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**LIST OF SYMBOLS AND ABBREVIATIONS**

AFLP	=	amplified fragment length polymorphism
ARC	=	Awassa Agricultural Research Center
ARTP	=	Agricultural Research and Training Project
$D^2$	=	Mahalanobis's distance
DNA	=	Deoxyribonucleic acid
EB	=	Extraction buffer
EIAR	=	Ethiopian Institute of Agricultural Research
EIBC	=	Ethiopian Institute of Biodiversity Conservation
$H'$	=	Shannon-Weaver diversity index
IBPGR	=	International Board for Plant Genetic Resources
MARC	=	Melkasa Agricultural Research Center
PCR	=	polymerase chain reaction
PIC	=	polymorphic information content
RAPD	=	random amplified polymorphic DNA
RCBD	=	randomized complete block design
RFLP	=	restriction fragment length polymorphism
rpm	=	rotation per minute
SARI	=	Southern Agricultural Research Institute
SSR	=	simple sequence repeat
UPGMA	=	unweighted pair-group methods arithmetic average

# **GENETIC DIVERSITY STUDY IN FINGER MILLET [*Eleusine coracana* (L.) Gaertn] LANDRACES USING MORPHOLOGICAL TRAITS, ISOZYME AND RAPD MARKERS**

## **INTRODUCTION**

Ethiopia is diverse country in terms of altitude, temperature, rainfall and soils types, one can sense such diversity within a short distance in a given locality. Such diversity of environmental elements was the cause for the existence of diverse vegetation, crop species and native varieties of crops that are observed in farmers' fields in most parts of the country (Vavilov, 1951).

According to N.I. Vavilov and other scientists, Ethiopia represents one of the major countries of genetic diversity. Vavilov (1951) has indicated that some thirty species are connected with Ethiopia as a primary or secondary gene center. Eleven crops are indicated as having their center of diversity in Ethiopia (Zonary, 1970). Crops such as barley (*Hordum vulgare*), sorghum (*Sorghum bicolar*), tef (*Eragrostis tef*), finger millet (*Eleusine coracana*), wheat (*Triticum* spp.), noug (*Guizotia abyssinica*) sesame (*Sesamum indicum*), castor bean (*Ricinius communis*), coffee (*Coffee arabica*), enset (*Ensete ventricosum*), chat (*Catha edulis*), and local domesticates are known to have a wide genetic diversity.

The long history of cultivation and the large agro-ecological and cultural diversity in the country have resulted in large number of landraces of finger millet. In Ethiopia, finger millet is grown as subsistence crops and usually marginal lands are allotted to it for growing. It is used for making the native bread, injera, porridge and genfo. It is also used for preparing of local drinks, such as tella and arekie. The straw is used as animal feed, fuel and thatching. It is an important crop for drought tolerance and does very well under poor soil conditions. . It also enables to protect against soil on-farm erosion because of better cover of the soil within a short period of time as common grasses do. It can be stored for a number of years up to ten without

appreciable loss and has comparable protein and even better calcium content than other cereal.

Despite its merits the cultivation of finger millet is being pushed to the more marginal areas, therefore, genetic erosion is believed to occur due to several factors. Nevertheless, operating for germplasm collecting has been undertaken from different areas of the country by the Ethiopian Institute of Biodiversity Conservation (EIBC), the then Plant Genetic Resource Center of Ethiopia (PGRC/E) and other organizations like Ethiopian Institute of Agricultural Research (EIAR) and some reports indicated that about 2,051 finger millet germplasm accessions have been collected and preserved.

Knowledge of the pattern of genetic variation for traits of economic interest is a paramount importance for efficient utilization of germplasm collections. i.e., subdividing the variation into its components may assist in genetic resource conservation and utilization by determining the relative contribution of the different levels of variability to the total diversity available in any one area. This would enable planning of future germplasm sampling, establishing *in situ* gene conservation, or use of appropriate gene pools in crop improvement for specific plant attribute.

More genes for better agronomic types, disease resistance, earliness, good quality, and higher biological yield are necessary for further progress in finger millet improvement. The availability of such genes depends on the identification of areas of concentration for various characters of agronomic value. The identification of these sites is a paramount importance for collection and for appropriate *in situ* site selection. Choices of sites for *in situ* conservation may depend on high diversity estimates based on markers and on knowledge of adaptive traits linked to certain ecological conditions (Negessa, 1986; Demisse and Bjornstad, 1996; Workeye 2002), e.g., co-evolving host-pathogen systems and adaptation to other stress conditions. *In situ* population of the crop species may not only maintain a large level of variation but also a high frequency of desirable genes (Van Leur *et al.* 1989).

Measurement and characterizations of genetic variation in germplasm have been great concern for genetic resources conservation and utilization over the years. The methods for detecting and assessing genetic diversity have extended from analysis of discrete morphological traits to biochemical and molecular traits. Studying the pattern of genetic variation and their differential distribution by using some markers like morphological characters, isozymes and DNA markers such as restricted fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) or microsatellite over the various microcenters could enable to determine genetic variability. In addition, prior knowledge of the nature and extent of genetic variation is crucial since successful conservation and utilization of germplasm depends on the proper assessment of variation within and among population (Tesemma and Belay, 1991).

Although a vast amount of materials are available, little is known about the major characters and potential usefulness of the individual accessions making up that store of materials. In other words, little of the materials residing the germplasm banks have been evaluated. In the absence of this knowledge, the breeder has no way of knowing which few among several hundred accessions will most likely provide the particular trait or traits needed in a breeding program.

Ethiopia being a center of diversity of many crops, the patterns of gene or character distribution within a center are hardly known. Apparently, germplasm collections will lack precisely defined target areas, and this limits the efficiency of its service to crop genetics and breeding.

A considerable number of diversity studies have been done in Ethiopia by different markers on different crops such as on tef (Bekele, 1983; Assefa *et al.* 1999; Kefyalew *et al.* 2000), barley (Zemedu, 1988, 1989; Demisse, 1996), wheat (Bekele, 1984; Negassa, 1986; Belay, 1997; Kebebew *et al.*, 2001) sorghum (Ayana and Bekele, 1998) and chickpea (Workye, 2002). However, a study in finger millet is limited.

Hence, this investigation was undertaken to study the diversity of finger millet landraces collected from Ethiopia and Eritrea using morphological traits, isozyme and random amplified polymorphic DNA (RAPD) markers.

## **OBJECTIVES**

### **Overall Objective**

To generate information on the diversity of finger millet landraces.

### **Specific Objectives**

1. To evaluate the diversity of finger millet with respect to some morphological traits, isozyme and RAPD markers.
2. To identify the sites of high genetic diversity for target collection of finger millet.
3. To estimate the nature and magnitude of variability for yield and yield related characters.
4. To understand the association among yield and yield related characters.
5. To study genetic affinity within the germplasm collection of finger millet in order to provide basis for selection of parents for hybridization.

## LITERATURE REVIEW

### **1. Finger millet**

Finger millet (*Eleusine coracana* L. subsp. *coracana*) and its wild relatives are the members of chloridoidea, one of the primary subfamilies of the grass (poaceae) family. Finger millet is an allotetraploid species with  $2n=4x=36$  chromosomes (Hilu and De Wet, 1976). It is predominantly self-fertilized (De Wet *et al.*, 1984).

The crop has several vernacular names in different parts of the world, some of them are finger millet, African millet, Koracan (English), pefit mil, eleusine cultivee, coracan, koracan (French), fingerhirse (Germany), wimbi, ulezie (Swahili), dagusa (Ethiopia, Amharic/Sodo), tokuso (Ethiopia, Amharic), barakiya (Ethiopia, Oromo), ragi (India), wimbi (Kenya, Kiswahili), mugmbi (Kenya, Kikuyu), mawere, lipoko, usange, khahawe, mulimbi, lupodo, males, mawi (Malawi), kodo (Nepal), tailbon (The Sudan, Arabic), ceyut (Sudan, Bari), muimbi, mbege (Tanzania), bulo (Uganda), kambale, lupoko, mawete, majolothin, checkmale, bule (Zambia), rapoko, zviyoinjera/rukuza, mazhovle, uphoko, poho (Zimbabwe).

Finger millet is an important staple food in parts of eastern and central Africa and India. In the semi-arid tropics of eastern Africa, it is a major staple food for million of resource poor people. This cereal plays an important role in the dietary habits and economy of subsistence farmers.

It is the principal cereal grain in northern and parts of western Uganda and northeastern Zambia. In India and Africa, two groups of finger millet are recognized. African highlands types with grains enclosed within the florets and Afro-Asiatic types with mature grains exposed outside the florets (CAC, 1990).

The height of cultivars varies from 40 cm to 1.5 m and the spike length ranges from 3 to 13 cm. The color of the grains may vary from white through orange-red

deep brown and purple, to almost black. The grains are smaller than those of pearl millet and the mean 1,000-seed weight is about 2.6 g.

It is a short-day plant, a 12 –hour photoperiod being optimum for the best known types. It has been successfully grown in the United States as far north as Davis, California (with considerable problems of photoperiod sensitivity), and it is widely grown in the Himalayas (30<sup>0</sup>N) latitude. However, it is mainly produced within 20<sup>0</sup>N and 20<sup>0</sup>S latitude. Day length-neutral types probably exist. It requires a moderate rainfall (500 - 1000 mm), well distributed during the growing season with an absence of prolonged drought. Dry weather is required for drying the grain at harvest.

Most of the world's finger millet is grown at intermediate elevations, between 500 and 2400 m, however, its actual altitude limits are unknown. The crop tolerates a cooler climate than other millets. Finger millet thrives under hot conditions. It can grow where temperature is as high as 35 <sup>0</sup>C. In Uganda, the crop grows best where the average minimum temperature does not fall below 18<sup>0</sup>C. The crop is grown on a variety of soils. It is frequently produced on reddish–brown latentic soils with good drainage but reasonable water holding.

### **1.1. Origin, taxonomy and distribution of finger millet**

The species was domesticated in Africa. Its closest wild relatives, *Eleusine coracana* subsp. *africana* is native to Africa (De Wet, *et al.*, 1984). It is common along the highlands of east Africa and the grasslands of the southern Africa. It was probably domesticated in area extending from western Uganda to the highlands of Ethiopia (Harlan, 1971), finger millet is grown extensively there. FAO (1998) also reported recently eastern Africa as a center of origin and diversity of finger millet.

Harlan *et al.* (1976) suggested that domestication of native African food plants started some 5000 years ago. Hilu *et al.* (1979) indicated that this cereal could have been grown in Ethiopia at that time. Archaeological material excavated at Axum

resembles *plana*, which is the principal finger millet still grown in Ethiopia. If this archaeological material dates from 3000 B.C. as suggested by Hilu *et al* (1979), the cereal must be substantially older as a crop in Africa. The cereal reached India during the first millennium B.C. (Vishnu, 1968). It became widely distributed in southern Africa during the expansion of iron working technology (Summers, 1958). A more likely date for this cereal assemblage in southern Africa is early Iron Age, some 800 years ago.

Finger millet in Africa and India are similar in adaptation and morphology (De Wet *et al*, 1984). Finger millets grown in the Ghata of India and highlands of east Africa or the coastal plains of east Africa and tropical south India belong to the same races, respectively (De Wet *et al*, 1984).

Subspecies *coracana* includes all cultivated finger millets. Plants are annual, tufted, and erect or with genticulately ascending culms that are up to 165 cm high and some times root from the lower nodes (Rao *et al.*, 1994). Culms are commonly branched from the upper nodes to produce secondary inflorescence. Leaf-blades are linear to linear-lanceolate up to 70 cm long and 20 mm wide. Inflorescences are digitate, often with one or more racemes some distance below the main cluster of 4 to 19 branches. Inflorescence branches are slender, or incurved at the tip when robust, sometimes with secondary branches. Spikelet has 6 to 9 florets and 6–10 mm long. Spikelets are overlapping and mostly arranged in two rows along one side of the rachis. Glumes are unequal and shorter than the spikelet. The grain is white, red, brown or black; up to 2 mm long, more or less globose, with the surface finely striated. Inflorescence shape is variable. The digitately arranged branches may spread out and became reflexed, or they may be erect and incurved, often forming a fist like structure.

## **1.2. Production and importance**

Finger millet is one of the few special species that currently support the world's food supplies. It is grown over 4 million hectares and is the primary food

source of millions of people in the dry land regions of east Africa, central Africa and southern India (OIA, 1996). In most parts of the world millets including finger millet are grown as subsistence crop for local consumption. In parts of eastern and southern Africa as well as in India, it became a staple upon million of tons of grain, of which Africa produces perhaps 2 million tons (OIA, 1996).

Of all major cereals, this crop is one of the most nutritious. In many parts of Africa, especially in dry areas, it is cultivated for its grain, which is higher in protein, fat and minerals than rice, corn and sorghum (Barbeau and Hilu, 1993). It is usually converted into flour for making chapattis, cakes, puddings or porridge. The crop has high levels of the essential amino acid, methionine. It provides a sustaining diet, particularly for people doing hard manual work. Grain may also be malted and flour of the malted grain used as a nourishing food for infants and invalids. It is considered as especially wholesome food for diabetics. In some parts of Africa and India the grain is also used for making beer and liquor called arak (Hilu and De Wet, 1976). Finger millet has also been reported to have some medicinal properties and is used as folk remedy for many diseases (Watt and Breyer-Brandwijk, 1962). It tolerates disease, drought, fungus, high pH, low pH, insects, laterite, mildew, salt, slope and virus (Duke, 1978).

If this crop within Africa is given proper attention, it has the following possibilities. In the humid tropic area it can have excellent prospects. Certain varieties are adapted to heat, humidity and tropical conditions. Given research recognition and sympathetic policies, production could expand dramatically (OIA, 1996).

Finger millet is indigenous to Ethiopia and occupies 304,758 ha of land with production of 305,101 tons (CSA, 2004). It is grown more or less throughout the country though mainly cultivated in the mid and low altitude of the region of Gonder, Gojam, Welega, and Tigray where it constitutes 10 to 20 per cent of the total cereal production (Kebede and Menkir, 1989). It has been also very important in central highlands of the country after the recent release of high yielding varieties for those areas (Gutema *et al.*, 2002).

### **1.3. Production constraints**

Millet production in the developing world, particularly in Africa, suffers from a number of constraints such as poor soil fertility, low and erratic rainfall, high temperatures, widespread *Striga* infestation, downy mildew disease and loss of grain to birds (FAO/ICRISAT, 1996).

Population pressures have led to a shortening of fallow periods, which in turn has accelerated the decline of soil fertility. These processes have also prompted the expansion of millet into more marginal lands. The impact is evident in the declining yields of millet in the major producing countries in Africa (Niger, Mali, and Nigeria) over the past 15 years. Similar trends also evident in the harsher millet production environment in Asia (e.g., western Rajasthan in India) (FAO/ICRISAT, 1996).

In addition, millets are cultivated on small, fragmented production units and are often intercropped (usually with legumes and sometimes with sorghum or maize). Unreliable precipitation tends to keep the use of inputs such as chemical fertilizer, pesticides and hired labor to a minimum. A limited commercial demand depresses the incentive to use purchased inputs (FAO/ICRISAT, 1996). Crop improvement is generally more difficult in millet than other crops, largely because of the nature of the environment in which they are grown. National millet improvement programs in different countries began much later and retained weaker than those for many other crops.

The major problems identified in Ethiopia as mentioned by Gutema *et al.* (2002) are drought in the dry lowland areas; moisture availability often limit successful production. Poor threshability of the crop associated with the nature of the crop. As a result, framers require spending a lot of time in threshing this crop. Head blast disease is the predominant biological constraint in the major millet producing area particularly in northwestern. Cutworms and stalk borers also cause trouble to farmers particularly in the central highlands where the newly introduced improved

varieties are being pushed by the extension program. As a small cereal, lodging is often an important problem with local cultivars. And the other important problem that consumers complain is the strong constipation that it causes.

## **2. Crop genetic diversity**

Crop genetic resources is described as the total of genetic diversity of cultivated species and their wild relatives which includes commercial varieties, landraces (farmers' varieties), special genetic stocks (mutants, breeder's lines etc.) and wild and weedy relatives of potential value to human (Worede, 1988). Genetic diversity is a ubiquitous property of all species in nature. The distribution and organization of genetic variation within and among population of species are the consequences of its evolution. The relentless process of mutation guarantees continuous input of new variants while the equally relentless of environmental adaptation and random genetic drift shape the distribution of genetic diversity in time and space. The extent to which populations or species can adapt to environmental challenges is determined by the store of genetic variation contained within local populations and shared among the networks of population. Similarly, the applied genetics still depends on genetic variants available within the gene pool of domesticated species and their close relatives as resource for crop improvement (Brown *et al.*, 1990).

Knowledge of the amount and distribution of genetic variability within species is a vital to plant breeding because it is an important consideration when selecting germplasm to be included in breeding program (Yu *et al.*, 2001). Also, it is helpful to geneticists in managing plant genetic resources and provides information for designing sampling protocols (Breiting and Wilrlechner, 1995)

Ethiopia is a major world center of genetic diversity for many important domesticated crop plant species largely represented in the country by landraces and wild types that are uniquely adapted, genetically diverse forms of these various crops. The genetic diversity found in Ethiopian landraces has been used worldwide in

developing new crop varieties and addressing acute yield constraints (Worede *et al.*, 2000).

The existence of genetic diversity has special significance for the maintenance and enhancement of productivity in agricultural crops in a country like Ethiopia, which is characterized by highly varied agro-climates and diverse growing conditions. Such diversity provides security for the farmers against diseases, pests, drought, and other stresses. Genetic diversity also allows farmers to exploit the full range of the country's highly varied micro-environments differing in characteristics such as soil, water, temperature, altitude, slope, and fertility. Diversity among species is especially significant to Ethiopia as it represents important resources to subsistence farming communities throughout the country. A wide variety of plant and animal species provide materials for food, feed, fiber and medicinal uses

Germplasm management is a multifaceted endeavor involving acquisition, maintenance and characterization such that plant genetic resources are conserved and utilized for crop improvement. Those of most immediate interest to plant breeding programs involve knowledge of the current genetic content of the collection-acquisition and distribution of genetic diversity among accessions, relationships of collections (new and old) to elite germplasm, and characterization of their potential genetic merit. Evaluation of numerous, highly similar accessions not only waste the plan of breeding resources but likely reduces the chance of identifying the truly unique and valuable accessions. Assessment of the genetic diversity of elite crop germplasm have been sought and used by plant breeders for numerous reasons – genetic relationships, parent selection, germplasm management and sampling, and germplasm protection.

### **3. Genetic erosion**

The current decline in biodiversity is largely the result of human activity and represents a serious threat to human development. But exactly what kinds of human activities are to blame and what are the other factors involved? Collectors need to

answer such questions so that they can target for priority collection regions and species that are particularly at risk. WCMC (1992) quoted the following factors as currently endangering biodiversity.

- Habitat loss or modification.
- Over exploitation for commercial or subsistence reasons.
- Introduction of exotic species, which may compete with, prey on or hybridize with native species.
- Incidental take.
- Disease infestation.
- Limited distribution.

Looking at the problem from the other side, Brush (1995) listed four factors which are important in preserving crop diversity or limiting the rate of genetic erosion: (i) fragmentation of farm holdings, allowing farmers to maintain landraces in at least one field; (ii) increasing cultivation of marginal land where landraces tend to have advantage over modern varieties; (iii) economic isolation, creating market distortions which give landraces a competitive advantage and (iv) cultural values and preferences for diversity. The contention is that in many cases adaptation of modern varieties does not result in the complete replacement of landraces but reaches an asymptote.

To day there is a great concern over the loss of genetic diversity, partly with the substitution of a diverse set of genetically variable crop landraces with few genetically uniform improved varieties (Brush, 1991; Harlan, 1992). This concern is especially valid in areas of crop domestication and a center of genetic diversity such as Ethiopia, where diversity of several crop species (e.g. durum wheat, barley, sorghum, coffee, finger millet etc.) is concentrated. Changes and development of hydroelectric or irrigation projects, highways, industrial sites and housing areas aggravated the loss of the wild and weed species of crop plants.

The broad range of genetic diversity existing in Ethiopia, particularly the primitive and wild gene pools, is presently subjected to serious genetic erosion and irreversible losses. This threat results from the interaction of several factors and is progressing at an alarming rate. The most crucial factors include the displacement of indigenous landraces by new, genetically uniform crop cultivars, changes and development in agriculture or land use, destruction of habitats and ecosystems and drought (Worede *et al.*, 2000). The extent to which the displacement of native seeds by exotic or improved materials occurred in Ethiopia has not been fully documented. Rates of displacement vary depending on regions and crops

The Ethiopian Sorghum Improvement Project (ESIP) has been doing extensive mass selection on sorghum and millet and, in some cases, selecting single lines or cultivars to develop elite materials with improved yield and/or disease (smut) and pest (stock borer) resistance. The distribution of these materials results in a gradual displacement of the original farmers' seed stock especially in the regions of Wello and southeast Shewa (Worede *et al.*, 2000). For crops such as sorghum, millet and pulses, for which there is no immediate threat of genetic erosion, there still exists a danger of their massive displacement in the future by expansion of other crops with better market values (e.g., maize, tef), monocropping, and shifts in cropping patterns that favor early maturing varieties.

#### **4. Genetic conservation**

The importance of crop genetic resources and the threats to them has led to the creation of conservation programs to preserve crop resources for future generations. One type of crop genetic conservation is *ex situ*- maintenance of genetic resources in gene banks, botanical gardens, and agricultural research stations (Plucknett *et al.*, 1987). Another type is *in situ* –maintenance of genetic resources on on-farm or in natural habitats (Brush, 1991; Maxted *et al.* 1997). In actuality, two types of *in situ* conservation can be distinguished. First, *in situ* habitats including areas where everyday practices of farmers maintain genetic diversity on their farms. This type is a historic phenomenon but it is now especially visible in regions where farmers

maintain local, diverse crop varieties (landraces), even though modern, broadly adapted or higher yielding varieties are available (Brush, 2000). Second, *in situ* conservation refers to specific projects and programs to support and promote the maintenance of crop diversity, sponsored by national governments, international programs, and private organizations.

In Ethiopia, a region representing a major world gene center, the various traditional agroecosystems constitute major *in situ* repositories of crop genetic diversity. Maintenance of species and genetic diversity in the field is one of the effective strategies whereby resource-poor farmers practice low input agricultural in marginal environment to create stable systems. The existence of such native germplasm is also to sustained provision of useful genetic material to breeding programs worldwide (Worede *et al.*, 2000).

Both strategies of germplasm conservation, *ex situ* and *in situ* systems are being followed in Ethiopia's crop genetic resource conservation. However, *in situ* which is commonly a community-based conservation of farmers' varieties in different agro-ecological regions is not rigorously undertaken as that of *ex situ* (Workeye, 2002).

Efforts to conserve crop diversity to-date have found on maintaining genetic diversity in *ex situ* gene banks. In Ethiopia, the Ethiopian Institute of Biodiversity Conservation (EIBC) have been practicing *ex situ* conservation system and undertaken systematic crop germplasm exploration and collection operations in the different administrative regions of the country covering a wide range of agro-ecological conditions. This method has, however, arrested the complex interaction of genetically diverse traditionally cultivated varieties (landraces) with their associated pests, predators, and pathogens (Worede *et al.*, 2000). *Ex situ* conservation also fails to retain traditional farmers' knowledge associated with landraces, knowledge that can be instrumental in utilization and development of new crop varieties from farmers' original varieties.

## **5. Genetic markers**

For genetic conservation work, determining the magnitude of genetic variability and its pattern of distribution in the different *in situ* crop conservation sites and altitude gradients is essential (Bekele, 1985). Such information could serve as a benchmark for future assessment of genetic erosion (Hammer *et al.*, 1996) and for proper management and better exploitation of the existing gene pool (Jain *et al.*, 1975; Bekele, 1984).

Morphological, biochemical and molecular markers are used to measure the pattern of genetic variation and this may assist in genetic resource conservation and utilization by determining the relative contribution of the different levels of variability to the total diversity available in the area of crop domestication and in center of genetic diversity (Kebebew *et al.*, 2001).

### **5.1. Morphological traits**

#### **5.1.1. Diversity of characters**

Since the early days of taxonomy, morphological characteristics have traditionally been used as a basis for classification and still overwhelming reliance is placed on morphological traits to produce practical classification. Characterization for agronomic and morphological traits is imperative for the plant genetic resource collections to be of practical value to plant breeders (Anderson and Fairbanks, 1990). A considerable number of researches have been done related to this aspect and some of the findings are reviewed below.

Negassa (1986) studied phenotypic diversity and breeding potential of an Ethiopian wheat collection consisting of tetraploid and hexaploids and scored for 14 qualitative and/or quantitative characters each having two or more phenotypic class. He found varied patterns of variation of the individual characters from region to region. Some characters showed localized concentration and others did not have any

clear pattern of distribution. A clinal pattern of variation was observed for resistance to powdery mildew. He also observed the frequency of resistance to get increased from north to south with a concentration of intermediate resistance on the Aresi-Bale highlands. Intermediate to high level of diversity was observed for all characters except for glume pubescence. Peceetti and Damania (1996) also found that the landraces of tetraploid wheat (*Triticum turgidum* spp. *turgidum*) collected from the two provinces of Ethiopia, Shewa and Tigray, to be distinctly different.

Tessema and Belay (1991) reported regional differences of diversity estimates for collections of Ethiopian tetraploid wheat from the central highlands; whereas Bechere *et al.* (1996) did not find such a difference for collections from the same area.

In Belay *et al.* (1997) study, thirty-four allotetraploid ( $2n=4x=28$ ) wheat (*Triticum turgidum* L.) landrace populations collected from four regions in Ethiopia consisting of large number of entries were characterized for glume color, glume pubescence, beak awn, seed color and spike density with the main objective of analyzing the diversity and distribution of these traits on the basis of administrative regions and altitude gradients. They observed all characters to be polymorphic in all regions and most altitude groups with the exception of spike density. However, clinal patterns were observed in only a few cases. The highest diversity was found in regions with relatively better climatic conditions and in optimal altitude ranges. On country basis, seed color and spike density displayed the highest and the lowest diversity indices, respectively. In their study spike density was the only character that exhibited significant differences both between regions and between altitudes.

Beuningen and Busch (1997) studied the genetic diversity among a broad collection of 289 north American spring wheat cultivars from the USA, Canada and Mexico using cluster analysis, all but six cultivars could be grouped into 17 major clusters. Major clusters grouped cultivars of common origin, parentage, and/or era of release.

Alemayehu and Parlevliet (1997) investigated the variation between and within 18 barley landraces collected from 18 localities of six barley growing regions of Ethiopia to find the variation in five quantitative traits and they found very large variation between and within landrace. The magnitude of variation was so large that most plants within a landrace had a different genotype. The landraces also varied in the degree of variation. Some landraces were more variable for most or all traits than other landraces.

Ayana and Bekele (1998) also evaluated sorghum (*Sorghum bicolor* L. Moench) accessions collected from different geographical regions in Ethiopia and Eritrea. They found high and comparable levels of phenotypic variation between the regions of origin and between the adaptation zones. However, significant levels of variation were found within the regions of origin and within adaptation zones. Partitioning of the variation into within and between regions as well as into within and between adapted zones further confirmed that a large portion of the total variation was found within regions of origin and within adaptation zones.

Bekele (1996) also evaluated accessions of *Eragrostis tef* (Zucc.) Trotter collected from 14 major sites in Ethiopia. The distribution patterns of the characters were found to be variable for some characters and constant for others. The results of principal component analysis revealed a marked regional pattern in grouping the characters studied. The various analyses showed that each of the regions contained a very large number of different genotypes.

### **5.1.2. Nature and magnitude of variation**

Primarily, biological variation presents in the plant population is of three types, viz., phenotypic, genotypic and environmental. Phenotypic variation is the total variability which is observable. It includes both genotypic and environmental variation and hence changes under different environmental conditions. Such variation is measured in terms of phenotypic variance. Genotypic variation is the inherent or genetic variability which remains unaltered by environmental conditions. This type of

variability is more useful to a plant breeder for exploitation in selection or hybridization. Such variation is measured in terms of genotypic variance. The genotypic variance consists of additive, dominance and epistatic components. Environmental variance refers to non-heritable variation which is entirely due to environmental effects and varies under different environmental conditions. This uncontrolled variation is measured in terms of error mean variance. The variation in true breeding parental line and their  $F_1$  is non-heritable.

