IDENTIFICATION AND CLASSIFICATION OF FINGER MILLET (ELEUSINE CORACANA) ACCESSIONS USING MORPHOLOGICAL TRAITS AND DNA FINGER PRINTING

MR. GOITSEONE MALAMBANE

A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

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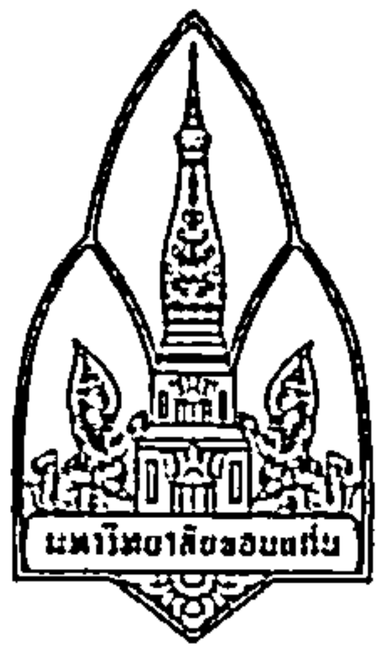

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บทคัดย่อ

ฐานข้อมูลความหลากหลายของเชื้อพันธุกรรมมีความสำคัญต่อโครงการปรับปรุงพันธุ์พืชมาก การประเมินและการจัดทำลักษณะประจำพันธุ์ของเชื้อพันธุกรรมจึงเป็นขั้นตอนแรกที่สำคัญในงานปรับปรุงพันธุ์โดยทั่วไป วิธีการจำแนกและจัดกลุ่มเชื้อพันธุกรรมพืชมีหลายวิธี ในอดีตใช้เพียงวิธีทางสัณฐานวิทยาเป็นหลัก แต่ปัจจุบันสามารถจำแนกโดยใช้ความรู้ด้านชีวเคมี และเครื่องหมายโมเลกุลซึ่งทำได้รวดเร็ว และแม่นยำมากกว่า ข้าวฟ่างนิ้วมือเป็นพืชที่ถูกละเลยจากนักปรับปรุงพันธุ์มาอย่างยาวนาน ส่งผลให้พืชนี้มีพื้นที่ปลูกและผลผลิตลดลงอย่างต่อเนื่อง ในขณะที่ปัจจุบันมีกระแสความตื่นตัวเกี่ยวกับอาหารเพื่อสุขภาพทำให้ข้าวฟ่างนิ้วมือซึ่งอุดมไปด้วยธาตุแคลเซียม และเหล็ก ได้รับความสนใจในการปรับปรุงพันธุ์ให้มีผลผลิตสูงขึ้นเหมือนธัญพืชชนิดอื่นๆ ผลสืบเนื่องจากการถูกละเลยในการพัฒนาพันธุ์พืชนี้มาอย่างยาวนาน จึงมีความจำเป็นต้องทำการประเมินเชื้อพันธุกรรมข้าวฟ่างนิ้วมือเพื่อใช้เป็นข้อมูลพื้นฐานในการปรับปรุงพันธุ์ให้ประสบความสำเร็จต่อไป

วัตถุประสงค์ของการศึกษาครั้งนี้ เพื่อจำแนกและจัดกลุ่มเชื้อพันธุกรรมข้าวฟ่างนิ้วมือ โดยอาศัยลักษณะทางสัณฐานวิทยาและดายพิมพ์ดีเอ็นเอ และศึกษาปฏิสัมพันธ์ระหว่างผลผลิตกับฤดูปลูก ทำการปลูกข้าวฟ่างนิ้วมือจำนวน 83 สายพันธุ์ ใน 2 ฤดู (ฤดูฝน และฤดูแล้ง) โดยวางแผนการทดลองแบบสุ่มสมบูรณ์ภายในบล็อก จำนวน 3 ซ้ำ ที่หมวดพืชไร่ คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่น ระหว่างปีเพาะปลูก 2553-2554 ทำการบันทึกข้อมูลผลผลิต องค์ประกอบผลผลิต และลักษณะทางการเกษตรต่างๆ รวมทั้งลักษณะทางสัณฐานวิทยาทั้ง ลักษณะทางปริมาณและลักษณะทางคุณภาพ ถูกใช้ในการจำแนกและจัดกลุ่มข้าวฟ่างนิ้วมือในครั้งนี้

รูปร่างของช่อดอกสามารถแบ่งข้าวฟ่างนิ้วมือได้เป็น 4 กลุ่ม ประกอบด้วย พันธุ์ป่า (subsp. *africana*) และพันธุ์ปลูก (subsp. *coracana*) ซึ่งสามารถจัดกลุ่มย่อยได้ 4 สายพันธุ์ ประกอบด้วย *vulgaris* (54.9%), *plana* (35.4%), *compacta* (7-3%) และ *elongeta* (2-4%) ตีของ

เมล็ดสามารถจัดกลุ่มได้ 6 กลุ่ม ประกอบด้วย สีน้ำตาลอ่อน 2.4 % สีน้ำตาล 4.9% สีน้ำตาลปนขาว 59.8% สีขาว 2.4% สีแดง 9.8% และสีม่วง 20.7% สีของใบสามารถจำแนกได้ 2 กลุ่ม คือ สีเขียว 63.4 % และสีม่วง 36.6% การหักล้มของลำต้น สามารถจำแนกเป็น สายพันธุ์ที่มีต้นล้ม 35.4% และต้นไม่ล้ม 64.6% การจำแนกโดยอาศัยทรงต้นและการเจริญเติบโตสามารถแบ่งเป็น 2 กลุ่ม คือ พวกลำต้นตั้งตรง 98.8% และลำต้นทอดเลื้อยไปตามผิวดิน 1.2% โดยอาศัยลักษณะทางสัณฐานวิทยาในการจัดกลุ่มเดนโดรแกรมสามารถจัดกลุ่มของ *E. coracana* ได้เป็น 4 กลุ่ม คือ กลุ่มที่ 1 มีจำนวน 6 สายพันธุ์ กลุ่มที่ 2 มีจำนวน 40 สายพันธุ์ กลุ่มที่ 3 มีจำนวน 22 สายพันธุ์ และกลุ่มที่ 4 มีจำนวน 14 สายพันธุ์

การจัดกลุ่มเชื้อพันธุกรรมข้าวฟ่างนิ้วมือโดยอาศัยเครื่องหมายโมเลกุล ด้วยการใช้ไพรเมอร์ชนิด Random Amplified Polymorphic DNA (RAPD) จำนวน 44 ไพรเมอร์ซึ่งให้ลายพิมพ์ดีเอ็นเอที่สามารถจำแนกความแตกต่างได้ บนแผ่นเจลชนิดอะกาโรส เมื่อถ่ายภาพด้วยกล้องระบบอัลตราไวโอเลต ได้แถบดีเอ็นเอจำนวน 255 แถบ ซึ่งมี 160 แถบที่บอกความแตกต่างระหว่างพันธุ์ของข้าวฟ่างนิ้วมือได้ กลุ่มแถบดีเอ็นเอดังกล่าวมีขนาดระหว่าง 400 - 2000 คู่เบส ไพรเมอร์ให้ลายพิมพ์แถบดีเอ็นเอในช่วง 4 – 15 แถบ เมื่อนำข้อมูลจากการอ่านผลแถบดีเอ็นเอมาวิเคราะห์จัดกลุ่มโดยวิธี Jacard's similarity coefficient และสร้างแผนภาพ เดนโดรแกรมด้วยวิธี UPGMA พบว่า เดนโดรแกรม สามารถแยกข้าวฟ่างนิ้วมือพันธุ์ป่า (*Eleusine coracana* subsp. *africana*) ออกจากข้าวฟ่างนิ้วมือพันธุ์ปลูก (*Eleusine coracana* subsp. *coracana*) ซึ่งประกอบด้วย 2 กลุ่ม นอกจากนี้กลุ่มใหญ่ยังประกอบด้วย 3 กลุ่มย่อย ส่วนกลุ่มเล็กจัดแบ่งได้เป็น 2 กลุ่มย่อยโดยมีลักษณะทางสัณฐานวิทยาที่คล้ายกันคือ กลุ่มที่ 1 มีการแตกกอดี มีจำนวนต้นมากกว่า 10 ต้นต่อกอ กลุ่มที่ 2 มีอายุเก็บเกี่ยวยาว กลุ่มที่ 3 มีสีของเมล็ดเป็น สีน้ำตาลปนขาว ส่วนกลุ่มที่ 4 มีต้นเตี้ยแคระมีความสูงไม่เกิน 100 เซนติเมตร และกลุ่มที่ 5 เป็นกลุ่มพันธุ์ที่มีจำนวนใบมาก นอกจากนี้ยังพบว่าข้าวฟ่างนิ้วมือ 2 พันธุ์คือ IE2043 และ IE2217 มีความใกล้ชิดทางพันธุกรรมมาก โดยมีค่าสัมประสิทธิ์ความเหมือน 0.91 มีความเหมือนกันของลักษณะอายุดอกบาน มีต้นเตี้ยแคระ อายุเก็บเกี่ยว และอายุดอกบาน และมาจากแหล่งปลูกเดียวกันคือประเทศอินเดีย การศึกษาครั้งนี้พบว่าแหล่งกำเนิดทางภูมิศาสตร์ไม่สามารถนำมาใช้จัดกลุ่มทางพันธุกรรมของข้าวฟ่างนิ้วมือได้

ข้าวฟ่างนิ้วมือจำนวน 35 สายพันธุ์ ถูกคัดเลือกเพื่อนำไปปลูกทดสอบใน 2 ฤดูปลูก คือ ฤดูแล้งปี 2553/54 และฤดูฝนปี 2554 ที่หมวดพืชไร่ คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่น โดยใช้แผนการทดลองแบบสุ่มสมบูรณ์ภายในบล็อก จำนวน 3 ซ้ำ ผลการทดลอง พบว่า มีความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างฤดูปลูก สายพันธุ์ และปฏิสัมพันธ์ระหว่างพันธุกรรมกับสภาพแวดล้อม ในลักษณะ น้ำหนัก 1,000 เมล็ด ความยาวของรวงช่อดอก ความสูง และอายุดอก

บาน โดยอิทธิพลของฤดูปลูกจะส่งผลต่อลักษณะผลผลิตต่อต้นมากที่สุด (53.09%) และมีอิทธิพลปานกลางต่อความสูงของต้น (32.28%) ส่วนอิทธิพลของพันธุกรรมจะแปรปรวนไปตามลักษณะต่างๆ ได้แก่ น้ำหนัก 1000 เมล็ด (38.34%) ความยาวของรวงช่อดอก (64.78%) จำนวนช่อดอก (36.80%) ความกว้างช่อดอก (50.84%) ความสูงของลำต้น (39.06%) และอายุดอกบาน (64.79%) ในขณะที่อิทธิพลของปฏิสัมพันธ์ระหว่างพันธุกรรมกับสภาพแวดล้อมต่อลักษณะผลผลิตเมล็ด จำนวนช่อดอก และความกว้างช่อดอกจะมีความแปรปรวนปานกลาง โดยมีค่าเท่ากับ 23.05, 29.98 และ 27.83% ตามลำดับ นอกจากนี้ยังพบว่า สายพันธุ์ IE2043 มีความแปรปรวนของผลผลิตต่อต้นต่ำทั้ง 2 ฤดู ในขณะที่ สายพันธุ์ IE518 มีความแปรปรวนสูงมาก จึงสรุปได้ว่า ข้าวฟ่างนิ้วมือสายพันธุ์ IE2043 เป็นสายพันธุ์ที่มีความสามารถในการปรับตัวดีและให้ผลผลิตสูงทั้ง 2 ฤดูปลูก

การศึกษาความสัมพันธ์ระหว่างลักษณะผลผลิตและองค์ประกอบผลผลิตต่างๆ พบว่า ผลผลิตกับจำนวนหน่อต่อต้น และผลผลิตกับน้ำหนัก 1,000 เมล็ด มีความสัมพันธ์ทางบวกอย่างมีนัยสำคัญทางสถิติ โดยมีค่าสัมประสิทธิ์สหสัมพันธ์เท่ากับ 0.29 และ 0.51 ตามลำดับ ในขณะที่ตรวจพบ ความสัมพันธ์ในทางลบอย่างมีนัยสำคัญทางสถิติระหว่างลักษณะต่างๆ ได้แก่ ผลผลิตกับความยาวช่อรวง ผลผลิตกับอายุดอกบาน และผลผลิตกับความสูงของลำต้น โดยมีค่าสัมประสิทธิ์สหสัมพันธ์เท่ากับ -0.17, -0.33 และ -0.41 ตามลำดับ สำหรับผลผลิตกับลักษณะทางการเกษตรอื่นๆ ที่เหลือ พบว่า ไม่มีความสัมพันธ์กัน

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Thesis Advisors: Assoc. Prof. Dr. Prasit Jaisil, Dr. Jirawat Sanitchon,
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ABSTRACT

Information on genetic diversity of germplasms is very important for every breeding program, thus evaluation and characterization of germplasms is the first and most important step for any breeding program. Different tools are now available for germplasm evaluation making the process rather quicker and less laborious like in the past where only morphological markers where the only markers available for evaluation, nowadays they are other advanced markers like biochemical and molecular markers which are much faster and more precise than morphological evaluation. Finger millet a crop which has been neglected for a very long time by breeders thus eliminating the crop to more undesirable environments on which its yield continued to fall, but with the current increased interest in the crop because of its health associated benefits like high calcium and iron has led to need for the crop yield to be improved to match other cereals yields. Because the crop breeding program has been neglected the need to evaluate the available germplasm has risen so that the advanced breeding of the crop can successfully be launched.

The objective of this study was to identify and classify finger millet accessions using morphological traits and DNA fingerprinting and further evaluate the effect of genotype, season and genotype by season interaction in yield performance of selected accessions. Eighty three finger millet accessions were planted to be evaluated over two season; to assess the performance of finger millet accessions the crops were grown in a randomized complete block design with three replications in the dry and rainy season, data was collected on yield, yield components and other agronomic traits performance over the two years under study in the Field Crops Experimental

Station, Faculty of Agriculture, Khon Kaen University, Thailand during 2010- 2011 growing season.

Qualitative and quantitative morphological characters were used to classify and group the finger millet accessions. Characters like panicle shape which is widely used to classify finger millet cluster the accessions into four clusters. The panicle shape classification identified the wild relative belong to the subsp *africana* and the cultivated (*coracana*) species were then grouped into four races; *vulgaris* (54.9%), *plana* (35.4%), *compacta* (7.3%) and *elongeta* (2.4%). Six different colours were observed throughout the accessions studied with the brown colour been the dominant colour which was the sub grouped into light brown (2.4%), brown (4.9%) and ragi brown (59.8%), the other colours observe were white (2.4%), red (9.8%), and purple (20.7%). Other qualitative traits like pigmentation grouped the accessions into two groups green (63.4%) and purple (36.6%), lodging susceptibility classified accessions into two groups being; susceptible (35.4%) and resistant (64.6%) while grouping based on growth habit the accessions were grouped into two groups; erect (98.8%) and decumbent (1.2%). Based on quantitative morphological traits data collected in the field for two seasons, a dendrogram constructed and the results showed that *E. coracana* formed 4 groups 1, 2, 3 and 4 having a total of 6, 40, 22 and 14 accessions respectively on largely distinct population subspecies at 79% similarity level with cophenetic correlation of 0.62 or 62%.

Laboratory work was also done to fingerprint the accession using RAPD markers. Forty four polymorphic RAPD markers were used to amplify the DNA which was later run in gel to separate the amplicons and the viewed under UV-light. Two hundred and fifty five bands were obtained from the amplification with 160 bands been polymorphic. The size range of bands was from 400-2000bp, with a number of bands per individual primer ranging from 4 to 15. The polymorphic bands were scored and cluster analysis based on Jaccard's similarity coefficient using UPGMA was performed and a dendrogram with correlation coefficient of 85% was produced. The dendrogram managed to separate *Eleusine coracana* subsp. *africana* a wild relative (IE 4709) from the cultivated finger millet (*Eleusine coracana* subsp. *coracana*), further subsp *coracana* was grouped into two major clusters. Further the main clusters formed three (3) and two (2) distinct sub cluster, respectively, with each

group having similar morphological traits; group 1 was made up of plants with high number of basal tillers all having more than ten tillers per plant, group 2 was made up of late maturing plants, whereas accessions in the 3rd group had same (ragi brown) colour, the 4th group was made up of dwarf plants all growing to less than 100 cm, while the last group was made up of plants with high leaf number. Maximum likehood or similarity of 0.91 was observed between IE 2043 and IE 2217 which showed very close morphological traits (days to flowering, dwarf, maturity, and days to maturity) and also from the same country (India). In this study the geographical origin of finger millet accession did not contribute much when it came to classification or grouping of finger millet accessions.

Thirty five finger millet accessions were evaluated in the dry season 2010/11 and the rainy season 2011 at the Field Crops Experiment Station, Khon Kaen, Thailand. A randomized complete block design with 3 replications was used in this study. Combined analyses showed significant differences among seasons, genotype and genotype by season interactions for yield per plant, 1000 seed weight, finger length, plant height and days to flowering. Season contributed to a large proportion of variations on yield per plant (53.09%) and was moderate for plant height (32.28%). However, variations due to genotype were varied for 1000 seed weight (38.34%), finger length (64.78%), finger number (36.80%), finger width (50.84%), plant height (39.06%) and days to flowering (64.79%) while variations due to genotype by season interaction were moderate for plant yield (23.05%), finger number (29.98%) and finger width (27.83%). Accession IE 2043 showed low variation in yield for the two seasons while IE 518 had the highest variations when evaluated in the two seasons for yield per plant. Because of high yield and low variation, IE 2043 is promising for production in the dry season and the rainy season.

Among the yield components (tiller number, finger length, finger number, finger width and 1000 seed weight) highly significant correlation at 95% level was observed for yield and number of tillers at 0.29 and for yield and 1000 seed weight with a highest positive of 0.51. Highly significant correlation was observed between yield and finger length at (-0.17). Non significant correlation was observed between yield and finger number at 0.12, also yield and finger width had a non significant correlation of 0.12 .All the agronomic traits (days to flowering, plant height and days

to flowering) had a highly significant negative correlation at 95% level with yield of plants. Highly significant negative correlation was observed between yield and days to flowering at (-0.33) and the highest negative correlation was between yield and plant height at (-0.41). Yield and days to maturity had showed the lowest correlation at (-0.08) which was non significant.

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Goitseone Malambane

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LIST OF ABBREVIATIONS

Asl	Above sea level
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of Variance
AP-PCR	Arbitrary primer - Polymorphism Chain Reaction
bp	Base pairs
cm (cm^2)	Centimetre (square centimetre)
C.V.	Coefficient of variation
DAF	DNA amplification fingerprinting
DF	Degree of freedom
DNA	Deoxyribonucleic acid
dH_2O	Distilled water
ddH_2O	Double distilled water
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
g	Grams
G x E	Genotype by Environment interaction
G x S	Genotype by Season interaction
H_2O	Water
Hrs	Hours
IBPGR	International Board for Plant Genetic Resources
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
ISSR	Inter simple sequence repeats
K	Potassium
kg	Kilogram
m (m^2)	Meter (square meter)
MAAP	Multiple Arbitrary Amplicons Profiling
Min	Minutes

LIST OF ABBREVIATIONS (cont.)

