150

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Comparative Morphological and Biochemical studies of Temporal Genetic Differentiation of *in-situ* (on-farm) Land races and *ex-situ* Accessions of Sorghum (*Sorghum bicolor* (L.) Moench) from North Shoa and South Welo

MEDHANIE ASSMELASH

Addis Ababa, June 2000

Comparative Morphological and Biochemical studies of Temporal Genetic Differentiation of *in-situ* (on-farm) Land races and *ex-situ* Accessions of Sorghum (*Sorghum bicolor* (L.) Moench) from North Shoa and South Welo

A Thesis submitted to the

School of Graduate Studies,

Addis Ababa University

In partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (*Applied Genetics*).

By

MEDHANIE ASSMELASH

Addis Ababa, June 2000.

This work is dedicated to My Parents,

Mom, Dad and brother,

who are currently under siege.

Acknowledgements

First and foremost, I would like to acknowledge my advisor, Prof. Endashaw Bekele for all the comments and support he had offered me.

I would like to extend my acknowledgement to the Department of Biology.

I also owe IPGRI (International Plant Genetic Resources Institute) and Dr. Awgechew Teshome, an inexpressible gratitude for offering me the chance to participate in their project. Without their assistance and financial support, I would not have been able to finish the thesis work.

I would like to extend my gratitude to Dr. Zemede Asfaw, for coordinating the financial support and supportive comments from the initial stage of this work.

Institute of Biodiversity Conservation and Research deserves an equivalent acknowledgement for their help, especially during the fieldwork.

The National Soil Laboratory (NSL), Ethiopian Agricultural Research Organization (EARO), also deserves acknowledgement for providing me the green house facility.

Friends, relatives and families, who have helped me directly or indirectly during the course of the work, are always remembered for their support and assistance.

Table of Contents

List of Tables

List of Figures

List of Appendices

ABSTRACT

*Morphological and biochemical (isozyme) study was conducted on sorghum (*Sorghum bicolor *L. Moench) in-situ (on-farm) land races and ex-situ accessions obtained from North Showa and South Welo and from the gene bank, respectively. Plants were sown and grown under controlled green house condition and the characters to be studied were scored at the appropriate time. On most of the quantitative characters scored, it was found that there is a significant difference in the means of each character scored, both at $P < 0.05$ and $P < 0.01$ significance levels. This excludes mean number of leaves per given plant, which is the only character for which no variation was observed. Distances between the different land races and accessions were calculated using the Euclidean distance measurement. Based on this distance, dendrograms were constructed for both the ex-situ accessions and in-situ land races. Biochemical study was undertaken for two enzyme systems - α and β-Esterase (EST) and acid phosphatase (ACP). There was no variation observed for these two enzyme systems and all the land races and accessions appear to have the same banding pattern.*

Key words: *in-situ, ex-situ, Sorghum bicolor*, land races, accessions, isozymes.

1.INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench), a cereal crop which is cultivated for its grain (Doggett, 1965; Purseglove, 1972; Hodgkin *et al.*, 1985) ranks fourth in cereal production, cultivation and utilization in the world following wheat, maize and rice (Doggett, 1988; Doggett, 1991). In Ethiopia, sorghum is the leading cereal in coverage (Doggett, 1991).

The presence of high diversity in sorghum has been noted by many authorities (Harlan, 1969; Purseglove, 1972). Vavilov (1951) has identified Ethiopia as the center of origin and domestication for sorghum while Harlan (1969) has noted Ethiopia as center of diversity.

Variability in cultivated land races or varieties gives extra advantage for different type of selection pressures confronted in a given agricultural ecosystem (Allard and Adams, 1969; Allard, 1988). The presence of land races (varieties) in multiple morphological forms (morphotypes) is due to the expression of different alleles (Lewontin, 1974), having different frequencies (Wright, 1965), under different ecological conditions. Such variation will lead to diversification of the species, ultimately leading to speciation, the basis for diversity of life (Barbault and Sastrapradja, 1995; Doggett and Prasada Rao, 1995).

The diversity in sorghum, has thus to be assessed and maintained, to keep an ecological balance (Lewontin, 1974) and to provide genetic resource for some breeding programs. Generally the maintenance of diversity can be done either by:

i. Maintaining the land races at their original farm site (ecological niche), i.e. *in-situ* conservation (on-farm) conservation, or

ii. Seeds, tissues, embryos, pollen of this crops can be kept under artificial conditions like field gene banks, temperature controlled gene banks, cryo-preservation and tissue culture rooms. i.e. *ex-situ* conservation (Frankel, 1978; Frankel, 1984; Frankel and Brown, 1984).

Prior to this, it is very important that the diversity should be assessed and quantified so that one can know areas of high diversity (hot spots) (Frankel, 1984), and thus either of the above genetic conservation schemes can be implemented. This can be done either by studying the quantitative characters of the different land races (Harlan, 1969; Falconer, 1965; Allard, 1988) or by going into the biochemical or molecular level and studying the different banding patterns either using isozymes (Lewontin, 1974), RAPDS (Randomly Amplified Polymorphic DNAs), RFLPS (Restriction Fragment Length Polymorphism) (May, 1992) or AFLPs (Arbitrarily amplified Fragment Length Polymorphism) (Hoelzel and Green, 1992; May, 1992).

Many diversity assessment works that have been done using morphological characterization methods include those of Tadesse Ebba (1978), Endashaw Bekele (1984) and Endashaw Bekele (1996), and in addition, works in strengthening and maintaining the diversity of sorghums in their natural ecosystem (Harlan and deWet, 1972; Rao and Rao, 1995; Awgechew Teshome *et al.*, 1999) have been promoted. Recent works done on morphological characterization of sorghum land races from Ethiopia include that of Amsalu Ayana and Endashaw Bekele (1999).

Biochemical studies have been conducted on sorghum to assess its diversity (Cardy *et al.*, 1983; Morden *et al.*, 1983; Morden *et al.*, 1987). However, in all of the above mentioned

studies comparative study on *ex-situ* and *in-situ* has not been treated. Thus the present study has been initiated with the following objectives.

2. OBJECTIVES

The present study considers the following as its **main objectives:**

- To study the diversity of both *in-situ* (on-farm) land races and *ex-situ* collections (from base collections in the gene bank) at morphological and (isozymes) levels for two selected enzyme systems.

The specific objectives include:

1. Identification of areas of high diversity.
2. Assessment of the difference in the diversity of the *ex-situ* accessions and *in-situ* (on-farm) land races collected from the same areas.

3. LITERATURE REVIEW

3.1. Taxonomy

Sorghum includes a large number of widely cultivated crops and grasses, known under a confusing variety of common and scientific names (Purseglove, 1972; Hodgkin *et al.*, 1985; Doggett, 1988). The classification followed is based on Dogett (1965). Sorghum belongs to the family Gramineae, tribe Andropogoneae, sub tribe Sorghastrae. The sub tribe has two genera: *Cleistachne* Benth., with four species in south Africa and India, and *Sorghum*, which has a wide distribution throughout the warmer region of the world (Purseglove, 1972). An examination on the phylogenetic tree indicates that *Sorghum* has got 21 cultivated 'snowdenian species', which could be grouped into three series consisting of 14, 6, and 1 'species' respectively (Rao and House. 1972). Several similarities rather than discrepancies wee observed between these three series and the Snowden's classification. For example, the first series corresponded to Snowden's series *sativa* composed of cultivated species and the second series to *spontanea* of Snowden embracing the wild species. The third series consisted of a simple species *S.aethiopicum* which appeared to be an intermediate species between the two series. These three series were again divided into subseries using the same method. The first series consisted of six sub-species, the second 3 sub-species and the third had one sub-species as follows:

Series I:

Sub-series 1. –*S. dochna, S.mellitum, S.conspicuum*

2.–*S.nervosum, S.caffrorum, S.milliforme, S.caudatum*

3.-*S.cernuum, S.durra*

4. -*S.nigricans, S.melakeucum*

5. –*S.bicolor, S.roxburghii*

6. -*S.niloticum*

Series II:

Sub-series 1. –*S.vigatum, S.lanceolatum, S.verticilliforum*

2. -*S.sudanense, S.arundinaceum*

3. -*S.drummondii*

Series III:

Sub-series 1. – *S.aethiopicum*

As to the floral behavior, the antithesis in sorghum starts from the top of the panicle and extends downwards gradually taking about a week for complete flowering (Berhane Gebrekidan, 1981). Sessile spikelets open first and then pedicellates. Cultivated sorghum is known to be a **protogynous** crop.

Sorghum is generally 95 % self-pollinated in the field. It has been reported that they cross readily with other varieties of sorghum or wild relatives (Berhane Gebrekidan, 1981; Berhane Gebrekidan and Abraham Menkir, 1979).

3.2. Cultivation and Cultural Practices

Sorghum is the fourth most important world cereal, following wheat, rice and maize (Purseglove, 1972). According to Purseglove (1972), the world acreage and production of sorghum indicates the cosmopolitan nature of this crop in different countries of the world. In Ethiopia, sorghum is a leading cereal both in production and area coverage (Doggett, 1991), and is found all over the country in different agro-ecological regions, showing the wide adaptivity of the crop to different environmental conditions.

Sorghum is a very sturdy crop. It can withstand a wide range of temperature varying from 15.5 °C to 40.5 °C, with a rainfall variation of 350 – 1500mm annually (Purseglove, 1972).

Sorghum is a short day plant; flowering and grain formation starts when day length shortens during winter (Dogett, 1988). In areas having annual rainfall exceeding 1000mm, people prefer to cultivate maize in uplands which takes less time to mature and yields more (Doggett, 1988).

Sorghum can be grown in a wide range of soil types, clay loam soil rich in humus to black cotton soil (pH 7 – 8). Its rate of planting depends on the variety grown the use and location. Generally, a planting distance of 40 – 42 inches is recommended (Hodgkin *et al.*, 1985).

As far as diseases are concerned, the most important diseases of sorghum are the loose and covered kernel smuts, head smut, and *Pythium* root rot (Agrios, 1987). Above all, the most important pest (weed) known in sorghum cultivation is striga (*Striga* Spp.), which is an annual flowering plant, which attacks the host by sending hausteria to the root part and absorbing the processed food from the host plant.

Another important feature pertinent to sorghum is their capability of producing sufficient prussic (hydrocyanic) acid to cause the death of live stock when pastured under certain conditions (Purseglove, 1972).

3.3. Diversity and genetic differentiation of sorghum

Cultivated plant species have been so drastically changed in comparison to their wild original ancestors (deWet *et al.*, 1971; Mulugeta Negassa, 1986), that they are no longer viable in nature but totally depend on humans for their survival (Blixt, 1988).

Sorghum, as a cultivated plant, fulfils such temporal differentiation of crops from its wild progenitors, due to disruptive or diversifying selection, thereby resulting in *morphotypes* (or varieties) with different morphological and biochemical features (Allard, 1988). According to Rao and Rao (1995), sorghum is one of the very diversified plant species, with the highest diversity in North of latitude 10°S and West of longitude 25°E. They have further summarized that sorghum can be sub divided into Milo, Kafir, Guinea, Caudatum, Dura and Bicolor. But with development of new varieties from crosses between types (Berhane Gebrekidan, 1981), this classification tends to breakdown. As a result, there would seem no reason why sorghum varieties can not be identified by their variety name alone. According to Rao and Rao (1995), such varieties include:

i. White grain sorghums: white Kafir, white Dura and other white grain, which constitute maximum 10 % of the others.

ii. Yellow grain sorghums: encompass yellow Milo and other yellow grain sorghums.

iii. Red grain sorghums: red Kafir and other Red grain sorghums.

iv. Brown grain sorghums

v. Mixed grain sorghums

Plant genetic resources need to be maintained under conditions that allow the formation of more diversity (Frankel, 1984). Frankel (1984) considers that such improvement of diversity can be achieved by:

- On-farm (*in-situ*) conservation strategies (Allard, 1988; Rao and Rao, 1995), where by the cultivars (cultivated varieties) are maintained in their agro-ecosystem (farm site), whereby farmers use and implement the different land races in hand (Awgechew Teshome *et al.*, 1999).

On the other hand there is a system of conservation as:

- Off-farm (*ex-situ*) conservation strategies (Barbault and Sastrapradja, 1995), whereby the cultivars are handled in gene banks through:
    a. Seed preservation
    b. Cryo-preservation
    c. Tissue culture, pollen, embryo conservation
    d. Others (botanical garden, field gene banks, arborations...)

The task of the gene bank is to collect, preserve, and make available for utilization certain genetic variation (Blixt, 1988). Most gene banks have been and still are in a stage of development where the emphasis is on rescuing threatened materials as well as to find the appropriate methods for storage and preservation and for handling the information.

3.3.1. Characterization of diversity based on biochemical data

Two measures of population variability can be made (Nei, 1973; Brown, 1983):

1. Proportion of loci polymorphic in the population.
2. Average proportion of loci heterozygous in an individual.

3.3.2. Isozyme studies in different organisms

Isozyme analysis has been applied in a number of organisms (Anderson, 1968; El-Kassaby, 1981; Cardy *et al.*, 1983; Weeden and Gotlieb, 1971). Isozyme analysis has been applied in horticultural crops (Wendel and Parks, 1971) like peach (Arulsekar *et al.*, 1986), wild banana (Jarret and Litz, 1986), and cabbage (Weeden and Robinson, 1986). Trees were also characterized for their isozyme variability – Pine (Yazdani and Rudin, 1982), and spruce trees (Cheliak and Pitel, 1984). Most of the isozyme works, since their first application in characterization and sub cellular localization by Shannon (1968), are on cultivated crops – maize (Ott and Scandalios, 1978; Gastony *et al.*, 1980; Kahler, 1983), soya beans (Kiang, 1981; Farinelli *et al.*, 1983), radish (Ellstrand and Marshall, 1985), tomato (Tanksley and Rick, 1980), Compositae (Warwick and Gotlieb, 1985). Isozymes were applied not only on plants but were also applied on other organisms like *Drosophila* (Prakash *et al.*, 1969), and human (Fildes and Harris, 1966), which was targeted at observing the genetic determination of adenylate kinase.

Well-detailed work on sorghum isozymes was that of Morden *et al.* (1988). They have on aconitase (ACO), to study its gene number (Nei, 1965), tissue specificity, subcellular localization, allelic variation, and genetic control. They have also worked on chloroplast extract of sorghum for aspartate amino transferase (AAT), 6-Phosphoglucose dehdrogenase (PGD), phosphoglucose isomerase (PGI), and triosphosphate isomerase (TPI) as markers. Similar work was also done by Doebley *et al.* (1986), to study the gene that modifies mitochondrial maltase dehdrogenase isozymes.

4. MATERIALS AND METHODS

The present study can broadly be categorized into three major activities. The activities and methods applied in each component can be summarized as follows.

4.1. Sample acquisition

The study sites include North Shoa and South Welo regions. The towns and villages included are given below:

Table 1. Sites, their altitude and distance form Addis Ababa.

Site	Distance from Addis Ababa	Altitude(a.s.l.)
1. Merewa Aderie (Efeson)	254 Km	1400 m
2. Layignaw Ataye	267 Km	1500 m
3. Korie Meida	275 Km	1500 m
4. Harbu Addis Mender 01	345 Km	1500 m
5. Fontenina	350 Km	1600 m
6. Bati	395 Km	1450 m
7. Hayk	431 Km	1900 m

These areas (Table 1) were selected for this study because it has been reported that they are areas of high sorghum diversity in Ethiopia (Awgechew Teshome *et al.*, 1999). Generally:

(i). Selection of appropriate sample sizes (number of farmers) from each area to have a representative farm size (after obtaining the list of farmers, samples were chosen randomly) (33 farmer were chosen from a total of 300 households).

(ii). For each selected farmer:

a. Transect lines were constructed, which are 10 m apart and plants after each 5 m per transect were selected and scored (Awgechew Teshome *et al.*, 1999), their frequencies were calculated later. This samples a total number of 200 plants per hectare (ha).
b. Panicles were randomly chosen and taken back for further morphological and biochemical study.
c. Ultimately, each farmer whose field had been sampled was interviewed for the selection priority and criteria used for the land races found in his / her farm (this information was not used for analysis).

(iii). Panicles were taken (after cutting using a cutter at about 15 – 20 cm below the neck of the peduncle) and labeled for the land race name, place of collection, altitude, and collector. Five panicles per land race were taken for study.

4.2. Morphological Study

The morphological aspect of this thesis work was carried out under green house condition in the National Soil Laboratory, Ethiopian Agricultural Research Organization (EARO).

According to IBPGR/ICRISAT (1993), the number of plants used for the characterization purpose of land races (both for their qualitative and quantitative characters) is 5, and thus, this number of plants per land race was adopted for this study.

Sorghum is not a typical greenhouse plant (Rao and Rao, 1995), and this is due to its length and difficulty of getting a medium to support this height. Sorghum is known to have good root extension and depth (Dogett, 1988), which requires large space and size of pot one can get. Thus, the pot size that was selected for this study is of a radius of 150 mm x 300 mm length, which is feasible enough to accommodate the root system and support the plant (green house space availability was also put into consideration). Each pot was labeled for the type of land race, plant number, planting date.

Sorghum can be grown in a wide range of soil types, sandy loam to clay loam (Purseglove, 1972). The soil chosen for this study was brought from a well-known Sorghum growing area, Arsi Negelle, in the Oromia Regional State. This area was chosen due to the additional factor of transport accessibility and ease of soil acquisition. The type of soil is clay loam, with slight sandy character. Fertilizer application of urea at the rate of 45 Kg ha^{-1} was used, mixing it thoroughly with the soil collected using hand shovel.

Pots were filled with equal weight of soil (4 .0 Kg), and each pot had an equal probability of attaining any position in the bench, which fulfils the definition of Completely Randomized Design (CRD). i.e. CRD was applied as a design for this greenhouse work, after obtaining computer generated random numbers.

Seeds attained from *in-situ* (17 land races) and *ex-situ* (13 accessions) were brought for planting (given in the results part). Four seeds per pot were planted, to increase the likelihood of having the desired plant (one plant) per pot, based on the previously determined germination tests, which yielded 80 % germination percentage for 95 % of the

land races concerned. Later, the plant closer to the center of the pot was consistently chosen and the rest were thinned out.

Watering (Distilled water) was carried out at each leap day to meet the desired amount of water required for the seeds to germinate (IBPGR/ICRISAT, 1993). Follow up was done on a daily basis and scoring were carried out in the appropriate time.

Quantitative morphological characters (Table 2) were scored to characterize a given land race or accession (Rao and Rao, 1995). These characters are also used for calculating diversity of the crop using different statistical packages (Harlan, 1961; Tadesse Ebba, 1978; Mulugeta Negassa, 1982; Endashaw Bekele, 1984). The following characters were scored in this study:

Table 2. The different characters scored along with their units of measurement.

Character and symbol	Measurement
1. Days to germination (DTG)	Number of days (1,2,3,)
2. Days to knee height stage (DTK)	Number of days (1,2,3)
3. Disease scoring (DSC)	0 to 9 scale (%)
4. Leaf number (LFN)	ordinal count (1, 2, 3,)
5. Leaf length (LFL)	Centimeters (cm)
6. Leaf width (LFW)	Centimeters (cm)
7. Leaf area (LFA)	Centimeter square (cm^2)
8. Leaf Sheath length (LSL)	Centimeters (cm)
9. Inter-node length (INL)	Centimeters (cm)
10.Stem thickness or diameter (SDI)	Centimeters (cm)
11.Plant height (PHT)	Centimeters (cm)

Mean days to germination was scored when 90 % of the planted seeds have germinated. Mean days to knee height stage were scored according to the standard length given by Rao and Rao (1995) which is about 35cm. Disease scoring was carried out according to

standardized scaling method adopted by IPIGRI descriptor (IBPGRI/ICRISAT, 1993), a scale of 0 up to 9, which is equal to decile presentation. i.e. each 10 % as a scale of 1. The leaf number is a count of the total number of leaves per plant, excluding the cotyledonary leaves. The leaf length is a measure of the leaf length starting from the end of the leaf sheath to the tip of the leaf. The leaf width was measured at half way from the leaf base to the leaf tip (IBPGRI/ICRISAT, 1993). The measurement was done on a centimeter scale. The mean leaf area was read using a leaf area reading meter. It should be noted that the leaf area could also be calculated from the formula given below:

$$A = (L \times W) - K$$

Where A = leaf area (cm^2)

L = leaf length (cm)

W = leaf width (cm)

K = correction factor for the difference of the rectangular area of L X W to find the true leaf area (Doggett, 1970).

Leaves for the area and other leaf characteristic measurement were carried out by randomly choosing a leaf from the mature leaves on the given plant (IBPGRI/ICRISAT, 1993). Leaf sheath length is a measure of the leaf sheath starting from the attachment of the leaf to the node to the starting of the true leaf. Inter-node length is a measurement of the inter-node distance between two nodes, taking the middle three nodes and taking their average (IBPGRI/ICRISAT, 1993). The diameter of the stalk was measured using a plastic ruler band, when the inflorescence starts to emerge (similarly, plant height was scored when the inflorescence starts to emerge). Each character was scored on five plants per land race, which makes a total of 150 plants. Statistical package (software) used to analyze these data was SPSS (SPSS Inc. Version 7.5, 1995). Descriptive graphs, Levene Statistic (test of homogeneity of variance, comparison of means using Analysis of Variance

(ANOVA), Chi-square test, discriminant analysis, Euclidean distance measurements (to calculate distance between different land races or accessions) and dendrograms (hierarchical cluster analysis) were constructed based on hierarchical cluster analysis.

