MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION, YIELD DETERMINATION AND PROTEOMIC ANALYSIS OF SELECTED BAMBARA GROUNDNUT LANDRACES IN LAKE VICTORIA BASIN, KENYA

BY

SELINE OMONDI

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DECLARATION

By student:

I declare that this thesis is my original work and therefore has not been previously submitted for the award of a degree in any other University. I have carried out the work reported herein, and all sources of information have been specifically acknowledged by means of references cited.

Seline Omondi

PhD/SC/00048/2017

Sign……. Date……..

By Supervisors:

This thesis has been submitted for examination with our approval as the University supervisors

Sign……. Date……..

Prof. J. C. Onyango

Department of Botany

Maseno University

Sign……. Date……..

Prof. David Miruka Onyango

Department of Zoology

Maseno University

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ABSTRACT

Bambara groundnut (BGN) (*Vigna subterranea* (L.) Verdc.) is a drought-resilient, nutrient-rich legume indigenous to Africa. Despite its nutritional and agronomic potential, BGN remains underutilized in Kenya's Lake Victoria Basin (LVB), where diverse landraces are grown but lack comprehensive characterization. Knowledge gaps in morphological traits, yield potential, and protein diversity hinder effective classification, breeding, and utilization, especially due to overlapping physical characteristics and varying farmer preferences. Moreover, low yields across zones, largely due to recycling of non-adapted germplasm, highlight the need for zone-specific yield assessments and genetic diversity studies. This study aimed to evaluate the genetic diversity of BGN in the LVB using morphological traits, yield performance, and protein profiling. Specifically, it focused on identifying morphological characteristics of selected landraces across different agro-ecological zones and greenhouse conditions, determining yield variation, and assessing protein composition in high-yielding accessions. Six landraces (K1, K2, B1, B4, M2 and M4) were cultivated at three KALRO research sites (Alupe, Kibos, Oyani) and under controlled greenhouse conditions at Maseno University. A randomized complete block design with three replications was used. Morphological characterization followed the *Vigna subterranea* descriptor list, yield was assessed using standard agronomic metrics, and protein profiling was conducted via Bradford assay and SDS-PAGE. The study revealed high phenotypic diversity, especially in pod color, leaflet shape, and seed testa patterns. Shannon-Weiner indices indicated significant morphological diversity ($H' > 1.6$), with terminal leaflet shape and seed eye pattern showing the highest diversity ($H' = 2.071$). Quantitative traits such as plant height, pod number, and seed weight exhibited significant variation within and between zones ($P \leq 0.05$), largely influenced by soil moisture availability. Accessions B1, M4, and M2 showed consistently high pod numbers. Cluster and principal component analyses differentiated landraces based on terminal leaflet area, seed weight, and pod number. Biplot analysis further identified B1 as a likely progenitor. Yield analysis revealed B4 (2.7 t/ha), M2 (3.3 t/ha), and M4 (1.6 t/ha) as the highest yielders at Alupe, Kibos, and Oyani, respectively. In greenhouse trials, B1 recorded the highest yield under irrigation (3.8 t/ha), while K1 excelled under drought (1.2 t/ha). Traits like flower number, pod number, and leaflet area correlated positively with yield ($r \geq 0.50$). Among the top performers, M2 had the highest total protein content (0.123 mg/mL), with albumins being the most abundant protein fraction. SDS-PAGE revealed seven shared protein bands across landraces, with M2 uniquely expressing a 180 kDa band, suggesting potential as a molecular marker. In conclusion, the study demonstrated significant morphological and molecular diversity among BGN landraces in the LVB, identified high-yielding accessions, and discovered a unique protein marker in M2. These findings support the targeted selection and breeding of adaptable, high-yielding BGN varieties. The study recommends focusing on M2 and B1 in future breeding efforts and integrating protein markers into classification and improvement programs for underutilized BGN in Kenya.

TABLE OF CONTENT

DECLARATION ii
ACKNOWLEDGEMENT iii
DEDICATION iii
ABSTRACT v
TABLE OF CONTENT vi
LIST OF AND ABBREVIATIONS xv
DEFINITIONS OF TERMS xvii
LIST OF TABLES xix
LIST OF FIGURES xxiii
LIST OF PLATES xxviii
LIST OF APPENDICES xxix
CHAPTER ONE: INTRODUCTION 1
1.1 Background of Study 1
1.2 Statement of the Problem 5
1.3 Justification 6
1.4 Objectives 8
1.4.1 General Objective 8
1.4.2 Specific Objectives 8
1.5 Null Hypotheses, No 8
CHAPTER TWO: LITERATURE REVIEW 9
2.1 Taxonomy of Bambara Groundnut 9
2.2 Distribution of Bambara groundnut in Africa 9
2.3 Multifunctional Uses of Bambara Groundnut 9
2.4 Morphological Characterization in Plants 10
2.4.1 General Morphological Characterization of the Bambara Groundnut 10
2.4.2 Efficacy of Morphological Characterization in the Taxonomic Hierarchy 12
2.4.3 Qualitative Morphological Characters of Bambara Groundnut 13
2.4.4. Quantitative morphological Characters of Bambara Groundnut 15
2.4.4.1 Plant height 15
2.4.4.2 Number of Pods with One or Two Seeds 16

2.4.4.3 Total Number of Pods per Plant 17
2.4.4.4 Seed Weight per Plant 18
2.4.4.5 Number of Flowers per Plant 19
2.4.4.6 Terminal Leaflet Area 21
2.4.5 Bambara Groundnut in the Lake Victoria Basin (LVB) 22
2.4.5.1Status and Distribution 22
2.4.5.2 Genetic Diversity and Landrace Conservation 22
2.4.5.3 Agronomic Practices and Production Trends 23
2.4.5.4 Challenges and Future Outlook 24
2.4.6 Yield and its component characters 25
2.4.6.1 Yield Production of Bambara Groundnuts in the Lake Victoria Basin 25
2.4.6.2 Yield of Bambara Groundnuts as Dependent on Seed Component Characters and Ecological Sites 26
2.5 Protein Biomolecules in Bambara Groundnuts 28
2.6 Climatic and Edaphic Factors Affecting Morphological Traits, Yield, and Protein Content 29
2.7 Phenetic Classification 30
CHAPTER THREE:MATERIALS AND METHODS 32
3.1 Study Area 32
3.2 Field Work and Field Data Collection 34
3.2.1 Collection of Germplasm for Planting 34
3.2.2 Land Preparation 35
3.2.3 Soil Sampling Procedures 35
3.2.3.1 Collection of Composite Samples 35
3.2.3.2 Soil Sample Processing 36
3.2.3.2.1 Drying 36
3.2.3.2.2 Grinding 36
3.2.3.2.3 Sieving 36
3.2.3.2.4 Extraction 36
3.2.3.3 Soil Sample Analysis 36
3.2.3.3.1 pH Analysis 36
3.2.3.3.2 Total Nitrogen Analysis 37

3.2.3.3.3 Analysis of Phosphorous....37
3.2.3.3.4 Analyses of Potassium, Calcium and Magnesium....37
3.2.4 Microclimate of the Zones....38
3.2.5 Bambara Groundnut Planting and Field Layout....38
3.3 Green House Work....40
3.3.1 Preparations in the Greenhouse....40
3.3.2 Experimental Design and Planting....40
3.4 Data Collection....41
3.4.1 Morphological Characterization....41
3.4.2 Data Analysis for Morphological Characterization....46
3.4.2.1 Data Analysis for Qualitative Morphological Characters....46
3.4.2.2 Data Analysis for Quantitative Morphological Characters....47
3.5 Yield....49
3.5.1 Data Analysis for Yield....49
3.6 Protein Analysis....49
3.6.1Sample Preparation....50
3.6.2 Extraction of Total Proteins....50
3.6.3 Extraction of Storage Protein Fractions....51
3.6.4 Quantification of Proteins using the Bradford Method....51
3.6.4.1 Preparation of the standard curve....51
3.6.5 Determination of Storage Protein Electrophoretic Banding Pattern....53
3.6.6 Protein Analysis....54
CHAPTER FOUR: RESULTS....55
4.1Microclimate of the study area....55
4.2 pH and Nutrient Composition of the Soils in the Greenhouse and at Alupe, Kibos and Oyani....56
4.3 Qualitative Morphological Characteristics of Bambara Groundnuts....58
4.3.1 Phenotypic Diversity....58
4.3.2 Qualitative Traits Diversity Indices....61
4.3.3 Cluster Analysis....62
4.4 Quantitative Morphological Characteristics of Bambara Groundnuts....62

4.4.1 Plant Height (PHT cm) 62
4.4.1.1 Plant Height Inter-Site Comparisons 62
4.4.1.1.1 K2 Plant Height (PHT cm) 62
4.4.1.1.2 B4 Plant Height (PHT cm) 63
4.4.1.1.3 B1 Plant Height (PHT cm) 64
4.4.1.1.4 K1 Plant Height (PHT cm) 65
4.4.1.1.5 M4 Plant Height (PHT cm) 66
4.4.1.1.6 M2 Plant Height (PHT cm) 67
4.4.1.2 Plant Height Intra-Site Comparisons 68
4.4.1.2.1 Plant Height Intra-Site Comparisons in Alupe 68
4.4.1.2.2 Plant Height Intra-Site Comparisons in Kibos 69
4.4.1.2.3 Plant Height Intra-Site Comparisons in Oyani 69
4.4.1.2.4 Plant Height Intra-Greenhouse Irrigated Comparisons 69
4.4.1.2.5 Plant Height Intra-Greenhouse Drought Comparisons 69
4.4.2 Number of Pods 72
4.4.2.1 Number of Pods Inter-sites Comparisons. 72
4.4.2.1.1 K2 Number of Pods 72
4.4.2.1.1.1 K2 Number of Pods with One Seed (N1S) 72
4.4.2.1.1.2 K2 Number of Pods with Two Seeds (N2S) 72
4.4.2.1.1.3 K2 Total Number of Pods (POD) 72
4.4.2.1.2 B4 Number of Pods 73
4.4.2.1.2.1 B4 Number of Pods with One Seed (N1S) 73
4.4.2.1.2.2 B4 Number of Pods with Two Seeds (N2S) 74
4.4.2.1.2.3 B4 Total number of Pods (POD) 74
4.4.2.1.3 B1 Number of Pods 75
4.4.2.1.3.1 B1 Number of Pods with One Seed (N1S) 75
4.4.2.1.3.2 B1 Total Number of Pods (POD) 76
4.4.2.1.4 K1 Number of Pods 77
4.4.2.1.4.1 K1 Number of Pods with One Seed (N1S) 77
4.4.2.1.4.2 K1 Number of Pods with Two Seeds (N2S) 77
4.4.2.1.4.3 K1 Total Number of Pods (POD) 77

4.4.2.1.5 M4 Number of Pods....78
4.4.2.1.5.1M4 Number of Pods with One Seed (N1S)....78
4.4.2.1.5.2 M4 Number of Pods with Two Seeds (N2S)....79
4.4.2.1.5.3 M4 Total number of Pods (POD) 79
4.4.2.1.6 M2 Number of Pods....80
4.4.2.1.6.1 M2 Number of Pods with One Seed (N1S)....80
4.4.2.1.6.2 M2 Number of Pods with Two Seeds (N2S)....80
4.4.2.1.6.3 M2 Total Number of Pods (POD) 80
4.4.2.2 Number of Pods Intra-site Comparisons.....82
4.4.2.2.1 Number of Pods Intra-site Comparisons in Alupe 82
4.4.2.2.1.1 Number of Pods with One Seed (N1S) intra-site comparisons in Alupe....82
4.4.2.2.1.2 Number of Pods with Two Seeds (N2S) Intra-Site Comparisons in Alupe....82
4.4.2.2.1.3 Total Number of Pods per Plant (POD) Intra-Site Comparisons in Alupe....82
4.4.2.2.2 Number of Pods Intra-site Comparisons in Kibos....83
4.4.2.2.2.1 Number of Pods with One Seed (N1S) Intra-Site Comparisons in Kibos 83
4.4.2.2.2.2 Number of Pods with Two Seeds (N2S) Intra-Site Comparisons in Kibos 84
4.4.2.2.2.3 Total Number of Pods per Plant (POD) Intra-Site Comparisons in Kibos 84
4.4.2.2.3 Number of Pods Intra-site Comparisons in Oyani. 85
4.4.2.2.3.1 Number of Pods with one seed (N1S) intra-site comparisons in Oyani 85
4.4.2.2.3.2 Number of Pods with Two Seeds (N2S) Intra-Site Comparison in Oyani 85
4.4.2.2.3.3 Total Number of Pods per Plant (POD) Intra-Site Comparison in Oyani 85
4.4.2.2.4 Number of Pods Intra-site Comparisons in Green House Irrigated Plants. 87
4.4.2.2.4.1 Number of Pods with One Seed (N1S) intra-GHI Comparisons....87
4.4.2.2.4.2 Total Number of Pods (POD) intra-GHI Comparisons 87
4.4.3 Terminal Leaflet Area (TLA cm2)....89
4.4.3.1 Terminal Leaflet Area (TLA cm2) Inter-Sites Comparisons....89
4.4.3.1.1 K2 Terminal Leaflet Area (TLA cm2) 89
4.4.3.1.2 B4 Terminal Leaflet Area (TLA cm2)....90
4.4.3.1.3 B1 Terminal Leaflet Area (TLA cm^2) 91
4.4.3.1.4 K1 Terminal Leaflet Area (TLA cm2) 92
4.4.3.1.5 M4 Terminal Leaflet Area (TLA cm2)....93

4.4.3.1.6 M2 Terminal Leaflet Area (TLA cm2) 94
4.4.3.2 Terminal Leaflet Area Intra-Sites Comparisons 95
4.4.3.2.1 Terminal Leaflet Area (TLA cm2) Intra-Site Comparisons in Alupe 95
4.4.3.2.2 Terminal Leaflet Area (TLA cm2) Intra-Site Comparisons in Kibos 95
4.4.3.2.3 Terminal Leaflet Area (TLA cm2) Intra-Site Comparisons in Oyani 96
4.4.3.2.4 Terminal Leaflet Area (TLA cm2) intra-Greenhouse Irrigated (GHI) Comparisons 96
4.4.3.2.5 Terminal Leaflet Area (TLA cm2) Intra-Greenhouse Droughted (GHD) Comparisons 96
4.4.4 Number of Flowers per Plant (NFP) 98
4.4.4.1 Number of Flowers per Plant (NFP) Inter-Sites Comparisons 98
4.4.4.1.1 K2 Number of Flowers per Plant (NFP) 98
4.4.4.1.2 B4 Number of Flowers per Plant (NFP) 99
4.4.4.1.3 B1 Number of Flowers per Plant 100
4.4.4.1.4 K1 Number of Flowers per Plant (NFP) 101
4.4.4.1.5 M4 Number of Flowers per Plant 102
4.4.4.1.6 M2 Number of Flowers per Plant 103
4.4.4.2 Number of Flowers per Plant (NFP) Intra-Sites Comparisons 104
4.4.4.2.1 Number of Flowers per Plant (NFP) Intra-Site Comparisons in Alupe 104
4.4.4.2.2 Number of Flowers per Plant (NFP) Intra-Site Comparisons in Kibos 104
4.4.4.2.3 Number of Flowers per Plant (NFP) Intra-Site Comparisons in Oyani 105
4.4.4.2.4 Number of Flowers (NFP) intra-Greenhouse Irrigated (GHI) Comparisons 105
4.4.4.2.5 Number of Flowers (NFP) intra-Greenhouse Droughted (GHD) Comparisons 105
4.4.5 Seed Weight per Plant (SWT g) 107
4.4.5.1 Seed Weight per Plant (SWT g) Inter-Sites Comparisons 107
4.4.5.1.1 K2 Seed Weight per Plant (SWT g) 107
4.4.5.1.2 B4 Seed Weight per Plant (SWT g) 108
4.4.5.1.3 B1 Seed Weight per Plant (g) 109
4.4.5.1.4 K1 Seed Weight per Plant 110
4.4.5.1.5 M4 Seed Weight per Plant 111
4.4.5.1.6 M2 Seed Weight per Plant 112
4.4.5.2 Seed Weight per Plant (SWT g) Intra-Sites Comparisons 113
4.4.5.2.1 Seed Weight per plant (SWT g) Intra-Site Comparisons in Alupe 113

4.4.5.2.2 Seed Weight per plant (SWT g) Intra-Site Comparisons in Kibos 113
4.4.5.2.3 Seed Weight per plant (SWT g) Intra-Site Comparisons in Oyani 114
4.4.5.2.4 Seed Weight per plant (SWT g) Intra-Greenhouse Irrigated (GHI) Comparisons..... 114
4.4.5.2.5 Seed Weight per plant (SWT g) Intra-Greenhouse Droughted (GHD Comparisons ... 114
4.5 Principle Component Analysis (PCA) for the Seven Quantitative Traits 116
4.6 Correlation Analysis of Traits..... 121
4.7 Yield 122
4.7.1 Yield inter-site comparisons 122
4.7.1.1 K2 yield (ton/ha) 122
4.7.1.2 B4 yield (ton/ha)..... 123
4.7.1.3 B1 yield (ton/ha)..... 124
4.7.1.4 K1 yield (ton/ha) 124
4.7.1.5 M4 yield (ton/ha)..... 125
4.7.1.6 M2 yield (ton/ha)..... 125
4.7.2 Yield Intra-Site Comparisons..... 129
4.7.2.1 Alupe Zone..... 129
4.7.2.2 Kibos Zone..... 129
4.7.2.3 Oyani Zone..... 129
4.7.2.4 Greenhouse Droughted (GHD) Zone 130
4.7.2.5 GreenhouseIrrigated (GHI) Zone 130
4.7.3 Correlation Coefficients of Yield and Yield Components 132
4.7.4 Selection of high yielding BGN accession per zone based on Rank Summation Index (RSI) 134
4.8 Protein Analysis 136
4.8.1 Total Protein Concentrations in the Accessions..... 136
4.8.2 Protein Fractions 137
4.8.2.1 Protein Fractions Concentrations Intra-Accession Sample Comparisons 137
4.8.2.1.1 K1 Sample..... 137
4.8.2.1.2 B4 Sample 137
4.8.2.1.3 B1 Sample 138
4.8.2.1.4 M2 Sample 139

4.8.2.1.5 M4 Sample 140
4.8.2.2 Protein Fraction Concentrations Inter-Accessions Sample Comparisons 140
4.8.2.2.1 Albumins 140
4.8.2.2.2 Globulins 141
4.8.2.2.3 Glutelins 141
4.8.2.2.4 Prolamins 141
4.8.3 Electrophoretic Banding Patterns 143
4.8.3.1 SDS-PAGE gel: Comparative Protein Peptides Profile in five BGN Accession Samples. 143
4.8.3.2 Electrophoretic Banding Patterns of Protein Fractions in Accessions Isolates 146
4.8.3.2.1 Electrophoretic Banding Patterns of Albumins in Accessions Isolates 149
CHAPTER FIVE: DISCUSSION 153
5.1 Qualitative morphological characters. 153
5.1.1 Frequency distribution of qualitative morphological characters 153
5.1.2 The Qualitative Morphological Traits Diversity Indices 155
5.1.3 Utilization ofQualitative Morphological Taxonomic Markers in Classification 157
5.2 Quantitative Morphological Traits 158
5.2.1 Plant Height 158
5.2.2 Number of Pods With One Seed (N1S) 160
5.2.3 Number of Pods with Two Seeds (N2S) 162
5.2.4 Total Number of Pods (POD) 163
5.2.5 Terminal Leaflet Area (TLA cm2) 164
5.2.6 Number of Flowers (NFP) 166
5.2.7 Seed Weight per Plant (SWT g) 168
5.3 Principal Component Analysis 172
5.4 Correlation Analysis 176
5.5 Yield 177
5.5.1 Coefficients of Variation of Yield 177
5.5.2 Yield Intra and Inter-Zonal Comparisons 178
5.5.3 Correlation Coefficients of Yield and Yield Components 179
5.5.4 Selection of High Yielding BGA per Zone based on Rank Summation Index (RSI) 180

5.6 Proteins 180
5.6.1 Protein Concentrations 180
5.6.2 Protein Electrophoretic Banding Pattern 184
5.6.3 Albumin and Globulin Electrophoretic Banding Pattern 185
5.6.4 Protein Banding Pattern and Phenetic Relationship of the Five Accessions 187
CHAPTER SIX: CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FUTURE RESEARCH 190
6.1 Conclusions 190
6.2 Recommendations 191
6.3 Suggestions for future research 192
REFERENCES 193
APPENDICES 217

LIST OF AND ABBREVIATIONS

AFLP	:	Amplified Fragment Length Polymorphism
ATP	:	Adenosine Triphosphate
BAMNET	:	Bambara Network
BGA	:	Bambara ground nut accession
BGN	:	Bambara groundnut
BSA	:	Bovine serum albumin
Ca	:	Calcium
DArT	:	Diversity Array Technology markers
DAS	:	Days after sowing
Drt	:	Droughted
GHD	:	Green house droughted
GHI	:	Green house irrigated
ICBN	:	International Code of Botanical Nomenclature
IITA	:	International Institute of Tropical Agriculture
IPGRI	:	International Plant Genetic Resources Institute
K	:	Potassium
KALRO	:	Kenya Agricultural Livestock Research Organization
kDa	:	kiloDalton
LL	:	Leaf length at maturity
LW	:	Leaf width at maturity
Mg	:	Magnessium
N	:	Nitrogen
N1S	:	Number of pods with 1 seed
N2S	:	Number of pods with 2 seeds
NFP	:	Number of flowers per plant
OTUs	:	Operational taxonomic units
P	:	Phosphorus
PAGE	:	PolyAcrylamide Gel Electrophoresis
PCA	:	Principal component analysis
PHT	:	Plant height at maturity

POC	:	Pod colour
POD	:	Number of pods per plant
POS	:	Pod shape
POT	:	Pod texture
RAPD	:	Random amplified polymorphic DNA markers
RCBD	:	Randomized complete block design
RSI	:	Rank summation index
RUBISCO	:	Ribulose bisphosphate carboxylase/oxygenase
SDS	:	Sodium dodecyl sulfate
SEP	:	Seed eye pattern
SSR	:	Simple sequences repeat markers
STC	:	Seed testa colour
STP	:	Seed testa pattern
SWT g	:	Seed weight per plant
TLA	:	Terminal leaflet area
TLS	:	Terminal leaflet shape at maturity
Ton	:	Tonnes
UBGM	:	University Botanic Garden Maseno
WAP	:	Weeks after planting

DEFINITIONS OF TERMS

Accession: Are plant materials collected from a particular area, hence adapted to a particular agro-ecological area.

Crop Yield: Refers to a standard measurement of the amount of agricultural production harvested per unit of land area.

Dendrogram: Refers to a tree diagram, especially one showing taxonomic relationships.

Genetic Diversity: Refers to the total number of genetic characteristics in the genetic make up of a species, and it includes the variations within species which encompasses differences in alleles and genes among individuals.

Germplasm: The term used to describe the seeds, plants or plant parts useful in crop breeding, research and conservation efforts.

Homology: Refers to a state of having the same or similar relation, relative position or structure.

Intercropping: Is an agricultural practice where two or more crops are cultivated simultaneously on the same piece of land.

Landrace: Is a cultivated, genetically heterogeneous variety that has evolved due to its traditional management practices in a certain ecogeographical area and is hence adapted to the edaphic and climatic conditions of that region.

Monocropping: Refers to planting of one crop in a piece of land in a given season.

Morphology: Refers to outward forms, shapes or structure of an organism.

Neglected crops: Are crops whose economic potentials have not fully been exploited and realized.

Operational taxonomic units: Are the selected taxa (or individuals) for study, usually the starting point units in phenetic classification.

Phenotypic plasticity: The ability of a single genotype to produce different phenotypes in response to varying environmental conditions.

Principal component analysis: Analysis of a data covariance matrix to identify the eigenvectors and eigenvalues which best describe the variation in data. Analysis begins with identifying the axis which encompasses most of the variation in the data and moves towards successively minor effects.

Proteins: Are primary metabolites made up of nitrogenous organic compounds that have large molecules composed of one or more long chains of amino acids and are essential part of all living systems.

Proteomics: Reffers to the systematic study of complete set of proteins produced by an organism or biological system.

SDS-PAGE Electrophoresis: Is a technique of separating complex mixtures of protein based on their molecular weights in an electric field.

Taxonomic character: Is a feature of an organism that is divisible into at least two states and that is used in constructing classifications and associated activities, principally identification.

Taxonomic marker: It is an identifiable structure or cellular component that can be used for the identification of a species or an organism.

Underutilized crop: Refers to the abandonment of a species by indigenous and international research communities.

LIST OF TABLES

Table 1: Socio-demographic table of Bambara groundnut farmers, in the selected Lake Victoria Basin counties of Western Kenya……..….34

Table 2: Some descriptors and classes of qualitative traits of Bambara groundnut…….43

Table 3: Quantitative traits recorded and brief description as listed from (IPGRI/IITA/BAMNET, 2000)……..…..44

Table 4: Landrace independent values for calculating Bambara groundnut leaf area ……46

Table 5: Standard curve samples for Bradford Protein Assay……..52

Table 6: The physio-chemical properties of soils in the green house at Maseno University research farms and in the different agro ecological zones (Kibos, Oyani and Alupe) in the Lake Victoria basin, Kenya……...…….…....57

Table 7: Percentage distribution of Qualitative traits in the six Bambara groundnut accessions.60

Table 8: Shannon-Weiner diversity index (H') showing the phenotypic diversity of six accessions……61

Table 9a: Plant Height Intra-Site Comparisons in Alupe zone…….……70

Table 9b: Plant Height Intra-Site Comparisons in Kibos zone…….70

Table 9c: Plant Height Intra-Site Comparisons in Oyani zone…….71

Table 9d: Plant Height Intra-Site Comparisons in Greenhouse irrigated zone…….71

Table 9e: Plant Height Intra-Site Comparisons in Greenhouse droughted zone……..71

Table 10a: Number of Pods with One Seed (N1S) intra-site comparisons in Alupe……83

Table 10b: Number of Pods with Two Seeds (N2S) intra-site comparisons in Alupe…….83

Table 10c: Total Number of Pods per Plant (POD) Intra-Site Comparisons in Alupe…….83

Table 11a: Number of Pods with One Seed (N1S) Intra-Site Comparisons in Kibos……..84

Table 11b: Number of Pods with Two Seeds (N2S) Intra-Site Comparisons in Kibos……84

Table 11c: Total Number of Pods (POD) Intra-Site Comparisons in Kibos……85

Table 12a: Number of Pods with one seed (N1S) intra-site comparisons in Oyani…….86

Table 12b: Number of Pods with two seeds (N2S) Intra-Site Comparison in Oyani……...…86

Table 12c: Total Number of Pods per Plant (POD) Intra-Site Comparison in Oyani……..86

Table 13a: Number of Pods with one seed (N1S) intra-GHI Comparisons……..87

Table 13b: Total Number of Pods (POD) intra-GHI Comparisons……..88

Table 14a: Number of Pods with One Seed (N1S) intra-GHD Comparisons……..……88

Table 14b: Total Number of Pods per Plant (POD) Intra-GHD Comparisons…….89
Table 15a: Terminal Leaflet Area (TLA cm^2) Intra-Site Comparisons in Alupe…….96
Table 15b: Terminal Leaflet Area (TLA cm^2) Intra-Site Comparisons in Kibos…….97
Table 15c: Terminal Leaflet Area (TLA cm^2) Intra-Site Comparisons in Oyani…….97
Table 15d: Terminal Leaflet Area (TLA cm^2) intra-Greenhouse Irrigated (GHI) Comparisons.97
Table 15e: Terminal Leaflet Area (TLA cm^2) intra-Intra-Green house Droughted (GHD) Comparisons……98
Table 16a: Number of Flowers per Plant (NFP) Intra-Site Comparisons in Alupe……105
Table 16b: Number of Flowers per Plant (NFP) Intra-Site Comparisons in Kibos……106
Table 16c: Number of Flowers per Plant (NFP) Intra-Site Comparisons in Oyani……106
Table 16d: Number of Flowers (NFP) intra-Greenhouse Irrigated (GHI) Comparisons……...106
Table 16e: Number of Flowers (NFP) intra-Greenhouse Droughted (GHD) Comparisons…...107
Table 17a: Seed Weight per plant (SWT g) Intra-Site Comparisons in Alupe……...115
Table 17b: Seed Weight per plant (SWT g) Intra-Site Comparisons in Kibos……..…115
Table 17c: Seed Weight per plant (SWT g) Intra-Site Comparisons in Oyani……..……115
Table 17d: Seed Weight per plant (SWT g) Intra-Greenhouse Irrigated (GHI) Comparisons...116
Table 17e: Seed Weight per plant (SWT g) Intra-Greenhouse Droughted (GHD) Comparisons……..….116
Table 18: Pearson's Correlation Matrix (r) for seven quantitative traits of Bambara groundnut accessions grown across three environments, and among the greenhouse drought-stressed and irrigated plants……...……121
Table 19a: Descriptive statistics of K2 Accession Inter-Site Yield (ton/ha) Comparisons……126
Table 19 b: Descriptive statistics of B4 accession inter-site yield (ton/ha) comparisons……...126
Table 19 c: Descriptive statistics of B1 accession inter-site yield (ton/ha) comparisons……...127
Table 19 d: Descriptive statistics of K1 accession inter-site yield (ton/ha) comparisons…..…127
Table 19e: Descriptive statistics of M4 accession inter-site yield (ton/ha) comparisons……...128
Table 19f: Descriptive statistics of M2 accession inter-site yield (ton/ha) comparisons……128
Table 20: Yield (ton/ha) of various accessions in different agro-ecological zones……128
Table 21a: Descriptive statistics of Alupe zone intra-site yield (ton/ha) comparisons of accessions K2, B4, B1, K1, M4 and M2……130

Table 21b: Descriptive statistics of Kibos zone intra-site yield (ton/ha) comparisons of accessions K2, B4, B1, K1, M4 and M2……..……131
Table 21c: Descriptive statistics of Oyani zone intra-site yield (ton/ha) comparisons of accessions K2, B4, B1, K1, M4 and M2……..…131
Table 21d: Descriptive statistics of GHD intra-site yield (ton/ha) comparisons of accessions K2, B4, B1, K1, M4 and M2…….131
Table 21e: Descriptive statistics of GHI intra-site yield (ton/ha) comparisons of accessions K2, B4, B1, K1, M4 and M2…….132
Table 22a: Pearson's correlation (r) coefficients for seven quantitative traits with yield of Bambara groundnut accessions in Alupe zone……...132
Table 22b: Pearson's correlation (r) coefficients for seven quantitative traits with yield of Bambara groundnut accessions in Kibos zone……..…133
Table 22c: Pearson's correlation (r) coefficients for seven quantitative traits with yield of Bambara groundnut accessions in Oyani zone……...133
Table 22d: Pearson's correlation (r) coefficients for seven quantitative traits with yield of Bambara groundnut accessions in GHD……..…133
Table 22e: Pearson's correlation (r) coefficients for seven quantitative traits with yield of Bambara groundnut accessions in GHI……...133
Table 23a: Mean values of yield-related traits and Rank Summation Index (RSI) of six BGN accessions at Alupe, Busia County……..…134
Table 23b: Mean values of yield-related traits and Rank Summation Index (RSI) of six BGN accessions at Kibos, Kisumu County……..…134
Table 23c: Mean values of yield-related traits and Rank Summation Index (RSI) of six BGN accessions at Oyani, Migori County……135
Table 23d: Mean values of yield-related traits and Rank Summation Index (RSI) of six BGN accessions under greenhouse drought stress (GHD)……...135
Table 23e: Mean values of yield-related traits and Rank Summation Index (RSI) of six BGN accessions under greenhouse irrigation (GHI)……..…135
Table 24: Protein concentrations (mg/mL) of five Bambara groundnut accessions……...143
Table 25: The protein peptides (kDa) present in each Bambara groundnut sample……...144
Table 26: The dissimilarity indices (r^2 values) of protein peptides based on Jaccard index…..144

Table 27: Molecular weights of Albumins fractions in various BGN accession samples……..149

Table 28: The dissimilarity indices (r^2 values) of albumin concentrations based on Jaccard index……..…149

Table 29: Molecular weights of different fractions in Globulins in various Bambara groundnut accessions samples……...151

Table 30: The dissimilarity indices (r^2 values) of globulins concentrations based on Jaccard index…….……151

LIST OF FIGURES

Figure 1: Map of Lake Victoria Basin showing study sites……..33
Figure 2: Landraces layout in field experiments ……....38
Figure 3: Sampling Matrix……42
Figure 4: The scree plot graph for PCA showing the explained variances……...48
Figure 5: A graph showing estimations of protein concentrations done by comparing the OD595 with the standard curve…..…….53
Figure: 6a: Monthly rainfall amounts (mm) and air temperatures in Alupe zone……55
6b: Monthly rainfall amounts (mm) and air temperatures in Kibos zone…….55
6c: Monthly rainfall amounts (mm) and air temperatures in Oyani zone…….55
Figure 7: Frequency distribution of qualitative traits in the Bambara accessions grown in various zones and in the greenhouse……..……58
Figure 8: A dendrogram showing the relationship among Bambara groundnut accessions based on their Similarity and Dissimilarity distances…….62
Figure 9: Plant height of K2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots)……..63
Figure 10: Plant height of B4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots)……..64
Figure 11: Plant height of B1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots)……..65
Figure 12: Plant height of K1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots)…….….66
Figure 13: Plant height of M4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)……...67
Figure 14: Plant height of M2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots)……..68
Figure 15 A: Number of pods with one seed (N1S) of K2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)…….73

Figure 15 B: Number of pods with two seeds (N2S) of K2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)…….……73

Figure 15 C: Total number of pods (POD) of K2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)…..73

Figure 16 A: Number of pods with one seed (N1S) of B4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)……..……75