MgCl2	Magnesium Chloride
mm	Millimeters
mM	Milli Molar
MS	Mean squares
N	Nitrogen
ng	Nanogram
NR	Nitrate reductase
OP	Operon Primer
OUT	Operational taxonomic units
P	Phosphorus
PCR	Polymerase chain reaction
pH	Power of Hydrogen
RAPD	Random amplified polymorphic DNA
RCBD	Randomised complete block design
Rpm	Revolutions per minute
SOV	Source of variation
SS	Sum squares
SSR	Simple sequence repeats
TBE	Tris-boric-EDTA
TE	Tris-EDTA
t/ha	Tones per hectare
UPGMA	Unweighted Pair Group Method Analysis
UV	Ultra-violet light
U *taq*	Units of *taq*
VNTR	Variable number tandem repeats
V	Volts
w/v	Weight (of solute) per volume (of solvent)
°C	Degrees Celsius
%	Percentage
µl	Micro litre

CHAPTER I
INTRODUCTION

1.1 Problem statement and justification

Finger millet (*Eleusine coracana)* is also known as bird foot millet, coracana, African millet (Vavidoo et al., 1998) and it is also referred to as ragi. Finger millet has outstanding properties for a sustainable staple food crop. It tastes very good and is an excellent source of methionine, calcium, iron, manganese, protein (Babu et al., 2007), high crude fiber content (3-4%) (Vavidoo et al., 1998). It also have some medical properties and is used as folk remedy for many diseases (Bisht and Mukai, 2000). The crop residues are excellent source of dry matter for livestock especially in dry season, the straw makes good fodder and contains up to 61% total digestible nutrients. Finger millet also contributes greatly to the incomes of rural households. It is brewed into local beer for sale or it is sold directly as grain in local markets where there is ready demand (Tenywa et al., 1999). Despite the great value associated with this crop its productivity (yield/unit area) has remained very low and exact yield has not being documented but only consolidated under the Millets group. Only estimated or potential yield are used.

Man has always strived to improve crop plants in regard to desirable traits such as yield, disease and insect resistance, adaptability to the environment, nutritional quality, palatability and others important traits. Therefore the needs of hybrids with improved traits are highly needed in improving food security and resolving energy crisis which is overwhelming the whole world. The basic information on the existence of genetic variability and diversity in a population and the relationships between different traits is essential for any successful plant breeding program.

Breeding efforts in finger millet have been very limited (Dida et al., 2006) thus knowledge on genetic variability of germplasm is vitally important for improvement of finger millet. Although novel methods such as molecular techniques are available for correct and more reliable assessment of the germplasm, conventional

evaluation of morphological and quantitative traits is still very important. Yield is known to be highly variable and selection for traits associated with yield should indirectly improve yield (Akinyele and Osekita, 2006; Nkongolo et al., 2008; Wilson et al., 2008). Because finger millet has long been neglected by plant breeders, the information on genetic variability and correlations among traits is still lacking, so an extensive study on variation of these traits that are associated with yield will help in identifying and selecting genotypes with superior yield related traits. Genetic evaluation can also be manipulated either for selecting superior genotypes or to be utilized as parents for the development of future cultivars through hybridization (Khan et al., 2011).

In the past morphological traits have been widely used to evaluate genetic variability in crops and although it is usually cost effective, morphological assessment may have its own limitations like insufficient variation among cultivars (especially if the cultivar to be compared shared a closely related pedigree), subjectivity in the analysis, influence of the environmental and managements practices and also expression of some characters are only visible at certain development stages. Upadhyaya et al. (2004) also reported that data on character associations could be used to identify a few traits, which are less relevant and could be of low priority in germplasm evolution. Upadhyaya et al. (2004) used morphological markers to assess the morphological diversity in finger millet germ-plasm and they successfully managed to group or cluster all the germplasms.

Nowadays, there are several different molecular methods for identification of cultivars. The mostly used molecular markers includes but not limited to ISSRs, SSRs, RFLP, AFLP and RAPDs, development on new and much precise molecular markers is still ongoing as more markers are been developed to get maximum results on molecular classification. Of these techniques, random amplified polymorphic DNAs (RAPDs) seem to be one of the most popular. RAPDs have been used for measuring genetic diversity in several plant species (Fernandez et al., 2002). RAPDs are fairly cheap, can be automated, they produce good polymorphisms and produce results quicker as compared to other techniques (Yumbi, 2010). Kumari and Pande (2010) reported that RAPD markers proved to be very informative and useful in monitoring the genetic diversity present in a sample of eleven finger millet

germplasm. RAPD is quite efficient in bringing out finger millet diversity at DNA level as compared to morphological characterization. The study identified diverse genotypes of GEC 182 and CO 12 for further use in hybridization program for finger millet improvement (Babu et al., 2007). In all the past researches, all the researchers were using either morphological markers or DNA markers none of them used both markers and correlated results of genetic variation.

To fully exploit genetic potential of finger millet, it is important to know the extent of the already existing genetic variability in the available accessions or landraces. This justifies the continued characterization and evaluation of different landraces or accessions and populations stored in germplasm banks in many research centers. In this study both markers will be used to identify the genetic similarity and dis-similarity of the germ-plasm which was not done before and correlate the results of the two markers.

1.2 Objectives of the research

The experiments were conducted in order:

1.2.1 To evaluate finger millet (*Eleusine coracana*) variation using morphological characters/traits and DNA fingerprint using RAPD markers

1.2.2 To evaluate genotype, season and genotype by season interaction among selected finger millet accession.

1.3 Research limitations

This study was conducted only on one location over two seasons where yield, yield related characters and agronomic traits were evaluated. The experiment was conducted at the research station of the Khon Kaen University where the soil was mainly sandy loam. The soil might have limited the phenotypic expression of the crops. DNA fingerprinting was done using the RAPDs markers which have proved to be very reliable when used to evaluate variation among cultivated crops even though the assays produced are not reproducible when re-run under the same conditions.

1.4 Scope of the research

The experiments were carried out over two season; dry season (November-March 2010/11) and rainy season (May-October 2011), where 83 accessions were evaluated in the field and leaf samples were collected to be used in DNA fingerprinting in the laboratory. RAPD molecular markers were used to fingerprint the 83 accessions.

CHAPTER II
LITERATURE REVIEW

2.1 Finger millet

2.1.1 The crops background

Finger millet (*Eleusine coracana* L. Gaerth) is a tetraploid crop (Dida et al., 2008; Panwar et al., 2010) that is 2n=4x=36, with a genome composition AABB and basic chromosome number of 9, belonging to the family Poaceae, subfamily Chloridoideae. It is commonly called Bird foot millet, Coracana, African millet, and Ragi (Dida et al., 2006; Vavidoo et al., 1998; Panwar et al., 2010). Finger millet was domesticated approximately 5000 years ago from its wild progenitor *E.coracana africana* (Dida et al., 2006) and it is believed to have originated from East Africa mainly Ethiopia. Finger millet is now mainly cultivated for human consumption in East Africa and southern India (Dida et al., 2008) and in other Asian countries like Sri Lanka (Vavidoo et al., 1998). The crop is widely adapted to wide regions in the world mainly in the arid and semi arid regions of the world (Panwar et al., 2010).

2.1.2 Growth habit and agronomic management

Finger millet is an annual robust grass (Upadhyaya et al., 2008). The crop seed lacks dormancy, it is mainly propagated by seeds, and the seeds take 6-10 days to germinate. Seedlings are sensitive to drought. The plant tillers strongly and root form from lower nodes, it takes 50-120 days emergence to flowering. Flowering on individual inflorescence last 8-10 days and proceeds from top to bottom on branches. Finger millet is predominantly self pollinated, with about 1% out-crossing. Heavy rains at flowering will greatly reduce seed set. The crop follows the C_4 photosynthesis pathway (de Wet, 2006).

Weeds are a major problem in finger millet. The first few weeks after germination being the critical stages for weed management in finger millet production (de Wet, 2006). A variety of weeds mainly grasses (*Eleusine indica, Digitaria* sp, *Cynodon dactylon, Cyperus* sp, *Panicum maximum*) were reported to be the most obnoxious in finger millet fields. Major broad-size weeds included *Euphorbia* sp, *Commelina* sp and *Bidensa pilosa* (Tenywa et al., 1999). Weeding can be done

exclusively manual, assisted by the use of small short-handed hoes. The efficiency of this method is very low because it is laborious and time consuming. Mechanical weed control like tractor drawn harrower can only be used if the crop is planted in rows. Use of herbicide is also very low because most weeds are grasses and they resemble the finger millet, so unless selective herbicides (which are more expensive) are used, therefore chemical control is avoided to safe production cost (de Wet, 2006). Finger millet responds well to fertilizers. The recommended rates of application are 40-60 kg N, 24-40 kg P and 30-50 kg K per hectare. The crop grows well at an average temperature of 23°C. The minimum rainfall required for the plant growth is 300-500 mm. Finger millet grows well on a wide range of, it can withstand high level of salinity (Dida et al., 2006), but it prefers fertile soils, well drained, sandy to sandy loam soils with reasonable water holding capacity (de Wet, 2006) and is also relatively resistant to water logging (Dida et al., 2006), it also prefers a pH of 5-7, but tolerates very alkaline (pH) soils

2.1.3 Yields and uses

Despite the great value associated with this crop, its productivity (yield per unit area) has remained low. Production figures consistently show increase in area under finger millet production, but paralleled by a decline in land productivity (Tenywa et al., 1999), precise global area under production is not known because this crop is often been grouped with other millets (Upadhyaya et al., 2004; Dida et al., 2008). However it is estimated that some 10% of the world's 30 million produced millets is finger millet. The yield of finger millet is in the range of 4-5 t/ha but yields vary greatly depending on the country and regions (Dida et al., 2008). Under irrigated conditions in field trials, the crop yields of up to 5-6 t/ha have been obtained. However yields in farmers fields usually sown with unimproved varieties, are commonly between 0.4 and 2 t/ha (Dida et al., 2006). The average finger millet grain yield under local practices of agriculture in tropical Africa is 0.25-1.5 t/ha. With improved cultivars, optimal weed control and use of fertilizer yields of the crop can go up to 5 t/ha under experimental conditions. Straw yields range from 1-1.5 t/ha for rain-fed crops and 9 t/ha for irrigated crops.

Finger millet ranks fourth among millets after sorghum, pearl millet and foxtail millet. It is a staple food crop in drought prone regions of the world and is

considered an important component of food security (Upadhyaya et al., 2008). Generally finger millet is consumed as a whole meal (Vavidoo et al., 1998). It is rich in protein (approximately 6-13%) and calcium (about 0.3-0.4%) (Panwar et al., 2010), fat and minerals (Bisht and Mukai, 2000), it contains high fibre which helps in slowing down the rate of digestion providing energy for a long time after consumption; peasants accustomed to eating the meal work all day on a single meal. The nutritional quality of finger millet grain makes it an ideal for infants and invalids (Vavidoo et al., 1998). The grain can also be made into flour for making chapattis, cakes, puddings or porridge. The crop has high levels of amino acids and methionine (Bisht and Mukai, 2000).

Finger millet has also been reported to have some medicinal properties and is used as a folk medicine for many diseases (Bisht and Mukai, 2000), medicinally the seeds are used as a prophylaxis for dysentery. In Africa the juice of a mixture of finger millet leaves and leaves of Plumbago are taken as an internal remedy for leprosy (de Wet, 2006). Finger millet is particularly rich in tryptophan, cysteine and total aromatic acid and has high iron content as compared to other cereals which highly helps in malnutrition (Upadhyaya et al., 2004). On the other hand some of the health benefits such as hypoglycemic, hypo-cholestrolemic and anti-ulcerative are associated with the millet (Chetan and Malleshi, 2007).

The other use of finger millet in Africa is to provide malt for making local beer and other alcoholic or non-alcoholic beverages (Upadhyaya et al., 2004), in some parts of India the grain is used for making beer and liquor called arak. In Uganda the by-products of finger millet beer production are fed to chickens, pigs and other animals. The crop straw is used for thatching and plaiting and in China is used for paper making. In Sudan the leaves are made into strings (de Wet, 2006). The crop residues are excellent source of dry matter for livestock especially in dry season. Finger millet makes good fodder and containing up to 61% total digestible nutrients (Upadhyaya et al., 2008).

2.2 Plant identification

The precise, fast, cost effective and reliable identification of important cultivars is important in agriculture in general as well as for practical breeding

purposes and related areas such plant proprietary rights protection. Traditional methods of cultivar identification frequently used are based on the evaluation sets of morphological characteristics. Although it is usually cost effective, morphological assessment may have their limitations like insufficient variation among cultivars (especially if the cultivar to be compared shared a closely related pedigree), subjectivity in the analysis, influence of the environmental and management practices and also expression of some characters are only visible at certain development stages. These limitations triggered the exploration of alternative means of cultivar identification like the allozymes analyses, cytogenetic, analysis of secondary metabolites and DNA profiling (Weising et al., 2005).

2.3 Genetic markers

Genetic markers are specific locations on a chromosome which serve as landmarks for genome analysis. Genetic markers are basically of two types being; morphological and molecular markers. Within the last twenty years, molecular biology has revolutionized conventional breeding techniques in all areas. Biochemical and molecular techniques have shortened the duration of breeding programs from years to months, weeks, or eliminated the need for them all together. The use of molecular markers in conventional breeding techniques has also improved the accuracy of crosses and allowed breeders to produce strains with combined traits that were impossible before the advent of DNA technology (Stuber et al., 1999).

Morphological and nuclear DNA markers are inherited in a Mendelian manner. In any genome, the number of morphological markers is limited as compared to DNA markers which are ubiquitous and numerous. Natural variation of conventional genes is limited. If morphological markers are to be used for this purpose, these variants will have to be produced by mutagenesis which often gives rise to lethal mutants. In DNA marker analysis, the natural variation in the DNA is made use of and no mutagenesis is required. Essentially all DNA markers have no effect on the phenotype because they are reflections of natural variation present in the DNA sequence. DNA marker analysis can be carried out at any stage of the life cycle of an organism and from almost any tissue including herbarium and mummified tissue. Morphological markers depend upon the expression of certain genes which in

turn are governed by environmental conditions, tissue specificity and development stage. Morphological markers have alleles that interact in a dominant/recessive manner; more over the PCR based makers require only a few nanograms of DNA for analysis. These properties of DNA markers make them ideal candidates for their use in linkage mapping analysis (Kumar, 1999).

Correspondence between morphological characters and DNA markers in terms of genetic distance between entries is sometimes surprisingly high, indicating that both types of data are usually good estimators of genetic relatedness. This is especially true if the morphological descriptors are represented by quantitative rather than qualitative data sets. Morphological variability due to qualitative traits, presumable governed by a single factor of a few gene mutations can actually be much more pronounced than the extent of variation indicated by random amplified polymorphic DNAs (RAPDs) or other molecular markers. Consequently certain groups of plants (e.g., cultivars, subspecies or species) appear to be considerably better defined through the analysis of qualitative morphological traits rather than by DNA markers. In the past morphological markers have been used rather extensively for discrimination of genotypes, but are now superseded by DNA markers because the latter is usually fast and timely (Weising et al., 2005).

2.3.1 Morphological markers

Morphological characters have long been used to identify species, genera and families, to evaluate systematic relationships; and to discriminate cultivars, breeding lines (Weising et al., 2005). In contrasts with molecular markers, morphological characters are often strongly influenced by the environment and consequently, special breeding programs and experimental designs are needed to distinguish genotypic from phenotypic variation. The inheritance of these markers can be monitored visually without biochemical or molecular techniques. Morphological traits that are controlled by a single locus can be used as genetic markers provided their expression is reproducible over a range of environments. Beside environments, the expression of such markers is also altered by epistatic and pleiotropic interaction. The number of morphological is very limited; their alleles interact in a dominant recessive manner, thereby making it impossible to distinguish the heterozygous individual from homozygous individual (Kumar, 1999).

There are several undesirable factors that are associated with morphological markers. The first is there high dependency on environmental factors. Often the conditions that a plant is grown in can influence the expression of these markers and lead to false determination. Second, these mutant traits often have undesirable features such as dwarfism or albinism. And lastly, performing breeding experiments with these markers is time consuming, labour intensive and the large populations of plants required need large plots of land and/or greenhouse space in which to be grown (Stuber et al., 1999).

Assessment of the genetic variability within cultivated crops has a strong impact on plant breeding strategies and conservation of genetics resources. It is particularly useful in the characteristics of individuals, accessions and cultivars in determining duplication in germplasm collections and for the choice of parental genotypes in breeding programs (Abu Assar et al., 2003).

Upadhyaya et al. (2004) successfully used morphological characters like pigmentation, growth habit, seed colour inflorescence length and width to classify the finger millet accession in the major genebank, all the morphological characters showed wide variations showing that most of them can be successfully be used in classification and genetic diversity studies and evaluation.

The morphological characters viz: the plant height, the stem diameter, leaf area and physiological characters like photosynthesis rate, chlorophyll fluorescence and total chlorophyll content and SPAD value as determined for the three varieties of finger millet at vegetative and reproductive stage were measured. The data clearly indicate close similarity of PRM 701 and PRM 801; similarly the analysis of nitrogen assimilating enzymes like nitrate reductase (NR) and glutamine synthase (GS) activity for these varieties at both stages also reveals maximum similarity between PRM 701 and PRM 801 (Gupta et al., 2010).

2.3.2 Biochemical marker

Markers that reveal polymorphism at the protein level are known as Biochemical markers (Kumar, 1999). Protein profiling determines the composition, abundance, modification and subcellular localization of proteins in a cell tissue and can be applied to cultivar characterization. Variety ID based on seed storage proteins patterns is now facilitated by the use of Matrix Assisted Laser Desorption Ionization-

Time of flight Mass Spectrometry (MALDITOF MS), which provide accurate molecular weight determination and is amenable to automation. The procedure includes seed grinding, protein extraction, generation of protein profiles, conversion of the results into a database-compatible format (Terzi et al., 2005).

Isozymes are used as biochemical markers in plant breeding. Isozymes are common enzymes expressed in the cells of plants. The enzymes are extracted, and run on denaturing electrophoresis gels. The denaturing component in the gels (usually SDS) unravels the secondary and tertiary structure of the enzymes and they are then separated on the basis of net charge and mass. Polymorphic differences occur on the amino acid level allowing singular peptide polymorphism to be detected and utilized as a polymorphic biochemical marker. Biochemical markers are superior to morphological markers in that they are generally independent of environmental growth conditions. The main advantages of allozymes markers are their codominant inheritance and the technical simplicity and low cost of the assay. Disadvantages include the restricted number of suitable allozyme loci in the genome, the requirement of fresh tissue, and the sometimes limited variation (Weising et al., 2005). The major problem with isozymes in MAS is that most cultivars (commercial breeds of plants) are genetically very similar and isozymes do not produce a great amount of polymorphism and polymorphism in the protein primary structure may still cause an alteration in protein function or expression.

Sometimes the terms isozymes and allozyme, incorrectly, are treated as interchangeable. Isozymes are enzymes that convert the same substrate, but are not necessarily products of the same gene. Isozymes may be active at different life stages or in different cell compartments. Allozymes are isozymes that are coded by orthologous genes, but differ by one or more amino acids due to allelic differences (Weising et al., 2005).