4.3.Biochemical Study

The isozyme analysis procedure followed here was adopted from different authorities in accordance to the type of enzyme selected. The enzyme systems assayed are Esterase (EST) (O'Malley *et al.* (1980) cited in Morden *et al.*, 1987) and acid phosphatase (ACP) (Cardy *et al.*, 1983). Since the enzymes assayed have identical sample extraction, and running procedures, the technique followed holds true for the two enzyme systems assayed.

4.3.1.Extraction of samples

Crude extracts for electrophoresis are prepared from etiolated seedlings (Morden *et al.*, 1987). As to Morden *et al.* (1987), for routine surveys of isozyme variation and segregation analyses, etiolated seedlings are employed because they require less space to grow, need less time to obtain desired size for grinding, and the tissue is easier to grind, than green house grown seedlings (Loomis and Battaile, 1966).

Sorghum seeds were rolled up in moistened paper towel, and later wrapped up in aluminum foil to exclude light and kept at room temperature, until the seedling shoot is about 7 to 9 cm long (typically in 4 to 7 days). The extraction buffer used (Anderson and Rowan, 1967; Anderson, 1968) was 600 mg of 42.3 mM Na_2HPO_4, 7.2 gm of 0.21 M Sucrose, 52 mg of 1.55 mM EDTA-Disodium salt, and 100 μl of 2 – mercaptoethanol

adjusted to pH 7.5 with NaOH, added to 100ml with distilled water (King, 1971; Cardy *et al.,* 1983; Morden *et al.*, 1987).

4.3.2. Preparing and running gels

For this study, 500-ml gel trays were employed (Cardy *et al.*, 1983). The gel surface was covered with a heat resistant plastic film to avoid the adherence of the gel to the tray, so that the gel will be easily removed from the tray. The plastic film is initially coated with ethanol using a rolling presser, having a final smooth surface, ready for the gel to be poured onto it (the gel tray was checked for its balance using a water level). The starch gel prepared was 12 percent starch (Morden *et al.*, 1987). Three-fourth of the gel buffer (375ml) was poured into an Erlenmeyer flask and was heated in a stove, until boiling. The remaining buffer (125ml) was mixed with the starch and swirled until the starch was completely suspended. The starch suspension and boiling buffer were mixed and vigorously agitated until the boiling buffer and starch suspension are thoroughly mixed. The starch mixture is then heated until bubbles emerged (Cardy *et al.*, 1983). The heated mixture is then degassed (Morden *et al.*, 1987) until all the air bubbles in the mixture are removed. Following degassing, the solution is poured into the 500-ml gel trays and left at room temperature for the following two hours (Morden *et al.*, 1988).

For this study, the enzyme systems assayed had the following staining chemicals:

1. Esterase (Shaw and Koen, 1968 cited in Cardy *et al.*, 1983; Tanksley and Orton, 1983)

   Staining chemicals: a. 0.2 g fast blue RR salt

   b. 1.5 ml 1% α-naphthyl acetate (1 gm in 100 ml acetone)

   c. 0.5 ml 1% β-naphthyl acetate (1 gm in 100 ml acetone)

d. Water (100 mls).

2. Phosphatase (Shaw and Koen (1968) cited in Cardy *et al.*, 1983)

Staining chemicals: a. 0.1 gm fast garnet GBC salt

b. 5 ml 1M sodium acetate buffer pH 5.0 (1M glacial acetic acid adjusted pH with NaOH)

c. 0.5 ml 10% $MgCl_2$ (10 gm $MgCl_2$ in 100 ml H_2O)

d. 2 ml 1% α-naphthyl acid phosphate, sodium salt (1 gm in 50 ml acetone / 50 ml H_2O)

e. Water was added to have a final volume of 100 mls.

The buffer system for running the gels was Tris-citrate (T) composed of:

1. Electrode buffer: 0.135 M Tris adjusted with citric acid to pH 7.0
2. Gel buffer: 1 part electrode buffer: 14 parts water
3. Starch: 64-g starch
4. Watts: 12.0

The wicks were blotted to remove excess homogenate and were loaded on the gel, on 2 – 3 mm spacing. Trays were filled with running buffer. The gel was run for 3 ½ hours.

4.3.3. Pre-staining and Staining

Fifteen minutes after the gel was removed from the running pack and sample wicks were taken out from the gel system, the gel was sliced using fishing line (Wendel and Weeden, 1988). The slices were stained on gel trays using the staining chemical mixture given in 3.3.2.

For the Esterase, the staining compounds were dissolved in water and the sliced gels were stained, by pouring over the stain onto the gel (Baraka, 1960). The staining continued for the next 30 minutes to 1-hr at room temperature. Later, the staining solution was decanted, then the gel was rinsed with distilled water, decanted and 10% acetic acid was added as a fixative.

For the Phosphatase, after dissolving the mixture in water, substrates were added just prior to staining. The staining continued for the next 10 – 15 minutes at room temperature. The staining solution was decanted, then the gel was rinsed, and fixed using 10 % acetic acid.

For both enzyme systems, the stained gel slices were scored, and packed using cellophane foods wrap and thus were stored.

5. RESULTS

The first part gives result of the morphological characters of the different land races and accessions (for both the *ex-situ* and *in-situ* conserved accessions and land races, respectively). The second part deals with the biochemical (isozyme study) result.

5.1. Morphological study

Morphological study that was carried out under green house condition has revealed the following results.

5.1.1. Days to germination

The land races (both *ex-situ* and *in-situ*) have shown that there is difference in their performance or ability to germinate. The results obtained are summarized and discussed as follows.

A. Ex-situ *accessions*

Mean days to germination and their corresponding standard deviations (Table 3) show that there is a difference in the ability of the accessions to germinate and this difference was found to be significant at P-levels of both $P < 0.05$ and $P < 0.01$ for one way analysis of variance (Appendix 1B). i.e. there is highly significant difference in the mean days to germination for the studied *ex-situ* accessions. Test was also carried out for the homogeneity of variance for the accessions (for their respective individual representatives) and compared accession-wise (Appendix 1A). Accessions had mean days to germination ranging from as short as 6 days to as long as 12 days. *Wancho* and *gedalech* have shown the longest mean days to germination (12 ± 0.7071 and 12 ± 1, respectively) (Table 3, and

Fig. 1). The difference in the ability to germinate from the mean days to germination (standard deviation) was as high as 2.3022 (for the accession *zenghada2*) and as close to the mean of the accessions as 0.7071 (*wancho*). Other land races lie between the above ranges, for both their mean days to germination and also for the deviation for their germination from the mean days to germination.

Table 3. Mean days to germination (Mean ±SD) of ex-situ *conserved accessions.*

Accessions (*ex-situ*)	Mean ±SD
wancho	12 ± 0.7071
gedalech	12 ±1
jamuyo	6 ± 2
grsh	6 ± 1.58
zenghada1	7 ± 1.414
mashila1	10.2 ± 1.304
mashila2	6.2 ± 1.64
mashila3	7.2 ± 1.924
cherekit	11.4 ± 2.3022
zenghada2	10.6 ±2.3022
mashila4	11 ± 0.8367
nech mashila	11 ± 1.225
marutie	9.8 ± 0.8367

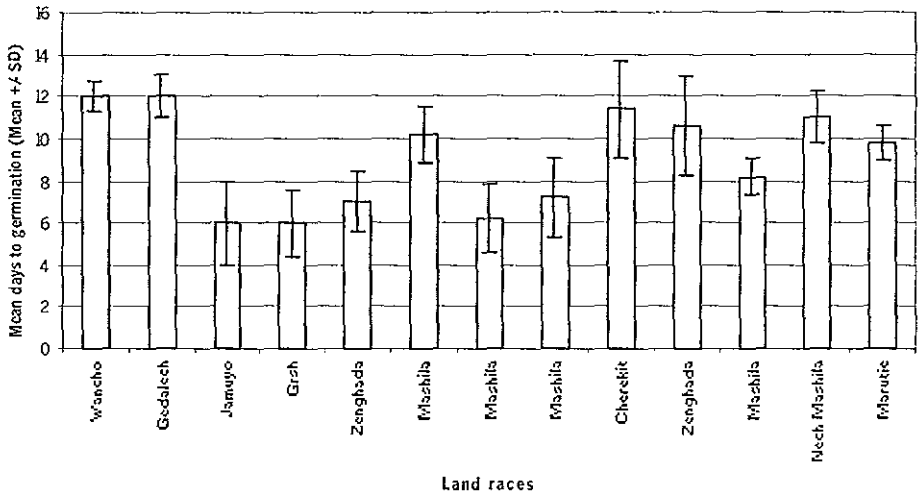


Fig 1. Mean days to germination (Mean ±SD) of ex-situ *conserved accessions.*

B. In-situ *land races*

Similar result was obtained for the *in-situ* (on-farm) land races. The result for mean days to germination showed that there are early germinating types as well as late germinating (as

early as 5 days and as late as 10 days) (Table 4 and Fig. 2). Analysis of variance has also proven this and has shown that there is significant difference in the mean germination days for the *in-situ* (on-farm) land races (at both P-levels, i.e. $P < 0.05$ and $P < 0.01$) (Appendix 1D). *In-situ* land races have non-significant ($P = 0.418$) variance heterogeneity (Appendix 1C). The land race that has achieved earliest germination was *ismaili* (5.2 ± 1.30). This land race was popularly known in the study area for its early-ness and quick maturity. The land race which has germinated latest of all was *zenghada*, having a mean days to germination of 9.8 ± 1.48. The rest of the land races have mean days to germination lying in the above listed range. On a land race level, deviations (from the mean for each land race) as large as 1.87 (*nech keteto*) and as small as 0.45 (*tuba*) were observed.

C. generalizations for mean days to germination

One way ANOVA (Appendix 1F) showed that there is significant difference in the mean days to germination between the *ex-situ* accessions and *in-situ* land races. Heterogeneity in their variances was also observed for their mean days to germination (Appendix 1E). ANOVA has shown that there is significance at $P < 0.05$ and at $P < 0.01$ (a P value of 0.001). It was also observed that there is significance (comparing P values 0.000 for both the *ex-situ* and *in-situ* conserved accessions and land races, respectively) compared to 0.001(the comparative result for the *ex-situ* and *in-situ* accessions and land races, respectively).

Table 4. Mean germination (Mean ±SD) of in-situ *conserved land races.*

Land races (*in-situ*)	Mean ± SD
wanchol	6 ± 1.24
delgome	5.6 ± 1.82
jamuyol	7.8 ± 1.48
goronjo	6.8 ± 1.48

dimetie	6.6 ± 0.55
wegerie	6.6 ± 1.14
nech keteto	7 ± 1.87
zenghadal	9.8 ± 1.48
tengeley	6.2 ± 1.10
tatigela	5.8 ± 1.64
tuba	6.8 ± 0.45
rayo	6.2 ± 1.64
jemaw	7.6 ± 0.55
jiru	7.4 ± 1.34
mokakie	6.4 ± 0.55
ismaili	5.2 ± 1.30
gubetie	5.8 ± 0.84

Continued...

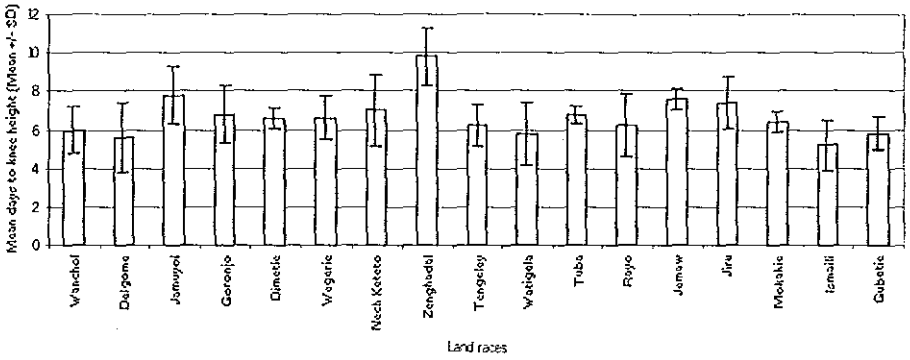


Fig. 2. Mean days to germination (Mean +/- SD) of the in-situ *conserved land races.*

5.1.2. Mean days to knee height stage

A. Ex-situ *accessions*

Cherekit was observed to have the longest mean days to knee height stage from the *ex-situ* group, 50 ± 1 (Table 5 and Fig 3). The lowest number of days to germination observed was that of *grsh*, which took 40.2 ± 0.84 mean days to knee height stage. The others lie between *grsh* and *cherekit*, and this difference in days to knee height stage between the different *ex-situ* accessions was found to be significant at P value of $P < 0.05$ and $P < 0.01$, after the homogeneity of variances was proven to be so at $P < 0.05$ but not at a $P <$ of 0.01(Appendix 2A and 2B). The highest standard deviation from the mean days to knee

height stage for each accession was that of *wancho* (2.12) and the lowest was that of *mashila* and *gedalech* (0.71).

Table 5. Mean days to knee height stage (Mean ±SD) of ex-situ *conserved land races.*

Accessions (ex-situ)	Mean ± SD
wancho	45 ± 2.12
gedalech	44 ± 0.71
jamuyo	47 ± 1
grsh	40.2 ± 0.84
zenghada1	44.8 ± 1.79
mashila1	50 ± 0.71
mashila2	48.2 ± 0.84
mashila3	48.6 ± 1.52
cherekit	50 ± 1
zenghada2	43.8 ± 1.30
mashila4	46.4 ± 1.14
nech mashila	48.8 ± 0.84
marutie	44.6 ± 0.55

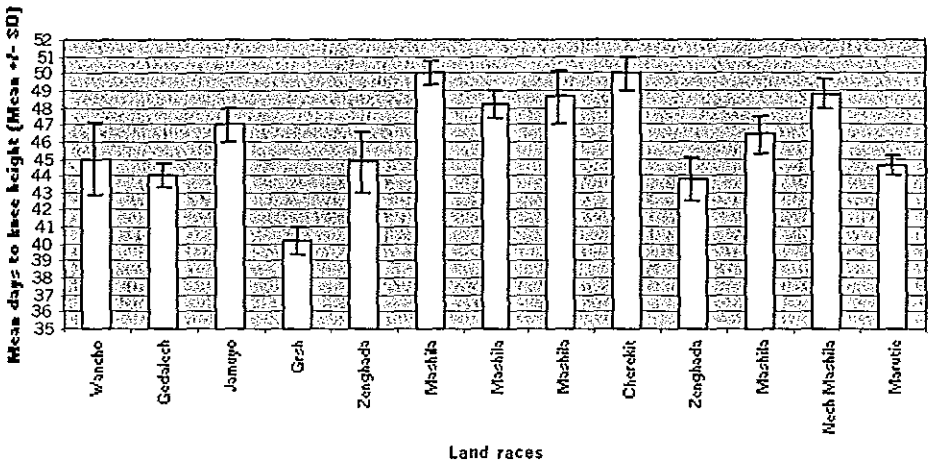


Fig 3. Mean days to knee height stage (Mean ±SD) of the ex-situ *conserved land races.*

B. In-situ *(on-farm) land races*

Land races were scored for their mean days to knee height stage and the resulting figures are given both in a tabular and graph form (Table 6 and Fig. 4). The fastest mean days to knee height stage scored was that of *ismaili* (38.75 ± 0.50). The longest mean days to

germination scored were that of *wancho* (46.4 ± 1.14). In addition, deviations from the mean were as large as 1.92 (*watigela*) and as close as 0.45 (*zenghadal*). Levene Statistic for testing the homogeneity of variance has proven that the means of days to germination for the *in-situ* land races was found to be homogenous at both 95 % and 99 % confidence intervals (P value of 0.131, a significant value at both $P < 0.05$ and $P < 0.01$ significance levels). This test followed one way ANOVA which revealed that there is highly significant difference in the mean days to knee height stage at $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.000$).

Table 6. Mean days to knee height stage (Mean ±SD) of the in-situ *conserved land races.*

Land races (*in-situ*)	Mean ± SD
wanchol	46.4 ± 1.14
delgome	44.8 ± 1.30
jamuyol	42.6 ± 1.52
goronjo	43.4 ± 0.55
dimetie	41.2 ± 0.84
wegerie	43.6 ± 1.34
nech keteto	45.6 ± 0.55
zenghadal	44.8 ± 0.45
tengeley	45.6 ± 0.55
watigela	38.8 ± 1.92
tuba	44.2 ± 0.84
rayo	41.6 ± 0.55
jemaw	42.4 ± 0.89
Jiru	45 ± 1.24
mokakie	47 ± 1
ismaili	38.75 ± 0.50
gubetie	42.33 ± 1.37

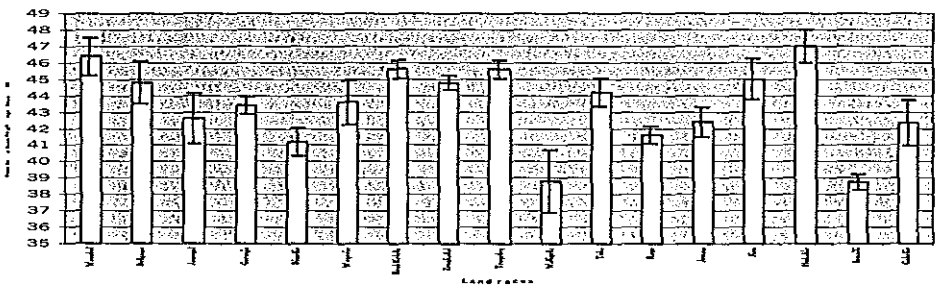


Fig 4. Mean days to knee height stage (Mean ± SD) of *in-situ* conserved land races.

C. Generalizations on the mean days to knee height stage

Test for homogeneity of variance and one way ANOVA was carried out to compare the *ex-situ* accessions and *in-situ* land races. The variances were observed to be homogenous at $P < 0.05$ and $P < 0.01$ significance levels. One way ANOVA showed that the difference in the mean days to knee height stage is significant at both $P < 0.05$ and $P < 0.01$ significance levels, giving a result of $P = 0.006$.

5.1.3. Disease scoring

The prevailing disease was only one type, which is a seed borne mosaic virus, that gives the leaves a mosaic appearance (variegated appearance), which gives a strip of yellowish green lying horizontally across the leaf width (Agrios, 1987). The scoring and the results refers to this disease.

A. Ex-situ *accessions*

Scoring showed that there were accessions that were not totally infected like *mashila1* and *nech mashila* (given in Table 7 and Fig 5). Other accessions had infections ranging from 0.2 ± 0.45 (*wancho, gedalech,* and *cherekit)* to as high as 2.8 ± 2.17 (*mashila4*). The highest deviation from the mean (Table 5), was found in *zenghada* and *mashila4* (±2.17), while the lowest were (excluding the 0 value of the non-infected ones) in *wancho, gedalech, mashila2,* and *cherekit.* A test for their homogeneity of variance was carried out and it resulted in a very significant heterogeneity at $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.000$, given in Appendix 3A). The same was also true for the one way

ANOVA calculated (Appendix 3B) resulting in the same P value as the test for the heterogeneity of variances.

Table 7. Mean percentage disease scoring (Mean ±SD) of ex-situ *conserved land races.*

Accessions (*ex-situ*)	Mean ± SD
wancho	0.2 ± 0.45
gedalech	0.2 ± 0.45
jamuyo	0.4 ± 0.55
grsh	0.6 ± 0.89
zenghada1	1.2 ± 1.30
mashila1	0 ± 0
mashila2	0.2 ± 0.45
mashila3	0.6 ± 0.55
cherekit	0.2 ± 0.45
zenghada2	2.2 ± 2.17
mashila4	2.8 ± 2.17
nech mashila	0 ± 0
marutie	3.8 ± 1.30

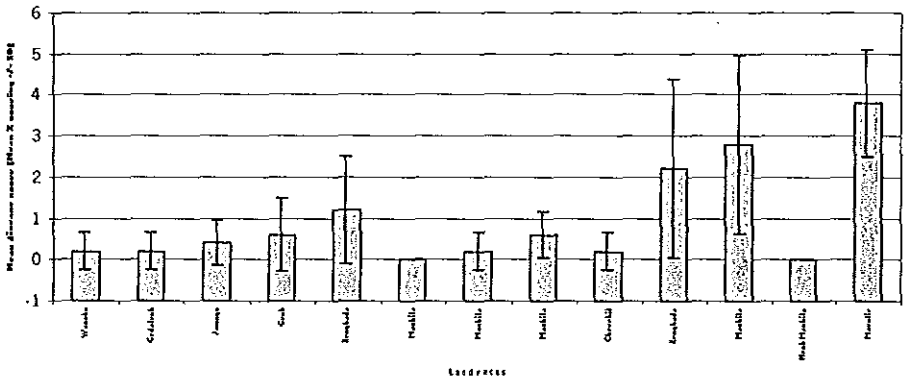


Fig 5. Mean percentage disease scoring (Mean ±SD) for the ex-situ *conserved land races.*

B. In-situ *land races*

Similar to that of *ex-situ* accessions, there were land races also in this group, which haven't shown any infection (Table 8 and Fig. 6). These are *nech keteto, tengeley, Watigela, jemaw, jiru, mokakie,* and *ismaili.* The land race, with the highest mean infection was *wanchol* (6.4 ± 2.074). The lowest was (excluding the non-infected ones) *delgome* (0.2 ± 0.447). The highest deviation from the mean was 2.074 (*wancho)* and lowest (0.447) was

that of *delgome*. Supporting this, test of homogeneity of variance and one way ANOVA revealed that there is high variance heterogeneity and significant difference in the level of infestation, respectively (both highly significant at $P < 0.05$ and $P < 0.01$ significance levels, resulting in $P = 0.000$).