Genetic variability is of immense importance to the breeder, because it can be transmitted to the progeny and the proper management of this diversity can produce permanent gain in the performance of the plant (Welsh, 1981). Moreover, identification of plants that exhibits variations for the trait or traits of interest to the plant breeder is the first step to the plant breeder (Stoskopf *et al.*, 1993).

Narasamha Rao and Parathasarathi (1968) evaluated 14 finger millet varieties and found a wide range of variations for plant height, finger number, grain yield and high coefficients of variation for plant height and grain yield. High coefficient of variations were also reported for grain yield in the study of 34 indigenous finger millet from sikkim along with 12 varieties selected from all-Indian coordinated variety trial (Goswami and Asthana, 1984) and in segregating populations of finger millet (Prabhakar and Prasad, 1984).

Kempanna and Govidu (1969) observed remarkable variation for plant height, maturity, structure and composition of the ears and grain colors among African collections of finger millet. The investigation of Walia and Solomon (1970) revealed a wide variability for weight of the main ear and number of grains in the main ear and they suggested that these characters can be used for genetic improvement of the grain. Kebede and Menkir (1989) reported the indigenous Ethiopian finger millet to have a vast range of genetic variability in seedling vigor, days to maturity, number of fingers and ears, length of fingers, plant height and seed color. The result of the study of Hussaini *et al.*, (1977) showed high variation among the entries from world collection of finger millet for 18 of the 29 characters assessed.

Abraham *et al.* (1989) evaluated 20 finger millet varieties and observed high genotypic and phenotypic coefficients of variation for effective tillers per plant, grain yield per plant, 1,000–grain weight and fingers per ear. Likewise, Debelo (1998) reported high phenotypic and genotypic coefficient of variations for biomass weight, ear weight, grain yield and productive tillers per plant and moderate for ear length. Whereas low genotypic coefficients of variation for fingers per plant, plant height and harvest index. Daba (2000) also recorded high genotypic and phenotypic coefficients of variation among 64 genotypes collected from Ethiopia and Zimbabwe for productive tillers per plant, ear weight per plant, ear length and grain weight per plant.

### **5.1.3. Heritability and genetic advance**

#### **5.1.3.1. Heritability**

Heritability and genetic advance are important selection parameters (Singh and Narayanan, 1993). Heritability is the ratio of genotypic variance to the phenotypic variance or the total variance (broad sense) or the ratio of additive genetic variance to the phenotypic variance (narrow sense) (Allard, 1960). It is generally expressed in per cent. Thus, heritability is the heritable portion of phenotypic variance. It is a good index of the transmission of characters from parents to their offspring (Falconer, 1981). The estimates of heritability help the plant breeder in selection of elite genotypes from diverse genetic population (Singh and Narayanan, 1993). The employment of heritability, particularly, in the broad sense is common in selection of individual characters in crop improvement. Heritability is of interest to the plant breeder mainly as a measure of the values of selection for a particular character and as index of transmissibility.

The investigation of Dhagate *et al.* (1972) showed high heritability in ragi for days to 50% flowering and maturity, number of tillers and low for grain yield. In contrast to this, Prabhakar and Prasad (1984) found high heritability for grain yield in segregating population of finger millet. High heritability estimate

values were also reported at different times by different authors for days to maturity (Reddy *et al.*, 1984); grain weight, plant height, and days to maturity (Shankar, 1985); days to maturity and flowering, finger number per plant and 1,000-grain weight (Abraham *et al.*, 1989). Moderate to high heritability estimates were recorded for number of total and productive tillers in four F<sub>2</sub> populations of finger millet (Ravikumar and Seetheram, 1994).

In genetical studies of the segregating population of finger millet derived from Indo-African crosses, Debelo (1998) found high heritability in a broad sense for days to 50% flowering, days to maturity, plant height and harvest index, while biomass, ear weight, grain yield, and productive tillers per plant revealed low to moderate value. The investigation of Daba (2000) also showed high estimates of heritability for ear weight per plant, ear length, biomass per plant, plant height, productive tillers per plant and fingers per main ear.

### **5.1.3.2. Genetic advance**

Genetic advance is an improvement in the mean genotypic value of selected plants over the parental population. It measures the expected genetic progress that would result from selecting the best performing genotypes for a character being evaluated. The success of genetic advance under selection depends on genetic variability, heritability and selection intensity (Allard, 1960).

High genetic advance values were recorded for straw yield in ragi (Chaudhari and Acharya, 1969) and for plant height (Goud *et al.* 1977). In the F<sub>3</sub> and F<sub>4</sub> inter varietal crosses of finger millet, grain yield exhibited high genetic advance (Prabhakar and Prasad, 1984). High genetic advance also reported for grain yield, 1,000-grain weight and fingers per ear (Abraham *et al.*, 1989); plant height, panicle length, straw yield and grain yield (Rao, 1991).

After they estimated the genetic advance in the indigenous varieties of finger millet in Sikkim, Goswam and Asthana (1984) suggested direct

selection for grain yield to be most effective followed by days to flowering, days to maturity, number of fingers on main ear and plant height. In other studies also grain yield and number of ears per plant showed high genetic advance (Verma, 1989) and moderate to high (Devkota and Mohapatra, 1991). Consequently, they concluded that phenotypic selection of these traits would be effective.

Populations derived from Indo-African crosses of finger millet revealed high genetic advance for biomass per plant, ear weight per plant and grain yield per plant and moderate to high for plant height (Debelo, 1998). Daba (2000) recorded high genetic advance for productive tillers per plant, biomass per plant, ear length and ear weight per plant.

#### **5.1.4. Interrelation among characters**

##### **5.1.4.1. Correlation**

Correlation coefficient is a statistical measure which is used to find out the degree (strength) and direction of relationships between two or more variables (Singh and Narayanan, 1993). In plant breeding, correlation coefficient analysis measure the mutual relationship between various plant characters and determines the component characters on which selection can be based for genetic improvement in yield. In other words, analysis of correlation coefficient at phenotypic, genotypic and environmental levels and path analysis for estimation of direct and indirect effects helps in understanding the association among yield and other traits for identifying yield and other developmental components for indirect selection (Dudly, 1997). Consequently, the identification of yield contributing plant characters helps in raising the breeding efficiency (Ebong, 1972).

Dhagate *et al.* (1972) observed the genetic correlation of grain yield with plant height, grain yield of main ear and 1,000-grain weight and phenotypic correlation of grain yield with maturity. In the late maturing strains of finger millet, Raji *et al.* (1973) recorded the highest association between the number

of tillers and yield. M'Shonga (1979) noted that high yielding finger millet varieties were characterized by early maturity and high value of grain weight per plant and number of grains per head. The other authors, Bhat and Shariff (1994) also mentioned the importance of selection for number of ears, ear weight, 1,000-grain weight and straw weight in enhancing grain yield in  $F_3$  generation of two crosses.

Shanthakumar and Gowda (1997) found that grain yield was positively associated with number of productive tillers per plant, number of fingers per main ear, number of grains per cm length of finger, ear weight per plant, plant height and length of finger. Marimuthu (1997) reported the highest positive association for grain yield with ear weight followed by number of productive tillers, finger numbers, finger length, plant height, days to 50% flowering and 1,000-grain weight.

The findings of Bandyopadhyay (1998) indicated negative and significant association between grain yield and days to maturity, and high harvest index and shortening of grain filling duration were found to enhance grain product at high altitude. Daba (2000) found positive and significant correlation for grain weight with days to maturity, plant height, productive tillers per plant and biomass per plant. This author also suggested selection for these traits in the materials assessed to be effective to improve grain yield per plant.

#### **5.1.4.2. Path-coefficient analysis**

Path-coefficient analysis is simply a standardized partial regression coefficient which splits the correlation coefficient into the measures of direct and indirect effects (Singh and Narayanan, 1993). It is quite possible that a trait showing positive direct effect on yield may have a negative indirect effect via other components. However, path analysis permits the examination of direct and indirect effects of various characters on yield as their indirect effect via other components. Thus, the estimates of direct and indirect effects help in determining the yield

components and provide bases for selection of superior genotypes from diverse breeding populations.

Productive tillers per plant and 1,000-grain weight were found to have the strongest direct effect on grain weight (Prabhakar and Prasad, 1983). The finding of Liu (1984) showed that the spike weight and plant height had positive indirect effect on grain weight. Ravindran *et al.* (1996) reported that the number of productive tillers per plant and fingers per main ear had high direct effect on grain yield, while plant height had a negative and indirect effect on yield. Consequently, these authors suggested selection based on the number of productive tillers per plant and finger number per main ear would be effective for grain yield improvement. As reported by Shanthakumar and Gowda (1997), ear weight per plant had the greatest direct effect on grain weight. Daba (2000) also reported that ear weight per plant had maximum positive direct effect on grain weight followed by plant height, harvest index, biomass per plant, 1,000-grain weight, days to flowering, fingers per main ear and productive tillers per plant. And he suggested single trait indirect selection based on ear weight to be more effective for improving yield in finger millet.

#### **5.1.5. Multivariate analysis**

Broadly speaking, multivariate analysis refers to all statistical methods that simultaneously analyze multiple measurements on each individual or object under investigation (Hair *et al.*, 1998). As such, many multivariate techniques are extension of univariate (analysis of single variable distribution) and bivariate analysis (correlation, analysis of variance, and simple regression used to analyze two variables). In many instances, multivariate techniques are a means of performing in a single analysis what once took multiple analysis using univariate techniques.

Deu *et al.* (1994) suggested that multivariate techniques might be helpful in quantifying the degree of relatedness among landraces. They allowed

efficient varietal classification for many crops. Hamman (1972) also indicated that multivariate techniques resolved several phenotypic measurements into fewer, more interpretable and more easily visualized dimensions. The method of cluster analysis could be one of the useful methods for determining the diversity among germplasm collection (Peeters and Martinelli, 1989) or the similarity of an entry relative to other entries. It can be used to assign entries to their specific group of similarity.

Germplasm curators as well as plant breeders have an interest in quantification and classification of genetic diversity. In germplasm collections, such a classification may help designate core collections to enhance efficiency of collection management and utilization. Transgressive segregation may be more likely to occur when parents in a cross are less similar, allowing different favorable alleles to be combined in the offspring (Cowen and Frey, 1987).

Ebba (1975) evaluated 35 tef cultivars and the cluster analysis grouped them into six major complexes unlike Costanza *et al.* (1979) who found only 3 groups for 36 accessions. Bekele (1986) evaluated different tef cultivars for 51 different morphological characters and more than 6 clusters were formed. In another investigation, the cluster analysis grouped the 320 tef genotypes into 14 major complexes consisting of one to 183 lines (Assefa *et al.*, 1999). Plant height, culm and panicle length, diameter of first and second internodes, main shoot panicle mass and grain yield, number of main shoot panicle branches and spikelets, and days to panicle emergence and maturity chiefly contributed for the gross variability among the 320 genotypes.

Hussaini *et al.* (1977) evaluated a world collection of 729 entries of cultivated *Eleusine coracana* (L.) Gaertn from Uganda, Ethiopia, Pakistan, Sikkim and 16 states of India and found 12 broad groups. In attempt to differentiate among the landraces, Wilson *et al.* (1990) assessed the S<sub>1</sub> bulks of 103 landraces of pearl millet [*Pennisetum glaucum* (L.) R.Br.] collected in central Burkina Faso. The landraces could be grouped into ten clusters of phenotypic diversity. Similarly, Ouendeba *et al.* (1995) evaluated ten pearl millet populations widely grown in several African

countries and experimental F1 hybrids and the principal component analysis grouped the 13 variables into 10 components with eigenvalues lying between 0.013 and 6.42. By considering only the four components with eigenvalues greater than 1, more than 92% of the total variation could be explained. Principal component 1 accounted for 49% of the total variation and it was equally associated with spike length, stem diameter, days to flowering and plant height. The cluster analysis revealed similarities between Niger and Senegal and between Niger and Nigerian landraces populations.

Van Beuningen and Busch (1997) assessed 289 spring wheat cultivars from USA, Canada, Mexico and grouped them into 17 major clusters. Ezeaku *et al.* (1999) conducted a classification study of sorghum germplasm accessions using two multivariate methods and their results revealed the existence of a considerable range of variability within the cultivated sorghums for 13 traits studied. Days to flowering represented 64.8% of the total variation observed within the 352 accessions while the remaining 12 traits accounted for 35.2%. Relatively high variation for these traits indicated greater contribution to phenetic diversity.

In investigation by Tsehaye and Kebebew (2002), the dendrogram obtained from the hierarchical cluster analysis grouped the 42 original finger millet populations sampled from 8 major growing regions into 7 clusters. A total of 11 principal components were extracted from 11 variables and found four principal components significant for that particular study. The four principal components explained 74% of the variation. The first principal component was strongly associated with the traits such as days to 50% flowering, days to 75% maturity, finger number, plant height and 100-seed weight. In chick pea, cluster analysis grouped the fifty populations sampled from ten regions of Ethiopia into seven clusters (Workeye 2002). A total of ten principal components were extracted, which accounted for the entire (100%) of the variability evident among the test genotypes. The first four which were significantly explained a cumulative of about 81.4% of the entire variability apparent among the test genotypes. The first principal component which alone explained for over one third of (about 44.8%) of the total variation had been due chiefly to variation

in number of primary branches, number of secondary branches, number of seeds per plant, plant height and grain yield.

## **5.2. Isozyme pattern**

Isozymes are a class of enzymes that share a common substrate but exhibit different electrophoretic mobilities (Market and Moller, 1959). When different electrophoresis are identified by genetic analysis as allelic, they are called allozymes.

Isozyme analysis was one of the most effective means to estimate the extent of genetic variations in native plant populations because most enzymes can be divided into some polymorphic forms called isozymes which can be separated by electrophoresis.

Isozyme analysis has also become a standard research tool of specific value for the assessment of phenetic relationship among populations and species demarcation, in part because the genotype to phenotype relationships are relatively simple (Das and Mukherjee, 1997)). Individual unit characters (loci) can be identified and screened in an individual organism (Bera and Mukherjee, 1992; Davis and Nixon, 1992) to provide the data that supplement conventional morphological parameters.

Developments in electrophoretic separation of proteins have dramatically increased the number of genetic markers available for use in plant breeding. The most widely used protein markers in plant breeding are isozymes (Tao *et al.*, 1999). Biochemical methods of investigation especially isozyme studies have provided valuable tools for rice geneticists. Electrophoretically identifiable isozymes have often been utilized for the classification of varieties within *O. sativa* (Oka, 1958; Shahi *et al.*, 1969; Pai *et al.*, 1975).

Study was carried by Suh *et al.* (1997) to identify the genetic basis of the weedy rice distributed in the various countries of the world. One hundred and fifty two strains collected from 10 countries were tested for variation in six morpho-

physiological characteristics and 14 isozyme loci. From the results of multivariate analysis based on the morpho-physiological characteristics and the isozymes, they classified weedy rice strains into *indica* and *japonica* types, and each type was further divided into forms resembling cultivated and wild rice.

Li and Rutger (2000) studied the genetic organization of isozyme variation in rice (*Oryza sativa* L.) based on 17 polymorphic isozymes loci using a sample of 511 accessions of worldwide origin. They found very high genetic diversity within the species as compared with most self-pollinated plant species. Three diversity centers were detected for isozyme variation including south Asia, China and southeast Asia.

Gao and Hong (2000) undertook a study in order to determine the genetic diversity and genetic structure of population in common wild rice *Oryza rufipogon*, an endangered species. They analyzed allozyme diversity using 22 loci in 607 individuals of 21 natural populations from the Guangxi, Guangdong, Hainan, Yunnan, Hunan, Jiangxi and Fujian provinces in China. Their study showed moderate allozyme variability which was relatively high for genus *Oryza*. The levels of genetic diversity for Guangxi and Guangdong were significantly higher than those for the other regions, and thus they concluded south China as a center of genetic diversity of *O. rufipogon* in China.

Posquet *et al.* (1999) evaluated domesticated and wild accessions of bambara groundnut populations for genetic diversity at 41 isozymes loci, representing 23 enzyme systems. Domesticated accessions were characterized by very low genetic diversity, while wild populations were characterized by higher genetic diversity. Intrapopulation diversity was comparatively high for domesticated and wild populations despite the near absence of heterozygous individuals which suggests a predominately selfing mode of pollination in both wild and domesticated Bambara ground nut. Overall isozyme diversity observed in their study was quite low confirming a previous study by Howell (1990).

Werth *et al.* (1994) compared 16 isozyme loci coding nine enzymes to evaluate the hypothesis that *Eleusine coracana* is an allotetraploid derived by hybridization between *E. indica* and unknown diploid and the result indicated genetic variability differed substantially among diploid species. The diploid tended to be genetically distinct. Both subspecies of the tetraploid *E. coracana* (*E. coracana* subsp.*coracana* and *E. coracana* subsp.*africana*) exhibited fixed heterozygosity at several loci. Both tetraploid also possessed *E. indica* marker alleles at all loci, corroborating ancestry by this taxon.

In a study conducted on *Eragrostis curveula* complex by Poverene and Voigt (1997), seed isozyme analysis of 52 strains including several morphological types and 24 hybrids provided useful descriptors. Distinct bands were found for each of those types and were used as isozyme markers. Clusters analysis revealed similarities within and among types in agreement with morphological characters. Results also provided new information about the genetic relationships of certain materials whose morphological characteristics were intermediate and allowed detection of misclassified strains. Moreover, the result of their study confirmed the validity of isozyme analysis as a complement to morphological classification and the isozyme markers proved useful as taxonomic descriptors.

### **5.3. Random amplified polymorphic DNA (RAPD) marker**

Protein-based polymorphism is a valuable tool in plant research, but the development of DNA-based technologies is providing a plentiful array of additional genomic markers. Restriction fragment length polymorphism (RFLP) analysis was applied to construct linkage maps in many species (Helentjaris, 1987; Landry *et al.*, 1987; Bonierbale *et al.*, 1988; McCouch *et al.*, 1988; Gebhardt, *et al.*, 1989; Heun *et al.*, 1991; Liu and Tsunewaki, 1991;), and the practical benefit of its application to plant breeding has been reviewed by Tanksley *et al.* (1989). Williams *et al.* (1990) proposed the use of random amplified polymorphic DNAs (RAPDs) as an additional form of molecular marker. The advantages of this technique over RFLPs include faster data production, a protocol that requires less DNA and radioactivity. Problems

concerning reliability can be alleviated by optimizing the experimental conditions and by following precisely a chosen experimental protocol. RAPD have been used to analyze genetic variation in several plant species (Halaward *et al.*, 1992; Hu and Quiros 1991; Vierling and Nguyen 1992).

Joshi and Nguyen (1993) conducted a study for detection of polymorphism among wild and cultivated tetraploid wheat using RAPD analysis of 40 primers in 20 accessions of wild tetraploid emmer wheat (*Triticum turgidum* L. ssp. *dicoccoides*) and ten genotypes of cultivated durum wheat (*Triticum turgidum* L. *durum*) selected from geographically diverse locations and they observed a higher level of polymorphism among different accessions of wild emmer wheat from Israel, Turkey and Jordan than the group of cultivated American, Turkish, and Syrian durum wheat. Moreover, based on RAPD product accessions from the same locality tended to cluster together.

M'Ribu and Hilu (1994) evaluated the potential use of RAPD as a source of genetic markers for studying variation among the four species of *Panicum* and within the crop species *P. miliacum* and *P. sumatrense* and they observed polymorphism in RAPD markers across and within species. Their study demonstrated that RAPD markers could be applied for studying genetic diversity, defining gene pools, and identifying cultivars for this group of millets. Cao *et al.* (1999) also suggested that RAPD analysis can be used to classify germplasm and to distinguish some species in *Triticum* after they conducted a study on 12 *Triticum* accessions suspected misclassified based on morphological characters. In similar type of studies, Connolly *et al.* (1994) found extremely high level of polymorphism within species of sweet potato (*Ipomoea batatas* L.) genotypes. The RAPD study also revealed genetic diversity in finger millet though the pattern of genetic variation in the crops was loosely correlated to geographical distribution (Hilu, 1995).

Salimath *et al.* (1995) assessed the genomic origins and genetic diversity in the genus *Eleusine* with three DNA markers technique, restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD) and inter

simple sequence repeat amplification (ISSR) and found 14, 10 and 26% polymorphism, respectively in 17 accessions of *E. coracana* from Africa and Asia. However, regardless of individual primers differed widely in their ability to detect polymorphism, Fakrudin *et al.* (2004) found 85.82% polymorphism using 37 RAPD primers in 12 selected finger millet accessions, representing different geographical origins and pedigree background.

Wachira *et al* (1995) used RAPD markers to estimate genetic diversity and taxonomic relationships in 38 clones belonging to the three tea species, *Camellia assamica*, *C. sinensis* and *C. assamica* ssp. *lasiocalyx*. Extensive genetic variability was detected between species that was partitioned into between and within population components. They detected 70% variation within populations. RAPD analysis also discriminated all of the 38 commercial clones, even those which cannot be distinguished on the basis of morphological and phenotypic traits.

In a study conducted to determine if RAPD markers could be used to assess the variability within and among species of annual *Medicago sativa*, Brummer *et al.* (1995) found variation among accessions of all species. Several accessions were considerably different from the others within species and in one of the species; four accessions were differentiated by both morphological characters and RAPD banding patterns from the other accessions.

## **MATERIALS AND METHODS**

### **1. Materials, equipments and chemicals**

#### **1.1. Plant materials**

Sixty-six finger millet accessions consisting of 64 landraces and 2 standard varieties (Paadet and Tadesse) were used in this study. The landraces were collected from 5 former regions of Ethiopia (Tigray, Gonder, Gojam, Welega, Gamo Gofa) and Eritrea covering different agro-ecologies (Table 1).

The landraces along with their passport data were obtained from the Ethiopian Institute of Biodiversity Conservation (EIBC). The improved varieties were provided by Melakasa Agricultural Research Center (MARC). However, the improved varieties were not included in the analysis of frequency distribution and Shannon-Weaver diversity index since they are introduction and only 2 in number.

#### **1.2. Equipments**

##### **1.2.1. Equipments for morphological diversity study**

- Digital balance
- Caliper
- Meter
- Glass lens

##### **1.2.2. Equipments for diversity study using isozyme markers**

- pH meter
- Electrophoresis apparatus
- Microwave

- Light box for gel analysis
- Microtube
- Refrigerator
- Gel cutter
- Digital balance
- Incubator
- Power supply
- Glass tray
- Porcelain spot plate

### **1.2.3. Equipments for diversity study using RAPD markers**

- Pipetman and tips
- Microcentrifuge tubes
- PCR machine (Thermo cycler)
- Gel electrophoresis tank
- Incubator
- Gel mould
- Electrophoresis apparatus
- Power supply
- Centrifuge
- Pestles and mortars
- Microwave
- Ultraviolet lamp and gel photography apparatus
- Computer
- Scanner

### **1.3. Chemicals**

#### **1.3.1. Chemicals for morphological diversity study**

- Fertilizer: diammonium phosphate (18:46:0) and urea (46:0:0)

#### **1.3.2. Chemicals for diversity study using isozyme markers**

- Mercaptoethanol
- Tris-citrate
- Sodium borate
- Hydrolyzed starch
- Staining solution (Appendix Table 2)
- Glycerol

#### **1.3.2. Chemicals for diversity study using RAPD markers**

- PCR (polymerase chain reaction) buffer
- MgCl<sub>2</sub>
- dNTPs (deoxyribonucleotide triphosphate)
- RAPD primers
- Taq* DNA polymerase
- DNA molecular marker standard
- Liquid nitrogen
- EDTA (disodium ethylenediaminetetra-acetate.2H<sub>2</sub>O)
- TBE (Tris-borate EDTA) buffer
- Ethidium bromide solution
- NaCl (sodium chloride)
- SDS (sodium dodecyl sulfate)
- Bromophenol blue

- Chloroform
- Isoamyl alcohol
- Isopropanol
- TE (Tris-EDTA)
- Sodium acetate
- Ethanol
- RNase (ribonuclease)
- Agarose

## **2. Methodology**

### **2.1. Morphological diversity study**

#### **2.1.1. Description of the area**

The field experiment was conducted at Aresi-Negele Research Sub-Center (Altitude, 1960 m.a.s.l., 7<sup>0</sup> 30' N and 39<sup>0</sup> 00'E) in Ethiopia during 2004 main cropping season. The soil is dark brown clay loam with pH of 6.3. The annual rainfall during the experimental season was about 980 mm (Appendix Table 1).

#### **2.1.2. Experimental design**

The Experiment was laid down in randomized complete block design (RCBD) with three replications. The seeds of each accession were mixed with sand at ratio of approximately 1:5 (seed: sand) to ensure uniform distribution. The seeds mixed with sand were drilled (Figure 1) on a plot of 4 m long in single row with a spacing of 75 cm between rows (plots). The spacing between plants within a row was maintained at 15 cm by thinning. The distance between blocks was 2.5 m. Each plot received 100 kg/ha DAP (18:46:0) at planting and 50 kg/ha urea (46:0:0) at early tillering stage on hectare basis. Weeding and other field management had been undertaken as frequent as required.

Five plants from each accession and replication (990 individuals) were randomly selected and tagged for data recording except for days to heading, maturity and grain-filling duration where they were on plot basis. Data on 21 morpho-agronomic characters (Table 2) were scored using International Board for Plant Genetic Resources descriptors for finger millet (IBPGR, 1985).

**Table 1** List of finger millet accessions with their respective former administrative region and district (woreda) and altitude.

Code	Accession no.	Local (vernacular) name	Region	Woreda	Altitude
1	100055	Dagussa	Gamo Gofa	Jinka	1450
2	100084	Dagussa	Gamo Gofa	-	1820
3	100094	Key dagussa	Welega	Diga	1290
4	100095	Dagussa	Welega	-	1480
5	204747	Dagussa	Eritrea	Debubawi Hamasen	2250
6	208444	Dagussa	Gonder	Dera	2500
7	208448	Dagussa	Gojam	Guangua	1250
8	208730	Daguja	Welega	Seyo	1900
9	211474	Baracha	Gamo Gofa	Konso	1560
10	213035	Bercha	Gamo Gofa	Gardula	1380
11	215841	Dagussa	Gojam	Bure and Shikudad	2050
12	215850	Dagussa	Gojam	Bahir Dar	1800
13	215867	Dagussa (Tikur)	Gojam	Bahir Dar	1990
14	215874	Dagussa	Gojam	Yilma and Densa	2350
15	215877	Dagussa	Gojam	Yilma and Densa	2230
16	215879	Dagussa	Gojam	Yilma and Densa	2400
17	215883	Dagussa	Gojam	Yilma and Densa	2400
18	215889	Dagussa (Nech)	Gojam	Bure and Shikudad	2100
19	215896	Dagussa	Gojam	Gwagusa and Wenber	2160
20	215973	Dagussa	Gonder	Gonder Zuriya	2090

**Table 1** (Cont'd).

Code	Accession no.	Local (vernacular) name	Region	Woreda	Altitude
21	215977	Dagussa	Gonder	Dembiya	1940
22	216024	Daguja	Welega	Gudru	2330
23	216025	Daguja	Welega	Gudru	2330
24	216028	Guracha Dagnja	Welega	Diga	2150
25	216041	Daguja	Welega	Nejo	1960
26	216043	Daguja	Welega	Nejo	1980
27	216045	Daguja	Welega	Nejo	1880
28	216051	Daguja	Welega	Lalo Asabi	1910
29	216052	Daguja	Welega	Lalo Asabi	1660
30	225895	Tikur Dagussa	Gojam	Mecha	1845
31	229723	Dagusa	Gojam	Mandura	1300
32	229726	-	Gojam	Dibate	1600
33	KNE#411	Paadet*	Kenya	-	-
34	229728	-	Gojam	-	1440
35	230101	Dagusa	Eritrea	Adikuala	1740
36	230117	Dagusa	Eritrea	Adikuala	1650
37	230130	-	Eritrea	Adikuala	1800
38	230136	-	Eritrea	Adikuala	1900
39	230714	Dagusa	Eritrea	Dekemehari	1950
40	230722	Dagusa	Eritrea	Dekemehari	2000
41	230724	Dagusa	Eritrea	Dekemehari	2040
42	234178	Dagusa	Tigray	-	1840
43	234205	Dagusa	Tigray	-	2100
44	235138	Dagusa	Gonder	-	2200
45	235141	Dagusa	Gonder	Wereta	1870
46	235700	Berecha	Gamo Gofa	Bako Gazer	1530
47	235830	-	Gonder	Dib Bahir	1640
48	235838	-	Gonder	Lay Armacheho	1290
49	235842	-	Gonder	Lay Armacheho	940

**Table 1** (Cont'd).

Code	Accession no.	Local (vernacular) name	Region	Woreda	Altitude
50	236447	Daguso	Welega	Sasiga	1630
51	236450	Daguso	Welega	Sasiga	2230
52	237447	Dagusa	Tigray	Werr	1570
53	237449	Dagusa	Tigray	Adi Arbate	1470
54	237451	Dagusa	Tigray	Adi Arbate	1450
55	237452	Dagusa	Tigray	Adi Arbate	1430
56	237459	Dagusa	Tigray	Adwa	1940
57	237462	Dagusa	Tigray	Tach Maychew	2100
58	237477	Dagusa	Tigray	Tsimbla	1710
59	238319	Dagusa	Tigray	Wemberta	2130
60	238331	Dagusa	Tigray	Adi Hageray	1300
61	238336	Dagusa	Tigray	Sheraro	1020
62	241769	Persheka	Gamo Gofa	Konso	1500
63	242123	Tikur Dagusa	Gonder	Lay Armacho	1720
64	242125	Tikur Dagusa	Gonder	Lay Armacho	2015
65	242131	Tikur Dagusa	Gonder	Dembia	2350
66	KNE#1098	Tadesse*	-	Kenya	-

- Information not available, \* Standard variety, developed from introduction.



