.32
103	95	159	3.30	3.30	67.05	5.48	6.08	4.45	3.70	3.95	2.87	1.01	2.80	91.49	22.38	24.46	27.50	0.32
104	90	153	7.18	7.10	78.70	10.93	6.85	6.08	1.68	4.65	2.29	0.65	2.30	69.84	25.96	37.17	73.75	0.32
105	88	160	3.15	3.15	60.15	5.73	6.63	3.80	4.35	4.00	2.39	1.06	2.90	76.92	19.53	25.39	35.00	0.31
106	84	158	7.00	7.00	57.55	9.00	6.93	3.65	1.53	5.00	2.16	0.69	2.40	73.81	29.52	39.99	62.50	0.19
107	97	161	3.53	3.53	67.80	4.78	7.58	3.90	3.48	4.00	2.58	0.93	3.40	113.21	29.52	26.08	17.50	0.25
108	86	155	4.60	4.60	67.95	11.30	9.83	4.35	2.30	5.00	2.55	0.74	2.40	57.14	19.26	33.71	60.00	0.35

Appendix 1. Continued

T. No	DH	DM	TTN	PTN	PLHT	FL	FN	CB	EW	NGPS	CD	FW	TGW	BWPPL	GYPPL	HI (%)	Lodg	Mean H'
109	90	155	5.50	5.50	75.65	10.58	7.93	4.60	1.83	3.95	2.47	0.72	2.40	98.68	33.53	33.98	50.00	0.18
110	95	163	5.23	5.23	74.55	10.33	8.98	2.88	3.13	5.15	2.46	0.83	2.30	129.41	26.57	20.53	55.00	0.15
111	103	167	6.55	6.43	67.05	10.10	7.70	2.70	2.05	3.20	1.92	0.63	1.40	107.69	16.98	15.77	67.50	0.34
112	101	158	3.85	3.75	78.15	6.88	7.18	3.38	5.53	5.55	3.28	0.95	3.50	104.29	18.34	17.59	8.75	0.29
113	97	158	5.78	5.65	60.95	9.83	7.23	4.15	1.53	3.75	2.79	0.60	2.00	85.96	15.75	18.32	77.50	0.27
114	102	161	10.15	9.63	93.43	7.70	10.25	3.20	3.43	4.75	2.52	0.72	2.10	112.77	26.53	23.53	77.50	0.39
115	112	165	7.03	7.03	94.25	8.23	7.05	4.38	1.83	3.40	2.64	0.82	2.40	158.33	20.02	12.64	43.75	0.26
116	93	160	6.05	5.98	72.50	10.70	8.18	3.20	2.08	5.45	2.38	0.79	2.00	114.00	26.94	23.63	66.25	0.23
117	98	161	5.93	5.93	62.70	6.85	7.45	2.70	3.10	4.30	2.39	0.93	2.50	119.51	26.18	21.91	47.50	0.36
118	92	158	5.38	5.38	64.35	5.88	8.10	1.68	5.48	5.60	2.84	1.00	2.60	92.00	17.65	19.18	21.25	0.28
119	86	154	4.35	4.35	69.50	9.63	6.70	2.05	2.15	3.60	2.60	0.85	2.40	88.89	23.21	26.11	56.25	0.28
120	90	155	4.63	4.63	68.98	7.15	6.43	2.25	4.83	5.90	2.49	0.80	3.30	81.13	24.64	30.37	47.50	0.37
121	92	160	6.98	6.98	67.20	10.73	7.23	2.95	1.75	3.15	2.06	0.69	1.80	129.63	25.35	19.56	70.00	0.25
122	99	158	4.43	4.43	59.35	5.68	7.30	1.93	4.13	5.30	2.70	0.80	2.50	124.32	25.27	20.33	48.75	0.29
123	99	163	3.10	3.10	67.05	5.90	6.68	1.68	4.50	4.00	2.59	0.99	2.20	127.59	18.80	14.73	31.25	0.35