Table 8. Mean percentage disease scoring (Mean ±SD) of the in-situ *conserved land races.*

Land races (*in-situ*)	Mean ± SD
wanchol	6.4 ± 2.074
delgome	0.2 ± 0.447
jamuyol	0.6 ± 0.548
goronjo	0.4 ± 0.548
dimetie	2.6 ± 1.673
wegerie	0.6 ± 0.548
nech keteto	0 ± 0
zenghadal	5 ± 0.707
tengeley	0 ± 0
watigela	0 ± 0
tuba	1.4 ± 0.548
rayo	2 ± 1.225
jemaw	0 ± 0
jiru	0 ± 0
mokakie	0 ± 0
ismaili	0 ± 0
gubetie	1.167 ± 0.983

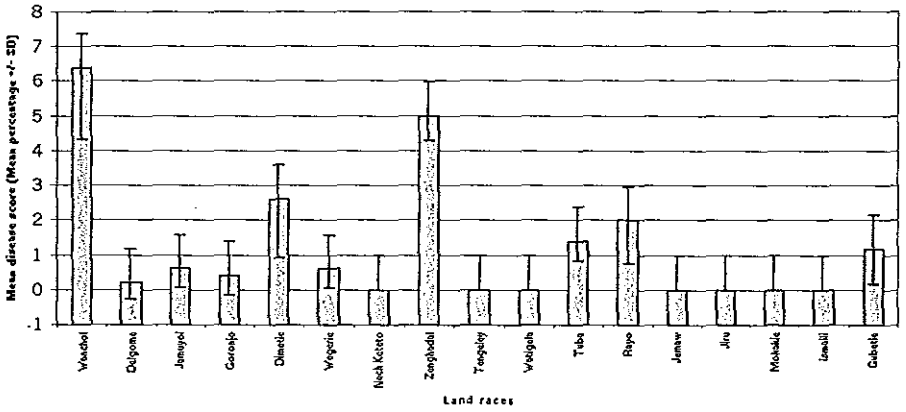


Fig 6. Mean percentage disease scoring (Mean ±SD) of the in-situ *(on-farm) conserved land races*

C. Generalizations on disease scoring

On the contrary to the above results, comparative tests done on the mean percentage disease scoring for the *ex-situ* accessions and *in-situ* land races showed that the variances are homogenous at both $P < 0.05$ and $P < 0.01$ levels ($P = 0.000$) (Appendix 3E). One way

ANOVA showed also that there is no significant difference in the level of infestation when the *ex-situ* accessions and the *in-situ* land races were compared, at both $P < 0.05$ and $P < 0.01$ significance levels (P value of 0.687) (Appendix 3F).

5.1.4.Mean leaf number

The number of leaves per a given plant was scored and these were later weighed to give the mean number of leaves for the given accession or land races.

A. Ex-situ *accessions*

For this character, it was observed that the range was narrow with the highest mean being 14 ± 0.548 (*wancho* and *mashila4*), and lowest 13 ± 1 (*zenghada*) (Table 9 and Fig. 7). The largest deviation that was observed was 1.00 (*zenghada1, zenghada2, nech mashila,* and *marutie*). The lowest deviation from the mean was 0.447 (*mashila1* and *cherekit*). Test for homogeneity of variance showed that the variances of means of leaf number are homogenous at $P < 0.05$ and $P < 0.01$ levels (P value of 0.180) (Appendix 4A). One way ANOVA revealed that the difference in the mean number of leaves is not significant both at $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.334$) (Appendix 4B).

Table 9. Mean leaf number (Mean ±SD) for the ex-situ *conserved land races.*

Accessions (*ex-situ*)	Mean ± SD
wancho	14 ± 0.548
gedalech	13 ± 0.894
jamuyo	13 ± 0.894
grsh	14 ± 0.447
zenghada1	13 ± 1.00
mashila1	14 ± 0.447
mashila2	13 ± 0.837
mashila3	13 ± 0.837
cherekit	14 ± 0.447
zenghada2	13 ± 1.00
mashila4	14 ± 0.548
nech mashila	13 ± 1.00
marutie	13 ± 1.00

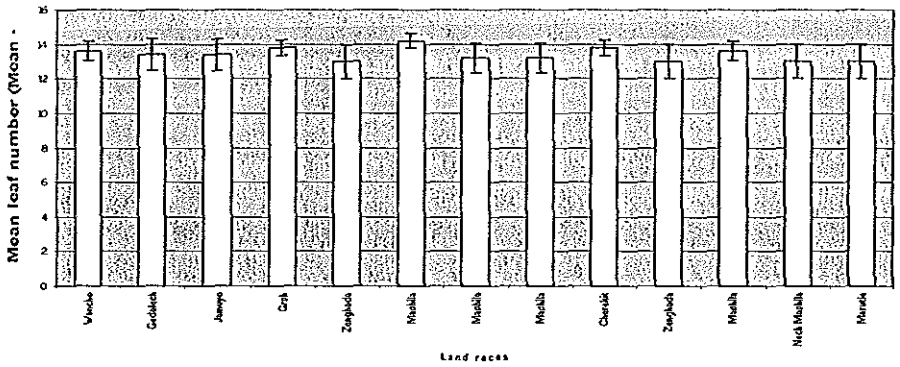


Fig 7. Mean leaf number (Mean ±SD) for the ex-situ *conserved land races.*

B. In-situ *(on-farm) land races*

Similarly, test of homogeneity of variance and one way ANOVA proved that the variances of means number of leaves (Table 10 and Fig. 8) for the land races are homogenous and that there is no significant difference in the mean number of leaves at both $P < 0.05$ and $P < 0.01$ significance level ($P = 0.498$ and $P = 0.314$), respectively (Appendix 4C and 4D).

Table 10. Mean leaf number for the in-situ *conserved land races.*

Land races (*in-situ*)	Mean ± SD
wanchol	14 ± 0.447
delgome	13 ± 0.548
jamuyol	14 ± 0.447
goronjo	14 ± 0.548
dimetie	13 ± 0.548
wegerie	14 ± 0.548
nech keteto	14 ± 0.894
zenghadal	14 ± 0.447
tengeley	14 ± 0.447
watigela	14 ± 0.837
tuba	13 ± 0.894
rayo	14 ± 0.548
jemaw	14 ± 0.707
jiru	14 ± 0.447
mokakie	14 ± 0.837
ismaili	14 ± 0.5
gubetie	13 ± 0.816

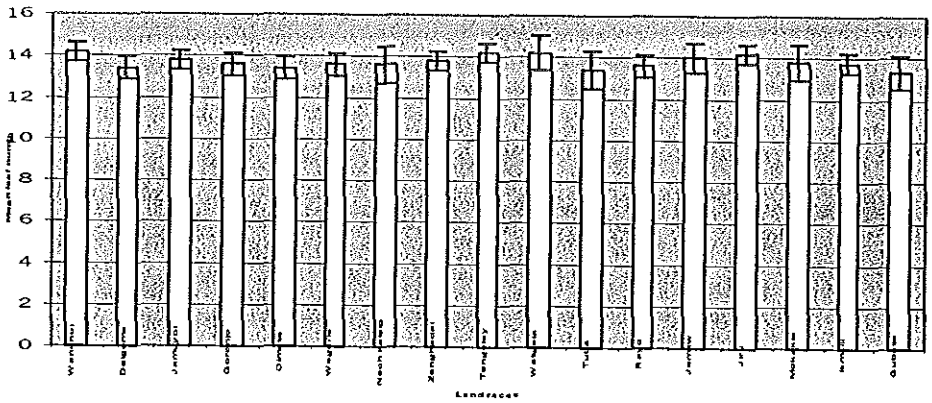


Fig 8. Mean leaf number (Mean ±SD) for the in-situ *(on-farm) conserved land races.*

C. Generalizations for mean number of leaves

When the *ex-situ* accessions and *in-situ* land races were compared, it was observed that there is no heterogeneity in their variances at both $P < 0.05$ and $P < 0.01$ ($P = 0.418$) (Appendix 4E). One way ANOVA showed that there is no significant difference in the mean leaf number between *ex-situ* accessions and *in-situ* land races, both at $P < 0.05$ and $P < 0.01$ (P value of 0.008) (Appendix 4F).

5.1.5. Mean leaf length

Mean leaf length was scored (cm) for both the *ex-situ* accessions and *in-situ* land races.

A. Ex-situ *accessions*

Wancho scored the highest mean leaf length (57 ± 1.581) (Table 11 and Figure 9). The lowest value observed for the mean leaf length was that of *marutie* (49.4 ± 0.894). The highest deviation from the mean was observed in *grsh* (2.387) and the lowest was in *mashila2* and *mashila4* (0.837). Test of homogeneity of variances was found to be homogenous at both $P < 0.05$ and $P < 0.01$ significance levels (0.137) (Appendix 5A). One

way ANOVA has revealed significant difference in the mean leaf length at $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.000$) (Appendix 5B).

Table 11. Mean leaf length (Mean ± SD) for the ex-situ *conserved land races.*

Accessions (ex-situ)	Mean ± SD
wancho	57 ± 1.581
gedalech	56.8 ± 1.095
jamuyo	53 ± 1
grsh	52.2 ± 2.387
zenghada1	50.8 ± 1.304
mashila1	50.4 ± 1.14
mashila2	51.8 ± 0.837
mashila3	51.6 ± 1.14
cherekit	51 ± 1.225
zenghada2	50 ± 0.707
mashila4	52.2 ± 0.837
nech mashila	53.2 ± 1.643
marutie	49.4 ± 0.894

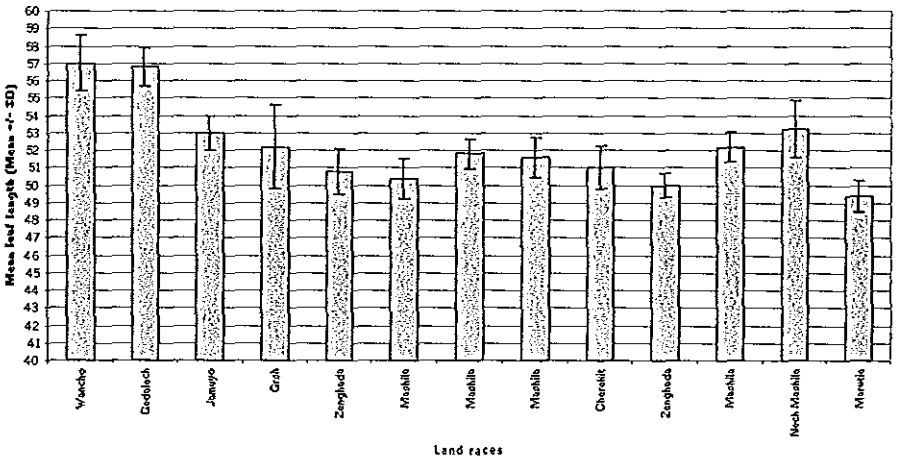


Fig 9. Mean leaf length (Mean ± SD) for the ex-situ *conserved land races.*

B. In-situ *(on-farm) land races*

wanchoI was the land race that was observed to have the highest mean leaf length (53.4 ± 1.342), while the land race with the shortest leaf length was identified to be *wategila* (49.2 ± 0.837) (Table 12 and Figure 10). Deviations from the mean for leaf lengths were

observed to be as high as 1.342 (*wanchol*), and as low as 0.707 (*jamuyo, tengeley* and *mokakie*). Test of homogeneity of variances carried out revealed that there is homogeneity of variances between the different land races studied (Appendix 5C). One way ANOVA, on the other hand, revealed significant difference in the mean leaf length at both $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.000$) (Appendix 5D).

Table 12. Mean leaf length (Mean ±SD) for the in-situ *(on-farm) conserved land races.*

Land races (*in-situ*)	Mean ± SD
wanchol	53.4 ± 1.342
delgome	52.6 ± 0.894
jamuyol	50 ± 0.707
goronjo	51.8 ± 0.837
dimetie	49.6 ± 0.548
wegerie	52.4 ± 0.548
nech keteto	51.4 ± 0.548
zenghadal	49.6 ± 0.548
tengeley	52 ± 0.707
watigela	49.2 ± 0.837
tuba	49.6 ± 0.548
rayo	51.8 ± 0.837
jemaw	51.2 ± 0.447
jiru	50.4 ± 1.14
mokakie	51 ± 0.707
ismaili	49.25 ± 1.258
gubetie	50.67 ± 1.033

Continued...

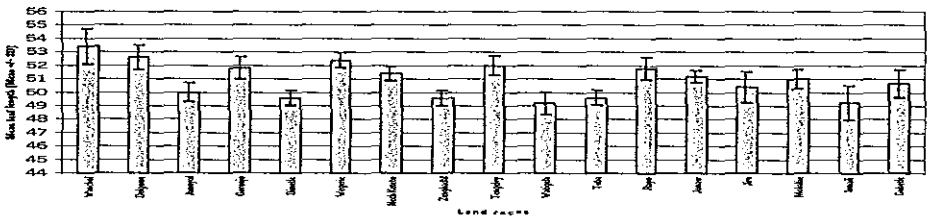


Figure 10. Mean leaf length (cm) (mean ± SD) for the *in-situ* (on-farm) land races

C. Generalizations on mean leaf length

Comparing the *ex-situ* accessions and *in-situ* land races, both test of homogeneity of variances and one way ANOVA revealed that there is homogeneity of variances at $P < 0.05$ and $P < 0.01$ ($P = 0.154$, Appendix 5E) and the difference between mean leaf lengths is not significant at both $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.057$) (Appendix 5F).

5.1.6. Mean leaf width

A. Ex-situ *accessions*

The widest mean leaf width scored was 7.4 ± 0.894 (*nech mashila*), while the accessions with the narrowest mean leaf width were *zenghada* and *mashila4* (5.6 ± 0.548) (Table 13 and Figure 11). Deviations from the mean leaf width for the accessions were ranging between 0.548 (*jamuyo, zenghada1, mashila1, mashila3,* and *mashila4*) and 1.414 (*mahsila2*). Test of homogeneity of variances showed that there is homogeneity of variance between the different accessions at both $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.075$) (Appendix 6A). One way ANOVA proven that there is significant difference in mean leaf width between the *ex-situ* accessions at $P < 0.05$ and $P < 0.01$ levels, yielding a P value of 0.006 (Appendix 6B).

Table 13. Mean width (cm) (Mean ±SD) for the ex-situ *land races.*

Accessions (*ex-situ*)	Mean ± SD
wancho	6.8 ± 0.837
gedalech	6.4 ± 0.894
jamuyo	6.4 ± 0.548
grsh	6.8 ± 0.837
zenghada1	5.6 ± 0.548
mashila1	7.4 ± 0.548
mashila2	6 ± 1.414
mashila3	6.4 ± 0.548
cherekit	6.4 ± 0.894
zenghada2	6 ± 0.707

mashila4	5.6 ± 0.548
nech mashila	7.4 ± 0.894
marutie	7 ± 0.707

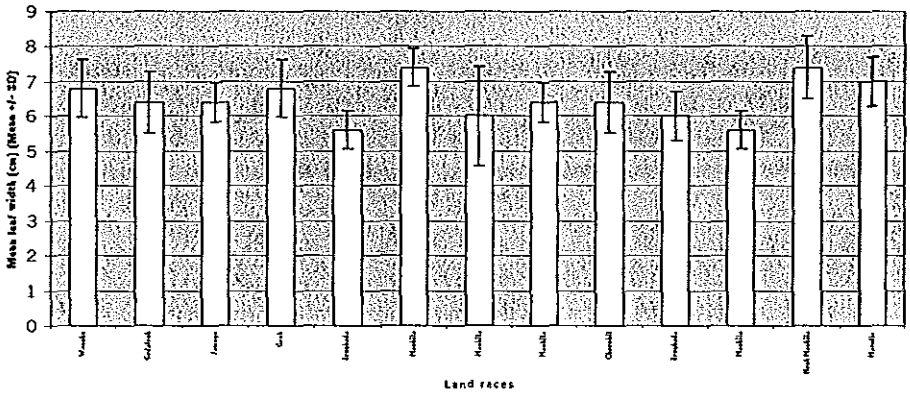


Fig 11. Mean leaf width (cm) (Mean ±SD) for the ex-situ *conserved land races.*

B. In-situ *(on-farm) land races*

Delgome and *nech keteto* were the land races with, comparatively, the widest leaf width (7.2 ± 0.837) (Table 14 and Figure 12). The smallest mean leaf width observed was 4.8 ± 0.837. The largest deviation from the mean leaf width observed was 1.14 (*zenghadal*) and the smallest was 0.5 (*ismaili*). Levene Statistic for testing homogeneity of variances between the different land races studied showed that the variances of the different land races were homogenous at both $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.775$) (Appendix 6C). The one way ANOVA revealed significant difference between the land races for the mean leaf width at $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.000$) (Appendix 6D).

Table 14. Mean leaf width (cm) (Mean ±SD) for the in-situ *conserved land races.*

Land races (*in-situ*)	Mean ± SD
wanchol	6.6 ± 0.548
delgome	7.2 ± 0.837
jamuyol	4.8 ± 0.837
goronjo	6.2 ± 0.837

dimetie	6.4 ± 0.548
wegerie	5.4 ± 0.548
nech keteto	7.2 ± 0.837
zenghadal	5.6 ± 1.14
tengeley	6.6 ± 0.548
watigela	6 ± 0.707
tuba	5.6 ± 0.894
rayo	6 ± 1
jemaw	6.2 ± 0.837
jiru	7 ± 0.707
mokakie	6.2 ± 0.837
ismaili	5.25 ± 0.5
gubetie	6.333 ± 0.516

Continued...

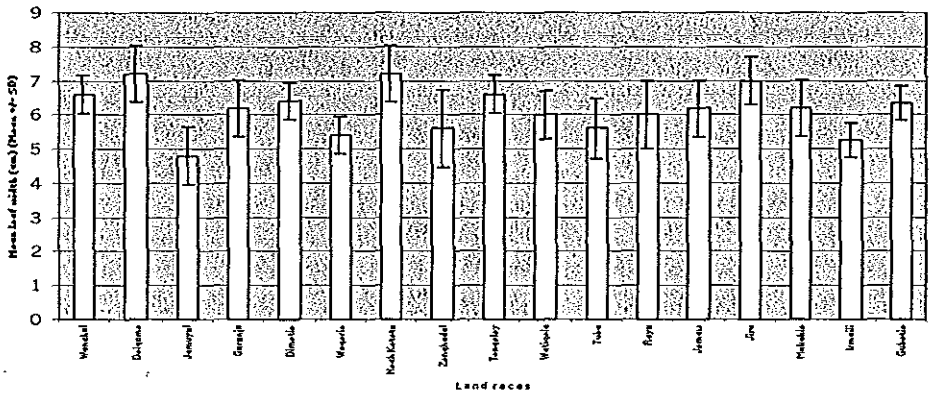


Fig 12. Mean leaf width for the in-situ *land races (cm) (Mean ±SD).*

C. Generalizations on mean leaf width

Test of homogeneity of variances revealed, just like in the independent tests, that the variances of *ex-situ* accessions and *in-situ* (on-farm) land races were found to be homogenous at $P < 0.05$ and $P < 0.01$ significance levels (Appendix 6E). One way ANOVA has revealed, on the other hand, that there is significant difference in the mean leaf widths between the *ex-situ* accessions and *in-situ* (on-farm) land races.

5.1.7. Mean leaf area

A. Ex-situ *accessions*

Nech mashila was observed to have the highest mean leaf area from the *ex-situ* accessions studied (343.6 ± 48.88) (Table 15 and Fig. 13). 234 ± 22.32 was the smallest mean leaf

area recorded (*zenghada1*). Mean leaf area with the highest deviation from the accession means was that of *mashila2* (75.12) and the deviation closest to the mean was 22.32 (*zenghada*). Test of homogeneity of variances for mean leaf area for the *ex-situ* accessions showed that there is heterogeneity in their variances at $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.006$) (Appendix 7A). One way ANOVA has revealed highly significant result in the differences in their means for leaf are between the different *ex-situ* accessions, at $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.000$) (Appendix 7B).

Table 15. Mean leaf area (cm^2) (mean ±SD) for the ex-situ *conserved accessions.*

Accessions (ex-situ)	Mean ± SD
wancho	337.2 ± 44.57
gedalech	313.4 ± 50.38
jamuyo	288.8 ± 23.13
grsh	304.2 ± 38.58
zenghada1	234 ± 22.32
mashila1	323 ± 29.43
mashila2	261.2 ± 75.12
mashila3	280 ± 25.5
cherekit	276.4 ± 46.76
zenghada2	249.6 ± 31.12
mashila4	242.2 ± 27.5
nech mashila	343.6 ± 48.88
marutie	295.6 ± 33.03

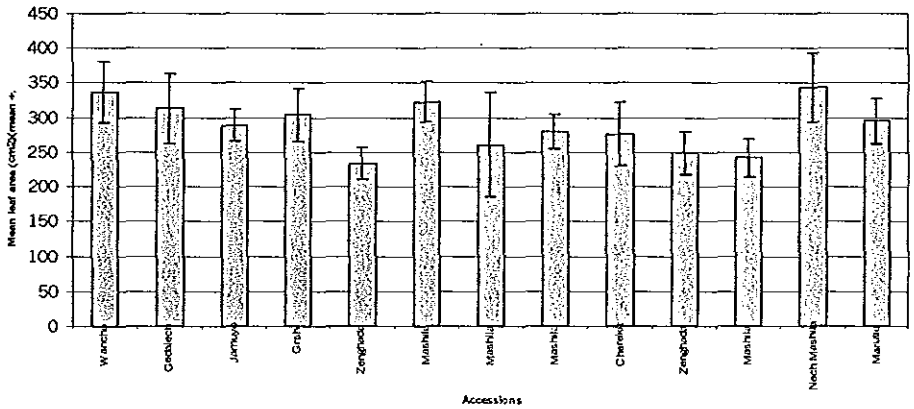


Fig 13. Mean leaf area (cm^2) for the ex-situ *accessions.*

B. In-situ *(on-farm) land races*

For the *in-situ* (on-farm) land races, the highest mean leaf area observed was 328.2 ± 38.89 (*delgome*) and the land race with the smallest leaf area was 208.3 ± 20.39 (*ismaili*) (Table 16 and Fig. 14). The deviations observed were as close to the mean as 20.39 (*ismaili*) and as far from the mean as 55.6 (*zenghada1*). Test on homogeneity of variance highly heterogeneous at both $P < 0.05$ and $P < 0.01$ (P=0.05) (Appendix 7C). One way ANOVA showed that there is significant difference between the *in-situ* land races for their mean difference in leaf area at $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.000$) (Appendix 7D).