Figure 16 B: Number of pods with two seeds (N2S) of B4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)……...75

Figure 16 C: Total number of pods (POD) of B4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)…..75

Figure 17 A: Number of pods with one seed (N1S) of B1 accession……...76

Figure 17 B: Total number of pods (POD) of B1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots)…76

Figure 18 A: Number of pods with one seed (N1S) of K1 accession……...78

Figure 18 B: Number of pods with two seeds (N2S) of K1 accession…….78

Figure 18 C: Total number of pods (POD) of K1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots). Observed…….…….78

Figure 19: A Number of pods with one seed (N1S) of M4 accession……..79

Figure 19 B: Number of pods with two seeds (N2S) of M4 accession……79

Figure 19 C: Total number of pods (POD) of M4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)……79

Figure 20 A: Number of pods with one seed (N1S) of M2 accession…….....81

Figure 20 B: Number of pods with two seeds (N2S) of M2 accession……81

Figure 20 C: Total number of pods (POD) of M2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)81

Figure 21: The Terminal leaflet Area of K2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots)……..90

Figure 22: The Terminal leaflet Area of B4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots)…….91

Figure 23: The Terminal leaflet Area of B1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots)……92

Figure 24: The Terminal leaflet Area of K1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots)……93

Figure 25: The Terminal leaflet Area of M4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots)…....94

Figure 26: The Terminal leaflet Area of M2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in Green house (Irrigated and Droughted pots)…...95

Figure 27: The Number of Flowers per Plant (NFP) of K2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)…….99

Figure 28: The Number of Flowers per Plant (NFP) of B4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)……100

Figure 29: The Number of Flowers per Plant (NFP) of B1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)……101

Figure 30: The Number of Flowers per Plant (NFP) of K1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)……..……...102

Figure 31: The Number of Flowers per Plant (NFP) of M4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)……103

Figure 32: The Number of Flowers per Plant (NFP) of M2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughtedplants)……..104

Figure 33: The Seed Weight per Plant (SWT g) of K2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)……108
Figure 34: The Seed Weight per Plant (SWT g) of B4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots)……..109
Figure 35: The Seed Weight per Plant (SWT g) of B1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)……110
Figure 36: The Seed Weight per Plant (SWT g) of K1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)……111
Figure 37: The Seed Weight per Plant (SWT g) of M4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)……112
Figure 38: The Seed Weight per Plant (SWT g) of M2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots)……113
Figure 39: Principal Component Analysis (PCA) of quantitative trait scores from three zones and greenhouse……...117
Figure 40A: PCA Biplot for Zones……118
Figure 40B: PCA Biplot for Accessions……118
Figure 41: Hierarchical clustering dendrogram showing the relationship among the six Bambara accessions based on their quantitative traits scored in the three field sites and in greenhouse……120
Figure 42: Correlations among the quantitative morphological traits……122
Figure 43: The five accessions (K1, B4, B1, M4 and M2) total protein concentrations……136
Figure 44: The four protein fractions (albumins, globulins, glutelins and prolamins) concentrations in K1 sample……..137
Figure 45: The four protein fractions (albumins, globulins, glutelins and prolamins) concentrations in B4 sample……..138

Figure 46: The four protein fractions (albumins, globulins, glutelins and prolamins) concentrations in B1 sample……...139
Figure 47: The four protein fractions (albumins, globulins, glutelins and prolamins) concentrations in M2 sample……...139
Figure 48: The four protein fractions (albumins, globulins, glutelins and prolamins) concentrations in M4 sample……...140
Figure 49a: The protein fraction albumins in the five accessions (K1, B4, B1, M2 and M4) samples.……...142
Figure 49b: Globulins in the five accessions (K1, B4, B1, M2 and M4) samples…….142
Figure 49c: Glutelins in the five accessions (K1, B4, B1, M2 and M4) samples……..…142
Figure 49d: Prolamins in the five accessions (K1, B4, B1, M2 and M4) samples…….142
Figure 50: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of Bambara groundnut isolates of total proteins……...143
Figure 51: Hierarchical clustering dendrogram showing the relationship amongst the five Bambara groundnut accessions based on their protein peptides profiles……..145
Figure 52: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) banding patterns of protein fractions of K1 isolates……146
Figure 53: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) banding patterns of protein fractions of B1 isolates……147
Figure 54: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) banding patterns of protein fractions of B4 isolates……147
Figure 55: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) banding patterns of protein fractions of M2 isolates……...148
Figure 56: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) banding patterns of protein fractions of M4 isolates……...148
Figure 57: Hierarchical clustering dendrogram showing the relationship amongst the five Bambara groundnut accessions based on their albumins profiles…….150
Figure 58: Hierarchical clustering dendrogram showing the relationship amongst the five Bambara groundnut accessions based on their globulins profiles……152

LIST OF PLATES

Plate 1: Morphological features of Bambara groundnut……..……11

Plate 2: Varieties of Bambara groundnut seeds……12

Plate 3: Bambara groundnut field established crop stand at Kenya Agricultural Livestock Research Organization (KALRO) Kibos farm, Kisumu County …..……39

Plate 4: Length and width measurements of a Bambara groundnut leaflet…….….45

Plate 5: The seed testa pattern and seed testa colour of the six Bambara groundnut germplasm used in the study…….61

LIST OF APPENDICES

Appendix 1: Soil sample collection at Alupe KALRO research farms, Busia County…….…217
Appendix 2: Randomization table……..218

CHAPTER ONE
INTRODUCTION

1.1 Background of Study

Bambara groundnut (BGN), hereafter referred to as Bambara (*Vigna subterranea* (L.) Verdc) is a herbaceous grain legume, belonging to the Fabaceae botanical family. Although Bambara (named after a tribe in Mali) is native to West Africa, the crop has spread widely over the continent's semi-arid region (Hillocks *et al*., 2012). Bambara is an economically important food crop in Africa and beyond. According to Paliwal *et al*., (2021), it is the third-most significant grain legume in Africa after groundnut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*), serving as a primary source of vegetable protein (Majola *et al*., 2021). Bambara is rich in nutrients, containing protein (18–24%), carbohydrates (51–71%), and fat (4–12%) (Muhammad *et al*., 2020). Its gross energy content surpasses that of other popular pulses including cowpea, lentils, and pigeon pea (Ajilogba *et al*., 2022). However, due to the introduction of exotic crops like the American groundnut (*Arachis hypogaea*), Bambara has been neglected (Jideani and Jideani, 2021). Neglected crops have the potential to enhance food and nutrition security, accessibility, and availability to local communities, but they require scientific attention to facilitate their reintegration into cropping systems (Ali and Bhattacharjee, 2023).

For many impoverished rural Africans who cannot afford expensive animal protein, Bambara serves as an alternative protein source (Wasula *et al*., 2014). Despite its potential to provide resource-poor families with vital nutrients, research on this crop has been minimal in many countries where it has been domesticated. The unrealized potential of Bambara in the Lake Victoria region is evident from the lack of comprehensive data on the agronomic and proteomic traits of the indigenous landraces. Bambara is cultivated by small-scale African farmers due to its numerous natural agronomic benefits, such as low input requirements, high nutritional value, drought resistance (Amwenyo *et al*., 2025), and its ability to yield well in soils considered too infertile for other, more favored species like common beans and groundnuts (Olanrewaju *et al*., 2022). Furthermore, Bambara's nitrogen-fixing capability makes it an advantageous addition to farming systems (Chelangat *et al*., 2023).

Knowledge of genetic diversity in crops is crucial as it indicates variability. In taxonomy, genetic diversity can be assessed at three major levels i.e phenotypic level using phenotypic descriptors

(Olukolu *et al*., 2012), biochemical level using biochemical markers such as isoenzymes (Pasquet *et al.,* 1999), and at molecular level using molecular markers (Molosiwa *et al.,* 2015). The variability observed through genetic diversity is essential for classification, as it reveals relationships between operational taxonomic units (OTUs). According to Oludare *et al*., (2017), genetic diversity evaluation is a pre-requisite for genetic improvement in underutilized crops like Bambara. Peddakasim *et al*., (2015) asserts that assessing genetic diversity among cultivars is necessary for identifying high performing parent plants.

Morphological characters of landraces can be used as taxonomic markers to establish phenetic relationships (Manoko, 2018). Taxonomic characters are fundamental in classification (Stuessy, 1990). In taxonomy, classification based solely on morphological characters is often inadequate, and superficial, because some morphological traits are plastic and lack a genetic basis. Invariant morphological characters have no value in determining relationships between sub taxa within a taxon. Only variant morphological characters with two or more states are taxonomically useful, as they help distinguish taxa at the next level of the hierarchy, provided that groups at that level have contrasting states (Stuessy, 1990). Moreover, morphological character analysis (including character selection and coding) is time consuming and requires numerous methodological decisions and assumptions at every step (Wiens, 2001). Therefore, cellular biomolecules such as proteins offer better classification markers.

Research on inter and intra-landrace variation for phenotypic descriptors has been extensively conducted in Bambara from other parts of the world (Onwubiko *et al*., 2019; Aliyu *et al.,* 2016; Ntundu *et al*. 2006). Most of these studies have been country-specific. The standard descriptors for Bambara (IPGRI/IITA/BAMNET 2000) have guided these phenotypic characterization exercises. However, in Kenya, specifically in the Lake Victoria basin region, no intensive phenotypic characterization of farmer-held landraces has been conducted to establish their variability. Aliyu *et al.,* (2016), reported that adequate variability within a species' germplasm is the most crucial resource for achieving breeding objectives in crop improvement programs. Additionally, a thorough understanding of germplasm variability is essential for conservation, management and future utilization (Aliyu *et al*., 2016). Odongo *et al*., (2015), phenotypically described some landraces in the Lake Basin region, but did not conduct a conclusive study. In their research focused only on seed testa colour, which is insufficient since morphological

characterization involves multiple traits. An in-depth morphological characterization of these farmer-held landraces in the Lake Victoria basin is needed, as various studies indicate that Bambara germplasm characterization influences farmer adoption and consumer uptake in Western Kenya (Wasula *et al*., 2014; Ngugi, 1995).

Research has also shown that variations in agronomic traits of Bambara, such as leaf length, number of flowers, seed weight, and pod dry weight, are influenced by independent ecological factors across different zones (Werner, 2006). Rainfall levels also affect most phenotypic characteristics (Werner, 2006). The influence of abiotic factors across different zones can lead to phenotypic plasticity among Bambara landraces (Valombola, *et al*., 2022). This phenotypic plasticity can, in turn, affect the phenetic classification pattern.

Most research on Bambara landraces from the Lake Victoria basin has focused on production trends, opportunities, and challenges affecting sustainable production and utilization (Wasula *et al*., 2014; Korir *et al*., 2011). In recent years, interest has grown in symbiotic characterization of root nodule bacteria isolated from Bambara roots in Western Kenya (Onyango *et al*., 2015) and yield determination in intercropping systems (Akuja *et al.,* 2016). However, relatively little attention has been given to yield determination in monocropping systems. Akuja *et al.* (2016) planted Bambara in monocropping systems as a control in their intercropping yield research and found that monocropped Bambara produced higher yields as compared to intercropped Bambara. There is therefore need of determination of Bambara landraces yields in mono cropping system, in order to determine the landraces that produce the highest yields. Therefore, further research is needed to determine the highest yielding landraces in the various zones within the Lake Victoria Basin, especially since Bambara yields in the region have declined in recent decades (Netondo and Maasai, 2013). Previous research by Ngugi (1995) highlighted that low Bambara groundnut yields within the Lake Victoria Basin are often linked to the use of germplasm from landraces poorly adapted to local environmental conditions, noting that each landrace tends to exhibit strong zonal adaptability. This underscores a critical gap in understanding the spatial suitability of landraces within the basin's diverse agro-ecological zones, which remains largely unexplored. Despite this constraint, Amwenyo *et al*. (2025) stated that, given the potential risk of prolonged droughts due to climate change, Bambara is likely to become more important in African agriculture. According to Majola *et al*. (2021), the current grain yield of the crop in Africa is

0.85 t ha^{-1}, despite its potential yield of over 3 t ha^{-1}. Bambara is therefore currently underutilized, highlighting the need for further research.

Soil nutrition also affects yield, even though research shows that Bambara is capable of meeting its nitrogen requirements through nitrogen fixation (Khan *et al*., 2022a). Bambara requires between 80-150 kg N ha^{-1} during its growing period, yet it can fix approximately 4-200 kg N ha^{-1} (Zoundji *et al*, 2022), depending on the type of rhizobia, as well as edaphic and environmental conditions (Fwanyanga *et al*., 2022). Although yield evaluations have been conducted in various agro-ecological zones elsewhere (Obidiebube *et al*., 2019), no evaluation of farmer-held landraces in monocropping systems across different agro-ecological zones in the Lake Victoria basin region has been conducted.

In the recent years, assessing Bambara's genetic diversity at the molecular level has generated a significant interest. DNA molecular markers have been used to determine variability in Bambara (Aliyu *et al.,* 2016). Ntundu *et al*. (2004) assessed the genetic diversity of 100 Bambara landraces from Tanzania using Amplified Fragment Length Polymorphism (AFLP) markers. The results indicated that the genetic distances between all accessions based on Jaccard's index ranged from 0.1 to 0.68, with a total average of 0.3, revealing a low genetic diversity index among the Bambara germplasm evaluated. Similarly, Odongo *et al*. (2015) assessed 105 landraces from Western Kenya counties and from National gene bank using 12 microsatellite markers. The gene diversity values averaged 0.35, indicating low allelic diversity. The low allelic diversity highlighted is a limitation in the use of DNA as a taxonomic marker since only variant characters are useful taxonomically in classification (Stuessy, 1990). Due to these limitations, there is need to assess the genetic diversity using other cellular markers, such as proteins which are more variant, stable and ubiquitous, making them more effective for taxonomic classification (Stuessy, 1990). In taxonomy, anatomical data generated from proteins are often useful in solving problems of relationships because they often suggest the homologies of morphological character state and can help in the interpretation of evolutionary directionality (Stuessy, 1990).

Protein biomolecules play a crucial role in molecular systematics as they are products of gene expressions (Saminu *et al*., 2021). Their presence in crops can be used to establish genetic

diversity. The studies of genetic diversity using proteins have been done although various techniques have been employed in isolation and separation of the proteins. Okpuzor *et al*., (2010), isolated and separated protein types in dry and malted Bambara seeds using LC-MALDI and found out that some protein types such as vicilin, seed storage protein B, and 8S globulin alpha subunit were in both dry and malted seeds whereas some were not, indicating that certain protein types exist in seeds based on their conditions and biological stresses they encounter. Okonkwo and Opara (2010), extracted the proteins from the Bambara using the soxhlet extractor. These different techniques used affect comparability across studies (Stuessy, 1990). It is on the basis of this backdrop that more precise methods, such as Sodium Dodecyl Sulfate-PolyAcrylamide Gel Electrophoresis (SDS-PAGE), have been used to isolate and extract proteins in Bambara (Saminu *et al*., 2021; Oludare *et al*., 2017). The SDS-PAGE technique separates proteins in an electrical field based on their molecular weights. However, in the Lake Victoria basin region, knowledge of protein types and their relative quantities in farmer-held landraces is virtually lacking. There is a need to determine their genetic diversity, as protein variability based on their molecular weights can serve as a basis for phenetic classification.

1.2 Statement of the Problem

In Kenya, several collections of Bambara groundnut landraces have been made over the years in the Lake Victoria Basin region (Odongo *et al*., 2015; Ngugi, 1995). However, in-depth knowledge of their genetic diversity at both the morphological and cellular levels remains limited. Such knowledge is essential, as it provides insights into the variability necessary for crop improvement. Comprehensive characterization of these landraces using both quantitative and qualitative morphological traits is lacking, which negatively affects farmer adoption, consumer acceptance, and broader utilization of the crop (Wasula *et al*., 2014).

Farmers often have specific trait preferences, for example, some prefer landraces with large leaves or black seed coats due to their perceived yield stability (Valombola *et al*, 2022). Similarly, consumers exhibit preferences based on seed coat color: cream-colored seeds are favored for their taste, while red seed-coated landraces are valued for their high iron content (Bamshaiye *et al*., 2011). Moreover, the phenetic relationships among these landraces remain unresolved, primarily because many exhibit similar morphological traits such as seed coat color, seed eye patterns, and pod color. This similarity has been a key impediment in classification, on

wether these accessions belong to the same species or not. These unresolved issues underscore the need to establish clear phenetic relationships among the collected landraces.

The similarity in morphological characteristics among Bambara groundnut landraces has posed a significant challenge in breeding efforts, as adequate variability within the germplasm is essential for achieving crop improvement objectives (Aliyu *et al*., 2016). This highlights the need to assess variation at the cellular level, where molecular markers such as proteins can offer more reliable traits for classification due to their inherent variability (Stuessy, 1990). Despite this potential, there is a notable lack of in-depth research on protein biomolecules in Bambara groundnut, both in Kenya and globally. Focusing on high-yielding accessions for protein analysis is particularly relevant, as these are the most valuable to farmers and consumers within the Lake Victoria Basin. Analyzing protein diversity in these accessions not only helps to reveal genetic variability and relationships among landraces, but also provides insights that can inform targeted breeding strategies. Furthermore, such analysis could offer clues to evolutionary divergence, potentially indicating early stages of speciation.

Bambara groundnut yields in the Lake Victoria Basin (LVB) region remain low, with limited available yield data. Onyango and Ogolla, (2020) observed that the Kakamega Cream accession exhibited superior yield traits but did not provide actual yield figures. Similarly, Korir *et al*. (2011) reported a yield of only 63.24 kg/ha, far below the 300–800 kg/ha typically achieved under traditional farming systems and the approximately 3,000 kg/ha attained under commercial production in other parts of sub-Saharan Africa (Majola *et al*., 2021). This highlights a critical need for comprehensive yield evaluation and improvement efforts in the Lake Victoria Basin. The low yields attained are partly due to uncontrolled germplasm recycling, where farmers plant the germplasm within their possession irrespective of their zonal adaptability in the various regions (Hall *et al*., 2014; Goli,*et al*., 1997).

1.3 Justification

Morphological characterization of Bambara groundnut in the Lake Victoria Basin is essential, as both farmer adoption and consumer acceptance of the crop are largely influenced by the phenotypic traits of the seed (Wasula *et al*., 2014; Ngugi, 1995). In Kenya, adoption levels of Bambara groundnut remain low (Wasula *et al*., 2014), partly because farmers prefer varieties that

exhibit specific morphological traits. The limited understanding and appreciation of the crop contribute to its underutilization, yet its potential to enhance food and nutrition security in local communities is significant.

Bambara groundnut is a vital protein source for many rural African communities who cannot afford animal-based protein (Wasula *et al*., 2014). Given the growing impacts of climate change, manifested in prolonged droughts and erratic rainfall patterns across the Lake Victoria Basin, there is an urgent need for climate-resilient crops. Bambara's drought tolerance and ability to yield under poor soil conditions make it a strong crop for improving food and nutritional security in the region. Evaluating the performance of different landraces across diverse agro-ecological zones with varying rainfall, soil types, and climatic parameters is necessary to identify optimal growing conditions. This knowledge will enable agronomists to understand how specific landraces respond to environmental variability and guide efforts to improve yields. Identifying high-yielding accessions within each agro-ecological zone will help farmers focus on the most productive varieties, thereby increasing overall production, a critical goal given the steady decline in Bambara cultivation in Kenya over the past few decades (Masayi and Netondo, 2013).

Characterization also holds taxonomic importance. While morphological traits have historically been used in classification, they are often insufficient due to superficial similarities across landraces. Cellular markers, especially seed protein profiles, provide more robust characters for classification. The unresolved phenetic relationships among Bambara landraces, largely due to similarities in traits such as seed coat color, eye patterns, and pod morphology, underscore the need for protein-based studies. Determining whether significant differences exist at the protein level is essential for resolving these taxonomic ambiguities and assessing whether new species may be emerging.

Genetic diversity is a cornerstone of crop improvement programs. Bambara groundnut in Kenya largely exists as uncharacterized landraces, with limited information on their protein-level diversity. Evaluating protein variation among high-yielding accessions will aid breeders in developing superior cultivars that combine both nutritional quality and high yield potential. This information will also enhance understanding of genetic relatedness among local landraces and

support targeted breeding strategies aimed at increasing productivity in specific agro-ecological zones.

Additionally, Bambara seed proteins offer multiple health benefits, including anti-hypertensive properties and roles in regulating food intake and body weight in humans (Okpuzor *et al*., 2010). Currently, information on the types of proteins present in different Bambara accessions is lacking. This gap limits the ability of nutritionists to recommend specific landraces with health-promoting protein profiles. Therefore, investigating the protein diversity of high-yielding accessions is not only vital for breeding and taxonomy but also for public health and nutritional planning, hence this study.

1.4 Objectives

1.4.1 General Objective

To determine the genetic diversity of the Bambara grown in Lake Victoria basin using morphological characters, yield and cellular proteins.

1.4.2 Specific Objectives

i. To identify the morphological characters of the selected Bambara landraces grown in different agro-ecological zones and in the green house.
ii. To determine the yield across the selected Bambara landraces grown in different agro-ecological zones and in the green house.
iii. To determine the protein types in the high yielding selected Bambara landraces grown in different agro-ecological zones and in the green house.

1.5 Null Hypotheses, No

i. There are no significant differences in the morphological characters of the selected Bambara landraces grown in different agro-ecological zones and in the green house.
ii. The yield across the selected Bambara landraces, grown in different agro-ecological zones and in the green house has no significant variation.
iii. There are no significant differences between protein biomolecules in all the selected high yielding Bambara landraces, grown in the different agro-ecological zones and in the green house.

CHAPTER TWO
LITERATURE REVIEW

2.1 Taxonomy of Bambara Groundnut

Bambara (*Vigna subterranea* L. Verdc) belongs to the genus *Vigna*, subtribe Phaseolinae, tribe Phaseoleae and family Fabaceae (Temegne *et al*., 2018). In 1763, Linnaeus described it in *Species Plantarum,* and named it *Glycine subterranea*, in accordance with his system of nomenclature (Mkandawire, 2006). DuPetit–Thouars in 1806 found the crop in Madagascar and proposed the name *Voandzeia subterranea* (L). Thouars, which was widely used by subsequent researchers for over a century. Detailed botanical studies were undertaken by Verdcourt, who in 1980, proposed the current name *Vigna subterranea* (L) Verdc. (Mkandawire, 2006).

2.2 Distribution of Bambara groundnut in Africa

Bambara groundnut (*Vigna subterranea*), believed to have originated in the Timbuktu region of central Mali in West Africa (Majola *et al*., 2021), has been widely cultivated in tropical regions of Africa since the seventeenth century. The crop is primarily grown in Niger, Burkina Faso, Ghana, Togo, and the Democratic Republic of Congo, with northeastern Nigeria and northern Cameroon identified as major centers of genetic diversity (Olanrewaju *et al*., 2022). South Africa, Botswana, Zimbabwe, Zambia, and Uganda are considered among the most suitable regions for Bambara production on the continent (Majola *et al*., 2021). This drought-tolerant legume, capable of thriving in nutrient-deficient soils (Ajilogba *et al*., 2022), is of increasing importance in addressing food and nutritional security in sub-Saharan Africa. Despite its broad cultivation, no distinct cultivars have been formally described, and efforts to develop new varieties remain limited. Consequently, all existing landraces are classified within a single species.

2.3 Multifunctional Uses of Bambara Groundnut

Bambara groundnut (*Vigna subterranea*) is primarily cultivated as a food crop, mainly for household consumption. It is widely recognized for its nutritional value and versatility in traditional diets. Bambara milk has been developed as a dairy alternative, with studies highlighting its potential as a probiotic beverage due to its rich nutritional profile (Murevanhema and Jideani, 2013). In the Lake Victoria Basin (LVB), its utilization includes boiling immature

pods, incorporating mature seeds into local dishes such as *nyoyo*, roasting, or combining them with sesame or meat stews (Ngugi, 1995).

Beyond its nutritional role, Bambara groundnut exhibits significant medicinal properties. Extracts from the seeds have demonstrated antimicrobial activity against various bacteria and fungi, attributed to their high phenolic and flavonoid content (Udeh *et al*., 2020). In Kenyan traditional medicine, the Luo community uses roasted Bambara seeds to treat polymenorrhea and cataracts. Additional uses include treatments for diarrhea, internal bruising, and as a biopesticide when mixed with *Lantana camara* (Ngugi, 1995).

As a legume, Bambara groundnut also contributes to soil fertility through biological nitrogen fixation, thereby enhancing soil health and supporting the productivity of subsequent crops (Ajilogba *et al*., 2022).

2.4 Morphological Characterization in Plants

2.4.1 General Morphological Characterization of the Bambara Groundnut

Bambara groundnut (*Vigna subterranea* [L.] Verdc.) is a herbaceous annual legume exhibiting both prostrate and erect growth forms, typically attaining a height of 30 to 35 cm (Khan *et al*., 2021a). The crop develops a compact and well-developed taproot system, with numerous short lateral stems, each supporting trifoliate leaves (Bamshaiye *et al*., 2011). Its general morphology is characterized by a bunched appearance, with leaf clusters emerging from branched stems that form a crown near the soil surface. These stems frequently root at the nodes, further contributing to the plant's dense, bushy growth habit (Khan *et al*., 2021a). Petioles are typically long (up to 15 cm), stiff, and grooved, with basal coloration that ranges from green to purple.

The plant's flowers, usually borne one to three per node, are papilionaceous and arranged in racemes on long, hairy peduncles arising from stem nodes (Chandra *et al*., 2019). Following fertilization, the flower stalk exhibits geotropism, elongating downward to bury the developing pod in the soil. The mature pods, measuring between 1.25 and 2.5 cm in diameter, are round and wrinkled, typically containing one or two seeds that are smooth and very hard when dried (Bamshaiye *et al*., 2011). Bambara groundnut seeds demonstrate considerable variability in shape, size, and seed coat colour. These features including testa colour, pigmentation around the

hilum, and growth habit (bunched or spreading), are often used to differentiate landraces (Aviara *et al*., 2013). Varietal classification is frequently based on seed characteristics and, in some cases, the geographic origin of collection (Olanrewaju *et al*., 2021a). Seed shapes may be round or elliptical, with weights ranging between 280 g and 320 g per 1000 seeds (Bamshaiye *et al*., 2011).

Despite the detailed morphological information available in literature, most studies are generalized or conducted in regions outside the Lake Victoria Basin (LVB). Given the climatic and edaphic diversity of the LVB, it is likely that landraces adapted to different agro-ecological zones exhibit significant morphological variation. However, localized morphological characterization of Bambara within the LVB remains scarce. This represents a critical knowledge gap, especially for genetic diversity assessment, breeding, conservation, and agronomic development efforts. A targeted morphological study that documents key traits, such as leaf, floral and seed characteristics, of landraces grown under both field and greenhouse conditions across various agro-ecologies in the LVB is therefore necessary.



Plate 1: Morphological features of Bambara groundnut: Source Karunaratne, A. S. (2009).



Plate 2. Varieties of Bambara groundnut seeds. The colour of the seeds range from cream, red, brown to purple. Source: Biodiversity international

2.4.2 Efficacy of Morphological Characterization in Bambara within the Taxonomic Hierarchy

Morphological characterization remains a foundational approach in plant taxonomy and systematics, offering a direct, observable basis for classification (Stuessy, 1990). In Bambara groundnut, the application of morphological traits, including leaf structure, seed shape, and floral traits, presents a valuable framework for understanding taxonomic relationships and varietal differentiation. Exomorphic features such as leaf, flowers, root, seedling, fruit, and seed morphologies are readily visible and thus allow for greater appreciation of phenotypic variability among accessions (Stuessy, 1990). This is particularly useful in the classification of angiosperms, where vegetative characters have proven effective in solving complex taxonomic issues (Manoko, 2018; Stuessy, 1990).

For Bambara, which is known for its wide morphological diversity influenced by environmental conditions (Olanrewaju *et al*., 2021a), these characteristics can be pivotal in distinguishing genotypes and supporting intraspecific classification. According to Manoko (2018), morphological data remain essential in taxa identification, description, and the development of taxonomic keys. Seed morphology, especially seed coat reticulations and pigmentation, has been

emphasized for its diagnostic value in taxonomy, including in Bambara, where testa color and seed shape often underpin landrace classification.

The International Code of Botanical Nomenclature (ICBN) stipulates that species names are derived from morphological character states, reinforcing the foundational role of morphological traits in formal classification (Manoko, 2018). Morphological data are applicable across all taxonomic ranks, from varietal to family level, and in some cases, families are distinguished almost entirely on vegetative characteristics (Stuessy, 1990).

Despite the acknowledged value of morphological data in taxonomy, there remains a notable gap in the comprehensive morphological characterization of Bambara landraces, particularly across diverse agro-ecological zones such as those in the Lake Victoria Basin (LVB). This gap is especially pronounced in the context of phenetic classification, where the use of morphological traits to group accessions based on overall similarity of character states remains largely unexplored. In a region like the LVB, where environmental gradients influence plant form and structure, the absence of phenetic studies grounded in morphological data hinders the development of a robust classification framework for local landraces. This limits not only the understanding of intraspecific diversity but also efforts toward conservation, breeding, and environmental adaptation strategies.

Therefore, a focused morphological characterization of Bambara grown in different agro-ecological zones of the LVB is not only necessary for enhancing taxonomic resolution but also essential for supporting phenetic classification. Such an approach can identify morphotypes adapted to specific environments, enhance the precision of varietal distinction, and provide a clearer understanding of genotype-environment interactions (Olanrewaju *et al*., 2021a).

2.4.3 Qualitative Morphological Characters of Bambara Groundnut

Terminal leaflet shape in Bambara groundnut (BGN) varies significantly among landraces, with common forms including ovate, lanceolate, and obovate shapes (Ntundu *et al*., 2006). These morphological variations are important for classification and have been linked to differences in photosynthetic efficiency and adaptability. According to Valombola *et al*. (2022), oval and

elliptic leaflets may enhance light capture and photosynthesis, a finding supported by Kidner and Umbreen (2010).

Pod characteristics such as colour and texture also exhibit considerable diversity. Colours range from yellowish-brown and brown to purple, influenced by genetic factors and maturity stage (Ntundu *et al*., 2006). Pod texture, ranging from smooth to grooved, serves as a key taxonomic trait (IPGRI/IITA/BAMNET, 2000). Valombola *et al*. (2022) emphasized that pod colour and texture play a crucial role in market appeal when Bambara is sold as a fresh vegetable.

Pod shape also varies, with types ranging from rounded forms to those with pointed ends (IPGRI/IITA/BAMNET, 2000), which may affect seed development and yield potential (Khan *et al*., 2021a). Seed eye patterns, such as black or dark red butterfly-like eyes, or no pattern, are useful in varietal identification. Similarly, testa patterns (mottled, marbled, or absent) are genetically inherited and influence marketability and consumer preferences (Mohammed *et al*., 2016a). Their study on 58 accessions from diverse origins underscored the importance of these traits in distinguishing germplasm and ensuring the production of true-to-type lines.

Testa colour, which ranges from cream and red to brown and black, is one of the most variable traits and is closely tied to consumer preference (Valombola *et al*., 2021; Wasula *et al*., 2014), but also, nutritional properties such as antioxidant and mineral content (Valombola *et al*., 2022). Dark seed coats, rich in polyphenols, are thought to confer greater drought tolerance and resistance to insect pests (Mabhaudhi *et al*., 2013). Conversely, light-coloured seeds, including cream and tan types, tend to have lower tannin levels, making them more susceptible to pest damage (Mubaiwa *et al*., 2018).

Despite substantial research on Bambara groundnut (BGN) diversity in other regions, in-depth assessment of qualitative morphological traits as a tool for evaluating genetic diversity remains critically underexplored within the Lake Victoria Basin (LVB) of Kenya**.** Although several landraces have been collected from the LVB (Ngugi, 1995; Odongo *et al*., 2015), systematic characterization based on observable traits, such as leaflet shape, pod morphology, seed coat colour, and eye pattern, has not been comprehensively carried out. These qualitative traits are valuable indicators of genetic variation and are essential for the identification, classification, and

selection of diverse landraces**.** The absence of such studies in the LVB presents a significant knowledge gap, which limits the ability to harness local genetic resources for breeding and adaptation purposes**.** Consequently, this restricts the development and promotion of superior landraces, negatively impacting farmer adoption, consumer uptake, and the overall utilization of Bambara groundnut in the region**.** Therefore, robust qualitative morphological characterization is urgently needed to assess genetic diversity, guide conservation strategies and inform breeding programs within the LVB.

2.4.4. Quantitative morphological Characters of Bambara Groundnut

2.4.4.1 Plant height

Plant height is a crucial agronomic trait in Bambara biomass accumulation and yield potential (Hlanga *et al*., 2024). BGN exhibits considerable variability in plant height, influenced by both genetic and environmental factors such as soil fertility, rainfall, and temperature (Olanrewaju *et al*., 2021b). For instance, a study analyzing twelve BGN genotypes and two F_2 bi-parental segregating populations reported plant heights ranging from approximately 14.23 cm to 36.9 cm, indicating substantial phenotypic diversity (Gao *et al*., 2020). Collinson *et al*. (1996) also found that soil moisture significantly affects BGN growth parameters, including plant height, suggesting that rainfall and water availability across agro-ecological zones can influence this trait.

Variation in plant height across agro-ecological zones is primarily driven by differences in climatic and edaphic conditions. Olanrewaju *et al*. (2021b) evaluated 95 BGN accessions in two Nigerian agro-ecological zones, the derived savannah (Ibadan) and the rainforest (Ikenne), and reported significant variation in plant height due to both genetic differences among accessions and environmental conditions. Notably, the interaction between location and accession had a highly significant effect on plant height ($p < 0.0001$), indicating genotype-specific responses to different environments.