Chemelik et al. (2002) identified several proteins extracted from barley grains using a combination of gel electrophoresis, MALDI-TOF MS and bioinformatics. However, the application of proteomics to varietal fingerprinting was limited by the level of variation in storage protein composition.

2.3.3 Molecular markers

Molecular markers are based on naturally occurring polymorphisms in DNA sequences (i.e.: base pair deletions, substitutions, additions or patterns) (Gupta et al., 1999). There are various methods to detect and amplify these polymorphisms so that they can be used for breeding analysis and these techniques will be the focus of this paper. Molecular markers are superior to other forms of genetic markers because they are relatively simple to detect, abundant throughout the genome even in highly bred cultivars, completely independent of environmental conditions and can be detected at virtually any stage of plant development.

A suitable molecular marker is usual characterized by basic five conditions that is; it must show high polymorphism, have co-dominant inheritance, must be distributed randomly and frequently throughout the genome, be easy and cheap to use, and must be reproducible. Most markers do not posses all the conditions so selecting a marker will be based on the one which show high number of required conditions. Molecular markers can be used for several different applications including: germplasm characterization, genetic diagnostics, characterization of transformants, study of genome organization and phylogenic analysis.

Molecular technologies provide almost unlimited numbers of potential markers at the genotypic level. Markers that reveal polymorphism at the DNA level are known as DNA markers. DNA markers can be classified in two categories depending upon how the polymorphism is revealed; Hybridization-based polymorphism and PCR-based polymorphisms. Hybridization based polymorphisms include RFLPs (restriction fragment length polymorphisms). PCR based polymorphisms include RAPDs (random amplified polymorphism DNA), MAAP (multiple arbitrary amplicons profiling), AP-PCR (arbitrary primer-PCR), VNTR (variable number tandem repeats) and DAF (DNA amplification fingerprinting) and more lately AFLP and ISSRs. The three steps of the PCR reaction (i.e. template denaturation, primer annealling and enzyme extension) are repeated many times to ensure high level of amplification of the intervening regions specified by the repeats. In each cycle the quantity of DNA is doubled resulting in an exponential increase in the amount of target DNA which can then be directly detected on gel (Kumar, 1999).

One of the PCR based molecular marker which is widely used for evaluating genetic diversity in plants is the rapid primers or RAPDs. The RAPD have all the characteristics for a simple marker and matches most of the characteristics mentioned above of a suitable marker and apart from those characters RAPDs have several advantages and that is why they are widely used in evaluating genetic variation in plants (Yumbi et al., 2010). The RAPD system has the advantage of being a first assay requiring only nanograms of genomic DNA and minimum apparatus. Therefore, it has been used to analyse the genetic relatedness in several crop species (Fakrudin et al., 2004).

2.4 RAPD markers

RAPD was the first PCR based molecular marker technique developed and it is by far the simplest (Williams et al., 1990). Short PCR primers (approximately 10 bases) are randomly and arbitrarily selected to amplify random DNA segments throughout the genome. The resulting amplification product is generated at the region flanking a part of the 10 bp priming sites in the appropriate orientation. RAPD often shows a dominant relationship due to primer being unable to bind (show 3:1 ration, unable to distinguish between homozygotes and heterozygotes (Yin et al., 2001)

The greatest advantage of the RAPD approach is its technical simplicity, paired with the independence of any prior DNA sequence information. Many researchers where enthusiastic about novel marker technique and myriad, RAPD studies were initiated in the 1990 (Williams et al., 1990). Main application areas include the identification of cultivars and clones, genetic mapping, marker assisted selection, population genetics and molecular systems at species level. One obvious advantage that RAPDs share with other molecular markers is their dominant nature, which limits their use for population genetics and mapping studies. RAPDs also turned out to be sensitive to slight changes in reaction conditions, which interfere with reproducibility of banding patterns between separate experiments, PCR istrumentation and laboratories. This high sensitivity is at least in part a consequence of the non-stringent PCR conditions, which are needed to allow for mismatch priming (Weising et al., 2005).

RAPD markers proved to be very informative and useful in monitoring the genetic diversity present in a sample of 12 selected accessions. Variations between finger millet accessions were observed with 35-37 selected primers (Fakrudin et al., 2004).

Agrama and Tuinstra, (2003) found out that genetic similarity among entries was higher when it was determined using RAPD markers (0.612). Genetic diversity of sorghum measured using SSR and RAPD markers exhibited highly significant associated with geographical origin and race classification ($P<0.01$).

2.5 Cluster analysis and grouping

A dendrogram is a genealogical diagram that resembles a tree; an evolutionary tree diagram that may order objects, individual genes on the basis of similarity using cluster analysis; a technique of statistical analysis in which similar variances are grouped or clustered together; and these results are shown in dendrograms particularly. In cross breeding, the cluster analysis is used in order to select most diverse parents for crossing (Schlegel, 2010). The aim of producing a dendrogram is to visualize the best representative of the phenotypic (overall similarity) or phylogenetic (evolutionary history) relationships among a group of so called operational taxonomic units (OTUs). These can be individuals, cultivars, populations or species. The most frequent used matrix algorithm is the so called un-weighted pair group method using arithmetic average (UPGMA). UPGMA assumes a rigid molecular clock, which means that the evolutionary rates along all branches of the tree needed to be identical (Weising et al., 2005). The dendrogram generated from the UPGMA clusters analysis based on Nei and LI similarity indices grouped all the 96 genotypes into two main clusters and 18 significant sub-clusters related to origin and morphological characters (Gupta et al., 2010).

The similarity coefficients were used as an input data for cluster performed by NTSYSpc program. The accessions clustered into two major groups, corresponding largely to African and Indian types at a similarity index corresponding to 0.55 with an intermix of few accessions. This is similar with Ishil's version (1996) in the rice where accessions with geographical proximity clustered together more frequently compared to the ones from different geographical locations. Advanced breeding lines

derived from different geographical parents (indica and japonica types) positioned into both clusters corresponding to indica and japonica groups. Two African types, GE 4865 and IE 2912 clustered with the Indian accessions while the two Indian types Indaf 9 and Sel 14 were grouped with African types (Fakrudin et al., 2004).

2.6 Genotype by season interaction (GXS)

Knowledge of genetic stability is the most important in plant improvement programs. Further, the breeding material should be evaluated under different environments, because in absence of that information, estimation of heritability and prediction of genetic advance become biased. The best genotype is the one that has consistent high performance over several environments (Allard and Bradshaw, 1964). Stability of yield of a cultivar across a range of production environments is very important for varietal recommendations. The cultivar must have the genetic potential for superior performance under ideal growing conditions, and must produce acceptable yields under less favourable environments (Fekadu et al., 2009) and the importance of testing genotypes over a range of environments is recognized as a prerequisite for crop improvement.

An ideal cultivation must maintain yield levels not only in the original environment but also in many other environments within its intended area of production (Monyo et al., 2003). Seasonal adaptation is also important because an ideal cultivar must be able to produce average yields in all others seasons than the normal growing season for it to be considered stable or superior to others.

Morakinyo and Ajibade (1998) reported that another variable of the environment which is comparable with years is season. That means that season can be included as a variable when analysing the genotype by environment (GxE) interactions for crops. Genotype by season (GxS) can record highly significant interaction ahead of genotype by location (GxL) showing that the evaluation on genotypes in different season is more important than the evaluation at various locations (Pereira et al., 2011).

Genotype by environment (GxE) interaction and yield stability analysis has continued to be important in measuring varietal stability and suitability for cultivation across seasons and ecological zones (Nassir and Ariyo, 2011) and the importance of

testing genotypes over a range of environments is recognized as a prerequisite for crop improvement for plant breeders. An ideal cultivation must maintain yields levels not only in the original environment but also in many other environments within its intended area of production (Monyo et al., 2003). The relative performance of cultivars for quantitative traits such as yield varies from one environment to another and there is general agreement amongst breeders that interaction between genotype x environment has an important impact on improvement of varieties (Shakoor et al., 1999)

CHAPTER III
MATERIALS AND METHODS

3.1 Experiment I: classification of finger millet accessions using morphological classification

Two consecutive field experiments were conducted in Field Crops Experiment Station of Khon Kaen University, Khon Kaen province, Thailand (latitude 16° 28′ N, longitude 102° 48′ E, 200 m above sea level). The first planting was carried out in dry season between November 2010 to March 2011 and the second planting was carried out on the rainy season between May 2011 and October 2011. Planting, agronomic management practices and data collection for the two seasons were similar.

3.1.1 Experimental design

The experiments were conducted in a Randomised Complete Block Design (RCBD), with 3 replications. Eighty three finger millet accessions were used as treatment thus making a total of 83 treatments.

3.1.2 Plant material

Eighty three finger millet accessions were acquired from International Crops Research Institute for the Semi Arid Tropics (ICRISAT) genebank in India (Table 1). The accessions had varying origins but mainly from two main producing continents; Africa and Asia.

3.1.3 Land preparation and planting

Land was prepared by double ploughing with a walk behind tractor. Double ploughed to reduce the incidence of weeds and breaking down the disease and pests cycles in the soil. The soil was then levelled before plots were demarcated and marked with wooden pegs. Plots were such they were two rows of 5 meters long and spacing between rows was 50 cm and between planting hills was 20 cm, thereby giving a plant population of 50 plants $plot^{-1}$

3.1.4 Crop management

Two weeks after seedling emergence, plants were thinned out to maintain one plant per hill. Only stronger and healthier seedlings were left to stand while the

weaker ones were thinned out. Split fertilizer of grade 15-15-15 was basally dressed and top dressed 40 days after seedling emergence at the rate of 70 kg ha^{-1} for each dressing. Supplementary irrigation was done during the dry season to provide adequate moisture for the crop growth. During the rainy season, the rainfall received was adequate thus no irrigation was undertaken. Weeding was done at two crop growth stages, the initial weeding was done 40 days after seedling emergence and the second weeding was done at flowering stage and long arm hoes were used for weeding. No disease outbreak was observed for both the two growing seasons. The major pest of millet which usually reduces the yields by more than half was controlled by erecting a non shading nylon net over the whole experimental area when early accessions started flowering.

3.1.5 Data collection

Data were recorded for quantitative and qualitative agronomic traits at different growth stages of the crop from five randomly selected plants in the middle of every plot. From the 83 accessions planted in the field only 82 accessions were used as one accession (IE 4816) showed poor plant stand and low yields for both growing seasons. Plant descriptors for finger millet were followed when data were collected (IBPGR, 1985). The three stages in which data was collected were at flowering stage, maturity stage and at final harvest. Flowering stage was defined as the stage when 50% of the main tillers in a plot have flowered, while maturity stage was defined as when 50% of the main tillers in plot have fully matured and final harvest referred to the stage when plants have been harvested and fully processed and been stored as seed or grain. Data collected at each different growth stage were as follows;

1. At flowering stage:
   - (i) Days to flowering: number of days from seedling emergence to when 50% of the main tillers in a plot have flowered
   - (ii) Number of productive tillers: a total number of tillers bearing a panicle per plant counted to get the total number of productive tillers per plant
   - (iii) Number of leaves on the main tiller: green leaves on the tallest and strongest tiller were counted to get total number of leaves per main tiller (dead or un-healthy leaves were not counted)

(iv) Flag leaf length: the length of the apex leaf or growth leaf on the main tiller measured from the tip to the base where it joins the stem

(v) Flag leaf width: width of the apex leaf or growth leaf measured from one margin to the other at the middle section of the leaf to get the leaf width

(vi) Blade leaf length: the fourth leaf from the apex leaf or flag leaf was measured from tip to base were the leaf offshoots from the stem to get blade leaf length

(vii) Blade leaf width: the width of the forth leaf from the apex or flag leaf measured from one margin to the other to get the blade leaf width

(viii) Pigmentation: any colour (mainly purple) change or no colour (green) change was observed and recorded as presence of pigmentation or absence of pigmentation either on the leaves or the stem

(ix) Growth habit: the form the plant takes when it grows up vegetative was observed and defined as either erect (growing up straight from the ground) or decumbent (first three-four nodes from the ground grow parallel to the ground then bend upward to give the plant an erect shape)

2. At maturity stage

(i) Days to maturity: number of days from seedling emergence to when 50% of panicles in main tillers of a plot have fully matured were recorded as number of days taken to reach maturity for every accession

(ii) Number of mature fingers: fully mature fingers in main tiller panicle were counted and recorded as number of mature tillers per panicle (immature fingers and fingers with no seeds or less than 50% of finger covered with seed were excluded in number of fingers per panicle)

(iii) Length of the longest finger: the longest finger in a panicle of the main tiller was measured from the base to the tip to get the length of the finger

(iv) Width of the main finger: the main finger or the longest finger in a panicle was measured for its thickness using a digital vernier calliper to the nearest millimetres

(v) Plant height: the height of the plant was collected from the main tiller which was the longest, measured from just above ground level to the tip of the longest finger

(vi) Stem diameter: the thickness of the plant stem was collected from the main tiller at the forth node above ground and was collected using a digital vernier calliper and recorded to the nearest millimetres.

(vii) Peduncle length: the last node of the tiller bearing the panicle referred to as the peduncle was measured from the start of the node to just below the panicle head and recorded as the peduncle length of the main tiller

(viii) Panicle shape: shape of mature panicle was observed before harvesting in its undisturbed form and compared to the shapes of panicles provided on the IBPGR, (1985).

(ix) Lodging susceptibility: plants ability to withstand heavy rainfall or heavy irrigation and heavy winds while bearing mature panicles were recorded as resistant to lodging

(x) At final harvest

(i) plant yield: all mature panicles harvested from the plant , threshed and cleaned were then weighed to get seed weight which was then recorded as individual plant yield

(ii) 1000 seed weight: 1000 healthy and whole seeds were manually counted and weighed to get mass of 1000 seeds

(iii) Seed colour: colour of seeds were observed after fully post harvest processing and compared to the description's provided in the finger millet descriptor IBPGR, (1985)

3.1.6 Data analysis

Combined analysis of variance was performed for two seasons for yields, yield components and agronomic traits using Mstat-C software. Means were compared by the least significant difference test (LSD) at 0.05 probability level.

Table 1 Origin of 83 accessions of finger millet used in the classification of crop using morphological traits and DNA fingerprinting

Continent	Country	No. of accessions
Africa	Burundi	1
	Kenya	8
	Malawi	4
	Nigeria	1
	Senegal	1
	Uganda	10
	Zimbabwe	21
	Zambia	3
America	USA	1
Asia	India	20
	Nepal	9
	Maldives	1
Europe	Germany	1
Unknown	unknown	2
	Total	83

Agronomic data collected from the three replications were averaged and each trait divided into 10 levels based on its mean value and standard deviation. Then the continuous agronomic data were scored 0 and 1 based on the 10 levels, the data was score as 1 where the agronomic trait value was at that level and score 0 where the agronomic trait value was not at that level. The genetic variations among accessions were evaluated by calculating the Jaccard's similarity coefficients for pairwise comparisons based on the presence and absence of morphological data scoring. NTSYS-Pc 2.1 was then used to construct a dendrogram.

3.2 Experiment II: DNA fingerprinting of finger millet accessions using RAPD markers

3.2.1 Plant samples

Plant samples were collected on the 83 accession planted in field for morphological evaluation during the dry season planting (November-March 2010/11), leaf samples were collected from seedlings seven days from emergence and the leaf samples were then stored in ice box and taken to laboratory. The samples were kept in the refrigerator at -20°C until DNA extraction.

3.2.2 DNA extraction

Leaf samples sampled from the field were taken from the field and frozen in liquid nitrogen where Dellaporta et al., (1983) method with minor improvements was used to extract DNA and the DNA extraction protocol was as follows;

(i) Weigh about 5-10 g of plant tissue

(ii) Place tissue on a clean mortar. Cut the leaves into smaller pieces.

(iii) Add liquid nitrogen and grind until fine powder is obtained (about 3g of tissue) the extraction of the nucleic acids is optimal when the powder is very fine.

(iv) Transfer the powder into a 1.5 ml microfuge tube.

(v) Add 500 µl of extraction buffer.

(vi) Incubate the tubes at 65°C in water bath for 15 min.

(vii) Add 250 µl of potassium acetate and mix vigorously

(viii) Centrifuge for 10 minutes at 13000 rpm then transfer supernatant into a new microfuge tube

(ix) Transfer tubes into freezer (-20°C) and incubate for 1 hour

(x) Centrifuge at 13000 rpm for 10 min at 4°C

(xi) Filter the mix into a tube containing 500µl of absolute ethanol at -20°C

(xii) Mix gentle

(xiii) Incubate tubes at -20°C for 15 min

(xiv) Centrifuge at 13000 rpm for 5 min at 4°C

(xv) Discard the supernatant

(xvi) Clean the pellet with70% alcohol and centrifuge for 2 minutes

(xvii) Dry the pellets overnight or at least 12 hours

(xviii) Dissolve the pellet with 30µl TE buffer or sterile water

(xix) Store at -20°C until use

3.2.3 DNA quality and quantity

The DNA quality and quantity was assessed using gel electrophoresis in 1% agarose gels and the concentrations were estimated by visual assessment compared with lambda (λ) DNA of known quantity and quality (50 and 100 ng/ul). DNA aliquots of 50ng were taken from large samples to be used as working concentration, if the DNA quantity was higher than 50ng the aliquots were diluted with double distilled H_2O (ddH_20). The protocol for checking the quality and quantity of DNA was as follows;

(i) Cast 1.0% (w/v) regular gel in 1X TBE

(ii) Dissolve agarose powder into 1x TBE buffer (1% gel= 0.4g agarose in 40ml TBE buffer)

(iii) Let the gel cool down in gel plate at-least for 30 minutes

(iv) Mix PCR product with 3X loading dye, load into gel at 2ul DNA sample to 4ul 3x dye

(v) Mix lambda DNA with dye and load

(vi) Run the gel for at-least 30 min at 100v

(vii) Stain gel in Ethidium bromide for at least 30 minutes

(viii) View results/picture under a UV light

3.2.4 PCR optimization and amplification

Ten RAPD primers were randomly selected to optimize the PCR amplification. Optimization was done by running PCR with varying annealing temperatures to get the best temperature that shows clear and distinct band. $MgCl_2$ concentrations were also varied to get the best amplified products. The PCR profile which gave the clearest bands was adopted and it is explained below.

The PCR assay was carried out in 20µl reaction volume containing 50ng of DNA, 5X buffer (4µl), 25mM $MgCl_2$ (2.4µl), 0.2mM each dNTP (0.4µl), 0.2mM random primer (0.8µl), 0.5 U *Taq* polymerase and topped up with dH_2O.

Amplification was performed using a Biometra with simulated tube. The amplifications were programmed for a certain time at a certain temperature for initial denaturation at 94°C for 5 minutes, then followed by 44 cycles of 30 seconds at 94°C

for denaturation, 30 seconds at 40°C for annealing and 30 seconds at 72°C for elongation, using the fastest possible transmission time possible between each temperature and then a final extension for 5 minutes at 72°C.