124	106	158	5.90	5.85	81.15	9.05	7.28	2.80	1.53	4.25	2.15	0.68	2.20	140.00	20.82	14.87	51.25	0.29
125	86	156	3.85	3.85	57.20	3.53	5.80	2.43	2.03	4.20	2.34	0.68	3.00	67.19	15.57	23.17	35.00	0.28
126	94	156	7.83	7.78	67.70	7.85	7.60	2.28	1.45	4.65	2.09	0.63	2.20	108.93	21.77	19.99	62.50	0.36
127	104	164	4.10	4.10	50.00	5.23	6.35	2.43	4.25	3.35	2.49	0.72	2.10	93.62	8.79	9.39	32.50	0.28
128	101	156	4.85	4.85	82.00	10.80	7.50	1.98	1.95	4.45	2.61	0.63	2.30	173.47	24.90	14.35	52.50	0.25
129	89	162	3.73	3.73	74.90	6.03	7.73	1.78	5.18	3.90	2.34	0.83	2.90	136.62	31.34	22.94	21.25	0.30
130	106	159	4.95	4.95	66.30	7.58	7.15	1.73	2.78	6.05	2.13	0.78	2.20	138.03	19.08	13.82	17.50	0.37
131	88	162	3.35	3.35	78.80	9.13	5.70	3.15	3.85	4.20	2.33	0.82	3.10	146.00	26.94	18.45	56.25	0.31
132	93	157	5.73	5.73	74.15	9.38	6.75	2.85	1.55	4.65	2.62	0.67	2.10	85.71	23.91	27.90	77.50	0.29
133	95	160	4.50	4.50	60.25	5.68	7.45	1.45	4.43	3.85	2.35	0.89	2.60	95.12	31.76	33.39	30.00	0.31
134	95	154	4.55	4.55	62.45	9.55	6.43	2.70	1.48	4.45	2.03	0.72	2.10	65.08	26.18	40.23	76.25	0.25
135	96	157	2.60	2.60	68.25	5.85	7.30	2.38	3.75	4.00	2.35	0.86	2.80	79.25	24.74	31.22	45.00	0.33
136	91	156	7.48	7.48	72.35	10.25	7.23	2.38	1.48	4.40	2.28	0.66	2.20	95.16	41.60	43.72	65.00	0.28
137	96	151	4.08	4.08	76.10	10.53	10.28	2.18	2.90	3.45	2.56	0.73	1.80	88.73	28.80	32.46	75.00	0.31
138	111	164	3.60	3.60	81.80	7.85	6.63	2.45	2.25	4.45	2.26	0.84	1.90	86.11	14.80	17.19	60.00	0.36
139	111	160	5.15	5.15	71.45	5.83	6.65	1.93	4.05	4.20	2.35	0.78	1.70	92.11	18.81	20.42	27.50	0.31
140	88	159	9.05	9.05	79.20	10.28	7.60	2.33	1.73	4.35	2.31	0.72	2.30	135.71	33.65	24.80	67.50	0.27
141	108	165	7.03	6.93	90.45	7.48	6.43	2.53	1.95	3.25	2.35	0.81	2.00	167.27	19.19	11.47	52.50	0.29
142	104	156	5.38	5.38	84.90	8.45	6.15	1.38	3.63	5.60	2.41	0.77	2.60	103.70	19.23	18.54	15.00	0.32
143	89	151	2.68	2.58	59.70	3.98	6.80	2.30	2.10	3.45	2.32	0.83	2.50	70.91	14.20	20.03	20.00	0.26
144	93	153	4.53	4.48	63.60	5.48	7.33	2.38	2.20	4.85	2.37	0.78	2.50	57.89	14.12	24.39	25.00	0.31
145	87	156	7.48	7.38	65.45	9.63	7.20	2.23	1.40	3.65	2.30	0.82	2.80	85.14	25.34	29.76	55.00	0.24