Table 16. Mean leaf area (cm^2) (mean ±SD) for the ex-situ *accessions.*

Land races (*in-situ*)	Mean ± SD
wanchol	302.6 ± 32.75
delgome	328.2 ± 38.89
jamuyol	189.8 ± 40.13
goronjo	271.2 ± 43.56
dimetie	267.6 ± 29.68
wegerie	233 ± 29.36
nech keteto	319.8 ± 40.18
zenghadal	227.6 ± 55.6
tengeley	293.4 ± 31.6
watigela	244.8 ± 30.55
tuba	228 ± 46.45
rayo	260.6 ± 50.44
jemaw	267.6 ± 44.42
jiru	302.6 ± 33.45
mokakie	266 ± 41.39
ismaili	208.3 ± 20.39
gubetie	270.7 ± 23.46

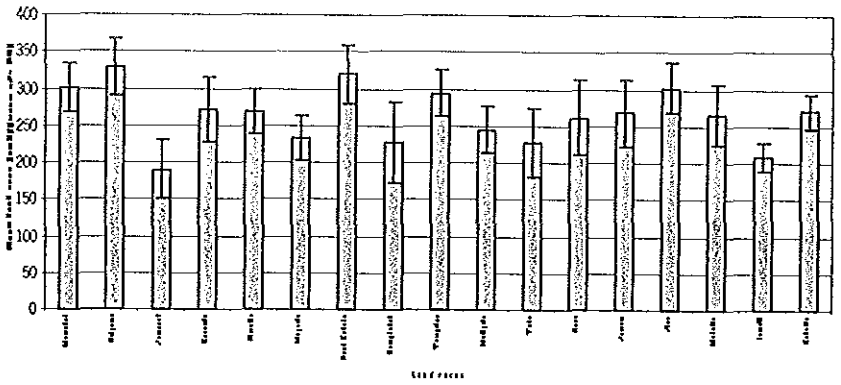


Fig 14. Mean leaf area (cm^2) (mean ±SD) for the in-situ *(on-farm) conserved land races.*

C. Generalizations on mean leaf area

Statistical tests carried out to compare the *ex-situ* accessions and *in-situ* land races revealed that there is no significant heterogeneity in the mean leaf area (the variances were found to be homogenous), at $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.897$) (Appendix 7E). it was also found that for the means compared (*ex-situ* accessions versus *in-situ* land races), that there is no significant difference in the mean leaf area ($P = 0.082$), which is true at either $P < 0.05$ or $P < 0.01$ significance levels.

5.1.8. Mean leaf sheath length (cm)

A. Ex-situ *accessions*

Nech mashila scored the highest mean leaf sheath length (12.2 ± 0.447) and on the other hand, 8.4 ± 0.548 was found to be the smallest leaf sheath length, belonging to *jamuyo* (Table 17 and Fig. 15). 0.447 was the smallest deviation observed for leaf sheath lengths (*nech mashila)* while the highest deviation observed was 1.304 (*grsh*). Test of homogeneity of variance revealed that there is homogeneity at $P < 0.05$ and $P < 0.01$ ($P =$

0.239) (Appendix 8A). One way ANOVA showed that (Appendix 8B) there is significant difference in the mean leaf sheath length at both $P < 0.05$ and $P < 0.01$ ($P = 0.000$).

Table 17. Mean leaf sheath length (cm) (Mean ±SD) for the ex-situ *accessions.*

Accessions (*ex-situ*)	Mean ± SD
wancho	**8.8 ± 0.837**
gedalech	**10.2 ± 0.837**
jamuyo	**8.4 ± 0.548**
grsh	**8.8 ± 1.304**
zenghada1	**10.4 ± 0.548**
mashila1	**9.8 ± 0.837**
mashila2	**10.8 ± 0.837**
mashila3	**11.4 ± 1.14**
cherekit	**9.6 ± 0.548**
zenghada2	**10 ± 0.707**
mashila4	**10.6 ± 1.14**
nech mashila	**12.2 ± 0.447**
marutie	**9.4 ± 0.548**

Continued...

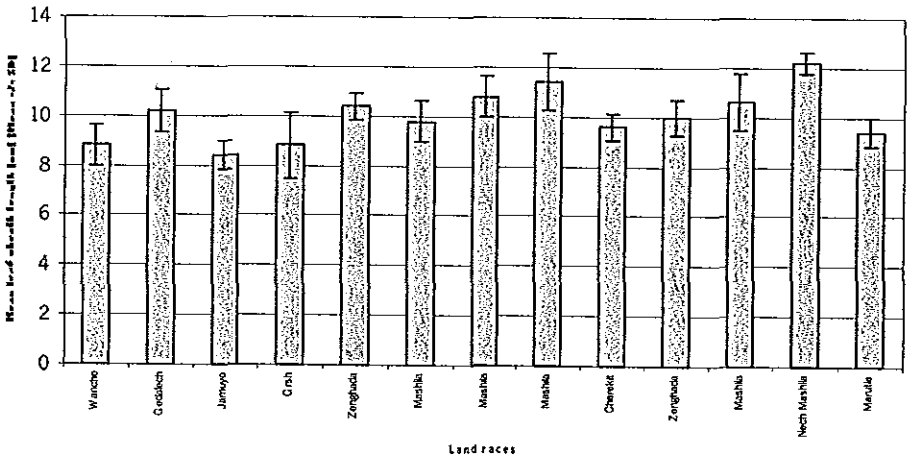


Fig 15. Mean leaf sheath length (cm) (Mean ±SD) of ex-situ *accessions.*

B. In-situ *(on-farm) land races*

Delgome showed the longest mean leaf sheath length (11.8 ± 0.837). The land race with the shortest mean leaf sheath length was *wancho* (9.8 ± 1.304) (Table 18 and Fig. 16). Deviations were also observed for the different land races, and as a result, 1.304 was the highest deviation from the mean (*wancho*) and 0.447 was the smallest deviation observed

(*zenghadal*). Test of homogeneity of variances was carried out and the variances were found to be homogenous at both $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.134$) (Appendix 8C). One way ANOVA revealed that there is significant difference in the mean leaf sheath length for the *in-situ* land races at $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.000$) (Appendix 8D).

Table 18. Mean leaf sheath length (cm) (Mean ±SD) of the in-situ *(On-farm) conserved land races.*

Land races (*in-situ*)	Mean ± SD
wanchol	9.8 ± 1.304
delgome	11.8 ± 0.837
jamuyol	9.4 ± 0.837
goronjo	10.8 ± 0.837
dimetie	9.8 ± 0.837
wegerie	10.8 ± 0.837
nech keteto	11.6 ± 1.14
Zenghadal	10.2 ± 0.447
Tengeley	12 ± 1.00
Watigela	10.8 ± 0.837
Tuba	10.4 ± 0.548
Rayo	11.6 ± 1.14
Jemaw	11.6 ± 1.517
Jiru	10.8 ± 0.837
Mokakie	10.8 ± 0.447
Ismaili	9.25 ± 0.5
Gubetie	11.17 ± 1.722

Continued...

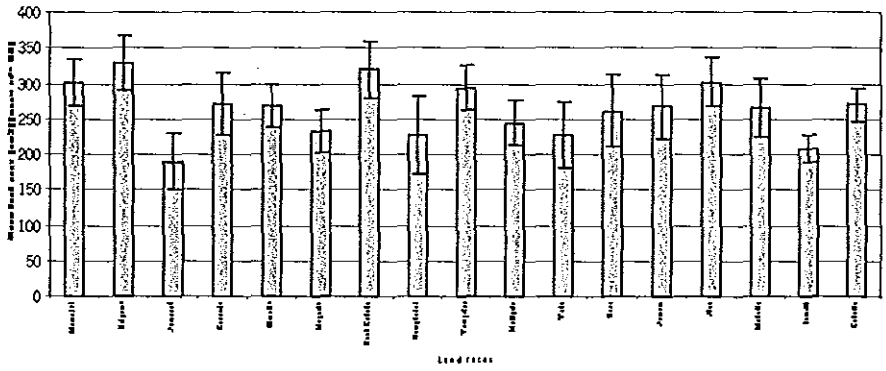


Fig 16. Mean leaf sheath length (Mean ±SD) for the in-situ *(on-farm) conserved land races.*

C. Generalizations for the means of leaf sheath

The *ex-situ* and *in-situ* sorghum accessions and land races were compared for their mean leaf sheath lengths and test of homogeneity of variance which showed that the variances of

the *ex-situ* and *in-situ* are homogenous (P = 0.409) at P < 0.05 and P < 0.01 significance levels (Appendix 8E). It can be observed also from Appendix 8F that there is no significant difference between the *ex-situ* accessions and *in-situ* land races in their mean leaf sheath lengths at both P < 0.05 and P < 0.01 significance levels (P = 0.052).

5.1.9.Mean inter-node length

A. Ex-situ *accessions*

Based on the result obtained, the mean inter-node length was found to be the longest for the accession *wancho* (18 ± 1.581). *Jamuyo* was the accession with the shortest inter-node length (12.8 ± 1.304) (Table 19 and Fig. 17). The range of deviations, as can be observed from Table 17 (or Fig. 17) lies between 1.581 (*wancho*) and 0.707 (*cherekit*). To see whether this difference in means and standard deviations is significant, test of variances was done which showed that there is homogeneity among the variances of the *ex-situ* accessions (P = 0.162), giving a figure which is significant at both P < 0.05 and P < 0.01 significance levels (Appendix 9A). It can be observed from Appendix 9B that one way ANOVA has revealed that there is significant difference between the accessions for their mean inter-node length at P < 0.05 and P < 0.01 significance levels (P = 0.000).

Table 19. Mean inter-node length (cm) (Mean ±SD) for the ex-situ *accessions.*

Accessions (*ex-situ*)	Mean ± SD
wancho	18 ± 1.581
gedalech	16.2 ± 0.837
jamuyo	12.8 ± 1.304
grsh	17.6 ± 1.14
zenghada1	15.2 ± 0.837
mashila1	16.4 ± 0.894
mashila2	15.8 ± 1.643
mashila3	16.6 ± 0.894
cherekit	14 ± 0.707
zenghada2	14.8 ± 0.837
mashila4	18 ± 1
nech mashila	18.6 ± 0.548
marutie	14.2 ± 0.837

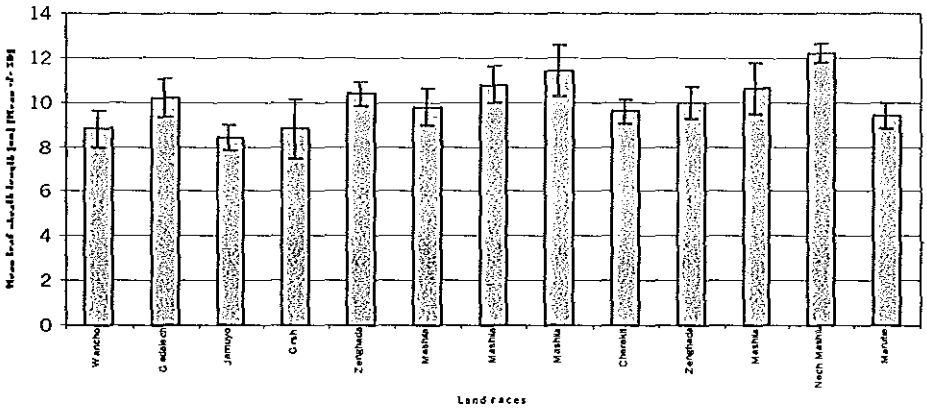


Fig 17. Mean intern-node length of the ex-situ *accessions.*

B. In-situ *(on-farm) land races*

Out of the 17 land races studied, the land races with the highest (longest) inter-node length was *Delgome* (19.2 ± 0.837) followed by the other different land races, having *jamuyoI* with the shortest inter-node length of mean 11.8 ± 1.304 (Table 20 and Figure 18). On the same table and figure, one can observe that 1.304 is the highest deviation from the mean, i.e. that of *jamuyoI.* The land races that showed the minimum deviation from their means for the inter-node length were *wanchoI, goronjo, wegerie, tengeley* and *watigela* (0.548). Testing this variability using Levene Statistic, the variances of the different land races were found to be homogenous at both $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.217$) (Appendix 9C). One way ANOVA to compare the means of inter-node length, proved that there is significant difference between the different land races for their means of inter-node lengths at $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.000$) (Appendix 9D).

Table 20. *Mean inter-node length (mean ±SD) for the* in-situ *(on-farm) land races.*

Land races (*in-situ*)	Mean ± SD
wanchol	17.4 ± 0.548
delgome	19.2 ± 0.837
jamuyol	11.8 ± 1.304
goronjo	17.4 ± 0.548
dimetie	18.2 ± 0.837

wegerie	17.6 ± 0.548
nech keteto	12.6 ± 1.14
zenghadal	14.8 ± 0.837
tengeley	17.4 ± 0.548
watigela	15.6 ± 0.548
tuba	15.4 ± 1.14
rayo	13.6 ± 1.342
jemaw	17.8 ± 0.837
jiru	18.2 ± 0.837
mokakie	14 ± 0.707
Ismaili	9.25 ± 1.258
gubetie	14.17 ± 1.472

Continued...

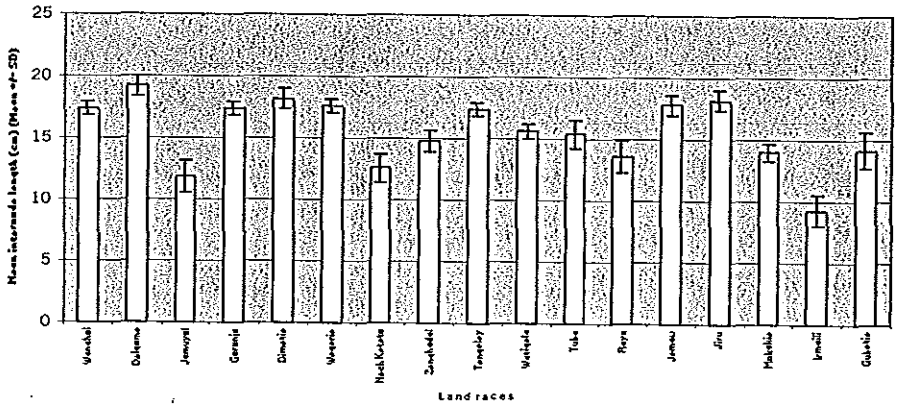


Fig 18. Mean inter-node length (mean ±SD) for the in-situ *(on-farm) conserved land races.*

C. *Generalizations on the mean inter-node length*

The *ex-situ* accessions and *in-situ* land races have shown homogeneity for their variances at both $P < 0.05$ and $P < 0.01$ significance levels (Appendix 9E). They were further tested for the difference in their means and this was found to be significant at $P < 0.05$ and $P < 0.01$ (Appendix 9F).

5.1.10. Mean stem diameter

A. Ex-situ *accessions*

Gedalech was the accession with the highest mean stem diameter, with a score of 1.528 ± 0.073, and the accession with the thinnest stem of all was found to be *jamuyo* (with a mean of 1.498 ± 0.008) (Table 21 and Figure 19). The deviations were also observed for the

different accessions, and, as a result, the highest deviation observed was that of *wancho* (0.073), and the deviation that was closest to the mean was that of *mashila4*. To test whether this difference is significant or not, Levene Statistic showed that there is heterogeneity among the variances at both $P < 0.05$ and $P < 0.01$ significance levels (Appendix 10A). Further more, one way ANOVA was done to test whether this difference in mean is significant or not, and as a result, the difference between the means was found to be highly significant at $P < 0.05$ and $P < 0.01$ significance levels (Appendix 11B).

Table 21. Mean stem diameter (mean ±SD) for the ex-situ *accessions.*

Land races (*in-situ*)	Mean ± SD
wancho	1.528 ± 0.073
gedalech	1.65 ± 0.067
jamuyo	1.498 ± 0.008
grsh	1.522 ± 0.015
zenghada1	1.51 ± 0.007
mashila1	1.532 ± 0.008
mashila2	1.508 ± 0.013
mashila3	1.532 ± 0.013
cherekit	1.532 ± 0.013
zenghada2	1.508 ± 0.016
mashila4	1.522 ± 0.004
nech mashila	1.56 ± 0.007
marutie	1.53 ± 0.017

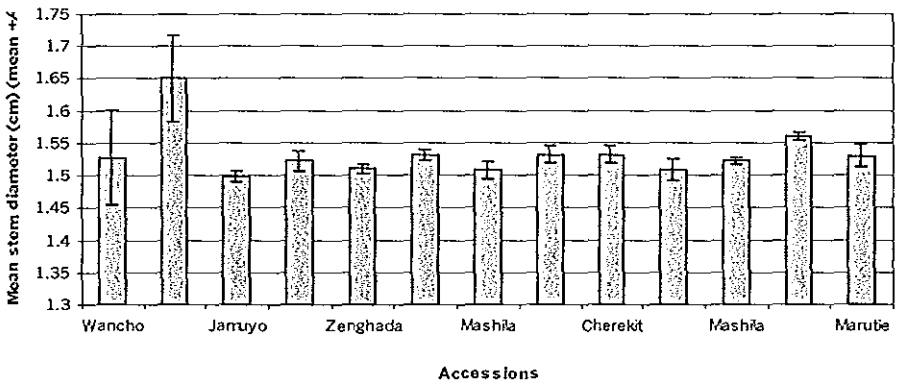


Fig 19. Mean stem diameter for the in-situ *(on-farm) land races.*

B. In-situ *(on-farm) land races*

For the mean stem diameter, *watigela* was observed to be the land race with the thickest stalk out of the *in-situ* land races. (Table 22 and Figure 20). On the other hand, the land

race with the thinnest stalk was *ismaili*, with a mean stem thickness of 1.49 ± 0.014. As far as their deviations from the mean are concerned, *rayo* (0.004) showed little deviation from its mean, while the most deviated recording were that of *gubetie* (0.045). Test of homogeneity of variance was carried out and it revealed that there is homogeneity among the variances at both $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.096$) (10C). One way ANOVA further clarified that there is significant difference between the means for stem diameter at both P levels (0.05 and 0.01) ($P = 0.000$) (Appendix 10D).

Table 22. Mean stem diameter (cm) (mean ±SD) for the ex-situ *accessions.*

Accessions (*ex-situ*)	Mean ± SD
wanchol	1.502 ± 0.008
delgome	1.54 ± 0.027
jamuyol	1.512 ± 0.028
goronjo	1.552 ± 0.008
dimetie	1.52 ± 0.012
wegerie	1.582 ± 0.019
nech keteto	1.526 ± 0.034
zenghadal	1.526 ± 0.011
tengeley	1.574 ± 0.011
watigela	1.588 ± 0.026
tuba	1.56 ± 0.012
rayo	1.538 ± 0.004
jemaw	1.536 ± 0.011
jiru	1.606 ± 0.03
mokakie	1.564 ± 0.032
ismaili	1.49 ± 0.014
gubetie	1.575 ± 0.045

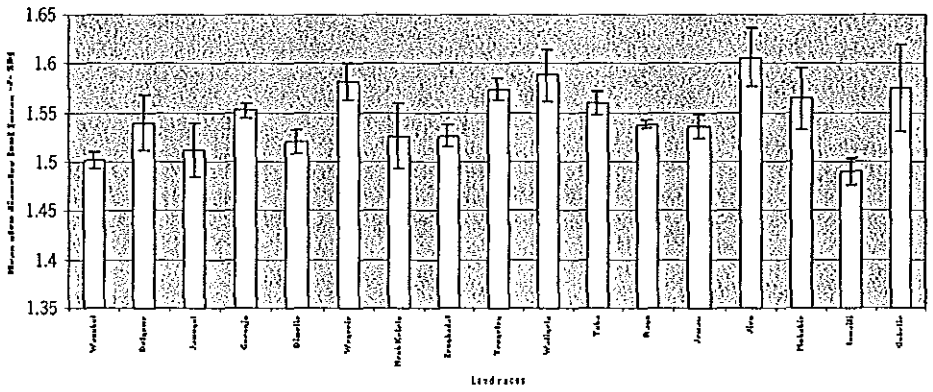


Fig 20. Mean stem diameter (Mean ±SD) for the in-situ *(on-farm) land races.*

C. Generalizations on mean stem diameter

Similar result as that obtained for the *in-situ* land races was observed that test of homogeneity of variances proved that there is no significant heterogeneity (the variances are homogenous) at $P < 0.05$ and $P < 0.01$ significance levels (Appendix 10E). One way ANOVA showed that there is significant difference in the means for stem diameter between the *ex-situ* accessions and *in-situ* land races, at $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.000$) (Appendix 10F).