**Figure 1** Drilling of the seeds mixed with sand on well prepared and leveled experimental plots.

**Table 2.** Morpho-agronomic characters recorded, their codes and description.

Character	Code	Description
Growth habit	GRH	40 days after sowing. 3=decumbent, 5=erect, 7=prostrate
Ear shape	ESHP	At dough stage. 1=droopy (fingers lax and drooping), 2=open (fingers straight), 3= semi-compact (tops of fingers curved), 4=compact (fingers incurved), 5=fist-like (fingers very incurved)
Grain shape	GSHP	At post harvest. 1=round, 2=reniform, 3=ovoid, 4=irregular
Grain surface	GSUR	At post-harvest. 1=smooth, 2=wrinkled
Grain color	GCL	At post-harvest. 1=white, 2=orange-red, 3=dark-brown

**Table 2** (Cont'd).

Character	Code	Description
Pericarp persistence	PPR	After threshing. 1=non-persistent, 2=intermediate- persistence, 3=persistence
Plant height (cm)	PH	From ground level to tip of finger (ear) at dough stage
Days to heading	DTH	From sowing to stage when ears have emerged from 50% of main tillers
Days to maturity	DTM	From sowing to stage when 50% of main tillers have matured ears
Grain filling duration	GFD	Days to maturity – days to heading
Productive tillers	PRT	Number of tillers which bear matured ears
Finger length (cm)	FL	From base to tip of longest spike (finger) on main tiller at dough stage
Finger width (mm)	FW	Measured across center of longest finger at dough stage
Finger number	FNU	On main ear at dough stage
Culm thickness (mm)	CT	Diameter of internode between third and fourth nodes from top at dough stage
Leaf number	LENU	Number of leaves on main tiller at heading
Leaf blade length (cm)	LEBL	Measured from ligule to tip of fourth leaf from top at heading
Leaf blade width (cm)	LEBW	Measured across center of fourth leaf blade from top at heading
No. of grains per spikelet	NGPSP	At harvesting
Thousand-grain weight (g)	TGW	At post harvest
Grain yield per plant (g)	GYPPL	Means of 5 plants at post harvest

### 2.1.3. Data analysis

The percentage frequencies of the phenotypic classes of each character, accession and region were calculated for 6 discrete characters (growth habit, ear shape, grain shape, grain surface, grain color and pericarp persistence). The Shannon-Weaver diversity index ( $H'$ ) was computed using the phenotypic frequencies to assess the overall phenotypic diversity for each character, region and countrywide. This parameter has been used extensively in evaluating genetic resources to measure phenotypic diversity for discrete characters both among (Jain *et al.*, 1975) and within country gene pools (Negassa, 1986; Bekele, 1996; Demissie and Bjornstad, 1996; Belay *et al.*, 1997; Ayana and Bekele; 1998; Kefyalew *et al.*, 2000; Tsehaye and Kebebew, 2002; Workeye, 2002). The Shannon-Weaver diversity index,  $H$  described by Hutchenson (1970) is given as follows:

$$H = -\sum_{i=1}^n P_i \log_e P_i$$

Where  $P_i$  is the proportion of individuals in the  $i^{\text{th}}$  class of an  $n$ -class character and  $n$  is the number of phenotypic classes for a given character. Each value of  $H$  was divided by its maximum value,  $\log_e n$ , and normalized in order to keep the values between zero and one and designated as  $H'$ . The non-normalized values  $H$  using regions as classifying variables were used for one-way analysis of variance of diversity for each character. The computations were conducted using MINITAB for windows release 14.3 (MINITAB, 2004) and Microsoft Excel computer software.

To examine the extent of variability among the finger millet accessions, the data of 15 morpho-agronomic characters (plant height, days to heading, days to maturity, grain- filling duration, productive tillers per plant, culm thickness, finger length, finger width, finger number per main ear, leaf number main tiller, leaf blade length, leaf blade width, 1,000- grain weight, number of grains per spikelet and grain yield per plant ) were subjected to analysis of variance using MSTATC software (Michigan State University, 1991).

The genotypic ( $\sigma^2_g$ ), phenotypic ( $\sigma^2_p$ ) and error ( $\sigma^2_e$ ) variances were computed using the formulae of Burton and De Vane (1953) as follows:

$$\sigma^2_g = \frac{MSg - MSe}{r}$$

$$\sigma^2_e = MSe$$

$$\sigma^2_p = \sigma^2_g + \sigma^2_e$$

Where: MSg = genotype mean square

MSe = environmental variance (error mean square)

r = the number of replications

The phenotypic (PCV), genotypic (GCV) and error (ECV) coefficients of variation were estimated following the procedure of Kumar *et al.* (1985):

$$PCV = \frac{\sigma_p}{\bar{X}} \times 100$$

$$GCV = \frac{\sigma_g}{\bar{X}} \times 100$$

$$ECV = \frac{\sigma_e}{\bar{X}} \times 100$$

Where:  $\sigma_p$  = phenotypic standard deviation

$\sigma_g$  = genotypic standard deviation

$\sigma_e$  = environmental standard deviation

$\bar{X}$  = character mean

Heritability ( $h^2$ ) in a broad sense was estimated by the formula of Allard (1960) as:

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Expected genetic advance (GA), assuming a selection intensity of 5% was estimated according to the method of Johnson *et al.* (1955):

$$GA = kh^2\sigma_p$$

Where: k is selection intensity

Genetic advance as percent of mean was calculated to compare the extent of predicted genetic advance of different characters under selection using the formula of Johnson *et al.* (1955):

$$GA \text{ as \% of mean} = \frac{GA}{\bar{X}} \times 100$$

Where:  $\bar{X}$  = mean value of the character concerned

Phenotypic ( $r_{pxy}$ ), genotypic ( $r_{gxy}$ ) and environmental ( $r_{exy}$ ) correlation coefficients were estimated by employing the formulae of AL-Jibouri *et al.* (1958) as:

$$r_{pxy} = \frac{CoV_{pxy}}{\sqrt{\sigma_{px}^2 \cdot \sigma_{py}^2}}$$

$$r_{gxy} = \frac{CoV_{gxy}}{\sqrt{\sigma_{gx}^2 \cdot \sigma_{gy}^2}}$$

$$r_{exy} = \frac{CoV_{exy}}{\sqrt{\sigma_{ex}^2 \cdot \sigma_{ey}^2}}$$

Where:  $CoV_{pxy}$  = phenotypic covariance of characters of x and y

$\sigma_{px}^2$  = phenotypic variance of character x

$\sigma_{py}^2$  = phenotypic variance of character y

$CoV_{gxy}$  = genotypic covariance of characters of x and y

$\sigma_{gx}^2$  = genotypic variance of character x

$\sigma_{gy}^2$  = genotypic variance of character y

$CoV_{exy}$  = environmental covariance of characters of x and y

$\sigma_{ex}^2$  = environmental variance of character x

$\sigma_{ey}^2$  = environmental variance of character y

Direct and indirect path-coefficients were calculated as described by Dewy and Lu (1959) using Agres-1 statistical package.

The fifteen morpho-agronomic (plant height, days to heading, days to maturity, grain- filling duration, productive tillers per plant, culm thickness, finger length, finger width, finger number per main ear, leaf number main tiller, leaf blade length, leaf blade width, 1,000- grain weight, number of grains per spikelet and grain yield per plant) mean data were standardized to eliminate the possible impact of different scale prior to multivariate analysis. Then, cluster analysis (Wards method) was performed on squared Euclidean distance matrix. This method, which minimize within cluster variance summed over all variables appear to give the most satisfactory clustering results with most cultivars included in cluster of similar size (Sneath and Sokal, 1973).

The relationships among the clusters were assessed by measuring the inter-clusters distances using Mahalanobis's distance ( $D^2$ ).  $D^2$  is a commonly used

measure of Euclidean that directly incorporates a standardization procedure (Hair *et al.*, 1998). The  $D^2$  values obtained from pairs of clusters were considered as the calculated value of chi-square ( $\chi^2$ ) and were tested for significance both at 1 and 5% probability level against tabulated values of ( $\chi^2$ ) for 'P' degrees of freedom, where P is the number of characters considered (P=15) (Singh and Chaudhary, 1985). Principal component analysis (PCA) using correlation matrix was performed to define the existing pattern of variation among population. Data were analyzed using MINITAB for windows release 14.3 (MINITAB, 2004).

## **2.2. Diversity study using isozyme markers**

The isozyme study was performed at the molecular genetics laboratory of Ethiopian Institute of Biodiversity Conservation (EIBC). Leaf and root tissue of 66 accessions was used to prepare homogenates by grinding leaf tissue in porcelain spot plates using Tris-HCl buffer and 0.1% v/v mercaptoethanol as previously described by Wendel (1989).

A Tris-citrate/ sodium borate system (electrode buffer: 0.06 M NaOH, 0.3 M boric acid, pH 7.8; gel buffer: 0.0019 M Tris, 0.005 M citric acid, pH 7.8) (Chamberlain, 1998) was used for assaying of 6 enzyme systems which were Esterase (EST), Aspartate aminotransferase (AAT), phosphoglucose isomerase (PGI) phosphoglucomutase (PGM), acid phosphatase (ACPH) and aldolase (ALD). Methods compiled by Muller-Starck (1999) for horizontal gel electrophoresis as indicated below were employed.

### **2.2.1. Gel preparation**

Two hundred and thirty milliliters (ml) of gel buffer was placed in 250 ml conical flask and boiled in the microwave oven on medium power first for 2 minutes and after shaking for one minute. The starch was suspended in 70 ml of gel buffer in a 1 liter Buchner flask. The boiling buffer was added in one movement to the starch solution and the Buchner flask was shaken vigorously in a circular motion. The

Buchner was kept again in the microwave oven and heated at medium power first 1 minute and after shaking for 2 minutes. Air bubbles in the gel matrix were removed using vacuum pump. The starch was poured quickly into the center of a glass gel mould and allowed to settle at room temperature for about 20 minutes and then placed at 4<sup>0</sup>C to cool completely.

### **2.2.2. Homogenization**

The samples were mechanically ruptured in extraction buffer (50 Mm Tris-HCl buffer and 0.1% v/v mercaptoethanol) using a grinding pestles. The crude extracts were absorbed simultaneously using 3MM filter paper strips (10x4 mm wide), each of which applied to a separate gel slice. To remove any excess homogenate, the paper strips were dried lightly on a paper towel, avoiding the contamination of others. To load the gel, the gel was cut approximately at 2 cm parallel to the cathodal end. Along this cut, up to 15 strips were inserted. The loading was completed by pushing the cathodal gel strip firmly back to its original position.

### **2.2.3. Electrophoresis**

Electrophoresis was carried out through 12 % starch gels. The gel mould was placed into the electrophoresis apparatus in which running buffer was filled. A drop of 1% aqueous bromophenol blue was added on the surface of the first and last wicks. The surface of the gel was covered with parafilm. Then, the gel and buffer was connected with cellulose sponge. The cooler plate was placed and the apparatus was adjusted at 4<sup>0</sup>C. Power was applied at 50 mA (200 V) to the system and left the gel to run for a few hours with close follow-up.

### **2.2.4. Staining and analysis of the zymogram**

Staining methodology followed Chamberlain (1998) and the staining solutions are listed in Appendix Table 2. After the electrophoresis, the starch gel was sliced horizontally using a thin copper wire and each of the slices were

transferred carefully to the individual staining trays containing the appropriate staining solution (Appendix Table 2) for each slice. Eventually zymograms were assessed. Data were not analyzed as there were no comparable data bases for the genetic variation for all enzyme system across all accessions that could make meaningful interpretation.

### **2.3. Diversity study using RAPD markers**

#### **2.3.1. DNA isolation**

The experiment was conducted at the Laboratory of Department of Genetics, Faculty of Science, Kasetsart University. The seedlings of 66 accessions were grown in pot for about 3 weeks and the leaves of each accession were harvested separately, and then washed and ground into a fine powder in liquid nitrogen and used directly for DNA extraction following the protocol of Dellaporta *et al.* (1983) as modified by Rueda *et al.* (1998) with further minor modification as follows.

- 0.5 g of leaf tissue was ground in liquid nitrogen with a pestle and mortar. A little more liquid nitrogen was added when necessary to keep the powder from thawing while grinding.
- The frozen powder was transferred using spatula into 1.5 ml eppendorf centrifuge tube containing 600 µl of extraction buffer (EB) (100 mM Tris, 50 mM EDTA, 500 mM NaCl, 1.25% SDS) and 10 mM β-mercaptoethanol.
- The mixture (the frozen powder in the EB) was vigorously shaken and incubated the tube at 65 °C for 30 minutes with inverting at 10 minutes interval for brief time.
- 600 µl chloroform/isoamyl alcohol (CIA, V/V=24:1) was then added to the tubes and centrifuged at 13,000 rpm for 15 minutes.
- The supernatant was transferred into clean 1.5 ml eppendorf tubes containing 600 µl of cold (-20 °C) isopropanol. Then, mixed gently and incubated at -20 °C for 30 minutes.

- The tubes were again centrifuged at 13,000 rpm for 5 minutes.
- The supernatant was poured off and the pellet was lightly dried by inverting the tubes on paper towel.
- The pellet was redissolved in 500  $\mu$ l of Tris-EDTA (TE 10:1) buffer at room temperature for 20-30 minutes till the entire pellet was dissolved and then the tube was centrifuged for 12 minutes at 10, 000 rpm to remove the debris.
- The supernatant was transferred to a new eppendorf tube, then 75  $\mu$ l 3 M sodium acetate and 500  $\mu$ l isopropanol were added and mixed well. The mixture was spun in microfuge.
- The supernatant was discarded while saving the pellet and washed with 700  $\mu$ l 80% cold (-20 °C) ethanol.
- After centrifuged for 5 minutes at 12,000 rpm, the ethanol was discarded and the pellet was air-dried to eliminate the alcohol completely.
- 500  $\mu$ l sterile ultrapure water was added and maintained at room temperature for 1 hr to redissolve the pellet.
- 5  $\mu$ l of RNase (10 mg/ml) solution was added and incubated for 1.5 hr at 37 °C to ensure that all the remaining RNA was digested.
- 500  $\mu$ l of CIA was added and centrifuged at 1,200 rpm for 10 minutes to separate the phases.
- The upper aqueous layer was recovered in a new eppendorf tube and 1/10 volume of 3 M sodium acetate and 2 volumes of cold (-20 °C) 100% ethanol was added and maintained for 1 hr at -20 °C.
- The mixture was centrifuged for 15 minutes at 12,000 rpm and the supernatant was discarded and then the DNA pellet was washed with 600  $\mu$ l of cold (-20 °C) 70% ethanol and air-dried to eliminate the alcohol.
- 200  $\mu$ l TE (10:0.1) was added to dissolve the DNA pellet.
- The DNA solution was monitored by subjecting each sample to 1% agarose gel electrophoresis in 1x TBE (Tris-Borate EDTA) buffer and the gel was stained in ethidium bromide. The visual bands under UV lamps were photographed and compared with standard DNA marker. The DNA concentration was adjusted to 100 ng/ $\mu$ l and then kept at -20 °C for further RAPD analysis.

### **2.3.2. PCR amplification and electrophoresis**

Primers were screened based on their ability to detect distinct and clearly resolved amplified products. PCR amplification and electrophoresis were undertaken to identify the polymorphism among the 66 finger millet accessions. The PCR was performed in a volume of 25  $\mu$ l containing 2.5  $\mu$ l of 10X PCR buffer, 1.0  $\mu$ l of 50 mM magnesium chloride, 0.5  $\mu$ l of each of the 2.5 mM deoxyribonucleotide triphosphates (dATP, dCTP, dGTP, dTTP), 1.5  $\mu$ l of a single PCR primer, 1.25 U of *Taq* DNA polymerase, approximately 15 ng of genomic DNA template and 16.75  $\mu$ l ddH<sub>2</sub>O. Amplification was carried out in programmable thermal cycler for one initial denaturation cycle at 94<sup>0</sup>C for 2 minutes followed by 40 cycles at 94<sup>0</sup>C for 30 seconds for denaturation, at 36<sup>0</sup>C for 30 seconds for annealing, at 72<sup>0</sup>c for 1 minute for extension and at 72<sup>0</sup>C for 3 minutes for final extension, then held at 4<sup>0</sup>C for 5 minutes. On completion of the cycles the samples were stored at 4<sup>0</sup>c before electrophoresis. The PCR amplified products were separated out through 1% agarose and visualized through staining with ethidium bromide and photographed under UV light.

### **2.3.3. RAPD data analysis**

Data were recorded as presence (1) or absence (0) of DNA band from the examination of photographic negatives. Each amplified fragment was named by the source of the primer, the kit number and its approximate size in base pairs. The percent of polymorphism was calculated as  $P\% = (P/n) \times 100$ , where: P is the number of polymorphic bands and n is the total number of bands produced from the reaction. The polymorphism information content (PIC) of each fragment was determined as described by Weir (1996) as  $PIC = 1 - \sum P_i^2$ . Where: P<sub>i</sub> is the frequency of the i<sup>th</sup> allele in the observed accessions. Genetic similarity between pairs of accessions was estimated using simple matching coefficient (Sneath and Sokal, 1973),  $SM = (a+d)/(a+b+c+d)$ . Where: a= number of 1,1 matches, b= number of 1,0 matches, c= number of 0,1 matches and d= number of 0,0 matches. Cluster analysis was carried out on similarity estimates using unweighted pair-group methods, arithmetic average (UPGMA) and

the resulting cluster was represented as dendrogram. Data were analyzed using NTSYS-pc version 2.01 computer program (Rohlf, 1997).

## RESULTS AND DISCUSSION

### 1. Morphological diversity study

#### 1.1. Character distribution

Frequencies of the phenotypic classes of the 6 discrete characters expressed in percentage are shown in Table 3. All the traits considered showed marked differences in their distribution and amount of variation. In no cases monomorphic phenotypic classes were observed.

Three phenotypic classes were recognized for growth habit. The variation in growth habit showed that the decumbent type was predominant in all regions followed by prostrate with overall frequency of 25% whereas erect type was found to concentrate mainly in Eritrea with a frequency of 34%. This implied that the former two types might be highly preferred by most farmers in different regions which could be due to their capacity to suppress weeds at early growth stage so that farmers would save their time and could spend it for activities on other crops. The other reason could be that finger millet with these two types of growth habit could relatively stand moisture stress. Such growth habit might cover the soil at early stage and thereby reduced the evapo-transpiration and could enable to perform better. In the areas of low rainfall, spreading types are also preferred in other crops such as chickpea (Singh and Saxena, 1999).

Variable ear shapes were observed in all regions (Figure 2). The droopy finger type was concentrated in Tigray, Gonder and Eritrea, indicating the predominance occurrence of race coracana in the northern part because droopy finger is a characteristic of race coracana (De Wet, 1989). Regions in the northern part, Tigray, Gondar and Eritrea are known to be relatively hot and dry and race coracana is drought tolerant and can compete with weeds (De Wet, 1989). Whereas, the open (straight) one was found at the higher frequency in all regions except Gamo Gofa. The

semi-compact, compact and fist like were concentrated mainly in Gamo Gofa. This result is generally in agreement with the findings of Tsehaye and Kebebew (2002) who reported high frequency for droopy finger types in Gojam, Gonder and Tigray and for semi-compact types in Gamo Gofa and Illubabur. The distribution of the different ear shape types reflects the distribution and the existence of different races of finger millet populations in Ethiopian collections (Tsehaye and Kebebew, 2002). The wide range of agro-climatic condition of the country could lead to the development of different races of finger millet. De Wet *et al.* (1984) indicated that inflorescence morphology was associated with grain yield and was used by the farmers to distinguish complexes of cultivars.

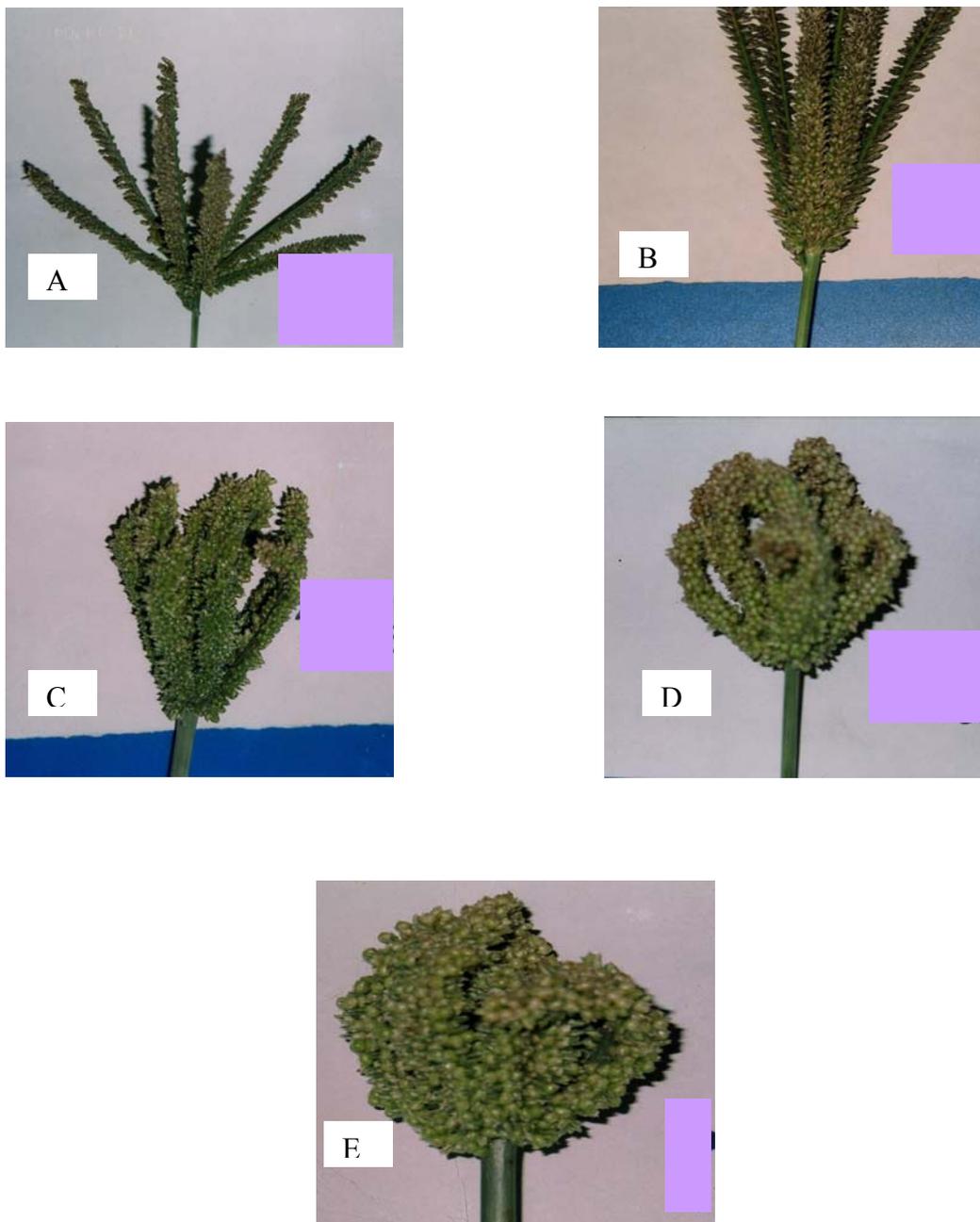
Round type of grain shape was predominant ( $\geq 50\%$ ) in all regions except in Eritrea where irregular grain shape was most frequent (46%). The reniform type was found at intermediate frequency (24%) in Gamo Gofa while ovoid type was observed mainly in Eritrea (22%).

The two classes of grain surface (smooth and wrinkled) were more or less distributed uniformly among all regions and did not show apparent trend relative to particular region manifesting there was no conscious human selection for these characters in subsistence agriculture. Though their adaptive significance is not known, grains with round shape and smooth surface are preferred by consumers.

**Table 3** Percentage frequencies of phenotypic classes of the 6 discrete characters in 64 finger millet accessions in the various regions and the overall collection.

Region	Growth habit			Ear shape					Grain shape				Grain surface		Grain color			Pericarp persistence		
	3 <sup>+</sup>	5	7	1	2	3	4	5	1	2	3	4	1	2	1	2	3	0	3	7
	Tigray	55	18	27	32	64	2	0	2	50	4	10	36	32	68	12	38	50	0	9
Gonder	42	32	26	30	62	8	0	0	50	10	13	27	40	60	0	27	73	0	20	80
Gojam	53	22	25	25	54	10	6	5	64	11	5	20	46	54	14	26	60	8	31	61
Welega	49	15	36	13	63	15	7	2	61	10	10	19	57	43	22	34	44	7	21	72
Gamo Gofa	62	23	15	7	20	23	20	30	57	24	10	19	51	49	0	26	74	2	46	52
Eritrea	54	34	12	37	60	0	0	3	32	0	22	46	38	62	0	38	62	0	14	86
Overall	52	23	25	24	57	9	5	5	54	9	10	27	44	56	10	32	58	3	22	75

<sup>+</sup> For phenotypic classes description codes see Table 2.



**Figure 2** The different types of ear shape observed in finger millet landraces: A=droopy (fingers lax and drooping), B=open (fingers straight), C=semi-compact (tops of fingers curved), D=compact (fingers incurved) and E=fist-like (fingers very incurved).

The three classes of grain color (white, orange-red and dark brown) were found in all regions except the white grain color, which was absent in Gonder, Gamo Gofa and Eritrea. The white grain color was rare with a maximum frequency of only 22% in Welega. The orange-red grain color showed intermediate frequency in all regions. The dark brown grain color was dominant in all regions reaching 73 and 74% in Gonder and Gamo Gofa, respectively. Similarly, Tsehaye and Kebebew (2002) in finger millet and Workeye (2002) in chickpea observed all classes of grain color in all regions from where the accessions were sampled.

Seed color is one of the most important characters which determines the quality and acceptance of cultivars. It has an economic value because it constitutes the basis for farmers' variety identification and commercial classification of different varieties of crops (Tsehaye and Kebebew, 2002). Farmers associated dark-colored with high-yield and hardiness with respect to climatic hazards such as poor soil fertility and they prefer it to grow even though it fetches less money as compared with white and red seed colored (Kefyalew and Ensermu, 1989). This could be the main reason for the preponderance of dark brown colored seed in all regions. Red colored varieties are good for making the local flat bread (injera). However, they require fertile soil that is free of water logging. In Ethiopia there exists an apparent association between phenotype and utility of, especially, grain color and human consumption (Zemedu, 1988).

The persistence pericarp type was predominant and concentrated in all regions reaching 91% in Tigray. The intermediate-persistence was also distributed moderately almost in all regions whereas the non-persistence type was absent in some regions but in Gojam, Welega and Gamo Gofa found at lower frequencies of 8, 7 and 2%, respectively. Overall, it comprised only 3%.