Appendix 1. Continued

T.No	DH	DM	TTN	PTN	PLHT	FL	FN	CB	EW	NGPS	CD	FW	TGW	BWPPL	GYPPL	HI (%)	Lodg	Mean H'
146	85	145	6.65	6.65	57.15	9.73	6.98	2.03	1.43	5.10	2.06	0.74	2.20	55.10	19.47	35.34	71.25	0.23
147	99	161	3.58	3.48	68.55	8.68	7.80	2.00	2.40	3.80	2.65	0.72	2.30	79.10	20.74	26.22	51.25	0.30
148	109	161	6.38	6.38	58.60	9.13	7.55	2.23	2.58	4.60	2.10	0.71	1.60	105.08	13.73	13.07	30.00	0.33
149	92	162	3.83	3.83	79.30	10.35	7.98	2.68	2.38	3.30	2.39	0.65	2.20	118.64	27.21	22.93	62.50	0.31
150	87	145	8.63	8.63	62.35	9.13	5.88	2.20	1.60	4.75	2.60	0.72	2.50	65.15	20.48	31.44	60.00	0.27

Key: DH=days to50% heading, DM= days to maturity, TTN=Total tiller number, PTN= productive tiller number, PLHT= plant height, FL= finger length, FN= finger number, CB=culm branch, EW= ear weight, NGPS=number of grain per spikelet, CD=culm diameter, FW=Finger width, TGW=thousand grain weight, BWPPL=Biomass weight per plant, GYPPL=grain yield per plant, HI=Harvest index (%), LOG= lodging index(%),Mean H'= mean Shannon diversity index

**= released varieties and thus has no Shannon index

Appendix 2: SSCP gel procedures and reagents used

SSCP GEL PROTOCOLS:

The PCR-SSCP procedure includes following steps viz; PCR amplification of the gene fragments, resolution in nondenaturing PAGE and visualization using silver staining.

1. The Single Strand Conformation Polymorphism analysis of amplified gene fragments is carried out using Bio-Rad Protein II xi Cell vertical gel electrophoresis unit (Bio-Rad laboratories). The two glass plates are washed thoroughly using tap water with detergent and rinsed initially under running tap water till no remains of detergent are left. The plates are wiped two times with tissue paper soaked in distilled water first, 70 percent alcohol and then air-dried. The similar thorough cleaning treatment is given to spacers and comb to ensure proper alignment of 20 cm glass plates.

2. The gel sandwich is assembled on a clean surface laying down the long rectangular plate first, then two spacers of equal thickness along the long edges of plate and the short plate is placed on the rectangular plate. The two glass plates with spacers between them are fitted well with proper alignment by tightening the bulldog clamps. The sandwiched gel plates are fitted in the stand with screw clamps. The cleaned comb (20 wells) is inserted from the topside of the gel sandwich and immediately bulldog clamps are applied over the plates containing comb to create sharp wells.
3. The bottom side of the gel sandwich is sealed using 10 ml of 12% gel mix. The gel sandwich is kept in slanting position and the solution mixed with 50 µl APS and 20 µl TEMED is injected between the two glass plates using syringe fitted with 10 µl tip and allowed to polymerize for 10 minutes.

4. After polymerization the assembled gel sandwich is placed in alignment slot of casting stand. The 12% native PAGE gel mix (25 ml) is prepared by adding APS (100 ul) and TEMED (40 µl) at a time and mixed well. This gel mix is filled from upper side of gel sandwich using syringe smoothly without any bubble and clamps are immediately applied over the comb to ensure sharp wells. The gel is kept undisturbed at least 45 minutes for polymerization.
5. After polymerization the comb is removed and wells are flushed with 0.5X buffer. The gel sandwich is placed in electrophoresis tank with notched plate facing towards the buffer reservoir.

The reservoir of the electrophoresis tank is filled with 0.5X TBE and the gel is given pre-run at 200 volts at constant temperature for minimum 45 minutes. Ice cooled water circulation with electric pump is applied to central cooling core of assembly to maintain constant temperature.