Similarly, Bonny *et al*. (2019) assessed the agromorphological divergence of BGN across four agro-ecological zones in Côte d'Ivoire and found that climatic and environmental variability significantly influenced plant height among accessions. These findings emphasize the role of environmental conditions in shaping BGN morphological traits. Furthermore, Olanrewaju *et al*.

(2021b) reported a broad-sense heritability estimate of approximately 29% for plant height, highlighting the combined influence of genetic and environmental factors.

Collectively, these studies underscore that plant height in BGN is significantly affected by both genetic makeup and environmental conditions such as soil type, nutrient availability, and climate. Although general variability in plant height among BGN landraces is well-documented, there remains a notable gap in research directly linking this trait to specific agro-ecological zones within the Lake Victoria Basin (LVB). Given its dual role as an indicator of both genetic variation and environmental adaptation, plant height remains a key trait in the morphological characterization of BGN.

2.4.4.2 Number of Pods with One or Two Seeds

Bambara number of pods, either with one or two seeds, are important traits that influence total pod yield and seed output. High variability in these parameters suggests potential for selection and breeding (Ntundu *et al*., 2006). Bambara pods typically contain one or two seeds, with the number of seeds per pod influenced by both genetic and environmental factors. Mabhaudhi and Modi (2013) observed that under optimal growing conditions, a greater proportion of two-seeded pods is produced. However, in drought-prone environments, reductions in pod and seed numbers are common due to stress-induced effects such as reduced flowering and seed abortion. These shifts are attributed to the crop's response to environmental stressors, which can alter pod development and seed formation.

Further supporting this, Mabitsela *et al*. (2024) conducted a meta-analysis on the response of Bambara to drought stress and reported significant declines in pod and seed numbers under short-term drought conditions. Pod number decreased with an effect size of –5.23, while seed number dropped with an effect size of –2.72, both statistically significant ($p < 0.0001$). These findings suggest that drought conditions can increase the prevalence of one-seeded pods, primarily due to seed abortion during reproductive stages.

The number of seeds per pod is not only important for yield but also reflects underlying genetic variability. Ouedraogo *et al*. (2008), in a study involving 310 accessions from Burkina Faso, found a positive correlation between the number of seeds per pod and yield per plant. Accessions

with more two-seeded pods tended to yield more, highlighting the importance of this trait for selection in breeding programs. While spacing significantly influenced overall yield, it did not significantly affect the number of seeds per pod, indicating that the trait is more genetically controlled than environmentally influenced.

Despite the recognition of pod seed number as a critical trait for yield and genetic diversity, no study has yet assessed the distribution of one- and two-seeded pods in farmer-held Bambara landraces from the Lake Victoria Basin (LVB). This represents a critical knowledge gap, especially considering the region's diverse agro-ecological conditions. Documenting such morphological characteristics in LVB landraces would provide vital insights into their genetic diversity and support breeding strategies for improved adaptation, productivity, and resilience in this underutilized legume crop

2.4.4.3 Total Number of Pods per Plant

The total number of pods per plant (POD) is a key agronomic trait in Bambara, widely used in the assessment of genetic diversity (Adu *et al*., 2023). This trait exhibits significant variability across agro-ecological zones (Gomoung *et al*., 2017), making it a valuable indicator of both environmental adaptation and underlying genetic differentiation among landraces. Adu *et al*. (2023) conducted a genome-wide association study on 100 Bambara landraces grown across multiple environments and reported substantial genetic variation in POD. The trait showed a broad-sense heritability of 79.39%, underscoring its genetic stability and suitability for selection in breeding programs. Furthermore, POD was positively correlated with days to maturity ($r = 0.60$), suggesting that longer growth durations may enhance pod development.

Likewise, Esan *et al*. (2023a) evaluated 15 genotypes across diverse environments and found significant differences in POD, reflecting a wide genetic base. Notably, genotype G8 consistently produced the highest number of pods across all environments, highlighting the potential for selecting high-yielding genotypes suited to specific agro-ecological zones. Similarly, Kambou *et al*. (2023) assessed 90 genotypes from three agro-climatic zones in Burkina Faso and reported highly significant variation ($p < 0.01$) in pod number per plant. Heritability estimates reached up to 97.95%, confirming that the trait is largely governed by genetic factors and is therefore highly amenable to selection.

At Bunda College, Chipeta and Gimode (2023) used both agro-morphological traits and DArTseq SNP markers to analyze Bambara genotypes. They found significant genetic variation ($p < 0.001$) in most traits, including POD, and identified pod number as a key trait for evaluating genetic diversity among accessions. Moreover, environmental conditions can modulate pod number. Mabhaudhi and Modi (2013) observed that irrigated conditions significantly increased pod number per plant compared to rain-fed environments, suggesting that environmental management can further optimize the expression of this genetically controlled trait.

Berchie *et al*. (2010) reported that the northern regions of Ghana are characterized with erratic rainfall patterns and higher temperatures, which are characteristic of semi-arid zones. These climatic conditions lead to shorter growing seasons and increased drought stress, resulting in a decreased number of pods per plant, suggesting that environmental stressors adversely affect pod development in this species.

Despite the importance of POD in assessing genetic diversity and yield potential, no known studies have specifically investigated this trait in farmer-held Bambara landraces from the Lake Victoria Basin (LVB). This represents a critical knowledge gap. Given the ecological variability of the LVB, documenting POD across landraces could provide valuable morphological markers for understanding genetic diversity and supporting conservation in this important agro-ecological region.

2.4.4.4 Seed Weight per Plant

Seed weight per plant is a vital yield component in Bambara influenced by a complex interplay of genetic, environmental, and agronomic factors. Studies have consistently shown considerable variability in seed weight among different genotypes, underscoring the crop's broad genetic base. For instance, Harry *et al*. (2023) reported significant variation in seed weight among eight Bambara accessions in Uyo which lies within the humid forest ecological zone, Nigeria, highlighting the genetic diversity present within the species.

Environmental conditions also play a pivotal role in modulating seed weight. In humid and sub-humid zones with adequate rainfall and fertile soils, seed weights tend to be higher. Xulu *et al*. (2024) observed seed weights ranging from 22.8 g to 79 g per plant, attributing these differences

to different environmental gradients across different zones. In their study, they attributed the seed weight differences to environmental factors such as temperature, soil type, and elevation. Conversely, in semi-arid regions, water stress during seed development has been associated with reduced seed weight. Abejide *et al*. (2018) found that under water-limited conditions, seed yield ranged from 6 to 28 seeds per plant, implying a likely corresponding decline in seed mass. This shows how seed weight is constrained in dry zones**.**

Soil quality, rainfall, and temperature are further recognized as major environmental determinants of yield traits. Bambara performs optimally in well-drained, sandy soils that facilitate underground pod development. Genotype × environment interactions have also been identified as significant contributors to seed weight variation, indicating the importance of matching genotypes to suitable agro-ecological conditions (Linus *et al*., 2023).

Hlanga (2020) conducted agro-morphological and genetic diversity analyses of 19 Bambara groundnut lines, revealing substantial differences in seed weight per plant, affirming the crop's immense genetic diversity and potential for selection of superior lines.

Despite this growing body of research, there remains a notable gap in knowledge regarding seed weight variation in farmer-held Bambara within the Lake Victoria Basin (LVB) of Kenya. No comprehensive morphological assessments targeting seed weight per plant have been reported in this region. Generating such data is essential, as it will contribute to a better understanding of the crop’s genetic diversity in the LVB and support the selection of high-yielding, locally adapted genotypes for breeding and conservation efforts.

2.4.4.5 Number of Flowers per Plant

Floral traits in Bambara are central to its reproductive development and play a direct role in pod and seed formation, which are critical determinants of yield. Among these traits, the number of flowers per plant stands out as a valuable morphological and agronomic trait, as it reflects the plant’s reproductive potential and may influence final seed output. However, not all flowers form pods due to factors such as flower abortion or failed fertilization, outcomes largely shaped by genotypic differences and environmental stressors including drought, high temperature, and soil nutrient limitations (Khan *et al*., 2021b).

The onset and quantity of flowers per plant are known to be sensitive to agro-ecological conditions such as photoperiod, altitude, temperature, and soil fertility (Mayes *et al*., 2019). These flowering dynamics vary across landraces and environments, making them important indicators of genotype adaptability and potential productivity under specific ecological zones. This highlights the utility of floral traits in evaluating genotype × environment interactions.

In controlled greenhouse environments, where external environmental fluctuations are minimized, flowering patterns tend to be more uniform. Such conditions provide a reliable baseline for assessing inherent genetic differences in floral traits, and when compared with field-grown plants across different agro-ecologies, can enhance our understanding of how genetic potential is expressed under variable conditions (Mayes *et al*., 2019). This comparative approach is particularly valuable for morphological characterization studies like the current one.

Multiple studies across sub-Saharan Africa have shown that flowering traits are useful proxies for genetic diversity in Bambara groundnut. For example, Kiryowa *et al*., (2022), in a study on 69 Bambara groundnut accessions in Uganda, observed variations in the number of flowers per peduncle (ranging from 1.2 to 1.4 across clusters), which signified distinct genetic groupings and adaptation strategies to different ecological settings. Similarly, Onwubiko *et al*. (2011) reported that the number of flower buds initiated per plant among different accessions ranged from 10 to 15, with some (e.g., Accession 02) demonstrating superior floral output, potentially linked to higher reproductive success.

Further emphasizing the impact of ecological factors, Xulu *et al*. (2023) found that flowering traits, including days to flowering, were significantly influenced by temperature, soil type, and altitude. These findings reinforce the necessity of contextualizing morphological traits within the ecological conditions under which they are expressed.

Gao *et al*. (2020) studied twelve Bambara groundnut genotypes and two F2 bi-parental populations, highlighting the extensive phenotypic variation in reproductive traits, including flowering characteristics. This variation was considered indicative of the crop's genetic resilience and potential to support food security in marginal environments, especially when matched to appropriate agro-ecological zones.

Despite this growing body of knowledge, a critical knowledge gap remains: no comprehensive study has yet assessed the number of flowers per plant among farmer-held Bambara landraces within the Lake Victoria Basin (LVB) of Kenya. Given the diverse climatic and edaphic conditions of this region, such information is crucial for evaluating the genetic diversity, adaptive capacity, and ecological suitability of local landraces. By systematically documenting this trait across landraces in both field and greenhouse settings, this study aims to contribute valuable insights for genetic diversity, conservation, and sustainable cultivation strategies in the LVB and beyond.

2.4.4.6 Terminal Leaflet Area

Terminal leaflet area (TLA) is a key morphological trait in Bambara, closely associated with photosynthetic efficiency, plant vigor, and overall yield potential (Olanrewaju *et al*., 2021b). The variability of this trait has significant implications for assessing genetic diversity and enhancing crop performance across diverse environments.

Previous studies have highlighted considerable variation in TLA among Bambara landraces. For example, Kiryowa *et al*. (2022) reported terminal leaflet lengths ranging from 52.3 mm to 68.2 mm and widths from 22.0 mm to 33.0 mm among 69 Ugandan accessions, underscoring the morphological diversity inherent in this crop. Similarly, Olanrewaju *et al*. (2021b), in a study involving 95 accessions across two agro-ecological zones in Nigeria, found that genotype× environment interactions significantly influenced leaf traits, including length and width, further emphasizing the role of environmental context in morphological expression.

Importantly, the relationship between TLA and yield has been supported by findings from Aliyu *et al*. (2016), who observed positive correlations between leaf area and yield components such as the number of pods per plant and seed weight. These observations affirm the potential of TLA as a selection criterion in breeding programs.

Greenhouse studies, in particular, provide a controlled setting where phenotypic variation in TLA can be more accurately attributed to genetic rather than environmental influences. As demonstrated by Mabhaudhi and Modi (2013), even under water stress, growth indices such as

leaf area index are responsive, reinforcing the utility of such environments for dissecting genotype-driven morphological expression.

Moreover, recent genomic analyses have identified specific loci linked to TLA, such as Vigun02g150200 and Vigun02g150500 (associated with leaflet length), and Vigun02g085200 and Vigun10g140300 (associated with leaflet width), providing molecular tools for diversity assessment and marker-assisted selection (Uba *et al*., 2023a). This genetic information complements morphological characterization and strengthens the case for integrating TLA data into breeding strategies and conservation planning.

Despite these advancements, no study has comprehensively assessed terminal leaflet area in farmer-held Bambara groundnut within the Lake Victoria Basin (LVB) of Kenya, an ecologically diverse region where understanding morphological variation could offer critical insights into the crop's adaptation and performance. Addressing this gap is essential, as such information will not only support efforts to assess genetic diversity within and between landraces but also guide the selection of ecologically adapted cultivars suited to both field and greenhouse environments.

2.4.5 Bambara Groundnut in the Lake Victoria Basin (LVB)

2.4.5.1 Status and Distribution

Bambara groundnut (*Vigna subterranea*) is predominantly grown by smallholder farmers within the Lake Victoria Basin (LVB), particularly in counties such as Kakamega, Vihiga, Bungoma, Siaya, Busia, Kisii, Kisumu, and Migori. These regions span diverse agro-ecological zones influenced by proximity to Lake Victoria, with high average temperatures of approximately 28.7°C and bimodal rainfall patterns, receiving around 1346 mm of precipitation annually (Lake Victoria Basin Commission and GRID-Arendal, 2017; Akuja *et al*., 2016).

2.4.5.2 Genetic Diversity and Landrace Conservation

In the LVB, the genotypes are distinguished on the basis of seed attributes such as seed testa colour and pigmentation around the eye (Ngugi, 1995). Most of the accessions have dark red, light red, black, brown or cream seed coats. Most Bambara accessions cultivated in the LVB are traditional landraces passed down through generations. Some documentation exists for counties like Busia, Kakamega, Bungoma, and Vihiga. For instance, 105 accessions from these counties,

and some from National gene bank, were analyzed molecularly, using Simple Sequence Repeat (SSR) markers, revealing low genetic diversity among farmer-held and genebank collections (Odongo *et al*., 2015). In these Counties, morphological characterization was based on seed testa alone, which is inadequate. However, there remains limited documentation for other key Bambara-growing areas in the Basin, specifically Kisumu and Migori Counties, despite their known involvement in Bambara cultivation.

This limited and uneven documentation presents a critical knowledge gap**:** the lack of systematic assessment of the crop's genetic diversity across different ecological zones within the LVB. Given the region's environmental heterogeneity, including differences in altitude, rainfall distribution, and soil characteristics, local landraces may have developed distinct adaptations. However, these variations remain largely unexplored. As such, there is an urgent need for comprehensive genetic characterization of Bambara landraces across LVB agro-ecological zones. This would not only support conservation efforts but also guide breeding programs aimed at improving yield stability, nutritional quality, and environmental resilience in the Basin.

2.4.5.3 Agronomic Practices and Production Trends

In the Lake Victoria Basin (LVB), Bambara groundnut is typically sown with the onset of the long rains in March and harvested around August, as short rain seasons are generally associated with lower yields (Ngugi, 1995). Cultivation practices vary, with farmers employing either row or random planting patterns. Intercropping with maize is common, though it often reduces Bambara yields unless spacing is carefully managed (Akuja *et al*., 2016). Monocropping is rarely practiced. The crop matures in about five to six months, with harvesting typically done manually once the foliage begins to senesce.

Despite its resilience and nutritional value, Bambara remains underutilized, with its production constrained by subsistence-level practices, inadequate agronomic knowledge, and limited land allocation. In counties like Busia and Kakamega, for instance, the average area allocated to Bambara per household is a mere 0.21 hectares, with poor yields largely attributed to low external input use and suboptimal management (Korir *et al*., 2011). Moreover, the cultivated area has been on a steady decline since the 1970s, influenced by shifting dietary habits, limited market access, and the unavailability of improved varieties (Masayi and Netondo, 2013).

These observations underscore a critical knowledge gap, the lack of comprehensive assessments on how agronomic practices and production trends vary across the LVB's diverse agroecological zones, and how these variations may influence or reflect the underlying genetic diversity of Bambara landraces. Understanding these dynamics is essential for designing targeted interventions that align agronomic performance with genetic potential, particularly in the context of climate-smart, sustainable intensification strategies. In this regard, evaluating the impact of monocropping systems, as opposed to traditional mixed-cropping practices, emerges as a vital consideration. Monocropping may provide insights into whether yield performance can be maximized by reducing interspecific competition and optimizing inputs for Bambara groundnut alone, thereby helping to determine its agronomic viability and contribution to regional food security.

2.4.5.4 Challenges and Future Outlook

Although pests and diseases are not a major limitation, post-harvest seed damage caused by bean weevils and cowpea bruchids (*Callosobruchus* spp.) reduces both storability and seed viability (Ngugi, 1995). More significantly, is the absence of improved or domesticated Bambara varieties for farmers in the Lake Victoria Basin (LVB), which hinders both productivity and adoption. The low production levels in the region are attributed to several factors, including the prevalence of calcareous soils unsuitable for many landraces, damage to flowers by moth beetles, leaf destruction by hailstorms, which limits photosynthesis and minimal input use (Akuja *et al*., 2016; Korir *et al*., 2011).

A major knowledge gap persists in the lack of ecological-zone-specific genetic diversity assessments of Bambara within the LVB. The genetic diversity study by Odongo *et al.,* (2015) was localized to Western Kenya Counties and do not reflect the full range of adaptive traits across the Basin's varied landscapes. Without this information, efforts towards elaborate assessment of genetic diversity, and crop improvement remain constrained.

Given the region's vulnerability to food insecurity, the high nutrient density and climate adaptability of Bambara groundnut make it a strong candidate for future food system resilience. To unlock its full potential, there is a pressing need for systematic germplasm collection in under represented LVB regions, and molecular and morphological characterization of accessions across

agro-ecological zones. These efforts would support genetic conservation, enhance crop improvement programs in the Lake Victoria Basin.

2.4.6 Yield and its component characters

2.4.6.1 Yield Production of Bambara Groundnuts in the Lake Victoria Basin

Despite its potential, Bambara groundnut production remains low in the Lake Victoria Basin (LVB), with limited comprehensive yield data available. Onyango and Ogolla (2020) noted that the Kakamega Cream accession exhibited promising yield traits; however, they did not report actual yield figures, underscoring the scarcity of quantitative yield information in the region. This gap is further emphasized by Akuja *et al*. (2016), who reported a maximum yield of only 310.84 kg/ha in a monocropping control trial. Yields were even lower under intercropping systems, dropping to as little as 38.80 kg/ha at a plant population density of 76,190 plants/ha with 45 cm spacing. Notably, most existing studies have focused on intercropping, with limited research dedicated to yield performance under monocropping conditions.

The issue of low production is also reflected in Wasula *et al*. (2014), who found that Bambara output in Western Kenya is too minimal to support structured marketing systems, largely due to the region's reliance on staple crops such as maize, beans, and sweet potatoes. Majola *et al*. (2021) similarly observed that Kenya is not listed among Africa's major producers of Bambara groundnut, highlighting its marginal production status. Korir *et al*. (2011) reported that although the crop is primarily grown in western Kenya, the average technical efficiency among farmers was only 38.4%, with just 13.79% achieving efficiency levels above 75%. The mean yield was approximately 63.42 kg/ha, far below the 300–850 kg/ha typically expected under traditional systems and significantly lower than the potential 3,000 kg/ha reported under optimized commercial cultivation (Gbaguidi *et al*., 2018; Berchie *et al*., 2016).

One of the critical agronomic constraints limiting Bambara productivity in the LVB is the poor adaptation of available landraces to the region's diverse agro-ecological environments (Hall *et al*., 2014; Ngugi, 1995). While Bambara is known for its overall hardiness, individual accessions often exhibit narrow environmental adaptability, leading to inconsistent yields across different growing conditions. Despite this challenge, very few studies have systematically assessed the yield performance of Bambara landraces in distinct agro-ecological zones within the LVB. Most

existing research, such as that by Akuja *et al*. (2016), has concentrated on intercropping systems or generalized yield outcomes, without isolating which specific landraces perform best in which environments under monocropping conditions.

This lack of localized adaptability data creates a significant knowledge gap that hinders efforts to improve Bambara productivity and uptake by farmers. Without clarity on which landraces are best suited to specific zones, farmers continue to rely on random selections from their collections, often resulting in suboptimal yields. To address this gap, the current study seeks to establish a clear linkage between specific landraces and the zones in which they perform best.

2.4.6.2 Yield of Bambara Groundnuts as Dependent on Seed Component Characters and Ecological Sites

Bambara groundnut yield is recognized as a complex, polygenic trait influenced by multiple interrelated components, majorly seed weight per plant and pod number (Oyiga and Uguru, 2011). Seed weight, commonly expressed as 100-seed weight or single seed weight, is a fundamental yield component in Bambara, with direct implications for biological productivity. Within the Lake Victoria Basin (LVB), where agro-ecological conditions range from lowland lake shores to mid-altitude inland zones, seed weight plays a vital role in shaping overall seed yield. Yield is typically determined by dividing seed weight by total number of plants harvested.

Seed weight is positively correlated with overall yield in Bambara groundnut. According to Khan *et al*. (2022a), traits such as dry seed weight show strong positive associations with yield per hectare, suggesting that selection for seed mass can be an effective strategy for yield improvement. Donkor *et al*. (2022) affirm that hundred seed weight exhibits high heritability and genetic variability, hence, often prioritized in breeding programs aimed at improving Bambara groundnut yield.

Research across similar agro-ecological zones in various parts of the world support the positive correlation between seed weight and total seed yield. For example, Khan *et al*. (2020) found a strong positive correlation ($r = 0.94$; $p \leq 0.001$) between dry-seed weight and yield in diverse landraces evaluated in Malaysia. Olanrewaju *et al*. (2021a) also found a strong positive

correlation (r = 0.86, $p < 0.001$) between seed weight and total yield in Nigeria, indicating its potential utility in selection for yield improvement.

Additionally, the number of pods per plant, especially those bearing one or two seeds, significantly influences total seed output, with reported correlations of $r = 0.62$ ($p < 0.01$), emphasizing the importance of pod number in yield determination (Olanrewaju *et al*., 2021a). Likewisely Khan *et al*. (2020) reported total number of pod had positive and highly significant association with yield ($r = 0.68$; $p \leq 0.001$). Plant height also shows a positive relationship with seed yield ($r = 0.24$; $p \leq 0.001$), suggesting that morphological vigor may translate into better reproductive performance (Khan *et al*., 2020).

Understanding these trait associations is crucial for Bambara groundnut improvement programs. Correlation analysis aids in identifying yield-determining components and guiding effective selection strategies (Oyiga and Uguru, 2011). Moreover, environmental conditions, particularly rainfall amount and distribution, are major factors influencing Bambara yield patterns (Werner, 2006). Multilocation trials in West Africa have shown that yield varies not only year-to-year but also across sites, highlighting strong genotype-by-environment interactions (Olanrewaju *et al*., 2021c).

Despite the wide genetic diversity within Bambara germplasm and the potential for breeding broadly adapted landraces (Karikari, 1996), yields remain inconsistent, even under favorable conditions. This variability has been attributed in part to heterogeneous growth and development among plants within landraces (Uba *et al*., 2023b), underscoring the need for more precise phenotypic and environmental matching.

However, while numerous studies have explored yield-component relationships in West Africa and in other parts of the world, there remains a distinct gap in such research within the Lake Victoria Basin (LVB), a region with unique agro-ecological dynamics. Understanding how yield components correlate under these specific local conditions is critical for guiding regionally adapted breeding and selection efforts. The lack of empirical data from the LVB limits the ability to develop robust, high-yielding cultivars tailored to this environment, thus presenting a key area for further investigation.

2.5 Protein Biomolecules in Bambara Groundnuts

Bambara groundnut (BGN) is a drought-tolerant legume valued for its high protein content, with reported concentrations ranging from 18.9–22.9% per 100 g (Mubaiwa *et al*., 2018). Traore-Barro *et al*. (2023), studying accessions from West Africa, primarily Burkina Faso, reported cultivar-dependent protein content is ranging from 9.6% to 40%. These differences are often influenced by genetic diversity and agro-ecological conditions, as well as protein estimation methods.

Bambara seed proteins include four major classes based on solubility: albumins (water-soluble), globulins (salt-soluble), glutelins (alkali-soluble), and prolamins (alcohol-soluble) (Silva and Dantas, 2016). These storage proteins, deposited during seed development, serve as vital amino acid reserves for germination (Alabi, 2023; Saminu *et al*., 2021). Their accumulation in cotyledon cells and their structural roles are comparable to seed proteins in other legumes. These proteins, second only to RuBisCO in abundance (Rasheed *et al*., 2020), are stored in protein bodies within vacuoles (Okpuzor *et al*., 2010).

Beyond nutrition, seed proteins are also indicators of genetic diversity. Previous research by Pasquet *et al*. (1999) using isozyme markers, enzymes that vary in amino acid sequence but perform the same catalytic function, revealed low overall isozyme diversity among 79 domesticated and 29 wild Bambara accessions. While useful, isozymes reflect only a subset of biochemical variation, and more refined approaches like SDS-PAGE protein profiling can provide deeper insights, especially across diverse agro-ecological settings.

Protein profiling via SDS-PAGE has been widely applied to characterize Bambara proteins. Chalid *et al*. (2015) used Tris-HCl buffer to extract proteins, identifying 14 bands with molecular weights ranging from 17 to 122 kDa. Arise *et al*. (2017) found four major bands in three Bambara landraces, with one broad band at 55 kDa and others at 62, 80, and 141 kDa. Saminu *et al*. (2021), analyzed six accessions from northern Nigeria, including Yobe and Kano states (semi-arid Sahel and Sudan savanna zones), using Bradford assay and SDS-PAGE. They identified consistent bands at 97.4, 45, and 18 kDa. Mukwevho and Emmambux (2024) reported bands at 118, 63, and 50 kDa in SDS-PAGE profiles of Bambara flour.

These studies demonstrate that protein composition and profiles are not only genetically determined but also influenced by environmental and agro-ecological factors. However, despite growing interest in Bambara in Africa, no comparable SDS-PAGE-based analysis has been conducted in Kenya, particularly in the diverse agro-ecological zones of the Lake Victoria Basin (LVB). Moreover, no documented research has evaluated the protein biomolecule diversity across farmer-held Bambara accessions grown in LVB agro-ecologies or under controlled greenhouse conditions. This represents a critical knowledge gap, as understanding how proteins vary across ecological zones can help in assessing genetic diversity of the Bambara, identifying adaptive traits and supporting varietal selection suited to specific environments within the LVB.

2.6 Climatic and Edaphic Factors Affecting Morphological Traits, Yield, and Protein Content

Climatic and edaphic factors play a fundamental role in shaping the growth, development, and productivity of Bambara groundnut. Variability in soil properties, such as texture, pH, and nutrient content, as well as climatic elements like rainfall, temperature, and elevation, significantly influence morphological traits, yield components, and protein profiles of different landraces. These environmental drivers are especially critical in the Lake Victoria Basin (LVB), where agro-ecological zones differ markedly in both soil characteristics and climate, thus exerting natural selection pressures that influence phenotypic expression and genetic adaptation over time.

Morphological variation in Bambara groundnut is closely linked to environmental conditions. For instance, landraces grown in loamy soils often exhibit better vegetative growth and higher pod yield due to superior nutrient and water retention, while those in sandy soils show reduced plant height and lower yields (Korir *et al*., 2011). Soil pH between 5.0 and 6.5 is optimal, whereas lower values, especially below 4.5, are associated with stunted growth and poor reproductive output (Majola *et al*., 2021; Akuja *et al*., 2015). Similarly, high nitrogen levels promote vegetative growth at the expense of pod formation, leading to imbalances between biomass production and reproductive success (Khan *et al*., 2024).

Climatic conditions further shape the phenotypic plasticity and performance of Bambara groundnut. Optimal elevations (1100–1600 meters above sea level) and average temperatures

ranging from 20°C to 28°C support balanced vegetative and reproductive growth (Majola *et al*., 2021). Rainfall plays a particularly pivotal role: moderate precipitation (600–750 mm) (Amwenyo *et al*., 2025) during the growing season enhances plant vigor and yield, while excessive rainfall during flowering and pod-filling stages negatively affects flower development, seed setting, and germination rates (Mabitsela *et al*., 2024). Conversely, under rain-fed conditions, drought stress can induce earlier flowering and shorten growth duration, often resulting in reduced plant stature and pod number (Mabhaudhi and Modi, 2013).

In addition to influencing morphology and yield, these environmental conditions also affect the nutritional quality of Bambara groundnut. Soil fertility and climatic stressors influence protein synthesis and accumulation in seeds, resulting in significant variation in total protein content and the proportions of different protein fractions (albumins, globulins, glutelins, and prolamins) among landraces (Traore-Barro *et al*., 2023).

Despite existing research, localized data on how climatic and edaphic factors influence these traits in the LVB is limited. This gap hinders our understanding of genotype-by-environment interactions and the adaptive capacity of local landraces. To address this, it is essential to conduct detailed soil analyses and systematically collect weather data across the studied agro-ecological zones and controlled greenhouse environments.

Collecting comprehensive environmental data will enable a nuanced interpretation of the observed phenotypic, agronomic, and biochemical differences, thereby enhancing our understanding of the environmental drivers of Bambara groundnut performance in the LVB.

2.7 Phenetic Classification

Despite its established role in systematics, phenetic classification remains underexplored in the study of trait diversity in underutilized crops such as Bambara groundnut. Phenetics is a method that classifies organisms based on overall similarity across observed characteristics, without prioritizing specific traits (Sokal, 1986). It begins with the identification of operational taxonomic units (OTUs) which, in this study, are Bambara groundnut landraces, and utilizes diverse data sources, including morphological and biochemical characteristics (Stuessy, 1990; Singh, 2009).

The procedure involves independently coding the selected traits, calculating similarity coefficients such as Jaccard's, Dice, Euclidean, or simple matching (Finch, 2005), and using clustering algorithms to construct dendrograms that visually represent groupings based on similarity (Manoko, 2018). Each dendrogram shows clusters of landraces that are more similar to each other than to those in other clusters.

A significant gap in the literature lies in the limited application of phenetic methods to separately classify the diverse trait sets of Bambara groundnut, specifically qualitative morphological traits, quantitative morphological traits, and protein profiles. Most previous studies tend to focus on a single trait category or utilize other classification approaches, leaving the phenetic potential to comprehensively explore trait-specific variation in Bambara groundnut largely untapped.

This study addresses that gap by applying phenetic classification independently to: qualitative morphological traits of Bambara groundnut, quantitative morphological traits of Bambara groundnut, and protein types and profiles of high-yielding Bambara groundnut landraces.

By analyzing these trait sets separately, the study will generate distinct phenetic groupings that reflect the variation and similarity among Bambara landraces grown across different agro-ecological zones and under greenhouse conditions. This approach will contribute to a more nuanced understanding of how phenotypic and biochemical traits are influenced by genetic and environmental factors, thereby informing targeted breeding, selection, and conservation of Bambara groundnut.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Area

Bambara to be used for this study were collected from ten randomly sampled farmers in the Lake Victoria basin region counties of Busia, Kisumu and Migori. Upon collection, the seeds were sorted out based on the accession and air-dried further to minimize dampness. The seeds were grown at four sites, KALRO-Alupe research-station in Busia County, KALRO - Kibos research station in Kisumu County, KALRO- Oyani research station in Migori County and at Maseno University research farms (greenhouse) within the diverse agro-ecological regions of Lake Victoria region (Figure 1). The selection of study site was by purposive sampling based on the fact that Bambara production in Kenya has been limited to former Western Province and to a lesser extent, former Coastal and Nyanza provinces (Wasula *et al.,* 2014).

KALRO - Kibos research fields are found in Kisumu County and occur at longitudes of 34^0 24”13’E and latitudes of 00^0 03” 01’S, within the lower midland ecological zone (LM 3) with heavy black clay soils (vertisols), fairly typical of the Kano plains (Jaetzold *et al*, 2009). The research fields lies at an altitude of 1173m above the sea level, and the mean annual rainfall is 1419mm (Mutembei *et al*., 2019) and temperature ranges between 16-31^{0}C (Jaetzold *et al*, 2009.

KALRO-Oyani research fields occur within the lower midland ecological zone (LM 2), at longitudes 34^0 28” 00’E and latitudes 01^0 04” 00’S. Soils of Oyani in Migori County are classified as andosols, being well drained, moderately deep, firm, clay soils on the valleys and poorly drained, deep, dark grey, brown, firm, clay soils in valley bottoms (Jaetzold *et al*, 2009). Altitude ranges between 1135-1200m above the sea level with mean temperatures of 22.7-23.3^{0}C. Annual rainfall ranges between 900-1100 mm and is bimodal with much (400-480mm) of it falling in the first season between March and May. KALRO-Alupe Sub-Station in Busia County is a low rainfall zone. The site receives annual rainfall of between 680.5 - 860mm, with daily temperature range of 16^{0}C-34^{0}C (Sikuku *et al*, 2015). The Alupe research fields are located on longitude 34^0 7” 50’E and latitude 0^0 30” 0 N, at an elevation of 1170 meters above sea level (Sikuku *et al*., 2015). The soils at Alupe have been characterized as ferralo-orthic Acrisol (FURP, 1987).

Maseno University Research farm is located within Maseno University, Kisumu County. The research farm lies at a latitude of S 0º 00' 19.8" and longitude of E 34º 35' 49.56" at altitude of 1500 m. Maseno area receives annual rainfall of about 1346 mm per year (Omondi, 2014) and the average temperature of Maseno University is 28.7^{0}C (Mwai *et al*., 2001).