3.2.5 Primer screening and selection

Initially 120 Operon (OP) primers were acquired from Biodesign Co., ltd and were screened to select the one which gave good banding. Five finger millet accessions were randomly selected for primer screening and used to run PCR for all the primers. A primer which showed good banding was then selected to be further screened for polymorphism. From the initial 120 primers, 80 showed good banding pattern. Five new randomly selected finger millet accessions were then selected to screen polymorphic primers. Primers which showed four or more bands and been polymorphic were selected to be used for DNA fingerprinting of 83 finger millet accessions. A total of 44 polymorphic primers were selected based on polymorphism.

3.2.6 DNA amplification

The amplification protocol adopted from PCR optimization was then used to amplify the DNA of 83 accessions of finger millet using the 44 polymorphic primers selected from primer screening. After amplification the amplified products were then separated on 2% agarose gel or stored in a refrigerator and used within 20 days.

3.2.7 Gel electrophoresis

The amplified products obtained after PCR were separated by gel electrophoresis in 2% agarose gels ran in 1xTBE buffer and electrophoresed at a constant voltage of 100v for 2:30hrs on Bio-Rad model 1000/500. The gel was stained with Ethidium bromide and DNA fragments were detected by UV trans-illumination and photographed under UV and picture of DNA fragments were captured using Sony Syngene gene flash camera. The sizes of the amplified fragments were estimated against the ladder.

3.2.8 Data scoring

Each unambiguous band was treated as an independent character or locus and assigned number in order of decreasing molecular weight. The size of each band was estimated using the lambda (λ) DNA molecular weight marker. A band was scored as present (1) and absent (0).

3.2.9 Data analysis

The genetic variations between accessions were evaluated by calculating the Jaccard's similarity coefficient for pairwise comparisons based on the proportion of shared band produced by primers. Data analysis was performed using the Numerical Taxonomy System (NTSYS-PC Version 2.11). Dendrogram was constructed using Un-weighted pair group method for arithmetic (UPGMA) mean method.

3.3 Experiment III: genotype, season and genotype X season interaction

This experiment used the data collected for experiment I (3.1), with only 35 accessions which produced sufficient plant and plot yield and maintained the maximum plant stand per plot for the two seasons were selected to be used in this experiment. The selected accessions represented the two main millet producing continents; Africa and Asia (Table 2). Ten yield, Yield components and economic agronomic traits were selected to be used in this research.

Table 2 Finger millet accession and their origin grown in dry season 2010/11 and rainy season 2011 for genotype by season interaction analysis

Accession	Origin	Accession	Origin	Accession	Origin
501	India	3470	India	4734	India
518	India	3475	India	4757	India
1055	Unknown	3614	Unknown	4797	Maldives
2034	India	3618	India	5066	Senegal
2042	India	3945	Uganda	5201	India
2043	India	3952	Uganda	5367	Kenya
2217	India	3973	Uganda	5537	Nepal
2430	Kenya	4028	Uganda	6059	Nepal
2457	Kenya	4121	Uganda	6165	Nepal
2872	Zambia	4565	Zimbabwe	7018	Kenya
3077	India	4671	India	7079	Kenya
3104	India	4673	India		

3.3.1 Data analysis

Data obtained for all ten traits of 35 accessions for the two seasons was subjected to a two way analysis of variance using Mstat-C computer program. Means were compared by the least significant difference (LSD) at 0.05 probability level. Further correlation analysis was done using data for two seasons with the Mstat-C computer program.

CHAPTER IV
RESULTS

4.1 Morphological classification

4.1.1 Qualitative traits

(i) Plant pigmentation: from a total of 82 accessions used in this study 52 (63.4%) accessions showed no pigmentation that is, the leaves and stems were all green with no any foreign colour while the other 30 (36.6%) accessions showed a purple colour either on the leaves or stems.

(ii) Growth habit: almost all accessions showed the similar pattern of growth showing an erect growth habit as a total of 81 (98.8%) were erect during the vegetative growth stage up while only one plant had a decumbent growth pattern and none had a prostate growth pattern (Table 3).

(iii) Inflorescence compactness: of the four patterns of the panicle shapes defined as inflorescence compactness a total of 45 (54.9%) plants had long and open panicles. The second largest group of accessions had a fisty panicle and a total of 29 (35.4%) accessions belonged to this group. The third group which was described by the compact panicle shape had 6 (7.3%) accessions falling under them and the group with least accessions was the one with pendulous panicles shape having only two accessions showing the similar shape.

(iv) Grain colour: a total of 6 different colours were observed through the 82 accessions evaluated in this study. The most dominant colour was the ragi brown colour as a total of 49 accessions (59.8%) were of the ragi brown colour. Purple brown grain colour was also dominant in the study with a total of 17 (20.7%) accession in the study showing the related colour, dark brown was also significant in the study as a total of 8 (9.8%) accession showed the colour close to dark brown or dark brown colour. The least dominant colours observed in this study were brown, light brown and white colour with 4, 2, 2 accessions in each group respectively.

(v) Lodging susceptibility: Plants that could not withstand the heavy rains or heavy winds when they beard mature panicles were observed and recorded as susceptible

(i) to lodging and in this study 29 (35.4%) accessions were susceptible to lodging while those that resisted falling down during heavy rains were observed and recorded as resistant to lodging and 53 (64.6%) accessions showed resistance to lodging.

Table 3 Morphological classification of accessions under qualitative traits

Trait	Number of accession	Percentage
Plant pigmentation	82	
No pigmentation	52	63.4
Purple	30	36.6
Growth habit	82	
Decumbent	1	1.2
Erect	81	98.8
Prostate	0	0
Inflorescence compactness	82	
Compact	6	7.3
Fisty	29	35.4
Long open	45	54.9
Pendulous	2	2.4
Grain colour	82	
White	2	2.4
Light brown	2	2.4
Brown	4	4.9
Ragi brown	49	59.8
Red/dark brown	8	89.8
Purple brown	17	20.7
Lodging susceptibility	82	
Susceptible	29	35.4
Resistant	53	64.6

4.1.2 Quantitative traits

Sixteen quantitative traits including growth, maturity, yield and yield component traits are presented in Table 4. Significant differences among finger millet accessions were observed for all characters under investigation. Variation in grain yield was high, ranging from 15.0-144.4 g plant^{-1}, and variation in grain size was also high, ranging from 0.5-5.4 g/1000 grains. There were also high variations for yield component traits such as

basal tillers (5-16), finger number (5-11), finger length (4.15-16.18 cm), finger width (1.35-13.40 mm) and peduncle length (4.35-13.4 cm).

Table 4 Population range and means for morphological quantitative traits studied under morphological classification

Traits	Range	Mean	Prob	F-test	C.V (%)
Flowering (days)	45-92	76.00	0.00	**	2.17
Maturity (days)	65-139	125.00	0.00	**	2.78
Leaf number	6-19	14.00	0 00	**	7.21
Flag leaf length (cm)	15.95-47.78	36.64	0 00	**	3.92
Flag leaf width (cm)	0.42-1.48	1.17	0.00	**	4 01
Blade leaf length (cm)	19.08-59.47	44.75	0.00	**	3.33
Blade leaf width (cm)	0.56-1.8	1.43	0.00	**	1.62
Stem diameter (mm)	1.6-10.41	10.27	0.00	**	4.24
Peduncle length (cm)	14.35-13.4	23.41	0.00	**	4.35
Plant height (cm)	25.3-128 01	91.10	0 00	**	4.18
Finger number	5-11	18.00	0 00	**	10.72
Finger length (cm)	4.15-16.18	6.71	0 00	**	5 82
Basal tillers	5-16	9 00	0 00	**	11.21
Finger width (mm)	1.35-13 40	10 52	0 00	**	4 27
Yield/plant (g)	15-144.38	74.16	0 00	**	7.18
1000 seed weight (g)	0.5-5 40	3.17	0 00	**	4.86

Days to flowering and days to maturity were in the ranges of 45-92 days and 65-139 days, respectively. These traits showed high variations and selection for maturity classes that are suitable for cropping systems is possible. Growth characters showed high variations, ranging from 6-19 leaves for leaf number, 15.95-47.78 cm for flag leaf length, 0.42-1.48 mm for flag leaf width, 19.08-59.47 cm for blade leaf length, 0.56-1.8 mm for blade leaf width and 25.3-128.01 cm for plant height.

4.1.3 Dendrogram grouping

A dendrogram (Figure 1) with correlation coefficient of 0.61 was successfully constructed with the UPGMA method with the NTSYS pc 2.1 program using the 16 quantitative traits. The dendrogram successfully managed to separate the wild species (*Eleusine coracana* subps *africana*) form the cultivated finger millet at 0.84 similarity

level. IE 501 was closely related to IE 4709 (wild species) and also separated from other cultivated finger millet accessions. The remaining 80 cultivated finger millet accessions were grouped into 5 main groups at 0.86 similarity level and the groups are as follows:

Group 1: the group was made up of 8 accessions from various countries with accession from India dominating the group with 5 out of 8 accessions originating from India. Maximum similarity in the group was observed among accession number IE 3475 and IE 4797 at 0.91 similarity level, the origin of the accessions can be traced to India and Maldives respectively. The group was made up of late flowering plants showing a group mean of 84 days taken to reach 50% of flowering and gave a range of 72-92 days. The group plants also showed high tillering, with plants in this group having a mean of 12 tillers per plants. Number of fingers for plants in this group was lower than for other groups with a 7 fingers per mature panicle.

Group 2: this group was the largest with a total of 28 accessions belonging to this group. A total of 10 countries were represented in this major group but when the group. The group further dissembled with two minor groups the accession were grouped according to continental origin with Asia (Nepal and India) group in the first group and the second group was made up of accessions from Africa (Zimbabwe, Kenya, Uganda and Maldives). One accession from USA (IE 2589) was grouped with accession from Africa. Maximum similarity for plants in this group was observed between two pairs of accessions at 0.92 similarity level and the pairs were of accession IE 3614 and IE 4073 while the other pair was between IE 2871 and IE 4795. Minimum similarity was observed between IE 2296 and IE 7018 at 0.88 similarity level. The group plants were characterized by long flag leaf with group mean of 45.51 cm and long blade leaf with a mean of 50.16 cm also having the largest blade leaf with the mean width of 1.53 cm. The plants in this group were taller than plants in all other groups recording a meat height of 98.19 cm.

Group 3: this group was made up of 16 finger millet accession. The major group had accessions originating from different countries, but when the main group dissembled down to two minor groups the accession grouping was more associated with continental origins of accession. The first minor group was made up with accessions from Asia mainly from India while the second minor group was made up of accessions from Africa

with Zimbabwe having majority of accessions in this group. Maximum similarity was observed between IE 3391 and IE 4545 at 0.92 similarity level while maximum dissimilarity was observed between IE 3104 and IE 4734 at 0.88 similarity level. The group was made up of accessions which were late maturing with a mean of 129 days taken to reach full maturity. The group plants also had a small blade at 1.33 cm wide, shorter fingers at 5.72 cm and shorter peduncle having a mean of 21.81 cm.

Group 4: The second largest group of the dendrogram with a total of 23 accessions falling under the group, this group was made up of accession from Africa with exception of 4 accessions, IE 2217, IE 3470, IE 4673 and IE 5201 originating from India. Two pairs of accessions showed maximum similarity and the pairs are IE 2710 and IE 6514 and the other pairs is IE 3317 and IE 3721 showing 0.92 similarity. Maximum dissimilarity was observed between IE 2572 and IE 6337 at 0.87 similarity level. The group was characterized by plants with high number of leaves at mean of 16 leaves per plant, biggest flag leaf at 1.51 cm wide, longest fingers at 7.55 cm and also they hard the thickest stems with mean stem diameter of 10.75 mm.

Group 5: the smallest group of the five groups from the dendrogram with only four accessions was clustered under this group. Three of the four accessions in this group originated from Nepal. The group plants were characterized by shortened period taken to reach 50% flowering with mean of 73 days taken to reach 50% flowering. Days to maturity recorded for these group plants was also lower than for other groups at 106 days taken for plants to reach maturity. The plants in these groups had the highest number of finger per mature panicle at 10 fingers.

Table 5 Group means from morphological classification dendrogram using quantitative traits

Traits	Group 1			Group 2			Group 3			Group 4			Group 5		
	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max
Flowering (days)	84.0±0.5	72.0	92.0	76±0.3	65 0	88.0	76.0±0.4	63.0	88.0	78.0±0.4	72.0	87.0	73.0±0.5	62.0	82.0
Number of tillers	12.0±0.3	7.0	12.0	9±0.2	6 0	13.0	9.0±0.4	6.0	11.0	8.0±0.3	6.0	9.0	7.0±0.7	5.0	8.0
leaf numbers	14.0±0.3	11.0	16.0	14±0.2	11.0	19.0	14.0±0.3	11.0	19.0	16.0±0.3	13.0	19.0	14.0±0.7	13.0	17.0
Flag length (cm)	34.1±0.5	27.31	47.78	45.51±0.24	28.56	47.01	32.56±0.39	30.49	38.12	35.95±0.44	29.58	39.23	33.98±0.61	31.38	36.81
Flag width (cm)	1.08±0.02	0.98	1.28	1.30±0.01	1.00	1.46	1.06±0 01	0.81	1.43	1.51±0.01	1.05	1.36	1.2±0.03	1.15	1.33
Blade length (cm)	42.4±0.60	36.76	51.76	50.2±0.30	40.13	59.5	40.2±0.40	33.58	45.32	42.93±0.40	38.77	49.27	40.41±0.76	36.27	44.55
Blade width (cm)	1.34±0.01	1.21	1.61	1.53±0 01	1.17	1.66	1.33±0 01	1.15	1.76	1.46±0.01	1.37	1.61	1.5±0.10	1.41	1.60
finger numbers	7.0±0.3	6.0	8.0	8.00±0.16	6 00	10 00	8 00±0.16	5.00	10.00	8.00±0.34	7.00	10.00	10±0.28	9.00	11.00
Finger length (cm)	6.74±0.09	5.3	5.3	7.29±0.10	4.00	16.33	5.72±0.12	4.15	8.58	7.55±0.11	5.63	10.72	5.83±0.19	4.55	7.79
Plant height (cm)	86.1±1.5	73.69	103.5	98.19±0.70	61.45	128 01	79.44±0 89	56.22	115.83	92.72±0.83	72.63	119.93	88.86±0.43	83.88	101.49
Peduncle (cm)	22.72±0.29	19.95	28.64	24.23±0.19	20 48	28.93	21.81±0.24	16.56	30.19	23.92±0.39	19.67	29.93	24.94±0.28	21.63	27.50
Finger width (mm)	10.12±0.21	7.87	11.56	10.95±0.07	7.32	13.34	10.43±0.12	8.79	13.17	10.94±0.14	9.80	12.69	10.07±0.16	9.44	10.45
Stem diameter (mm)	9.98±0.20	8.53	11.85	10.53±0.08	7.87	14.01	10.12±0.11	8.19	11.65	10.75±0.16	7.85	12.76	10.33±0.14	8.24	11.23
Days to maturity	125.0±1.6	92.0	138.0	127.0±0.7	98 0	139.0	12.09±0.8	124.1	133.2	128±1.0	112.0	138.0	106.0±1.4	76 0	125.0
Yield/plant (g)	79.53±1.62	53.01	144.38	75.8±0.1	44.4	112.4	74.59±1.39	50.92	108.86	76.2±1.55	39.87	97.82	76.71±3.06	45.49	112.12
1000 seed weight	3.01±0.00	2.53	4.13	3.20±0 00	2.60	4.00	3.11±0 01	2.77	3.73	3.29±0.00	2.53	5.00	3.23±0.01	2.60	4.23

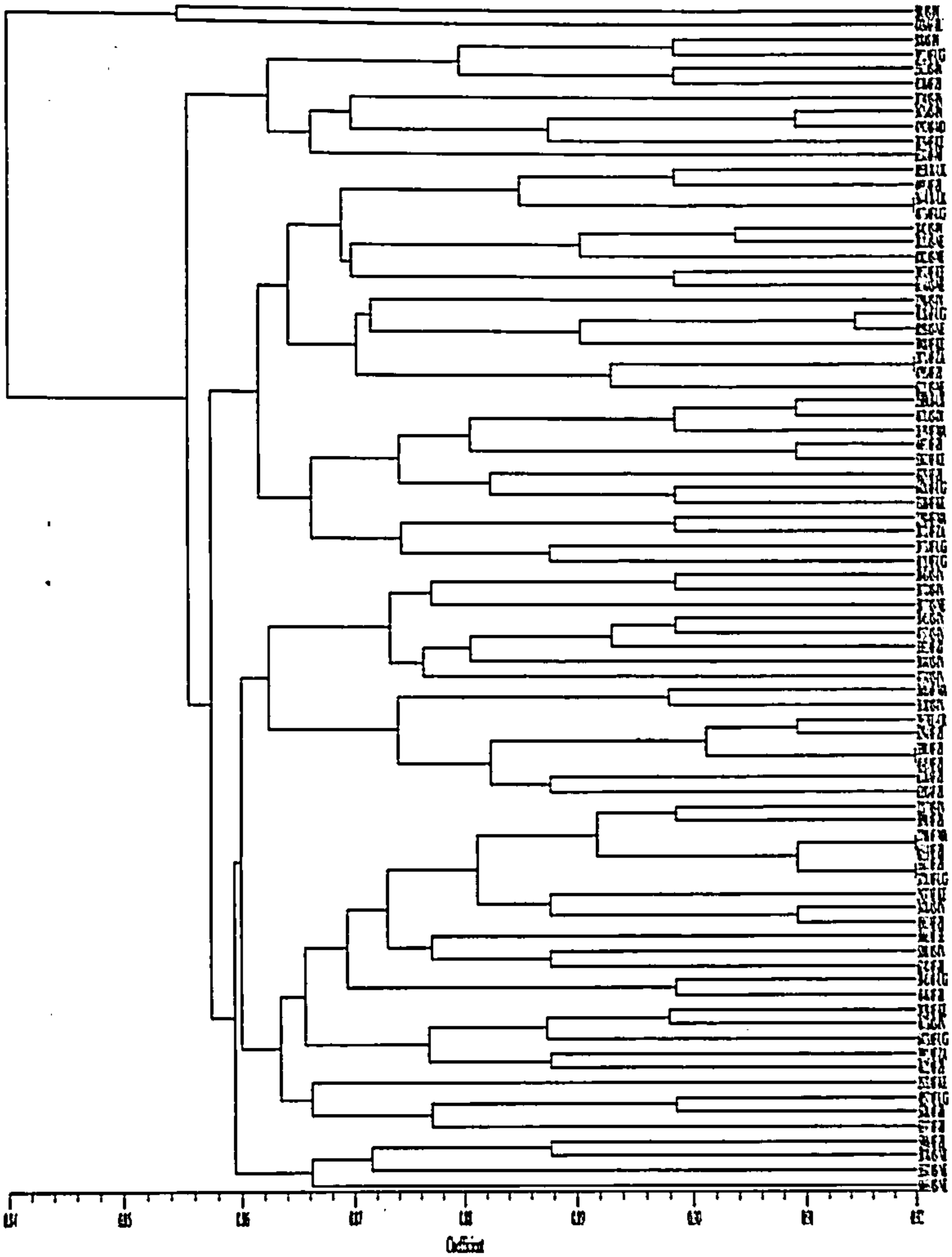

Figure 1 Dendrogram showing genetic similarity of 82 finger millet accession revealed by UPGMA cluster analysis based on morphological traits

4.1.4 Comparison between dendrogram groups

(i) Days to flowering: The entire groups showed means more than 76 days to reach 50% flowering except for the fifth group plant which took exactly 73 days to flower. Group one plants took longer days to reach flowering with mean of 84 days. The other three groups recorded means of 76, 76 and 78 days for group two, three and four, respectively (Table 5).