5.1.11. Mean plant height

A. Ex-situ *accessions*

Wancho was the accession with the highest mean plant height (205.8 ± 7.895), followed by the other accessions, with the smallest reading for *marutie* (168.2 ± 2.049) (Table 23 and Figure 21). Test of homogeneity of variances revealed that there is heterogeneity at $P < 0.01$ level but not at $P < 0.05$ level. i.e. there is homogeneity at $P < 0.05$ (0.038) (Appendix 11A). Further more, one way ANOVA showed that there is significant difference at $P < 0.05$ and $P < 0.01$ significance levels ($P = 0.000$) (Appendix 11B).

Table 23. Mean plant height (cm) (mean ±SD) for the ex-situ *accessions.*

Accessions (*ex-situ*)	Mean ± SD
wancho	205.8 ± 7.895
gedalech	194.7 ± 3.701
jamuyo	170.7 ± 2.729
grsh	179.7 ± 1.987
zenghada1	181 ± 2.208
mashila1	184 ± 4.373
mashila2	183.1 ± 4.6
mashila3	186.5 ± 4.817
cherekit	184.4 ± 3.616
zenghada2	174.1 ± 3.13
mashila4	178.3 ± 3.384
nech mashila	178.9 ± 4.029
marutie	168.2 ± 2.049

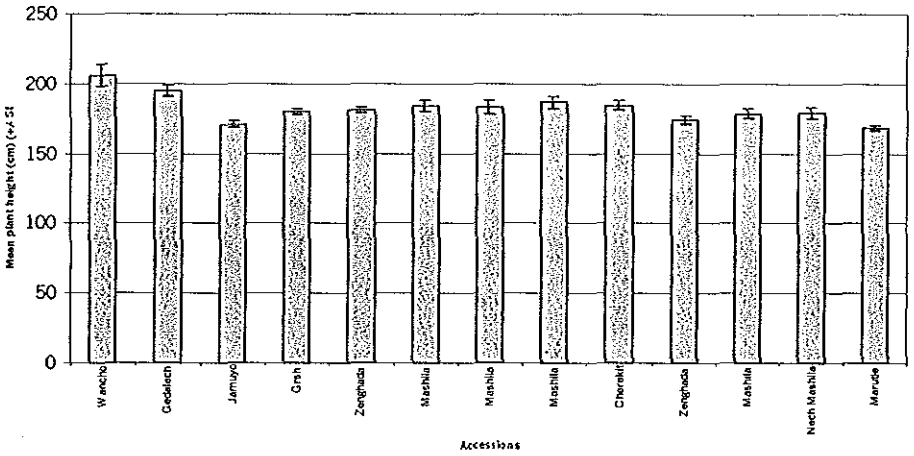


Figure 21. Mean plant height (Mean ±SD) for the ex-situ *accessions.*

B. In-situ *(on-farm) land races*

Here again, *wancho* was the land races with the highest score for mean plant height among the *ex-situ* accession (192 ± 2.775) and the shortest of all was *Ismaili* (158.3 ± 3.21) (Table 24 and Figure 22). 5.049 was the largest deviation that was observed (*jamuyol*) and the smallest deviation observed was 1.121 (*watigela*). To test whether this difference is significant or not, first, homogeneity of variance was carried out and as a result, 0.201 was the P value observed and this shows that there is homogeneity among the variances of the *in-situ* land races. One way ANOVA showed that there is significant difference between the means for the plant heights at both $P < 0.05$ and $P < 0.01$ ($P = 0.000$) (Appendix 11D).

Table 24. Mean plant height (mean ±SD) for the in-situ *(on-farm) conserved land races.*

In-situ (on-farm) land races	Mean ± SD
wanchol	192.2 ± 2.775
delgome	172.2 ± 2.122
jamuyol	165.6 ± 5.049
goronjo	175.3 ± 3.307
dimetie	172.5 ± 1.706
wegerie	178.8 ± 3.732
nech keteto	178.2 ± 4.143
zenghadal	168 ± 1.899

tengeley	185.1 ± 2.537
watigela	177.1 ± 1.121
tuba	171.8 ± 1.242
rayo	171.8 ± 4.869
jemaw	177.9 ± 2.783
jiru	188.6 ± 2.457
mokakie	176.2 ± 1.665
ismalli	158.3 ± 3.21
gubetie	174.1 ± 7.12

Continued...

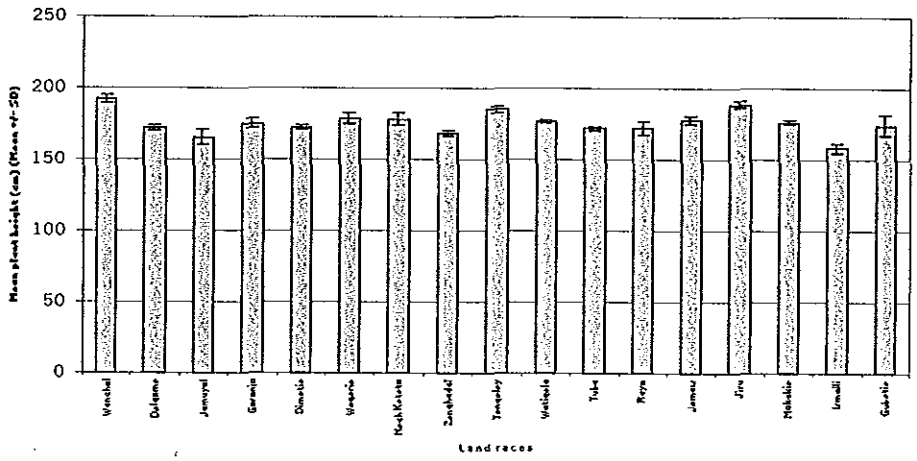


Figure 22. Mean plant height (cm) (mean ±SD) for the in-situ *(on-farm) land races.*

C. Generalizations on mean plant height

Test of homogeneity of variance and one way ANOVA was carried out for comparing *ex-situ* and *in-situ* land races. Accordingly, P = 0.649 was the P value obtained for test of homogeneity of variance using Levene Statistic, and as a result, the variances were observed were found to be homogenous (Appendix 11E). One way ANOVA showed that the difference between the means is in-significant at both $P < 0.05$ and $P < 0.01$ significance levels (P = 0.05) (Appendix 11F).

5.1.12. Statistical analysis

Based on the means of each character scored (for which χ^2 test was done to see the results whether they fit the expectation to fit to a given distribution and for which there was no significant difference between the expected and actual readings, given in Table 25, and the

covariance analysis was also done for the *ex-situ* accessions and *in-situ* accessions (to see the covariation of some characters, given in Table 26). The covariance matrix has given the relationship between the different characters scored for the *ex-situ* accessions. It can be observed that characters like inter-node length highly co-vary positively with other characters like mean plant height. It was found out that most of the characters co-vary neutrally to each other. The same is also true in the case of covariance table generated by readings from the *in-situ* land races.

Table 25. Chi-square ($\chi 2$) test for the test to see the deviation of results from expected values.

Test Statistics

	DSC	DTG	DTK	ILT	LFA	LFL	LFN	LFW	LSL	PHT	SDI
Chi-Squa	4.846	1.385	.846	.846	.000	.846	2.231	2.231	.846	.000	2.923
df	7	10	11	11	12	11	5	5	11	12	8
Asymp. S	.679	.999	1.000	1.000	1.000	1.000	.816	.816	1.000	1.000	.939

a.8 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 1.6.

b.11 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 1.2

c.12 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 1.1.

d.13 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 1.0

e.6 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 2.2.

f.9 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 1.4.

Table 26. Covariance matrices of the different characters from the ex-situ *data.*

Covariance Matrices

LANDR		DSC	DTG	DTK	ILT	LFA	LFL	LFW	LSL	NLF	PHT	SDI
Total	DSC	2.326	4E-02	-.847	-.218	5.419	1.300	-.134	4E-02	-.247	6.333	1E-03
	DTG	4E-02	7.138	1.535	.390	8.856	1.597	.743	.139	8E-02	8.372	9E-02
	DTK	-.847	1.535	8.852	-.363	7.034	-.944	.436	1.257	.206	-.246	6E-04
	ILT	-.218	.390	-.363	3.828	9.275	1.527	.164	.890	.213	7.860	4E-02
	LFA	5.419	8.856	7.034	9.275	9.931	7.894	3.588	4.919	5.728	2.246	.997
	LFL	1.300	1.597	-.944	1.527	7.894	6.477	4E-02	2E-03	.191	6.205	2E-02
	LFW	-.134	.743	.436	.164	3.588	4E-02	.847	-.109	0E-02	-.110	2E-02
	LSL	4E-02	.139	1.257	.890	4.919	2E-03	-.109	1.655	-.247	-.350	0E-02
	NLF	-.247	8E-02	.206	.213	5.728	.191	0E-02	-.247	.650	1.007	9E-03
	PHT	6.333	8.372	-.246	7.860	2.246	6.205	-.110	-.350	1.007	4.431	.121
	SDI	1E-03	9E-02	6E-04	4E-02	.997	2E-02	2E-02	0E-02	9E-03	.121	0E-03

a.The total covariance matrix has 64 degrees of freedom.

Discriminant analysis was used and various outputs generated are described below. Eigenvalues (Table 27) generated showed that the highest percentage of variance was contributed by function 1, determined mainly by the character plant height (56.7 % of the variance and a cumulative of 56.7 %), as one can observe from Table 28, giving an absolute correlation with function 1 of 0.393. The second highest character contributing to the variation contributes 26.5 % of the variance, with a cumulative percentage of 83.3. This character can be identified from Table 28, and it has got a correlation of 0.620 with function 2 (the character is days to knee height stage).

Table 27. Eigenvalues for the different morphological parameters for the ex-situ *accessions.*

Eigenvalues

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	27.717[a]	56.7	56.7	.982
2	12.966[a]	26.5	83.3	.964
3	2.893[a]	5.9	89.2	.862
4	2.205[a]	4.5	93.7	.829
5	1.299[a]	2.7	96.4	.752
6	.938[a]	1.9	98.3	.696
7	.558[a]	1.1	99.4	.598
8	.277[a]	.6	100.0	.466

a. First 8 canonical discriminant functions were used in the analysis.

Table 28. Functions from the canonical discriminant analysis for the characters scored.

Structure Matrix

	Function							
	1	2	3	4	5	6	7	8
DTK	-.206	.620*	-.019	.278	.128	.521	.344	.300
ILT	.185	.099	.744*	-.460	-.141	.222	.068	.341
LSL	-.031	.170	.604*	.098	.465	-.275	.336	-.438
DTG	.168	.115	.069	.778*	-.231	.494	-.021	-.224
LFW[a]	.037	-.018	-.032	.384*	.130	.083	-.135	.319
LFA[a]	.113	.010	-.078	.326*	.224	.130	-.009	.251
SDI	.180	.049	.055	.357	.679*	-.067	-.001	.608
PHT	.393	.370	-.175	-.206	-.516	-.604*	.030	-.058
NLF[a]	.028	.018	-.017	-.224	-.057	.254*	-.063	-.005
DSC	-.067	-.219	.173	.280	-.276	-.042	.772*	.408
LFL	.316	.102	-.269	-.323	.344	.255	.581*	-.439

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions
Variables ordered by absolute size of correlation within function.

*. Largest absolute correlation between each variable and any discriminant function

a. This variable not used in the analysis.

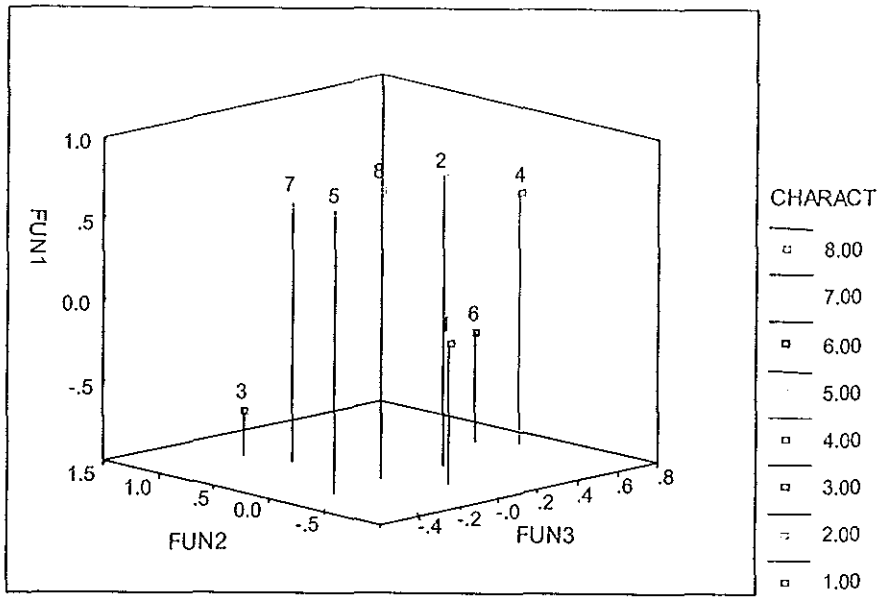


Figure 23. Three-dimensional chart showing the role of each character in generating the function (based on ex-situ data (N.B. The characters asterisked in the previous table are not in the graph).

Fig. 23 gives the summary of the characters contribution towards significance of the selected three functions –function 1, function 2, and function 3. Character 2 (days to knee height stage) and characters 4 (leaf number) are the characters that have led to the simultaneous significant contribution of the three functions.

The same analysis was carried out for the *in-situ* (on-farm) land races. Covariance matrix produced (Table 29) that, according to the *in-situ* land races, both positively co-varying (leaf width and leaf area , which is approx. 48), neutrally co-varying (stem diameter and plant height (0.112)) and negatively co-varying (leaf area and days to germination (approx. –13) characters can be scored.

Table 29 Covariance of the different characters as scored from in-situ *land races data.*

Covariance Matrices

LANDR/		DSC	DTG	DTK	ILT	LFA	LFL	LFW	LSL	NLF	PHT	SDI
Total	DSC	3.907	.711	1.092	.488	2.993	.427	02E-04	-.592	58E-03	2.400	44E-02
	DTG	.711	2.434	.707	69E-02	13.299	-.539	-.197	-.207	35E-02	-.967	98E-05
	DTK	1.092	.707	6.180	1.954	43.664	1.510	.674	.740	37E-02	10.296	19E-02
	ILT	.488	69E-02	1.954	7.312	49.108	1.470	.791	.931	70E-02	12.858	67E-02
	LFA	2.993	13.299	43.664	49.108	68.496	26.136	47.356	15.777	1.222	93.011	.167
	LFL	.427	-.539	1.510	1.470	26.136	2.093	.258	.584	57E-02	5.706	56E-04
	LFW	02E-04	-.197	.674	.791	47.356	.258	.901	.242	37E-02	3.125	07E-03
	LSL	-.592	-.207	.740	.931	15.777	.584	.242	1.468	69E-02	2.780	72E-02
	NLF	58E-03	35E-02	37E-02	70E-02	1.222	57E-02	37E-02	69E-02	.426	1.069	44E-03
	PHT	2.400	-.967	10.296	12.858	93.011	5.706	3.125	2.780	1.069	70.044	.112
	SDI	44E-02	98E-05	19E-02	67E-02	.167	56E-04	07E-03	72E-02	44E-03	.112	00E-03

a.The total covariance matrix has 84 degrees of freedom.

The Eigenvalue calculated from the data for the *in-situ* land races have produced 6 functions and their relative importance towards the variation produced in the *in-situ* (on-farm) land races. Function 1 is responsible for the 34.4 % of the variation observed, with a cumulative variance of 34.4 % (Table 30). Going to Table 31, one can read that the character that has got a determinant absolute correlation value with function 1 is inter-node length, with an absolute correlation value of 0.870. The second most important morphological value contributing towards the variability among the different land races is, based on the Eigenvalue for function 2 (with a percentage variance contribution of 26 and a cumulative percentage of 60.4) is leaf width, having an absolute correlation value of -0.157.

*Table 30. Eigenvalues producing 6 functions (*in-situ*) for the different characters scored..*

Eigenvalues

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	10.379[a]	34.4	34.4	.955
2	7.837[a]	26.0	60.4	.942
3	6.821[a]	22.6	83.1	.934
4	2.878[a]	9.5	92.6	.861
5	1.584[a]	5.3	97.9	.783
6	.646[a]	2.1	100.0	.627

a. First 6 canonical discriminant functions were used in the analysis.

Table 31. Structure matrix of the functions produced as correlated to the characters.

Structure Matrix

	Function					
	1	2	3	4	5	6
ILT	.870*	-.124	-.272	-.380	.096	-.023
LSL[a]	.329*	.232	.046	-.031	.065	.125
NLF[a]	-.136*	.084	-.062	-.058	-.112	.100
LFW[a]	-.040	-.157*	.131	.089	.150	-.108
DSC	.182	-.506	.714*	.074	.194	.397
LFA[a]	.004	-.132	.156*	.079	.054	-.034
PHT	.630	.209	.103	.714*	-.012	-.197
LFL	.310	.229	.214	-.090	-.727*	.519
DTK	.361	.504	.502	-.188	.573*	-.017
DTG[a]	.069	-.178	.012	.022	.300*	.078
SDI	.165	.165	-.351	.271	.467	.728*

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions
Variables ordered by absolute size of correlation within function.

*. Largest absolute correlation between each variable and any discriminant function

a. This variable not used in the analysis.

The simultaneous contribution of the different characters as related to the first three most important functions in the generation of variability between the land races, is given graphically in Fig. 24.

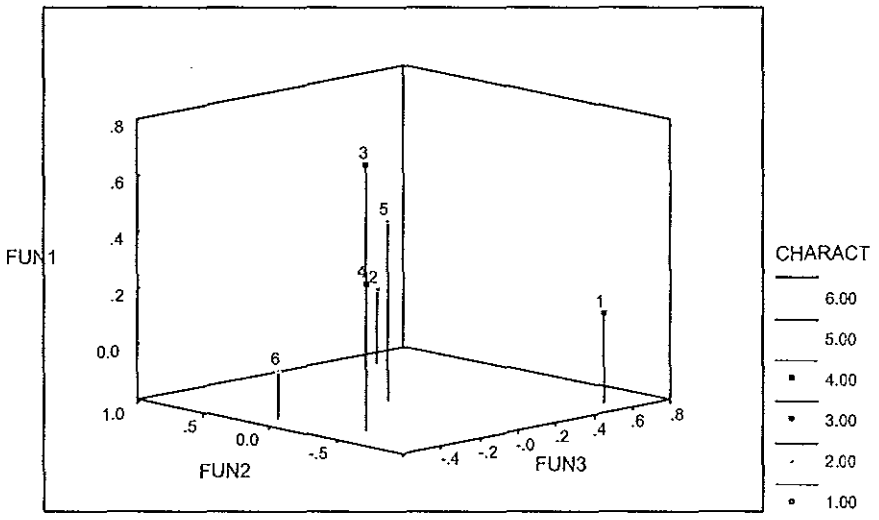


Figure 24. Three-dimensional representation of the simultaneous contribution towards the variability generating functions.

According to the result given in Fig. 24, function 5 (leaf length and days to knee height), function 2 (leaf width) and function 4 (plant height) are characters that are simultaneous determinants contributing to the significance of the first three functions in showing variability among the different land races.

Distance matrix was calculated to see the difference or similarity between the different land races or accessions. This distance matrix can also be calculated from diversity indices, like Shannon-weaver diversity index (Shannon, 1968). But whichever way one follows to calculate the distance, be it Euclidean (Rolf, 1970) Mahalanobis (Amsalu Ayana and Endashaw Bekele, 1999), the relative positioning of the given land races (accessions) will be identical (Rolf, 1970). Due to the easy accessibility of SPSS program for windows (Version 7.5), Euclidean distance measurement was used. The distance from the centroid (Predicted value) of the different accessions and land races is given in Table 32 (the distance calculation is based on Mahanlobis distance matrix). The covariance matrix (Table 33), generalized means for the different characters for *ex-situ* and *in-situ* (Table 34 and 35 respectively) are given sequentially. The Euclidean distance matrix measurement result generated is given on Table 36 and Table 37. Accessions (land races) are given in the first column, while the different land races (accessions) are given on the first row.

Distances between the different accessions (land races) were plotted on a dendrogram produced by the same statistical package mentioned above (Fig 25 and 26). Dendrogram was also constructed combining both the *ex-situ* and *in-situ* accessions and land races (Fig. 27). According to the dendrogram produced (for the *ex-situ* accessions and *in-situ* land races) the result was as follows:

Ex-situ accessions: *mashila3* and *cherekit* were the first accessions to be brought together into one cluster (re-adjusted scale of 1). *jamuyo* and *marutie*, *zenghada1* and *marutie* joined each other at about the same distance, 2.5. *zenghada1* group was clustered with *zenghada2* at a distance of about 4. *grsh* joined the *jamuyo* group, *mashila2* clustered into the *mashila3* group, *gedalech* with *mashila1* – all were clustered with the respective groups mentioned at a distance of 5. The *mashila3* group joined the *jamuyo* group at a distance of 10, taking along the *zenghada1* group at a distance of 17, and ultimately joined by the *gedalech* group at a distance of 25. Thus, accessions like *mashila3* are less related to other accessions like *wancho* or *gedalech*.

In-situ (on-farm) accessions: *jemaw* and *dimetie*, *zenghada1* and *tuba* –clustered together at a distance of 1.5. *Wancho1, jiru, tengeley, delgome,* and *nech keteto* all came to a single cluster at distance of 4. The first two clusters (the Dimetie group and *zenghada1* group came as one at a distance of 12.5, joining the *jamuyo1* group at a distance of 22, ultimately joined by the *wancho1* group at a distance of 25.