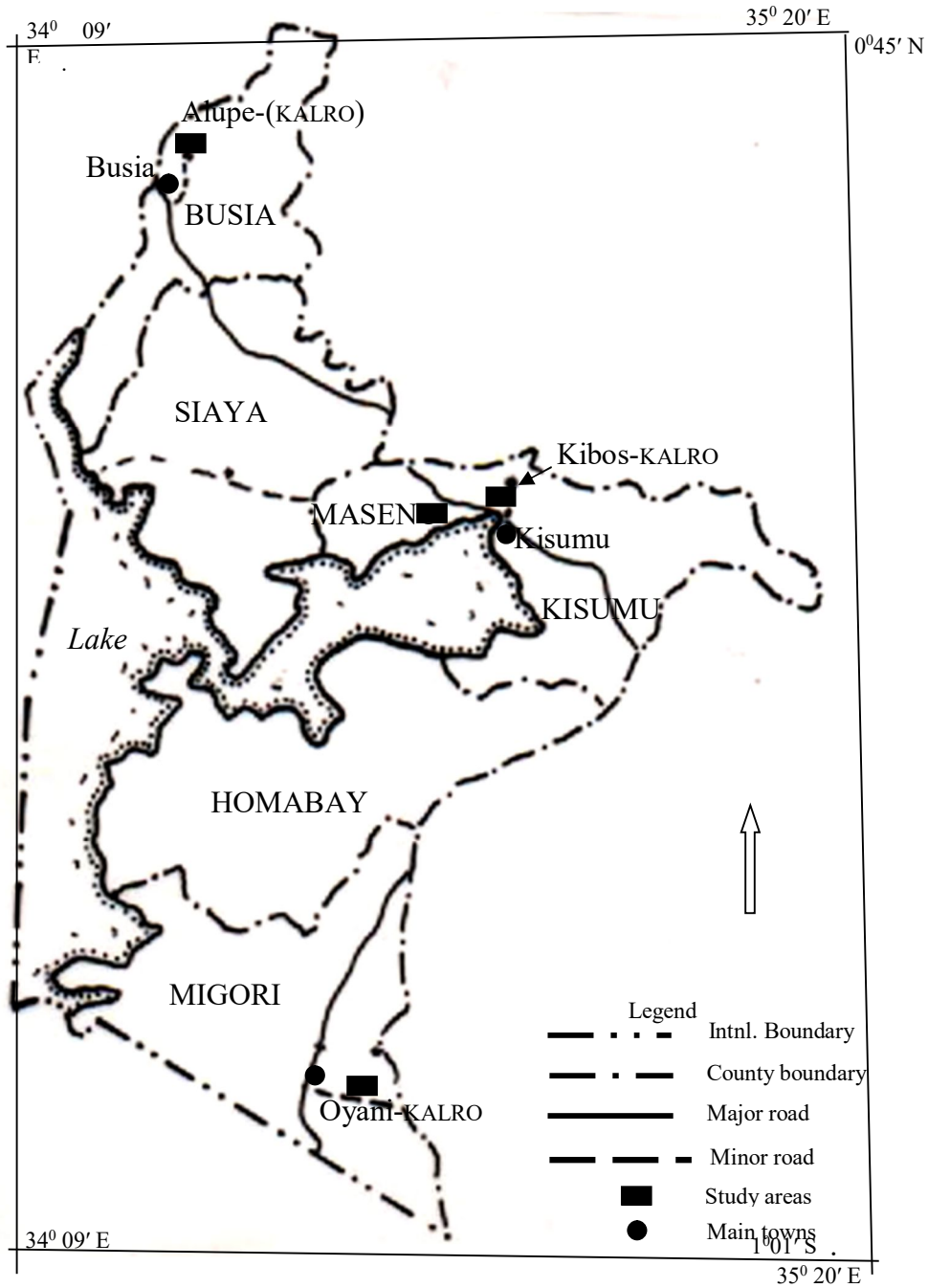


Figure 1: Map of Lake Victoria Basin showing study sites.
(Courtesy of Department of Geography, Maseno University, 2018)

3.2 Field Work and Field Data Collection

3.2.1 Collection of Germplasm for Planting

Bambara accessions used for this study were collected from ten randomly sampled farmers in the Lake Victoria Basin region Counties of Busia, Kisumu and Migori between October and December 2018, just two months after the harvesting period. The selection of the three counties was through purposive sampling as the Bambara is reported to be grown in the three counties (Onyango *et al*., 2015). Four farmers were sampled from Busia, whereas three farmers each were sampled from both Kisumu and Migori (Table 1).

Table 1: Socio-demographic table of Bambara groundnut farmers, in the selected Lake Victoria Basin counties of Western Kenya

Accessions	County	Sub - county	Exact Locality of collection	No. of Farmers per locality	No. of Farmers per county	Gender	Age
K2	Kisumu	Kisumu West	Holo	1	3	F	20-34
K1	Kisumu	Nyando	Ahero	1		F	>50
		Kisumu West	Holo*				
		Kisumu East	Kajulu	1		F	>50
B4	Busia	Matayos	Busende	1	4	F	35-49
		Butula	Bumala	1		M	>50
			Busire	1		F	>50
		Teso South	Amoni	1		F	35-49
B1	Busia	Matayos	Busende*				
		Butula	Bumala*				
M4	Migori	Suna East	God Jope	1	3	M	>50
			Anjego	1		F	35-49
M2	Migori	Uriri	Bware	1		F	>50

Key: 20-34=youthful farmers, 35-49= middle aged farmers, >50 elderly farmers
*means the collection locality similar for both accessions from same county. F (Female), M (Male).

In Busia more farmers were sampled because Busia County has the largest number of Bambara accessions at thirty-nine as compared to any other western Kenya county (Odongo *et al.,* 2015). The targeted farmers had to be farmers who had planted the Bambara on one or more acres of land. Germplasm selection was based on the germplasm within the farmers' possession as at the time of collection. From each farmer, four kilograms of the Bambara were collected. Upon collection, the seeds were sorted out based on the accession and air-dried further to minimize dampness and attain a longer post-harvest life. A total of six Bambara accessions (Table 1.) were collected and identified at East African Herbarium, Nairobi.

3.2.2 Land Preparation

Field preparations and evaluation of the accessions was done in the three agro-ecological sites at KALRO Alupe, Kibos and Oyani (Figure 1). At each planting site, an area of 17m by 8m was selected and prepared. The acreage of land prepared depended on the number of accessions collected from farmers which were six in number. Field preparations were done according to Brink *et al.,* (2006), where manual groundbreaking was done after clearing the land. Tillage operations involved loosening the soil to a depth of approximately 15 cm (Eifediyi *et al*., 2020) to support effective root penetration and pod development. Hillocks *et al*. (2012) emphasize that the soil should be well-drained and deep enough for root growth. The soil was subsequently harrowed twice upto a depth of 15cm to improve tilth, as Bambara performs best in light-textured soils (Brink *et al*., 2006). Proper soil loosening also facilitates pod burial during fructification.

3.2.3 Soil Sampling Procedures

3.2.3.1 Collection of Composite Samples

Soil sampling was done to test for the soil pH and soil nutrient composition prior to planting and after harvesting in order to access level of various nutrient uptake by the Bambara, and hence their influence on performance of Bambara morphological traits, yield and protein contents in various zones. In each study site, soil samples were obtained by zig-zag sampling techniques using conventional soil sampling tools of soil auger and garden trowel. Each farm was surveyed then marked into fields depending on observable variations such as soil colour, slope and topography. Soil sampling was done by taking courses of soil from the experimental fields from a depth 0-25 cm using soil auger and avoiding under trees (Appendix 1). The courses were then

transferred and mixed together in a trough using a trowel. Composite samples were made from between eight to twelve courses by quartering method. Samples of about 500g were packed and stapled in brown sampling bags, marked with the plot name, county, date of sampling using marker pens, then taken to KALRO-Alupe soil laboratory for analysis.

3.2.3.2 Soil Sample Processing

The soil processing was done according to the method described by Anderson and Ingram (1993) and it involved four major processes;

3.2.3.2.1 Drying

In the laboratory the samples were air-dried by leaving them in the sample bags for one month, and by turning them with hand twice in a day until they were fully dry. The samples were kept in the bags throughout to minimize contamination.

3.2.3.2.2 Grinding

This was done by taking portions of samples from the sample bags and putting in a mortar and crushing using the pestle until it was coarsely finer.

3.2.3.2.3 Sieving

This was done using a soil sieve with 2mm mesh. The sieved sub-sample was put in a zip-lock plastic bags and stored ready for analysis.

3.2.3.2.4 Extraction

The sub-samples were divided into 2 portions; one portion was used in the determination of pH and the remaining portion was used for further nutrients analysis as the plant yield is reported to be affected by soil nutrients and soil pH (Majola *et al*., 2021).

3.2.3.3 Soil Sample Analysis

3.2.3.3.1 pH Analysis

Soil pH was determined according to the method described by McLean (1982).

Using calibrated analytical balance, 10g sample was accurately weighed on Aluminium foil, transferred into a 100cm^3 plastic shaker bottle and dissolved in 25cm^3 of deionized water. This was clamped onto a horizontal mechanical shaker and shaken for 5 minutes at 100 revolutions

per minute after which it was made to settle for one hour before reading the pH using a bench calibrated pH-meter.

3.2.3.3.2 Total Nitrogen Analysis

Total Nitrogen Analysis was done according to a method of Bremner (1965), with slight modifications in quantification using a Vapodest Nitrogen Analyzer. 1g sample of soil was weighed in a clean Aluminium foil, transferred into glass digestion tubes and calculated amount of extractant added and digested for 1hour at 300^0C. The digest was cooled and filtered. The clear extract was fed into Kjeldahl Vapodest Nitrogen Analyzer which gave readings in percentage total Nitrogen in soil sample.

The analytical procedures of phosphorus, calcium, potassium and magnesium were done according to Mehlich 3 Chemical Methods of Analyses (Mehlich, 1984).

3.2.3.3.3 Analysis of Phosphorous

1g sample of soil was weighed in a clean Aluminium foil, transferred into glass digestion tubes and calculated amount of extractant added and digested for 1hour at 300^0C. The digest was cooled and filtered. The clear extract was put into sample Cuvettes and inserted into the Uv-Vis Spectrophotometer that gave readings in Concentration of Phosphorous in parts per million (ppm) against absorbance values for the wavelength range within which Phosphorous (400nm) absorbs light.

3.2.3.3.4 Analyses of Potassium, Calcium and Magnesium

1g sample of soil was accurately weighed, put into100cm^3 plastic shaker bottles, mixed with extractant and clamped to shake in the horizontal mechanical shaker at 100 revolutions per minute for one hour. The extract was allowed to settle for one hour then filtered. The clear filtrate was fed to sample vials of the Auto sampler of the Atomic Absorption Spectrophotometer. The concentration values were given in readings in percentage against absorbance values of the specific elements (Magnessium 285.2nm, Calcium 422.7nm and Potassium 766.5nm).

3.2.4 Microclimate of the Zones

The temperature and rainfall amounts received in the zones during the growing season (April 2019 – August 2019) were collected and recorded inorder to access their influence on the performance of morphological quantitative traits, yield and protein contents in various zones.

During the experimental period, weather parameters were continuously monitored using automatic weather stations (AWS-GP1, Delta-T Devices, Cambridge, UK) installed in each of the KALRO sub-stations in an open area to avoid interference from trees. Rainfall and temperature parameters were continuously monitored. Measurements were taken monthly during the growing period, with daily rainfall per month summed to get total monthly rainfall. Diurnal temperature ranges were also recorded. Daily minimum temperatures were averaged to obtain the monthly minimum temperature, and daily maximum temperatures were averaged to obtain the monthly maximum temperature.

3.2.5 Bambara Groundnut Planting and Field Layout

Planting in the three field sites was done in early April 2019 at the onset of long rains and not in March as normally is the case. This was as a result of delayance in the onset of rainfall due to the erratic weather patterns experienced in recent years.

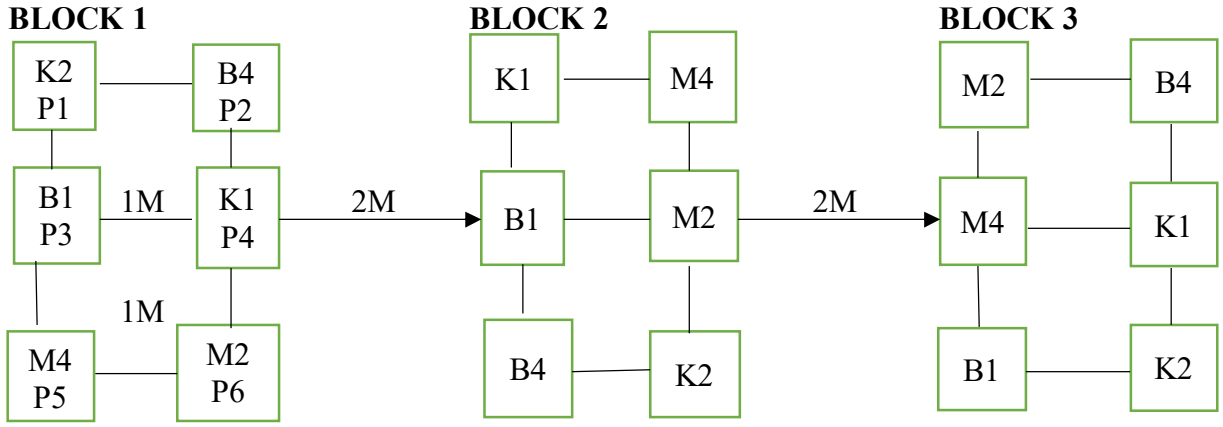


Figure 2: Landraces layout in field experiments

Key: P stands for plot number.

Each accession was planted in a five-row, three-replicate plot. Each zone had three blocks with eighteen experimental plots (Plate 3). A randomized complete block design was used (Appendix 2), with accessions as the key factor. The field layout for each accession covered 1.44 m², with

rows measuring 1.2 m in length, 30 cm inter-row spacing, and 20 cm intra-row spacing, with one plant per hill (Figure 3). Two seeds were sown per hill, with holes for dibbling Bambara seeds dug to a depth of 3 cm using a hoe (Nweke and Emeh, 2013). The seedlings were thinned to one plant per hill at 21 days after planting, giving a plant population of about 35 plants per plot. Rows 1 and 5 were guard rows and therefore plants from these rows were not used in sampling. All the twenty border plants were not used in sampling; hence only fifteen plants were available, of which ten were sampled in the study. Individual plots within a block were separated by 1m, while the blocks were separated from each other by a distance of 2m (Figure 2). The plants were weeded manually using hoe and to keep weed pressure low, at 3 weeks, 5 weeks and 8 weeks after emergence. Earthing up to cover the young pods was done with a hoe at 3 weeks and 8 weeks after emergence as this improves yield, but is labor intensive (INERA, 2012). To control insects, alpha-cypermethrin 10% EC (Sikko Industries Ltd, India) at a rate of $2ml1^{-1}$ was used two weeks after emergence. To control fungal diseases, dithane M-45 (Greenriver Industry Co., Ltd, China) was applied at the rate of $3g\ 1^{-1}$ at 4 weeks, 6 weeks and at 8 weeks after planting, using knapsack sprayer.



Plate 3: Bambara groundnut field established crop stand at Kenya Agricultural Livestock Research Organization (KALRO) Kibos farm, Kisumu County.

No fertilizer application was made as the research endeavored to establish which accession produces the highest yield in the natural agronomic environments. Bambara is also known to do

well with little fertilizer input as its nitrogen requirement is met by natural biological nitrogen fixation (Ajilogba *et al*., 2022) since Bambara requires between 80-150 kg N ha^{-1} during its growing period, yet it can fix approximately 4-200 kg N ha^{-1} (Zoundji *et al*., 2022). Harvesting was done manually at physiological maturity, after about 5 months in August during dry season when the entire foliage dried up or turned yellow (Mapeli, 2020) and pods started to dry up. The Bambara at KALRO Oyani senesced first and was harvested in early August. Harvesting at KALRO Alupe took place in mid-August, while at KALRO Kibos, it was done at the end of August.

3.3 Green House Work

3.3.1 Preparations in the Greenhouse

Greenhouse trials were integrated into the study to complement field evaluations and serve as a controlled reference environment. While field experiments across different agro-ecological zones captured the effects of environmental variability on morphological traits, yield performance, and protein composition, greenhouse conditions offered a standardized setting by minimizing confounding factors such as rainfall variability and soil heterogeneity. This controlled environment facilitated a more accurate assessment of the inherent genetic differences among Bambara landraces.

A suitably large greenhouse located within the University research farm, capable of accommodating all the accessions, was utilized for the trials. Soil for the experiment was sourced from a designated area within the farm, thoroughly mixed, and screened to remove stones and debris. The prepared soil was then subjected to pH and nutrient analysis, following the same procedures used in the field experiments.

3.3.2 Experimental Design and Planting

Planting was done in March, concurrent to planting time in the field experiments. The experimental design was randomized complete block design with three replications (Figure 2) similar to that used in the fields. The factors were six Bambara accessions and water.

About 15kg of soil sample was filled in each of the 20 L PVC pot (Ø 30cm and 30cm height). Two seeds of each landrace were planted at a recommended depth of 3cm. The pots were arranged in rows separated from each other, 1m by 1m apart. Within the rows, pots were

arranged 30cm apart. The irrigated and droughted pots were separated by 2m. The landraces were under two moisture regimes (fully irrigated and droughted) to investigate their growth response to soil moisture since various researches report soil moisture to be a key agronomic factor that affects both phenotypic characters and yield (Azam-Ali *et al.,* 2001; Werner, 2006). Drought-stressed plants were irrigated with 0.5 litres of water per plant daily for the first four weeks to establish seedlings, after which irrigation was withheld. Fully irrigated plants received 0.5 litres of water per plant daily until 42 days after sowing (DAS), when 50% flowering was achieved. Thereafter, they were watered with 1 litre per plant twice weekly (on Sundays and Wednesdays) until physiological maturity, marked by complete drying of the foliage. Irrigation was done as the plant does well in moderate water environments (Werner, 2006) and this was to minimize water stress in the plant. The watering was done at 6pm to minimize loss of water through evaporation. The daily mean greenhouse temperature was maintained at 29^0C with diurnal amplitude of $\pm5^0$C (Ombima, 2016), since average temperature of Maseno University is 28.7^0C (Mwai *et al*., 2001).

In both the set ups, the seedlings were thinned to one plant per pot 21 days after sowing (DAS), hence only a single plant developed to maturity. All the other agronomic practices (weeding, earthing up, insecticide and fungicide applications) were done similar to those in the field.

Harvesting of irrigated plants was done manually at physiological maturity, after about 5 months at the end of August when the entire foliage dried up or turned yellow and pods started to dry up. Basically, the droughted plants senescenced earlier than the irrigated plants.

3.4 Data Collection

3.4.1 Morphological Characterization

A sampling matrix was designed to enhance efficiency in the collection of data in the field (Figure 3) and to ensure selection was non-biased, random and to avoid border effects.

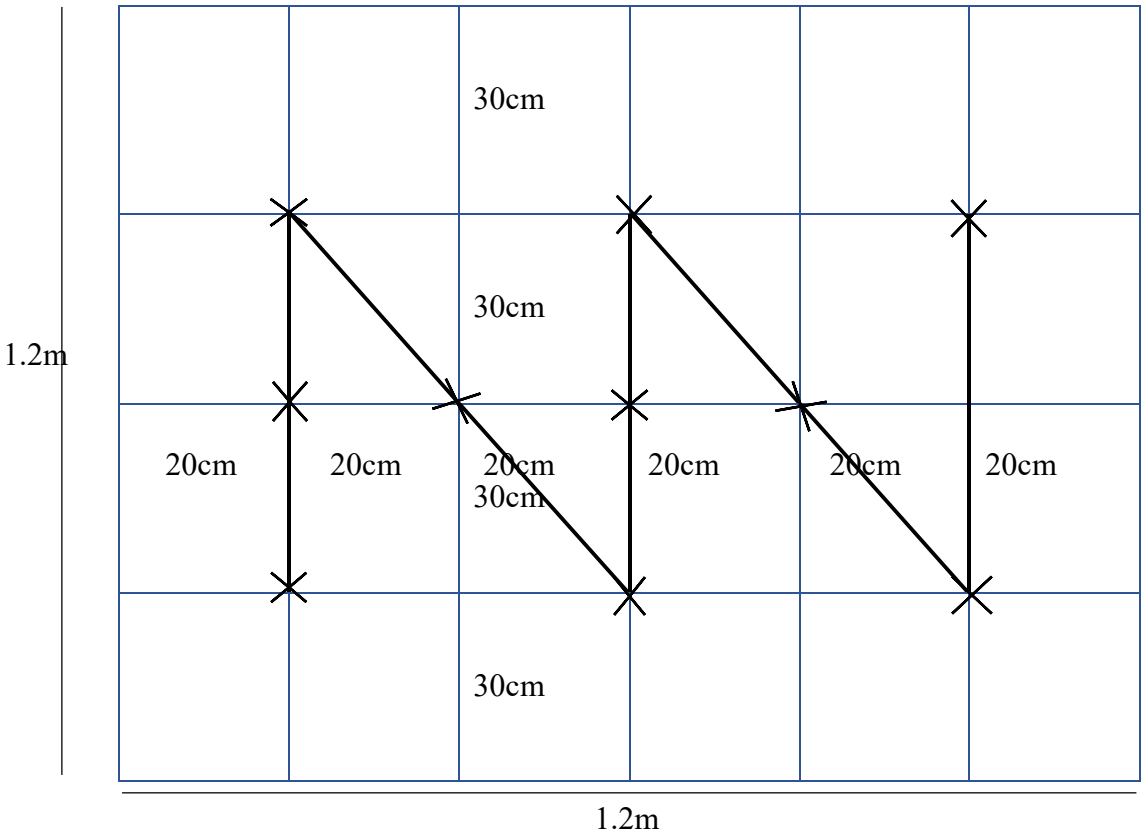


Figure 3: Sampling Matrix

Morphological characterization of all the pod and seed traits were recorded two months after harvesting, as recommended by IPGRI/IITA/BAMNET (2000) while the terminal leaflet traits characterization were done in the tenth week after planting (WAP) at the fourth node. Seven qualitative characters related to leaves, pods, and seeds such as Terminal Leaflet shape (TLS), Pod Colour (POC), Pod Texture (POT), Pod shape (POS), Seed Eye Pattern (SEP), Seed Testa Pattern (STP) and Seed Testa Colour (STC) were evaluated using the six landraces harvested from experimental plots.

The qualitative characters were scored using ten simple randomly selected plants per plot. The standard descriptor list of *V. subterranea* edited by the International Plant Genetic Resources Institute (IPGRI/IITA/BAMNET 2000) was used as a guideline in these phenotypic characterization exercises as described in Table 2.

Table 2: Some descriptors and classes of qualitative traits of Bambara groundnut Source: (IPGRI/IITA/BAMNET 2000)

Descriptor and Classes	Classes
Terminal leaflet shape (TLS)	1=Round 2=Oval 3=Lanceolate 4=Elliptic 99=Other
Pod Colour (POC)	1= Yellowish-brown 2= Brown 3= Reddish-brown 4=Purple 5= Black 99= Other
Pod Texture (POT)	1= Smooth 2=Little grooves 3=Much grooved 4= Much folded
Pod Shape (POS)	1=Without a point 2=Round on the other side 3=With nook on the other side 4=Two points on each side 99=Other
Seed Eye Pattern (SEP)	0=No eye pattern 1=Black butterfly-like eye 2=Dark red butterfly-like eye 3= Grey-butterfly like eye 99=Other
Seed Testa Pattern (STP)	0= No testa pattern(plain) 1= Black small dotted spots 2=Dark brown small dotted 3=Black and grey mottles 4=Black and Brown mottles 5=Black marbled spots on cream background 99=Black marbled spots on brown background
Seed Testa Colour (STC)	1)Cream 2)Grey 3)Light red 4)Dark red 5)Light brownish red 6) Dark brown 7) Dark purple 8) Black 99) Other

Seven quantitative characters such as plant height at maturity (PHT, cm), number of pods with 1 seed (N1S), number of pods with 2 seeds (N2S), Number of pods per plant (POD), Terminal Leaflet Area (cm^2), Number of flowers per plant(NFP) and Seed weight per plant/ (SWT g) were also evaluated. The traits were recorded as per the Table 3 below.

Table 3: Quantitative traits of Bambara groundnut recorded and brief description as listed from IPGRI/IITA/BAMNET (2000)

Characters	Characters and description
Plant height at maturity (PHT, cm),	Measured from the ground level to the tip of the highest point, recorded at 10 WAP.
Number of flowers per plant(NFP)	Average of 10 plants recorded per plot recorded from the onset of flowering period up to about eighty days after sowing.
Number of pods with 1 seed (N1S)	Average of 10 plants recorded per plot recorded within two months of harvest.
Number of pods with 2 seeds (N2S),	Average of 10 plants recorded per plot recorded within two months of harvest.
Number of pods per plant (POD),	Average of 10 plants recorded per plot recorded within two months of harvest.
Seed weight (yield) per plant/ (SWT g).	Average weight of seed from 10 plants taken within a plot after drying, within two months of harvest.
Terminal Leaf Area (cm^2),	Multiply leaflet width X length and number of leave(s) and use a formula in equation 4 below At 10WAP

According to IPGRI/IITA/BAMNET (2000), plant height at maturity was measured using a ruler in centimeters from the shoot region just above the soil level to the shoot tip. Number of flowers per plant were counted from the onset of flowering period (between 35-48 DAS) up to when flowering started subsiding (at about eighty days after sowing) (IPGRI/IITA/BAMNET., 2000); Masindeni, 2006). Number of pods per sampled plant were counted and recorded (IPGRI/IITA/BAMNET., 2000).

The number of pods per plant were then air dried. After air drying, during shelling, the number of pods with 2 seeds (N2S) and with I seed (N1S) were counted and recorded (IPGRI/IITA/BAMNET., 2000). After shelling the pods, the dry seeds were then dried further in an oven (Carbolite high temperature laboratory oven PF60 Model) at 110^{o}C until a constant weight was obtained (Ndidi *et al.,* 2014). The mass of oven dried seeds were determined using electronic weighing balance (CS200 Ohaus Compact scale) and recorded. Seed weight per plant was calculated by dividing the total seed weight (g) from ten plants per plot by ten, representing the number of harvested plants (IPGRI/IITA/BAMNET., 2000).

Being trifoliate, and for consistency purposes, non-destructive leaf area assessment was determined using the leaflet length and leaflet width measurements on median (terminal) leaflet at the fourth node recorded at 10 WAP. Leaflet length per plant in centimeters was measured from median leaflet apex to base of petiole. Leaflet width per plant was measured in centimeters across the median leaflet lamina at the widest area (IPGRI/IITA/BAMNET., 2000).

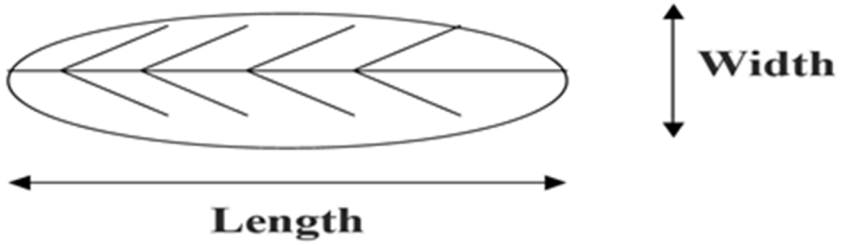


Plate 4: Length and width measurements of a Bambara groundnut leaflet

The terminal leaflet length and the terminal leaflet width was used in calculating the terminal leaflet area.

Plant Terminal leaflet area ($\mathbf{A_{plant}}$) was measured using the model assumptions of Deswarte (2001) and Cornelissen (2005) as described below:

The Bambara groundnut has leaves with a shape very close to an ellipse (plate 3)

Area for the ellipse: $\mathbf{A = L * W * \pi /4}$…… **Equation (1)**

Equation to estimate the leaflet area: $\mathbf{A = \sigma * L * W * \pi /4}$……**Equation (2)**

Where: **L** = Length of the leaflet (cm), **W** = Width of the leaflet (cm), $\boldsymbol{\pi = 3.1416}$ and

$\boldsymbol{\sigma}$ = correction factor (to account for the difference between the actual shape and an ellipse)

The size of the lateral leaflets is usually closely related to the size of the middle leaflet. The plant leaf area is a function of the leaf number and the single leaf area. Bambara groundnut has trifoliate leaves (see plate 1), although these three leaflets have the same shape, they do not always have the same dimensions or leaf area. In order to compensate for these differences an extra parameter $\boldsymbol{\tau}$ is added to Equation 2:

$\mathbf{A = \tau * 3 (\sigma L * W * \pi /4)}$…… **Equation (3)**

The step to leaf area of the whole plant seems to be simply multiplying Equation 3 with the total number of leaves ($\mathbf{N_1}$); however, an extra parameter ($\boldsymbol{\upsilon}$) needs to be added to compensate for inaccuracy in sample methods. Young not fully unfolded leaves and leaves that look significantly

smaller than others are rejected. This can lead to under or overestimation of the leaf area. The equation becomes:

A_{plant} = υ * N_1 [τ * 3 (σ * Length * Width * π /4)]…….. Equation (4)

Deswarte (2001) calculated landrace independent values for **σ**, **τ** and **υ**, given in (Table 4) and the relation between actual and estimated leaf area.

Table 4: Landrace independent values for calculating Bambara groundnut leaf area

Model Parameters	Landrace independent values
σ	0.95
τ	0.91
υ	0.86
R^2	90.90%

Source: Deswarte (2001)

For practical purposes, equation 4 can be simplified by subsuming **σ**, **τ** and **υ** into **δ (δ=0.74):**

Leaf Area **(A_{plant}) = 0.74 * 3 * N_l (leaflet length * W * π /4)**

This equation has been used by several plant researchers in their estimation of Bambara leaf area. Some of these researchers include Molosiwa, (2012), who applied it in glasshouse experiments in the UK and by Ombima, (2016), in greenhouse experiments at Maseno University, Kenya.

In the greenhouse, irrigated and drought-stressed plants from each of the three replicates underwent morphological characterization, using the same qualitative and quantitative procedures as in the field. Analyses were based on three plants per accession consistent with field methodology.

3.4.2 Data Analysis for Morphological Characterization

3.4.2.1 Data Analysis for Qualitative Morphological Characters

The phenotypic diversity of the accessions both in the field and in the greenhouse were computed using the frequency distribution (Khan *et al*., 2021b), of agronomic data on the qualitative morphological characters of the landraces (such as Terminal leaflet shape at maturity (TLS), Pod colour (POC), Pod Texture (POT), Pod shape (POS), Seed eye pattern (SEP), Seed Testa pattern (STP), Seed Testa colour (STC) using the formula;

$$f_i = \frac{n_i}{N} = \frac{n_i}{\sum_i n_i}$$

Where *fi* is the frequency, *ni* is the number of accessions with a particular trait, and *N* is the total number of accessions under study. Their percentages were computed using SPSS package version 21, to show how diverse the accessions were phenotypically.

The Trait Diversity Index of the qualitative characters accessed both in the field and in the greenhouse plants were estimated using the Shannon-Weiner diversity index (H') (Olukolu *et al*., 2012) to show how diverse the phenotypic qualitative traits were. The estimation was based on the phenotypic frequencies for each trait. The Shannon-Weiner diversity Index formula;

$$-\sum_{i-1}^{s}(PilnPi)$$

was used where:

Pi is the proportion of accessions (*n/N*) of individuals of one particular character found (*n*) divided by the number of individuals found (*N*), multiplied by the natural logarithm (ln) summation of the calculation, and *s* is the number of traits. The diversity index was done using R statistical software version 3.3.1. Phenotypic similarity based on seven qualitative traits was assessed across all six accessions.

To determine similarity and putative relationships among the six Bambara accessions, cluster analysis was performed. Qualitative data were nominalized, and similarity indices were calculated using the Euclidean distance method. Hierarchical clustering was conducted using the complete linkage method, and a dendrogram was generated with R software version 3.3.1 (R Development Core Team, 2016).

3.4.2.2 Data Analysis for Quantitative Morphological Characters

Quantitative morphological traits in both field and greenhouse plants, including plant height at maturity (PHT, cm), number of pods with one seed (N1S), number of pods with two seeds (N2S), total number of pods per plant (POD), terminal leaflet area (cm^2), number of flowers per plant (NFP), and seed weight per plant (SWT, g) were analyzed. Analysis of variance (ANOVA) was conducted to compare intra- and inter-site differences, followed by Tukey's multiple comparisons test. All analyses and error bar plots were generated using GraphPad Prism (version 8.0.2).

Quantitative morphological traits were further analyzed using Principal Component Analysis (PCA) in R software version 3.3.1 (R Development Core Team, 2016) to assess the influence of zones and accessions (independent variables) on the correlation and clustering of traits (dependent variables) across multiple environments. PCA also identified the traits contributing most to the variation among accessions. A scree plot (Figure 4) was used to determine the optimal number of principal components, selecting the point just before the curve levels off.