(ii) Basal tillers: the highest mean number of tillers recorded was 12 tillers per plant and this was observed for group one plants while the lowest was observed for group five plants with mean of 7 tillers per plant stand. Group two and three plants recorded mean tiller per plant of 9 tillers each while group four plants recorded a mean of 8 tillers per plant.

(iii) Number of leaves: the highest mean number of leaves on the main tiller recorded in this study was 16 leaves per tiller and this was observed in group four plants, while group one, two, three and five recorded mean number of leaves of 14 for all the groups.

(iv) Flag leaf length (cm): the longest mean flag leaf recorded was 45.51 cm for group two plants while the shortest was 32.56cm recorded for group three plants while the other three groups recorded flag leaf length of 34.1, 38.95 and 33.98 cm for group one, four and five plants.

(v) Flag leaf width: group four plants of the dendrogram recorded the biggest mean flag leaf in terms of width at 1.51 cm wide while the smallest mean flag leaf was observed in group three plants which were 1.06 cm wide. The other three groups, one, two and five recorded mean flag leaf width of 1.08, 1.3 and 1.2 cm, respectively.

(vi) Blade leaf length: at 50.16 cm group two plants had the longest mean blade leaf length of the five groups and at 40.22 cm group three plants were having short blade leaves as compared to others. Group one, four and five plants recorded a mean blade leaf length of 42.43, 42.93 and 40.41 cm, respectively.

(vii) Blade leaf width: at 1.33 cm group three plants were the showed the smallest blade leaf width of the five dendrogram groups while group two plants had the thickest blade leaf which was 1.53 cm wide and the other three groups recorded blade leaf width of 1.34, 1.46 and 1.5 cm for group one, four and five, respectively.

(viii) Finger number: group five recorded the highest number of fingers per panicle with a mean of 10 fingers per panicle while group two, three and four having mean of 8 finger numbers per panicle while group one recorded a mean of 7 fingers per panicle.

(ix) Finger length: with the mean of, 7.55 cm was the longest finger length recorded in these study and the plants in group four in the dendrogram were the ones with the longest fingers of all the groups. The shortest finger length was observed among group three plants recording a mean length of 5.72 cm while group one, two and five plants had a mean length of 6.74, 7.29 and 5.83 cm, respectively.

(x) Plant height: group two plants were the tallest plants in these research with a mean height of 98.19 cm as the other groups' plants recorded a mean height less than group two plants. The shortest plants in this study belonged to group three plants in the dendrogram with mean height of 79.44cm. The other three groups recorded height between the two groups with a mean height of 86.11, 92.72 and 88.86 cm for group one, four and five plants, respectively.

(xi) Peduncle length: the longest peduncle was recorded for group five plants with peduncle length of 24.94 cm while the shortest peduncle was observed in group three plants with mean peduncle length of 21.81 cm and other three groups of the dendrogram had a mean peduncle length of 22.72, 24.23 and 23.92 for group one, two and four, respectively.

(xii) Finger width: the biggest finger was observed for group two plants which had a mean finger width of 10.95 mm and the smallest finger was among group five plants with a mean width of 10.07 mm and mean width of 10.43, 10.94 and 10.41 mm was observed among group one, three and four plants, respectively.

(xiii) Stem diameter: the thickest stem of all the groups was observed among group four plants with stem diameter of 10.75 mm and group one plants had the smallest stems among other plants recording stems of 9.98 mm while the other three groups mean stem diameter was 10.53, 10.12 and 10.53 mm for group two three and five plants, respectively.

(xiv) Days to maturity: the early maturing plants belonged to group five in the dendrogram as they had the least number of days taken to reach maturity having matured

in within 106 days after emergence. Group three plants took the longest period to reach the maturity with plants in this group maturing after 129 days. The other three groups reached maturity after 123, 127 and 128 days for group one, two and four, respectively.

(xv) Yield per plant: at 79.53 g group one plant were the highest yielding plants compared to other group plants in the dendrogram as they recorded a mean yield way above other plants. Group three plants were low yielding among the groups with a mean yield of 74.59 g plant^{-1}. The other three groups recorded mean yield per plant of 75.82, 76.2 and 76.71 g plant^{-1} for group two, four and five plants, respectively.

(xvi) 1000 seed weight: not much difference was observed among the groups as all the groups recorded mean seed weight with little difference. The highest mean 1000 seed weight was observed among group four plants with a mean weight of 3.29 g while group one plants recorded the lowest mean weight of 3.01g. Mean weight of 3.2, 3.11 and 3.23 g was recorded for group two, three and five plants, respectively.

4.2 DNA fingerprinting

4.2.1 Primers

Initially a total of 120 Operon primers were screened to select the ones that give high amplification and produce constant banding when repeated under the same PCR conditions. From the initial 120 only 80 primers were selected for further analysis on polymorphism and production of good amplification. Primers which produced at least four or more bands and showed clear banding pattern and most important showing good polymorphism from the 80 primers taken through to the second screening were selected to be used in fingerprinting. A total of 44 Operon (OP) RAPD primers were selected to be used in this study for fingerprinting the 83 finger millet accessions.

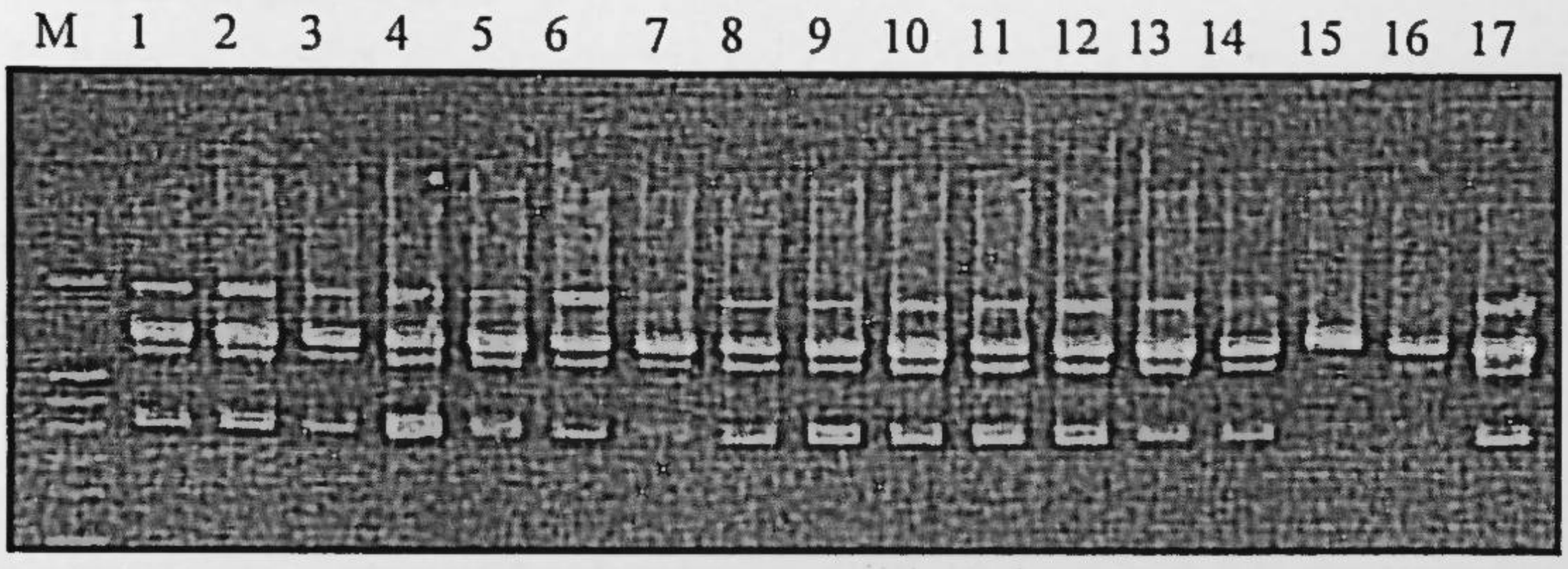

Figure 2 OPF 4 showing the lowest banding pattern of finger millet accessions during DNA amplification

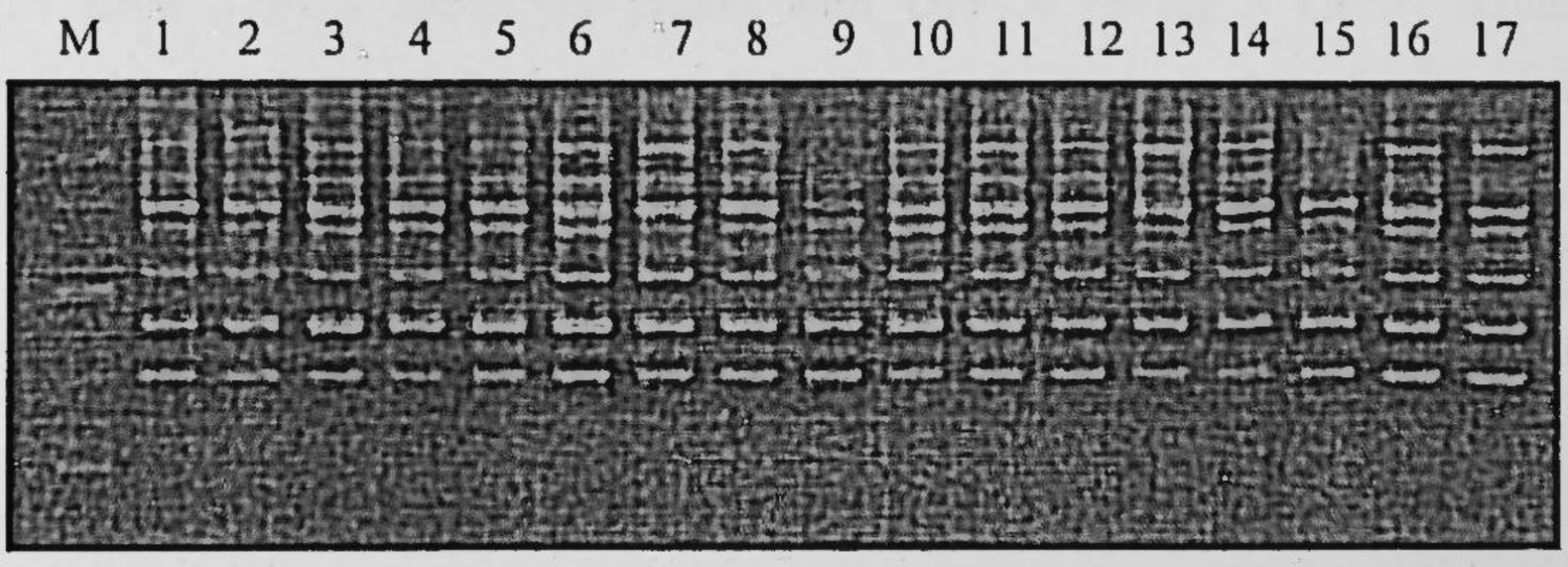

Figure 3 OPN 2 showing monomorphic banding pattern of finger millet accessions during DNA amplification

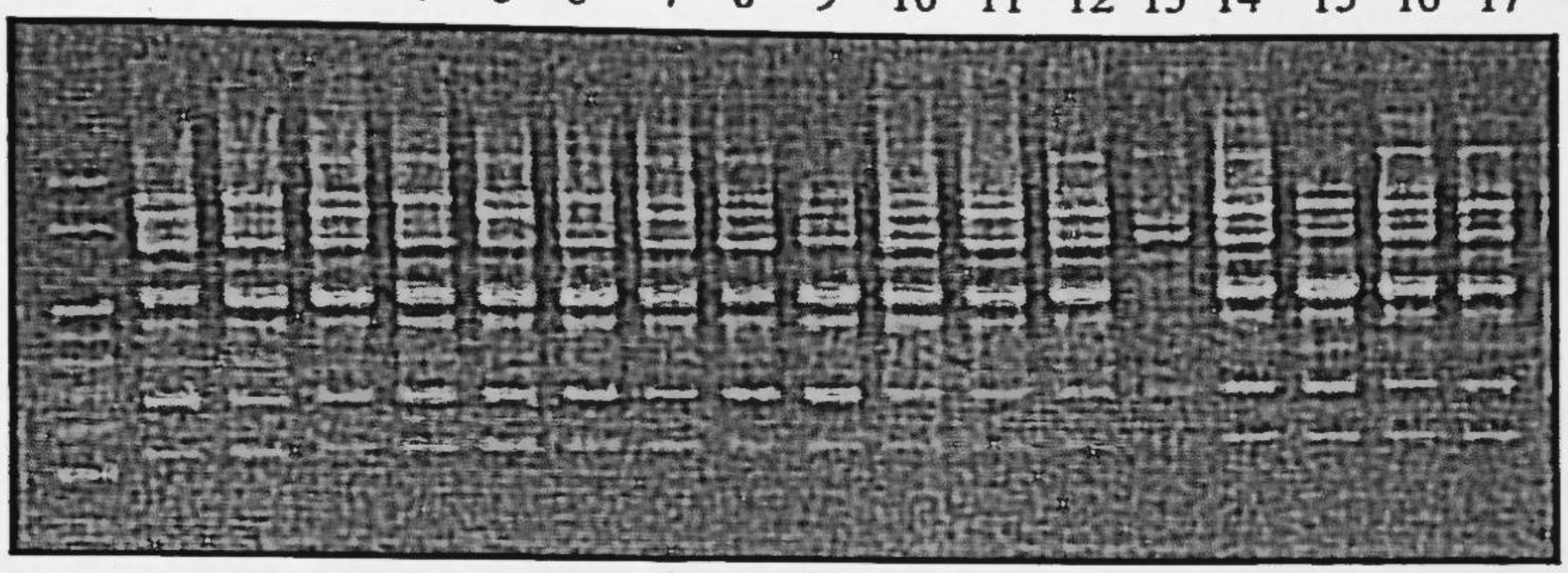

Figure 4 OPN 3 showing polymorphic banding pattern of finger millet accessions during DNA amplification

4.2.2 Amplification

Primers with 3 or polymorphic band were selected as polymorphic primers; the ones with two or less polymorphic bands were discarded as monomorphic as two primers are not informative enough to be used for fingerprinting. From the 120 primers only 80 were selected for further analysis with only 44 primers being polymorphic and were selected to be used to fingerprint the finger millet accessions. Number of bands per primer ranged from 4-13 amplicons with OPF 4 (Figure 2) having the least number of bands whereas OPF 10 having the highest number of scorable bands. A total of 255 amplicons were scored from all the 44 polymorphic primers used, this translating into 5.8 amplicons per primer. Polymorphic bands ranged from 3 to 8 bands per primer with OPF 4 having 3 clearly scorable polymorphic bands and OPN 3 (Figure 4) having the highest number of polymorphic bands.

Banding of amplicons ranged from 400bp to 2000bp and from the total 255 amplicons 160 were polymorphic. The number of monomorphic (Figure 3) primers was very low with only 16 primers showing monomorphism when used to amplify finger millet accession and from the 255 scorable bands from polymorphic primers less than 50% of the bands were monomorphic. The lower number of monomorphic primers and bands per primer reflects that the selected primers were highly informative. as they showed high polymorphism, also with a low number of

monomorphic bands per primer the primers can be said to be good when used to fingerprint the finger millet crop.

4.2.3 Dendrogram

A dendrogram (un-weighted pair group method analysis) with cophenetic correlation of 0.85 (Figure 5) managed to separate *E. coracana* subsp *africana* (IE 4709) from 82 accession of *E.coracana* subsp *coracana* at 50% similarity level further proving that the cultivated finger millet origins can be traced to the wild progenitor, further subsp *coracana* was grouped into two major clusters. Panwar et al. (2010) Dendrogram clustered 83 accession of finger millet into two major clusters. Further the two main clusters had three (3) and two (2) distinct sub cluster respectively, with each group having similar morphological traits, group 1 was made up of high number of basal tillers all having more than ten tillers per plant, group 2 was made up of late maturing plants, whereas accessions in the 3rd group had same (Ragi brown) colour, the 4th group was made up of dwarf plants all growing to less than 100 cm, while the last group was made up of plants with high leaf number. Maximum like-hood or similarity 0.91 was observed between IE 2043 and IE 2217 which showed very close morphological traits (days to flowering, dwarf, maturity, and days to maturity) and also from the same country (India). Maximum dis-similarity was observed between accession 2872 and 5066 at 0.74 similarity level.

Country or continent of origin did not have effect in grouping the finger millet accessions, as all the five groups of the dendrogram were made up of mixture of many accession originating from diverse parts of the world. Only the two closely similar accession were from the same origin or country which also have the same morphological traits, and it can be said they might have been a mistake when collected, with the same accession collected twice and given two different identities, which then calls for further evaluation of all accession in the gene bank to check if double collection have not taken place.

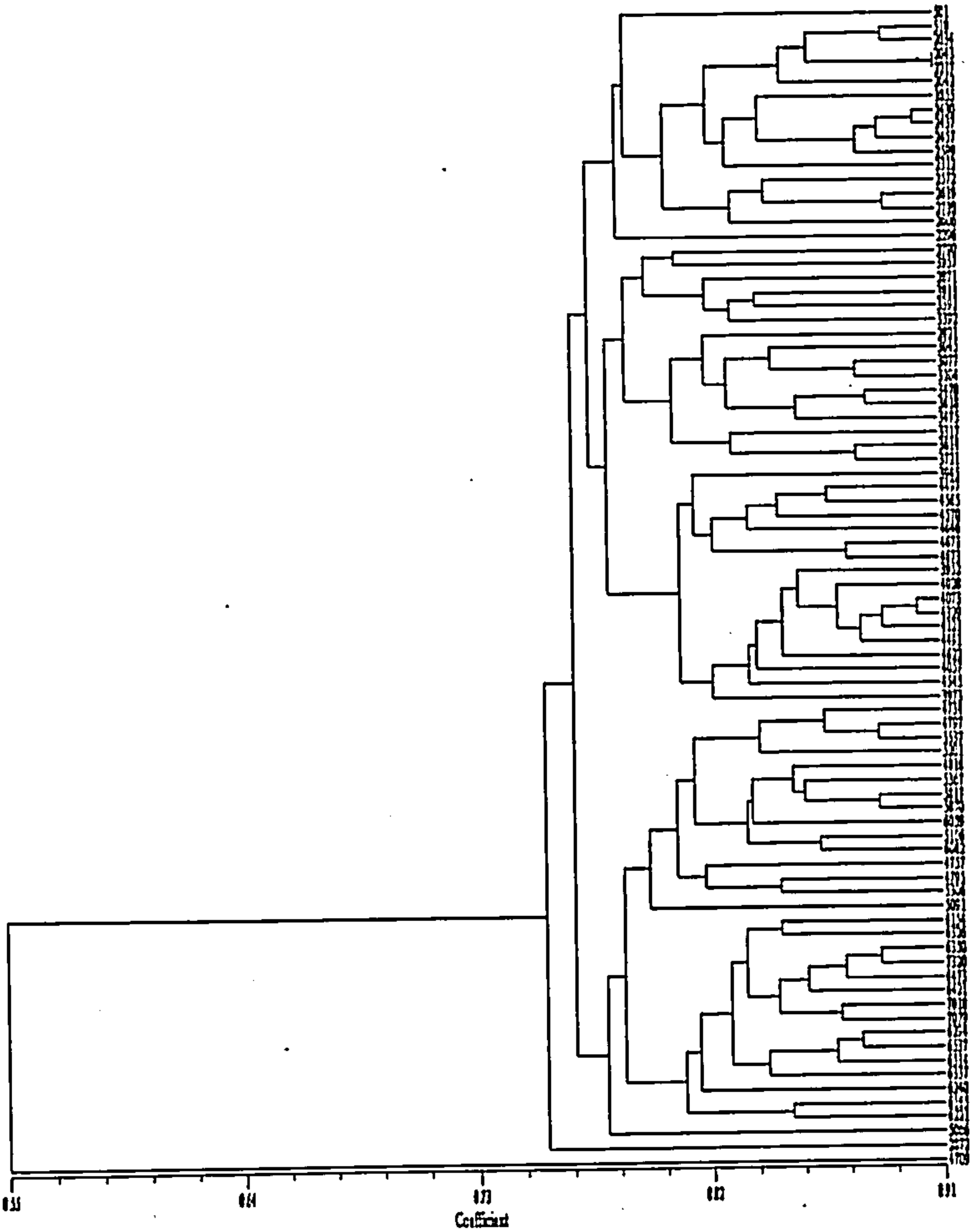

Figure 5 Dendrogram showing genetic similarity of 83 finger millet accession revealed by UPGMA cluster analysis based on RAPD fingerprinting

4.3 Genotype, season and GxS interaction

4.3.1 Yield, yield components and agronomic traits means

Days to flowering: early flowering was observed during the rainy season with accession IE 501 flowering just after 47 days while the same accession during the dry season flowered just after 56 days giving a difference of almost 10 days during the two different growing seasons. The same accession was also the earliest to flower during the dry season showing 50% mature main tillers flowering just after 56 days; (Table 6).