Comparing *ex-situ* and *in-situ*: most of the *ex-situ* accessions were grouped together and the same was true for the *in-situ* land races (Fig. 27). But it was also observed that some accessions and land races seem to be related. For example, *zenghada1* and *wegerie* were related at a distance of 0. They all came to one cluster at a higher Euclidean distance (24) than done for either of the *ex-situ* and *in-situ* independently.

It can be observed from the clustering done and dendrogram constructed based on Euclidean distance, the number of independent clusters at a distance of 0 is 1 for the *ex-situ* while it is 4 for the *in-situ*. Similarly, there is a higher distance where all come together for the *in-situ* (22) than for the *ex-situ* (17), producing a lesser cluster under the *ex-situ* condition. i.e. the number of clusters observed at a longer Euclidean distance is higher for the *in-situ* land races than *ex-situ* accessions. This can account for the higher diversity at

the *in-situ* level than at the *ex-situ* level. This can be explained by the presence of diversifying selection (Lewontin, 1974) pressure under the field condition (to which the *in-situ* land races are exposed) which is not present under the artificial gene bank. This is to say that the presence of diversifying selection tends to let organisms adapt to their immediate environment, during which it applies different selection pressures to the different ecosites (under agricultural scheme), producing different morphotypes with different morphological and biochemical properties, leading to diversity.

Table 32.Highest group, second highest group, and mahanolbis distance to centroid (for both) for ex-situ versus in-situ.

Casewise Statistics

	Case Number	Actual Group	Highest Group					Second Highest Group			Discriminant Scores
			Predicted Group	P(D>d | G=g)		P(G=g | D=d)	Squared Mahalanobis Distance to Centroid	Group	P(G=g | D=d)	Squared Mahalanobis Distance to Centroid	Function 1
				p	df						
Original	1	1	1	.008	1	1.000	7.045	2	.000	35.421	-4.523
	2	1	1	.727	1	.986	.122	2	.014	8.692	-1.519
	3	1	1	.868	1	.997	.028	2	.003	11.998	-2.035
	4	1	1	.103	1	.515	2.659	2	.485	2.778	-.238
	5	1	1	.981	1	.995	.001	2	.005	10.714	-1.844
	6	1	1	.801	1	.990	.063	2	.010	9.276	-1.617
	7	1	1	.995	1	.996	.000	2	.004	10.832	-1.862
	8	1	1	.848	1	.992	.037	2	.008	9.647	-1.677
	9	1	1	.399	1	1.000	.711	2	.000	17.143	-2.712
	10	1	1	.679	1	.999	.172	2	.001	13.775	-2.283
	11	1	2**	.139	1	.635	2.193	1	.365	3.300	-.052
	12	1	1	.788	1	.998	.072	2	.002	12.719	-2.138
	13	1	1	.938	1	.994	.006	2	.006	10.363	-1.790
	14	2	2	.995	1	.996	.000	1	.004	10.911	1.435
	15	2	2	.587	1	.975	.295	1	.025	7.586	.886
	16	2	2	.542	1	.969	.371	1	.031	7.227	.820
	17	2	2	.494	1	.960	.468	1	.040	6.827	.744
	18	2	2	.160	1	.691	1.974	1	.309	3.581	.024
	19	2	2	.695	1	.984	.154	1	.016	8.440	1.037
	20	2	2	.482	1	.958	.495	1	.042	6.728	.725
	21	2	2	.969	1	.996	.001	1	.004	11.127	1.467
	22	2	2	.251	1	1.000	1.319	1	.000	19.765	2.577
	23	2	2	.033	1	1.000	4.559	1	.000	29.511	3.564
	24	2	2	.309	1	.889	1.034	1	.111	5.200	.412
	25	2	2	.150	1	1.000	2.075	1	.000	22.446	2.869
	26	2	2	.604	1	.999	.269	1	.001	14.561	1.947
	27	2	2	.817	1	.991	.053	1	.009	9.401	1.198
	28	2	2	.624	1	.978	.241	1	.022	7.876	.938
	29	2	2	.495	1	1.000	.466	1	.000	15.840	2.112
	30	2	2	.915	1	.997	.011	1	.003	11.587	1.536

**. Misclassified case

Table 33. *Covariance matrices for the different morphological characters (for both* ex-situ *and* in-situ*) (negative result show negative co-variation, and vise versa).*

Covariance Matrices[a]

TYPE		DSC	DTG	DTK	ILT	LFA	LFL	LFW	LSL	NLF	PHT	SDI
Total	DSC	2.578	.130	-.117	.131	-10.365	-.436	-.146	-.340	3.04E-02	-1.712	1.82E-02
	DTG	.130	4.333	2.523	.639	25.652	1.366	.326	-.418	-.156	7.986	.199E-02
	DTK	-.117	2.523	8.643	1.337	47.760	1.412	.764	.361	-.138	11.347	7.38E-03
	ILT	.131	.639	1.337	5.417	44.760	1.743	.663	.751	.403E-02	12.103	.667E-02
	LFA	-10.365	25.652	47.760	44.760	1496.721	42.768	23.970	5.508	.780E-02	215.306	.310
	LFL	-.436	1.366	1.412	1.743	42.768	3.618	.381	6.35E-02	5.71E-02	12.807	.724E-02
	LFW	-.146	.326	.764	.663	23.970	.381	.423	.117	.387E-03	2.617	.015E-03
	LSL	-.340	-.418	.361	.751	5.508	6.35E-02	.117	1.001	1.17E-02	.204	.421E-02
	NLF	3.04E-02	-.156	-.138	.403E-02	.780E-02	5.71E-02	.387E-03	1.17E-02	.145	.656	.328E-03
	PHT	-1.712	7.986	11.347	12.103	215.306	12.807	2.617	.204	.656	88.701	.112
	SDI	1.82E-02	.199E-02	7.38E-03	.667E-02	.310	.724E-02	.015E-03	.421E-02	.328E-03	.112	.233E-03

a. The total covariance matrix has 29 degrees of freedom.

Table 34. Generalized means for each ex-situ *accessions against the 11 metric characters.*

Char.	Accessions												
	wancho	*gedalech*	*jamuyo*	*grsh*	*zenghada*	*mashila*	*mashila*	*mashila*	*cherekit*	*zenghada*	*mashila*	*nech mashila*	*marutie*
DSC	0.2	0.2	0.4	0.6	1.2	0	0.2	0.6	0.2	2.2	2.8	0	3.8
DTG	12	12	6	6	7	10	6.2	7.2	11	10.6	8.2	11	9.8
DTK	45	44	47	40	45	50	48	49	50	43.8	46.4	48.8	45
ILT	8.8	10.2	8.4	8.8	10	9.8	11	11	9.6	10	10.6	12.2	9.4
LFA	337	313	289	304	234	323	261	280	276	250	242.2	344	296
LFL	57	56.8	53	52	51	50	52	52	51	50	52.2	53.2	49
LFN	14	13.4	13.4	14	13	14	13	13	14	13	13.6	13	13
LFW	6.8	6.4	6.4	6.8	5.6	7.4	6	6.4	6.4	6	5.6	7.4	7
LSL	18	16.2	12.8	18	15	16	16	17	14	14.8	18	18.6	14
PHT	206	195	171	180	181	184	183	187	184	174	178.3	179	168
SDI	1.5	1.65	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.51	1.522	1.56	1.5

Table 35. Mean of characters scored for each in-situ *accession.*

Char.	L.races																
	wanchol	*delgome*	*jamuyol*	*goronjo*	*dimetie*	*wegerie*	*nech keteto*	*zenghadal*	*tengeley*	*watigela*	*tuba*	*rayo*	*jemaw*	*jiru*	*mokakie*	*ismaili*	*gubetie*
DSC	6.4	0.2	0.6	0.4	2.6	0.6	0	5	0	0	1.4	2	0	0	0	0	1.166667
DTG	6	5.6	7.8	6.8	6.6	6.6	7	9.8	6.2	5.8	6.8	6.2	7.6	7.4	6.4	5.2	5.8
DTK	46.4	44.8	42.6	43.4	41.2	43.6	45.6	44.8	45.6	38.8	44.2	41.6	42.4	45	47	38.75	42.3333
ILT	9.8	11.8	9.4	10.8	9.8	10.8	11.6	10.2	12	10.8	10.4	11.6	11.6	10.8	10.8	9.25	11.16667
LFA	302.6	328.2	189.8	271.2	267.6	233	319.8	227.6	293.4	244.8	228	260.6	267.6	302.6	266	208.25	270.6667
LFL	53.4	52.6	50	51.8	49.6	52.4	51.4	49.6	52	49.2	49.6	51.8	51.2	50.4	51	49.25	50.6667
LFN	14.2	13.4	13.8	13.6	13.4	13.6	13.6	13.8	14.2	14.2	13.4	13.6	14	14.2	13.8	13.75	13.3333
LFW	6.6	7.2	4.8	6.2	6.4	5.4	7.2	5.6	6.6	6	5.6	6	6.2	7	6.2	5.25	6.333333
LSL	17.4	19.2	11.8	17.4	18.2	17.6	12.6	14.8	17.4	15.6	15.4	13.6	17.8	18.2	14	9.25	14.16667
PHT	192.2	172.24	165.64	175.32	172.48	178.84	178.18	168	185.14	177.08	171.76	171.82	177.94	188.56	176.22	158.275	174.1333
SDI	1.502	1.54	1.512	1.552	1.52	1.582	1.526	1.526	1.574	1.588	1.56	1.538	1.536	1.606	1.564	1.49	1.575

Table 36. Euclidean distance matrix (Suiter et al, *1983) for the* ex-situ *accessions, from which the dendrogram in Fig.25 is constructed.*

Proximity Matrix												
	Euclidean Distance											
Accessions	***wancho***	***gedalech***	***jamuyo***	***grsh***	***zenghada***	***mashila***	***mashila***	***mashila***	***cherekit***	***zenghada***	***mashila***	***nech mashila***
wancho												
gedalech	26.38											
jamuyo	60.48	35.43										
grsh	43.04	19.64	19.71									
zenghada	106.50	80.97	55.96	70.46								
mashila	27.44	16.99	37.37	22.18	89.30							
mashila	79.83	54.19	30.54	43.97	27.55	62.01						
mashila	60.97	35.43	18.92	26.70	46.54	43.28	19.18					
cherekit	65.07	39.38	19.65	30.58	43.13	46.70	16.40	6.94				
zenghada	93.53	67.45	39.98	55.36	17.52	74.40	16.21	33.53	29.49			
mashila	99.16	73.42	47.68	62.44	9.55	81.22	20.08	38.87	35.44	10.06		
nech mashila	28.39	34.77	56.11	40.83	109.88	21.73	82.73	64.23	67.69	94.45	101.54	
marutie	56.88	33.12	10.03	16.45	63.08	32.43	38.14	25.05	26.05	46.44	54.65	49.94
This is a dissimilarity matrix												

Table 35. Euclidean distance (Suiter *et al*, 1983) calculated from the 11 quantitative characters for the *in-situ* (on-farm) land races (see Fig 26).

Proximity Matrix																	
	Euclidean Distance																
	wanchol	*delgome*	*jamuyol*	*goronjo*	*dimetie*	*wegerie*	*nech keteto*	*zenghadal*	*tengeley*	*watigela*	*tuba*	*rayo*	*jemaw*	*jiru*	*mokakie*	*ismaili*	
wanchol																	
delgome	33.22																
jamuyol	116.31	138.86															
goronjo	36.34	57.17	82.22														
dimetie	40.88	60.88	78.43	6.11													
wegerie	71.19	95.48	45.66	38.38	35.46												
nech keteto	23.79	12.39	130.69	48.99	53.12	87.00											
zenghadal	79.07	101.06	38.38	44.71	40.76	13.92	93.01										
tengeley	13.53	37.19	105.69	24.43	29.40	60.79	27.75	68.40									
watigela	60.75	83.92	56.51	27.07	23.73	13.47	75.43	21.37	49.86								
tuba	77.68	100.36	38.93	43.47	39.85	9.44	92.13	6.10	66.89	18.54							
rayo	47.36	67.95	71.20	12.08	8.92	28.89	59.74	33.83	35.90	17.35	32.86						
jemaw	38.68	60.99	79.05	4.79	6.74	34.69	52.57	41.81	27.03	23.37	40.28	10.55					
jiru	8.30	30.54	115.34	34.18	38.83	70.35	20.92	78.06	10.15	59.35	76.56	45.68	36.70				
mokakie	40.68	62.63	77.14	7.31	8.82	33.52	53.89	39.84	29.10	22.89	38.45	9.12	6.58	38.93			
ismaili	101.19	121.41	20.59	65.98	61.81	33.81	113.64	24.16	90.02	41.63	25.41	54.49	63.34	99.75	61.28		
gubetie	37.56	57.89	81.41	4.05	5.95	38.20	49.48	43.96	25.77	26.40	42.83	10.49	6.53	35.45	7.08	64.75	
This is a dissimilarity matrix																	

* * * * * * H I E R A R C H I C A L C L U S T E R A N A L Y S I S * * * * * *

Dendrogram using Average Linkage (Between Groups)

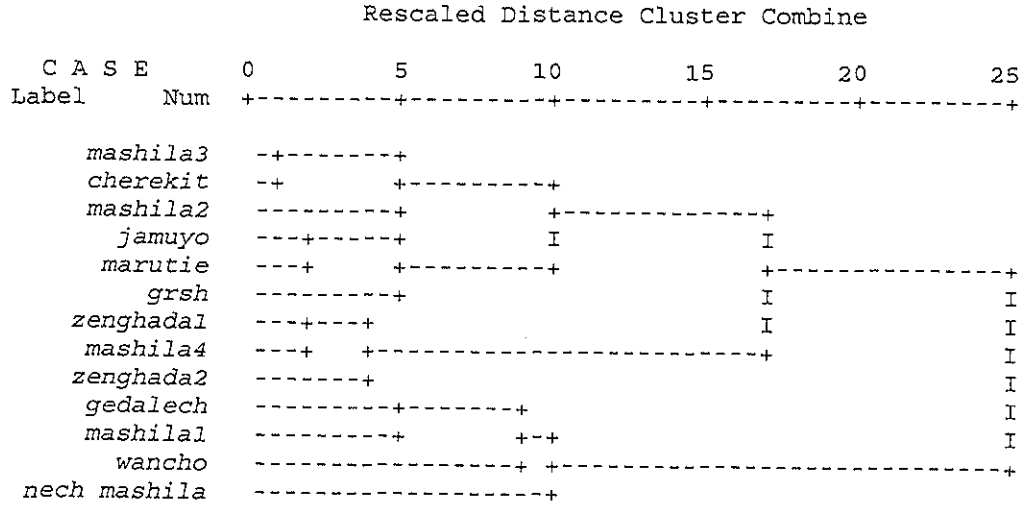


Figure 25. Dendrogram (on a rescaled scale) for the ex-situ *accessions, calculated from the distance of dissimilarity matrix.*

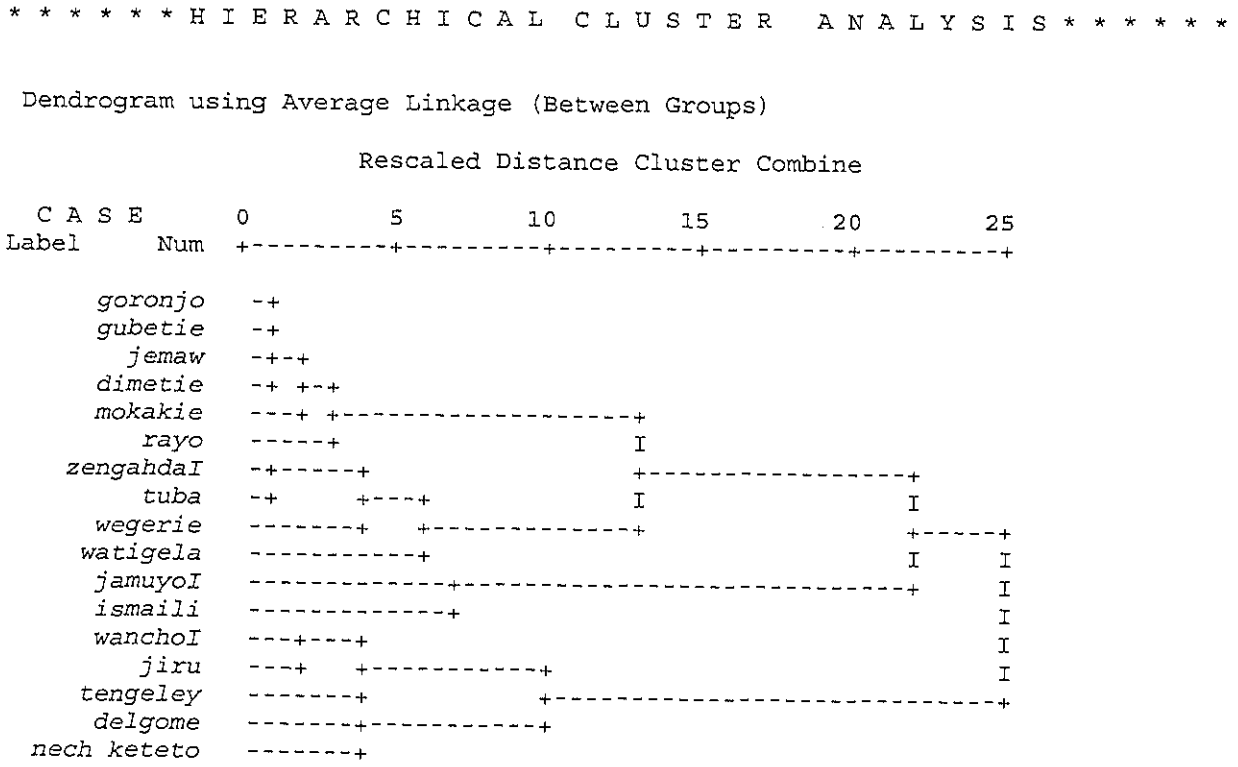


Figure 26. Dendrogram showing the relations ship between the in-situ *(on-farm) land races.*

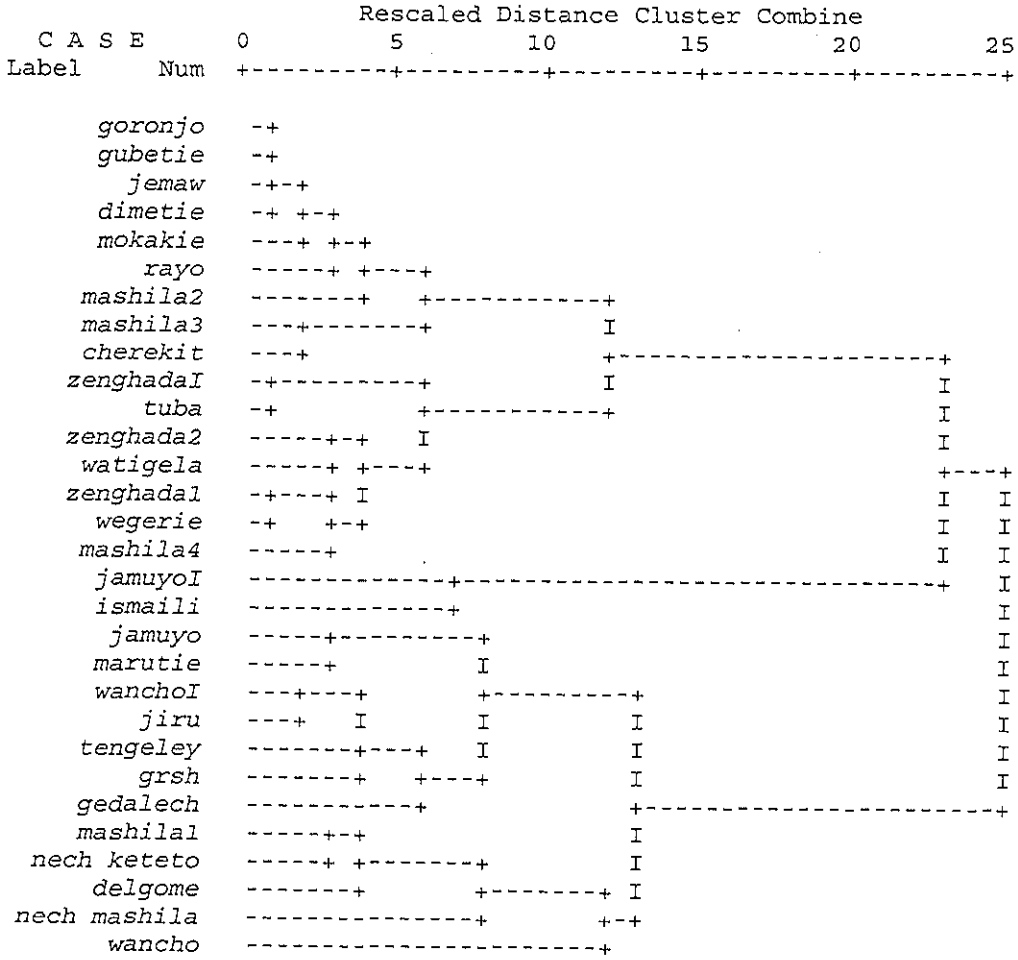


Figure 27. Combined dendrogram for both the ex-situ and in-situ accessions and land races.