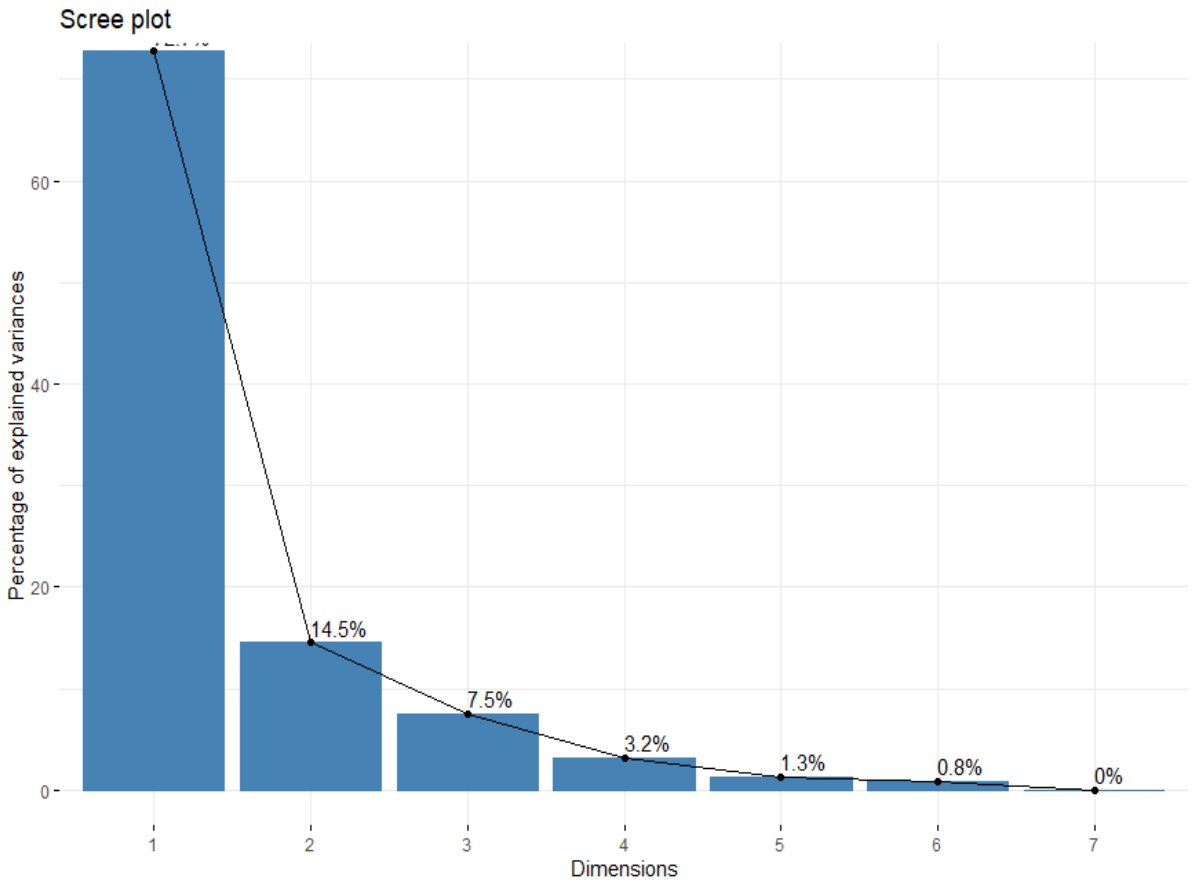


Figure 4: The scree plot graph for PCA showing the explained variances

Pearson's correlation coefficients (r) were calculated to assess relationships among seven quantitative morphological traits of Bambara groundnut across the three zones and in the greenhouse, using a correlation matrix. Analyses were performed in R software version 3.3.1 (R Development Core Team, 2016). Additionally, a dendrogram illustrating the phenetic relationships among the six accessions was constructed based on the seven traits using complete linkage cluster analysis, which grouped accessions according to their similarity distances.

3.5 Yield

Field yield was determined using seeds from pods of ten randomly selected plants per plot within each block. Border plants were excluded to minimize environmental variation and bias. The pods were air-dried, shelled, and the seeds further dried in a Carbolite high-temperature laboratory oven (PF60 model) at 110 °C until a constant weight was achieved (Ndidi *et al*., 2014). The mass of the oven-dried seeds from the ten selected plants per plot was then recorded.

3.5.1 Data Analysis for Yield

The seed weight per plant in grams for the ten randomly selected plants per plot in the fields were determined by dividing the total weight of seeds (g) by the number of plants harvested per plot. Yield per square meter (YMS, g/m²) was then computed for each accession in each agro-ecological zone. The seed yield was calculated as follows:

$Y = W/NP$

Y= Pod or Grain *yield (g/plant)*

W = Total Pod or Grain weight (g)

NP = Total Number of plants harvested

The crop yield in tonnes per ha was then calculated based on grain yield (g/plant) using the formula

$$\text{Tonnes per ha} = \frac{10000\text{m}^2 \times 35 \text{ plants per plot} \times \text{seed weight (g/plant)}}{1.44\ \text{m}^2 \times 1000 \times 1000}$$

Quantitative yield data from the field, as well as from irrigated and drought-stressed greenhouse plants, were subjected to one-way analysis of variance (ANOVA), and means were separated using Least Significant Difference (LSD, $P \leq 0.05$) in GraphPad Prism (Version 8.0.2). Pearson's correlation coefficients between yield (ton/ha) and yield components, such as seed weight per plant and number of pods per plant, were calculated using R software version 3.3.1 (R Development Core Team, 2016). Correlation analysis was conducted for all quantitative traits to identify those that varied positively or negatively with seed yield

Traits positively correlated with yield in each zone were used to compute a Rank Summation Index (RSI), following the method of Mulamba and Mock (1978), to guide the selection of high-yielding accessions. In each zone, accessions were ranked based on their mean values for these traits, with a rank of 1 assigned to the highest mean and 7 to the lowest. The ranks were then

summed to determine each accession's overall performance, with a lower RSI score indicating superior yield potential.

3.6 Protein Analysis

Protein analysis samples were selected from the high-yielding accessions in each zone, including both irrigated and drought-stressed Bambara accessions. Selection was based on the Rank Summation Index (RSI). To quantify total protein content and the concentrations of four protein fractions in each selected accession, the Bradford assay was used. This method is a quick and accurate spectroscopic procedure for measuring protein concentrations in solution. The process involved:

3.6.1 Sample Preparation

Sample preparation followed the method of Saminu *et al*. (2021). Twenty (20) randomly selected seeds from each landrace (K1, B1, B4, M2 and M4) were cleaned by washing with distilled water, oven-dried at 110^0C for ten minutes and whole sample materials milled to powder (0.60 mm) form with a high-speed blender (Braun KMM 30 mill), type 3045, CombiMax (Germany). The fine flour was stored in airtight plastic containers. The use of fine powdered plant tissue is crucial as it helps minimize proteolysis and protein modification that may occur during tissue disruption. About 10g of fine powdered flour from each landrace was transferred into a 250 mL beaker and defatted with n-hexane (1:3 w/v) at room temperature by continuous shaking for thirty minutes and then allowed to settle. The hexane was then removed by decantation and the defatted flour was air dried at room temperature (25 ± 2°C) and stored in an airtight plastic container and kept in a refrigerator of about 4°C prior to analysis.

3.6.2 Extraction of Total Proteins

Total protein extraction was performed following the method of Hameed *et al*. (2009). Seed flour (1 g) was dissolved in a 10 mL extraction buffer (50 mM phosphate buffer, pH 7.8). The homogenate was then vortexed and centrifuged at 14,000 rpm for 10 minutes at 4 °C, and supernatant collected. The supernatant was used for estimating total protein concentrations by dye-binding assay as described in section 3.6.4 and in Sodium Dodecyl Sulphate PolyAcrylamide Gel Electrophoresis (SDS-PAGE) analysis as described in section 3.6.5.

3.6.3 Extraction of Storage Protein Fractions

Storage protein fractions were sequentially extracted based on their solubility. The extraction was done according to the method of Silva and Dantas, (2016). Seeds flour (1g) was dissolved in 10 mL distilled water, the homogenate incubated in the oven for 30 minutes at 30 °C and subsequently centrifuged using Thermo Scientific Centrifuge (Sorvall, Model ST16R, Germany) at 3000 x g for 20 minutes at 4°C. The supernatant was recovered and the pellet resuspended in 5 mL distilled water, incubation, centrifugation and supernatant recovery procedures were repeated as in step one above. The supernatants were combined as albumin fraction. The residue obtained after albumin extraction, was used in the extraction of globulins, glutelins and prolamins protein fractions in that order. The methodology used was similar to that used in albumins extraction, only the extraction solvents differed. Globulins fraction was extracted in a similar manner using 1% NaCl, glutelins fraction was extracted with 0.1 M NaOH while prolamins fraction was extracted with 70% ethanol.

3.6.4 Quantification of Proteins using the Bradford Method

The Bradford method (Bradford, 1976) which is a quantitative protein assay method, based on the binding of Coomassie Brilliant Blue dye, to a protein sample, and comparing this binding to a standard curve generated by the reaction of known amounts of a standard protein, usually Bovine serum albumin (BSA) was used. The requirements for the Bradford method were; BSA standard solution (1 mg/ml), Bradford assay dye reagent (Bio-Rad Protein Assay Dye Reagent Concentrate, cat. no. 500-0006) and the Protein dilution buffer (kit). For this assay, protein samples were diluted in an appropriate protein dilution buffer (generally the same buffer in which they were dissolved). The BSA standard curve was prepared using the same buffer.

The concentration of the BSA standard solution was measured photometrically. 1 mg/ml solution of BSA had an (Absorbance) A280 of 0.66. The absorbance and protein concentration reading was done using a UV-VIS Spectrophotometer (Bibby Scientific Ltd, UK, Jenway, Model 6800).

3.6.4.1 Preparation of the standard curve

A standard curve was prepared by pipetting together carefully the solution volumes listed in Table 5, corresponding to 0, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml BSA. The end volume of all samples was maintained at 200 µl. An aliquot of the dye reagent concentrate was diluted in a

ratio of 1:5 with distilled water. 20 µl of each BSA dilution was pipetted into rows of 96 wells plate, and 50µl diluted dye reagent added, mixed well, and incubated for 5 min at room temperature.

The optical density (OD) was measured at 595 nanometers (nm) for each sample, and the standard curve plotted. Dilutions of the protein sample of interest were prepared and 20 µl aliquots tested in triplicate as above. The protein samples to be tested were handled in exactly the same manner as the samples used in generating the standard curves. The protein concentration of the test samples was calculated by comparing the OD595 with the standard curve, taking into account dilution factors.

Table 5: Standard curve samples for Bradford Protein Assay

BSA concentration (mg/ml)	Volume of BSA standard solution (µl)	Volume of proteins dilution buffer (µl)
0	-	200
0.05	10	190
0.1	20	180
0.2	40	160
0.3	60	140
0.4	80	120
0.5	100	100
0.6	120	80
0.7	140	60
0.8	160	40
1.0	200	-

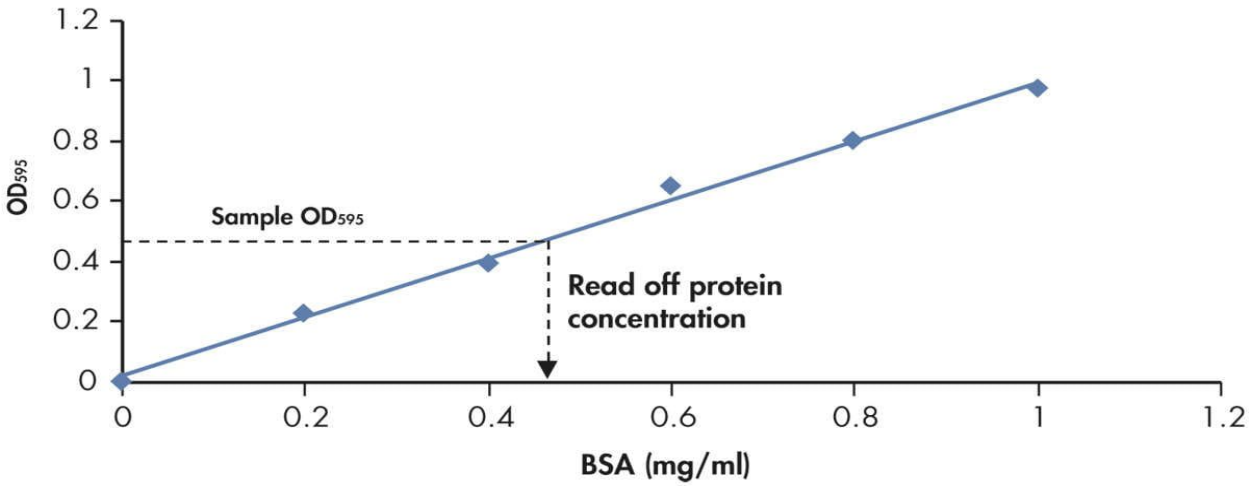


Figure 5: A graph showing estimations of protein concentrations done by comparing the OD595 with the standard curve(s)

3.6.5 Determination of Storage Protein Electrophoretic Banding Pattern

The supernatant collected was mixed (4:1) with cracking solution which comprised of 10 ml containing 1g SDS, 0.01g bromphenol blue, 2ml Mercaptoethanol, 1.5ml 0.5M tris, pH 6.8, 5g sucrose and 6.5 ml water. The mixture of cracking solution and the supernatant were put in a vortex mixer and heated in a boiling water bath for five minutes to denature the proteins. Denaturation is important as it disrupts the three-dimensional structure of proteins by altering its conformation. This ensures the proteins become more soluble in the extraction buffer as well as prevent protein aggregation, thereby facilitating their separation and extraction.

SDS-PAGE analysis of total storage proteins and the various protein fractions from the five Bambara landraces was carried out according to the method of Saminu *et al*., (2021). SDS-PAGE analysis of total storage proteins was carried out in 12% polyacrylamide slab gels. Ten microliters (10µl) each of the protein ladder (acculadder TM Protein size marker,11 kDa to 250 kDa) and samples were loaded into the wells of stacking gel. Electrophoresis was done at 150 V for 1 hour until the bromophenol blue run to the bottom of the gel. After electrophoresis, the gels were stained with 2% coomassie blue for 1 hour and destained by solution containing 5% (v/v) acetic acid, 20% (v/v) methanol and distilled water in the ratio of 5:20:75 (v/v) for 2

hours. Finally, destained gels were photographed using UVI pro-platinum gel documentation system (UVItec UK).

3.6.6 Protein Analysis

Total protein content was analyzed using one-way ANOVA ($P \leq 0.05$), followed by Tukey's multiple comparisons test to compare protein quantities across accessions. Coefficients of variation were calculated to assess heterogeneity among the accessions. The analyses were conducted using GraphPad Prism (Version 8.0.2), and bar graphs were plotted.

Protein fraction data were also analyzed using one-way ANOVA ($P \leq 0.05$) for intra- and inter-accession comparisons, with Tukey's test used to compare specific protein fraction quantities. Coefficients of variation were computed to evaluate heterogeneity of protein fractions among accessions. GraphPad Prism (Version 8.0.2) was used for analysis, and error bar diagrams were generated.

The total protein, albumin, and globulin peptide profiles were analyzed using Jaccard's coefficient to calculate similarity matrices among the five Bambara groundnut accessions. A hierarchical clustering dendrogram showing the putative relationships among the accessions was generated using Jaccard indices in R software version 3.3.1 (R Development Core Team, 2016).

CHAPTER FOUR

RESULTS

4.1 Microclimate of the study area

During the study period, rainfall distribution varied across the three zones (Figure 6a-6c). Total rainfall amounts from April to August 2019 were 882.5 mm, 600.1 mm, and 480.6 mm for Alupe, Kibos, and Oyani, respectively. Air temperatures generally increased during the dry period and decreased during the wet growing season.

Temperature ranges during the growing season were as follows: at Alupe, the minimum was 21°C in July and the maximum was 30.5°C in April; at Kibos, the minimum was 17.9°C in August and the maximum was 31.6°C in July; at Oyani, the minimum was 20.7°C in June and the maximum was 29.6°C in July.

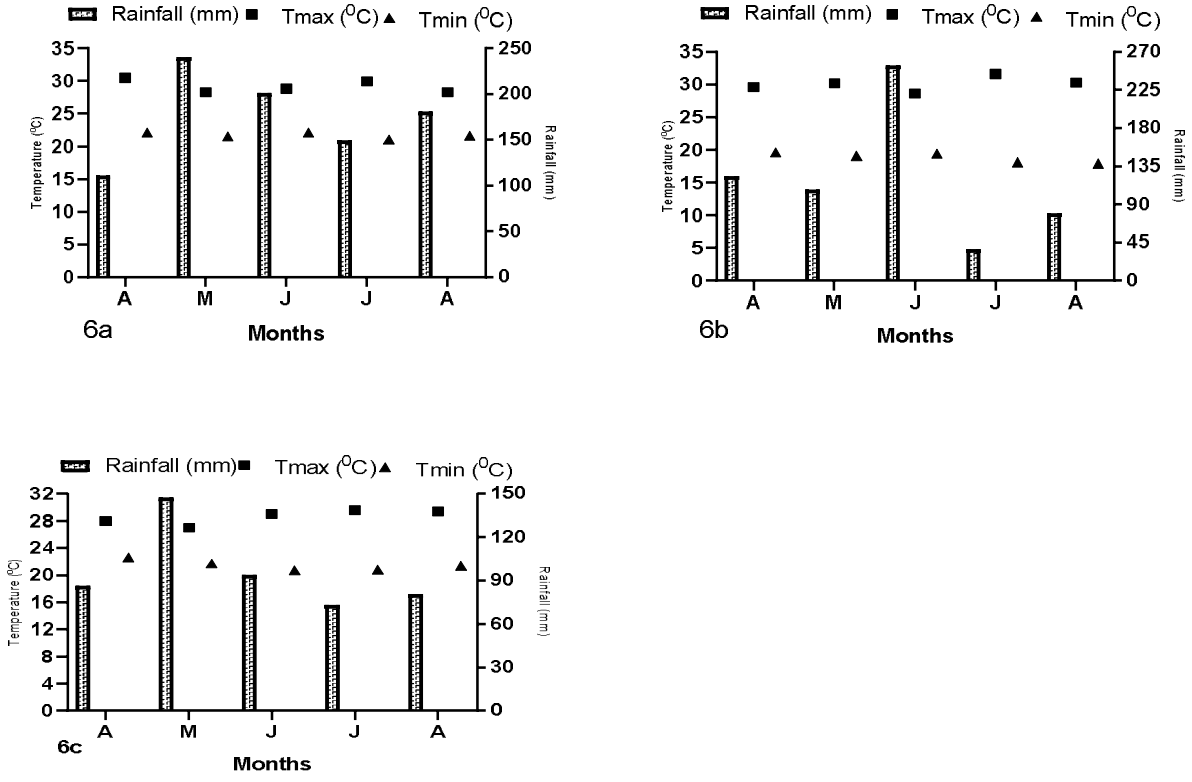


Figure: 6a, 6b and 6c. Monthly rainfall amounts (mm) for the five months (April – August) and maximum and minimum air temperatures, T_{air} (^{0}C) recorded during the same period in the agro ecological zones (Alupe, Kibos and Oyani respectively) in 2019 during growing season.

4.2 pH and Nutrient Composition of the Soils in the Greenhouse and at Alupe, Kibos and Oyani

In all zones and in the greenhouse, soil pH values were in the acidic range. In Oyani and the greenhouse, soil pH was moderately acidic (>5.0) both before and after planting (Table 6). Soils in Alupe and Kibos were strongly acidic (Table 6). Nitrogen (N) levels were deficient (<0.2%) in all soils before planting but above deficiency levels (≥0.2%) after planting (Table 6). Phosphorus (P) levels were deficient (<30 ppm) in all soils both before planting and after harvesting (Table 6). Before planting, phosphorus was moderately low (≥25 ppm) but did not reach the minimum critical level of 30 ppm required for optimal Bambara growth and development.

Potassium (K^+) was above the deficiency level (≥0.24me %) in all the soils, before and after planting (Table 6). Calcium (Ca^{2+}) was above the deficiency level (≥ 2.0me %) in the soils of Kibos and Oyani before planting. This mineral element was however deficient (< 2.0 me %) in the Kibos and Oyani soils after harvesting. It was also below the deficiency level (< 2.0 me %) in Alupe and greenhouse soils before and after planting (Table 6). Soil analysis showed that Magnessium element was above the deficiency level (≥ 1.0 me %) in all the soils before planting except in the Oyani soils (Table 6). It was however deficient (< 1.0 me %) in the Kibos and the Oyani soils after harvesting. From the analysis, the soil textures from various zones were varied. The soils of Kibos and greenhouse had clay loam texture, the soils of Oyani had silty clay texture, whereas the soils of Alupe had loamy silt texture (Table 6). Based on these analyses, and on the deficiency levels of each nutrient in the soil (Table 6), none of the soils had sufficient macro nutrients (N, K, P, Ca and Mg) required by the Bambara.

Table 6: The physio-chemical properties of soils in the greenhouse at Maseno University research farms and in the different agro ecological zones (Kibos, Oyani and Alupe) in the Lake Victoria basin, Kenya

	KIBOS		**OYANI**		**ALUPE**		**GREEN HOUSE**		
	Before planting	After planting	Before planting	After Planting	Before Planting	After planting	Before planting	After Planting (irrigated plots)	After Planting (Droughted plots)
Soil depth (cm)	0cm-15cm	0cm-15cm	0cm-15cm	0cm-15cm	0cm-15cm	0cm-15cm	0cm-15cm	0cm-15cm	0cm-15cm
pH	4.6	4.2	6.0	5.6	4.7	4.5	6.1	5.4	5.9
Nitrogen (%)	0.1	0.3	0.1	0.2	0.1	0.4	0.1	0.4	0.2
Phosphorus (ppm)	27.0	10.0	25.0	10.0	28.0	11.0	25.0	8.0	15.0
Potassium (me %)	1.3	1.1	1.2	1.1	1.5	1.4	1.3	0.8	1.2
Calcium (me %)	2.1	1.4	2.2	1.5	1.4	0.8	1.2	0.3	1.0
Magnesium (me %)	1.1	0.4	0.4	0.1	2.0	1.4	1.6	1.1	1.5
Soil Texture	Clay loam	Clay loam	Silty clay	Silty clay	Loamy silt	Loamy silt	Clay loam	Clay loam	Clay loam

The deficiency level of Nitrogen is any value below 0.2 %. The deficiency level of Phosphorus is any value below 30ppm. The deficiency level of Potassium is any value below 0.24me%. The deficiency level of Calcium is any value below 2.0me%. The deficiency level of Magnesium is any value below 1.0 me %.

4.3 Qualitative Morphological Characteristics of Bambara Groundnuts

4.3.1 Phenotypic Diversity

Seven qualitative characters related to leaves, pods and seeds such as Terminal Leaflet shape (TLS), Pod Colour (POC), Pod Texture (POT), Pod shape (POS), Seed Eye Pattern (SEP), Seed Testa Pattern (STP) and Seed Testa Colour (STC) were evaluated. The qualitative morphological traits of Bambara were similar across all zones and in the greenhouse (Figure 7), indicating no zonation effect on these traits. The seven qualitative traits evaluated (Table 7) revealed high phenotypic diversity among the six Bambara accessions. Among the traits, oval terminal leaflet shape was the most common, occurring in three accessions. Brown was the dominant pod colour, observed in four accessions, and the majority of pods (4 out of 6) had a shape that ended in a point, rounded on the othe side. Most seeds (4 out of 6) lacked a testa pattern, while cream was the predominant seed coat colour, found in three accessions (Figure 7).

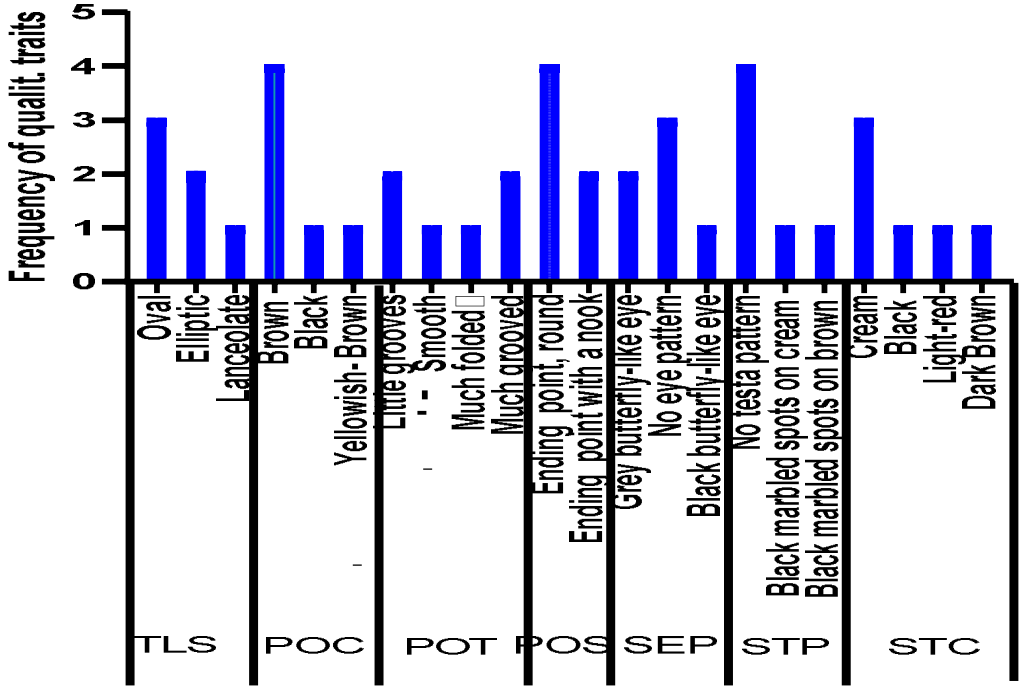


Figure 7: Frequency distribution of qualitative traits in the Bambara accessions grown in various zones and in the greenhouse.

KEY: TLS-Terminal leaflet shape, POC-Pod Colour, POT-Pod Texture, POS-Pod Shape, SEP-Seed Eye Pattern, STP-Seed Testa Pattern, STC-Seed Testa Colour.

Specifically, among the accessions evaluated, 50% had oval-shaped terminal leaflets, 33.3% had elliptic-shaped terminal leaflets, and 16.6% had lanceolate-shaped terminal leaflets. For pod colour, 66.7% were brown, while black and yellowish-brown each accounted for 16.7% (Table 7). Pod texture was divided equally between little-grooved and much-grooved pods (33.3% each), with smooth and much-folded textures each observed in 16.7% of the accessions.

Regarding pod shape, 66.7% of accessions had pods ending in a point and rounded on the opposite side, while 33.3% had pods ending in a point with a nook on the other side. Analysis of seed eye pattern indicated that 50% had no eye pattern (simple), 33.3% had a grey butterfly-like eye, and 16.7% had a black butterfly-like eye. For seed testa pattern, 66.7% had no pattern (plain), while black marbled spots on a cream background with grey butterfly-like eyes and black marbled spots on a brown background without an eye each accounted for 16.7%.

In terms of seed testa colour, 50% were cream, while black, light red, and dark brown each represented 16.7% (Plate 5). Overall, no single trait was uniformly distributed among all accessions, reflecting a high level of morphological variation.

Table 7: Percentage distribution of Qualitative traits in the six Bambara groundnut accessions grown in various zones and in the greenhouse.

Character	Description of traits	Percentages (%)
Terminal leaflet Shape	Oval	50.0
	Elliptic	33.3
	Lanceolate	16.7
Pod Colour	Brown	66.7
	Black	16.7
	Yellowish Brown	16.7
Pod Texture	Smooth	16.7
	Little Grooves	33.3
	Much Grooved	33.3
	Much Folded	16.7
Pod shape	Ending point, Round	66.7
	Ending point with a nook	33.3
Seed Eye Pattern	No eye Pattern	50.0
	Grey-butterfly like eye pattern	33.3
	Black- butterfly like eye pattern	16.7
Seed Testa Pattern	No testa pattern	66.7
	Black marbled spots on cream background with grey butterfly like eyes	16.7
	Black marbled spots on brown background without eye.	16.7
Seed Testa Colour	Cream	50.0
	Black	16.7
	Dark Brown	16.7
	Light red	16.7



Plate 5: The seed testa pattern and seed testa colour of the six Bambara groundnut germplasm used in the study

4.3.2 Qualitative Traits Diversity Indices

The Shannon-Weiner diversity index (H') was calculated on the qualitative characters in order to access the phenotypic diversity among the seven traits. The estimated Shannon-Weiner diversity index (H') for the Bambara groundnut qualitative traits are shown in Table 8. All traits showed a high level of polymorphism (H'>0.500). The indices ranged from H'=1.6501 (seed testa pattern) which was the least to H'=2.0708 (terminal leaflet shape and seed eye pattern) which were the highest, with an average of H'=1.8973. All the traits therefore exhibited the polymorphism phenomena.

Table 8: Shannon-Weiner diversity index (H') showing the phenotypic diversity of seven qualitative characters for the studied landraces in the Lake Victoria Basin, Kenya.

Qualitative Trait	Shannon-Weiner Index (H')
Terminal Leaflet Shape (TLS)	2.0708
Pod Colour (POC)	1.6784
Pod Texture (POT)	2.0620
Pod Shape (POS)	1.8136
Seed Eye Pattern (SEP)	2.0708
Seed Testa Pattern (STP)	1.6501
Seed Testa Colour (STC)	1.9356
Average diversity index	1.8973

4.3.3 Cluster Analysis

The seven qualitative traits were used for the hierarchical cluster analysis of six germplasm accessions. The dendrogram revealed two main clusters for the germplasm accessions (Figure 8). The accessions from Migori County showed similarity as they belong to the same cluster group. Consequently, the Kisumu and the Busia accessions were broadly clustered together, even though K2 and B4 appeared to be more similar, while B1 and K1 also tended to be similar. The Euclidean distance ranged from 0.2 to 1.2 with an increase in dissimilarity.

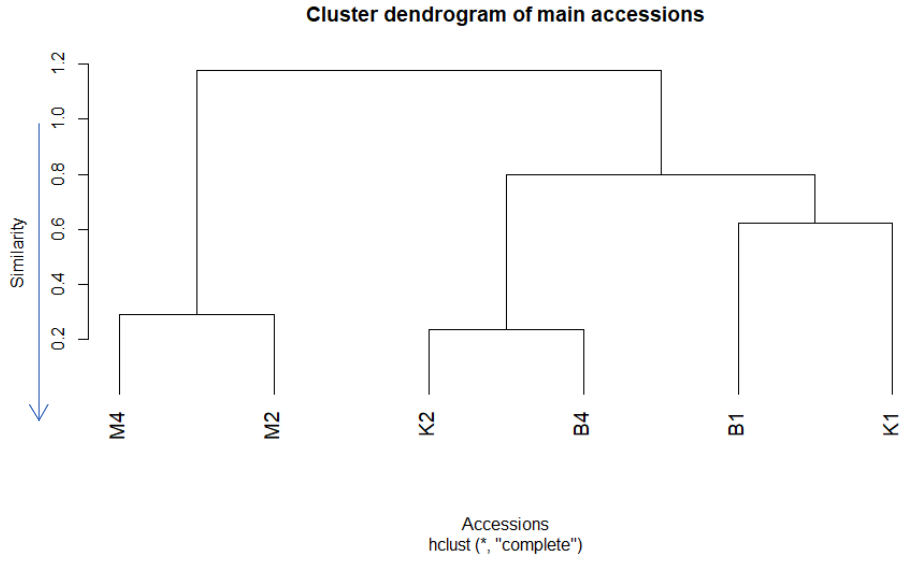


Figure 8: A dendrogram showing the relationship among Bambara groundnut accessions based on their Similarity and Dissimilarity distances.

4.4 Quantitative Morphological Characteristics of Bambara Groundnuts

4.4.1 Plant Height (PHT cm)

4.4.1.1 Plant Height Inter-Site Comparisons

4.4.1.1.1 K2 Plant Height (PHT cm)

The plant height (PHT) mean of K2 Bambara accession was compared in three different agro ecological zones (Alupe, Kibos and Oyani). Tukey's multiple comparisons test showed that K2 PHT had significant difference (P=0.0050) between Alupe (19.08±0.3547) and Kibos (17.27±0.1856), and between Alupe (19.08±0.3547) and Oyani (17.20±0.4314), where P=0.0039. In the greenhouse, Tukey's multiple comparisons test showed that there was strong significant difference (P<0.0001) in height between K2 greenhouse irrigated (GHI)

(20.87±0.1004) and K2 greenhouse droughted (GHD) (14.62±0.02082) Bambara accession (BGA). The K2 irrigated plants (20.87±0.1004) had strong significant difference (P<0.0001) in height between Oyani (17.20±0.4314) and Kibos (17.27±0.1856) K2 plants. However, between Alupe (19.08±0.3547) and GHI (20.87±0.1004), P=0.0057. The comparisons test also showed that there were significant differences (P≤0.05) between K2 PHT in all the three field sites and the GHD plants (14.62±0.02082) (Figure 9).

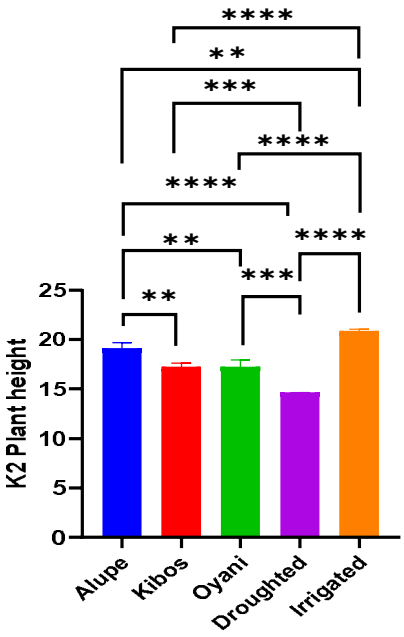


Figure 9: Plant height of K2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of K2 plant height between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, P<0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated imply that there was no significant difference.

4.4.1.1.2 B4 Plant Height (PHT cm)

Comparisons of B4 plant heights in the greenhouse and in each of the three agro-ecological zones are shown (Figure 10). The highest B4 plant heights were found in the Alupe zone, at 21.29 cm, and in GHI, at 19.57 cm. Based on the results of the Tukey's multiple comparisons test, there were significant differences (P≤0.05) in B4 PHT between all the three agro-ecological zones. The significant difference of P<0.0001 was observed between Alupe (21.29±0.0617) and Oyani (14.86±0.3320), and between Kibos (19.35±0.3735) and Oyani (14.86±0.3320). However, between Alupe (21.29±0.0617) and Kibos (19.35±0.3735), P=0.0050. In the greenhouse, great

variation was observed in height between, B4 GHI (19.57±0.2333) and B4 GHD (14.53±0.3180) BGA, where P<0.0001. Amongst the zones, only Alupe, (21.29±0.0617) and Oyani (14.86±0.3320) showed significant difference (P<0.05) with GHI (19.57±0.2333). The B4 droughted plants had significant difference (P<0.0001) with Alupe (21.29±0.0617) and Kibos (19.35±0.3735).