Late flowering for the rainy season was observed for IE 5066 as it reached 50% flowering for main tillers just over 107 days during the rainy season while during the dry season the same accession reached 50% flowering of main tillers after 79 days after emergence making it one of the mid flowering plants during the dry season. The late flowering accession during the dry season was IE 4797 flowering at just over 88 days after emergence and during the rainy season the very same accession reached 50% flowering of main tillers at exact same days taken in rainy season pegging it against the mid season flowering plants in the rainy season.

The average days to flowering for the dry season was recorded at 75 days while the average days to flowering during the dry season was much higher than that of the rainy season with plants reaching flowering point 83 days after emergence.

Number of tillers: little difference was observed for tillering pattern during the two different growing seasons. Most plants had the same number of tillers or having a small difference of plus or minus 1 for the two growing seasons: 12 accessions showed a difference of three or more tillers for the two growing season and the accessions are in no particular order; IE 518, 1055, 2872, 3104, 3475, 3952, 3973, 4028, 4757, 4797, 5537 and 7018.

The highest number of tillers was observed for accession IE 2872 and 5537 plants with 12 tillers plant^{-1} each during the rainy season and the same accession showed the highest difference of tillers in two seasons with the dry season having 6 and 8 tillers plant^{-1} respectively for the same accession. The highest number of tillers during the dry season was 12 recorded for accession IE 501 and 518 tillers per plants each with the same accession having 11 and 8 tillers plant^{-1} during the rainy season, respectively.

The accession with the lowest accession during the dry season planting was accession IE 4565 having recorded a minimum of 5 tillers plant^{-1} while the same accession during the rainy season recording at least 6 tillers plant^{-1}. The lowest tillering plants during the rainy season belonged to accession IE 3104 plants with a low of 5 tillers per plant stand. The average for the two seasons for number of tillers per plant was 9 and 8 for dry and rainy season, respectively.

Plant height: the results showed that plants were taller during the rainy season with all accessions showing a slight to high difference in height between the dry and rainy season. The tallest plants during the dry period belonged to accession IE 3945 plants with a height of 121.5 cm with the same plant recording a height of 126.5 cm during the rainy season being one of the tallest plants in both season. During the rainy season the tallest plants was observed in group of IE 2043 recording a height of 132.2 cm and having being one of the shortest plants during the rainy season with a height of 64.3 cm.

The shortest plants for the dry season belonged to accession number IE 4734 with a low height of 56.4 cm and recording a height of 94.8 cm during the rainy season. During the rainy season the shortest plants belonged to accession IE 501 plants recording a height of 80.4 cm and the plants heights during the dry season was recorded at 63.0 cm being one of the shortest plants in the dry season. The average height recorded for the two seasons was 88.4 cm and 110.5 cm for the dry and rainy season respectively showing a great plant height difference for the two growing season.

Table 6 Mean performance of yield, yield components and agronomic traits of 35 finger millet accessions

Acc No.	Days to flowering			Basal tillers			Plant height (cm)		
	dry	rainy	mean	dry	rainy	mean	dry	rainy	mean
501	56	47	52	12	11	12	63.03	80.37	71.70
518	74	79	77	12	8	10	86.13	135.63	110.88
1055	77	79	78	11	7	9	95.27	104.33	99.80
2034	92	104	98	21	9	15	72.67	104.67	88.67
2042	66	70	68	8	6	7	114.10	114.40	114.25
2043	84	89	86	11	9	10	64.33	132.20	98.27
2217	86	79	83	8	6	7	71.00	104.57	87.78
2430	82	89	85	9	8	8	117.13	129.00	123.07
2457	75	96	85	8	8	8	87.37	109.60	98.48
2872	76	87	82	6	12	9	78.93	114.90	96.92
3077	80	79	79	11	10	11	61.50	118.80	90.15
3104	75	69	72	8	5	6	76.47	82.57	79.52
3470	75	81	78	7	8	8	96.90	106.60	101.75
3475	86	90	88	11	7	9	71.97	100.97	86.47
3614	77	90	83	7	8	7	121.00	112.57	116.78
3618	71	81	76	11	11	11	79.27	120.67	99.97
3945	82	93	87	7	8	8	121.50	126.47	123.98
3952	80	94	87	9	6	8	95.47	114.33	104.90
3973	85	90	87	11	8	10	104.43	108.60	106.52
4028	67	89	78	9	6	7	115.40	117.17	116.28
4121	68	79	74	7	8	8	90.47	118.70	104.58
4565	75	70	73	5	6	5	91.47	100.13	95.80
4671	76	68	72	8	9	9	84.83	95.47	90.15
4673	68	69	69	9	8	9	82.30	100.97	91.63
4734	67	52	60	8	7	7	56.40	94.80	75.60
4757	77	79	78	8	11	9	61.80	127.83	94.82
4797	88	88	88	11	10	10	79.37	105.27	92.32
5066	79	107	93	9	6	7	115.40	120.43	117.92
5201	73	94	84	11	11	11	93.33	125.53	109.43
5367	82	87	84	8	8	8	82.30	105.60	93.95
5537	81	90	85	8	12	10	85.33	119.80	102.57
6059	71	94	83	9	8	8	103.13	105.10	104.12
6165	61	91	76	8	8	8	99.57	100.53	100.05
7018	82	95	88	10	6	8	102.10	113.77	107.93
7079	87	79	83	7	9	8	72.43	108.27	90.35
Means	75	83	80	9	8	9	88.40	110.47	99.64
LSD 0.05	3.82	3.82		1.67	1.24		10.16	8.98	
C.V.(%)	3.11	2.82		11.14	9.62		7.06	4.97	

Table 6 Mean performance of yield, yield components and agronomic traits of 35 finger millet accessions (cont.)

Acc No	Finger length (cm)			Finger numbers			Finger width (mm)			Days to maturity		
	dry	rainy	mean	dry	rainy	mean	dry	rainy	mean	dry	rainy	mean
501	5.05	6.74	5.90	7	7	7	8.32	8.59	8.45	112	81	97
518	5.77	8.56	7.16	7	7	7	10.2	11.3	10.8	125	113	119
1055	6.48	7.73	7.11	8	6	7	12.4	10.2	11.3	125	113	119
2034	5.04	6.44	5.74	8	7	8	9.10	10.0	9.55	136	133	135
2042	6.38	9.48	7.93	8	11	10	10.6	10.6	10.6	110	105	108
2043	5.21	7.16	6.18	8	7	7	9.77	11.0	10.4	135	129	132
2217	4.47	6.82	5.65	7	9	8	9.55	10.2	9.86	135	113	124
2430	6.03	6.93	6.48	7	6	6	12.7	10.6	11.7	117	127	122
2457	8.02	7.13	7.58	7	5	6	12.6	10.4	11.5	116	133	125
2872	8.42	8.28	8.35	8	8	8	12.8	10.5	11.7	134	127	130
3077	5.01	7.43	6.22	5	7	6	9.88	9.73	9.81	130	123	126
3104	5.64	8.50	7.07	9	12	10	9.37	9.34	9.36	120	104	112
3470	5.24	7.75	6.49	6	7	6	10.5	9.82	10.2	116	113	115
3475	5.30	7.25	6.28	6	12	9	9.54	10.3	9.92	133	128	130
3614	9.76	9.31	9.54	6	6	6	10.2	11.1	10.7	130	129	129
3618	6.11	7.76	6.93	7	8	8	8.69	10.5	9.60	125	113	119
3945	5.49	6.33	5.91	8	6	7	10.2	10.6	10.4	130	133	131
3952	7.56	6.40	6.98	6	6	6	11.9	10.4	11.2	92	132	112
3973	6.09	7.13	6.61	9	6	8	12.6	10.6	11.6	121	129	125
4028	6.78	7.42	7.10	8	7	7	10.4	9.27	9.83	128	130	129
4121	6.33	7.31	6.82	8	6	7	12.4	10.8	11.6	126	120	123
4565	10.7	8.82	9.78	8	8	8	11.5	11.1	11.3	128	105	117
4671	7.42	10.9	9.19	8	8	8	12.5	12.4	12.5	134	105	120
4673	9.32	10.7	10.0	8	8	8	9.40	10.5	9.96	127	100	114
4734	4.89	6.30	5.60	6	7	7	10.3	8.52	9.39	114	82	98
4757	4.87	6.66	5.77	6	7	7	9.34	9.75	9.54	130	113	121
4797	5.67	8.21	6.94	7	7	7	9.81	10.2	10.0	132	124	128
5066	7.17	8.51	7.84	8	7	7	9.47	11.7	10.6	132	143	138
5201	10.1	11.6	10.8	7	7	7	8.81	10.2	9.49	126	128	127
5367	6.63	9.12	7.87	9	7	8	10.3	10.8	10.5	133	117	125
5537	7.46	9.08	8.27	9	9	9	10.1	10.1	10.1	114	127	120
6059	6.61	6.43	6.52	9	5	7	12.9	10.1	11.5	127	133	130
6165	4.72	6.89	5.81	11	7	9	10.6	8.68	9.61	76	131	104
7018	7.44	7.60	7.52	7	6	7	9.63	10.6	10.1	126	133	130
7079	7.84	9.57	8.71	7	6	6	11.1	11.7	11.6	128	113	121
Means	6.60	7.95	7.28	8	7	7	10.5	10.3	10.5	123	119	121
LSD0.05	0.80	0.80		1.2	2.50		1.34	0.77		4.9	3.26	
C.V. (%)	7.12	6.13		9.6	21.0		7.77	4.56		9.9	1.70	

Table 6 Mean performance of yield, yield components and agronomic traits of 35 finger millet accessions (cont.)

Acc No.	Yield plant^{-1} (g)			Yield plot^{-1} (g)			1000 seeds weight (g)		
	dry	rainy	mean	dry	rainy	mean	dry	rainy	mean
501	52.7	40.7	46.7	466.3	453.2	459.8	3.51	3.21	6.72
518	144.4	29.7	87.1	509.2	647.7	578.5	2.63	2.61	5.24
1055	75.8	28.0	51.9	359.1	207.5	283.3	3.46	2.61	6.07
2034	77.0	6.2	41.6	335.7	49.0	192.4	4.10	2.05	6.15
2042	68.1	39.9	54.0	395.3	407.4	401.4	3.77	2.65	6.41
2043	106.8	72.8	89.8	531.2	710.6	620.9	3.61	3.36	6.97
2217	56.3	35.2	45.8	314.7	562.3	438.5	3.80	3.13	6.93
2430	80.0	23.4	51.7	692.8	158.7	425.7	3.03	2.55	5.58
2457	73.1	18.4	45.8	590.6	162.9	376.7	2.95	2.16	5.11
2872	80.8	46.4	63.6	464.6	639.1	551.9	3.45	2.91	6.37
3077	66.8	19.7	43.3	223.7	534.9	379.3	3.42	2.87	6.29
3104	52.1	12.5	32.3	220.7	52.0	136.3	1.90	2.52	4.42
3470	51.2	35.8	43.5	604.8	476.2	540.5	3.20	2.84	6.04
3475	69.6	14.3	41.9	559.5	80.3	319.9	3.40	2.43	5.83
3614	61.7	10.4	36.0	222.0	110.7	166.3	3.51	2.58	6.09
3618	50.9	72.7	61.8	954.6	831.7	893.2	3.62	3.11	6.73
3945	55.7	7.4	31.6	252.9	54.7	153.8	2.94	2.52	5.46
3952	102.2	22.6	62.4	132.2	198.9	165.5	2.65	1.99	4.64
3973	80.9	19.4	50.1	630.1	337.3	483.7	2.91	2.59	5.51
4028	50.0	7.3	28.7	424.4	75.8	250.1	3.30	2.82	6.12
4121	60.1	37.0	48.6	572.5	272.3	422.4	2.89	2.56	5.45
4565	67.3	43.5	55.4	673.1	91.1	382.1	3.33	2.73	6.06
4671	80.0	64.1	72.1	623.9	796.5	710.2	3.13	2.81	5.94
4673	78.2	44.9	61.6	242.1	361.3	301.7	3.44	2.73	6.17
4734	44.8	38.0	41.4	199.1	536.7	367.9	3.10	2.67	5.77
4757	73.8	21.6	47.7	274.1	519.5	396.8	3.44	2.89	6.33
4797	62.0	38.5	50.3	588.8	269.6	429.2	2.91	2.25	5.16
5066	59.3	31.0	45.2	513.9	317.3	415.6	2.95	2.66	5.61
5201	133.4	15.1	74.2	509.5	153.1	331.3	3.14	2.45	5.59
5367	84.7	26.2	55.5	555.1	508.3	531.7	3.52	2.59	6.11
5537	88.0	11.3	49.7	487.8	214.6	351.2	4.27	2.46	6.73
6059	44.0	16.4	30.2	194.5	132.0	163.2	2.50	2.36	4.86
6165	106.0	6.0	56.0	290.1	38.8	164.5	2.58	1.99	4.57
7018	102.4	23.0	62.7	285.5	188.0	236.7	3.05	2.03	5.08
7079	61.0	42.9	52.0	332.6	463.7	398.2	2.56	2.66	5.22
Means	74.3	29.2	51.8	435.4	331.8	383.4	3.20	2.61	5.81
LSD 0.05	12.94	7.67		140.90	77.31		0.40	0.32	
C.V. (%)	10.69	16.12		19.88	14.34		7.67	7.68	

Finger length: almost all the fingers were longer during the rainy season than in the dry season except for only 6 accessions which recorded a reverse in finger length for the dry and rainy season. The longest finger for the dry season and rainy season belonged to accession IE 5201 plants. IE 5201 recorded a finger length of

11.61 cm for the rainy season and also recorded the tallest finger length during the dry season at 10.84 cm.

The shortest fingers during the dry season was observed among accession IE 2217 plants with a mean length of 4.47 cm and the same accession gave a finger length of 6.82 cm during the rainy season making it one of the medium long fingers. IE 4734 was the accession which had the shortest finger length at 6.30 cm during the rainy season. The average finger length recorded for both seasons was 7.28 cm and average for each season was 6.60 and 7.92 cm for dry and rainy season, respectively.

Finger number: the total average finger number for the two seasons was 7 and 8 fingers per mature panicle for dry and rainy season respectively. For the dry season the finger number ranged from 5 to 11 fingers per mature plant panicle with IE 3077 recording the least fingers and during the rainy season the same accession recorded one of the lowest numbers of fingers per panicle again with 7 fingers per mature panicle. The highest finger number per panicle for the dry season was observed in accession IE 6165 plants at 11 fingers per mature panicle with the same accession recording the least number of fingers during the rainy season at 7 fingers per panicle.

The finger numbers per mature panicle for the rainy season ranged from 5 to 12 fingers per mature main panicle. The accession with low numbers of fingers per panicle was IE 6059 and IE 2457 both recording 5 fingers each. The accession with high fingers was IE 3104 and IE 3475 both having an average of 12 finger per panicle.

Finger width: the thickest finger for the dry season was observed in accession IE 6165 plants with finger thickness of 12.88 mm and the same accession showed one of the highest finger widths also for the rainy season with finger width of 10.11 mm. During the rainy season accession IE 4671 had the thickest finger of all the other accession with a width of 12.40 mm and the same accession showed one of the highest finger widths during the dry season recording a finger width of 12.51 mm.

The smallest finger width during the dry season was recorded at 8.32 mm for accession IE 501 plants; the same accession during rainy season also recorded one of the smallest finger width compared to other accessions with a finger width of 8.59 mm. For the rainy season accession IE 4734 had the smallest fingers as compared to

all other plants with a finger width of 8.52 mm and at 10.26 mm the same accession was one of the medium fingers during the dry season. The average mean finger width for the dry season and rainy season were very close with a little difference, showing the width of 10.52 and 10.34 mm for the dry and rainy season, respectively.

Days to maturity: days to maturity for both season had the greatest variation with dry season having a range of 76-136 days to reach full maturity and for the rainy season the range was 81-143 days to reach full maturity. The range was much similar with both seasons having maturity period spanning for about 60 days from the earliest accession to the late maturing accession.

The earliest accession to reach full maturity during the dry season was IE 6165 reaching maturity just after 76 days from seedling emergence, the same accession during the rainy season took one of the longest periods to reach maturity taking at least 131 days to reach full maturity of main tillers. During the rainy season the earliest accession to reach maturity was IE 501 reaching maturity after just 81 days from seedling emergence; it took more than 112 days for the same accession to reach full maturity during the dry season.

The late maturing accession for the dry season was IE 2034 reaching maturity well after 136 days from seedling emergence and the same accession was one of the late maturing again during the rainy season. During the rainy season the late accession to reach maturity was IE 5066 taking at least 143 days to reach maturity and the same accession when evaluated during the dry season it reached maturity in lesser days taking only 132 days to reach maturity. The average mean day taken to reach maturity for the two seasons was not much different with the dry season plants having average maturity days of 123 and the rainy season having average maturity days of 119 from seedling emergence.

Yield per plant: this was one of the traits which showed high variation between the two seasons with the dry season outperforming the rainy season, with some accession on the rainy season recording less than 10% yield when compared to the yield recorded during the dry season. This could be attributed to the effect of rain on plants during flowering and seed set period. Only one accession recorded a higher

yield in rainy season as compared to dry season, IE 3618 recorded yield per plant of 72.7 g for the rainy season while the dry season it recorded a low yield of 50.9 g.