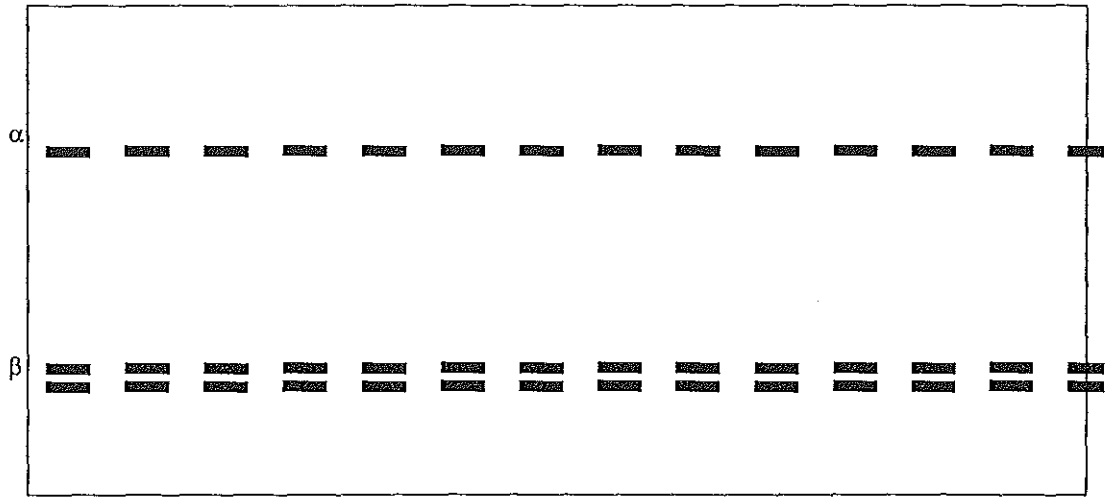
5.2 BIOCHEMICAL STUDY

Bands were observed for both assay systems. The relative migration of the bands were observed (both the *ex-situ* accessions and *in-situ* land races were run on the same gel frame and tested for their isozyme activity).

Esterase (α and β): two bands were observed for both the *ex-situ* accessions and *in-situ* land races. Both bands were observed to be identical throughout the loaded sample, be it an *ex-situ* sample or *in-situ* sample. The upper band (α) was blur and was difficult to score, but all the bands lie on the same line. This shows that there is no variation in the relative migration of the enzymes for the compared land races and accessions (Fig. 28).

Acid Phosphatase (ACP): Bands were more faint than the esterase, but it was observable that there was no difference in the relative migration of the bands, for both the *ex-situ* accessions and *in-situ* land races (Figure 29). The bands were observed to be two, for all of the cases.

Esterase - Lane 1 to Lane 15 (*ex-situ* and *in-situ* accessions were run on the same gel).



Esterase - Lane 15 to Lane 30 (*ex-situ* and *in-situ* combined).

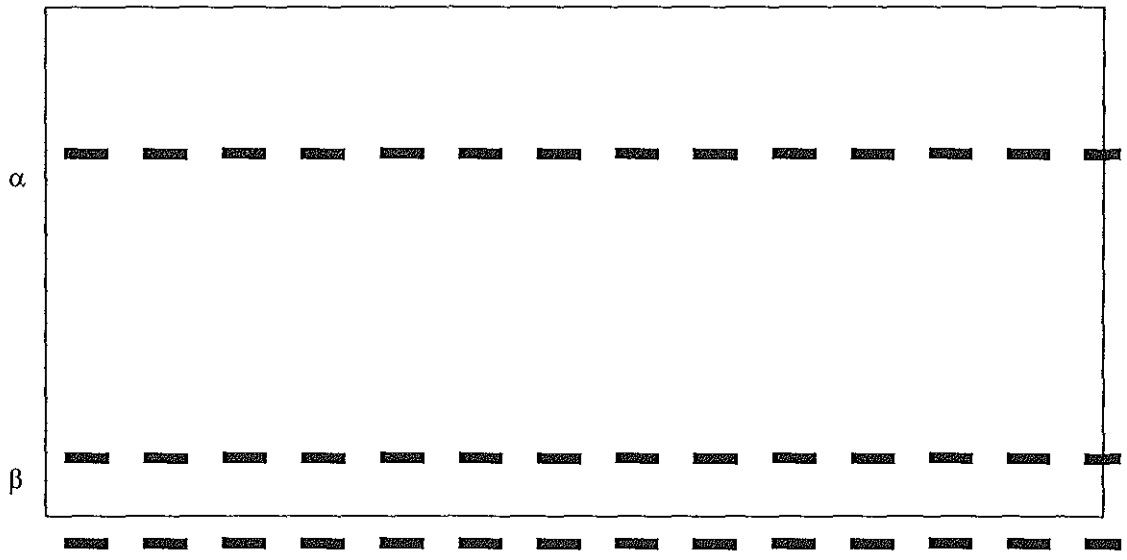
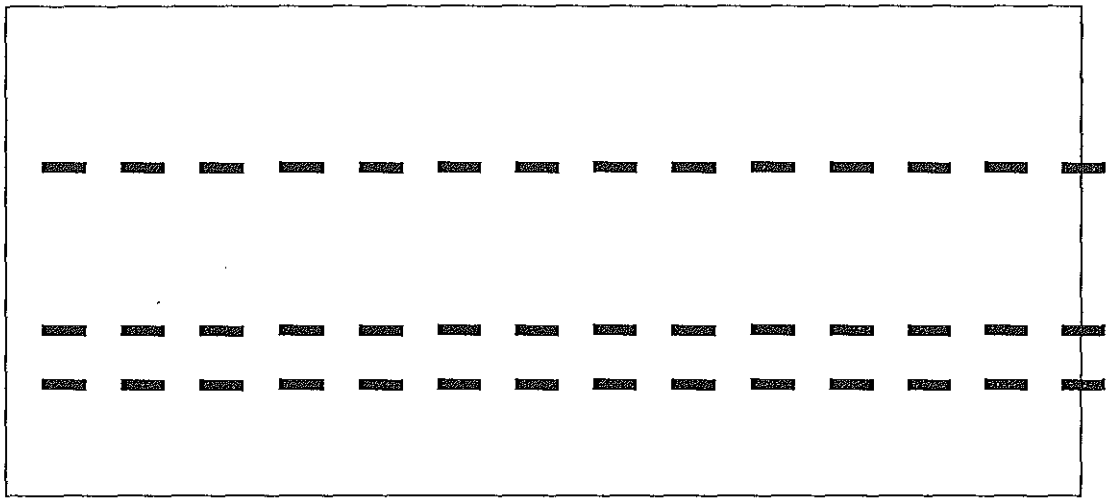


Fig 28. Isozyme bands (esterase) observed for both the ex-situ *accessions and* in-situ *land races.*

Acid Phosphatase (ACP): Lane 1 to Lane 15 (*ex-situ* and *in-situ* combined).



Acid Phosphatase (ACP): Lane 15 to Lane 30 (*ex-situ* and *in-situ* combined).

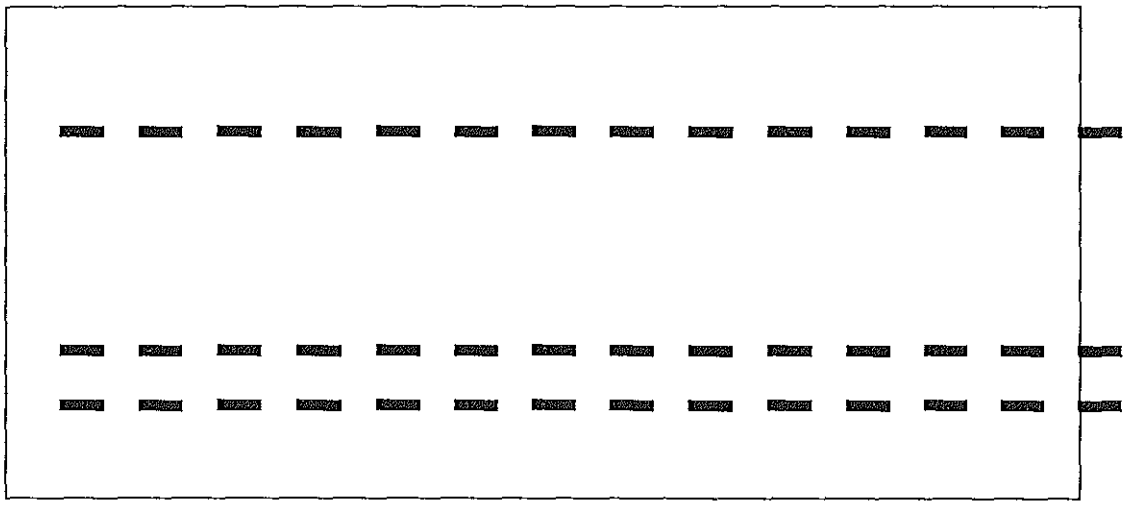


Fig 29. Isozyme bands for acid Phosphatase (Acp) for both in-situ *and* ex-situ *accessions.*

6. DISCUSSIONS

6.1. MORPHOLOGICAL STUDY

A number of works have been done on sorghum characterization and evaluation (Rao and Rao, 1995; Amsalu Ayana and Endashaw Bekele, 1999). Generally, quantitative and qualitative data have been generated, and most of the works revealed that there was little or no variation at the morphological level (Rao and Rao, 1995). This goes in line with some of the results obtained in this study. Significant differences obtained in this work include days to germination, days to knee height stage, disease scoring, leaf length, leaf width, leaf area, and plant height. Presence of purely or distinctly different morphotypes under Ethiopian condition was mentioned by Awgechew Teshome *et al.* (1999), remarking the presence of more than 60 to 70 different land races. On the contrary to the general conclusion that there is variation for most of the morphological characters, there was a character, mean leaf number, for which no variation was observed. It was observed here that there was no variation among the different land races and accessions studied, as far as the mean leaf number is concerned.

Amsalu Ayana and Endashaw Bekele (1999) have done detailed morphological variation assessment. They have taken regions as a classification factor and compared the land races of sorghum from all over Ethiopia for their morphological variation, using statistical tools like discriminant analysis and different scaling methods. The present work compares the land races and accessions at *in-situ* and *ex-situ* using morphological and biochemical characters and compare the two for any differences shown and account for these differences.

It has been pointed out by (Doggett, 1965; Doggett, 1970) that the inherent ability to germinate depends on the genetic make up (heterozygote condition for enzymes required during germination, like hydrolase, give advantage) and also the prevailing environmental condition. In addition, it has been reported by Frankel (1978) that with prolonged storage of genetic resources in the gene bank (*ex-situ* conservation), materials tend to:

1. Loss of germination viability and vigor
2. Chromosome breakage and fusion
3. Increment of interference of radical effects

Thus, the three factors can account for differential germination capacity of the different *ex-situ* accessions observed in the course of this study, i.e. the *ex-situ* accessions themselves can have, inherently, different response to prolonged storage exposure. Thus, the accession scoring the earliest days to germination, *jamuyo* (for both the *ex-situ* and *in-situ* land races), is a land race found dominantly in the Hayk area, Welo, which is one of the drought stricken areas. This area, as found from the interview with the farmers, is a drought stricken area. Thus, one can assume that land races found in those areas should be characterized by their early maturity and drought tolerance. Allard (1988) has shown that heritablility of certain characters in cultivated crops or any other organism increases with the persistence of certain environmental influence. This could explain, in a certain way, the condition observed in most of the cases in this study. *Wancho* and *gedalech* showed the longest days to germination. Wancho is a land race, which is not planted intentionally by the farmer, and is thus almost a wild relative of the crop. Such wild progenitors, as to Allard (1988), are bridge to gene flow between the differently co-evolved land race and its extreme wild progenitors. *Gedalech* is an accession which is not currently grown in the area but may be related to one of the land races, for example, *jiru* (compare 1.65 ± 0.067 to

1.606 ± 0.03 mean stem diameters of the accession and land race, respectively). Similar explanation can be given to days to knee height stage.

Disease scoring conducted did not follow intentional inoculation (Agrios, 1987). i.e. the prevailing disease was seed borne mosaic virus, which is a common seed borne virus in most cereals and pulses (Agrios, 1987). Its severity might vary from plant to plant and also the level of infection (Agrios, 1987). Different performances were observed by the different land races and accessions, but this performances might not necessarily be due to the inherent disease resistance capacity of the accession or land race, because the level of infection has not been factored out. This by itself requires independent work.

The other metric characters, excluding mean leaf number, were observed to be inter-related. For example, accessions (land races) which are observed to have longer inter-node length are also plants with longer plant height.

Further analysis of metric characters has revealed important information in disclosing each metric character's contribution towards the significant variability observed, as given in the ANOVA tables in the appendix. The tests were independently done for the *ex-situ* accessions and *in-situ* land races. Primarily, χ^2 test was carried out to test the deviation of scorings from the predicted χ^2 values, and it was found that there is no significant difference from the expected values for all characters. This is an important feature pertinent in such cases, where one should test the appropriateness of the readings. Some authorities (Berhane Gebrekidan, 1981; Blixt, 1988) have applied this test and have put it as a pre-requisite for tests of homogeneity, test of model fitness. Berhane Gebrekidan (1981) has used it in testing the fitness of character scorings onto filial generation's

segregational character, to test fitness of data onto Mendelian characters on a given sorghum breeding scheme.

Covariance analysis (Falconer, 1965; Nei, 1965) was conducted to see the co-variation of the morphological characters. Characters like plant height and inter-node length were observed to co-vary positively. Covariance analysis has been implemented widely in plant breeding and diversity assessment (Harlan, 1972; Harlan, 1975). Harlan (1975) has studied these characters and similar results were obtained.

Discriminant analysis was adopted (Fisher, 1936; Amsalu Ayana and Endashaw Bekele, 1999) and in this study, the Eigenvalues were calculated for each character and each function. Based on the Eigenvalues, the structure matrix produced the absolute correlation coefficient, to see the correlation between the accession and land races. As a result, for the *ex-situ* accessions, it was observed that (from the 3D graph produced) the character days to knee height was placed as a simultaneous determinant of the three given functions. Next to plant height, the simultaneous determinant of the first three functions is days to knee height stage. Thus, these metric characters have determined the variability observed. As to the *ex-situ* data, leaf length and days to knee height, leaf width and plant height are noted to have determinant of the variability noted. As can be observed from *ex-situ* accessions and *in-situ* land races, plant height and days to knee height are the major contributors towards the variability observed. This supports the removal of disease scoring from the analysis due to the heterogeneity in the variances observed for this character and this character contributes little to the overall observed variability. Amsalu Ayana and Endashaw Bekele (1999) have concluded based on discriminant analysis result that discrimination of accessions was more

they were able to observe little variation for the 16 enzyme systems out of the 19 enzyme systems they assayed. Doelbey *et al.* (1986) has found no variation for the maltate dehydrogenase (MDH) chloroplast isozymes. Morden *et al.* (1987) have worked on sub-cellular localization of aconitase (ACO), and has observed little variation.

Esterase (EST) has shown no variation both at α and β position. Esterase have been widely adopted on the gramineae family (like wheat and barley) (Linde-Laursen *et al.*, 1987). Good variation is observed in such crops like wheat. Little is known about sorghum behavior in esterase isozyme, except for the work done by Morden *et al.* (1987) and Morden *et al.*, (1988).

The analysis of acid phosphatase (ACP), the other enzyme system used here, had showed no variation. As described by Morden *et al.* (1988), when they have developed a protocol for sorghum isozymes, they haven't used acid Phosphatase for analysis of variation of sorghum. Thus, the present work has proven the identical banding pattern for phosphatase isozymes for sorghum collected form North Shoa and South Welo. i.e. for the enzyme systems assayed, no variation in banding pattern was observed.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

Generally, the work conducted on the morphological characters has shown that there is genetic variation among the different land races (*in-situ*) and accessions (*ex-situ*). The variation is not significant as such, and this may be due to the selfing nature of the crop and/or of the similarity in the natural environment (Wright, 1965). Green house condition has reduced (minimized) the effect of environmental influence on the expression of certain morphological characters. Thus, whatever can be observed as different or variation (range) of morphological characteristics are mainly genotypic (Falconer, 1965), i.e.

$$V_P = V_G + V_E$$

Under the above condition, if the variation that occurs due to environmental effect is minimized, then,

$$V_P \approx V_G$$

Overall, there is no land race or accession that can be identified as a quality land race or accession, performing best in all characters scored. *Wancho* and *wanchoI* are land race and accession, respectively, observed to be best performers in plant height. Fast days to germination and days to knee height stage can be given to the land races collected from the drier areas, and this are *isamaili, jamuyo* and *jamuyoI*. Good stem thickness goes to accessions like *grsh*. One can thus recommend the various accessions to be considered in the breeding program to enhance performance.

Biochemical data has shown, on the contrary, that there is no variation either for the *ex-situ* and *in-situ* or comparatively between *ex-situ* and *in-situ* land races. The enzymes assayed (α and β esterase) and acid Phosphatase are metabolic pathway enzymes (Kahler, 1983).

7.2. Recommendations

Based on the results of this study, it was observed that there is higher diversity (from the number of the clusters formed) in the field (*in-situ*) compared to the *ex-situ* accessions, and thus, the area is an area recommendable for *in-situ* conservation strategy.

The *ex-situ* accessions were observed to have a differential germination capacity, which could be due to the prolonged storage and even the storage condition itself. This asks for certain studies to improve the storage condition and keep the viability and vigorousity for a long time.

To do perfectly well designed comparative study, to compare gene bank seeds against field land races for temporal genetic differentiation, it is quite clear that one needs to observe them (both being of the same original identity), on a given time scale, after which one can decide whether *ex-situ* conservation arrests evolution or not. Thus, based on the present results, it would be very difficult to decide whether there is temporal genetic differentiation or not, if one doesn't apply the above time scale follow up. But the present study and the results obtained can be used in comparison with the future similar studies to be conducted to see the exact magnitude of differences of *ex-situ* and *in-situ* genetic resources.

8. REFERECES

Agrios, (1987). *Plant Pathology*. John Wiley Publishers, New York, USA.pp: 315-316.

Allard, R. W. (1988). Genetic changes associated with the evolution of adaptedness in cultivated plants and their wild progenitors. *Journal of Heredity* **79**: 225 – 238.

Allard, R. W. and Adams, J. (1969). The role of intergenotypic interactions in plant breeding. *Proceedings of the XII International Congress on Genetics*. **3**: 349 – 370.

Amsalu Ayana and Endashaw Bekele (1999): Multivariate analysis of morphological variation in sorghum (*Sorghum bicolor* (L.) Moench) germplasm from Ethiopia and Eritrea. *Genetic Resources and Crop Evolution* **46**: 273 – 284.

Anderson, J. W. (1968). Extraction of enzymes and subcellular organelles from plant tissues. *Phytochemistry* **7**: 1973 – 1988.

Anderson, J. W. and K. S. Rowan (1967): Extraction of soluble leaf enzymes with thiols and other reducing agents. *Phytochemistry* **6**: 1047 – 1056

Arulsekar, S. D. E. Parfitt, W. B. and Hansche, P. E. (1986). Genetics of malate dehydrogenase isozymes in the peach. *J. Heredity* **77**: 49 – 51.

Awgechew Teshome, Fahrig, L., Torrance, J. K., Lambert, J. D., Arnason, T. J. and Baum, B. R. (1999). Maintenance of sorghum (*Sorghum bicolor*, Poaceae) landrace diversity by farmers' selection in Ethiopia. *Economic Botany* **53**: 79 – 88.

Baraka, T. (1960). A simple azo-dye method for histochemical demonstration of acid phosphatase. *Nature* **187**: 248 – 249.

Barbault, R. and Sastrapradja, S. D. (1995). Generation, maintenance and loss of biodiversity. In: *Global biodiversity assessment*. Watson, R. T. and Heywood, V. H. (Eds.). Cambridge University Press, Cambridge, England. 750 pp.

Berhane Gebrekidan (1981). Salient features of the sorghum breeding strategies used in Ethiopia. *Eth. J. Agric. Sci.* 3: 97 – 104.

Berhane Gebrekidan and Abraham Menkir (1979). *Ethiopian Sorghum improvement Project Progress Report No. 5.* pp.: 60. College of Agriculture, Addis Ababa University, Addis Ababa, Ethiopia.

Blixt, S. (1988). Genetic resources at work: practical examples of the importance of gene bank work regarding agriculture, horticulture and forestry. *Hereditas* **108**: 271 – 273.

Brown, A. H. D. (1983). Barley. In: *Isozymes in plant genetics and breeding*, Part B. Tanksley, S. D. and Orton, T. J. (eds.).pp: 245-248. Elsevier, Amsterdam, pp. 57 – 77.

Cardy, B. J., Stuber, C. W., Wendel, J. F., Goodman, M. M. (1983). *Techniques for starch gel electrophoresis of enzymes from maize (Zea mays L.).* pp: 170-171. Institute of Statistics Mimeograph Series No. 1317, North Carolina State University, Raleigh, NC.

Cheliak, W. M. and Pitel, J. A. (1984). Genetic control of allozyme variants in mature tissues of white spruce trees. *J. Heredity* **75**: 34 – 40.

deWet, J. M. J., Harlan, J. R. and Kurmorohita, B. (1971). Origin and domestication of *Sorghum bicolor*. *Econ. Botany* **25**:128 – 135

Doggett, H. (1965). The development of cultivated sorghums. In: *Essays on Crop Evolution*. Hutchinson, S. J. B. (Ed). pp: 50 –69. Cambridge University Press, Cambridge.

Doggett, H. (1988). *Sorghum*, 2nd edition. Longman, UK. 550 pp.

Doggett, H. (1991). Sorghum history in relation to Ethiopia. In: *Plant Genetic Resources of Ethiopia*. pp: 55-60. Engles, J. M. M., Hawkes, J. G. and Melaku Worede (Eds.). Cambridge University Press, Cambridge.