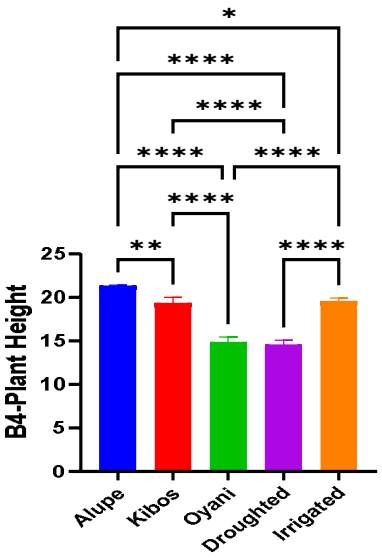


Figure 10: Plant height of B4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots). Comparisons of B4 plant height between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, P<0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated imply that there was no significant difference.

4.4.1.1.3 B1 Plant Height (PHT cm)

In the B1 accession, Kibos (19.45±0.1882) had significantly (P<0.05) higher PHT than in other zones and in the greenhouse (Figure 11). The B1 PHT ranged from 19.45cm in Kibos to 13.97cm in the droughted plants. The Tukey's multiple comparisons test showed that there was strong significant difference (P<0.0001) between Alupe (15.14±0.1233) and Kibos (19.45±0.1882), and between Kibos (19.45±0.1882) and Oyani (17.03±0.1943). However, between Alupe (15.14±0.1233) and Oyani (17.03±0.1943), the significant differerence was P=0.0007. In the greenhouse, the comparisons test showed that there was strong significant difference (P<0.0001) in height between B1 GHI (18.57±0.2333) and B1 GHD (13.97±0.2906) plants. The

comparisons test also showed that B4 GHI (18.57±0.2333) had significant difference (P≤0.05) in height with Alupe (15.14±0.1233) and Oyani (17.03±0.1943). The B1 droughted plants had strong significant difference (P<0.0001) in height with Oyani (17.03±0.1943) Kibos (19.45±0.1882). However, the significant difference between Alupe (15.14±0.1233) and GHD (13.97±0.2906) was P=0.0194.

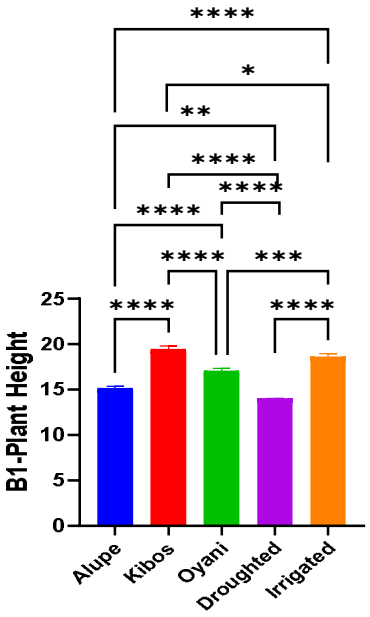


Figure 11: Plant height of B1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of B1 plant height between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, P<0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combination with no level of significance indicated, imply that there was no significant difference.

4.4.1.1.4 K1 Plant Height (PHT cm)

In the K1 BGA, there was significant difference (P=0.0037) in height between Alupe (16.40±0.6245) and Kibos (14.03±0.2728), and between Alupe (16.40±0.6245) and Oyani (13.30±0.1528), where P=0.0005. The comparative test showed that, although the GHI PHT was greater than the GHD, there was no significant difference in height between the K1 GHI (14.97±0.2603) and K1 GHD (14.80±0.1732). According to the comparisons test, among the field sites, only the height of K1 plants in Oyani (13.30±0.1528) differed significantly (P=0.0343) with that of GHI (14.97±0.2603). Alupe (16.40±0.6245) was the sole field site with a significant difference (P=0.0427) with the droughted (14.80±0.1732) plants (Figure 12).

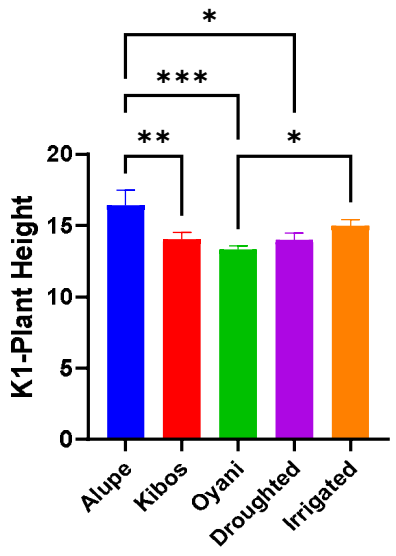


Figure 12: Plant height of K1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of K1 plant height between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, P<0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combination with no level of significance indicated, imply that there was no significant difference.

4.4.1.1.5 M4 Plant Height (PHT cm)

The M4 accession PHT across the three zones and in the greenhouse is presented in figure 13. GHI recorded the highest height, followed by Kibos at 13.73cm and 13.30cm respectively. The M4 PHT had no significant difference (P>0.05) between all the zones. In the greenhouse, Tukey's multiple comparisons test showed that there was significant difference (P=0.0057) in height between M4 GHI (13.73±0.2906) and M4 GHD (12.11±0.3541) plants. The comparisons test showed that only Alupe, (12.27±0.2254) and Oyani (12.28±0.1563) plants had significant difference (P≤0.05) with GHI (13.73±0.2906) plants. The droughted plants had significant difference (P=0.0378) in height with Kibos (13.30±0.1102) plants.

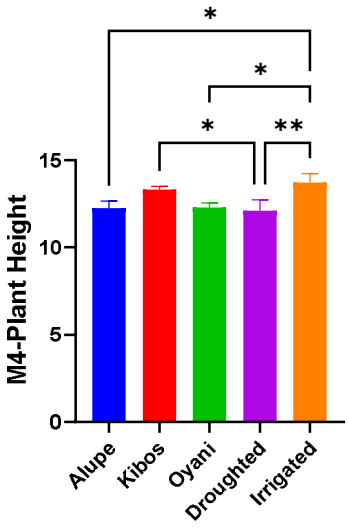


Figure 13: Plant height of M4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of M4 plant height between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, P<0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combination with no level of significance indicated; imply that there was no significant difference.

4.4.1.1.6 M2 Plant Height (PHT cm)

The M2 accession PHT showed a significant difference in height across all agro-ecological zones (one-way ANOVA, $P < 0.05$; Figure 14). A highly significant difference ($P < 0.0001$) was observed between Alupe (14.57 ± 0.1453) and Kibos (19.62 ± 0.1856), as well as between Kibos and Oyani (13.50 ± 0.1528). The difference between Alupe and Oyani was also significant ($P = 0.0424$).

In the greenhouse, a comparison test revealed a highly significant difference in plant height between M2 GHI (15.87 ± 0.2404) and M2 GHD (13.17 ± 0.1453) plants ($P < 0.0001$). Additionally, M2 GHI plants were significantly taller ($P \leq 0.05$) than those grown at all field sites. The drought-stressed M2 plants also differed significantly in height ($P \leq 0.05$) from those grown in Alupe and Kibos.

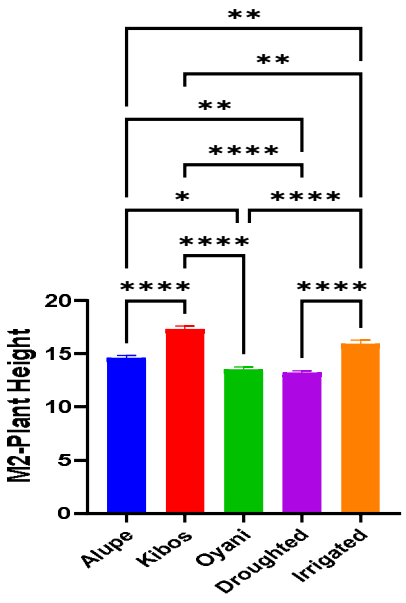


Figure 14: Plant height of M2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of M2 plant height between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, P<0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combination with no level of significance indicated, imply that there was no significant difference.

4.4.1.2 Plant Height Intra-Site Comparisons

4.4.1.2.1 Plant Height Intra-Site Comparisons in Alupe

At Alupe zone, PHT means for the accessions grown ranged from 21.29cm to 12.27cm. In this zone, B4 had the highest plant height mean (21.29±0.0617), followed by K2 (19.08±0.3547) and then K1 (16.40±0.6245). On the contrary M4 had the lowest mean (12.27±0.2254). The analysis (one-way ANOVA, P≤0.05; Tukey's multiple comparisons test) showed that there was strong significant difference (P<0.0001) in most of the accessions comparisons, except in two comparisons, between B1 (15.14±0.1233) and K1 (16.40±0.6245), and between B1 (15.14±0.1233) and M2 (14.57±0.1453) where the significant differences were P>0.05 (Table 9a).

4.4.1.2.2 Plant Height Intra-Site Comparisons in Kibos

In Kibos, K2 had the highest PHT mean (19.62±0.3509), followed by B1 (19.45±0.1882) and then B4 (19.35±0.3735). On the contrary M4 had the lowest mean (13.30±0.1102). The analysis showed that plant height was significantly different (one-way ANOVA, $P \leq 0.05$; Tukey's multiple comparisons test) in all the accessions comparisons, except in four comparisons where there were no significant differences (Table 9b).

4.4.1.2.3 Plant Height Intra-Site Comparisons in Oyani

At Oyani, PHT means for the accessions grown ranged from 17.20 cm to 12.28 cm. The K2 accession had the highest plant height mean (17.20±0.4314), followed by B1 (17.03±0.1943) and then B4 (14.86±0.3320). On the contrary M4 had the lowest mean (12.28±0.1563). The analysis result showed that plant height was significantly different (one-way ANOVA, $P \leq 0.05$; Tukey's multiple comparisons test) in most of the accessions comparisons except in few comparisons where there were no significant differences. There were no variations in height between four comparisons such as between K2 (17.20±0.4314) and B1 (17.03±0.1943), between M4 (12.28±0.1563) and M2 (13.50±0.1528), between K1 (13.30±0.1528) and M2 (13.50±0.1528), and between K1 (13.30±0.1528) and M4 (12.28±0.1563) (Table 9c).

4.4.1.2.4 Plant Height Intra-Greenhouse Irrigated Comparisons

K2 accession recorded significantly ($P<0.05$) higher plant height than all the other five accessions in this zone. The PHT in this zone varied from 20.87 cm to 13.73 cm, with K2 performing best (20.87±0.1004), followed by B4 (19.57±0.2333) and then B1 (18.57±0.2333). The results revealed that M4 had the lowest mean (13.73±0.2906). The analysis result showed that plant height was significantly different (one-way ANOVA, $P \leq 0.05$; Tukey's multiple comparisons test) between all of the accessions comparisons except between B4 (19.57±0.2333) and B1 (18.57±0.2333) and between K1 (14.97±0.2603) and M2 (15.87±0.2404) comparisons where there were no significant differences ($P>0.05$) (Table 9d).

4.4.1.2.5 Plant Height Intra-Greenhouse Drought Comparisons

K1 accession had the highest plant height mean (14.80±0.1732), followed by K2 (14.62±0.02082) and then B4 (14.53±0.3180). The results showed that M4 had the lowest PHT mean (12.11±0.3541) amongst the greenhouse droughted plants. The analysis showed that plant

height was significantly different (one-way ANOVA, P≤0.05, Tukey's multiple comparisons test) between seven of the accessions comparisons. There was however no significant differences between eight comparisons (Table 9e).

Table 9a: Plant Height Intra-Site Comparisons in Alupe zone

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Alupe	19.08± 0.3547	21.29± 0.0617	15.14± 0.1233	16.40± 0.6245	12.27± 0.2254	14.57± 0.1453	K2 vs. M4 B4 vs. B1 K1 vs. M4 K1 vs. M2 K2 vs. B4	<0.0001 <0.0001 <0.0001 0.0149 0.0037

Acc accession. Apart from B1 vs K1 and B1 vs M2 where there were no significant differences (P≥0.05), all comparisons were highly significant at P≤0.0001 except K2 vs B4 where P≤0.0037, B1 vs M4 where P≤0.0004, K1 vs M2 where P≤0.0149, M4 vs M2 where P≤0.0027 and K2 vs K1 where P≤ 0.0007. Plant height expressed as Mean±Standard Error of Mean (SEM).

Table 9b: Plant Height Intra-Site Comparisons in Kibos zone.

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Kibos	19.62± 0.3509	19.35± 0.3735	19.45± 0.1882	14.03± o.2728	13.30± 0.1102	17.27± 0.1856	K2 vs. K1 B1 vs. K1 M4 vs.M2 B4 vs. M2 B1 vs. M2	<0.0001 <0.0001 <0.0001 0.0013 0.0009

Acc accession. Apart from K2 vs B4, K2 vs B1, B4 vs B1 and K1 vs M4 where there were no significant differences (P≥0.05), all comparisons were highly significant at P≤0.0001 except K2 vs. M2 where P≤ 0.0004, B4 vs. M2 where P≤0.0013 and B1 vs. M2 where P≤0.0009. Plant height expressed as Mean±Standard Error of Mean (SEM).

Table 9c: Plant Height Intra-Site Comparisons in Oyani zone.

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Oyani	17.20± 0.4314	14.86± 0.3320	17.03± 0.1943	13.30± 0.1528	12.28± 0.1563	13.50± 0.1528	K2 vs. K1 B1 vs. K1 B1 vs. M2 K2 vs. M2 K2 vs. B4	<0.0001 <0.0001 <0.0001 <0.0001 0.0004

Acc accession. Apart from K2 vs B1, K1 vs M4, K1 vs M2 and M4 vs M2 where there were no significant differences (P≥0.05), all comparisons were highly significant at P≤0.0001 except K2 vs. B4 where P≤0.0004, B4 vs. B1 where P≤0.0008, B4 vs. K1 where P≤0.0114, B4 vs. M4 where P≤0.0002 and B4 vs. M2 where P≤0.0286. Plant height expressed as Mean±Standard Error of Mean (SEM).

Table 9d: Plant Height Intra-Site Comparisons in Greenhouse irrigated zone.

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
GHI	20.87± 0.1004	19.57± 0.2333	18.57± 0.2333	14.97± 0.2603	13.73± 0.2906	15.87± 0.2404	K2 vs. K1 B4 vs. M4 B1 vs. K1 B1 vs. M4 K2 vs. M2	<0.0001 <0.0001 <0.0001 <0.0001 <0.0001

Acc accession. Apart from B4 vs. B1 and K1 vs. M2 where there were no significant differences (P≥0.05), all comparisons were highly significant at P≤0.0001 except K2 vs. B4 where P≤0.0192, K2 vs. B1 where P≤0.0002, K1 vs. M4 where P≤0.0270 and M4 vs. M2 where P≤0.0004. Plant height expressed as Mean±Standard Error of Mean (SEM).

Table 9e: Plant Height Intra-Site Comparisons in Greenhouse droughted zone.

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
GHI	14.62± 0.0208	14.53± 0.3180	13.97± 0.2906	14.80± 0.1732	12.11± 0.3541	13.17± 0.1453	K2 vs. M4 B4 vs. M2 B1 vs. M4 K1 vs. M4 K1 vs. M2 K2 vs M2 B4 vs M4	0.0001 0.0207 0.0021 <0.0001 0.0058 0.0137 0.0002

Acc accession. Apart from K2 vs. B4, K2 vs. B1, K2 vs. K1, B4 vs. B1, B4 vs. K1, B1 vs. K1, B1 vs. M2 and M4 vs. M2 where there were no significant differences (P>0.05), the remaining seven comparisons were significant (P≤0.05).

4.4.2 Number of Pods

4.4.2.1 Number of Pods Inter-sites Comparisons.

4.4.2.1.1 K2 Number of Pods

4.4.2.1.1.1 K2 Number of Pods with One Seed (N1S)

In K2 accession, the analysis result showed that there were significant differences ($P \leq 0.05$) in N1S between all the sites. Between Alupe (12.50±0.7638) and Kibos (17.67±0.8819), $P \leq 0.0020$, between Alupe (12.50±0.7638) and Oyani (9.33±0.3333), $P=0.0465$, whereas between Kibos (17.67±0.8819) and Oyani (9.33±0.3333), $P<0.0001$ (Figure 15 A). In the greenhouse, the comparisons test showed that there was strong significant difference ($P<0.0001$) in N1S between K2 GHI (21.33±0.6667) and K2 GHD (6.00±0.5774). The comparisons test also showed that there were significant differences ($P \leq 0.05$) between all the field sites and the GHI (21.33±0.6667), and between all the field sites and GHD (6.00±0.5774) (Figure 15A).

4.4.2.1.1.2 K2 Number of Pods with Two Seeds (N2S)

In K2 BGA, the comparisons test showed that only Alupe (4.83±0.1667) and Oyani (3.00±0.1155) plants had significant difference ($P=0.0244$). In the greenhouse, Tukey's multiple comparisons test showed that there was significant difference ($P=0.0415$) in N2S between the irrigated K2 (4.00±0.5774) and droughted K2 (2.33±0.3333) plants. The comparisons test showed that amongst the zones, only Alupe K2 plants (4.83±0.1667) had significant difference ($P=0.0032$) with GHD (2.33±0.3333) plants (Figure 15B).

4.4.2.1.1.3 K2 Total Number of Pods (POD)

The mean total number of pods (POD) of K2 BGA was compared in three different zones, and there was significant difference ($P \leq 0.05$) between all the zones. Between Alupe (17.33±0.8819) and Kibos (21.33±0.8819), $P=0.0079$, between Alupe (17.33±0.8819) and Oyani (12.33±0.2404) $P=0.0016$, whereas between Kibos (21.33±0.8819) and Oyani (12.33±0.2404), $P<0.0001$. In the greenhouse, Tukey's multiple comparisons test showed that there was strong significant difference ($P<0.0001$) in POD between K2 GHI (25.00±0.5000) and K2 GHD (8.33±0.3333) BGA. The comparisons test also showed that there was significant difference ($P \leq 0.05$) between all the field sites and the K2 GHI (25.00±0.5000), and between all the field sites and K2 GHD (8.33±0.3333) (Figure 15C).

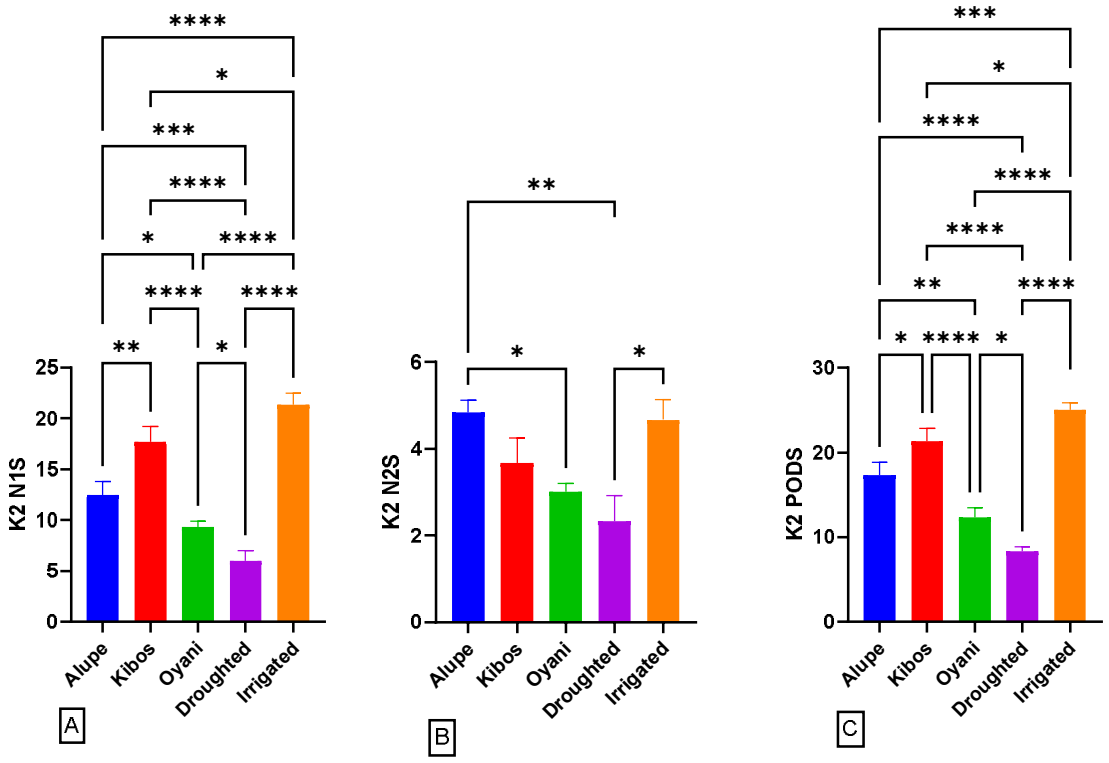


Figure 15A. Number of pods with one seed (N1S) of K2 accession. Figure 15B. Number of pods with two seeds (N2S) of K2 accession. Figure 15C. Total number of pods (POD) of K2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of K2 N1S, N2S and POD between the zones and in greenhouse based on Tukey's multiple comparisons test (one-way ANOVA, P≤0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significances indicated imply that there were no significant differences observed.

4.4.2.1.2 B4 Number of Pods

4.4.2.1.2.1 B4 Number of Pods with One Seed (N1S)

The B4 Number of Pods with one seed (N1S) comparisons across the three agro ecological zones and in the greenhouse is presented (Figure 16A). The comparisons test showed that B4 N1S had significant difference (P=0.0004) between Alupe (23.00±1.155) and Oyani (14.00±0.5774), and between Kibos (21.67±0.8819) and Oyani (14.00±0.5774), where P=0.0015. In the greenhouse, the comparisons test showed that there was significant difference (P=0.0008) in N1S between B4 GHI (20.33±0.8819) and B4 GHD (12.00±1.155) plants. The B4 irrigated plants had significant difference (P=0.0059) in N1S with only B4 plants in Oyani (14.00±0.5774). The comparisons

test also showed that only Alupe and Kibos B4 N1S had significant difference (P≤0.05) with GHD. Strong significant difference (P<0.0001) was however observed between the GHD (12.00±1.155) and Alupe (23.00±1.155), and between GHD (12.00±1.155) and Kibos (21.67±0.8819) where P=0.0002.

4.4.2.1.2.2 B4 Number of Pods with Two Seeds (N2S)

Amongst the zones, Tukey's multiple comparisons test showed that N2S had significant difference (P=0.0013) between Alupe (2.67±0.1453) and Kibos (5.67±0.2333), and between Kibos (5.67±0.2333) and Oyani (3.33±0.2028), where P=0.0079. In the greenhouse, the comparisons test showed that there was significant difference (P=0.0006) in N2S between B4 GHI (5.67±0.6667) and B4 GHD (2.33±0.3333) plants. Only Alupe (2.67±0.1453) and Oyani (3.33±0.2028) N2S had significant difference (P≤0.05) with GHI (5.67±0.6667), whereas only Kibos (5.67±0.2333) had significant difference (P=0.0006) with GHD (2.33±0.3333) BGA (Figure 16B).

4.4.2.1.2.3 B4 Total number of Pods (POD)

In B4 BGA, Tukey's multiple comparisons test showed that POD had significant difference (P=0.0008) between Alupe (25.67±1.299) and Oyani (17.33±0.3756), and between Kibos (27.33±1.011) and Oyani (17.33±0.3756), where P=0.0002. In the greenhouse, the analysis showed that there was strong significant difference (P<0.0001) in POD between B4 GHI (26.00±0.5774) and B4 GHD (14.33±1.202) BGA. The comparisons test showed that only Oyani (17.33±0.3756), had significant difference (P=0.0006) with GHI (26.00±0.5774). Strong significant difference (P<0.0001) was also observed between Alupe (25.67±1.299) and GHD (14.33±1.202), and between Kibos (27.33±1.011) and GHD (14.33±1.202). However, no variation was observed between Oyani (17.33±0.3756) and GHD (14.33±1.202) (Figure 16C).

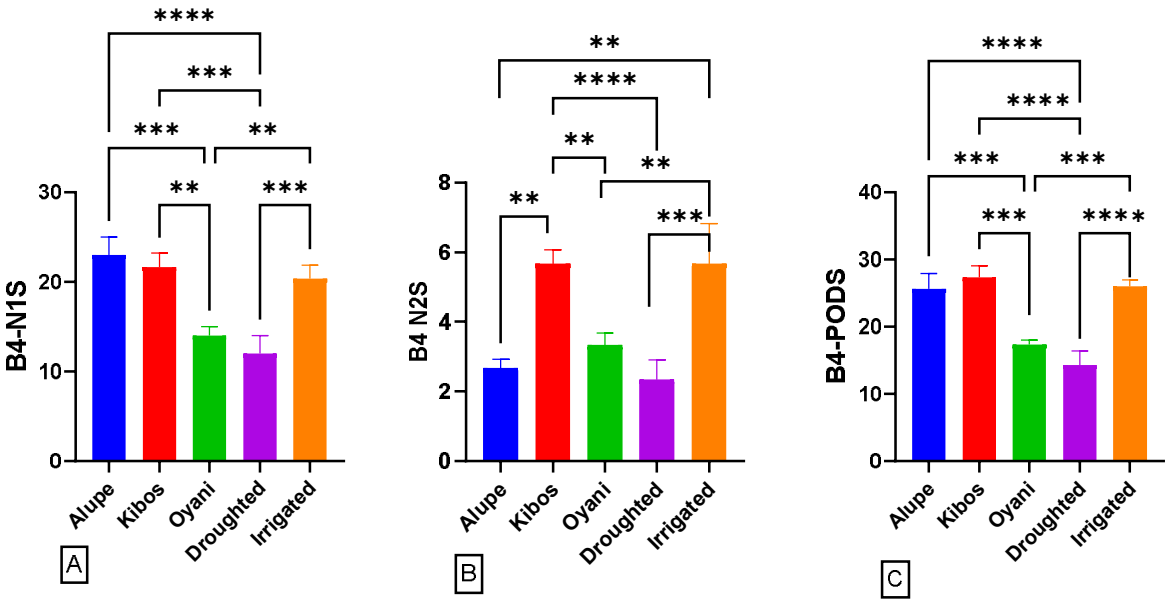


Figure 16A: Number of pods with one seed (N1S) of B4 accession. Figure 16B. Number of pods with two seeds (N2S) of B4 accession. Figure 16C. Total number of pods (POD) of B4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of B4 N1S, N2S and POD between the zones and in greenhouse based on Tukey's multiple comparisons test (one-way ANOVA, P≤0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences observed.

4.4.2.1.3 B1 Number of Pods

4.4.2.1.3.1 B1 Number of Pods with One Seed (N1S)

Amongst the zones, Tukey's multiple comparisons test showed that N1S had significant difference (P=0.0191) between Alupe (19.33±0.6667) and Kibos (26.33±2.333). The significant difference (P=0.0028) was also observed between Kibos (26.33±2.333) and Oyani (17.00±0.5774). In the greenhouse, significant difference (P=0.0003) in N1S was observed between B1 GHI (28.00±1.000) and B1 GHD (15.33±0.8819) plants. The N1S of B1 plants at Alupe and Oyani zones had significant difference (P≤0.05) with GHI plants. The comparisons test showed that amongst the zones, only Kibos (26.33±2.333) had significant difference (P=0.0008) with GHD (15.33±0.8819) B1 plants (Figure 17A).

4.4.2.1.3.2 B1 Total Number of Pods (POD)

The B1 total number of pods (POD) comparisons across the three agro ecological zones and in the greenhouse are presented (Figure 17B). Tukey's multiple comparisons test showed that POD had significant difference (P=0.0270) only between Alupe (23.67±0.8819) and Kibos (30.67±1.856), and between Kibos (30.67±1.856) and Oyani (20.00±0.5774), where P=0.0016. There was significant difference (P=0.0001) in POD between B1 GHI (33.67±1.764) and B1 GHD (19.00±1.155) BGA in the greenhouse. The comparisons test showed that GHI (33.67±1.764) POD had significant difference (P=0.0026) with Alupe (23.67±0.8819), and Oyani (20.00±0.5774) where P=0.0002. Kibos (30.67±1.856) was the only zone that recorded significant difference (P=0.0008) with GHD (19.00±1.155) B1 plants. There was no significant difference in N2S among the Alupe, Kibos, and Oyani zones, nor between the greenhouse treatments (GHI and GHD) and any of the field zones.

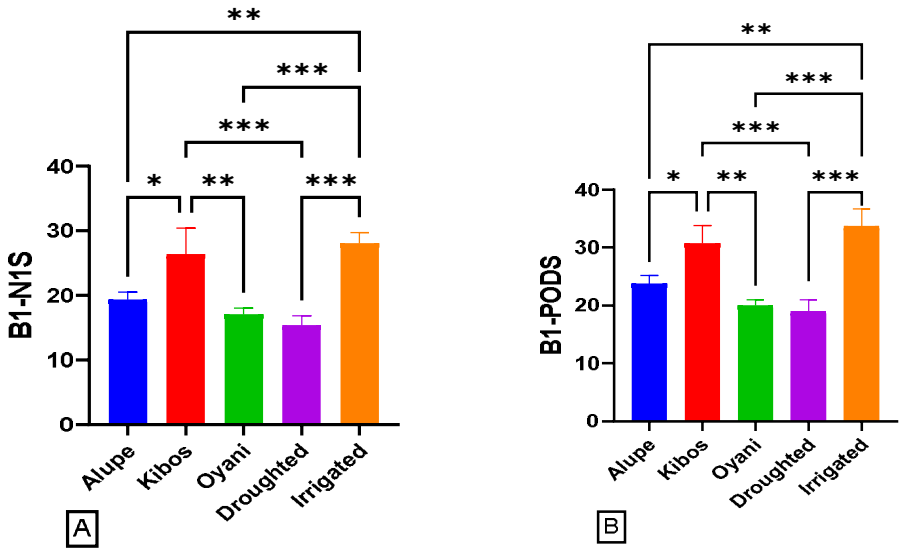


Figure 17A: Number of pods with one seed (N1S) of B1 accession. Figure 17B. Total number of pods (POD) of B1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in green house (Irrigated and Droughted pots). Comparisons of B1 N1S and POD between the zones and in greenhouse based on Tukey's multiple comparisons test (one-way ANOVA, P≤0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences observed.

4.4.2.1.4 K1 Number of Pods

4.4.2.1.4.1 K1 Number of Pods with One Seed (N1S)

Figure 18A summarizes the mean performance of K1 number of pods with one seed (N1S) across the field zones and greenhouse conditions. Among the field sites, the comparison test showed significant differences in N1S between Alupe (18.93 ± 0.8090) and Kibos (27.00 ± 1.528) ($P = 0.0015$), and between Kibos and Oyani (17.00 ± 1.155) ($P = 0.0003$).

In the greenhouse, a significant difference in N1S was observed between GHI (24.67 ± 0.6667) and GHD (15.00 ± 0.5774) ($P = 0.0004$). Additionally, GHI plants differed significantly from those in Alupe ($P = 0.0163$) and Oyani ($P = 0.0023$). A highly significant difference ($P < 0.0001$) was also recorded between Kibos (27.00 ± 1.528) and GHD (15.00 ± 0.5774).

4.4.2.1.4.2 K1 Number of Pods with Two Seeds (N2S)

Amongst the zones, N2S had significant difference (P=0.0122) only between Alupe (2.33±0.1333) and Kibos (5.00±0.2887), and between Kibos (5.00±0.2887), and Oyani (2.00±0.1155), where P=0.0056. In the greenhouse, Tukey's multiple comparisons test showed that there was significant difference (P=0.0056) in N2S between K1 GHI (5.67±0.8819) and K1 GHD (2.67±0.3333) BGA. The significant difference (P=0.0026) was also observed between Alupe (2.33±0.1333) and GHI (5.67±0.8819), and between Oyani (2.00±0.1155) and GHI (5.67±0.8819), where P=0.0013. Amongst the K1 field plants, the comparisons test showed that there was significant difference (P=0.0272) only between Kibos (5.00±0.2887) and GHD (2.67±0.3333) (Figure 18B).

4.4.2.1.4.3 K1 Total Number of Pods (POD)

Figure 18C summarizes the mean performance of K1 total number of pods (POD) across the field zones and greenhouse conditions. Tukey's multiple comparisons test revealed significant differences in POD between Alupe (21.27 ± 0.8253) and Kibos (32.00 ± 1.258) ($P = 0.0002$), and between Kibos and Oyani (19.00 ± 1.217) ($P < 0.0001$).

In the greenhouse, a strong significant difference in POD was observed between K1 GHI (30.33 ± 1.202) and K1 GHD (17.67 ± 0.3333) ($P < 0.0001$). GHI plants also showed significant differences with Alupe ($P = 0.0007$) and Oyani ($P = 0.0001$), but not with Kibos.

Tukey's multiple comparisons test revealed that only Alupe (21.27 ± 0.8253) and Oyani (19.00 ± 1.217) differed significantly from GHI (30.33 ± 1.202), with $P = 0.0007$ and $P = 0.0001$, respectively. When comparing POD across the three field zones and GHD, only Kibos (32.00 ± 1.258) showed a highly significant difference with GHD (17.67 ± 0.3333) (one-way ANOVA, $P < 0.0001$).