6. About 4 µl PCR product and 12 µl of a formamide dye is prepared in PCR tube and denatured at 95°C for 10 minutes in the Biometra PCR machine. After denaturation the samples are immediately kept in ice-chilled box and kept in -20°C deep freeze for 10 minutes.
7. After completion of pre-run the wells are flushed again using buffer. The samples are loaded on a nondenaturing 12% acry1amide: bis-acrylamide (49:l) gel with gel loading tip and immediately electrophoresis is performed in 0.5 X Tris borate (pH 8.3)-EDTA buffer at 10-12.5 volts/cm for 3-24 hr at room temperature depending on the optimized conditions for each primer.
8. After completion of the electrophoresis for required time the glass plates are removed from the assembly. There after gel is subjected to silver staining to visualize SSCP band patterns.
9. In order to stain the gel it is immersed in a tray of appropriate size filled with 10% acetic acid (500ml) for at least 30 minutes for fixing DNA bands in gel so as to prevent diffusion of the DNA bands (Care has to be taken so that gel remains dipped well in solution). The gel is agitated slowly for 30 minutes or until the tracking dye is no longer visible.
10. The acetic acid is decanted and 500 ml of distilled water is poured in the tray and rinsed thoroughly by placing the tray on oscillatory automatic shaker for 20 minutes.
11. Meanwhile 500 ml of 0.1% silver nitrate solution is prepared in amber color bottle and 750 µl of 37% formaldehyde is added and mixed. Distilled water is gently decanted from tray. The gel is stained for 45 minutes in silver nitrate solution with constant shaking in a dark room or covering the tray with black cloth.
12. Then the gel is rinsed briefly for 25 seconds in distilled water.
13. Working quickly distilled water is decanted from the tray. The freshly prepared and chilled 3% sodium carbonate solution (3% Na_2CO_3 and 750µl of 37% formaldehyde + 1% sodium thiosulfate) is transferred to the tray. The gel is kept immersed until bands get developed sharply.
14. The gel is given 10 percent acetic acid (stop solution) treatment for 10 minutes. Then 500 ml distilled water is added to tray.
15. The gel is transferred gently on the transparency; excess water is soaked with tissue paper, and air dried for half an hour.

SSCP GEL CHEMICALS AND STAINING PROCEDURES

A. SSCP GEL CHEMICALS

i. Fixer (1.8 ml of H_2O + 200 ml glacial acetic acid)

ii. Silver stain – (2L H_2O 12 ml 1N $AgNO_3$ + 3 ml Formaldehyde).

iii. Developer – (60g Na_2CO_3 in 2 L of H_2O +3 ml Formaldehyde + 300 µl Na_2SO_3 thiosulphate).

NB: Keep in the refrigerator to cool and add 3 ml Formaldehyde + 300 µl Na_2SO_3 thiosulphate to the Na_2CO_3 solution 5 minutes before developing the gel.

iv. Water

B. PLATE TREATMENT

a. Long plate

- Rinse with distilled water
- Rinse with alcohol
- Apply repel silane
- Rinse with water

b. Short plate

- Rinse with alcohol
- Apply bind silane (28 µl)
- Rinse with water
- Rinse with alcohol

C. GEL MIX (6%)

a. 60ml

- 41.4 ml d H_2O
- 3.6 ml 10 x TEB buffer
- 15 ml 2 X MDE gel solution
- 240 µl 10% APS*
- 24 µl TEMED*

*=add at the same time

b. 80 ml

- 55.2 ml d H_2O
- 4.8 ml 10 x TEB buffer

- 20 ml 2 X MDE gel solution
- 320 µl 10% APS*
- 32 µl TEMED*

  *=add at the same time

D. SSCP Gel staining procedure

- Fix gel plate in fixer on shaker for 40-45 minutes
- Wash in 2L of water on shaker for 15-20 minutes
- Stain in silver-stain for 20-30 minutes and wash in water for 20 seconds
- Immerse stained plate in developer and rock to and fro till band appear
- Stop developer with fixer when band appear clearer
- When the bubbles disappear, rinse in water
- Allow to dry