Finger millet seed is a challenge to threshing and milling because of its small size and its seed coat is bound tightly to the edible part (endosperm) inside (Tsehaye and Kebebew, 2002). Therefore, the entries having low pericarp-persistence (non-persistence type) could be a good source of genes for improving the threshability

and milling quality of this crop. Regions such as Gojam, Welega and Gamo Gofa could be considered as center of concentration for this character.

Morphological traits studied except open (straight), compact and fist like ear shapes, white grain color and non-pericarp persistence types were not conspicuously unique to any single region (Table 3). This could be attributed to germplasm exchange (gene flow) which renders regional boundaries irrelevant. Another human factor that might have played a role as, for example, the various seed colors are traditionally preferred to specialized consumption purposes (Belay *et al.*, 1997). Nevertheless, the predominance of some phenotypic classes might indicate the adaptive role as mentioned above for ear shape and growth habit which have a role in stress tolerance and weed suppression.

## **1.2. Estimates of diversity**

Table 4 shows the estimates of Shannon-Weaver diversity index for the 6 discrete characters by region. Overall, all characters revealed intermediate to high diversity ranging from 0.60 for pericarp persistence to 0.99 for grain surface. Polymorphism was common for most traits within accessions indicating the widely distributed intrapopulation variability for most of the characters. However, monomorphism ( $H' < 0.10$ ) was also observed in 6, 4, 11, 9, 13 and 34 accessions for growth habit, ear shape, grain shape, grain surface, grain color and pericarp persistence, respectively (Appendix Table 3). Monomorphism in some accession could be due to either drift or loss of genetic integrity caused by selection forces (Hammer *et al.*, 1996).

Individual characters showed different levels of diversity index in different regions (Table 4). Grain surface with two phenotypic classes exhibited higher diversity index in most regions than other characters with more than two phenotypic classes. Higher diversity index for characters with less number of phenotypic classes was also reported by Ayana and Bekele (1998) in sorghum. The lower diversity index of characters having greater than two phenotypic classes as

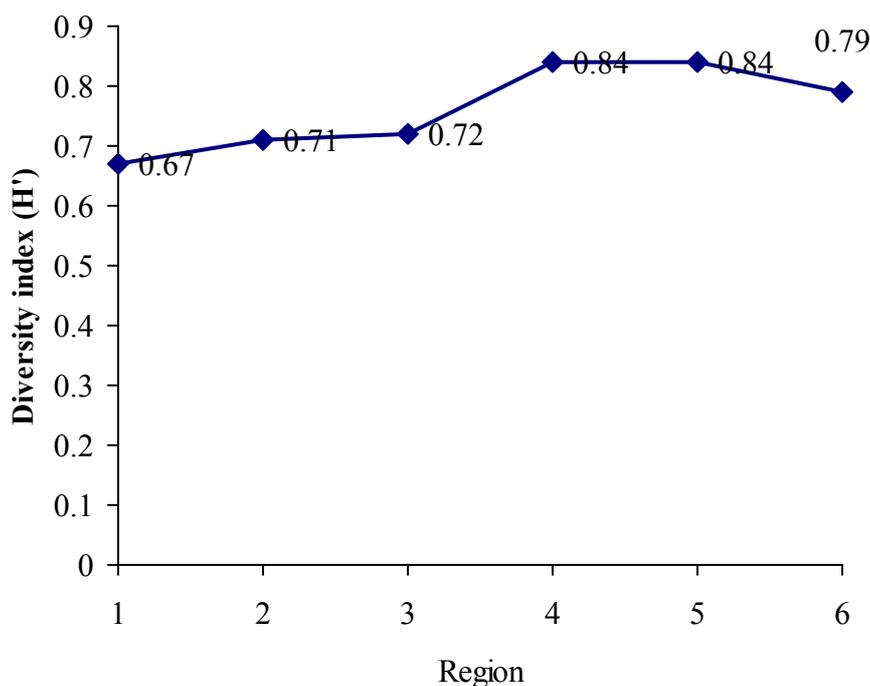
compared with characters with two phenotypic classes could be attributed to unequal distribution of the accessions of every region over all the phenotypic classes of the character rather than lack of variation for the character. Hence, it might be misleading to compare the value of diversity index ( $H'$ ) from characters having different classes of phenotypes (Ayana and Bekele, 1998). As reminded by Negassa (1985) caution should be made while interpreting the estimates of diversity of different characters with different phenotypic classes as measured by Shannon –Weaver diversity index.

**Table 4** Estimates of the Shannon-Weaver diversity index ( $H'$ ), mean of diversity and standard error in finger millet by region.

Region	Growth habit	Ear shape	Grain shape	Grain surface	Grain color	Pericarp persistence	Mean $H \pm SE$
Tigray	0.90	0.50	0.77	0.90	0.88	0.28	0.71 $\pm$ 0.11
Gonder	0.98	0.53	0.86	0.97	0.53	0.46	0.72 $\pm$ 0.10
Gojam	0.92	0.76	0.72	1.00	0.85	0.78	0.84 $\pm$ 0.04
Welega	0.91	0.69	0.78	0.98	0.97	0.68	0.84 $\pm$ 0.06
Gamo Gofa	0.83	0.95	0.71	1.00	0.52	0.71	0.79 $\pm$ 0.07
Eritrea	0.86	0.49	0.76	0.95	0.61	0.37	0.67 $\pm$ 0.09
Overall	0.93	0.73	0.82	0.99	0.83	0.60	0.82 $\pm$ 0.06

The highest mean diversity indices pooled over characters within regions were recorded for accessions sampled from Gojam ( $H'=0.84\pm 0.04$ ) and Welega ( $H'=0.84\pm 0.06$ ) followed by Gamo Gofa ( $H'=0.79\pm 0.07$ ) whereas accessions from Eritrea and Tigray showed relatively lower diversity estimates of  $0.67\pm 0.09$  and  $0.71\pm 0.11$ , respectively. As reported by Demissie and Bjornstad (1996) for Ethiopian barley landraces and by Belay *et al.*, (1997) for tetraploid Ethiopian wheat landraces, in this study also the maximum diversity was observed in regions relatively with favorable environments. There was an increasing trend from north to south (Figure 3). Bekele (1996) in tef and Tsehaye and Kebebew (2002) in finger millet also observed similar trend of diversity indices. The former author indicated that migration effect of the Ethiopian people from the diverse central and northern part of the country into the south and south west carrying their seed stocks with themselves might have partly

resulted in increasing diversity towards south. He further elaborated that the diversity of habitats in addition to being wetter could result in accommodating many genotypes.



**Figure 3** Overall mean diversity index (H') of 64 finger millet accessions across characters in 5 former regions of Ethiopia and Eritrea. In north-south sequence: 1=Eritrea, 2=Tigray, 3=Gonder, 4= Gojam, 5=Welega and 6=Gamo Gofa.

The over all mean diversity estimate in this study ( $0.82 \pm 0.06$ ) was high and almost similar to estimate ( $0.73 \pm 0.07$ ) of Tsehaye and Kebebew (2002) for those finger millet in another set of germplasm. The high level of diversity in finger millet landraces heralds the presence of many important genes for breeding.

## 1.2. Analyses of diversity

The analysis of variance (ANOVA) of the non-normalized diversity index (H) for individual characters is presented in Table 5. The analysis revealed similar

levels of diversity among regions for three of the characters (growth habit, grain color and pericarp persistence). Similar results were observed in sorghum (Ayana and Bekele, 1998), chickpea (Workeye, 2002) and finger millet (Tsehaye and Kebebew, 2002). The variances were attributed to among accessions rather than between regions. This cautions that future sampling for these characters (or other characters linked to them) should not make discrimination among regions. Kefyalew *et al.* (2000) in tef observed variation among the regions for some characters as found in this study for ear shape ( $P \leq 0.01$ ), grain shape ( $P \leq 0.05$ ) and grain surface ( $P \leq 0.01$ ) among regions. Accordingly, Gamo Gofa for ear shape ( $H' = 0.95$ ), Gojam and Gamo Gofa for grain surface ( $H' = 1.0$ ) and Gonder for grain shape provided the highest diversity (Table 4).

**Table 5** Mean squares for variations between regions from one-way analysis of variance of diversity index (H) for individual characters.

Character	Between regions (df=5)	Between accessions within region (df=58)
Growth habit	0.06	0.08
Ear shape	0.30**	0.07
Grain shape	0.29*	0.10
Grain surface	0.20**	0.04
Grain color	0.09	0.07
Pericarp persistence	0.08	0.06

\*, \*\* Significant at 5% and 1% level, respectively; df= Degree of freedom

#### 1.4. Analysis of variance

Analysis of variance for the 15 morpho-agronomic characters indicated that the genotypic mean square values were highly significant for all characters (Table 6), implying that the landraces tested were highly variable. Substantial variations in finger millet have been also reported in previous studies (Naik *et al.*, 1994; Prasada Rio *et al.*, 1994; Daba, 2000).

**Table 6** Mean square values and coefficient of variations in morpho-agronomic characters of finger millet.

Character	Mean squares			C.V.(%)
	Replication (df=2) <sup>+</sup>	Genotype (df=65)	Error (df=130)	
Plant height (cm)	1902.98 <sup>**</sup>	536.78 <sup>**</sup>	134.50	13.49
Days to heading	129.96 <sup>**</sup>	448.47 <sup>**</sup>	26.98	4.94
Days to maturity	592.75 <sup>**</sup>	429.61 <sup>**</sup>	63.80	4.72
Grain-filling duration	701.09 <sup>**</sup>	151.79 <sup>**</sup>	87.12	14.57
Productive tillers/plant	2.92 <sup>NS</sup>	15.53 <sup>**</sup>	3.76	17.38
Culm thickness (mm)	6.43 <sup>**</sup>	2.67 <sup>**</sup>	0.46	14.24
Leaf number/main tiller	14.94 <sup>**</sup>	5.42 <sup>**</sup>	1.34	14.03
Leaf blade length (cm)	31.96 <sup>NS</sup>	41.57 <sup>**</sup>	14.44	10.17
Leaf blade width (cm)	0.13 <sup>**</sup>	0.04 <sup>**</sup>	0.01	9.84
Finger length (cm)	7.50 <sup>**</sup>	11.17 <sup>**</sup>	1.35	13.93
Finger width (mm)	0.50 <sup>NS</sup>	3.41 <sup>**</sup>	0.27	12.21
Finger number/main ear	2.68 <sup>NS</sup>	4.52 <sup>**</sup>	1.80	15.12
No. of grains/spikelet	9.79 <sup>**</sup>	2.10 <sup>**</sup>	0.77	16.14
Thousand-grain weight (g)	0.96 <sup>**</sup>	0.33 <sup>**</sup>	0.10	13.35
Grain yield/plant (g)	9.04 <sup>NS</sup>	32.86 <sup>**</sup>	5.95	19.73

\* \*\* Significant at 5% and 1%, respectively; NS =non-significant.

<sup>+</sup>Numbers in parenthesis are degree of freedom.

Most of the characters except productive tillers per plant, leaf blade length, finger width, finger number per main ear and grain yield per plant also showed highly significant differences due to environmental effects (replication). The coefficient of variation ranged from 4.72% for days maturity to 19.73% for grain yield per plant.

### **1.5. Estimates of mean and range**

The performance of most accessions for characters of grain filling duration, productive tillers per plant, finger length and finger number per main ear

and some accessions for plant height, days to heading, days to maturity, leaf number per main tiller, leaf blade length, leaf blade width, number of grains per spikelet and grain yield per plant exceeded the performance of the commercial varieties (Paadet and/or Tadesse) (Appendix Table 4). This situation ensures the existence of base population for improving the character of interest in either of the directions depending upon the objectives to be met.

The ranges along with the contrasting extreme genotypes and the means for the 15 finger millet morpho-agronomic characters are summarized in Table 7. Regardless of the variation in the relative magnitude of the ranges, the means of the genotypes generally displayed considerable differences between the minimal and maximal values for all the traits evaluated. For the traits such as productive tillers per plant, culm thickness, finger length, finger width, finger number main ear, 1,000-grain weight and grain yield per plant, the ranges between the minimal and maximal values exceeded the corresponding minimal values recorded.

Grain yield per plant exhibited the widest range (4.87 – 21.21g) followed by finger length (3.67 – 14.6 cm) and finger width (3.13 – 9.13 cm) with the values of 336, 298 and 192% that the maximal value exceeded the corresponding minimal values, respectively. Relatively, low ranges with 40, 53, 60, 61 and 69% differences between the corresponding minimal and maximal values were recorded for days to maturity, leaf blade width, grain filling duration, days to heading and leaf blade length, respectively. Similar to the result of this study, a wide range of variations for plant height and grain yield per plant (Narasmba Rao and Parathasarathi, 1968), for finger length and number (Kebede and Menkir, 1989), for plant height and productive tillers (Prasada Rao *et al.*, 1994) and for most traits studied (Daba, 2000), were reported. Such broad difference apparent among the finger millet landraces tested would provide ample opportunities for the genetic improvement of the crop through selection directly from the landraces and/or following traits recombination through intra-specific hybridization of desirable traits.

**Table 7** Mean maximum (max.), minimum (min.) and difference values of morph-agronomic characters in finger millet.

Character	Mean	Min.	Max.	Difference	Difference as % of minimum
Plant height (cm)	86.0	61.3(61) <sup>+</sup>	116.7(28)	55.4	90
Days to heading	105	84(46)	135(31)	51	61
Days to maturity	169	143(46)	200(50)	57	40
Grain-filling duration	64	48(53)	77(12)	29	60
Productive tillers/plant	11.15	8.00(2)	17.33(38)	9.33	117
Culm thickness (mm)	4.75	3.47(6,14)	7.87(66)	4.40	127
Leaf number/main tiller	8.26	6.07(12,37)	11.07(64)	5.00	82
Leaf blade length (cm)	37.4	27.27(61)	46.10(20)	18.8	69
Leaf blade width (cm)	1.09	0.93(58)	1.42(2)	0.49	53
Finger length (cm)	8.33	3.67(46)	14.60(20)	10.93	298
Finger width (mm)	4.26	3.13(54)	9.13(66)	6.00	192
Finger number/main ear	8.87	5.87(33)	12.00(23)	6.13	104
No. of grains/per spikelet	5.44	3.93(50)	7.20(46)	3.27	83
Thousand-grain weight (g)	2.41	1.53(7)	3.20(66)	1.67	109
Grain yield/plant (g)	12.37	4.87(50)	21.21(36)	16.34	336

<sup>+</sup> Numbers in parenthesis are accessions codes: see Table 1 for accessions codes.

### **1.6. Estimates of phenotypic, genotypic and environmental variability**

As shown in Table 8, high phenotypic and genotypic variances were depicted by plant height, days to heading and days to maturity whereas the lowest ones were found for leaf blade width followed by 1,000-grain weight. In characters such as days to heading, days to maturity, productive tillers per plant, culm thickness, leaf number main tiller, finger length, finger width, and grain yield per plant a large portion of the phenotypic variance was accounted by the genetic component (Table 8). This indicated the existence of immense inherent variability for these characters that remained unaltered by environmental conditions among the genotypes, which in turn is more useful for exploitation in hybridization and/or selection.

**Table 8** Phenotypic, genotypic and error variance of morpho-agronomic characters in finger millet.

Character	Phenotypic variance( $\sigma^2_p$ )	Genotypic variance( $\sigma^2_g$ )	Error variance( $\sigma^2_e$ )
Plant height(cm)	268.595	134.092	134.503
Days to heading	167.477	140.497	26.980
Days to maturity	185.734	121.937	63.797
Grain filling duration	108.676	21.559	87.117
Productive tillers/plant	7.680	3.925	3.755
Culm thickness (mm)	1.196	0.739	0.457
Leaf number/main tiller	2.700	1.358	1.342
Leaf blade length (cm)	23.484	9.042	14.442
Leaf blade width (cm)	0.020	0.008	0.012
Finger length (cm)	4.623	3.276	1.347
Finger width (mm)	1.317	1.046	0.271
Finger number/main ear	2.703	0.907	1.796
No. of grains/spikelet	1.213	0.442	0.772
Thousand-grain weight (g)	0.179	0.075	0.104
Grain yield per plant (g)	14.923	8.968	5.954

The phenotypic (PCV) and genotypic (GCV) coefficient of variations of the various finger millet characters computed based on analysis of variance are presented in Table 9. For all the 15 traits, the PCV and GCV ranged in the order of 8.05 to 31.23% and 6.52 to 24.21% in both cases for days to maturity and grain yield per plant, respectively. Generally, the PCV estimates were higher than the GCVs as found by Daba (2000) showing that the apparent variation was not only due to genotypes but also to the influence of environment. Selection for such traits is sometimes misleading. However, for majority of the traits including days to heading, days to maturity, productive tillers per plant, culm thickness, leaf number main tiller, finger length, finger width, and grain yield per plant the environmental coefficients of variation (ECV) estimates were lower than from both GCV and PCV. This implied that the environmental role was less for the expression of such characters (Singh and

Narayana, 1993). Thus, selection for such characters is expected to be effective. Relatively, the PCV estimates were high for culm thickness, productive tillers per plant, finger length and width and grain yield per plant, which varied from 23.05 – 31.23%. Grain filling duration, 1,000-grain weight, finger number per main ear, plant height, leaf number per main tiller and number of grains per spikelet exhibited moderate PCV estimates ranging from 16.27 to 20.24% whereas low PCV (8.05-12.88%) estimates were recorded for days to heading, days to maturity, leaf blade length and leaf blade width.

**Table 9** Phenotypic (PCV), genotypic (GCV) and error (ECV) coefficient of variability, heritability in broad sense ( $h^2$ ) and genetic advance (GA) of morpho-agronomic characters in finger millet.

Character	PCV	GCV	ECV	$h^2$	GA	GA as % of mean
Plant height (cm)	19.07	13.47	13.49	50	16.88	19.63
Days to heading	12.30	11.27	4.94	84	22.40	21.33
Days to maturity	8.05	6.52	4.72	66	18.46	10.92
Grain-filling duration	16.27	7.25	14.57	20	4.27	6.67
Productive tillers/plant	24.85	17.77	17.38	51	2.92	26.20
Culm thickness (mm)	23.05	18.12	14.24	62	1.39	29.35
Leaf number/main tiller	19.90	14.12	14.03	50	1.70	20.64
Leaf blade length (cm)	12.97	8.05	10.17	39	3.85	10.29
Leaf blade width (cm)	12.88	8.32	9.84	41	0.12	10.71
Finger length (cm)	25.81	21.72	13.93	71	3.14	37.73
Finger width (mm)	26.92	23.99	12.21	80	1.88	44.14
Finger number/main ear	18.55	10.74	15.12	34	1.14	12.83
No. of grains/spikelet	20.24	12.21	16.14	37	0.83	15.22
Thousand-grain weight (g)	17.52	11.34	13.35	42	0.37	15.17
Grain yield/plant (g)	31.23	24.21	19.73	60	4.79	38.72

Three of the traits, namely finger length, finger width and grain yield per plant exhibited relatively high GCV values of 21.72, 23.99 and 24.21%, respectively. In contrast, low GCV values ranging from 6.52 to 12.21% were recorded for days to maturity, grain filling duration, leaf blade length, leaf blade width, finger number per main ear, days to heading, 1,000-grain weight and number of grains per spikelet. Whereas plant height, leaf number per main tiller, productive tillers per plant and culm thickness exhibited intermediate (13.47–18.12%) GCV values. Similarly, high genotypic and phenotypic coefficient of variation were also found for productive tillers, finger length and grain weight per plant (Abraham *et al.*, 1989), for finger length (Goswami and Asthana, 1984) and for grain weight per plant (Prabhakar and Prasad, 1983). In contrast to the current finding, high genotypic coefficients of variations were reported for finger number and 1,000-grain weight (Abraham *et al.*, 1989) and for days to flowering and maturity (Patnaik and Jana, 1973).

### **1.7. Estimates of heritability in broad sense and expected genetic advance**

Heritability which is the heritable portion of phenotypic variance is a good index of transmission of characters from parents to offspring (Falconer, 1981). In this study, heritability ( $h^2$ ) estimates ranged from 20% for grain filling duration to 84% for days to heading (Table 9). Overall, comparatively days to maturity, finger length, finger width and days to heading revealed high  $h^2$  values ranging from 66-84% as opposed to low  $h^2$  values of 20- 41% recorded for grain filling duration, finger number per main ear, number of grains per spikelet, and leaf blade length and leaf blade width. The values were intermediate (42-62%) for 1,000-grain weight, plant height, leaf number per main tiller, productive tillers per plant, grain yield per plant and culm thickness. Likewise, high heritability estimates for days to flowering and maturity (Dhagate *et al.*, 1972) and finger length (Daba, 2000) and low for 1,000-grain weight and finger number (Patnaik, 1968) were reported. On the other hand, low heritability estimates for days to flowering and high heritability estimates for finger number (Daba, 2000) and for 1,000-grain weight (Abraham *et al.*, 1989) were also reported.

Estimates of genetic advance (as percentage of the mean) expected from selecting 5% of the best genotypes are given in Table 9. As percentage of mean, the genetic advance estimates varied from 6.67-44.14% for grain-filling duration to finger width, respectively. On the whole, grain-filling duration, leaf blade length, days to maturity, leaf blade width, finger number per main ear, 1,000-grain weight and number of grains per spikelet demonstrated relatively low genetic advance estimates of 6.67-15.22%. In contrast, comparatively high values (37.73-44.14%) were observed for three of the characters involving finger length, grain yield per plant and finger width. Intermediate estimates of 19.63-26.20% were obtained for plant height, leaf number per main tiller, days to heading and productive tillers per plant.

Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection than heritability estimates alone. However, it is not necessary that a character showing high heritability will also exhibit high genetic advance (Johnson *et al.*, 1955). High heritability with high genetic advance (as percentage of the mean) was observed for finger length and width as found by Daba (2000) for productive tillers per plant, ear weight per plant and biomass per plant. Such conditions most likely caused by additive gene action, thereby, reflecting the efficiency of selection for the improvement of these traits.

### **1.8. Correlation among characters**

The phenotypic correlation ( $r_p$ ) among traits is influenced by genotypes and environment. Genotypic correlation ( $r_g$ ) is usually attributed to pleiotropy (Falconer, 1981) whereas environmental correlation ( $r_e$ ) is entirely due to environment and is not heritable and stable. It reflects a similarity or dissimilarity in the response of the two traits to a common environment. The estimates of phenotypic, genotypic and environmental correlation coefficients are displayed in Table 10. The strongest positive association was observed between culm thickness and leaf blade length ( $r_g=0.98$ ) followed by days to heading and maturity ( $r_g=0.92$ ) and culm thickness and finger width ( $r_g=0.89$ ), while the strongest negative association was observed between 1,000-grain weight and finger number per main ear ( $r_g=-0.77$ ) followed by finger

width and grain filling duration ( $r_g=-0.74$ ) and culm thickness and grain filling duration ( $r_g=0.72$ ). Significant negative and positive associations were also observed among other traits. Regardless of the direction and the strength of association days to heading showed significant genotypic correlation with all characters evaluated whereas finger number per main ear correlated relatively with few of them.

Grain yield per plant, which is a trait of primary interest, had significant positive associations with productive tillers per plant ( $r_g= 0.49$ ), number of grains per spikelet ( $r_g=0.45$ ), 1,000-grain weight ( $r_g=0.38$ ) and with finger number main ear ( $r_g= 0.25$ ). The high association of grain yield per plant with productive tillers per plant was considered to have been occurred due to favorable influence of the environment, as the value of  $r_e$  between them was greater than both  $r_p$  and  $r_g$ . Such association would probably be changed with a change in environment. It was also associated significantly but negatively with both days to heading ( $r_g= -0.46$ ), days to maturity ( $r_g= -0.49$ ) and leaf number per main tiller ( $r_g= -0.25$ )

Likewise, positive associations of grain yield per plant with 1,000-grain weight (Dhagate *et al.*, 1972) and with productive tillers (Gowda and Lakshimi, 1977; Daba, 2000) were reported. In contrast to the result of this study, significant and positive associations of grain yield per plant with days to maturity and ear length (Daba, 2000), with plant height (Dhanakodi, 1988; Daba, 2000) and with finger number and ear length (Gowda and Lakshimi, 1997; Daba, 2000) were also reported.

Generally, the correlation results revealed that besides selection for grain yield per plant *per se*, indirect selection for 1,000-grain weight, number of grains per spikelet, finger number per main ear and productive tillers per plant might lead to the improvement of grain yield per plant since they exhibited significantly positive correlation with grain yield per plant. However, there should be a balance among characters in selection particularly between 1,000-grain weight and finger number per main ear as they had strong inverse association.

**Table 10** Phenotypic ( $r_p$ ), genotypic ( $r_g$ ) and environmental ( $r_e$ ) correlations among 15 morpho-agronomic characters in finger millet.

Character		DTH	DTM	GFD	PRT	CT	LENU	LEBL	LEBW	FL	FW	FNU	NGPSP	TGW	GYPPL
PH	$r_p$	0.34**	0.32**	0.01	-0.14	-0.02	0.32*	0.45**	-0.06	0.25*	-0.01	0.27*	-0.27*	-0.11	-0.01
	$r_g$	0.52**	0.63**	0.18	0.06	0.02	0.28*	0.73**	0.00	0.37**	0.07	0.22	-0.53**	-0.37**	-0.14
	$r_e$	0.06	-0.09	-0.08	-0.34**	-0.07	0.35**	0.25**	-0.10	0.08	-0.16	0.31**	-0.07	0.11	0.09
DTH	$r_p$		0.70**	-0.34**	-0.15	0.31*	0.52**	0.42**	0.27*	-0.19	0.23	0.13	-0.30	-0.31*	-0.36**
	$r_g$		0.92**	-0.36**	-0.25*	0.41**	0.79**	0.69**	0.44**	-0.27*	0.30*	0.31*	-0.45**	-0.50**	-0.46**
	$r_e$		0.04	-0.52**	0.04	0.05	0.04	0.09	0.03	0.08	-0.03	-0.11	-0.17	-0.04	-0.12
DTM	$r_p$			0.45**	-0.02	0.12	0.37**	0.40**	0.12	0.07	-0.01	0.15	-0.39**	-0.34**	-0.30*
	$r_g$			0.23**	-0.03	0.14	0.58**	0.71**	0.22	0.08	0.00	0.36**	-0.71**	-0.62**	-0.49**
	$r_e$			0.03	-0.01	0.09	0.10	0.09	0.01	0.05	-0.05	-0.05	-0.09	-0.03	0.02
GFD	$r_p$				0.16	-0.22	-0.17	0.00	-0.18	0.33**	-0.30*	0.03	-0.13	-0.05	0.05
	$r_g$				0.56**	-0.72**	-0.64**	-0.07	-0.61**	0.87**	-0.74**	0.07	-0.54**	-0.18	0.02
	$r_e$				-0.04	0.05	0.06	0.02	-0.00	-0.00	-0.03	0.02	0.02	-0.00	0.08
PRT	$r_p$					-0.21	-0.27*	-0.15	-0.20	0.22	-0.08	-0.07	-0.07	0.06	0.50**
	$r_g$					-0.37**	-0.39**	-0.26*	-0.54**	0.22	-0.19	-0.14	-0.24	0.17	0.49**
	$r_e$					-0.02	-0.26*	-0.06	0.08	0.03	0.11	-0.03	0.06	-0.05	0.50**
CT	$r_p$						0.47**	0.30*	0.65**	-0.34**	0.68**	0.05	0.30*	0.13	0.11
	$r_g$						0.89**	0.46**	0.98**	-0.58**	0.89**	0.21	0.43**	0.15	0.18
	$r_e$						-0.05	0.15	0.33**	0.13	0.22	-0.09	0.17	0.11	0.02
LENU	$r_p$							0.31*	0.35**	-0.36**	0.40**	0.17	0.04	-0.03	-0.07
	$r_g$							0.58**	0.86**	-0.57**	0.68**	0.26*	0.21	-0.23	-0.25*
	$r_e$							0.10	-0.08	-0.07	-0.09	0.10	-0.10	0.14	0.16

**Table 10** (Cont'd).

Character	DTH	DTM	GFD	PRT	CT	LENU	LEBL	LEBW	FL	FW	FNU	NGPSP	TGW	GYPPL
LEBL	r <sub>p</sub>							0.30	0.21	0.17	0.20	-0.21	-0.14	-0.02
	r <sub>g</sub>							0.57**	0.15	0.27*	0.38**	-0.50**	-0.36**	-0.04
	r <sub>c</sub>							0.12	0.30**	0.05	0.10	-0.03	0.01	0.00
LEBW	r <sub>p</sub>								-0.24	0.51**	0.07	0.15	0.04	0.07
	r <sub>g</sub>								-0.57**	0.84**	0.23	0.33**	0.11	0.01
	r <sub>c</sub>								0.16	0.07	-0.03	0.03	-0.00	0.12
FL	r <sub>p</sub>									-0.52**	0.11	-0.23	0.03	0.05
	r <sub>g</sub>									-0.72**	0.20	-0.40**	0.01	0.10
	r <sub>c</sub>									0.07	0.03	-0.07	-0.07	-0.06
FW	r <sub>p</sub>										-0.13	0.21	0.20	0.20
	r <sub>g</sub>										-0.21	0.39**	0.37**	0.24
	r <sub>c</sub>										-0.07	0.01	-0.05	0.11
FNU	r <sub>p</sub>											-0.07	-0.24	0.14
	r <sub>g</sub>											-0.17	-0.77**	0.25*
	r <sub>c</sub>											0.07	0.09	0.05
NGPSP	r <sub>p</sub>												0.21	0.22
	r <sub>g</sub>												0.46**	0.45**
	r <sub>c</sub>												0.04	0.01
TGW	r <sub>p</sub>													0.16
	r <sub>g</sub>													0.38**
	r <sub>c</sub>													-0.07

\*, \*\* Significant at 5% and 1%, respectively.