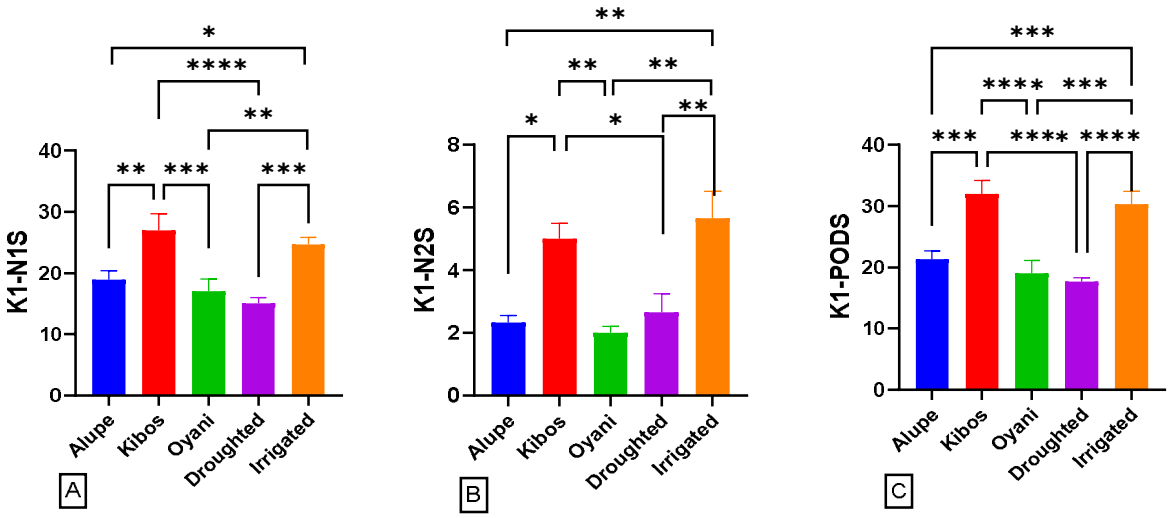


Figure 18A: Number of pods with one seed (N1S) of K1 accession. Figure 18B. Number of pods with two seeds (N2S) of K1 accession. Figure 18C. Total number of pods (POD) of K1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of K1 N1S, N2S and POD between the zones and in greenhouse based on Tukey's multiple comparison test (one-way ANOVA, P≤0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations withno level of significance indicated, imply that there were no significant differences observed.

4.4.2.1.5 M4 Number of Pods

4.4.2.1.5.1 M4 Number of Pods with One Seed (N1S)

When N1S of M4 BGA was compared for the three field zones, using Tukey's multiple comparisons test, the results showed that only Alupe (19.33±0.3333) and Kibos (26.67±2.028) had significant difference (P=0.0213). In the greenhouse, the comparisons test showed that there was significant difference (P=0.0096) in N1S between M4 GHI (24.67±1.202) and M4 GHD (16.33±1.764) BGA. There was no variation between all the field sites and the GHI. Variation however was observed between Kibos (26.67±2.028) and GHD (16.33±1.764), where significant difference was P=0.0021 (Figure 19A).

4.4.2.1.5.2 M4 Number of Pods with Two Seeds (N2S)

In M4 BGA, Tukey's multiple comparisons test showed that N2S had no significant difference (P>0.05) between all the sites. In the greenhouse, the comparisons test showed that there was significant difference (P=0.0101) in N2S between M4 GHI (4.33±0.3333) and M4 GHD (2.33±0.3333) BGA. No variation was observed between all the field sites and the GHI. Variation was only observed between Kibos (4.67±0.2906) and GHD (2.33±0.3333), where P=0.0035 (Figure 19B).

4.4.2.1.5.3 M4 Total number of Pods (POD)

When POD of M4 BGA was compared across the three field zones using Tukey's multiple comparisons test, significant differences were observed between Alupe (22.67±0.2848) and Kibos (31.33±1.737) (P = 0.0047), and between Kibos and Oyani (24.33±0.6667) (P = 0.0190). No significant difference was found between Alupe and Oyani (P = 0.8780). In the greenhouse, a significant difference in POD was observed between M4 GHI (29.00±1.528) and M4 GHD (18.67±1.453) (P = 0.0013). Among the field sites, only Alupe (22.67±0.2848) showed a significant difference when compared with GHI (P = 0.0338). Tukey's test also revealed a significant difference between Kibos (31.33±1.737) and GHD (18.67±1.453) (P = 0.0003) (Figure 19C).

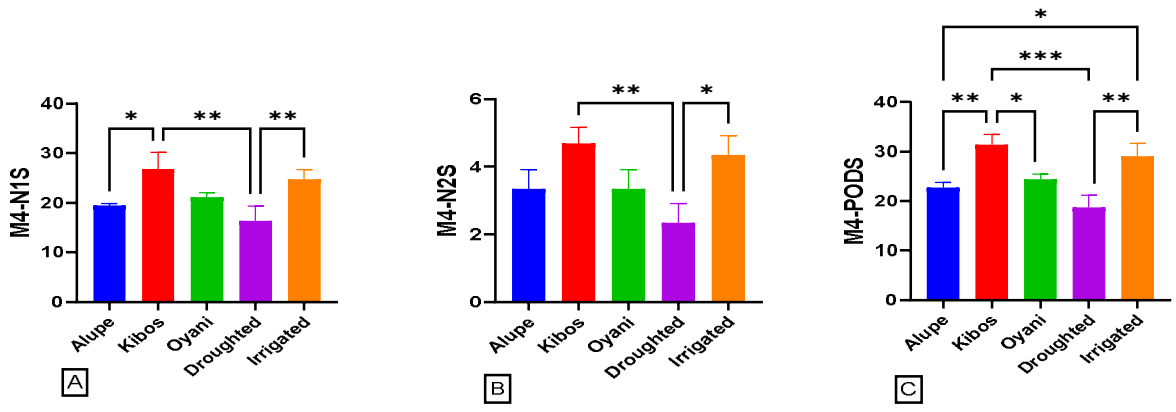


Figure 19A: Number of pods with one seed (N1S) of M4 accession. Figure 19B. Number of pods with two seeds (N2S) of M4 accession. Figure 19C. Total number of pods (POD) of M4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of M4 N1S, N2S and POD between the zones and in greenhouse based on Tukey's multiple comparisons test (one-way ANOVA, P≤0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences observed.

4.4.2.1.6 M2 Number of Pods

4.4.2.1.6.1 M2 Number of Pods with One Seed (N1S)

Figure 20A summarizes the mean performance of M2 number of pods with one seed (N1S) across the field zones and in the greenhouse. Tukey's multiple comparisons test showed a significant difference in N1S between Alupe (18.67±0.3333) and Kibos (27.33±1.764) (P = 0.0037), and between Kibos (27.33±1.764) and Oyani (18.33±1.453) (P = 0.0028).

In the greenhouse, Tukey's test revealed a strong significant difference (P < 0.0001) in N1S between M2 GHI (22.67±1.333) and M2 GHD (9.00±0.5774). No significant difference (P > 0.05) was observed between GHI and any of the field sites. However, significant differences (P < 0.05) were recorded between GHD and all field sites. Specifically, the difference between Alupe (18.67±0.3333) and GHD (9.00±0.5774) was P = 0.0016, between Kibos (27.33±1.764) and GHD (9.00±0.5774) was P < 0.0001, and between Oyani (18.33±1.453) and GHD (9.00±0.5774) was P = 0.0021.

4.4.2.1.6.2 M2 Number of Pods with Two Seeds (N2S)

In M2 BGA, the comparison test showed significant differences in N2S among all three field sites. Between Alupe (3.00±0.1732) and Kibos (4.33±0.2404), P = 0.0297; between Alupe (3.00±0.1732) and Oyani (1.67±0.1667), P = 0.0297; and between Kibos (4.33±0.2404) and Oyani (1.67±0.1667), P = 0.0002.

Significant variation in N2S was also observed between M2 GHI (4.33±0.3333) and M2 GHD (2.33±0.3333), with P = 0.0020. The comparison test further showed that only Alupe (3.00±0.1732) and Oyani (1.67±0.1667) had significant differences (P < 0.05) when compared with GHI (4.33±0.3333). Additionally, significant variation was observed between Kibos (4.33±0.2404) and GHD (2.33±0.3333), with P = 0.0020 (Figure 20B).

4.4.2.1.6.3 M2 Total Number of Pods (POD)

When POD in M2 BGA was compared across the three field zones, the analysis showed significant differences: between Alupe (21.67±0.3756) and Kibos (31.67±1.568), P = 0.0013, and between Kibos (31.67±1.568) and Oyani (20.00±1.443), P = 0.0004.

Among the greenhouse plants, the comparison test revealed a strong significant difference ($P < 0.0001$) in POD between M2 GHI (27.00±1.528) and M2 GHD (11.33±0.6667). Only Oyani (20.00±1.443) showed a significant difference ($P = 0.0154$) when compared with GHI (27.00±1.528).

The comparison test also indicated significant differences ($P \leq 0.05$) between all field sites and GHD. Specifically, between Alupe (21.67±0.3756) and GHD (11.33±0.6667), $P = 0.0010$; between Kibos (31.67±1.568) and GHD (11.33±0.6667), $P < 0.0001$; and between Oyani (20.00±1.443) and GHD (11.33±0.6667), $P = 0.0037$ (Figure 20C).

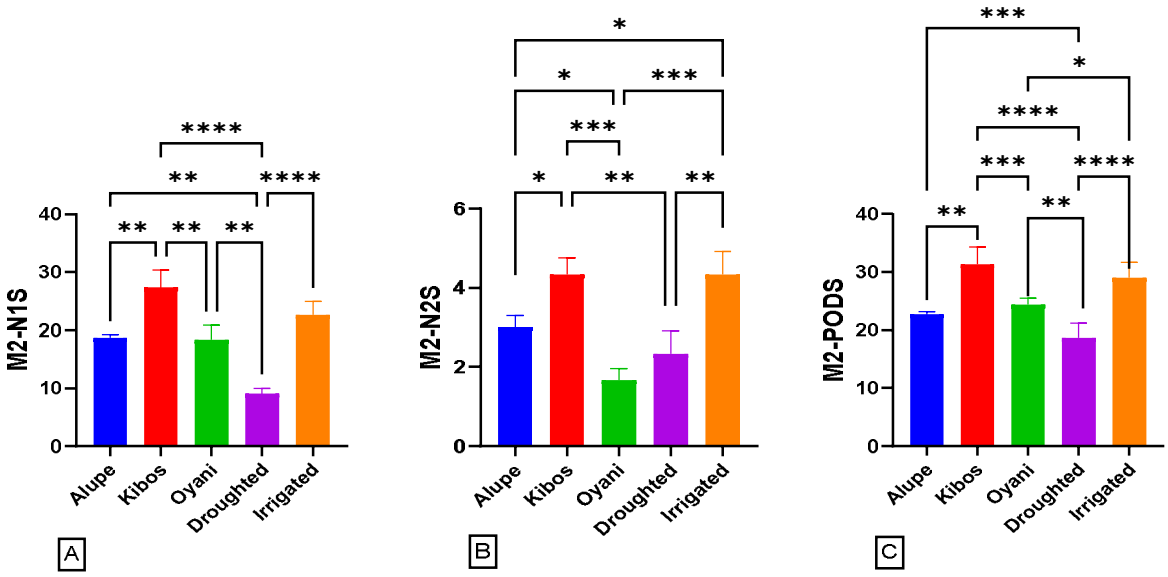


Figure 20A: Number of pods with one seed (N1S) of M2 accession. Figure 20B. Number of pods with two seeds (N2S) of M2 accession. Figure 20C. Total number of pods (POD) of M2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of M2 N1S, N2S and POD between the zones and in greenhouse based on Tukey's multiple comparisons test (one-way ANOVA, P≤0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences observed.

4.4.2.2 Number of Pods Intra-site Comparisons.

4.4.2.2.1 Number of Pods Intra-site Comparisons in Alupe

4.4.2.2.1.1 Number of Pods with One Seed (N1S) intra-site comparisons in Alupe

In Alupe, B4 had the highest number of pods with one seed (N1S) (23.00±1.155), followed by B1 (19.33±0.6667) and then M4 (19.33±0.3333). On the contrary K2 (12.50±0.7638) had the lowest means. The analysis (one-way ANOVA, $P<0.05$, Tukey's multiple comparisons test) showed that there was significant difference ($P\leq0.05$) in most of the accessions comparisons, except in six comparisons wherethere were no significant differences (Table 10a).

4.4.2.2.1.2 Number of Pods with Two Seeds (N2S) Intra-Site Comparisons in Alupe

At Alupe zone, K2 accession had the highest Number of Pods with two seeds (N2S) (4.83±0.1667), followed by B1 (4.33±0.2028) and then M4 (3.33±0.1453), whereas K1 had the lowest number (2.33±0.1333) of pods with two seeds. The analysis (one-way ANOVA, $P\leq0.05$, Tukey's multiple comparisons test) showed that there were significant differences in some of the accessions comparisons for instance between K2 (4.83±0.1667) and B4 (2.67±0.1453), and between K2 (4.83±0.1667) and M2 (3.00±0.1732) where $P<0.0001$. (Table 10b).

4.4.2.2.1.3 Total Number of Pods per Plant (POD) Intra-Site Comparisons in Alupe

In this zone, B4, B1 and M4 accessions had the highest numbers of POD at 25.67±1.299, 23.67±0.8819 and 22.67±0.2848 respectively. On the contrary K2 had the lowest POD (17.33±0.8819). The analysis (one-way ANOVA, $P\leq0.05$, Tukey's multiple comparisons test) showed that there was significant difference ($P\leq0.05$) in six accessions comparisons (Table 10c).

Table 10a: Number of Pods with One Seed (N1S) intra-site comparisons in Alupe

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Alupe	12.50± 0.7638	23.00± 1.155	19.33± 0.6667	18.93± 0.8090	19.33± 0.3333	18.67± 0.3333	K2 vs. B4 K2 vs. B1 K2 vs. M2 B4 vs. K1 B4 vs. M2 K2 vs. K1 K2 vs. M4 B4 vs. B1 B4 vs. M4	<0.0001 0.0003 0.0008 0.0196 0.0127 0.0005 0.0003 0.0375 0.0375

Acc accession. All other nine comparisons were highly significant at P≤0.05, apart from B1 vs. K1, B1 vs. M4, B1 vs. M2, K1 vs. M4, K1 vs. M2 and M4 vs. M2 where there were no significant differences (P>0.05). Number of Pods with One Seed expressed as Mean±Standard Error of Mean (SEM).

Table 10b: Number of Pods with Two Seeds (N2S) intra-site comparisons in Alupe

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Alupe	4.83± 0.1667	2.67± 0.1453	4.33± 0.2028	2.33± 0.1333	3.33± 0.1453	3.00± 0.1732	K2 vs. B1 B1 vs. M4 B1 vs. K1 K1 vs. M4 B1 vs. M2	0.6561 0.0833 0.0006 0.0833 0.0151

Acc accession. The comparisons K2 vs. B4, K2 vs. M4, K2 vs. M2, B4 vs. B1, B1 vs. K1, K2 vs. K1 and B1 vs. M2were significant at P≤0.05. The other eight comparisons had no significant differences (P>0.05). Number of Pods with two seedsexpressed as Mean±Standard Error of Mean (SEM).

Table 10c: Total Number of Pods per Plant (POD) Intra-Site Comparisons in Alupe

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Alupe	17.33± 0.8819	25.67± 1.299	23.67± 0.8819	21.27± 0.8253	22.67± 0.2848	21.67± 0.3756	K2 vs. K1 B4 vs. B1 K1 vs. M2 K1 vs. M4 M4 vs. M2	0.0511 0.5555 0.9992 0.8331 0.9514

Acc accession. Most comparisons had no significant differences (P>0.05) apart from K2 vs. B4where P≤0.0001, K2 vs. B1 where P≤0.0017, K2 vs. M4 where P≤0.0069, B4 vs. K1 where P≤0.0262, K2 vs. M2 where P≤0.0288 and B4 vs. M2 where P≤0.0465. Total Number of Pods expressed as Mean±Standard Error of Mean (SEM).

4.4.2.2.2 Number of Pods Intra-site Comparisons in Kibos

4.4.2.2.2.1 Number of Pods with One Seed (N1S) Intra-Site Comparisons in Kibos

In Kibos, M2 had the highest Number of Pods with one seed (N1S) (27.33±1.764), followed by K1 (27.00±1.528) and then M4 (26.67±2.028). On the contrary K2 (17.67±0.8819) and B4 (21.67±0.8819) had the lowest means. The comparisons testshowed that there were no significant differences (P>0.05) in most of the accessions comparisons, except in few comparisons where there were significant differences. (Table 11a).

4.4.2.2.2.2 Number of Pods with Two Seeds (N2S) Intra-Site Comparisons in Kibos

In Kibos zone, B4 had the highest number of pods with two seeds (N2S) (5.67±0.2333), followed by K1 (5.00±0.2887) and then M4 (4.67±0.2906). The K2 accession had the lowest number of pods with two seeds at a mean of 3.67±0.3333. The comparisons testshowed that there were no significant differences (P>0.05) in all of the accessions comparisons except in one comparison, between K2 (3.67±0.3333) and B4 (5.67±0.2333), where the significant difference was P≤0.0242 (Table 11b).

4.4.2.2.2.3 Total Number of Pods per Plant (POD) Intra-Site Comparisons in Kibos

In this zone, K1 had the highest POD (32.00±1.258), followed by M2 (31.67±1.568) and then M4 (31.33±1.737). On the contrary K2 had the lowest POD at a mean of 21.33±0.8819. The comparisons test showed that there were no significant differences (P>0.05) in most of the accessions comparison, except in four comparisons (Table 11c).

Table 11a: Number of Pods with One Seed (N1S) Intra-Site Comparisons in Kibos

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Kibos	17.67± 0.8819	21.67± 0.8819	26.33± 2.333	27.00± 1.528)	26.67± 2.028	27.33± 1.764	K2 vs. B1 B4 vs. M2 M4 vs. M2 K2 vs. M4 K1 vs. M4	0.0287 0.2259 0.9997 0.0226 >0.9999

Acc accession. Most comparisons had no significant differences (P>0.05) apart from K2 vs. B1 where P≤0.0287, K2 vs. K1 where P≤0.0177, K2 vs. M4 where P≤0.0226 and K2 vs. M2 where P≤0.0140. Number of Pods with one seed expressed as Mean±Standard Error of Mean (SEM).

Table 11b: Number of Pods with Two Seeds (N2S) Intra-Site Comparisons in Kibos

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Kibos	3.67± 0.3333	5.67± 0.2333	4.33± 0.2404	5.00± 0.2887	4.67± 0.2906	4.33± 0.2404	K2 vs. B1 B4 vs. B1 B1 vs. K1 K1 vs. M K2 vs. B4	0.7985 0.1908 0.7985 0.7985 0.0242

Acc accession. Allcomparisons had no significant differences (P>0.05) apart from K2 vs B4 where the significant difference was P≤0.0242. Number of Pods with two seeds expressed as Mean±Standard Error of Mean (SEM).

Table 11c: Total Number of Pods (POD) Intra-Site Comparisons in Kibos

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Kibos	21.33± 0.8819	27.33± 1.011	30.67± 1.856	32.00± 1.258	31.33± 1.737	31.67± 1.568	K2 vs. B1 K2 vs. K1 K2 vs. M4 K2 vs. M2	0.0061 0.0021 0.0036 0.0027

Acc accession. Most comparisons had no significant differences (P>0.05) apart from the four comparisons highlighted. Total Number of Pods expressed as Mean±Standard Error of Mean (SEM).

4.4.2.2.3 Number of Pods Intra-site Comparisons in Oyani.

4.4.2.2.3.1 Number of Pods with one seed (N1S) intra-site comparisons in Oyani

In this zone, M4 had the highest number of pods with one seed (N1S) (21.00±0.5774) followed by M2 (18.33±1.453) and then B1 (17.00±0.5774). The K2 accession had the lowest mean (9.333±0.3333). The analysis (one-way ANOVA, P<0.05, Tukey's multiple comparisons test) showed that there was no significant difference (P>0.05) in most of the accessions comparison, except in few comparisons where there were significant differences (Table 12a).

4.4.2.2.3.2 Number of Pods with Two Seeds (N2S) Intra-Site Comparison in Oyani

In this zone, B4 (3.33±0.2028), and M4 (3.33±0.3333), had the highest Number of pods with two seeds (N2S), while M2 had the lowest number of N2S at a mean of 1.67±0.1667. The analysis (one-way ANOVA, P<0.05, Tukey's multiple comparisons test) showed that there was significant difference (P<0.05) in only two of the fifteen accessions comparisons (Table 12b).

4.4.2.2.3.3 Total Number of Pods per Plant (POD) Intra-Site Comparison in Oyani

In Oyani, M4 had the highest total number of pods (POD) (24.33±0.6667), followed by M2 (20.00±1.443) and B1 (20.00±0.5774), while K2 had the lowest total number of pods at a mean of 12.33±0.2404. The comparisons testshowed that there were significant differences (P<0.05) in most of the accessions comparisons, except in six comparisons where there were no significant differences (Table 12c).

Table 12a: Number of Pods with one seed (N1S) intra-site comparisons in Oyani

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Oyani	9.333± 0.3333	14.00± 0.5774	17.00± 0.5774	17.00± 1.155	21.00± 0.5774	18.33± 1.453	K2 vs. B4 K2 vs. B1 K2 vs. M4 K2 vs. M2 B4 vs. M4 K2 vs. K1	0.0243 0.0005 <0.0001 0.0001 0.0011 0.0005

Acc accession. Most comparisons had no significant differences (P>0.05) apart from the six comparisons highlighted. Total Number of Pods expressed as Mean±Standard Error of Mean (SEM).

Table 12b: Number of Pods with two seeds (N2S) Intra-Site Comparison in Oyani

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Oyani	3.000± 0.1155	3.333± 0.2028	3.000± 0.5774	2.000± 0.1155	3.33± 0.3333	1.67± 0.1667	K2 vs. B4 K2 vs. M2 B4 vs. M2 B1 vs. M2 M4 vs. M2	0.9648 0.0717 0.0191 0.0717 0.0191

Acc accession. All the comparisons had no significant differences (P>0.05) apart from B4 vs M2 and M4 vs. M2 comparisons where the significant differences were P<0.05. Number of Pods with two seeds expressed as Mean±Standard Error of Mean (SEM).

Table 12c: Total Number of Pods per Plant (POD) Intra-Site Comparison in Oyani

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Oyani	12.33± 0.2404	17.33± 0.3756	20.00± 0.5774	19.00± 1.217	24.33± 0.6667	20.00± 1.443	K2 vs. B4 K2 vs. B1 K2 vs. K1 K2 vs. M4 K2 vs. M2 B4 vs. M4 B1 vs. M4 K1 vs. M4 M4 vs. M2	0.0152 0.0005 0.0017 <0.0001 0.0005 0.0011 0.0380 0.0097 0.0380

Acc accession. Nine comparisons had significant differences (P<0.05) apart from six comparisons which had no significant differences (P>0.05). Total number of pods expressed as Mean±Standard Error of Mean (SEM).

4.4.2.2.4 Number of Pods Intra-site Comparisons in Green House Irrigated Plants.

4.4.2.2.4.1 Number of Pods with One Seed (N1S) intra-GHI Comparisons

In GHI, B1 recorded the highest N1S (28.00±1.000), followed by K1 (24.67±0.6667) and M4 (24.67±1.202), while B4 (20.33±0.8819) had the lowest number of pods with one seed. The analysis (one-way ANOVA, $P \leq 0.05$; Tukey's multiple comparisons test) showed no significant differences ($P > 0.05$) in most accession comparisons, except for three where significant differences ($P \leq 0.05$) were observed: between K2 (21.33±0.6667) and B1 (28.00±1.000), P = 0.0048; between B4 (20.33±0.8819) and B1 (28.00±1.000), P = 0.0015; and between B1 (28.00±1.000) and M2 (22.67±1.333), P = 0.0235 (Table 13a).

For the number of pods with two seeds (N2S), comparisons among all accessions showed no significant differences ($P > 0.05$) and were therefore not tabulated.

4.4.2.2.4.2 Total Number of Pods (POD) intra-GHI Comparisons

Amongst the irrigated plants, B1 had the highest POD (33.67±1.764), followed by K1 (30.33±1.202) and then M4 (29.00±1.528), whereas K2 had the lowest POD (25.00±0.5000). The comparisons testshowed that there were no significant differences (P>0.05) in most of the accessions comparisons, except in three comparisons where there were significant differences. For instance, there were significant differences between K2 (25.00±0.5000) and B1 (33.67±1.764) where P=0.0045, between B4 (26.00±0.5774) and B1 (33.67±1.764) where P=0.0113 and between B1 (33.67±1.764) and M2 (27.00±1.528) where P=0.0288 (Table 13b).

Table 13a: Number of Pods with one seed (N1S) intra-GHI Comparisons

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
GHI	21.33± 0.6667	20.33± 0.8819	28.00± 1.000	24.67± 0.6667	24.67± 1.202	22.67± 1.333	K2 vs. B4 B4 vs. M4 B1 vs. M2 K1 vs. M2 B4 vs. M2	0.9765 0.0778 0.0235 0.7116 0.5759

Acc accession. All comparisons had no significant differences (P>0.05) apart from K2 vs. B1 where P≤0.0048, B4 vs. B1 where P≤0.0015 and B1 vs. M2 where P≤0.0235. Number of Pods with one seed expressed as Mean±Standard Error of Mean (SEM).

Table 13b: Total Number of Pods (POD) intra-GHI Comparisons

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
GHI	25.00± 0.5000	26.00± 0.5774	33.67± 1.764	30.33± 1.202	29.00± 1.528	27.00± 1.528	K2 vs. B1 B1 vs. M2 B4 vs. B1	0.0066 0.0306 0.0121

Acc accession. Most comparisons had no significant differences (P>0.05) apart from the three comparisons highlighted. Total Number of Pods expressed as Mean±Standard Error of Mean (SEM).

4.4.2.2.5 Number of Pods Intra-site Comparisons in Green House Droughted (GHD) Plants

4.4.2.2.5.1 Number of Pods with One Seed (N1S) intra-GHD Comparisons

In GHD, M4 had the highest N1S (16.33±1.764), followed by B1 (15.33±0.8819) and then K1 (15.00±0.5774), while K2 (6.00±0.5774) had the lowest number of pods with one seed. The comparisons test showed that there were significant differences ($P \leq 0.05$) in seven of the accessions comparisons, while in eight comparisons there were no significant differences (Table 14a). For the number of pods with two seeds (N2S), comparisons among all accessions showed no significant differences ($P > 0.05$) and were therefore not tabulated.

4.4.2.2.5.2 Total Number of Pods per Plant (POD) Intra-GHD Comparisons

Amongst the droughted plants, B1 accession had the highest POD (19.00±1.155), followed by M4 (18.67±1.453). On the contrary K2 had the lowest POD mean (8.333±0.3333). The analysis (one-way ANOVA, $P \leq 0.05$, Tukey's multiple comparisons test) showed that there were significant differences in most of the accessions comparisons (Table 14b).

Table 14a: Number of Pods with One Seed (N1S) intra-GHD Comparisons

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
GHD	6.00± 0.5774	12.00± 1.155	15.33± 0.8819	15.00± 0.5774	16.33± 1.764	9.000± 0.5774	K2 vs. B4 K2 vs. B1 K2 vs. K1 K2 vs. M4 B1 vs. M2 K1 vs. M2 M4 vs. M2	0.0128 0.0003 0.0005 0.0001 0.0087 0.0128 0.0028

Acc accession. Most comparisons had no significant differences (P>0.05) apart from the seven comparisons highlighted. Number of Pods with one seed expressed as Mean±Standard Error of Mean (SEM).

Table 14b: Total Number of Pods per Plant (POD) Intra-GHD Comparisons

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
GHI	8.333± 0.3333	14.33± 1.202	19.00± 1.155	17.67± 0.3333	18.67± 1.453	11.33± 0.6667	K2 vs. B4 K2 vs. B1 K2 vs. K1 K2 vs. M4 B4 vs. B1 B1 vs. M2 K1 vs. M2 M4 vs. M2	0.0085 <0.0001 0.0002 <0.0001 0.0444 0.0012 0.0057 0.0017

Acc accession. Eight comparisons highlighted had significant differences (P<0.05) apart from seven comparisons which had no significant differences (P>0.05). Total Number of Pods expressed as Mean±Standard Error of Mean (SEM).

4.4.3 Terminal Leaflet Area (TLA cm2)

4.4.3.1 Terminal Leaflet Area (TLA cm2) Inter-Sites Comparisons

4.4.3.1.1 K2 Terminal Leaflet Area (TLA cm2)

In K2 BGA, Tukey's multiple comparisons test showed that terminal leaflet area (TLA cm^2) differed significantly among all three field sites. The analysis revealed a strong significant difference ($P < 0.0001$) between Alupe (24.28±0.3079) and Oyani (16.78±0.03512), and between Kibos (25.82±0.09866) and Oyani (16.78±0.03512). A smaller but still significant difference was observed between Alupe (24.28±0.3079) and Kibos (25.82±0.09866), $P = 0.0137$.

Among the greenhouse plants, a strong significant difference ($P < 0.0001$) in TLA was observed between K2 GHI (29.04±0.4915) and K2 GHD (12.33±0.02333). Similarly, strong significant differences ($P < 0.0001$) were recorded between all field sites and GHI (29.04±0.4915), as well as between all field sites and GHD (12.33±0.02333) (Figure 21).

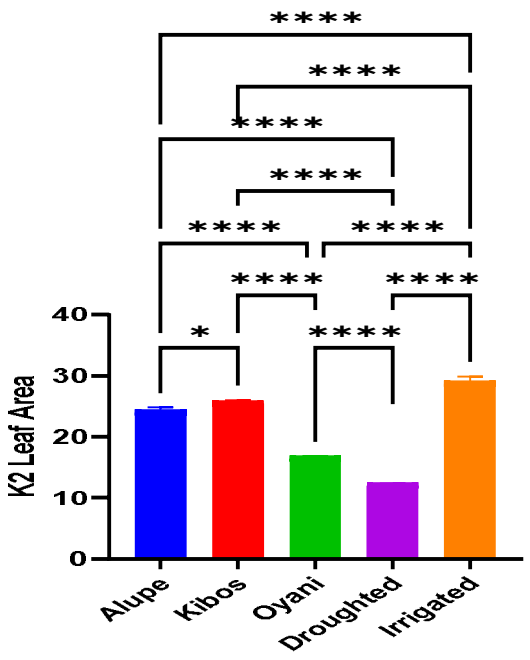


Figure 21: The Terminal leaflet Area of K2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of K2 Terminal leaflet Area between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, *P*≤0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.3.1.2 B4 Terminal Leaflet Area (TLA cm2)

Figure 22 summarizes the mean performance of the B4 accession TLA in the three zones and in the greenhouse. Tukey's multiple comparisons test showed that TLA had strong significant difference ($P<0.0001$) between Alupe (26.05±0.07638) and Oyani (18.08±0.08327), and between Kibos (26.41±0.3378) and Oyani (18.08±0.08327). The strong significant difference ($P<0.0001$) in TLA was also observed between B4 GHI (27.89±0.9682) and B4 GHD (15.63±0.01453) BGA. Only B4 accession at Oyani had significant difference ($P<0.05$) with GHI. The comparisons test showed that TLA had significant difference between all the sites and GHD (15.63±0.01453).

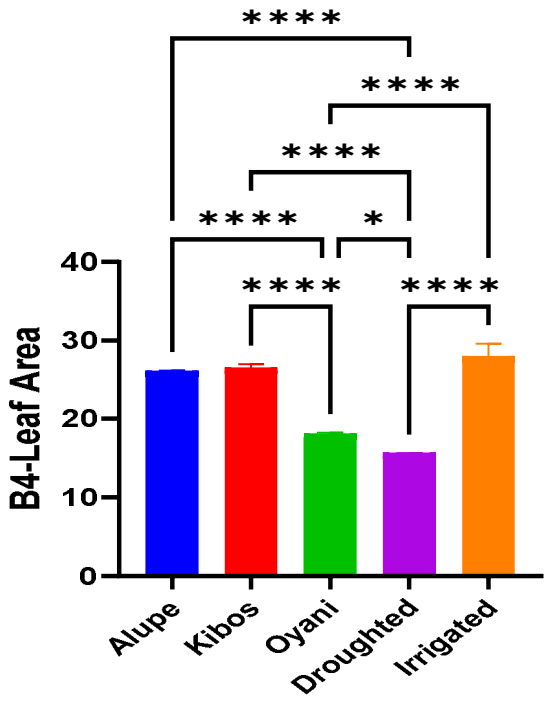


Figure 22: The Terminal leaflet Area of B4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of B4 Terminal leaflet Area between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, $P \leq 0.05$) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.3.1.3 B1 Terminal Leaflet Area (TLA cm^2)

In B1 BGA, Tukey's multiple comparisons test showed a strong significant difference ($P < 0.0001$) in TLA between Alupe (24.01±0.2354) and Oyani (20.52±0.2643), and between Kibos (25.04±0.1504) and Oyani (20.52±0.2643). However, no significant difference was observed between Alupe (24.01±0.2354) and Kibos (25.04±0.1504).

The comparisons test also revealed a significant difference ($P < 0.0001$) in TLA between B1 GHI (31.57±0.4256) and B1 GHD (21.86±0.0153) BGA. Significant differences ($P \leq 0.05$) were observed between all the field sites and GHI (31.57±0.4256), as well as between all the field sites and GHD (Figure 23).

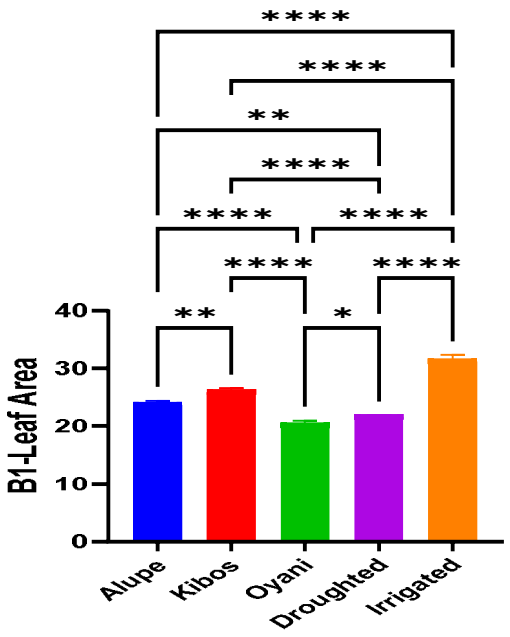


Figure 23: The Terminal leaflet Area of B1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of B1 Terminal leaflet Area between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, $P \leq 0.05$) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.3.1.4 K1 Terminal Leaflet Area (TLA cm2)

The TLA of K1 accession across the three zones and in the greenhouse is presented in Figure 24. GHI recorded the largest TLA (25.04 cm²), followed by Kibos (22.16 cm²). Tukey's multiple comparisons test showed a strong significant difference ($P < 0.0001$) in TLA between all the field sites: Alupe (15.85±0.2674), Kibos (22.16±0.2318), and Oyani (18.78±0.02646). A strong significant difference ($P < 0.0001$) was also observed between K1 GHI (25.04±0.3496) and K1 GHD (20.26±0.01732) BGA.