IE 518 had the highest yield per plant during the dry season recording plant yield of 144.4 g plant^{-1} and the lowest yielding crops in the same season belonged to accession IE 6059 with a yield of 44.0 g plant^{-1}. IE 518 had a great difference in yield for the two seasons evaluated on the rainy and dry season, IE 4734 had a much stable yield showing a little difference between the two growing season. The mean average yield for the dry season was 75.9 g plant^{-1}.

During the rainy season low yields were recorded for most if not all crops. The highest yielding accession IE 2043 with a mean yield of 72.8 g and the lowest yield at 6.2 g plant^{-1} was observed in accession IE 2034 plants. IE 4565 had a little more stable yield as compared to IE 2034 which showered a dramatic decrease of yield from 77 g plants^{-1} to a low of 6.2 g plant^{-1} for the dry season to rainy season. The mean yield for rainy season was very low just recording a low of 29.2 g plant^{-1} as compared to 74.3 g plant^{-1} for the dry season.

Yield per plot: yield plot^{-1} which was much associated with yield plant^{-1} also showed the same pattern as in yield plant^{-1} with high yield recorded in the dry season and lower to very low yields observed in the rainy season. Of the 35 accessions evaluated only 9 (IE 518, 2042, 2043, 2217, 2872, 3077, 3952, 4671, 4673, 4734, 4757 and 7079) showed higher yields during the rainy season as compared to dry season.

For the dry season the highest yielding accession per plot was IE 3618 recording a high yield of 954.6 g plot^{-1} or 1909.2 kg ha^{-1} and the lowest yield was for IE 3952 plants with plot yield of 132.2 g plot^{-1} or 264.4 kg ha^{-1}. Both accessions showed much stability over the two seasons with IE 3618 showing only a little decrease in yield to 831.7 g plot^{-1} for the rainy season while IE 3952 showed a little increase with its yield going up to 198.9g plot^{-1} for the rainy season.

During the rainy season the highest yielding crops belonged to accession IE 3618 which also had the highest yield during the dry season recording a high of 831.8 g during the rainy season and the low yielding accession was IE 6165 with a low of

38.8 g plot^{-1} showing a dramatic drop in yield from the dry season which had an average yield of 290.1 g plot^{-1}.

1000 seed weight: the mass of 1000 seed weight for the dry season ranged from 1.90 g to 4.27 g with an average mean weight of 3.20 g. The highest seed weight was observed in accession IE 5537 plants with average of 4.27 g while the lowest was observed in accession IE 3104 plants with average of 1.90 g.

The seed weight range for the rainy season planting was 1.99 to 3.36 g and showing a mean average of 2.61 g for the entire season. The highest weight was observed in IE 2043 plants with an average of 3.36 g/1000 seed weight and the lowest weight was observed in IE 3952 and 6165 plants with a low of 1.99 g each. 1000 seed weight during the rainy season was much lower than in the dry season and this could have had an effect on low yields in the rainy season for both yield per plant and yield per plot.

4.3.2 Analysis of variance

Most of the traits analysed showed significant to highly significant mean squares for seasons, genotypes and the genotype x season effect

i) Seasonal effect

The seasonal effect on yield was highly significant at 95% level (Table 7), with seasonal effect being the major factor affecting performance of accession with seasonal effect contributing 53.09% as compared to other factors analysed. Seasonal effect of 1000 seed weight was also highly significant (95%) contributing 30.25% to variation. Finger length had highly significant mean squares and seasonal effect contributed 16.38% to variation. The results were non significant for seasonal effect on finger number and finger width showing that the effect of season was very low at 0.42% and 0.84%, respectively . The seasonal effect was highly significant with lower percentage sum squares of 32.28% and 9.29% for plant height and days to flowering, respectively.

ii) Genotype effect

The genotype effect for all traits showed high significance at 95% level. The genotypic effect contributed highly as compared to seasonal effect and that of season by genotype interaction for all the characters under study except for yield plant^{-1}.

Genotypic contribution was higher for finger length (64.46%), finger width (50.34%) and days to flowering (64.79%). The genotypic factor also showed moderate effect on other traits under study like; 1000 seed weight (38.31%), finger numbers (36.80%) and plant height (39.06%). The genotypic effect on yield plant^{-1} was very low recording percentage sum square of 20.56% which was lower than the seasonal effect and that of seasonal by genotype interaction.

iii) Genotype x season

Even though all traits showed highly significance at 95% level, the combined effect of genotype and season was lower as compared to the seasonal and genotype effect for all the traits except for yield plant^{-1} where G x S contributed higher than genotypic effect showing percentage sum square of 23.05%, the other traits showing moderate effect of GxS with lower percentage sum squares like; finger number (29.98%), finger width (27.83%), plant height (22.55%) and days to flowering (22.92%). 1000 seed weight and finger length showed lower sum squares percentages confirming the lower contribution of GxS contribution on those traits with 19.35% and 13.21% for 1000 seed weight and finger length, respectively.

4.3.3 Correlation between characters

i) Yield and yield components

Among the yield components (tiller number, finger length, finger number, finger width and 1000 seed weight) highly significant correlation at 95% level was observed for yield and number of tillers at 0.29** and for yield and 1000 seed weight with a highest positive correlation of 0.51**. Highly significant correlation was observed between yield and finger length at (-0.17**). Non significant correlation was observed between yield and finger number at 0.12, also yield and finger width had a non significant correlation of 0.12 (Table 8).

ii) Agronomic traits and yield

All the agronomic traits (days to flowering, plant height and days to flowering) had a highly significant negative correlation at 95% level with yield of plants. Highly significant negative correlation was observed between yield and days to flowering at (-0.33**) and the highest negative correlation was between yield and

plant height at (-0.41**). Yield and days to maturity had showed the lowest correlation at (-0.08) which was non significant.

iii) Among yield components

Correlation between yield components was very low even though highly significant correlation was recorded among yield components. Highly significant positive correlation was observed between number of tillers and 1000 seed weight at 0.28**, finger length and finger width also recorded a positive correlation of 0.23**. Highly significant negative correlation was observed between number of tillers and finger length at (-0.19**), number of tillers and finger width together with finger number and 1000 seed weight at (-0.20**) each. Positive non significant correlation was observed between numbers of fingers and finger length at 0.01 and finger number and 1000 seed weight at 0.03. Negative non significant correlation was observed for number of tillers and number of fingers at (-0.01) and finger width and 1000 seed weight at (-0.09).

iv) Agronomic traits

Highly significant correlation was observed among some of the correlations between the agronomic traits. Highly significant positive correlation was observed between days to flowering and days to maturity at 0.68** and days to flowering and plant height at 0.33**. Plant height and maturity also recorded non significant positive correlation of 0.02.

v) Agronomic traits and yield components

Highly significant positive correlation was observed between plant height and finger width at 0.18** and between plant height and finger length at 0.37**. Highly significant negative correlation was observed between days to flowering and finger number (-0.24**), 1000 seed weight (-0.32**); plant height and number of tillers at (-0.17**), 1000 seed weight at (-0.39**): days to maturity and finger number at (-0.24**).

Non significant positive correlation was observed between days to flowering and number of tillers at 0.01, finger length at 0.05, finger width at 0.09; days to maturity and finger width at 0.06, 1000 seed weight at 0.04, number of tillers at 0.08.

Negative non significant correlations was observed between plant height and finger number at (-0.05) and days to maturity and finger length at (-0.07).

Table 7 Analysis of variance with mean squares (% sum squares) for yield, yield components and agronomic traits of 35 finger millet accessions.

SOV		Yield/plant		1000 seed weight		Finger length		Finger no		Finger width	
	DF	MS	%SS	MS	%SS	MS	%SS	MS	%SS	MS	%SS
Season	1	106778**	53.09	18.2**	30.25	16.4**	16.38	2.5	0.42	2.8	0.84
Genotype	34	1216**	20.56	0.7**	38.31	1.9**	64.46	6.5**	36.80	4.9**	50.34
GxS	34	1363**	23.05	0.3**	19.25	0.4**	13.51	5.3**	29.98	2.7**	27.83
error	136	43	2.88	0.1	11.37	0.0	5.32	1.4	31.86	0.5	18.45
C.V. (%)		12.61		7.72		6.58		16.18		6.40	

Table 7 Analysis of variance with mean squares (% sum squares) for yield, yield components and agronomic traits of 35 finger millet accessions (cont.)

SOV		Days to flowering		Days to maturity		Basal tillers		Stem diameter		Yield per plot	
	DF	MS	%SS	MS	%SS	MS	%SS	MS	%SS	MS	%SS
Season	1	2427.6**	9.29	828.0**	2.08	49.5**	3.84	18.2**	30.25	559400**	5.5
Genotype	34	498.2**	64.79	566.6**	48.39	18.9**	49.79	0.7**	38.31	170752**	57.1
GxS	34	176.2**	22.92	496.4**	42.38	13.9**	36.75	0.3**	19.25	91719**	30.7
error	136	5.58	2.90	20.8	7.11	0.9	9.32	0.1	11.37	4871	6.51
C.V. (%)		2.96		3.76		10.82		7.72		18.20	

*,**indicates significant at 0.05 and 0.01significance level respectively.

Table 8 Correlations coefficients between yield, yield components and agronomic traits of 35 finger millet accessions

Traits	Days to flowering	Number of tillers	Plant Height (cm)	Finger Length (cm)	Finger number	Finger Width (mm)	Days to maturity	Yield per plant (g)
Tillers	0.01ns							
Plant height	0.33**	-0.17*						
Finger length	0.05ns	-0.19**	0.37*					
Finger number	-0.24**	-0.10ns	-0.05ns	0.08ns				
Finger width	0.09ns	-0.20**	0.18**	0.23**	0.00ns			
Maturity (days)	0.68**	0.08ns	0.02ns	-0.07ns	-0.24**	0.06ns		
Yield per plant	-0.33**	0.29**	-0.41**	-0.17*	0.12ns	0.12ns	-0.08ns	
1000seeds	-0.32**	0.28**	-0.39**	-0.20**	0.03ns	-0.09ns	0.04ns	0.51**

Ns indicates non significant while *,** is significant at 0.05 and 0.01significance level respectively.

CHAPTER V
DISCUSSION

5.1 Evaluation of finger millet based on morphological characters

Inflorescence compactness (panicle shape) further proved that it could be used successfully to classify finger millet accessions, in this study the four panicle shapes were observed and classified cultivated finger millet (subsp *coracana*) into 4 groups. The largest group had long-open (*vulgaris*) inflorescences followed by the smaller group with fisty (*plana*) inflorescences, and the last two groups had compact (*compacta*) inflorescence and pendulous (*elongeta*) inflorescences. The only wild relative in this study was identified to belong to subsp *africana* because of its panicle shape. According to de Wet, (2006), finger millet accessions can be characterized by spreading inflorescence, advanced cultivars have highly proliferated inflorescence branches that are clumped together to form fist-like structure and the most commonly grown cultivars have much smaller inflorescences with more or less spreading branches that may become somewhat incurved or reflexed at maturity. This concedes with the results of this study as the long and open inflorescence dominated the accessions evaluated in this research. The species *E. coracana* consists of two subspecies, *africana* and *coracana*. The subspecies *africana* consists of two wild species *africana* and *spontanea* while the cultivated subspecies *coracana* consists of four species namely *elongeta*, *plana*, *compacta* and *vulgaris* (Rao and de wet, 1997; Upadhyaya et.al., 2008).

Based on seed colour, finger millet was classified into 6 different colours (ranging from white to purple); with the colour brown being the dominant colour for the evaluated accessions and the colour brown was further classified into light brown, brown, ragi brown which was the most dominant having more than half of the evaluated accessions being ragi brown in seed colour, other seed colours observed were white, red and purple brown. Traditionally, finger millet farmers usually or most of the time use seed colour to classify millet and other crops, they even associate colour with palatability, whereby light or white colour is thought to be more palatable

than the darker or red/purple brown colours.

Continuous evaluation of phenotypic traits is very critical for any breeding program because they are greatly associated with traditional farmers' adoption of new varieties. Traditional farmers adoption is mainly based on yield but other phenotypic characteristics of plants like seed colour, pigmentation and lodging susceptibility contributes to the decision by farmers to adopt or not. Available evidence indicates that farmers use certain phenotypic features of plants for selection and identification (Jarvis et al., 2000) and on farm evaluation is important also to select genotypes that farmer prefer the most and that will help in increasing production.

Sixteen quantitative traits including growth, maturity, yield and yield component traits were also used to classify finger millet accessions. The results showed high variation in almost all of the characters under study. The highest variation was observed in grain yield, and variation in grain size was also significantly high. The other characters like yield components such as basal tillers, finger number, finger length, and finger width and peduncle length showed moderate high variations. The other growth characters observed in this study also showed moderate to high variation.

High yield plant^{-1} variation in this study could be attributed to high variation in genetic make-up of the accession studied thus proving that finger millet has high genetic diversity among its accessions. Grain yield being a complex trait is highly influenced by various environmental factors including biotic and abiotic factors that means the high variations in yield could also be attributed to environmental changes, therefore further evaluation on different environment has to be done to substantiate the variations. It is also interplay of various morphological characters which either favours of worsen final yield. All the traits studied in this research showed considerable variation, (Khan et al., 2011; Ahmad et al., 2011; Lang et al., 2009). Lang et al. (2009) also observed high variability on most of the quantitative traits under their study. High variability existing in these accessions brings forward the much needed information for the genetic improvement program of finger millet.

Studying the variations among agronomic traits is very important for every breeding program as most of them are highly correlated and have a direct effect on yield, they can either affect yield positively or negative depending on their variations.

An understanding of each trait and how it affects the crop performance especially yield is critical for every crop improvement. Ahmad et al. (2011) observed that plant height, days to silking and days to maturity are more related to yield outcome of maize. Among the sixteen plant traits Galarreta and Alvarez (2001) studied, they found out that plant height was the most important for discriminant analysis in maize.

Based on 16 quantitative traits a dendrogram was constructed using the UPGMA method with the NTSYS pc 2.10 program. The dendrogram successfully managed to separate the wild species (*Eleusine coracana* subsp *africana*) form the cultivated finger millet at 0.84 similarity level. IE 501 was closely related to IE 4709 (wild species) and also separated from other cultivated finger millet accessions. The remaining 80 cultivated finger millet accessions were grouped into 5 main groups at 0.86 similarity level.

In these study the main grouping was not associated with geographical diversity as it comprised of accessions from different origin, but when 3 main groups clustered into minor groups the accessions were grouped along continental origins, with the two main continents (Africa and Asia) dominating the minor groupings. Relationship between genetic diversity and geographical diversity has been a point of debate in the past, classification of accessions does not necessarily cluster in relation to geographical diversity because genetic drift and selection in different environments could cause higher variation that geographical distance (Murthy and Arunanchalam, 1996). Lang et al. (2009) reported that although the varieties came from different places, they can be grouped together because of close similarities in terms of qualitative traits; in contradiction Rohman et al. (2004) reported that genetic diversity is generally associated with geographical diversity.

Grouping accessions with related morphological traits is very critical in every breeding program so as to understand and to have basic information on which and how many accessions posses' traits of importance. Group information on which a superior accession with economic traits belong will in future help to check more accessions from the same group with similar or closely related economic traits and further be used in finger millet breeding program (Upadhyaya et al., 2004).

Evaluation of genetic variation based on morphological characters has proved to be very informative enough and can also be manipulated into either selecting superior

accessions or to be utilized to select parents for a breeding program (Khan et al., 2011; Lang et al., 2009). Mannetji, (1984) argued that genetic diversity based on standard morphological markers has proved to be inadequate because of wide spectrum of phenotype and other interaction with the environment.

5.2 DNA fingerprinting of finger millet using RAPD markers

With a total of 44 of 60 (73%) primers used in this research being polymorphic with more than 4 major bands used these proved very informative. A total of 255 scorable bands had been obtained which translated to 5.8 amplicons per primer and showing a polymorphism of 63% (160 polymorphic bands). The mean number of RAPD bands per primer in this study is higher as compared to that reported by Kumari and Pande, (2010), but lower than that reported by Fakrudin et al. (2004).. The level of polymorphism in this study is higher as compared to that reported by Panwar et al. (2010) and Gupta et al. (2010) but lower than that reported by Babu et al. (2005).

A dendrogram (unweighted pair group method analysis) with cophenetic correlation of 0.85 managed to separate *E. coracana* subsp *africana* (IE 4709) from 82 accessions of *E. coracana* subsp *coracana*, further subsp *coracana* was grouped into two major clusters. Panwar et al. (2010) clustered 83 accession of finger millet into two major clusters.

Further the two main clusters had three (3) and two (2) distinct sub cluster respectively, with each group having similar morphological traits, group was made up of high number of basal tillers all having more than ten tillers per plant, group was made up of late maturing plants, whereas accessions in group had same (ragi brown) colour, the group was made up of dwarf plants all growing to less than 100 cm, while the last group was made up of plants with high leaf number.

Maximum like-hood or similarity 0.91 was observed between IE 2043 and IE 2217 which showed very close morphological traits (days to flowering, dwarf, maturity, and days to maturity) and also from the same country (India) while IE 2296, IE 2872 and IE 5066 showed maximum variation from the rest of the accession and this shows that they have distinct germplasm compared to other accession. This

confirms that large amount of genetic variation exists among finger millet accession and this can be utilized in the breeding program to improve the crop.

Understanding the genetic relatedness and genetic diversity is critical for every breeding program, selecting parents is mostly based on genetic variation of genotypes. Breeding program for every crop seeks genetically diverse parents which are also morphological diverse to transfer the genes lacking from one genotypes to another so as to improve the qualities of the selected or the most superior genotypes. Basic understanding of genetic variation is most critical to improve the promising or superior genotypes and molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations (Karim et al., 2010).

In this study the geographical origin of finger millet accession did not contribute much when it came to classification or grouping of finger millet accessions. Relationship between genetic diversity and geographical diversity has been a point of debate in the past, classification of accessions does not necessarily cluster in relation to geographical diversity because genetic drift and selection in different environments could cause higher variation that geographical distance (Murthy and Arunanchalam, 1996). Lang et al. (2009) reported that although the varieties came from different places, they can be grouped together because of close similarities in terms of qualitative traits; in contradiction Rohman et al. (2004) reported that genetic diversity is generally associated with geographical diversity. RAPD showed weak association with regional or racial diversity (Agrama and Tuinstra, 2004; Dalkilic et al., 2011) found out that the genotypes collected from same regions were placed in different groups in dendrogram. Fahima et al. (1999) results showed no association with geographical distance between wheat population sites of origin and RAPD markers.

5.3 Genotype, season and genotype by season interaction

Climatic conditions show that the two seasons had a wide varying conditions for all the factors recorded. During the dry season very low amounts of rainfall were recorded with an average of 13.5 mm whereas during the rainy season high volumes of rainfall were recorded with a total of 1283.5 mm precipitation received. The high

amounts of rain fall in the rainy season significantly reduced the yields of finger millet by more than half of the dry season, de Wet, 2006 reported that heavy rains flowering and seed set greatly reduce yields of finger millet. Because heavy rains were received throughout the rainy season it can be concluded that rainfall contributed to the low yields recorded during rainy season. Worku et al. (2001) also found out that distribution of rainfall during the growing period is the determining factor for performance of maize genotypes.