<sup>+</sup> PH=Plant height (cm), DTH=days to heading, DTM=days to maturity, GFD=grain filling duration, PRT=productive tillers/plant, CT=culm thickness (mm), LENU=leaf no./main tiller, LEBL=leaf blade length (cm), LEBW=leaf blade width (cm), FL=finger length (cm), FW=finger width (mm), FNU=finger no./main ear, NGPSP=no. of grains per spikelet, TGW=1,000-grain weight (g), GYPPL=grain yield per plant (g).

Most of the materials tested including the commercial varieties were late in maturity, which required on average 169 days to mature (Table 7 and Appendix Table 4) and finger millet is cultivated in the area where moisture stress is prevalent and the growing period is not as long as required for late maturing varieties. Hence, the very strong negative association between days to maturity and grain yield per plant give an opportunity to develop relatively early maturing varieties with better yield potential.

### **1.9. Path-coefficient analysis**

The information obtained from path-coefficient analysis, which splits the correlation coefficient into the measures of direct and indirect effects helps in indirect selection for genetic improvement of yield.

In this investigation, the genotypic correlation coefficient was further divided into direct and indirect effects using path-coefficient analysis. In computing the path analysis, grain yield per plant was considered as resultant (dependable) variable while the rest of the variables that were significantly correlated with grain yield per plant (days to heading, days to maturity, productive tillers per plant, leaf number per main tiller, finger number per main ear, number of grains per spikelet, and 1,000-grain weight) were used as causal (independent) variables.

As shown in Table 11, among the seven causal (independent) variables five of them including days to heading (0.670), productive tillers per plant (0.701), leaf number per main tiller (0.659) and finger number per main ear (1.212), and 1,000-grain weight (0.858) had positive direct effect whereas days to maturity (-1.932), number of grains per spikelet (-0.784) showed negative direct effect.

**Table 11** Direct and indirect genetic effects via various paths of seven characters on grain yield per plant.

Character	Direct effect	Indirect effect via							Total correlation with grain yield / plant
		Days to heading	Days to maturity	Productive tillers/ plant	Leaf no./main tiller	Finger. no./main ear	No. of grains/ spikelet	Thousand -grain weight	
Days to heading	0.670	-	-1.777	-0.172	0.518	0.377	0.353	-0.432	-0.463
Days to maturity	-1.932	0.616	-	-0.019	0.379	0.441	0.557	-0.529	-0.487
Productive tillers/plant	0.701	-0.164	0.052	-	-0.255	-0.172	0.186	0.145	0.493
Leaf number/main tiller	0.659	0.526	-1.111	-0.271	-	0.313	-0.164	-0.198	-0.246
Finger number/main ear	1.212	0.208	-0.703	-0.100	0.170	-	0.129	-0.663	0.254
No. of grains/spikelet	-0.784	-0.301	1.372	-0.166	0.138	-0.200	-	0.396	0.454
Thousand-grain weight (g)	0.858	-0.337	1.192	0.119	-0.152	-0.937	-0.362	-	0.380

Residual effect (h) =0.304

Finger number per main ear exerted the highest positive direct effect (1.212) upon grain yield per plant. It had also positive indirect effect via days to heading (0.208), leaf number per main tiller (0.170) and number of grains per spikelet (0.129). However, the positive direct effect of finger number per main ear was counterbalanced by relatively high negative indirect effect via days to maturity (-0.703), 1,000-grain weight (-0.663), and productive tillers per plant (-0.10) which resulted in lesser correlation with grain yield per plant ( $r_g=0.254$ ) as compared with its highest positive direct effect.

Thousand-grain weight as an important component of yield exerted the second highest positive direct effect (0.858) on grain yield per plant. It also exhibited very high positive indirect effect (1.192) via days to maturity and low positive indirect effect (0.119) through productive tillers per plant. Though, 1,000-grain weight exhibited negative indirect effect through majority of the characters, its association with grain yield per plant remained positive and highly significant ( $r_g=0.380$ ). Regardless of the unfavorable indirect effect of productive tillers per plant, its association with grain yield per plant was positive and highly significant ( $r_g=0.493$ ) due to its positive high direct effect (0.701) and indirect positive effect through number of grains per spikelet (0.186), 1,000-grain weight (0.145) and days to maturity (0.052).

Days to heading had high favorable direct effect (0.670) and high favorable indirect effect via leaf number per main tiller (0.518), finger number per main ear (0.377) and number of grain per spikelet (0.353). However, it exerted the highest indirect effect (-1.777) via days to maturity. This along with the negative indirect effect via 1,000-grain weight (-0.432) and productive tillers per plant (-0.172) contributed to its negative highly significant association with grain yield per plant ( $r_g=-0.463$ ).

Similar to days to heading, the high favorable direct effect (0.659) of leaf number per main tiller and its indirect favorable effect via days to heading (0.526) and finger number per main ear (0.313) was counterbalanced by relatively higher

unfavorable indirect effect through days to maturity (-1.111), productive tillers per plant (-0.271), 1,000-grain weight (-0.198) and number of grains per spikelet (-0.164) and resulted in negative association between leaf number main tiller and grain yield per plant ( $r_g=-0.246$ ).

In line to the finding of the present study, strong direct effects on grain yield per plant were reported for productive tillers (Mahudeswaran and Marugesan, 1973), productive tillers and 1,000-grain weight (Prabhakar and Prasad 1983), productive tillers and finger number (Ravindran *et al.*, 1996) and finger number, productive tillers and 1,000-grain weight (Daba, 2000). As opposed to this, Reddy *et al.* (1995) recorded negative direct effect for productive tillers and Daba (2000) for days to heading.

The direct effect of days to maturity on grain yield per plant was negative and very high (-1.932). Besides, it exerted negative indirect effect via 1,000-grain weight (-0.529) and productive tillers per plant (-0.019). Hence, these effects led to negative and highly significant association with grain yield per plant ( $r_g=-0.487$ ). Regardless of positive association with grain yield per plant, negative direct effect of days to maturity on grain yield per plant was reported by Daba (2000). The other character which exerted high and negative direct effect (-0.784) on grain yield per plant was number of grains per spikelet. It also exhibited negative indirect effect via days to heading (-0.301), finger number per main ear (-0.200) and productive tillers per plant (-0.166). The favorable indirect effect particularly via days to maturity (1.372) counterbalanced its unfavorable effect and its association with grain yield per plant found to be highly significant and positive ( $r_g=0.454$ ).

In this study, the positive direct effect of 1,000-grain weight, finger number and productive tillers per plant on grain yield per plant and their significant positive association at genotypic level with grain yield per plant revealed that these characters were the major contributors to grain yield per plant. Hence, apart selection for grain yield per plant *per se*, selection of individuals for these characters might be effective to the improvement of finger millet.

### **1.10. Cluster analysis**

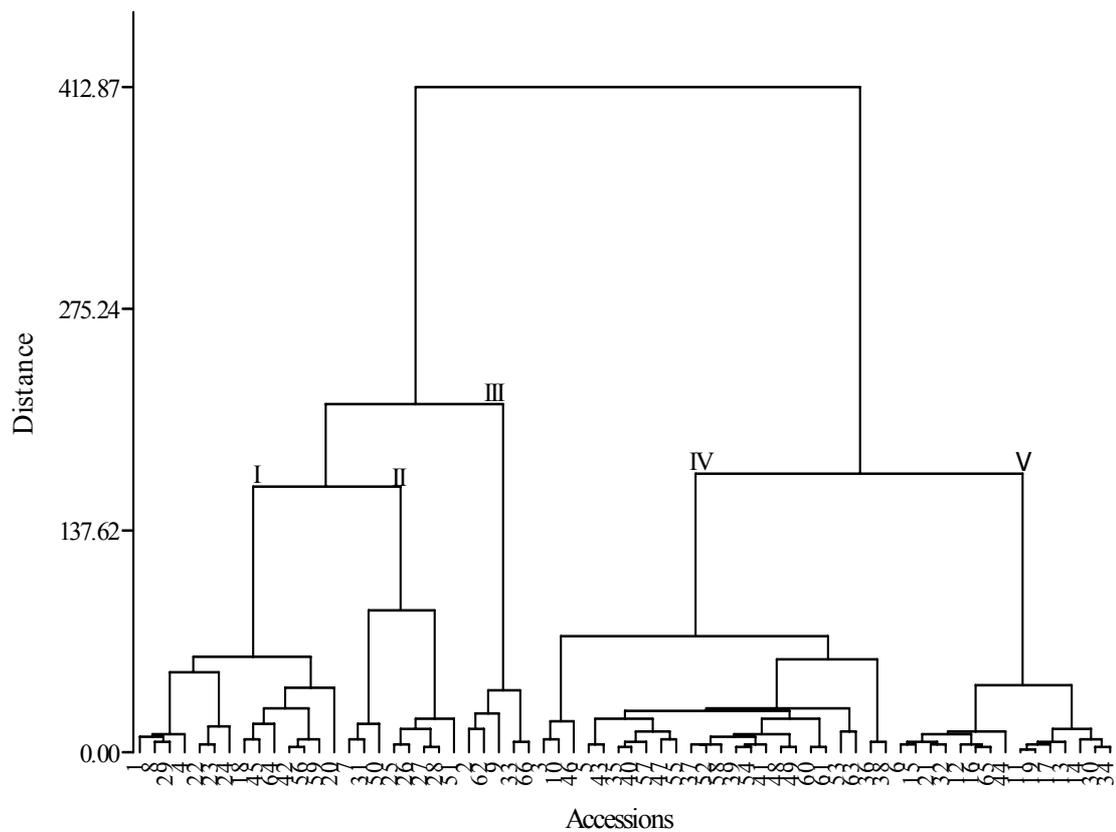
Cluster analysis grouped the 66 accessions into 5 clusters (Figure 4 and Table 12) using standardized mean of 15 morpho-agronomic characters as an input. However, Tsehaye and Kebebew (2002) grouped the 42 accessions of finger millet into 7 clusters. The means of 15 morpho-agronomic characters over the clusters are presented in Table 13.

The number of accessions in clusters varied from 5 in cluster III to 24 in cluster IV. Cluster I consisted of 14 accessions, which were sampled from all regions except from Eritrea. This group was found to be a good source of desirable gene for grain yield per plant and finger number per main ear (Table 13). Two accessions from Gojam and 6 from Welega formed cluster II. They were characterized by being latest to head and mature. They had also the longest leaf and tall stature but they possessed the fewest number of productive tillers per plant and grains per spikelet and the lowest grain yield per plant.

Cluster III grouped 5 accessions including the 3 landraces and 2 standard varieties, Paadet and Tadesse. All the 3 landraces in this cluster originated from Gamo Gofa region. They were short in stature and had the shortest grain filling duration, thickest finger and culm with the highest leaf number per main tiller, widest leaf, high number of grains per spikelet, heaviest grain weight and high grain yield per plant. They were also characterized by their few and shortest finger and long leaf length. Populations of this group could be a good source of genes for number of grains per spikelet, grain weight, grain yield per plant in the improvement program since they were known to have higher values for these mentioned and other characters.

Cluster IV consisted of the maximum number of accessions (24) collected from all regions except from Gojam. Accessions with the shortest stature and the earliest to head and mature and with the fewest and shortest leaf per main tiller were grouped in this cluster. Hence, this group could be utilized in the breeding

program aiming at developing early maturing varieties. The members of cluster V were 15, which originated only from two regions, Gonder and Gojam. They exhibited the longest grain filling duration and finger length and the highest number of productive tillers per plant with the thinnest culm.



**Figure 4** Dendrogram constructed using Ward's method based on 15 morpho-agronomic characters of 66 finger millet accessions.

**Table 12** Number of finger millet accessions in each cluster and region of origin.

Cluster	Total number of accessions/cluster	Region of origin	Number of accessions/region
I	14	Tigray	3
		Gonder	3
		Gojam	1
		Welega	6
		Gamo Gofa	1
II	8	Gojam	2
		Welega	6
III	5	Gamo Gofa	3
		Standard varieties*	2
IV	24	Tigray	9
		Gonder	4
		Welega	1
		Gamo Gofa	2
		Eritrea	8
V	15	Gonder	4
		Gojam	11

\*Developed from introduction.

The cluster analysis revealed that accessions from the same origin of region were grouped in different clusters and conversely accessions from different regions were grouped in the same cluster as found by Kefene *et al.* (1997) in sunflower and by Workeye (2002) in chick pea. This implied that genetic diversity and geographic diversity were not necessarily related. This was also in agreement with the findings of Sabharwal *et al.* (1995) who concluded that there was no association between clustering pattern and eco-geographical distribution of forage sorghum accessions. In contrary, Wilson *et al.* (1990) reported correspondence between the geographic collection sites of landraces and their inclusion in particular clusters.

**Table 13** Mean values and standard deviations (SD) of 15 morpho-agronomic characters over the 5 clusters of finger millet.

Character	Cluster									
	I		II		III		IV		V	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plant height (cm)	94.4	10.35	94.2	23.06	84.5	10.75	75.4	6.14	91.1	6.14
Days to heading	108	10.12	127	5.59	112	8.96	97	8.46	103	8.46
Days to maturity	171	10.01	190	7.55	169	5.22	160	6.46	172	6.46
Grain filling duration	63	5.83	63	5.18	57	9.57	63	6.87	70	6.87
Productive tillers/plant	10.38	1.73	9.63	1.87	10.06	1.59	11.33	2.32	12.76	2.32
Culm thickness (mm)	5.33	0.60	4.93	0.99	6.78	0.84	4.33	0.41	4.09	0.41
Leaf no./main tiller	9.33	1.13	9.37	0.79	10.24	0.53	7.38	0.82	7.41	0.82
Leaf blade length(cm)	39.36	2.79	40.26	2.60	40.24	3.49	33.94	2.68	38.48	2.68
Leaf blade width (cm)	1.14	0.1	1.14	0.12	1.33	0.07	1.04	0.05	1.03	0.05
Finger length (cm)	8.91	2.20	7.70	1.66	4.91	0.40	8.04	1.56	9.74	1.56
Finger width (mm)	4.30	0.51	4.10	0.64	7.31	1.32	3.98	0.54	3.75	0.54
Finger no./main ear	10.27	1.07	8.87	1.33	7.91	1.52	8.24	0.7	8.87	0.7
No. of grains/spikelet	5.93	0.74	4.46	0.54	5.65	0.61	5.92	0.55	4.68	0.55
Thousand-grain weight (g)	2.31	0.29	2.03	0.27	2.85	0.32	2.56	0.27	2.33	0.27
Grain yield/plant (g)	14.51	2.45	6.85	1.37	13.72	0.85	12.33	3.20	12.92	3.2

### 1.11. Inter-cluster divergence

A commonly used measure of Euclidean distance that directly incorporates a standardization process is the Mahalanobis distance ( $D^2$ ) (Hair *et al.*, 1998). Hence, the cluster analysis results were confirmed by distance analysis (Mahalanobis distance,  $D^2$ ) among clusters. Table 14 shows the inter-cluster distance. Maximum inter-cluster divergence occurred between cluster I and III ( $D^2=81.47$ ) and the inter-cluster divergence was least between cluster IV and V ( $D^2=11.30$ ). The distance between clusters I and V ( $D^2=22.72$ ); II and V ( $D^2=23.34$ ); and cluster IV and V ( $D^2=11.30$ ) were low and non significant. Similarly, in the previous studies  $D^2$  as low as 18.3 and as high as 159.8 in finger millet (Tsehaye and Kebebew, 2002) and as low as 9.63 and as high as 249.14 in chickpeas (Workye, 2002) were reported.

Distance measures are actually a measure of dissimilarity, with larger values denoting lesser similarity (Hair *et al.*, 1998). Lower inter-cluster distance indicates the similarity of populations in their genetic make up, thus crossing between them expected not to produce desirable recombinants. The distance between clusters I and II ( $D^2=36.41$ ) and I and IV ( $D^2=27.20$ ) were relatively low though they were significantly different from each other. Cluster III revealed maximum and almost equal distance with Cluster I ( $D^2=81.47$ ) and IV ( $D^2=80.90$ ). The  $D^2$  values helped to identify the diverse accessions from the evaluated germplasm, which will be useful in future breeding work. Hence, the crossing of parents selected from Cluster III particular with those parents selected from clusters I and IV is likely to generate desirable recombinants since parents with more diversity among them are expected to exhibit a broad spectrum of variability in segregating generations. Besides, Cluster III with clusters II ( $D^2=68.89$ ) and with V ( $D^2=68.19$ ) and Cluster II with Cluster IV ( $D^2=43.48$ ) showed significant and fairly large distances and hence hybridization of parents from these clusters also expected to produce segregants with moderate to high variation. Higher genetic variability likely to be created when the crosses are made between selected parents in different clusters than within cluster (Gardner, 1972; Ezeaku and Awopetu, 1992).

**Table 14** Mahalonobis's distance among clusters of finger millet.

Cluster	I	II	III	IV	V
I	-	36.41**	81.47**	27.20*	22.72 <sup>NS</sup>
II		-	68.89**	43.48**	23.34 <sup>NS</sup>
III			-	80.92**	68.19**
IV				-	11.30 <sup>NS</sup>
V					-

\*, \*\* Significant at 5% and 1% level, respectively; NS=non significant.

### **1.12. Principal component analysis**

The principal component analysis grouped the 15 variables into 14 components which accounted for the entire (100%) of the variability (Appendix Table 5). The eigenvectors, the eigenvalues representing the variance of the principal components and the cumulative percent of the eigenvalues indicating percentage contribution to the total variance attributable to each of principal components accounted for 80.7% are given in Table 15. In latent root criterion, factors having latent roots or eigenvalues greater than 1 are considered significant (Hair *et al.*, 1998). Accordingly, the first 5 principal components, which had eigenvalues greater than unity were retained for this study and accounted for cumulative of 80.7% of the variation among the test genotypes.

The first and the second principal components accounted for a cumulative of 54.5% of the overall variability evident. The first principal component which alone explained 30.6% of the gross variability among the genotypes had been mainly to variations in days to heading, culm thickness, leaf number per main tiller, leaf blade width and finger width, all of them with positive loading. Unlike the result of this study, Tsehaye and Kebebew (2002) found finger number, plant height, 100-seed weight along with days to flowering to be the major contributors for the variation. Factor loadings greater than  $\pm 0.30$  are considered to meet the minimum level and the signs are interpreted as just as with any other correlation coefficients. On each factor, like signs mean the variables are positively related and opposite signs mean the variables are negatively related (Hair *et al.*, 1998). In orthogonal solutions, the factors are independent of one another. Therefore, the signs for factor loadings relate only to the factor on which they are appearing not to other factors in the solutions. The sign of the loading also indicates the direction of the relationship between the components and the variables (Seiler and Stafford 1985). The second principal component accounted for 23.9% of the total variation, which originated chiefly from plant height and days to maturity with negative-loading and number of grains per spikelet with positive-loading. In the third principal component, grain yield per plant with negative-loading and in the fourth component, finger number per main

ear with positive-loading and 1,000-grain weight with negative-loading accounted for 11.3% and 8.2% of the variation, respectively whereas finger length and leaf blade length with positive-loading and productive tillers per plant with negative-loading in the fifth components accounted for 6.8% of the variation.

**Table 15** Eigenvectors and eigenvalues of the first 5 principal components for 15 morpho-agronomic characters in 66 finger millet accessions.

Character	PC1	PC2	PC3	PC4	PC5
Plant height (cm)	0.140	-0.315	-0.288	-0.141	0.206
Days to heading	0.359	-0.250	0.135	-0.138	-0.197
Days to maturity	0.236	-0.393	0.030	-0.181	-0.165
Grain filling duration	-0.221	-0.236	-0.184	-0.062	0.046
Productive tillers/plant	-0.205	-0.028	-0.363	-0.359	-0.583
Culm thickness (mm)	0.379	0.212	-0.172	0.026	0.050
Leaf number/main tiller	0.409	-0.003	-0.002	0.001	-0.082
Leaf blade length (cm)	0.279	-0.246	-0.272	-0.067	0.318
Leaf blade width (cm)	0.361	0.160	-0.112	0.063	0.159
Finger length (cm)	-0.237	-0.279	-0.262	0.035	0.457
Finger width (mm)	0.324	0.280	-0.143	-0.256	-0.066
Finger number/main ear	0.102	-0.191	-0.276	0.664	-0.152
No. of grains/spikelet	0.015	0.403	-0.068	0.307	0.050
Thousand-grain weight (g)	-0.083	0.322	-0.137	-0.423	0.374
Grain yield/plant (g)	-0.083	0.192	-0.654	0.072	-0.189
Eigenvalues	4.587	3.582	1.691	1.232	1.021
Percent variation explained	30.6	23.9	11.3	8.2	6.8
Cumulative percent of total variance explained	30.6	54.5	65.7	73.9	80.7

## **2. Diversity study using isozyme markers**

The enzyme system assayed and the numbers of bands observed are shown in Table 16. Accessions were monomorphic for all enzyme system assayed. As a result, the assay did not discriminate among finger millet accessions revealing the isozyme system were less sensitive and the failure of allozymes frequencies to differentiate among the landraces of finger millet.

**Table 16** Enzyme systems assayed and the number of bands observed.

Enzyme system	Code	No. of Bands	Description
Esterase	EST	6	Dominantly faint
Aspartate aminotransferase	AAT	2	Good resolution and very clear
Phosphoglucose isomerase	PGI	2	Good resolution, dense and clear
phosphoglucose mutase	PGM	2	Poor resolution and diffused
Acid phosphate	ACP	1	Faint band
Aldolase	ALD	0	Not observed

Esterase (EST) pattern showed 6 bands, which were homogeneous in all tested materials. Among the 6 bands, Est-3 and Est-4 were clear and distinct whereas the remaining bands except Est-6 which was moderately clear, Est-1, Est-2, and Est-5 were faint and difficult to observe. All bands were homozygous except Est-6. Gel stained for enzyme system of aspartate aminotransferase (AAT) revealed clear and distinct two bands. They were homozygous and homogeneous across the materials tested. Likewise, each of phosphoglucose isomerase (PGI) and phosphoglucose mutase (PGM) produced two zones, which were homozygous and homogeneous for all materials. In case of PGI, the fast moving Pgi-2 was clear and distinct whereas Pgi-1 was dense and thicker. Both Pgm-1 and Pgm-2 for PGM enzyme system were diffused and could not be distinctively observed. Acid phosphate (ACP) enzyme

revealed homogeneous single band which was very faint and could be seen with difficulty whereas aldolase (ALD) was found to produce no band.

The absence of polymorphism for isozyme analysis in this study was parallel to finding of Werth *et al.* (1994) who reported identical isozyme genotype of *E. coracana* subsp. *coracana* and much lower variation in the three diploid species of *E. jaegeri*, *E. multiflora* and *E. trisacchia*. Workeye (2002) also found no polymorphism in the isozyme assay using different enzyme system and electrophoretic methods for Ethiopian chickpea landraces. In contrast, substantial genetic variation was encountered among accessions of *E. coracana* subsp. *africana* and in the diploid species of *E. indica* and *E. floccifolia* (Werth *et al.*, 1994).

### **3. Diversity study using RAPD markers**

#### **3.1. Primer screening**

A total of 164 RAPD primers were screened for amplification and 111 primers generated amplified bands, while 53 primers did not generate amplified bands. Based on consistent production of strong amplification and uniform reproducible products, 23 primers were chosen and used for further RAPD analysis in all the 66 accessions. Among the 23 primers employed for RAPD analysis, five of them showed monomorphic bands across all accessions and another three were not consistent. As a result, the remaining 15 primers (Table 17) were used for the genetic analysis.

#### **3.2. RAPD polymorphism and power of discrimination**

Using the 15 RAPD primers, the RAPD analysis generated a total of 123 RAPD fragments (Table 17, 18, 19). The band size, detected by comparing the amplified fragments with Gene Ruler™ DNA Ladder mix ranged from about 200 bp (OPD-12) to 2400 bp (OPA-18) in length. The RAPD markers were found to be informative markers in assessing the genetic diversity of 66 finger millet accessions.

Examples of the banding pattern and polymorphism detected with RAPD primers OPA-11 and OPD-08 are shown in Figure 5 and 6, respectively.

Each primer produced a different set of polymorphism with average number of  $8.2 \pm 2.833$  bands per primer (Table 17 and 19). Primer OPD-08 generated the maximum (16) number of scorable bands all of them being polymorphic (100%). The minimum number (5) of bands was generated by primer OPC-02 with only 1 monomorphic band presented across all accessions and the other 4 were polymorphic bands with 80% polymorphism. Though primer OPE-1 produced 10 scorable bands, the majority of bands (60%) were monomorphic across all accessions.

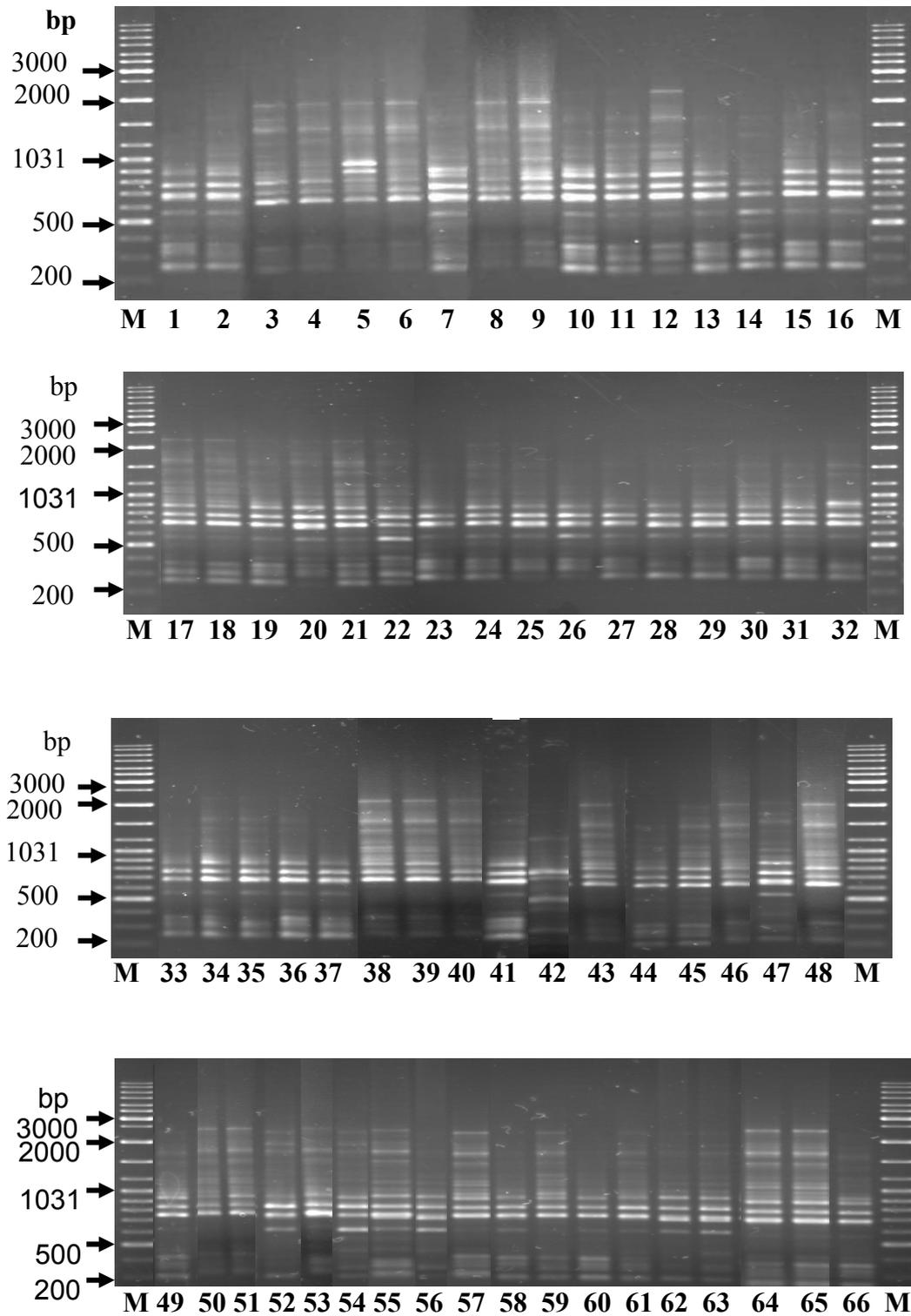
The average number of bands per primer of  $8.2 \pm 2.833$  observed in this study was relatively high compared with 6.86 reported by Fakrudin *et al.* (2004) in finger millet from Africa and India but almost similar with the report of Salimath (1995) who found 8 bands per primers in finger millet from Africa, Asia and Brazil. This difference could be attributed to the genotypes evaluated, the primers used and the selection of scorable bands in each study (Cansian and Echeverrigarya, 2000).