Doggett, H. and Prasada Rao, K. E. (1995). Sorghum. In: *Evolution of Crop Plants*. 2nd Ed. Smartt, J. and Simmonds, N. W. (Eds.). pp.: 173 – 180. Longman, UK.

Doebley, J., Morden, C. W. and Schertz, K. F. (1986). A gene modifying mitochondrial malate dehydrogenase isozymes in *Sorghum* (Graminae). *Biochem. Genet.* **24**: 813 – 819.

El-Kassaby (1981). Genetic interpretation of malate dehydrogenase isozymes in some conifer species. *J. Heredity.* **72**:451 – 452

Ellstrand, N. C. and Marshall, D. L. (1985). The impact of domestication on distribution of allozyme variation within and among cultivars of radish, *Raphanus sativus* L. *Theor. Appl. Genet.* **69**: 393 – 398.

Endashaw Bekele (1984). Analysis of regional patterns of phenotypic diversity in the Ethiopian tetraploid and hexaploid wheats. *Hereditas* **100**: 131 – 154.

Endashaw Bekele (1996). Morphological analysis of *Eragrostis tef*: detection for regional patterns of variation. *Sinet: Eth. J. Sci.* **19**: 117 – 140.

Falconer, R. (1965). *Quantitative Genetics*. Longman, UK.

Farinelli, M. P., Fry, D. W. and Richardson, K. E. (1983). Isolation, purification, and partial characterization of formate dehydrogenase from Soya bean seed. *Pl. Physol.* **73**: 858 – 859.

Fildes, R. A. and Harris, H. (1966). Genetically determined variation of adenylate kinase in man. *Nature* **209**: 262 – 263.

Fisher, R. A. (1936). The use of multiple measurements in taxonomic problems. In:*Multi variate statistical methods:Among groups covariation.* Atcheley, W. R. and Bryan, E. H. (eds.). Dowden, Hutchinson and Ross, Inc., Pennsylvania, USA.

Frankel, O. H. (1978). Conservation of plant genetic resources. *Biol. Conserv.* 14: 178 – 182

Frankel, O. H. (1984). Genetic perspectives of germplasm conservation. In: *Genetic Manipulation: Impact on Man and Society.* K. Limensee, W. K., Peacock and Starlinker (Eds.).pp.: 161 – 170. Cambridge University Press. Cambrige.

Gastony, G. J., Gotlieb, L. D., Lee, C. N. and Johnson, F. M. (1980). Genetic control of malate dehdrogenase isozymes in maize. *Genetics* **94**: 153 – 168.

Goodman, M. M. (1968). The races of maize: II. Use of multivariate analysis of variance to measure morphological similarity. *Crop. Sci.* 8: 693 – 698.

Harlan, J. R. (1969). Ethiopia: A center of diversity. *Econ. Bot.* **23**: 309 – 314.

Harlan, J. R. (1975). Geographic patterns of variation in some cultivated plants. *J. Heredity* **66**: 182 – 191.

Harlan, J. R. De Wet, J. M. J. (1972). A simple classification of cultivated sorghum. *Crop. Sci.* **2**: 172 – 176.

Hodgkin, T., Brown, A. H. D., Vanhintum, Th. J. L. and Morales, E. A.V. (1985). *Sorghum.* Pp.: 109 - 117. John Wiley & Sons, UK.

Hoelzel, A. R. and Green, A. (1992). Population-level variation by sequencing PCR-amplified DNA and isozymes. In: *Molecular genetic analysis of populations: A practical approach.* Hoelzel, A. R. (Ed.) pp.: 154 – 181. Oxford University Press, USA.

IBPGR/ICRISAT (1993). Descriptors for sorghum (*Sorghum bicolor* (L) Moench). International Board for Plant Genetic Resources, Rome, Italy: International Crops Research Institute for Semi Arid Tropics, Patancheru, India.

Jarret, R. L. and Litz, R. E. (1986). Enzyme polymorphism in *Musa acuminata* Colla. *J. Heredity* **77**:183 – 188.

Kahler, A. L. (1983). Inheritance and linkage of acid phosphatase locus Acp4 in maize. *J. Heredity* **74**: 239 – 246.

Kiang, Y. T. (1981). Inheritance and variation of amylase in cultivated and wild soybeans and their wild relatives. *J. Heredity* **72**: 382 – 386.

King, E. E. (1971). Extraction of cotton leaf enzymes with borate. *Phytochemistry* **10**: 2337 – 2341.

Lewontin, R. C. (1974). *The genetic basis of evolutionary change*. Columbia University Press, NY, USA.

Linde-Laursen, I., Nielsen, G. and Johansen, V. (1987). Distribution of isoenzyme markers at 37 loci in a pedigree of European spring barley. *Hereditas* **106**: 241 – 251.

Loomis, W. D. and Battaile, J. (1966). Plant phenolic compounds and the isolation of plant enzymes. *Phytochemistry* **5**:423 – 438.

May, B. (1992). Starch gel electrophoresis of allozymes. In*: Molecular genetic analysis of populations: A practical approach*. Hoelzel, A. R. (Ed.) pp.: 1- 26. Oxford University Press, USA.220 pp.

Morden, C. W., Doelbey, J. F. and Schertz, K. F. (1987). *A manual of Techniques for Starch gel Electrophoresis of Sorghum Isozymes*. Texas A & M University, College Station, Texas, USA.45 pp.

Morden, C. W., Doelbey, J. F. and Schertz, K. F. (1988). Genetic control and sub cellular localization of Aconitase isozymes in Sorghum. *Journal of Heredity* **79**: 295 – 299

Markert, C. L. and Moller, F. (1959). Multiple forms of enzymes: tissue, ontogenetic and species specific patterns. *Proc. Natl. Acad. Sci. USA* 45: 753 – 763.

Mulugeta Negassa (1986). Estimates of phenotyic diversity. *Hereditas* **104**: 41 - 48

Nei, M. (1965). Variation and Co-variation of gene frequencies in subdivided populations. *Evolution* **19**:256 – 258

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proc.Natl. Acad. Sci.* **70**:3321 – 3323.

Ott, L. and Scandalios (1978). Genetic control and linkage relationships among aminopeptidases of maize. *Genetics* **89**: 137 – 146.

Poulik, M. D. (1957). Starch gel electrophoresis in a discontinuous system of buffers. *Nature* **180**: 1477 – 1479.

Prakash, S., R. C. Lewontin and Hubby, J. L. (1969). A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations of *Drosophila pseudobscura. Genetics* **61**: 841 - 858

Purseglove, I. W. (1972). *Tropical Crops: Monocotyledons*. Longman groups limited, London, England. 420 pp.

Rao, K. E. P. and Rao, V. R. (1995). The use of characterization data in developing a core collection of sorghum. In: *Core collection of plant Genetic Resources*. John Wiley & Sons, NY, USA. 270 pp.

Rolf, F. J. (1970). Adaptive hierarchical clustering schemes. *Syst. Zool.*, **19**: 58 – 82.

Shannon, L. M. (1968). Plant isozymes. *Ann. Rev. Pl. Physiol.* **19**: 187 – 210.

SPSS (1995). *SPSS on line help*. Release version 7.5. SPSS company, USA.

Suiter, K. A., Wendel, J. F. and Case, J. S. (1983). Linkage 1:a Pascal computer program for the detection and analysis of genetic linkage. *J. Heredity* **74**: 203 – 204.

Tadesse Ebba (1978). Plant Genetic Resources Center/Ethiopia: Activities and Programs. In: *Crop Genetic Resources in Africa*, pp. 173 – 180. Longman, UK.

Tanksley, S. D. and Orton, T. J. (1983). Isozymes in plant genetics and breeding. Elsevier, Amsterdam.378 pp.

Tanksley, S. D. and Rick, C. M. (1980). Genetics of esterases in species of *Lycopersicon*. *Theor. Appl. Genet.* **56**:209 – 219.

Vavilov, N. I. (1951). The origin, variation, immunity and breeding of cultivated plants. *Chron. Bot.* **13**: 1- 3.

Warwick, S. I. Gotlieb, L. D. (1985). Genetic divergence and geographic speciation in *Layia* (Compositae). *Evolution* **39**: 1236 – 1241.

Weeden, N. F. and Gotlieb, L. D. (1971): Isolation of cytoplasmic enzymes from pollen. *Pl. Physiol.* **66**: 400 – 403.

Weeden, N. F. and Robinson, R. W. (1986). Allozyme segregation ratios in the interspecific cross *Cucurbita maxima X C. ecuadorensis* suggest that hybrid breakdown is not caused by minor alterations in chromosome structure. *Genetics* **114**: 593 – 609.

Wendel, J. F. and Parks, C. R. (1982). Genetic control of isozyme variation in *Camellia japonica* L. *J. Heredity* **73**: 197 – 204.

Wendel, J. F. and Weeden, N. F. (1988). *Visualization and interpretation of plant isozymes*. Cornell University, USA.

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

Yazdani, R., and Rudin, D. (1982). Inheritance of fluorescent esterase and β-galactosidase in haploid and diploid tissues of Pinus sylvestris L. Hereditas 96: 191 – 194.

9.Appendices

ppendix 1. Test of homogeneity of variances and one way ANOVA for mean days to germination.

1A. Test of homogeneity of variance for Mean days to germination of *ex-situ* conserved land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
DTG	1.155	12	52	.339

1B. One way ANOVA for the mean days to germination of *ex-situ* conserved land races – Significant.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
DTG	Between Groups	330.462	12	27.538	11.329	.000
	Within Groups	126.400	52	2.431		
	Total	456.862	64			

1C.Test of Homogeneity of Variances for the *in-situ* (on-farm) land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
DTG	1.003	16	68	.464

1D. One way ANOVA analysis for the *in-situ* (on-farm) conserved land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
DTG	Between Groups	91.124	16	5.695	3.418	.000
	Within Groups	113.300	68	1.666		
	Total	204.424	84			

1E. Test of Homogeneity of Variances for comparing *ex-situ* and the *in-situ* (on-farm) conserved land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
DTG	21.765	1	28	.000

1F. One way ANOVA comparing the means of days to germination for the *ex-situ* and *in-situ* (on-farm) land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
DTG	Between Groups	41.162	1	41.162	13.640	.001
	Within Groups	84.497	28	3.018		
	Total	125.659	29			

Appendix 2. Test of homogeneity of variances and one way ANOVA for mean days to knee height.

2A. Test of homogeneity of variance for the *ex-situ* conserved land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
DTK	2.222	12	52	.024

2B. One way ANOVA for days to knee height stage of *ex-situ* conserved land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
DTK	Between Groups	492.954	12	41.079	29.024	.000
	Within Groups	73.600	52	1.415		
	Total	566.554	64			

2C. Test of homogeneity of variance for the *in-situ* (on-farm) conserved land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
DTK	1.485	16	68	.131

2D. One ways ANOVA for days to knee height stage of the *in-situ* conserved land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
DTK	Between Groups	441.423	16	27.589	24.150	.000
	Within Groups	77.683	68	1.142		
	Total	519.106	84			

2E. Test of homogeneity of variance for comparative mean days to knee height stage.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
DTK	.632	1	28	.433

2F. One way ANOVA for the comparison of means for days to germination.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
DTK	Between Groups	59.621	1	59.621	8.739	.006
	Within Groups	191.026	28	6.822		
	Total	250.647	29			

Appendix 3. Test of homogeneity of variances and one way ANOVA for disease scoring.

3A. Test for homogeneity of variance for disease scoring for the *ex-situ* land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
DSC	5.345	12	52	.000

3B. One way ANOVA for mean disease scoring for the *ex-situ* conserved land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
DSC	Between Groups	88.862	12	7.405	6.418	.000
	Within Groups	60.000	52	1.154		
	Total	148.862	64			

3C. Test of homogeneity of variance for disease scoring for the *in-situ* conserved land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
DSC	7.366	16	68	.000

3D.One way ANOVA for disease scoring for the *in-situ* conserved land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
DSC	Between Groups	281.355	16	17.585	25.532	.000
	Within Groups	46.833	68	.689		
	Total	328.188	84			

3E. Test of homogeneity of variance comparing the *ex-situ* versus *in-situ* (on-farm) land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
DSC	.987	1	28	.329

3F. One way ANOVA for disease scoring comparing *ex-situ* versus *in-situ* land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
DSC	Between Groups	.439	1	.439	.165	.687
	Within Groups	74.333	28	2.655		
	Total	74.773	29			

Appendix 4. Test of homogeneity of variances and one way ANOVA for mean leaf number.

4A. Test for the homogeneity of variances for the leaf number of *ex-situ* land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
NLF	1.436	12	52	.180

4B. One way ANOVA for the mean number of leaves for the *ex-situ* conserved land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
NLF	Between Groups	8.800	12	.733	1.163	.334
	Within Groups	32.800	52	.631		
	Total	41.600	64			

4C. Test for the homogeneity of variance for mean leaf number for the *in-situ* conserved land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
NLF	.970	16	68	.498

4D. One way ANOVA for the mean leaf number for the *in-situ* conserved land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
NLF	Between Groups	7.728	16	.483	1.170	.314
	Within Groups	28.083	68	.413		
	Total	35.812	84			

4E. Test of homogeneity of variance comparing *ex-situ* versus *in-situ*.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
NLF	.677	1	28	.418

4F. One way ANOVA for mean leaf number comparing *in-situ* versus *ex-situ* land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
NLF	Between Groups	.943	1	.943	8.077	.008
	Within Groups	3.270	28	.117		
	Total	4.213	29			

ppendix 5.Test of homogeneity of variances and one way ANOVA for mean leaf length.

5A. Test for the homogeneity of variance for mean leaf length for *ex-situ* land races.

5B. One way ANOVA for mean leaf length for the *ex-situ* conserved land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LFL	Between Groups	328.154	12	27.346	16.458	.000
	Within Groups	86.400	52	1.662		
	Total	414.554	64			

5C. Test of homogeneity of variance for the mean leaf length *in-situ* conserved land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
LFL	1.239	16	68	.263

5D. One way ANOVA for mean leaf length for the *in-situ* land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LFL	Between Groups	128.928	16	8.058	11.687	.000
	Within Groups	46.883	68	.689		
	Total	175.812	84			

5E. Test of homogeneity for variances of mean leaf length *in-situ* and *ex-situ* land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
LFL	2.148	1	28	.154

5F. One way ANOVA to compare *in-situ* versus *ex-situ* for mean leaf length.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LFL	Between Groups	12.938	1	12.938	3.939	.057
	Within Groups	91.975	28	3.285		
	Total	104.914	29			

Appendix 6. Test of homogeneity of variance and one way ANOVA for mean leaf width.

6A. Test of homogeneity of variance for the *ex-situ* land races for their mean leaf width.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
LFW	1.787	12	52	.075

6B. One way ANOVA for the *ex-situ* conserved land races for their mean leaf width.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LFW	Between Groups	21.015	12	1.751	2.743	.006
	Within Groups	33.200	52	.638		
	Total	54.215	64			

6C. Test of homogeneity of variance for leaf width (cm) for *in-situ* conserved land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
LFW	.710	16	68	.775

6D. One way ANOVA for mean leaf width (cm) for *in-situ* conserved land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LFW	Between Groups	35.611	16	2.226	3.776	.000
	Within Groups	40.083	68	.589		
	Total	75.694	84			

6E. Test of homogeneity of variance for leaf width (cm) for comparing *ex-situ* versus *in-situ* land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
LFW	.154	1	28	.698

6F. One way ANOVA for mean leaf width (cm) comparing *in-situ* versus *ex-situ* land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LFW	Between Groups	35.611	16	2.226	3.776	.000
	Within Groups	40.083	68	.589		
	Total	75.694	84			

Appendix 7. Test of homogeneity of variance and one way ANOVA for mean leaf area.

7A. Test of homogeneity of variance for the mean leaf are of the *ex-situ* accessions.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
LFA	2.723	12	52	.006

7B. One way ANOVA for the mean leaf area for the *ex-situ* accessions.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LFA	Between Groups	5529.600	12	6294.133	3.789	.000
	Within Groups	6386.000	52	1661.269		
	Total	161915.6	64			

7C. Test of homogeneity of variance for the *in-situ* (on-farm) land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
LFA	.877	16	68	.597

7D. One way ANOVA for the mean leaf area for the *in-situ* (on-farm) land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LFA	Between Groups	115833.2	16	7239.576	4.927	.000
	Within Groups	9920.483	68	1469.419		
	Total	215753.7	84			

7E. Test of homogeneity of variances for the comparison of *ex-situ* accessions and *in-situ* (on-farm) land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
LFA	.017	1	28	.897

7F. One way ANOVA for the comparative difference between the *ex-situ* accessions and *in-situ* (on-farm) land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LFA	Between Groups	4519.697	1	4519.697	3.254	.082
	Within Groups	8885.225	28	1388.758		
	Total	3404.921	29			

Appendix 8. Test of homogeneity of variance and one way ANOVA for mean leaf sheath length.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
LSL	1.314	12	52	.239

8A. Test of homogeneity of variance for means of leaf sheath length for the *ex-situ* accessions.

8B. One way ANOVA for means of leaf sheath length for the *ex-situ* accessions.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LSL	Between Groups	69.938	12	5.828	8.419	.000
	Within Groups	36.000	52	.692		
	Total	105.938	64			

8C. Test of homogeneity of variance for the means of leaf sheath length for the *in-situ* (on-farm) land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
LSL	1.410	16	68	.164

8D. One way ANOVA for the means of leaf sheath length for the *in-situ* (on-farm) land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LSL	Between Groups	54.511	16	3.407	3.368	.000
	Within Groups	68.783	68	1.012		
	Total	123.294	84			

8E. Test of homogeneity of variance for means of leaf sheath length comparing *ex-situ* accessions versus *in-situ* land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
LSL	.702	1	28	.409

8F. One-way ANOVA comparing *ex-situ* accessions against *in-situ* land races for means of leaf sheath length.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LSL	Between Groups	3.728	1	3.728	4.125	.052
	Within Groups	25.308	28	.904		
	Total	29.036	29			

Appendix 9. Test of homogeneity of variance and one way ANOVA for mean inter-node length.

9A. Test of homogeneity of variance for means of inter-node length for the *ex-situ* accessions.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
ILT	1.479	12	52	.162

9B. One way ANOVA for means of inter-node length for the *ex-situ* land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
ILT	Between Groups	187.385	12	15.615	14.097	.000
	Within Groups	57.600	52	1.108		
	Total	244.985	64			

9C. Test of homogeneity of variance for means inter-node length for the *in-situ* (on-farm) land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
ILT	1.309	16	68	.217

9D. One way ANOVA for means inter node length for the *in-situ* (on-farm) land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
ILT	Between Groups	552.205	16	34.513	37.863	.000
	Within Groups	61.983	68	.912		
	Total	614.188	84			

9E. Test of homogeneity of variance for means of inter-node length comparing *ex-situ* accessions versus *in-situ* (on-farm) land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
ILT	2.775	1	28	.107

9F. One way ANOVA for means of inter-node length comparing *ex-situ* accessions *in-situ* (on-farm) land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
ILT	Between Groups	1.569	1	1.569	.282	.599
	Within Groups	155.538	28	5.555		
	Total	157.106	29			

Appendix 10. Test of homogeneity of variance and one way ANOVA for mean stem diameter.

10 A. Test of homogeneity of variance for mean stem diameter (cm) for the *ex-situ* accessions.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
SDI	5.547	12	52	.000

10 B. One way ANOVA to test whether there is significant difference between the *ex-situ* accessions.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SDI	Between Groups	8.850E-02	12	7.375E-03	8.352	.000
	Within Groups	4.592E-02	52	8.831E-04		
	Total	.134	64			

10 C. Test of homogeneity of variance for the *in-situ* (on-farm) land races for their mean stem diameter.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
SDI	1.587	16	68	.096

10 D. One way ANOVA for mean stem diameter for the *in-situ* (on-farm) land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SDI	Between Groups	8.123E-02	16	5.077E-03	9.497	.000
	Within Groups	3.635E-02	68	5.346E-04		
	Total	.118	84			

10E. Test of homogeneity of variance to compare the *ex-situ* accessions against *in-situ* (on-farm) land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
SDI	1.587	16	68	.096

10F. One way ANOVA to compare *ex-situ* accessions against *in-situ* land races for mean stem diameter.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SDI	Between Groups	9.123E-02	16	5.077E-03	9.497	.000
	Within Groups	3.635E-02	68	5.346E-04		
	Total	.118	84			

Appendix 11. Test of homogeneity of variance and one way ANOVA for mean plant height.

11A. Test of homogeneity of variance for the means of plant height for the *ex-situ* accessions.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
PHT	2.049	12	52	.038

11B. One way ANOVA for the means of plant height for the *ex-situ* accessions.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PHT	Between Groups	5841.395	12	486.783	30.056	.000
	Within Groups	842.176	52	16.196		
	Total	6683.571	64			

11C. Test of homogeneity of variance for the means of plant height for the *in-situ* (on-farm) land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
PHT	1.338	16	68	.201

11D. One way ANOVA for the means of plant height for the *in-situ* (on-farm) land races.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PHT	Between Groups	5046.754	16	315.422	25.627	.000
	Within Groups	836.953	68	12.308		
	Total	5883.707	84			

11E. Test of homogeneity of variance for the means of plant height comparing *ex-situ* accessions against *in-situ* land races.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
PHT	.212	1	28	.649

11F. One way ANOVA comparing *ex-situ* accessions against *in-situ* land races for their mean plant height.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PHT	Between Groups	335.024	1	335.024	4.193	.050
	Within Groups	2237.311	28	79.904		
	Total	2572.335	29			