Significant differences were found among seasons, genotypes and their interactions for yield plant^{-1}. Seasonal variation showed that it contributed highly to variation in yield plant^{-1} as compared to the genotypic variation and genotype by season interaction. The high contribution of variation can be attributed to varying rainfall amounts in the two season, heavy rains were recorded during the rainy season and these could have affected the flowering and seed set of finger millet as de Wet, (2006) reported that that yields of millet are greatly reduced if heavy rains are experienced during flowering and seed set stage of finger millet.

A thousand seed weight, finger length and agronomic traits recorded highly significant mean squares for season, genotype and genotype by season effect. Seasonal effect was non significant for finger number and significant finger width whereas both genotype and genotype by season recorded highly significant for all other traits studied. The sum of squares showed that seasonal effect had the highest impact on yield plant^{-1}, on the other hand differences in finger millet agronomic traits growth and development are more associated and affected by the differences in genotypes as most of the traits except yield plant^{-1}showed that the genotypic factor was the main contributory factor to their variation. This then certifies the importance of season and genotype interaction studies in every crop improvement process (Pereira et al., 2011). Genotype by season had the moderate interaction but lower than the contribution of genotype and season as showed by the lowest the sum squares for all the traits under study. Similar results were reported by Sharathbabu et al. (2008), with environment and genotype by environment showing highly significant interaction for all traits under study but lower contribution to variation for the evaluated traits.

Mean square for days to flowering was also highly significant for all factors (season, genotype, genotype by season). The sum squares showed that the genotypic factor had the highest effect compared to seasonal and genotype by season effect, respectively. The results showed moderate contribution of seasonal factor in flowering pattern of finger millet with some accessions showing similar flowering pattern for both season. The results showed that flowering for some accession was not associated with seasonal than genotype and these results are in contradiction with de Wet, (2006) that all finger millet is a photoperiod sensitive crop; with flowering of evaluated accessions being affected by genotypic factor than seasonal it can concluded that not all finger millet accessions are photoperiod sensitive.

Highly significant interaction of genotypes by season shows that genotype evaluated in two different season performs very differently for expression of character of interest. Sharathbabu et al. (2008) also found out that because of variations in weather conditions which prevailed during experimentations at three locations showed highly significant variance for almost all the traits studied signifying considerable differences among the environment and their predominant effects on characters.

Accession IE 518 showed the highest mean grain yield per plant for the dry season while IE 2043 recorded the highest mean grain yield per plant for the rainy season. The lowest mean grain per plant for the dry season and rainy season was recorded for accession IE 6059 and IE 6165, respectively. Even though high yield is very economic important, but accession with high variation for yield on different season will not be suitable to be adopted and distributed to farmer, Beiragi et al. (2011) reported that farmers are more interested in cultivars that produce consistent yields under their growing conditions, that means when breeders select they have to select cultivar which have low variation in yields on different locations or seasons. IE 518 had the highest variations as it recorded the highest mean yield in dry season and one of the lowest yields in the rainy season. IE 2043 showed minimum variation as it recorded one of the highest yields for both seasons with little difference in mean yields. This results shows that it can be competitive in any environmental conditions.

Days to flowering are very critical when more desirable genotypes are to be selected as response to photoperiod of any crop is considered one of the important

aspects of crop improvement. Genotypes with little variation in days to flowering will be considered more suitable for breeding program. IE 5066, 5201, 6059 and IE 6165 had the biggest variation as they recorded a big difference in days to flowering for the two seasons. Five accessions IE 1055, 3077, 4673, 4757, and 4797 showed low variations for days to flowering as they took almost the same number of days to flower in both seasons with IE 4797 flowering after exactly same days for both seasons. Flowering is very important agronomic traits as it highly related with yield outcome of many crops, it can either reduce on influence yield positively depending on the length taken to reach flowering point, any positive improvement will also result in positive of yield (Nadini et al., 2010; Bezalawtew et al., 2006; Wolie and Dessalegn et al., 2011).

Yield per plant showed highly significant correlations with most of the traits under study except for finger number, finger width and days to maturity. Highly significant positive correlation was observed for grain yield per plant and number of tillers. Wolie and Dessalegn et al. (2011) also reported correlation between number of tillers and grain yield per plot, grain yield per plant also showed positive correlation with 1000 seed weight and the same correlation was reported by Nadini et al. (2010) thus suggesting that any positive improvement on this traits will results in positive results for both grain yield per plot and grain yield per plant. Grain yield per plant showed highly significant negative correlation with days to flowering, similarly, Bezalawtew et al. (2006); Wolie and Dessalegn et al. (2011), found finger millet grain yield to be negative highly significantly correlated to days to flowering. The results show that breeding to reduce number of days to reach 50% flowering and maturity is critical for improvement of finger millet yield. Significant studies and evaluation of all traits related or affecting yield of finger millet have to be extensively done before going into breeding of the crop; an adequate understanding of traits which affect yield will greatly come in handy and make the breeding very easy and quick because more effort will be directed to the characters of importance.

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Morphological classification proved to be highly reliable even though most of the accessions of finger millet had very close morphological traits like: plant height, days to flowering and days to maturity. Use of morphological traits for classification of finger millet proved to be important as it showed that it can also be manipulated to identify the performance of characters of interest. The grouping in this study can be further used for selection of parental stocks based on economic traits like yield, days to flowering and maturity which are the most important taxonomic features in breeding.

On the other hand the large variation indicates that a good progress could be obtained through intra-population selection progress. These populations could enhance the genetic base of breeding program in other countries and produce hybrids adapted to many other areas. The research has shown that grouping can be done for each morphological trait either qualitatively or quantitatively and to get more precise results the accessions have to be evaluated in varying locations and environment as most of the characters are influenced by environmental changes.

Results of the evaluated finger millet shows that even though the crop has small genetic base molecular markers (RAPD) successfully managed to classify the accession and shows that it can further be extended to additional finger millet accession especially those which have not being identified already. Molecular fingerprinting is very important to help separate accession which are morphologically closely related and can not be easily be classified using morphological classified.

High variability existed among finger millet accession for almost all the traits under the study. Overall the study on yield and days to flowering showed that IE 2043 was more stable when it came to producing stable yields over the two seasons and IE 518 was poorly stable as it recorded a wide varying yield for the two seasons. IE 5066, 5201, 6059 and IE 6165 can be said to be unstable when days to flowering a

considered as the two accessions had a big difference between days to flowering for dry and rainy season, while IE 1055, 3077, 4673, 4757 and 4797 can be said to much more stable as the recorded almost the same number of days to flowering for both seasons. With some accessions having varying days to flowering while others had similar number of days to flowering, it can be concluded that not all finger millet crops are photoperiod sensitive.

6.2 Recommendations

To validate the results obtained from this study more evaluation has to be done on different locations and environments.

Morphological evaluation should be done on more than one environment and locations because results from diverse environments and locations will give a much better understanding as the effect of environment will be eliminated when the classification of accessions is done. Farmers fields can also be included in further classification of finger millet, with the crops in farmers farms been given the same agronomic management as other farmers crops in the field, this will also aid in getting classification of the crop under different management level and the results will be very good to be used to finally satisfactorily classify finger millet accession before any parental selection of diverse accession can be selected for breeding program.

Mainly because finger millet have being bypassed for a long time it could be possible that farmers have developed germplasms by themselves which need identification and RAPD DNA fingerprinting will come in handy. With many other and much advanced molecular markers available, they can further be used also the further fingerprint the accession mainly for confirmation of results obtained when RAPD markers are used to classify finger millet.

The interaction of season and genotypes as a form of evaluation can also be run parallel with morphological classification of finger millet accession. The evaluation can be taken further and be assessed over different locations, seasons, and environments and also on farm evaluation can be done to get the performance of the accession when they are evaluated under farmers management which will be more important to understand, because usually evaluation is done under research station which have a totally different management level when compared to commercial

farmers and also subsistence farmers. Accession which will have less variation for all the economic traits when evaluated in the different locations and environment can then be selected and be used as parental lines for a breeding program.

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APPENDICES

APPENDIX A

Weather data

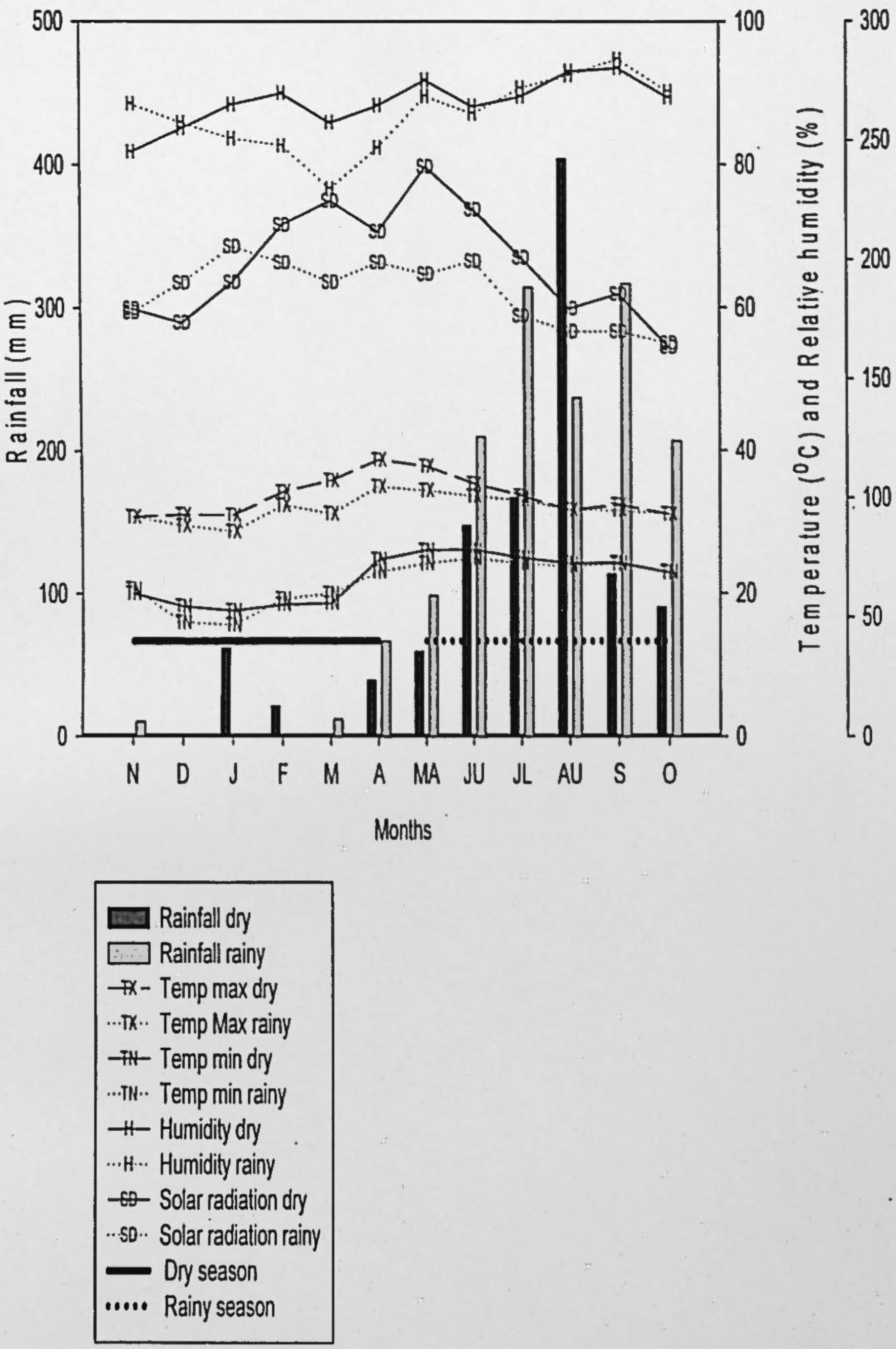

Figure 6 Weather data for the dry (2010/11) and rainy (2011) growing season .

APPENDIX B

Primers

Table 9 RAPD Operon primers and there sequence used in DNA fingerprinting

PRIMER		SEQUENCE	PRIMER		SEQUENCE
OPA	1	CAGGCCCTTC	OPP	2	TCGGCACGCA
OPA	2	TGCCGAGCTG	OPP	4	GTGTCTCAGG
OPA	11	CAATCGCCGT	OPP	5	CCCCGGTAAC
OPA	13	CAGCACCCAC	OPP	6	GTGGGCTGAC
OPA	15	TTCCGAACCC	OPP	9	GTGGTCCGCA
OPB	5	TGCGCCCTTC	OPP	10	TCCCGCCTAC
OPB	18	CCACAGCAGT	OPQ	2	TCTGTCGGTC
OPB	20	GGACCCTTAC	OPQ	3	GGTCACCTCA
OPC	5	GATGACCGCC	OPQ	4	AGTGCGCTGA
OPC	7	GTCCCGACGA	OPQ	5	CCGCGTCTTG
OPC	12	TGTCATCCCC	OPQ	7	CCCCGATGGT
OPC	15	GACGGATCAG	OPS	1	CTACTGCGCT
OPC	20	ACTTCGCCAC	OPS	2	CCTCTGACTG
OPE	16	GGTGACTGTG	OPS	3	CAGAGGTCCC
OPE	19	ACGGCGTATG	OPS	5	TTTGGGGCCT
OPF	1	ACGGATCCTG	OPS	7	TCCGATGCTG
OPF	2	GAGGATCCCT	OPS	8	TTCAGGGTGG
OPF	3	CCTGATCACC	OPS	9	TCCTGGTCCC
OPF	4	GGTGATCAGG	OPX	4	CCGCTACCGA
OPF	6	GGGAATTCGG	OPX	13	ACGGGAGCAA
OPF	9	CCAAGCTTCC	OPX	17	GACACGGACC
OPF	10	GGAAGCTTGG	OPX	19	TGGCAAGGCA
OPF	12	ACGGTACCAG	OPY	2	CATCGCCGCA
OPF	13	GGCTGCAGAA	OPY	17	GACGTGGTGA
OPG	1	CTACGGAGGA	OPY	18	GTGGAGTCAG
OPG	3	GAGCCCTCCA			
OPG	5	CTGAGACGGA			
OPG	6	GTGCCTAACC			
OPN	2	ACCAGGGGCA			
OPN	3	GGTACTCCCC			
OPN	4	GACCGACCCA			
OPN	5	ACTGAACGCC			
OPN	6	GAGACGCACA			
OPN	9	TGCCGGCTTG			
OPN	10	ACAACTGGGG			

APPENDIX C

Plant material

Table 10 Collection data of all finger millet accessions used in the research

Accession Identifier	Collectors number	Sources country	FAO in trust
IE 501	Tenda Mandia	India	yes
IE 518	Bati Mandia	India	yes
IE 1055	IE 1055	Unknown	yes.
IE 2034	KEP 534	India	yes
IE 2042	PPR 1989	India	yes
IE 2043	PR 202	India	yes
IE 2217	MS 9272	India	yes
IE 2296	PR 4617	India	yes
IE 2312	PR 4755	India	yes
IE 2430	FAO #49465	Kenya	yes
IE 2437	FAO #49474	Kenya	yes
IE 2457	FAO #49491	Kenya	yes
IE 2572	FAO 49660	Kenya	yes
IE 2589	EC 130708	USA	yes
IE 2606	SAD 173	Malawi	yes
IE 2619	SAD 342	Malawi	yes
IE 2710	SAD 767 A	Malawi	yes
IE 2790	SAD 1069	Malawi	yes
IE 2821	EC 132101	Nepal	yes
IE 2871	ZM 548; EC 138375	Zambia	yes
IE 2872	ZM 552; EC 138376	Zambia	yes
IE 2911	ZM 676; EC 138417	Zambia	yes
IE 2957	EC 140211	Germany	yes
IE 3045	DEP 63	India	yes
IE 3077	DEP 132	India	yes
IE 3104	RPSP 738	India	yes
IE 3317	TGR 819; EC 153221	Zimbabwe	yes
IE 3391	TGR 1593; EC 153330	Zimbabwe	yes
IE 3392	TGR 1599; EC 153319	Zimbabwe	yes

Table 10 Collection data of all finger millet accessions used in the research (cont.)

Accession Identifier	Collectors number	Sources country	FAO in trust
IE 3470	JM 4997	India	yes
IE 3475	PR 6038	India	yes
IE 3614	IE 1024-2	Unknown	yes
IE 3618	RAU 8	India	yes
IE 3721	UM 106; Kallango	Uganda	yes
IE 3945	UM 345; Lopus	Uganda	yes
IE 3952	UM 353; Nanyama	Uganda	yes
IE 3973	UM 373	Uganda	yes
IE 4028	UM 432	Uganda	yes
IE 4057	UM 462; Rvshari	Uganda	yes
IE 4073	UM 479	Uganda	yes
IE 4121	UM 532	Uganda	yes
IE 4329	AMM 1465; EC 173966	Zimbabwe	yes
IE 4491	AMM 197	Zimbabwe	yes
IE 4497	AMM 224	Zimbabwe	yes
IE 4545	AMM 679	Zimbabwe	yes
IE 4565	AMM 963	Zimbabwe	yes
IE 4570	AMM 983	Zimbabwe	yes
IE 4622	AMM 1252	Zimbabwe	yes
IE 4646	AMM 1487	Zimbabwe	yes
IE 4671	VL 137	India	yes
IE 4673	VL 149	India	yes
IE 4709	EZ 212863; MTB 80	Burundi	yes
IE 4734	AS 67; Ragi	India	yes
IE 4757	AS 106; Ragi	India	yes
IE 4795	E 1379 (SAR)	Zimbabwe	yes
IE 4797	KLM 1868	Maldives	yes
IE 4816	RV 96	India	yes
IE 5066	SDFM208	Senegal	yes
IE 5091	SDFM 313	Zimbabwe	yes

Table 10 Collection data of all finger millet accessions used in the research (cont.)

Accession Identifier	Collectors number	Source country	FAO in trust
IE 5106	SDFM 356	Zimbabwe	yes
IE 5201	SDFM 951	India	yes
IE 5306	SDFM 1705	Zimbabwe	yes
IE 5367	SDFM 1922	Kenya	yes
IE 5537	ACC No. 443	Nepal	yes
IE 5817	ACC No. 2667	Nepal	yes
IE 5870	ACC No. 2720	Nepal	yes
IE 6059	ACC No. 2912	Nepal	yes
IE 6082	ACC No. 2935	Nepal	yes
IE 6154	ACC No. 6393	Nepal	yes
IE 6165	ACC No. 6404	Nepal	yes
IE 6221	ACC No. 6461	Nepal	yes
IE 6240	AMM 41	Zimbabwe	yes
IE 6294	AMM 392	Zimbabwe	yes
IE 6326	AMM 840	Zimbabwe	yes
IE 6337	AMM 965	Zimbabwe	yes
IE 6350	AMM 1027	Zimbabwe	yes
IE6421	RGS 23; Kabatanguri	Uganda	yes
IE6473	RGS 133; Rwabukidia	Uganda	yes
IE6514	AMZ 9	Zimbabwe	yes
IE6537	AOC 116	Nigeria	yes
IE7018	SDFM 1879; 423	Kenya	yes
IE7079	SDFM 1987; 18816	Kenya	yes
IE7320	SDFM 2279; 476	Kenya	yes

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