As shown in Table 17 and 19, among the 123 RAPD fragments, 89 (72.35%) were polymorphic and 34 (27.64%) were monomorphic. This is an evidence for high degree of variability among all accessions. The high polymorphic rate (72.35%) was in agreement with the findings of Fakrudin *et al.* (2004) who reported polymorphic rate of 85.82%. However, Salimath (1995) reported only 10% polymorphism in 17 accessions of finger millet from Africa, Asia and Brazil. The polymorphism observed in RAPD markers among finger millet accession observed in this study demonstrated the effectiveness of RAPD technique (method) in determining intraspecific variation.

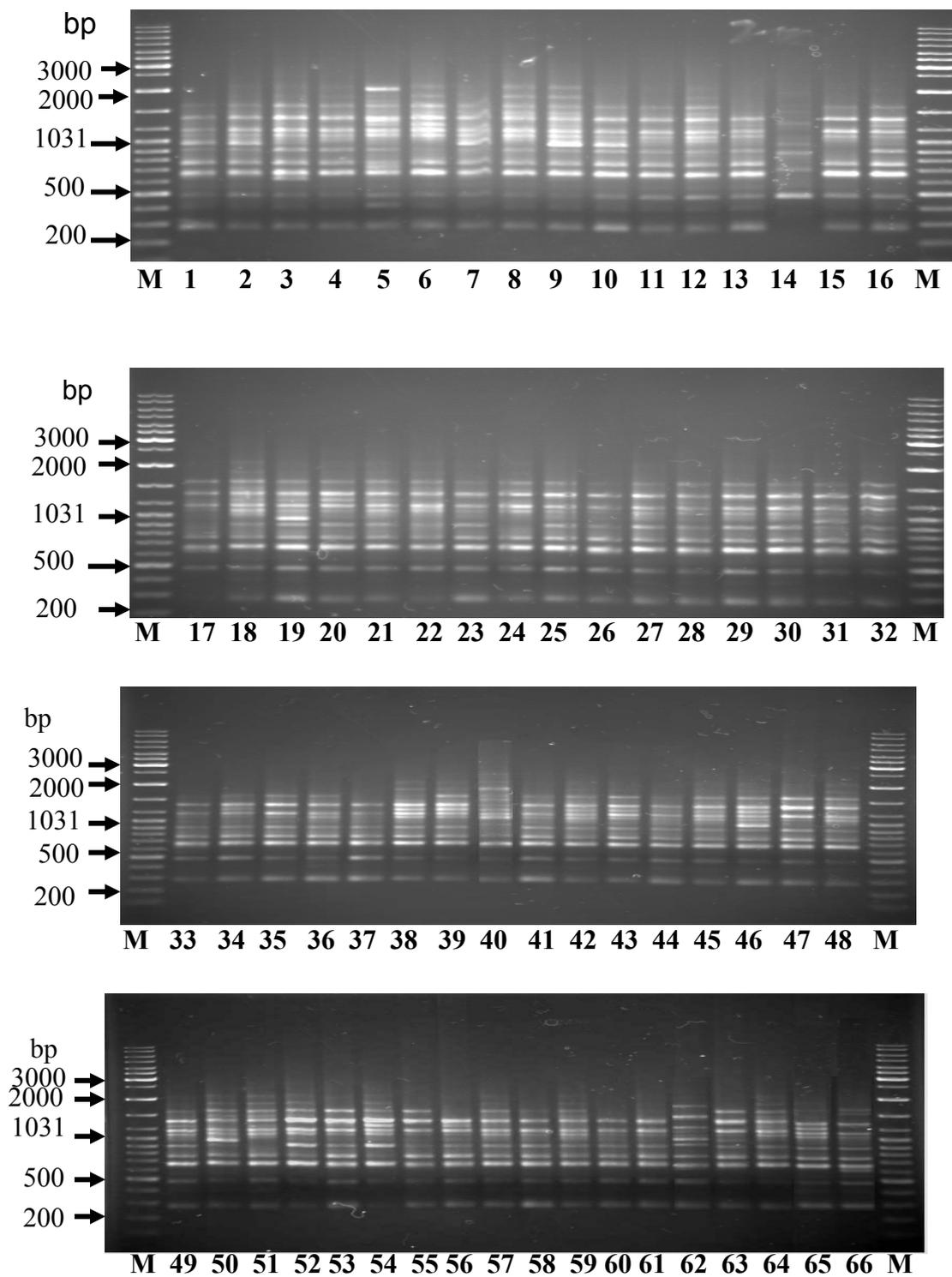
**Table 17** List of selected RAPD primers, their sequence, number of bands and polymorphic (%) of the RAPD analysis result in 66 finger millet accessions.

Primer no.	Primer sequence	Amplified DNA bands			Polymorphic (%)
		Polymorphic	Monomorphic	Total	
OPA-09	5'GGGTAACGCC <sup>3'</sup>	6	0	6	100
OPA-10	5'CAATCGCCGT <sup>3'</sup>	5	3	8	62.5
OPA-11	5'GTGATCGCAG <sup>3'</sup>	7	3	10	70.0
OPA-18	5'AGGTGACCGT <sup>3'</sup>	6	2	8	75.0
OPAA-03	5'TTAGCGCCCC <sup>3'</sup>	4	2	6	66.8
OPC-02	5'GTGAGGCGTC <sup>3'</sup>	4	1	5	80.0
OPC-05	5'GATGACCGCC <sup>3'</sup>	5	2	7	71.4
OPC-20	5'ACTTCGCCAC <sup>3'</sup>	3	6	9	33.3
OPD-08	5'GTGTGCCCCA <sup>3'</sup>	16	0	16	100
OPD-12	5'CACCGTATCC <sup>3'</sup>	11	0	11	100
OPD-18	5'GAGAGCCAAC <sup>3'</sup>	5	1	6	83.3
OPE-01	5'CCCAAGGTCC <sup>3'</sup>	4	6	10	40.0
OPX-05	5'CCTTTCCCTC <sup>3'</sup>	5	1	6	83.3
OPY-02	5'CATCGCCGCA <sup>3'</sup>	3	3	6	50.0
UBC-06*	5'GGGCCGTTTA <sup>3'</sup>	5	4	9	55.6
Total		89	34	123	
Average		5.93	2.27	8.20	72.35
SD		3.390	1.944	2.833	

\* UBC =University of British Columbia, Source: Tseng *et al.* (1999)



**Figure 5** DNA pattern of primer OPA-11. M is a standard marker and lane numbers refer to the accession members in Table 1.



**Figure 6** DNA pattern of primer OPD-08. M is a standard marker and lane numbers refer to the accession members in Table 1.

**Table 18** RAPD primers, size of RAPD fragments and polymorphic information content (PIC) of the RAPD analysis result in 66 finger millet accessions.

RAPD primer	DNA fragment size	PIC
OPA-09	OPA-09-570	0.298
	OPA-09-620	0.114
	OPA-09-730	0.463
	OPA-09-980	0.059
	OPA-09-2050	0.257
	OPA-09-2100	0.351
OPA-10	OPA-10-340	0.334
	OPA-10-540	0.000
	OPA-10-690	0.000
	OPA-10-750	0.000
	OPA-10-900	0.140
	OPA-10-1150	0.165
	OPA-10-1300	0.213
	OPA-10-1400	0.114
OPA-11	OPA-11-250	0.030
	OPA-11-320	0.000
	OPA-11-360	0.278
	OPA-11-400	0.454
	OPA-11-550	0.410
	OPA-11-680	0.000
	OPA-11-770	0.000
	OPA-11-890	0.059
	OPA-11-1700	0.278
	OPA-11-2250	0.471
OPA-18	OPA-18-550	0.030
	OPA-18-610	0.000
	OPA-18-750	0.000
	OPA-18-850	0.483
	OPA-18-1250	0.257
	OPA-18-1750	0.278
	OPA-18-2000	0.140
	OPA-18-2400	0.165
OPAA-03	OPAA-03-550	0.000
	OPAA-03-650	0.000
	OPAA-03-750	0.454
	OPAA-03-850	0.030
	OPAA-03-920	0.030
	OPAA-03-1300	0.030

**Table 18 (Cont'd)**

RAPD primer	DNA fragment size	PIC
OPC-02	OPC-02-610	0.213
	OPC-02-710	0.000
	OPC-02-1050	0.114
	OPC-02-1400	0.059
	OPC-02-1600	0.471
OPC-05	OPC-05-710	0.471
	OPC-05-900	0.140
	OPC-05-940	0.000
	OPC-05-1031	0.000
	OPC-05-1200	0.213
	OPC-05-1300	0.257
	OPC-05-1700	0.140
OPC-20	OPC-20-600	0.059
	OPC-20-650	0.493
	OPC-20-710	0.000
	OPC-20-850	0.000
	OPC-20-950	0.000
	OPC-20-1031	0.000
	OPC-20-1120	0.000
	OPC-20-1200	0.000
	OPC-20-1550	0.059
OPE-01	OPE-01-500	0.000
	OPE-01-550	0.382
	OPE-01-640	0.410
	OPE-01-750	0.000
	OPE-01-900	0.000
	OPE-01-1200	0.000
	OPE-01-1300	0.000
	OPE-01-1700	0.483
	OPE-01-1800	0.000
OPE-01-2200	0.213	
OPD-08	OPD-08-300	0.059
	OPD-08-400	0.444
	OPD-08-490	0.030
	OPD-08-640	0.059
	OPD-08-660	0.030
	OPD-08-700	0.059
	OPD-08-750	0.030
	OPD-08-900	0.087
	OPD-08-930	0.298
	OPD-08-1100	0.030
	OPD-08-1180	0.030
	OPD-08-1300	0.367
	OPD-08-1400	0.030

**Table 18 (Cont'd)**

RAPD primer	DNA fragment size	PIC
OPD-08	OPD-08-1600	0.140
	OPD-08-1800	0.500
	OPD-08-2050	0.498
OPD-12	OPD-12-200	0.030
	OPD-12-280	0.030
	OPD-12-300	0.059
	OPD-12-340	0.410
	OPD-12-350	0.410
	OPD-12-450	0.114
	OPD-12-660	0.059
	OPD-12-700	0.489
	OPD-12-800	0.278
	OPD-12-1300	0.422
	OPD-12-1400	0.030
OPD-18	OPD-18-500	0.190
	OPD-18-580	0.213
	OPD-18-660	0.000
	OPD-18-740	0.190
	OPD-18-1100	0.114
	OPD-18-1300	0.114
OPX-05	OPX-05-550	0.114
	OPX-05-690	0.493
	OPX-05-840	0.087
	OPX-05-1100	0.000
	OPX-05-1400	0.257
	OPX-05-2000	0.478
OPY-02	OPY-02-650	0.030
	OPY-02-800	0.000
	OPY-02-980	0.087
	OPY-02-1250	0.000
	OPY-02-1350	0.000
	OPY-02-1700	0.140
UBC-06*	UBC-06-350	0.000
	UBC-06-500	0.493
	UBC-06-560	0.334
	UBC-06-640	0.114
	UBC-06-660	0.000
	UBC-06-750	0.000
	UBC-06-920	0.000
	UBC-06-1140	0.454
UBC-06-1800	0.496	
Average±SD		0.162±0.175

\* UBC =University of British Columbia, Source: Tseng *et al.* (1999)

The polymorphic rate for each primer is shown in Table 17. The polymorphic rate ranged from 33.3 to 100%. Primer OPc-20 exhibited the lowest (40%) polymorphism whereas three primers, namely OPA-09, OPD-08 and OPD-12 exhibited 100% polymorphism despite the fact that each of them generated variable number of RAPD fragments. The overall average percentage of polymorphism (72.35) was high, indicating the existence of a very high level of DNA sequence variability in finger millet landraces from Ethiopia and Eritrea. This in turn revealed the existence of high variability.

Comparison of more primers generally provides additional confirmatory evidence for genetic variation; however, due to the existence of wide gene pool of Ethiopian and Eritrean finger millet, 89 polymorphic bands generated by 15 primers could distinguish the accession evaluated in this study. Hence, this revealed the ability of RAPD to discriminate among the accessions and suggested its application for diversity study.

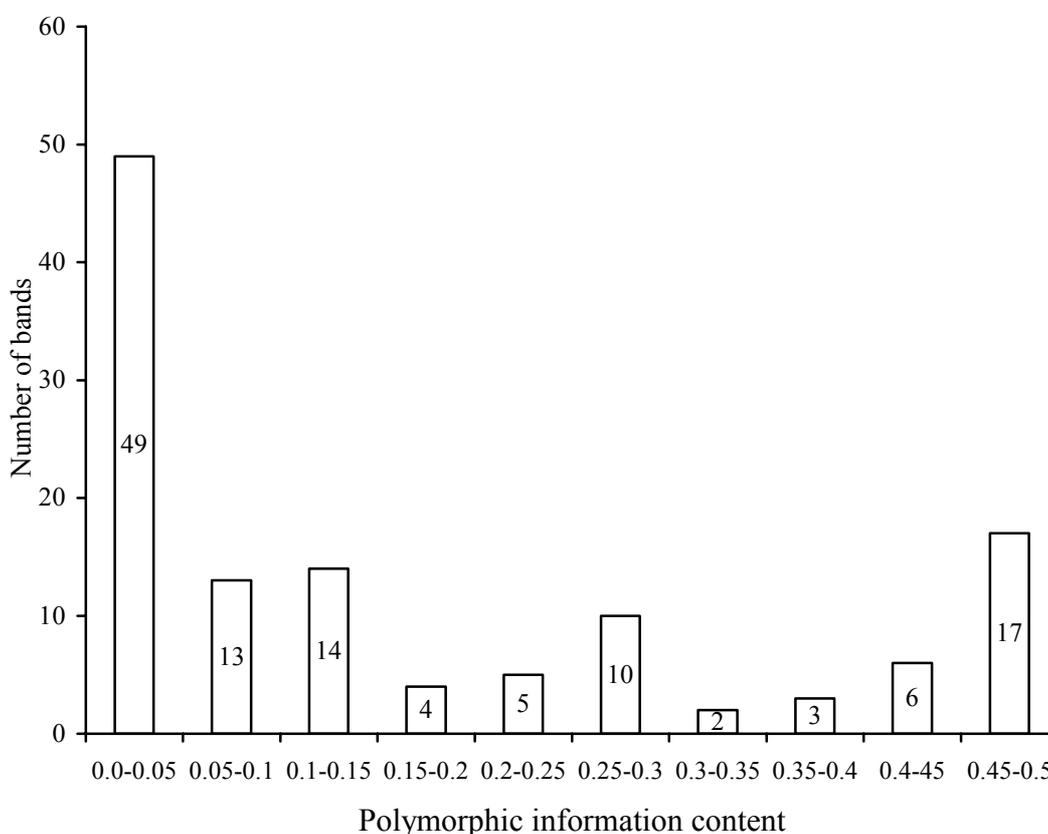
**Table 19 Summary** of statistics of RAPD analysis of 66 finger millet accessions.

Parameter	Estimates
Total marker	123
Total number of polymorphic bands	89
Maximum number of bands produced by a primer	16
Minimum number of bands produced by a primer	5
Average number of polymorphic bands per primer $\pm$ SD	5.93 $\pm$ 3.39
Average number of bands produced by primer $\pm$ SD	8.2 $\pm$ 2.833
Average polymorphic information content (PIC) $\pm$ SD	0.162 $\pm$ 0.175
Average polymorphism rate	72.35

The discrimination power of each marker was estimated by polymorphic information content (PIC) (Table 18). The PIC scores ranged from 0.0 (OPA-10-540, OPA-10-690, OPA-10-750, OPA-11-320, OPA-11-680, OPA-11-770, OPA-18-610, OPA-18-750, OPAA-03-550, OPAA-03-650, OPC-02-710, OPC-05-940, OPC-05-1031, OPC-20-710, OPC-20-850, OPC-20-950, OPC-20-1031, OPC-201120, OPC-

20-1200, OPE-01-500, OPE-01-750, OPE-01-900, OPE-01-1200, OPE-01-1300, OPE-01-1800, OPD-18-660, OPX-05-1100, OPY-02-800, OPY-02-1250, OPY-02-1350, UBC-06-350, UBC-06-660, UBC-06-750, and UBC-06-920) to 0.500 (OPD-08-1800) with a mean value  $\pm$ SD of  $0.0.162\pm 0.175$ .

The RAPD markers showed heterogeneous distribution of PIC scores (Figure 7) and about 23% of markers had a high discrimination power of  $\geq 0.30$  (Table 18 and Figure 7).



**Figure 7** Distribution of polymorphic information content scores for 123 RAPD markers among 66 finger millet accessions.

### **3.3. Estimates of genetic similarity**

Similarity matrix based on the simple matching coefficient (Sneath and Sokal, 1973) using the RAPD data was used to assess the extent (level) of genetic

relatedness among the 66 finger millet accessions (Appendix Table 6). The similarity coefficient value ranged from 0.585 to 0.984 among the finger millet accessions assessed in this study. The most similar genotypes appeared to be accessions 234205 (43) from Tigray, 230722 (40) and 230714 (39) both from Eritrea. Of the 123, amplified products, 121 products (98.4%) showed the same presence or absence behavior whereas the remaining two products (1.6%) exhibited different presence or absence behavior in these accessions.

The minimum genetic similarity 0.585 was between accession 215874 (14) from Gojam with accessions 211474(9) from Gamo Gofa, 236447 (50) from Welega and 238319 (59) from Tigray. Accession 215874 (14) showed 41.5 % difference of absence or presence behavior for the amplified products with these three accessions.

Relatively the small number of pair wise differences in traits/markers (high genetic similarity values) among some accessions is likely their genetic relatedness. On the other hand, large number of pair wise differences (low genetic similarity values) revealed that accessions are genetically far distant.

### **3.4. RAPD clustering**

The cluster analysis using the simple matching similarity coefficient was performed by NTSYpc 2.01 computer program. The dendrogram constructed from cluster analysis and the number of accessions in each cluster and their origin of regions are shown in Figure 8 and Table 20, respectively. The cluster analysis separated the accessions into two major groups at similarity scale of 0.79. In the first group only 1 accession, 215874 (14) from Gojam was clearly distinguished from all other accessions, implying that this accession was genetically more distinct from other accessions. However, its exclusion from others in the RAPD dendrogram was rather difficult to conclude whether this was due to morphological difference, as it was closely clustered with other 14 accessions in cluster V in the morphological dendrogram (Figure 4). The second group contained all the remaining 65 accessions,

which could be further grouped into several sub-clusters depending on the similarity index employed.

All accessions were successfully separated from one another with the highest similarity value of approximately 0.98. Hence, this again confirmed RAPD could be used routinely by plant breeders to identify genetic variation (Keil and Griffin, 1994), locate region of genome linked to agronomically important genes (Pillay and Kenny, 1996) and facilitate introgression of desirable genes to commercial accessions (Stuber, 1992).



**Figure 8** Dendrogram showing genetic relationship among 66 finger millet accessions generated by UPGMA cluster using 123 RAPD markers amplified by 15 RAPD primers.

**Table 20** Number of finger millet accessions in each cluster and region of origin.

Cluster	Total number of accessions/cluster	Region of origin	Number of accessions/region
I	1	Gojam	1
II	1	Gonder	1
III	4	Tigray	2
		Gonder	1
		Gamo Gofa	1
IV	2	Tigray	1
		Gonder	1
	1	Gojam	1
VI	1	Improved Variety*	1
VII	2	Tigray	2
VIII	49	Tigray	7
		Gonder	7
		Gojam	13
		Welega	10
		Gamo Gofa	3
		Eritrea	8
		Improved variety	1
IX	5	Gonder	1
		Welega	2
		Gamo Gofa	2

\* Developed from introduction

At similarity index corresponding approximately to 0.83, the 66 accessions were clustered into 9 groups. Clusters I, II, V and VI constituted only 1 accession each. Accession 215824 (14) in cluster I and accession 215877 (15) in cluster V originated from Gojam whereas accession 242131 (65) in cluster II was from Gonder. The improved variety, Tadesse, originally from Kenya was grouped in cluster VI.

Four accessions, 242123 (63) from Gonder, 241769 (62) from Gamo Gofa and both 237459 (56) and 237449 (53) from Tigray made cluster III. Cluster IV and VII each constituted only 2 accessions. Accessions 237452(55) and 235830(47), which were sampled from Tigray and Gonder, respectively made cluster IV whereas accessions, 237451 (54) and 237447 (52) both from Tigray were member of cluster VII.

The majority of the accessions including 1 improved variety (Paadet) were grouped in cluster VIII. Forty-nine accessions that was about 74.24% fell in this group. However, none of them were found to be absolutely similar. Eight accessions from Eritrea, 7 from Tigray, 7 from Gonder, 13 from Gojam, 10 from Welega, 3 from Gamo Gofa and 1 improved variety, Paadet, were included in cluster VIII. One accession, 235138 (44) from Gonder, 2 accessions from Welega, 216024 (22) and 216025 (23), 2 accessions from Gamo Gofa, 100084 (2) and 100055 (1) made-up cluster IX.

Unlike the findings of Fakrudin *et al.* (2004) who found that cluster analysis based unweighted pair-group method using arithmetic average to have clear apportionment of finger millet accessions in concordance with geographical origin and pedigree history, the RAPD data result in the present study did not provide a clear-cut separation among finger millet accessions in relation to the origin of their respective geographical region although there was irregular trend that accessions from the same region were clustered together. M'Ribu and Hilu (1994) reported that the RAPD data grouped proso millet cultivars according to their geographical origin. Cultivars from each country grouped together and those representing geographic regions showed high affinity. Lack of a clear pattern of variation in relation to geographic origin in this study was in agreement with the result of Hilu (1995) where RAPD data did not discriminate between African and Indian finger millet cultivars.

Though the accessions assessed in this study mainly represented landraces from different geographical regions of Ethiopia and Eritrea, the analysis of RAPD data did not show a clear-cut pattern of variation in relation to geographical region

could be due to the long history of domestication and cultivation of finger millet that might be contributed to the dispersion of alleles through out the country, lessening the influence of geography on pattern of variation among them.

Grouping based on the RAPD variation did not fully agree with that based on morphological traits. The typical example is the two improved varieties, Paadet and Tadesse, were closely grouped in the morphological dendrogram but they were grouped in different cluster in the RAPD dendrogram. This could be due to the fact that the morphological traits could vary with the prevailing environment, but most likely related to their genetic resemblance, while RAPD analysis estimates overall dissimilarity at DNA level with each PCR product potentially a different locus (Liu, 1997).

## SUMMARY AND CONCLUSION

Quantification and classification of diversity in germplasm collection is important in plant breeding since effective utilization of germplasm in breeding program is enhanced if the variation is properly characterized and described. It would also enable planning of future germplasm sampling, establishing *in situ* gene conservation, or use of appropriate gene pools in crop improvement for specific attributes. In this regard, genetic diversity study using morphological traits, isozyme and RAPD markers was conducted to generate information on the diversity of finger millet landraces.

Sixty-six accessions composed of 2 standard varieties (Paadet and Tadesse) and 64 landraces collected from 5 former regions of Ethiopia and Eritrea were used. The morphological diversity was conducted at Aresi-Negele Research Center during main cropping season of 2004. The accessions were evaluated in randomized complete block design (RCBD) with three replications. Data on 21 morpho-agronomic characters were scored using International Board for Plant Genetic Resources descriptors for finger millet (IBPGR, 1985).

The frequency distribution of 6 discrete morphological characters showed marked differences in their distribution and amount of variation. Monomorphic phenotypic classes were not observed at regional or country level. There was an increasing trend in diversity from north to south, the highest diversity indices pooled over characters being for accessions sampled from Gojam ( $H' = 0.84 \pm 0.04$ ) and Welega ( $H' = 0.84 \pm 0.06$ ) where as the lowest was for those from Eritrea ( $H' = 0.67 \pm 0.09$ ). The overall diversity ( $0.82 \pm 0.06$ ) of finger millet observed in this study was high and in agreement with the finding of Tsehaye and Kebebew (2002) who reported the existence of a vast range of genetic variability in the indigenous Ethiopian finger millet germplasm. The analysis of variance of diversity for individual characters revealed similar levels of diversity among regions for three of the discrete

characters (growth habit, grain color and pericarp persistence) whereas variations among regions were observed for ear shape, grain shape and grain surface.

Analysis of variance for the 15 morpho-agronomic characters showed significant variation for all characters among accessions. High phenotypic and genotypic variances were depicted by plant height, days to maturity and days to heading whereas the lowest ones were found for leaf blade width followed by 1,000-grain weight. The phenotypic (PCV) and genotypic (GCV) coefficient of variations ranged in the order of 8.05 to 31.23% and 6.52 to 24.21% in both cases for days to maturity to grain yield per plant, respectively.

As an index of transmission of characters from parents to offspring the heritability ( $h^2$ ) estimates in this study ranged from 20% for grain filling duration to 84% for days to heading, while the genetic advance estimates varied from 6.67 to 44.14% for grain filling duration to finger width, respectively. Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection than heritability estimates.

The genotypic correlation analysis indicated that grain yield per plant had significant positive association with productive tillers per plant ( $r_g=0.49$ ), number of grains per spikelet ( $r_g=0.45$ ), 1,000-grain weight ( $r_g=0.38$ ) and finger number per main ear ( $r_g=0.25$ ). It was also associated significantly but negatively with days to heading ( $r_g=-0.46$ ), days to maturity ( $r_g=-0.49$ ) and leaf number per main tiller ( $r_g=-0.25$ ). The strongest positive association was observed between leaf blade width and culm thickness ( $r_g=0.98$ ) and the strongest negative association between 1,000-grain weight and finger number per main ear ( $r_g=-0.77$ ). The genotypic correlation was further divided into direct and indirect effects using path-coefficients analysis, grain yield as resultant (dependable) variable while the variables that were significantly correlated with grain yield as causal (independent) variables. The path-coefficient analysis revealed days to heading (0.670), productive tillers per plant (0.701), leaf number per main tiller (0.659), finger number per main ear (1.212) and 1,000-grain weight (0.858) had positive direct effect whereas days to maturity (-1.932) and

number of grains per spikelet (-0.784) showed negative direct effect. The correlation and path analyses indicated apart selection for grain yield per plant *per se*, indirect selection for 1,000-grain weight, finger number per main tiller and productive tillers per plant could be applied in the improvement of finger millet.

The morphological cluster grouped the 66 accessions in to 5 clusters. However, accessions from same region were grouped into different clusters and conversely accessions from different regions were grouped in the same cluster implying there was no clear correspondence between geographic collections sites of landraces and their inclusion in particular clusters. The Mahalanobis's ( $D^2$ ) analysis confirmed that there was considerable variability among finger millet accessions that could be selected as parental lines for hybridization and improvement of the crop. Principal component analysis revealed that days to heading, culm thickness, leaf number per main tiller, leaf blade length and finger width all of them with positive loading were the main contributors for the variation among accessions.

The isozyme analysis study was conducted at the molecular genetics laboratory of Ethiopian Institute of Biodiversity Conservation in Ethiopia. The isozyme assay for 6 enzyme systems did not show polymorphism. As a result, the assay did not discriminate among finger millet accessions revealing the isozyme system are less sensitive and the failure of allozymes frequencies to differentiate among the landraces of finger millet.

The RAPD analysis using 15 RAPD primers was executed at the laboratory of Department of Genetics, Faculty of Science, Kasetsart University. The RAPD analysis was found to be effective in assessing the genetic diversity in finger millet. The number of scorable bands generated ranged from 5 (OPC-02) to 16 (OPD-08) with average number of 8.2. Among the 123 RAPD fragment, 89 (72.35%) were polymorphic, demonstrating the effectiveness of RAPD technique (method) to detect intraspecific variations. The polymorphic rate for each primer ranged from 33.3% (OPC-20) to 100% (OPA-09, OPD-08 and OPD-12). The overall average percent of polymorphism was 72.35%, indicating the existence of a very high level of DNA

sequence variability among finger millet landraces from Ethiopian and Eritrean. The polymorphic information content (PIC) as a measure of discrimination power, ranged from 0 to 0.50 with heterogeneous distribution and about 23% of the markers with a high discrimination power of  $\geq 0.30$ . The Genetic similarity between accessions estimated with simple matching coefficients ranged from 0.585 to 0.984 among finger millet accessions assessed in this study. RAPD cluster analysis successfully separated all accessions with the highest similarity value of approximately 98% revealing that RAPD can be used routinely by plant breeders to identify genetic variation. The 66 accession were grouped into 9 clusters at similarity index of approximately 0.83. Similar to the morphological clustering, the RAPD clustering did not provide a clear-cut separation among finger millet accessions in relation to the origin of their respective geographic region. Grouping based on the RAPD data did not agree with that based on morphological traits.

To generalize, the morphological traits and RAPD markers were better indicator of the existing diversity in the landraces of Ethiopian finger millet as opposed to the isozyme marker. The result of the present study revealed the existence of ample variability and potential in the landraces of finger millet that could be employed in the genetic improvement. The most divergent materials and those having complementary characters could be employed in the improvement program. The existence of tremendous variability and the high diversity index observed in this study support the hypothesis that eastern Africa is a center of origin and diversity for finger millet. The high diversity index also implied that *in situ* conservation has been in place and genetic erosion has not been wide spread. Moreover, the result of the present study could also help in planning future germplasm collection.

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## APPENDIX

**Appendix Table 1** Monthly rainfall (mm) at Aresi-Negele Research Sub-Center in 2004.

Date	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1	0.0	0.0	0.0	0.0	4.1	8.5	3.5	0.0	3.5	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.3	0.0	2.5	4.0	9.2	4.0	0.0	0.0
3	0.0	0.0	0.0	0.0	9.3	0.0	0.0	11.5	0.0	0.0	0.0	0.0
4	0.0	7.5	0.0	0.0	3.3	22.7	1.3	4.4	3.3	19.5	0.0	0.0
5	0.0	8.5	0.0	39.0	16.2	0.0	0.0	0.0	5.4	6.3	0.0	0.0
6	0.0	0.0	0.0	3.9	0.0	41.5	0.0	2.2	34.0	0.0	0.0	0.0
7	0.0	0.0	0.0	4.4	0.0	1.7	5.2	15.0	4.8	2.8	0.0	0.0
8	0.0	0.0	0.0	12.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	6.9	0.0	0.0	0.0	10.5	28.0	10.2	0.0	0.0
10	0.0	0.0	0.0	7.5	0.0	0.0	0.0	6.5	0.0	2.4	0.0	0.0
11	0.0	0.0	0.0	6.5	0.0	0.0	0.0	9.7	0.0	7.0	0.0	0.0
12	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0	7.5	0.0	0.0
13	0.0	0.0	0.0	1.5	0.0	0.5	0.0	0.0	22.2	5.5	0.0	0.0
14	0.0	0.0	0.7	0.0	0.0	6.0	16.0	0.0	30.0	7.5	0.0	0.0
15	8.5	0.0	0.0	4.5	0.0	1.5	0.0	43.0	37.0	0.0	0.0	0.0
16	0.0	0.0	3.0	4.4	0.0	1.7	0.6	5.5	0.0	0.0	0.0	0.0
17	0.0	0.0	0.0	0.0	0.0	15.3	4.5	0.0	15.0	0.0	0.0	0.0
18	0.0	0.0	6.7	0.0	0.0	7.9	3.4	7.5	2.6	0.0	0.0	0.0
19	2.5	0.0	4.5	0.0	0.0	0.0	0.0	0.5	4.5	0.0	0.0	0.0
20	0.0	0.0	0.0	0.0	0.0	18.5	4.5	1.7	0.0	0.0	0.0	0.0
21	7.2	0.0	0.0	0.0	0.0	0.0	8.5	5.8	0.0	0.0	0.0	0.0
22	0.0	0.0	0.0	0.0	0.0	1.5	4.2	14.0	0.5	0.0	0.0	0.0
23	52.5	0.0	0.0	0.0	0.0	0.0	13.4	0.0	4.5	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	6.4	0.0	0.0	0.0	3.0	0.0
25	0.0	0.0	0.0	0.0	0.0	4.5	8.0	12.5	0.0	0.0	0.0	0.0
26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.5	0.0	0.0	0.0	0.5
27	0.0	0.0	0.1	0.0	5.0	0.0	12.5	0.0	8.5	0.0	0.0	0.0
28	0.0	0.0	5.1	0.0	13.7	1.0	28.0	0.0	0.0	0.0	0.0	0.0
29	0.0	0.0	6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30	0.0		3.5	0.0	6.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31	0.0		5.1		0.0		0.0	0.0		0.0		0.0
Total	70.7	16	35.2	92.8	58.7	132.8	122.5	161.8	213	72.7	3.0	0.5

Source: Aresi-Negele Weather Station.

**Appendix Table 2.** Staining solution for selected isozyme system.

No.	Enzyme system, EC No.	Staining solution (buffer/ pH)	Ingredients
1	Esterase (EST) EC 3.1.1.-	20 ml H <sub>2</sub> O 20 ml 0.2 M NaH <sub>2</sub> PO <sub>4</sub> 10 ml 0.2 M Na <sub>2</sub> HPO <sub>4</sub>	2 ml 1% $\alpha$ -naphthyl acetate in acetone, 125 mg fast blue BB salt, 1 ml acetone
2	Aspartate aminotransferase (AAT) EC 2.6.1.1	50 ml 0.1 M Tris-HCl, pH 8.5	18 mg $\alpha$ -ketoglutaric acid, 65 mg L-aspartic acid, 5 mg pyridoxal-5-phosphate, 250 mg PVP-40T, 50 mg disodium EDTA, 710 mg Na <sub>2</sub> HPO <sub>4</sub> , 200 mg fast blue BB salt
3	Phosphoglucose isomerase (PGI) EC 5.3.1.9	50 ml 0.1 M Tris-HCl, pH 7.5	20 mg disodium fructose-6-phosphate, 0.4 $\mu$ l glucose-6-phosphate dehydrogenase, 7 Mg NADP, 1.2 ml MTT, 300 $\mu$ l PMS, 0.5 10% MgCl <sub>2</sub>
4	Phosphoglucomutase (PGM) EC 2.7.5.1	50 ml 0.1 M Tris-HCl, pH 7.5	80 mg disodium glucose-1-phosphate, 4 $\mu$ l glucose-6-phosphate dehydrogenase, 10 mg NADP, 1.5 ml MTT, 1 $\mu$ l PMS, 0.5 ml 10% MgCl <sub>2</sub>
5	Acid phosphatase (ACP) EC 3.1.3.2	50 ml 0.4 M sodium acetate buffer pH 5.0 (pre-soak) 50 ml 0.2 M sodium acetate buffer pH 5.0	50 mg $\alpha$ -naphthyl acid phosphate, 50 mg fast garnet GBC salt, .5 ml 0.5 % ml 10% MgCl <sub>2</sub>
6	Aldolase (ALD) EC 4.1.2.13	50 ml 0.05 M Tris-HCl, pH 8.0	200 mg fructose-1,6-biphosphate, sodium salt, 75 mg sodium arsenate, 10 mg NAD, 10 mg MTT, 2 mg PMS

**Appendix Table 3** Estimates of Shannon-Weaver diversity index ( $H'$ ), mean  $H'$  and standard error ( $\pm SE$ ) in each accession based on 6 discrete morphological traits.

Accession	Adm.	GRHA <sup>+</sup>	ESHP	GSHP	GSUR	GCL	PPR	Mean
	region.							H $\pm$ SE
100055	Gamo Gofa	0.000	0.548	0.361	0.567	0.528	0.000	0.334 $\pm$ 0.110
100084	Gamo Gofa	0.579	0.774	0.283	0.722	0.455	0.883	0.616 $\pm$ 0.090
100094	Welega	0.613	0.603	0.418	0.000	0.357	0.223	0.369 $\pm$ 0.096
100095	Welega	0.629	0.530	0.000	0.000	0.000	0.000	0.193 $\pm$ 0.123
204747	Eritrea	0.613	0.244	0.000	0.567	0.223	0.000	0.275 $\pm$ 0.109
208444	Gonder	0.613	0.152	0.715	0.567	0.223	0.000	0.378 $\pm$ 0.119
208448	Gojam	0.613	0.548	0.000	0.353	0.000	0.000	0.252 $\pm$ 0.118
208730	Welega	0.988	0.360	0.000	0.353	0.613	0.000	0.386 $\pm$ 0.154
211474	Gamo Gofa	0.988	0.778	0.485	0.722	0.579	0.000	0.592 $\pm$ 0.138
213035	Gamo Gofa	0.731	0.576	0.000	0.567	0.000	0.000	0.312 $\pm$ 0.142
215841	Gojam	0.579	0.613	0.459	0.997	0.629	0.000	0.546 $\pm$ 0.132
215850	Gojam	1.000	0.395	0.000	0.837	0.000	0.000	0.372 $\pm$ 0.185
215867	Gojam	0.613	0.395	0.000	0.000	0.000	0.455	0.244 $\pm$ 0.113
215874	Gojam	0.988	0.603	0.459	0.000	0.000	0.082	0.355 $\pm$ 0.163
215877	Gojam	0.629	0.360	0.000	0.000	0.357	0.000	0.224 $\pm$ 0.108
215879	Gojam	0.613	0.395	0.728	0.837	0.223	0.000	0.466 $\pm$ 0.130
215883	Gojam	0.988	0.000	0.753	0.837	0.455	0.357	0.565 $\pm$ 0.149
215889	Gojam	0.000	0.535	0.000	0.000	0.000	0.000	0.089 $\pm$ 0.089
215896	Gojam	0.988	0.360	0.485	0.000	0.988	0.357	0.530 $\pm$ 0.159
215973	Gonder	0.629	0.244	0.000	0.722	0.223	0.000	0.303 $\pm$ 0.126
215977	Gonder	0.579	0.475	0.177	0.722	0.223	0.000	0.303 $\pm$ 0.126
216024	Welega	0.442	0.244	0.418	0.971	0.000	0.000	0.346 $\pm$ 0.148
216025	Welega	0.579	0.576	0.350	0.353	0.442	0.000	0.346 $\pm$ 0.148

**Appendix Table 3** (Cont'd).

Accession	Adm. region	GRHA <sup>+</sup>	ESHP	GSHP	GSUR	GCL	PPR	Mean
								H±SE
216028	Welega	0.883	0.301	0.283	0.567	0.357	0.579	0.495±0.094
216041	Welega	0.629	0.000	0.418	0.837	0.357	0.000	0.374±0.137
216043	Welega	0.613	0.244	0.361	0.918	0.455	0.357	0.491±0.099
216045	Welega	0.629	0.152	0.418	0.567	0.357	0.223	0.391±0.076
216051	Welega	0.000	0.311	0.552	0.567	0.528	0.000	0.326±0.110
216052	Welega	0.455	0.311	0.283	0.837	0.000	0.357	0.374±0.112
225895	Gojam	0.613	0.832	0.669	0.000	0.528	0.455	0.516±0.116
229723	Gojam	0.613	0.418	0.000	0.000	0.000	0.528	0.260±0.119
229726	Gojam	0.455	0.311	0.459	0.918	0.579	0.000	0.454±0.123
229728	Gojam	0.528	0.429	0.621	0.971	0.613	0.000	0.527±0.129
230101	Eritrea	0.579	0.674	0.728	0.567	0.455	0.000	0.501±0.107
230117	Eritrea	1.000	0.418	0.485	0.997	0.223	0.000	0.521±0.166
230130	Eritrea	0.000	0.360	0.753	0.997	0.613	0.000	0.454±0.166
230136	Eritrea	0.629	0.395	0.485	0.837	0.455	0.000	0.467±0.114
230714	Eritrea	0.613	0.360	0.485	0.971	0.528	0.000	0.493±0.130
230722	Eritrea	0.528	0.311	0.498	0.997	0.528	0.000	0.477±0.133
230724	Eritrea	0.455	0.360	0.728	0.918	0.629	0.357	0.575±0.092
234178	Tigray	0.528	0.576	0.418	0.722	0.528	0.000	0.462±0.101
234205	Tigray	1.000	0.152	0.700	0.250	0.218	0.223	0.424±0.141
235138	Gonder	0.455	0.418	0.498	0.837	0.579	0.357	0.524±0.070
235141	Gonder	0.528	0.360	0.459	0.997	0.000	0.528	0.479±0.131
235700	Gamo Gofa	0.613	0.603	0.459	0.722	0.528	0.357	0.547±0.052
235830	Gonder	0.455	0.395	0.783	0.837	0.613	0.357	0.573±0.083
235838	Gonder	0.000	0.311	0.669	0.971	0.629	0.223	0.467±0.144
235842	Gonder	0.613	0.603	0.715	0.567	0.528	0.357	0.564±0.049

**Appendix Table 3** (Cont'd).

Accession	Adm. region	GRHA <sup>+</sup>	ESHP	GSHP	GSUR	GCL	PPR	Mean
								H'±SE
236447	Welega	0.000	0.360	0.485	0.567	0.000	0.455	0.311±0.102
236450	Welega	0.613	0.000	0.283	0.567	0.528	0.000	0.332±0.115
237447	Tigray	0.988	0.395	0.753	0.918	0.629	0.223	0.651±0.122
237449	Tigray	0.613	0.616	0.459	0.971	0.455	0.000	0.651±0.122
237451	Tigray	0.629	0.418	0.485	0.971	0.613	0.000	0.519±0.129
237452	Tigray	0.528	0.499	0.715	0.918	0.528	0.223	0.569±0.095
237459	Tigray	0.579	0.311	0.669	0.837	0.950	0.528	0.646±0.093
237462	Tigray	0.629	0.360	0.621	0.918	0.528	0.000	0.509±0.126
237477	Tigray	0.613	0.000	0.418	0.918	0.357	0.223	0.422±0.130
238319	Tigray	0.988	0.418	0.636	0.971	0.223	0.455	0.615±0.127
238331	Tigray	0.629	0.152	0.418	0.567	0.455	0.000	0.370±0.100
238336	Tigray	0.455	0.311	0.845	0.837	0.579	0.613	0.607±0.086
241769	Gamo Gofa	0.629	0.674	0.485	0.567	0.000	0.528	0.481±0.100
242123	Gonder	0.579	0.360	0.498	0.971	0.357	0.455	0.537±0.093
242125	Gonder	0.696	0.301	0.526	0.971	0.223	0.357	0.512±0.115
242131	Gonder	0.579	0.530	0.459	0.997	0.357	0.357	0.547±0.097
Overall		0.931	0.732	0.820	0.990	0.825	0.604	0.817±0.056

<sup>+</sup> GRHA= growth habit, ESHP=ear shape, GSHP=grain shape, GSUR=grain surface  
GCL=grain color, PPR=pericarp persistence.

**Appendix Table 4** Mean values of 15 morph-agronomic characters in 66 finger millet accessions.

Accession	PH <sup>+</sup>	DTH	DTM	GFD	PRT	CT	LENU	LEBL	LEBW	FL	FW	FNU	NGPSP	TGW	GYPPL
100055	84.1	110	164	54	9.33	5.93	10.07	38.0	1.23	5.73	5.27	9.93	6.90	2.33	15.48
100084	78.3	115	166	51	8.00	6.27	10.07	45.5	1.42	4.63	6.07	10.00	4.97	2.80	12.85
100094	80.7	113	162	49	11.67	4.47	8.67	31.5	1.08	5.07	4.80	7.33	6.73	2.27	11.55
100095	102.3	112	177	65	14.67	5.60	10.93	40.8	1.15	5.97	5.00	10.40	6.77	2.40	18.09
204747	81.7	102	173	71	10.33	3.93	6.13	37.0	1.07	9.53	3.80	9.00	5.90	1.93	11.69
208444	96.7	108	184	76	12.67	3.47	8.13	38.1	0.97	9.77	3.53	8.73	4.90	2.40	12.43
208448	64.3	127	187	60	9.33	6.87	10.80	38.4	1.17	4.87	4.67	9.20	5.44	1.53	6.29
208730	108.4	113	173	60	9.67	5.73	9.87	37.7	1.10	7.37	4.93	11.07	6.47	2.20	16.82
211474	69.7	96	169	73	10.33	6.00	9.87	36.1	1.25	4.80	6.00	8.53	5.50	2.73	13.75
213035	89.3	108	162	55	9.00	5.20	9.00	35.8	1.07	4.90	5.47	8.80	6.10	2.00	11.23
215841	100.0	103	169	66	12.33	4.73	7.53	39.6	0.97	9.47	4.13	9.53	4.78	2.40	13.85
215850	93.0	99	175	77	16.67	3.60	6.07	37.3	0.99	9.57	3.60	8.27	4.06	2.60	16.48
215867	94.7	100	165	65	10.67	3.80	7.80	39.5	1.01	8.90	3.27	10.67	5.23	2.27	10.07
215874	90.7	100	169	69	9.67	3.47	7.20	39.6	1.03	9.77	3.20	8.00	4.07	2.13	9.79
215877	95.3	104	172	67	13.67	4.13	7.53	38.9	1.02	10.77	3.60	8.60	4.73	2.33	10.67
215879	93.3	103	177	74	14.00	4.13	7.40	35.4	1.04	9.93	3.93	9.53	4.53	2.60	15.61
215883	95.1	103	164	61	9.67	4.60	6.80	36.0	1.03	9.67	3.73	9.87	4.44	2.20	13.83
215889	93.0	102	171	69	11.67	5.93	8.00	38.3	1.13	9.87	4.40	10.47	5.23	2.67	14.39
215896	99.7	102	169	67	10.67	4.47	7.87	40.2	1.08	9.90	4.33	9.53	5.10	2.40	14.59
215973	106.0	103	175	72	8.33	6.00	8.33	46.1	1.37	14.60	4.27	11.47	6.20	2.47	15.01
215977	88.1	99	171	72	12.67	3.60	8.27	34.1	1.00	8.57	3.60	8.60	4.17	2.33	14.11
216024	97.0	108	173	66	12.33	4.60	7.47	39.5	1.01	8.23	4.00	11.67	4.87	1.67	17.65
216025	103.3	109	176	67	9.67	4.93	9.07	37.4	1.10	9.07	4.00	12.00	4.97	1.73	15.24
216028	107.8	119	178	59	11.33	3.73	10.07	39.4	0.96	9.27	3.53	10.80	4.80	2.13	10.19
216041	116.3	126	192	66	11.67	4.13	9.00	38.3	0.99	9.67	3.33	7.93	4.18	2.20	5.39
216043	106.1	129	190	62	13.33	4.73	9.33	40.4	1.03	9.17	4.13	8.07	4.17	2.47	7.52
216045	110.1	129	191	62	8.00	4.20	9.20	45.9	1.09	7.63	4.30	7.73	4.20	2.07	8.96

**Appendix Table 4** (Cont'd).

Accession	PH <sup>+</sup>	DTH	DTM	GFD	PRT	CT	LENU	LEBL	LEBW	FL	FW	FNU	NGPSP	TGW	GYPPL
216051	116.7	126	195	69	8.67	4.27	9.60	40.9	1.05	7.77	4.73	7.67	4.23	2.13	7.85
216052	97.7	121	178	57	11.33	5.40	10.53	40.6	1.16	6.73	4.67	10.40	6.00	2.27	12.39
225895	85.0	102	164	62	11.67	4.07	6.53	39.6	1.01	10.83	4.27	8.07	4.57	2.33	11.65
229723	62.3	135	192	57	9.00	5.73	9.20	39.1	1.25	5.70	4.80	11.23	5.17	1.93	6.32
229726	80.7	101	176	75	14.00	4.07	8.00	37.1	1.07	9.20	3.87	8.13	4.37	2.10	10.63
Paadet	96.0	114	169	54	9.33	7.47	10.80	40.0	1.29	5.37	7.93	5.87	6.53	3.13	13.12
229728	82.7	101	166	65	11.67	4.47	7.33	42.7	1.07	10.13	3.77	8.13	5.18	2.60	12.29
230101	81.7	93	158	65	10.67	4.40	8.60	37.7	1.03	9.00	3.87	8.33	5.33	2.53	11.20
230117	79.3	94	157	63	15.67	4.67	6.47	36.5	1.01	9.73	3.80	8.07	6.27	2.67	21.21
230130	77.0	96	157	61	9.00	4.60	6.07	36.0	1.05	8.67	4.00	8.53	5.63	2.80	10.17
230136	68.7	97	157	60	17.33	4.80	7.13	32.4	1.13	7.90	4.63	9.20	6.20	2.80	21.09
230714	73.0	86	158	72	11.33	4.20	7.33	33.0	1.05	8.23	3.53	8.27	6.10	2.60	11.95
230722	76.3	94	158	63	11.00	4.20	7.07	36.6	0.96	9.43	3.60	7.53	5.47	2.73	11.49
230724	79.7	92	159	67	10.67	4.87	7.93	34.1	1.12	9.33	4.03	7.20	6.13	2.73	11.41
234178	84.7	97	158	61	9.67	5.20	8.87	36.9	1.12	9.37	3.80	9.27	6.50	2.40	14.24
234205	77.3	93	164	72	10.33	3.80	7.07	33.3	0.97	9.83	3.60	8.33	5.93	2.20	11.31
235138	83.0	112	188	76	15.67	5.00	7.40	41.0	1.12	9.67	3.67	8.53	5.17	2.07	12.73
235141	93.3	110	177	66	8.33	5.53	9.07	44.2	1.12	10.23	3.93	9.27	5.57	2.40	14.49
235700	62.1	84	143	59	9.33	5.13	7.40	32.2	1.17	3.67	5.13	8.00	7.20	2.13	12.24
235830	78.0	99	161	62	13.33	4.20	8.20	32.2	1.01	7.97	3.93	10.13	5.30	2.47	13.52
235838	77.0	103	169	66	9.33	4.53	8.33	34.1	1.05	7.97	4.07	7.53	6.27	2.87	7.95
235842	73.0	106	167	61	9.33	4.47	8.00	33.7	1.06	7.07	3.77	8.07	5.67	2.60	9.53
236447	75.3	128	200	72	8.33	5.40	9.80	41.2	1.25	8.03	3.47	10.40	3.93	1.87	4.87
236450	102.3	115	174	59	8.67	4.13	8.00	37.9	1.29	8.77	3.33	8.73	4.33	2.07	7.59
237447	77.0	90	158	67	8.67	3.93	7.27	34.1	1.00	8.80	3.20	9.53	5.73	2.67	10.43

**Appendix Table 4** (Cont.d).

Accession	PH <sup>+</sup>	DTH	DTM	GFD	PRT	CT	LENU	LEBL	LEBW	FL	FW	FNU	NGPSP	TGW	GYPPL
237449	78.7	99	147	48	9.33	4.13	6.27	33.1	1.01	7.80	3.73	7.93	5.17	2.87	11.69
237451	72.3	89	160	71	9.33	3.87	7.73	31.9	1.03	8.00	3.13	7.87	6.33	2.87	9.60
237452	75.7	91	157	66	15.33	4.60	7.47	35.4	1.01	8.47	4.13	7.93	4.93	2.80	15.77
237459	83.7	91	160	69	9.00	5.33	9.13	36.4	1.17	9.53	3.93	8.47	6.80	2.47	12.21
237462	73.0	93	158	65	12.33	3.93	7.00	39.7	1.05	8.67	3.80	7.67	6.30	2.40	14.63
237477	74.3	99	160	61	10.67	4.00	7.13	32.5	0.93	9.37	4.00	8.60	6.27	2.67	10.35
238319	82.7	94	147	54	10.00	5.27	8.13	38.3	1.16	9.73	4.53	9.00	6.10	2.60	16.43
238331	68.3	87	159	72	13.33	4.13	6.80	29.7	1.02	7.17	3.87	8.00	6.37	2.67	13.80
238336	61.3	90	159	69	11.67	3.73	6.33	27.3	1.05	8.00	4.07	8.20	5.80	2.67	12.05
241769	85.3	114	163	49	12.33	6.27	9.67	38.5	1.31	4.47	7.40	7.80	5.30	2.40	13.83
242123	73.3	118	171	53	13.00	4.13	7.60	34.7	1.05	8.27	3.67	7.73	4.97	2.47	10.07
242125	78.0	126	184	58	10.00	5.47	11.07	37.5	1.19	9.10	4.00	9.53	5.83	2.53	10.53
242131	88.3	102	176	74	15.67	3.67	7.33	38.1	1.03	9.97	3.73	8.87	4.87	2.20	15.12
Tadesse	93.0	119	177	58	10.33	7.87	10.80	41.1	1.39	5.27	9.13	7.33	5.97	3.20	15.04
Mean	86.0	105	169	64	11.15	4.75	8.26	37.4	1.09	8.33	4.26	8.87	5.44	2.41	12.37
SE	6.70	3.00	4.61	5.39	1.12	0.39	0.67	2.19	0.06	0.67	0.30	0.77	0.51	0.18	1.41
CV%	13.49	4.94	4.72	14.57	17.38	14.24	14.03	10.17	9.84	13.93	12.21	15.12	16.14	13.35	19.73
LSD0.05	18.73	8.39	12.90	15.08	3.13	1.09	1.80	6.14	0.17	1.87	0.84	2.16	1.42	0.52	3.94
LSD0.01	24.75	11.08	17.05	19.92	4.14	1.44	2.47	8.11	0.23	2.48	1.11	2.87	1.87	0.69	5.21

<sup>+</sup> PH=Plant height (cm), DTH=days to heading, DTM=days to maturity, GFD=grain filling duration, PRT=productive tillers/plant, CT=culm thickness (mm), LENU=leaf no./main tiller, LEBL=leaf blade length (cm), LEBW=leaf blade width (cm), FL=finger length (cm), FW=finger width (mm), FNU=finger no./main ear, NGPSP=no. of grains per spikelet, TGW=1,000-grain weight (g), GYPPL=grain yield per plant (g).

**Appendix Table 5** Principal components (PC) extracted from 15 variables of 66 finger millet accessions.

Variable <sup>+</sup>	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14
PH	0.140	-0.315	-0.288	-0.141	0.206	0.451	0.431	-0.259	-0.185	-0.261	0.207	-0.074	-0.224	-0.282
DTH	0.359	-0.25	0.135	-0.138	-0.197	0.139	-0.02	0.313	0.155	-0.195	-0.148	-0.236	0.202	0.016
DTM	0.236	-0.393	0.03	-0.181	-0.165	-0.287	0.146	0.169	0.051	-0.154	-0.193	-0.277	0.195	-0.004
GFD	-0.221	-0.236	-0.184	-0.062	0.046	-0.718	0.277	-0.264	-0.184	0.059	-0.066	-0.063	-0.023	-0.026
PRT	-0.205	-0.028	-0.363	-0.359	-0.583	-0.028	-0.083	0.223	0.237	0.032	0.202	0.117	-0.43	-0.047
FL	-0.237	-0.279	-0.262	0.035	0.457	-0.026	-0.047	0.321	0.28	-0.36	-0.006	0.366	0.066	0.368
FW	0.324	0.28	-0.143	-0.256	-0.066	0.004	-0.03	-0.307	-0.189	-0.244	-0.331	0.026	-0.238	0.607
CT	0.379	0.212	-0.172	0.026	0.05	-0.203	-0.054	0.06	0.062	-0.165	-0.351	0.504	-0.028	-0.567
LNU	0.409	-0.003	-0.002	0.001	-0.082	-0.054	0.447	0.188	-0.087	0.408	0.364	0.42	0.17	0.275
LEBL	0.279	-0.246	-0.272	-0.067	0.318	0.038	-0.272	-0.167	0.369	0.602	-0.154	-0.163	-0.17	0.021
LEBW	0.361	0.16	-0.112	0.063	0.159	-0.329	-0.353	-0.012	0.016	-0.286	0.656	-0.236	-0.038	-0.027
FNU	0.102	-0.191	-0.276	0.664	-0.152	0.018	-0.099	0.315	-0.415	0.047	-0.143	-0.106	-0.302	0.084
NGPSP	0.015	0.403	-0.068	0.307	0.05	-0.072	0.538	0.099	0.527	-0.076	-0.077	-0.324	-0.192	0.033
TGW	-0.083	0.322	-0.137	-0.423	0.374	-0.015	0.062	0.546	-0.371	0.173	-0.083	-0.255	-0.037	-0.088
GYPPL	-0.083	0.192	-0.654	0.072	-0.189	0.14	-0.035	-0.127	-0.01	0.024	-0.02	-0.123	0.66	0.004
Eigenvalues	4.587	3.582	1.691	1.232	1.021	0.825	0.557	0.438	0.361	0.215	0.172	0.149	0.103	0.069
Proportion	0.306	0.239	0.113	0.082	0.068	0.055	0.037	0.029	0.024	0.014	0.011	0.010	0.007	0.005
Cumulative	0.306	0.545	0.657	0.739	0.807	0.863	0.900	0.929	0.953	0.967	0.979	0.989	0.995	1.000

<sup>+</sup> PH=Plant height (cm), DTH=days to heading, DTM=days to maturity, GFD=grain filling duration, PRT=productive tillers/plant, CT=culm thickness (mm), LENU=leaf no./main tiller, LEBL=leaf blade length (cm), LEBW=leaf blade width (cm), FL=finger length (cm), FW=finger width (mm), FNU=finger no./main ear, NGPSP=no. of grains per spikelet, TGW=1,000-grain weight (g), GYPPL=grain yield per plant (g).

**Appendix Table 6** Simple matching similarity indices between pairs of 66 finger millet accessions.

Accession	1	2	3	4	5	6	7	8	9	10
1. 100055										
2. 100084	0.886									
3. 100094	0.821	0.870								
4. 100095	0.797	0.846	0.911							
5. 204747	0.780	0.829	0.878	0.935						
6. 208444	0.764	0.813	0.878	0.967	0.935					
7. 208448	0.821	0.854	0.805	0.829	0.846	0.797				
8. 208730	0.780	0.829	0.878	0.967	0.935	0.935	0.829			
9. 211474	0.805	0.870	0.886	0.943	0.911	0.911	0.870	0.943		
10. 213035	0.846	0.894	0.862	0.886	0.870	0.870	0.911	0.870	0.911	
11. 215841	0.797	0.813	0.829	0.854	0.805	0.821	0.780	0.837	0.846	0.837
12. 215850	0.797	0.878	0.829	0.854	0.821	0.837	0.829	0.854	0.846	0.902
13. 215867	0.821	0.886	0.886	0.911	0.862	0.878	0.854	0.878	0.886	0.911
14. 215874	0.650	0.618	0.667	0.626	0.610	0.593	0.618	0.610	0.585	0.593
15. 215877	0.813	0.829	0.797	0.821	0.772	0.805	0.748	0.789	0.813	0.805
16. 215879	0.813	0.846	0.829	0.854	0.854	0.854	0.813	0.854	0.829	0.870
17. 215883	0.829	0.846	0.846	0.886	0.854	0.854	0.813	0.902	0.878	0.837
18. 215889	0.805	0.837	0.837	0.878	0.829	0.862	0.789	0.846	0.870	0.862
19. 215896	0.837	0.886	0.886	0.911	0.894	0.894	0.837	0.878	0.902	0.911
20. 215973	0.805	0.870	0.902	0.911	0.878	0.894	0.805	0.878	0.870	0.862
21. 215977	0.772	0.854	0.837	0.846	0.797	0.813	0.805	0.829	0.837	0.829
22. 216024	0.821	0.870	0.821	0.846	0.813	0.813	0.821	0.813	0.854	0.862
23. 216025	0.829	0.894	0.829	0.821	0.805	0.789	0.829	0.821	0.813	0.870
24. 216028	0.821	0.854	0.837	0.878	0.846	0.846	0.837	0.862	0.886	0.894
25. 216041	0.813	0.846	0.846	0.854	0.837	0.821	0.846	0.854	0.829	0.886
26. 216043	0.846	0.862	0.846	0.837	0.805	0.805	0.813	0.821	0.829	0.870
27. 216045	0.813	0.829	0.813	0.837	0.821	0.805	0.829	0.837	0.862	0.854
28. 216051	0.821	0.821	0.821	0.846	0.829	0.813	0.821	0.846	0.821	0.829
29. 216052	0.805	0.821	0.805	0.846	0.846	0.813	0.870	0.862	0.837	0.878
30. 225895	0.870	0.886	0.854	0.862	0.846	0.829	0.821	0.862	0.870	0.894
31. 229723	0.854	0.870	0.821	0.829	0.829	0.797	0.870	0.829	0.854	0.911
32. 229726	0.797	0.829	0.846	0.870	0.854	0.837	0.813	0.886	0.862	0.870
33. Paadet	0.837	0.837	0.837	0.829	0.813	0.797	0.789	0.829	0.805	0.829

**Appendix Table 6** (Cont'd).

Accession	1	2	3	4	5	6	7	8	9	10
34. 229728	0.789	0.821	0.805	0.813	0.829	0.797	0.821	0.813	0.821	0.862
35. 230101	0.805	0.821	0.837	0.846	0.829	0.813	0.805	0.846	0.837	0.846
36. 230117	0.821	0.837	0.821	0.846	0.813	0.813	0.870	0.829	0.854	0.894
37. 230130	0.862	0.862	0.829	0.854	0.854	0.821	0.894	0.854	0.862	0.919
38. 230136	0.821	0.837	0.870	0.894	0.911	0.878	0.821	0.911	0.902	0.862
39. 230714	0.805	0.854	0.902	0.943	0.959	0.927	0.854	0.943	0.935	0.894
40. 230722	0.805	0.854	0.902	0.959	0.943	0.927	0.854	0.959	0.951	0.894
41. 230724	0.821	0.870	0.854	0.894	0.862	0.862	0.902	0.878	0.902	0.943
42. 234178	0.813	0.862	0.813	0.854	0.821	0.837	0.846	0.854	0.862	0.854
43. 234205	0.789	0.837	0.886	0.943	0.943	0.927	0.837	0.959	0.951	0.878
44. 235138	0.837	0.837	0.821	0.846	0.797	0.813	0.789	0.829	0.821	0.846
45. 235141	0.829	0.846	0.846	0.886	0.870	0.870	0.813	0.886	0.894	0.886
46. 235700	0.797	0.846	0.878	0.935	0.902	0.919	0.829	0.902	0.943	0.902
47. 235830	0.715	0.764	0.748	0.789	0.772	0.756	0.829	0.789	0.797	0.821
48. 235838	0.780	0.846	0.894	0.935	0.886	0.902	0.862	0.919	0.927	0.870
49. 235842	0.813	0.878	0.862	0.870	0.870	0.854	0.846	0.870	0.878	0.886
50. 236447	0.772	0.837	0.854	0.911	0.878	0.878	0.870	0.927	0.935	0.878
51. 236450	0.805	0.870	0.886	0.927	0.894	0.894	0.837	0.927	0.935	0.878
52. 237447	0.748	0.797	0.829	0.854	0.870	0.854	0.797	0.870	0.862	0.837
53. 237449	0.732	0.715	0.780	0.756	0.805	0.756	0.732	0.789	0.764	0.756
54. 237451	0.772	0.837	0.805	0.813	0.829	0.813	0.772	0.846	0.837	0.829
55. 237452	0.756	0.772	0.789	0.829	0.813	0.797	0.837	0.846	0.854	0.829
56. 237459	0.764	0.764	0.797	0.756	0.756	0.740	0.715	0.756	0.748	0.756
57. 237462	0.797	0.846	0.878	0.886	0.870	0.886	0.846	0.870	0.911	0.886
58. 237477	0.821	0.837	0.821	0.862	0.862	0.829	0.854	0.894	0.902	0.878
59. 238319	0.772	0.854	0.854	0.911	0.878	0.894	0.837	0.911	0.919	0.878
60. 238331	0.821	0.837	0.789	0.813	0.813	0.780	0.837	0.846	0.854	0.829
61. 238336	0.862	0.862	0.862	0.870	0.870	0.837	0.846	0.886	0.878	0.870
62. 241769	0.780	0.780	0.813	0.805	0.805	0.772	0.764	0.805	0.829	0.805
63. 242123	0.756	0.756	0.772	0.764	0.764	0.732	0.756	0.780	0.789	0.780
64. 242125	0.772	0.837	0.854	0.894	0.862	0.878	0.854	0.894	0.902	0.878
65. 242131	0.724	0.789	0.805	0.813	0.813	0.813	0.756	0.813	0.854	0.829
66. Tadesse	0.789	0.789	0.821	0.797	0.780	0.764	0.821	0.813	0.805	0.862

**Appendix Table 6** (Cont'd).

Accession	11	12	13	14	15	16	17	18	19	20
11. 215841										
12. 215850	0.870									
13. 215867	0.862	0.878								
14. 215874	0.659	0.610	0.634							
15. 215877	0.805	0.821	0.862	0.593						
16. 215879	0.821	0.854	0.862	0.642	0.805					
17. 215883	0.854	0.854	0.878	0.626	0.837	0.837				
18. 215889	0.911	0.878	0.870	0.618	0.846	0.862	0.878			
19. 215896	0.846	0.846	0.919	0.634	0.829	0.911	0.878	0.886		
20. 215973	0.846	0.862	0.902	0.634	0.829	0.862	0.878	0.886	0.919	
21. 215977	0.862	0.862	0.854	0.650	0.780	0.813	0.846	0.870	0.837	0.854
22. 216024	0.829	0.846	0.870	0.618	0.829	0.862	0.813	0.870	0.886	0.837
23. 216025	0.789	0.854	0.878	0.593	0.789	0.837	0.821	0.829	0.846	0.846
24. 216028	0.894	0.894	0.854	0.618	0.829	0.878	0.878	0.935	0.886	0.854
25. 216041	0.837	0.870	0.894	0.626	0.789	0.886	0.837	0.862	0.894	0.862
26. 216043	0.870	0.902	0.911	0.626	0.837	0.870	0.837	0.894	0.878	0.862
27. 216045	0.886	0.870	0.862	0.642	0.821	0.870	0.854	0.894	0.894	0.829
28. 216051	0.862	0.862	0.886	0.667	0.829	0.878	0.862	0.870	0.870	0.854
29. 216052	0.829	0.894	0.886	0.618	0.813	0.862	0.862	0.854	0.870	0.837
30. 225895	0.894	0.927	0.870	0.634	0.846	0.894	0.862	0.902	0.902	0.870
31. 229723	0.829	0.878	0.870	0.634	0.797	0.846	0.862	0.854	0.902	0.821
32. 229726	0.870	0.902	0.862	0.642	0.821	0.870	0.886	0.911	0.878	0.862
33. Paadet	0.813	0.846	0.870	0.650	0.829	0.846	0.878	0.870	0.854	0.837
34. 229728	0.829	0.846	0.837	0.650	0.797	0.862	0.846	0.886	0.854	0.854
35. 230101	0.846	0.846	0.821	0.699	0.780	0.878	0.846	0.870	0.870	0.854
36. 230117	0.862	0.878	0.886	0.618	0.813	0.813	0.846	0.886	0.837	0.837
37. 230130	0.837	0.870	0.894	0.642	0.821	0.870	0.870	0.846	0.878	0.829
38. 230136	0.829	0.862	0.870	0.650	0.829	0.878	0.894	0.870	0.902	0.870

**Appendix Table 6** (Cont'd).

Accession	11	12	13	14	15	16	17	18	19	20
39. 230714	0.829	0.862	0.886	0.618	0.797	0.878	0.878	0.870	0.919	0.902
40. 230722	0.846	0.862	0.886	0.618	0.797	0.862	0.894	0.870	0.902	0.886
41. 230724	0.862	0.878	0.935	0.618	0.829	0.862	0.846	0.870	0.902	0.870
42. 234178	0.870	0.870	0.862	0.642	0.821	0.837	0.870	0.894	0.846	0.846
43. 234205	0.829	0.846	0.870	0.602	0.780	0.862	0.894	0.870	0.902	0.886
44. 235138	0.846	0.862	0.886	0.618	0.797	0.829	0.797	0.837	0.870	0.837
45. 235141	0.870	0.886	0.846	0.626	0.805	0.870	0.886	0.911	0.894	0.862
46. 235700	0.870	0.837	0.878	0.593	0.789	0.854	0.837	0.894	0.927	0.878
47. 235830	0.837	0.789	0.797	0.642	0.707	0.772	0.756	0.780	0.780	0.780
48. 235838	0.837	0.854	0.894	0.593	0.789	0.805	0.854	0.846	0.862	0.878
49. 235842	0.837	0.837	0.846	0.610	0.756	0.854	0.837	0.846	0.878	0.862
50. 236447	0.813	0.846	0.870	0.585	0.764	0.797	0.862	0.837	0.854	0.837
51. 236450	0.862	0.862	0.886	0.602	0.813	0.829	0.878	0.902	0.854	0.870
52. 237447	0.789	0.821	0.813	0.593	0.724	0.837	0.821	0.797	0.829	0.829
53. 237449	0.772	0.772	0.732	0.659	0.659	0.772	0.756	0.748	0.732	0.748
54. 237451	0.780	0.846	0.805	0.618	0.732	0.829	0.813	0.805	0.837	0.837
55. 237452	0.797	0.813	0.789	0.667	0.732	0.797	0.829	0.821	0.789	0.789
56. 237459	0.805	0.756	0.764	0.707	0.691	0.789	0.756	0.764	0.780	0.780
57. 237462	0.854	0.837	0.862	0.642	0.789	0.821	0.837	0.862	0.846	0.846
58. 237477	0.878	0.878	0.854	0.634	0.813	0.878	0.862	0.870	0.870	0.821
59. 238319	0.829	0.862	0.854	0.585	0.764	0.846	0.862	0.837	0.854	0.837
60. 238331	0.829	0.829	0.821	0.650	0.797	0.846	0.846	0.821	0.821	0.789
61. 238336	0.854	0.854	0.846	0.642	0.805	0.870	0.886	0.862	0.862	0.846
62. 241769	0.772	0.756	0.780	0.626	0.691	0.756	0.789	0.748	0.813	0.797
63. 242123	0.780	0.764	0.756	0.650	0.683	0.748	0.764	0.756	0.756	0.756
64. 242125	0.829	0.829	0.854	0.618	0.764	0.829	0.846	0.854	0.837	0.837
65. 242131	0.813	0.764	0.772	0.618	0.715	0.732	0.764	0.805	0.772	0.756
66. Tadesse	0.780	0.829	0.821	0.618	0.732	0.780	0.813	0.805	0.805	0.772

**Appendix Table 6** (Cont'd).

Accessions	21	22	23	24	25	26	27	28	29	30
21. 215977										
22. 216024	0.854									
23. 216025	0.829	0.846								
24. 216028	0.886	0.902	0.829							
25. 216041	0.846	0.878	0.870	0.911						
26. 216043	0.829	0.878	0.870	0.894	0.935					
27. 216045	0.846	0.911	0.821	0.927	0.886	0.902				
28. 216051	0.805	0.854	0.846	0.870	0.911	0.927	0.894			
29. 216052	0.789	0.837	0.846	0.870	0.894	0.911	0.894	0.935		
30. 225895	0.854	0.886	0.846	0.935	0.894	0.927	0.943	0.886	0.902	
31. 229723	0.805	0.854	0.878	0.886	0.878	0.878	0.911	0.870	0.902	0.902
32. 229726	0.862	0.862	0.837	0.927	0.886	0.902	0.919	0.911	0.927	0.927
33. Paadet	0.837	0.837	0.862	0.870	0.878	0.894	0.862	0.902	0.870	0.870
34. 229728	0.821	0.837	0.846	0.902	0.878	0.878	0.878	0.870	0.854	0.870
35. 230101	0.805	0.854	0.846	0.902	0.878	0.878	0.911	0.902	0.870	0.886
36. 230117	0.837	0.854	0.878	0.886	0.878	0.894	0.878	0.886	0.886	0.854
37. 230130	0.797	0.846	0.854	0.894	0.886	0.886	0.886	0.878	0.911	0.894
38. 230136	0.805	0.837	0.813	0.886	0.862	0.862	0.878	0.870	0.870	0.902
39. 230714	0.837	0.854	0.829	0.886	0.878	0.846	0.862	0.854	0.854	0.886
40. 230722	0.854	0.854	0.829	0.902	0.878	0.846	0.862	0.854	0.854	0.886
41. 230724	0.837	0.886	0.894	0.886	0.894	0.878	0.894	0.886	0.902	0.886
42. 234178	0.862	0.862	0.837	0.894	0.837	0.837	0.870	0.846	0.846	0.878
43. 234205	0.837	0.837	0.813	0.886	0.862	0.829	0.846	0.837	0.837	0.870

**Appendix Table 6** (Cont'd).

Accession	21	22	23	24	25	26	27	28	29	30
44. 235138	0.837	0.870	0.862	0.837	0.862	0.878	0.878	0.854	0.821	0.886
45. 235141	0.862	0.862	0.821	0.927	0.854	0.854	0.870	0.846	0.846	0.911
46. 235700	0.829	0.862	0.789	0.894	0.854	0.854	0.854	0.829	0.829	0.878
47. 235830	0.748	0.797	0.805	0.797	0.789	0.772	0.821	0.797	0.813	0.797
48. 235838	0.829	0.829	0.854	0.846	0.821	0.837	0.837	0.829	0.862	0.846
49. 235842	0.862	0.846	0.854	0.878	0.837	0.821	0.837	0.813	0.829	0.862
50. 236447	0.821	0.821	0.829	0.854	0.829	0.813	0.829	0.821	0.854	0.837
51. 236450	0.870	0.837	0.846	0.886	0.846	0.829	0.846	0.837	0.837	0.870
52. 237447	0.829	0.813	0.821	0.846	0.837	0.805	0.821	0.797	0.780	0.829
53. 237449	0.732	0.715	0.756	0.780	0.805	0.805	0.772	0.813	0.797	0.780
54. 237451	0.821	0.805	0.829	0.821	0.813	0.813	0.846	0.772	0.789	0.854
55. 237452	0.789	0.805	0.813	0.870	0.813	0.797	0.846	0.805	0.821	0.821
56. 237459	0.732	0.732	0.756	0.764	0.789	0.805	0.772	0.797	0.764	0.797
57. 237462	0.862	0.846	0.805	0.878	0.837	0.821	0.854	0.829	0.797	0.846
58. 237477	0.821	0.886	0.813	0.919	0.878	0.878	0.943	0.886	0.902	0.935
59. 238319	0.854	0.854	0.780	0.886	0.846	0.813	0.829	0.805	0.805	0.854
60. 238331	0.772	0.821	0.813	0.854	0.829	0.829	0.878	0.837	0.854	0.870
61. 238336	0.797	0.829	0.854	0.878	0.854	0.854	0.886	0.862	0.878	0.894
62. 241769	0.699	0.748	0.772	0.764	0.756	0.772	0.756	0.764	0.780	0.780
63. 242123	0.691	0.772	0.764	0.772	0.748	0.797	0.797	0.756	0.805	0.789
64. 242125	0.854	0.854	0.813	0.886	0.862	0.829	0.846	0.805	0.805	0.837
65. 242131	0.805	0.756	0.732	0.805	0.764	0.748	0.748	0.707	0.707	0.772
66. Tadesse	0.789	0.756	0.797	0.821	0.813	0.829	0.813	0.805	0.854	0.821

**Appendix Table 6** (Cont'd).

Accession	31	32	33	34	35	36	37	38	39
31. 229723									
32. 229726	0.878								
33. Paadet	0.870	0.911							
34. 229728	0.870	0.894	0.870						
35. 230101	0.886	0.927	0.854	0.919					
36. 230117	0.870	0.878	0.854	0.886	0.870				
37. 230130	0.911	0.886	0.894	0.894	0.878	0.911			
38. 230136	0.886	0.894	0.870	0.886	0.870	0.837	0.894		
39. 230714	0.870	0.894	0.854	0.870	0.870	0.837	0.878	0.951	
40. 230722	0.870	0.894	0.854	0.854	0.870	0.837	0.878	0.935	0.984
41. 230724	0.902	0.878	0.854	0.870	0.870	0.935	0.943	0.870	0.886
42. 234178	0.862	0.854	0.829	0.862	0.829	0.878	0.870	0.894	0.862
43. 234205	0.854	0.878	0.837	0.854	0.854	0.821	0.862	0.951	0.984
44. 235138	0.837	0.846	0.854	0.789	0.821	0.837	0.829	0.821	0.837
45. 235141	0.878	0.886	0.862	0.862	0.862	0.862	0.870	0.927	0.911
46. 235700	0.846	0.870	0.813	0.846	0.846	0.829	0.854	0.894	0.943
47. 235830	0.797	0.821	0.748	0.797	0.846	0.829	0.821	0.764	0.797
48. 235838	0.846	0.854	0.813	0.813	0.829	0.846	0.854	0.862	0.911
49. 235842	0.846	0.854	0.813	0.862	0.862	0.862	0.886	0.862	0.894
50. 236447	0.870	0.862	0.821	0.821	0.821	0.821	0.846	0.886	0.919
51. 236450	0.854	0.878	0.837	0.837	0.837	0.854	0.846	0.886	0.935
52. 237447	0.797	0.837	0.813	0.829	0.846	0.797	0.821	0.862	0.894
53. 237449	0.764	0.805	0.764	0.813	0.846	0.797	0.772	0.797	0.797
54. 237451	0.821	0.846	0.789	0.854	0.870	0.789	0.813	0.837	0.870
55. 237452	0.854	0.846	0.789	0.854	0.886	0.854	0.846	0.854	0.854
56. 237459	0.780	0.789	0.780	0.797	0.846	0.780	0.805	0.797	0.780
57. 237462	0.829	0.837	0.797	0.846	0.846	0.862	0.854	0.862	0.894
58. 237477	0.902	0.911	0.854	0.870	0.886	0.870	0.911	0.919	0.902
59. 238319	0.805	0.846	0.805	0.821	0.821	0.821	0.862	0.886	0.919
60. 238331	0.837	0.846	0.837	0.837	0.837	0.837	0.894	0.854	0.837
61. 238336	0.894	0.886	0.862	0.862	0.894	0.862	0.886	0.878	0.894
62. 241769	0.797	0.789	0.748	0.748	0.813	0.780	0.805	0.797	0.813
63. 242123	0.789	0.813	0.740	0.789	0.854	0.805	0.813	0.772	0.772
64. 242125	0.821	0.846	0.805	0.854	0.837	0.837	0.846	0.870	0.902
65. 242131	0.756	0.780	0.756	0.772	0.740	0.756	0.748	0.805	0.821
66. Tadesse	0.837	0.878	0.870	0.837	0.821	0.854	0.878	0.805	0.805

**Appendix Table 6** (Cont'd).

Accession	40	41	42	43	44	45	46	47	48
40. 230722									
41. 230724	0.886								
42. 234178	0.862	0.878							
43. 234205	0.984	0.870	0.878						
44. 235138	0.837	0.870	0.813	0.821					
45. 235141	0.911	0.862	0.919	0.927	0.846				
46. 235700	0.943	0.878	0.854	0.943	0.829	0.902			
47. 235830	0.797	0.862	0.805	0.780	0.764	0.772	0.805		
48. 235838	0.927	0.894	0.854	0.911	0.829	0.837	0.902	0.805	
49. 235842	0.894	0.894	0.870	0.894	0.829	0.902	0.886	0.789	0.886
50. 236447	0.935	0.870	0.878	0.935	0.805	0.862	0.911	0.797	0.959
51. 236450	0.951	0.886	0.878	0.935	0.821	0.878	0.911	0.797	0.927
52. 237447	0.894	0.829	0.805	0.894	0.813	0.854	0.837	0.772	0.837
53. 237449	0.797	0.748	0.740	0.797	0.715	0.789	0.756	0.772	0.756
54. 237451	0.854	0.805	0.813	0.870	0.837	0.829	0.829	0.764	0.829
55. 237452	0.870	0.837	0.878	0.870	0.756	0.862	0.813	0.846	0.829
56. 237459	0.764	0.780	0.789	0.780	0.748	0.805	0.789	0.821	0.740
57. 237462	0.911	0.894	0.870	0.894	0.797	0.854	0.886	0.805	0.870
58. 237477	0.902	0.902	0.911	0.902	0.837	0.911	0.878	0.829	0.862
59. 238319	0.935	0.854	0.878	0.935	0.805	0.894	0.911	0.780	0.878
60. 238331	0.837	0.854	0.878	0.837	0.789	0.846	0.813	0.829	0.829
61. 238336	0.894	0.862	0.854	0.878	0.813	0.886	0.854	0.821	0.870
62. 241769	0.813	0.797	0.724	0.813	0.732	0.789	0.821	0.821	0.805
63. 242123	0.772	0.789	0.748	0.772	0.724	0.764	0.780	0.846	0.797
64. 242125	0.919	0.854	0.911	0.919	0.789	0.862	0.894	0.797	0.894
65. 242131	0.837	0.772	0.797	0.837	0.740	0.813	0.846	0.748	0.780
66. Tadesse	0.805	0.854	0.780	0.789	0.772	0.813	0.797	0.764	0.797

**Appendix Table 6** (Cont'd).

Accession	49	50	51	52	53	54	55	56	57
49. 235842									
50. 236447	0.862								
51. 236450	0.878	0.935							
52. 237447	0.870	0.846	0.846						
53. 237449	0.772	0.764	0.780	0.837					
54. 237451	0.862	0.837	0.837	0.862	0.797				
55. 237452	0.813	0.854	0.837	0.846	0.846	0.821			
56. 237459	0.805	0.732	0.748	0.789	0.870	0.780	0.829		
57. 237462	0.870	0.862	0.911	0.854	0.789	0.813	0.846	0.756	
58. 237477	0.862	0.886	0.886	0.862	0.813	0.837	0.886	0.813	0.862
59. 238319	0.878	0.902	0.902	0.878	0.780	0.854	0.837	0.764	0.894
60. 238331	0.813	0.837	0.837	0.813	0.780	0.805	0.854	0.797	0.813
61. 238336	0.854	0.862	0.894	0.837	0.821	0.829	0.862	0.821	0.837
62. 241769	0.789	0.797	0.780	0.789	0.821	0.764	0.813	0.854	0.756
63. 242123	0.780	0.772	0.756	0.780	0.846	0.805	0.854	0.878	0.748
64. 242125	0.862	0.919	0.902	0.878	0.797	0.854	0.886	0.764	0.911
65. 242131	0.797	0.821	0.837	0.829	0.780	0.772	0.789	0.780	0.862
66. Tadesse	0.813	0.805	0.789	0.764	0.764	0.756	0.789	0.764	0.797

**Appendix Table 6** (Cont'd).

Accession	58	59	60	61	62	63	64	65
58. 237477								
59. 238319	0.886							
60. 238331	0.935	0.837						
61. 238336	0.927	0.846	0.927					
62. 241769	0.797	0.780	0.764	0.821				
63. 242123	0.821	0.756	0.805	0.846	0.894			
64. 242125	0.886	0.935	0.854	0.846	0.748	0.772		
65. 242131	0.789	0.837	0.740	0.764	0.748	0.740	0.870	
66. Tadesse	0.821	0.789	0.789	0.829	0.764	0.789	0.772	0.756