TLA values from all field sites showed strong significant differences ($P < 0.0001$) when compared with GHI (25.04±0.3496). Additionally, a strong significant difference was found between Alupe (15.85±0.2674) and GHD (20.26±0.01732) ($P < 0.0001$). Significant differences were also observed between Kibos (22.16±0.2318) and GHD ($P = 0.0009$), and between Oyani (18.78±0.02646) and GHD ($P = 0.0059$).

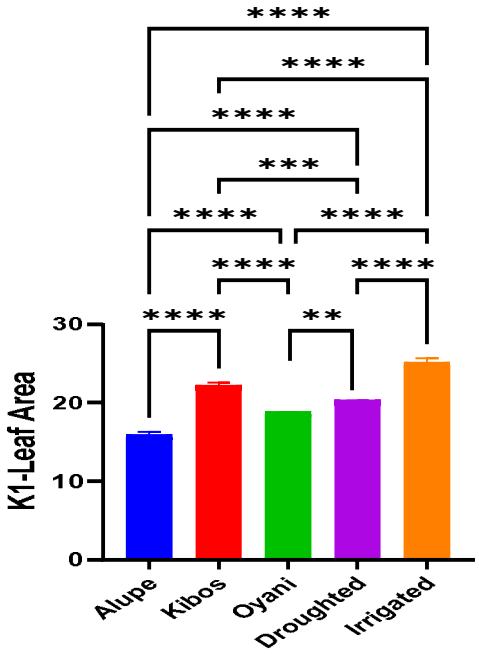


Figure 24: The Terminal leaflet Area of K1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of K1 Terminal leaflet Area between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, *P*≤0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.3.1.5 M4 Terminal Leaflet Area (TLA cm2)

In M4 BGA, Tukey's multiple comparisons test showed a strong significant difference ($P < 0.0001$) in TLA between Alupe (17.79±0.3819) and Kibos (23.26±0.2619), and between Alupe (17.79±0.3819) and Oyani (25.76±0.01528). The difference between Kibos (23.26±0.2619) and Oyani (25.76±0.01528) was also significant ($P = 0.0002$). A strong significant difference ($P < 0.0001$) was observed between M4 GHI (27.48±0.1513) and M4 GHD (12.61±0.2310) BGA.

TLA values from Alupe (17.79±0.3819) and Kibos (23.26±0.2619) showed strong significant differences ($P < 0.0001$) when compared with GHI (27.48±0.1513). Oyani (25.76±0.01528) also differed significantly from GHI ($P = 0.0036$). Additionally, M4 accession TLA showed strong significant differences ($P < 0.0001$) between all field sites and GHD (12.61±0.2310) (Figure 25).

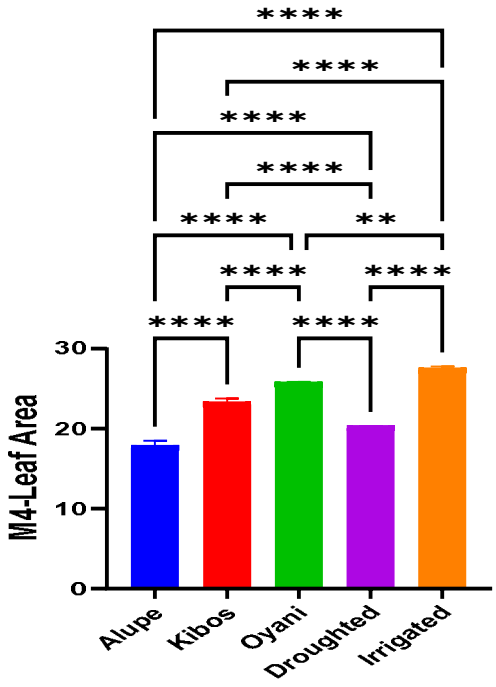


Figure 25: The Terminal leaflet Area of M4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of M4 Terminal leaflet Area between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, $P \leq 0.05$) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.3.1.6 M2 Terminal Leaflet Area (TLA cm2)

In M2 accession, Tukey's multiple comparisons test showed that TLA had significant difference (P=0.0113) between Alupe (24.00±0.5774) and Oyani (22.28±0.0200), and between Kibos (26.21±0.1903) and Oyani (22.28±0.02000) where P<0.0001. Strong significant difference (P<0.0001) in TLA was observed between M2 GHI (28.23±0.07211) and M2 GHD (12.94±0.1802) BGA. The significant difference ($P \leq 0.05$) in TLA was also observed between all the field sites and greenhouse irrigated (28.23±0.07211) and between all the field sites and droughted M2 (12.94±0.1802) plants (Figure 26).

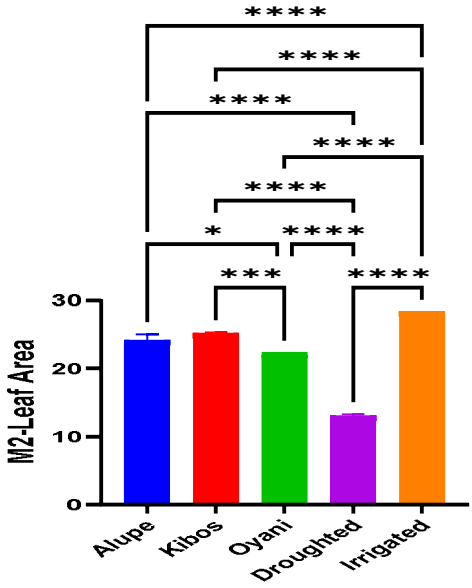


Figure 26: The Terminal leaflet Area of M2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of M2 Terminal leaflet Area between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, $P \leq 0.05$) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.3.2 Terminal Leaflet Area Intra-Sites Comparisons

4.4.3.2.1 Terminal Leaflet Area (TLA cm2) Intra-Site Comparisons in Alupe

In Alupe, B4 had the highest TLA cm^2 (26.05±0.0764), followed by K2 (24.28±0.3079) and then B1 (24.01±0.2354). On the contrary K1 (15.85±0.2674) had the lowest mean. The analysis (one-way ANOVA, $P \leq 0.05$, Tukey's multiple comparisons test) showed there were significant differences ($P \leq 0.05$) in most of the accessions comparisons, except in three (Table 15a).

4.4.3.2.2 Terminal Leaflet Area (TLA cm2) Intra-Site Comparisons in Kibos

In Kibos, B4 had the highest TLA cm^2 (26.41±0.3378), followed by M2 (26.21±0.1903) and then K2 (25.82±0.0987), whereas K1 (22.16±0.2318) had the lowest mean. The analysis (one-way ANOVA, $P \leq 0.05$, Tukey's multiple comparisons test) showed that there were significant differences ($P < 0.05$) in most of the accessions comparisons except between four comparisons (Table 15b).

4.4.3.2.3 Terminal Leaflet Area (TLA cm2) Intra-Site Comparisons in Oyani

In Oyani, M4 accession had the highest TLA cm^2 (25.76±0.0153), followed by M2 (22.28±0.0200) and then B1 (20.52±0.2643), while K2 (16.78±0.0351) had the lowest mean. The comparisons test showed that there was strong significant difference (P<0.0001) in all the accessions comparisons except in one (Table 15c).

4.4.3.2.4 Terminal Leaflet Area (TLA cm2) intra-Greenhouse Irrigated (GHI) Comparisons

In GHI, B1 had the highest TLA cm^2 (31.57±0.4256), followed by K2 (29.04±0.4915) and then M2 (28.23±0.0721). On the contrary K1 (25.04±0.3496) had the lowest mean. The analysis (one-way ANOVA, P≤0.05, Tukey's multiple comparisons test) showed that there were significant differences (P<0.05) in some of the accessions comparisons (Table 15d).

4.4.3.2.5 Terminal Leaflet Area (TLA cm2) Intra-Greenhouse Droughted (GHD) Comparisons

Among the droughted plants, B1 had the highest TLA (21.86±0.0153 cm^2), followed by K1 (20.26±0.01732), with B4 having a mean of 15.63±0.01453. In contrast, K2 recorded the lowest mean (12.33±0.02333). Tukey's multiple comparisons test showed strong significant differences ($P < 0.0001$) across all accession comparisons, except between K2 (12.33±0.02333) and M2 (12.94±0.1802), where $P = 0.0345$. However, no significant difference was observed between K2 (12.33±0.02333) and M4 (12.61±0.2310) ($P = 0.5888$), or between M4 (12.61±0.2310) and M2 (12.94±0.1802) ($P = 0.4275$) (Table 15e).

Table 15a: Terminal Leaflet Area (TLA cm^2) Intra-Site Comparisons in Alupe

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Alupe	24.28± 0.3079	26.05± 0.0764	24.01± 0.2354	15.85± 0.2674	17.79± 0.3819	24.00± 0.5774	K2 vs. K1 K2 vs. M4 B1 vs. K1 B1 vs. M4 K1 vs. M4	<0.0001 <0.0001 <0.0001 <0.0001 0.0172

Acc accession. Other than K2 vs. B1, K2 vs. M2 and B1 vs. M2 where there were no significant differences ($P \geq 0.05$), all other comparisons were highly significant at $P \leq 0.0001$ except K2 vs. B4 where $P \leq 0.0308$, B4 vs. B1 where $P \leq 0.0122$, B4 vs. M2 where $P \leq 0.0116$ and K1 vs. M4 where $P \leq 0.0172$. Terminal leaflet area expressed as Mean±Standard Error of Mean (SEM).

Table 15b: Terminal Leaflet Area (TLA cm^2) Intra-Site Comparisons in Kibos

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Kibos	25.82± 0.0987	26.41± 0.3378	25.04± 0.1504	22.16± 0.2318	23.46± 0.2619	26.21± 0.1903	K2 vs. K1 K2 vs. M4 B4 vs. K1 B4 vs. M4 K1 vs. M2	<0.0001 <0.0001 <0.0001 <0.0001 <0.0001

Acc accession. Apart from K2 vs. B4, K2 vs. B1, K2 vs. M2 and B4 vs. M2 where there were no significant differences (P>0.05), all other comparisons were highly significant at P<0.0001 except B4 vs. B1 where P≤0.0103, B1 vs. M4 where P≤0.0013, B1 vs. M2 where P≤0.0291 and K1 vs. M4 where P≤ 0.0437. Terminal leaflet area expressed as Mean±Standard Error of Mean (SEM).

Table 15c: Terminal Leaflet Area (TLA cm^2) Intra-Site Comparisons in Oyani

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Oyani	16.78± 0.0351	18.08± 0.0833	20.52± 0.2643	18.78± 0.0265	25.76± 0.0153	22.28± 0.0200	K2 vs. B4 K2 vs. M2 B4 vs. M2 K1 vs. M4 M4 vs. M2	<0.0001 <0.0001 <0.0001 <0.0001 <0.0001

Acc accession. All comparisons were highly significant at P≤0.0001 except B4 vs. K1 where P≤0.0102. Terminal leaflet area expressed as Mean±Standard Error of Mean (SEM).

Table 15d: Terminal Leaflet Area (TLA cm^2) intra-Greenhouse Irrigated (GHI) Comparisons

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
GHI	29.04± 0.4915	27.89± 0.9682	31.57± 0.4256	25.04± 0.3496	27.48± 0.1513	28.23± 0.0721	K2 vs. B1 K2 vs. K1 B4 vs. B1 B4 vs. K1 B1 vs. K1 B1 vs. M4 B1 vs. M2 K1 vs. M4 K1 vs. M2	0.0356 0.0012 0.0024 0.0165 <0.0001 0.0010 0.0053 0.0437 0.0074

Acc accession. The nine comparisons highlighted had significant differences (P<0.05), whereas six comparisons had no significant differences (P>0.05). Terminal leaflet area expressed as Mean±Standard Error of Mean (SEM).

Table 15e: Terminal Leaflet Area (TLA cm^2) intra-Intra-Green house Droughted (GHD) Comparisons

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
GHD	12.33± 0.0233	15.63± 0.0145	21.86± 0.0153	20.26± 0.0173	12.61± 0.2310	12.94± 0.1802	K2 vs. K1 B4 vs. B1 B4 vs. M4 B1 vs. M2 K1 vs. M2	<0.0001 <0.0001 <0.0001 <0.0001 <0.0001

Acc accession. Apart from K2 vs. M4 and M4 vs. M2 where there were no significant differences (P>0.05), all other comparisons were highly significant at P<0.0001 except K2 vs. M2 where P≤0.0345. Terminal leaflet areaexpressed as Mean±Standard Error of Mean (SEM).

4.4.4 Number of Flowers per Plant (NFP)

4.4.4.1 Number of Flowers per Plant (NFP) Inter-Sites Comparisons

4.4.4.1.1 K2 Number of Flowers per Plant (NFP)

In K2 BGA, Tukey's multiple comparisons test revealed significant differences (P<0.05) in the number of flowers (NFP) across all field sites. Between Alupe (45.00±0.5774) and Kibos (56.33±1.856), the difference was significant (P=0.0004). A significant difference was also observed between Alupe (45.00±0.5774) and Oyani (33.30±1.305), with P=0.0003, and between Kibos (56.33±1.856) and Oyani (33.30±1.305), with P<0.0001.

A strong significant difference (P<0.0001) in NFP was observed between K2 GHI (64.00±1.155) and K2 GHD (20.33±0.3499), as well as between Alupe (45.00±0.5774) and GHI (64.00±1.155), and between Oyani (33.30±1.305) and GHI (64.00±1.155). The difference between Kibos (56.33±1.856) and GHI (64.00±1.155) was also significant (P=0.0068).

Additionally, strong significant differences (P<0.0001) were observed between Alupe (45.00±0.5774) and GHD (20.33±0.3499), and between Kibos (56.33±1.856) and GHD (20.33±0.3499). The difference between Oyani (33.30±1.305) and GHD (20.33±0.3499) was also significant, with P=0.0001 (Figure 27).

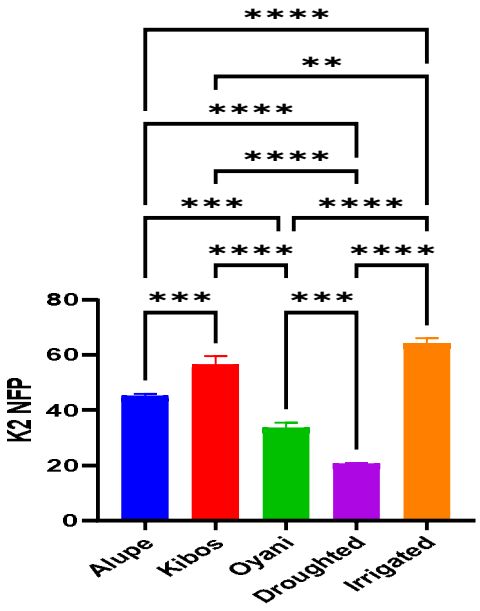


Figure 27: The Number of Flowers per Plant (NFP) of K2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of K2 number of flowers per plant between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, *P*≤0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.4.1.2 B4 Number of Flowers per Plant (NFP)

The B4 accession NFP across the three zones and in the greenhouse is presented in Figure 28. Kibos recorded the highest number of flowers, followed by Alupe and the GHI at 59.67, 58.17 and 55.80 respectively. Tukey's multiple comparisons test showed that NFP had strong significant difference (*P*<0.0001) between Alupe (58.17±1.167) and Oyani (31.67±0.8819), and between Kibos (59.67±1.453) and Oyani (31.67±0.8819). There was great variation observed between B4 GHI (55.80±1.474) and B4 GHD (28.00±1.000) where *P*<0.0001. Amongst all the field sites, only Oyani (31.67±0.8819) had significant difference (*P*<0.0001) with GHI (55.80±1.474). The significant difference (P<0.0001) was also observed between Alupe (58.17±1.167) and GHD (28.00±1.000), and between Kibos (59.67±1.453) and GHD (28.00±1.000) (Figure 28).

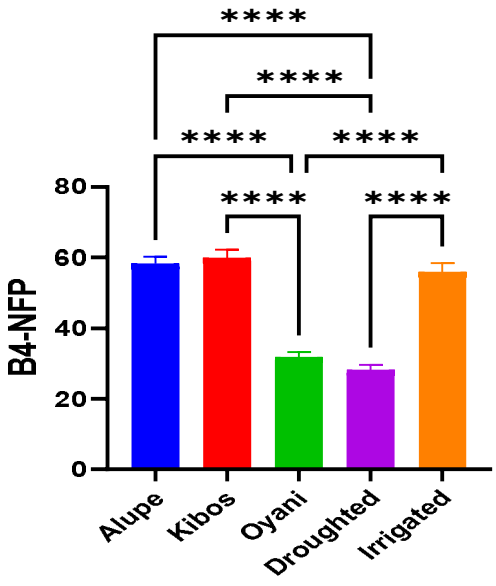


Figure 28: The Number of Flowers per Plant (NFP) of B4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of B4 number of flowers per plant between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, $P \leq 0.05$) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.4.1.3 B1 Number of Flowers per Plant

In B1 BGA, Tukey's multiple comparisons test showed that NFP had strong significant difference ($P<0.0001$) between Alupe (62.00±1.153) and Oyani (44.03±0.5487), and between Kibos (69.17±1.014) and Oyani (44.03±0.5487). The analysis showed that the significant difference between Alupe (62.00±1.153) and Kibos (69.17±1.014) was $P=0.0051$. There was strong significant difference ($P<0.0001$) in NFP between B1 GHI (75.00±1.258) and B1 GHD (30.00±1.155). All the field sites had significant difference ($P<0.05$) in NFP with B1 GHI and GHD plants (Figure 29).

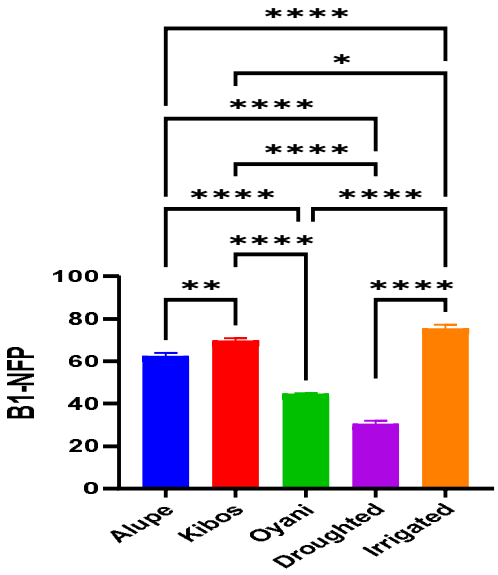


Figure 29: The Number of Flowers per Plant (NFP) of B1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of B1 number of flowers per plant between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, $P \leq 0.05$) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.4.1.4 K1 Number of Flowers per Plant (NFP)

The K1 accession NFP across the three zones and in the greenhouse is presented in Figure 30. GHI recorded the highest number of flowers, followed by Kibos at 70.00 and 66.73 respectively. Tukey's multiple comparisons test showed that NFP had strong significant difference ($P<0.0001$) between Alupe (47.00±1.155) and Kibos (66.73±1.425), and between Kibos (66.73±1.425) and Oyani (41.00±1.528). The strong significant difference (P<0.0001) in NFP was also observed between K1 GHI (70.00±1.155) and K1 GHD (43.50±1.443) plants. Amongst all the field sites, only Alupe (47.00±1.155) and Oyani (41.00±1.528) K1 plants had significant difference (P<0.0001) with GHI (70.00±1.155), only K1 plants at Kibos had significant difference (P<0.0001) with GHD (43.50±1.443).

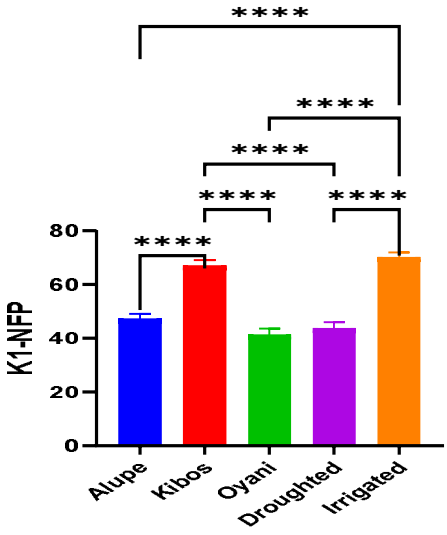


Figure 30: The Number of Flowers per Plant (NFP) of K1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of K1 number of flowers per plant between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, *P*≤0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.4.1.5 M4 Number of Flowers per Plant

In M4 BGA, Tukey's multiple comparisons test showed that NFP had significant difference (P<0.0001) between Alupe (51.67±1.764) and Kibos (74.87±1.304), and between Kibos (74.87±1.304) and Oyani (53.17±0.8333). The strong significant difference (*P*<0.0001) in NFP was also observed between M4 GHI (72.30±1.350) and M4 GHD (35.00±1.155) plants. Only Alupe (51.67±1.764) and Oyani (53.17±0.8333) M4 accessions had significant difference (P<0.0001) with GHI (72.30±1.350). The M4 NFP from all the field sites had significant difference (P<0.0001) with the GHD (35.00±1.155) (Figure 31).

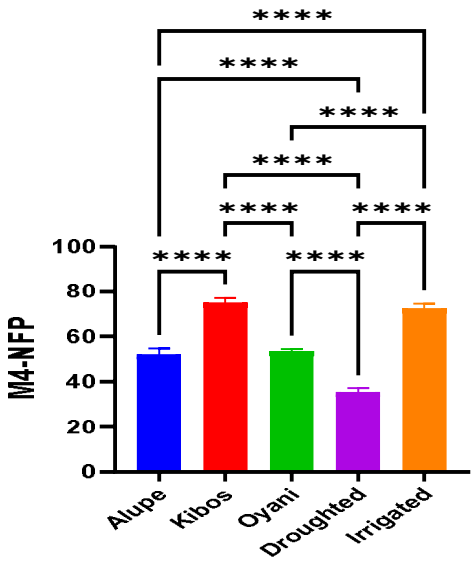


Figure 31: The Number of Flowers per Plant (NFP) of M4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of M4 number of flowers per plant between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, $P \leq 0.05$) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.4.1.6 M2 Number of Flowers per Plant

The M2 BGA number of flowers per plant (NFP) across the three field zones and in the greenhouse is presented in Figure 32. Kibos recorded the highest number of flowers (73.37), followed by GHI (62.27). Tukey's multiple comparisons test showed that M2 NFP had significant differences ($P<0.0001$) between Alupe (50.00±1.041) and Kibos (73.37±1.941), as well as between Kibos (73.37±1.941) and Oyani (45.63±1.810).

In the greenhouse, the test revealed a significant difference ($P<0.0001$) in NFP between M2 GHI (62.27±1.462) and M2 GHD (26.33±1.856). Additionally, M2 GHI plants showed significant differences ($P<0.05$) in NFP when compared with all field sites. All field site M2 NFP values also showed strong significant differences ($P<0.0001$) when compared with GHD (26.33±1.856) plants.

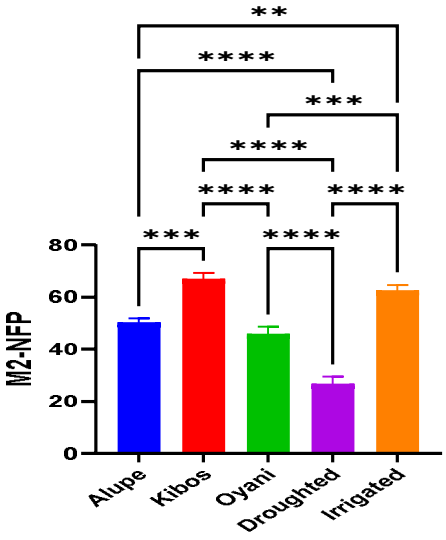


Figure 32: The Number of Flowers per Plant (NFP) of M2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughtedplants).Comparisons of M2 number of flowers per plant between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, *P*≤0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.4.2 Number of Flowers per Plant (NFP) Intra-Sites Comparisons

4.4.4.2.1 Number of Flowers per Plant (NFP) Intra-Site Comparisons in Alupe

In Alupe, B1 accession had the highest number of flowers per plant (NFP) (62.00±1.153), followed by B4 (58.17±1.167) and then M4 (51.67±1.764). On the contrary K2 (45.00±0.5774) had the lowest number of flowers per plant. The analysis (one-way ANOVA, P≤0.05, Tukey's multiple comparisons test) showed that there were significant differences (*P*≤0.05) in majority of the accession comparisons (Table 16a).

4.4.4.2.2 Number of Flowers per Plant (NFP) Intra-Site Comparisons in Kibos

In Kibos zone, M4 had recorded the highest NFP, followed by M2 accession at 74.87 and 73.37 respectively. The K2 accession (56.33±1.856) recorded the least number of flowers per plant. The analysis (one-way ANOVA, *P*≤0.05, Tukey's multiple comparisons test) showed that there were significant differences (*P*≤0.05) in some of the accessions comparisons (Table 16b).

4.4.4.2.3 Number of Flowers per Plant (NFP) Intra-Site Comparisons in Oyani

In Oyani, M4 had the highest NFP (53.17±0.8333), followed by M2 (45.63±1.810) and then B1 (44.03±0.5487), whereas B4 (31.67±0.8819) had the lowest NFP. The analysis (one-way ANOVA, $P \leq 0.05$, Tukey's multiple comparisons test) showed that there were significant differences ($P<0.05$) in all the accessions comparisons, except in four comparisons where there were no significant differences (Table 16c).

4.4.4.2.4 Number of Flowers (NFP) intra-Greenhouse Irrigated (GHI) Comparisons

In GHI, B1 accession had the highest number of flowers per plant (75.00±1.258), followed by M4 (72.30±1.350) and then K1 (70.00±1.155). On the contrary B4 (55.80±1.474) had the lowest number of flowers per plant. The comparisons test showed that there were significant differences ($P \leq 0.05$) in majority of the accessions comparisons (Table 16d).

4.4.4.2.5 Number of Flowers (NFP) intra-Greenhouse Droughted (GHD) Comparisons

In GHD, K1 (43.50±1.443) had the highest NFP, followed by M4 (35.00±1.155) and then B1 (30.00±1.155). The K2 (20.33±0.3499) had the lowest number of flowers per plant. The analysis (one-way ANOVA, $P \leq 0.05$, Tukey's multiple comparisons test) showed that there were significant differences ($P \leq 0.05$) in majority of the accessions comparisons, for instance there were strong significant differences ($P<0.0001$) between K2 (20.33±0.3499) and K1 (43.50±1.443), and between K2 (20.33±0.3499) and M4 (35.00±1.155) (Table 16e).

Table 16a: **Number of Flowers per Plant (NFP) Intra-Site Comparisons in Alupe**

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Alupe	45.00± 0.5774	58.17± 1.167	62.00± 1.153	47.00± 1.155	51.67± 1.764	50.00± 1.041	K2 vs. B4 K2 vs. B1 B1 vs. K1 B1 vs. M4 B1 vs. M2 B4 vs. M2 B4 vs. M4 B4 vs. K1 K2 vs. M4	<0.0001 <0.0001 <0.0001 0.0006 0.0001 0.0042 0.0219 0.0003 0.0185

Acc accession. The nine comparisons tabulated had significant differences ($P<0.05$), except six comparisons (K2 vs. K1, K2 vs. M2, B4 vs. B1, K1 vs. M4, K1 vs. M2 and M4 vs. M2) which had no significant differences ($P>0.05$). Number of flowers expressed as Mean±Standard Error of Mean (SEM).

Table 16b: Number of Flowers per Plant (NFP) Intra-Site Comparisons in Kibos

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Kibos	56.33± 1.856	59.67± 1.453	69.17± 1.014	66.73± 1.425	74.87± 1.304	73.37± 1.941	K2 vs. B1 K2 vs. K1 K2 vs. M4 K2 vs. M2 B4 vs. B1 B4 vs. M4 B4 vs. M2 K1 vs. M4	0.0008 0.0045 <0.0001 <0.0001 0.0089 0.0002 0.0004 0.0257

Acc accession. Eight comparisons tabulatedwere statistically significant at $P<0.05$, except seven comparisons (K2 vs. B4, B4 vs. K1, B1 vs. K1, B1 vs. M4, B1 vs. M2, K1 vs. M2 and M4 vs. M2) which had no significant differences ($P>0.05$). Number of flowers expressed as Mean±Standard Error of Mean (SEM).

Table 16c: Number of Flowers per Plant (NFP) Intra-Site Comparisons in Oyani

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisonstest	Adjusted *p* value
Oyani	33.30± 1.305	31.67± 0.8819	44.03± 0.5487	41.00± 1.528	53.17± 0.8333	45.63± 1.810	K2 vs. B1 K2 vs. M4 B4 vs. B1 B4 vs. M2 B1 vs. M4 M4 vs. M2	0.0005 <0.0001 0.0001 <0.0001 0.0022 0.0098

Acc accession. All comparisons were statistically significant at $P<0.05$ except K2 vs. B4, B1 vs. K1, B1 vs. M2 and K1 vs. M2 which had no significant differences ($P>0.05$). Number of flowers expressed as Mean±Standard Error of Mean (SEM).

Table 16d: Number of Flowers (NFP) intra-Greenhouse Irrigated (GHI) Comparisons

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
GHI	64.00 1.155	55.80±1 .474	75.00±1 .258	70.00±1 .155	72.30±1 .350	62.27 1.462	B4 vs. B1 K2 vs. B4 K2 vs. B1 K2 vs. M4 B4 vs. K1 B4 vs. M4 B4 vs. M2 B1 vs. M2 K1 vs. M2 M4 vs. M2	<0.0001 0.0086 0.0008 0.0078 <0.0001 <0.0001 0.0411 0.0002 0.0130 0.0017

Acc accession. Ten comparisons were statistically significant at $P<0.05$ except five comparisons (K1 vs. M4, B1 vs. M4, B1 vs. K1, K2 vs. M2 and K2 vs. K1) which had no significant differences ($P>0.05$). Number of flowers expressed as Mean±Standard Error of Mean (SEM).

Table 16e: Number of Flowers (NFP) intra-Greenhouse Droughted (GHD) Comparisons

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
GHD	20.33± 0.3499	28.00± 1.000	30.00± 1.155	43.50± 1.443	35.00± 1.155	26.33± 1.856	K2 vs. K1 K2 vs. M4 B4 vs. M4 K1 vs. M2 M4 vs. M2 K1 vs. M4 B1 vs. K1 B4 vs. M4 K2 vs. M2 K2 vs. B1 K2 vs. B4	<0.0001 <0.0001 0.0178 <0.0001 0.0037 0.0043 <0.0001 0.0178 0.0462 0.0015 0.0094

Acc accession. Eleven comparisons tabulated had significant differences (P<0.05), except four comparisons (B4 vs. B1, B4 vs. M2, B1 vs. M4 and B1 vs. M2) which had no significant differences (P>0.05). Number of flowers expressed as Mean±Standard Error of Mean (SEM).

4.4.5 Seed Weight per Plant (SWT g)

4.4.5.1 Seed Weight per Plant (SWT g) Inter-Sites Comparisons

4.4.5.1.1 K2 Seed Weight per Plant (SWT g)

In K2 BGA, Tukey's multiple comparisons test showed that seed weight (SWT, g) differed significantly (P<0.0001) among all three field sites. Alupe had a mean seed weight of 5.75g, Kibos 10.24g, and Oyani 2.77g. A large variation in seed weight was observed between the irrigated (11.91±0.1485) and droughted (0.45±0.0764) K2 plants, with a highly significant difference (P<0.0001).

The GHI seed weight (11.91±0.1485) differed significantly (P<0.0001) from both Alupe (5.75±0.3769) and Oyani (2.77±0.1873). The difference between Kibos (10.24±0.1480) and GHI (11.91±0.1485) was also significant, with P=0.0018. Furthermore, seed weights from both Alupe and Kibos K2 accessions showed strong significant differences (P<0.0001) when compared to GHD (0.45±0.0764) (Figure 33).

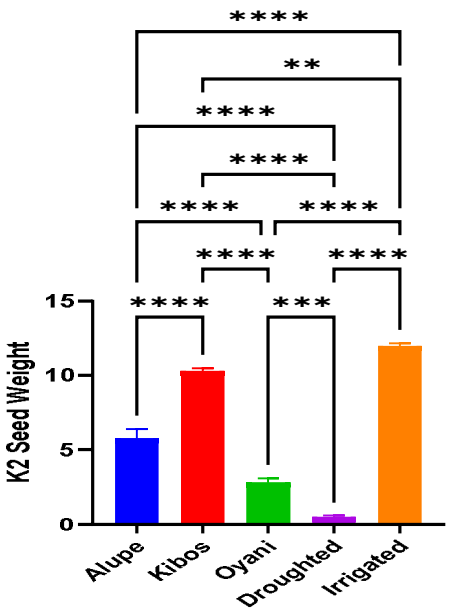


Figure 33: The Seed Weight per Plant (SWT g) of K2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of K2 seed weight per plant between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, $P \leq 0.05$) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.5.1.2 B4 Seed Weight per Plant (SWT g)

The B4 accession SWT across the three field zones and in the greenhouse is presented in Figure 34. Alupe recorded the highest seed weight per plant, followed by Kibos at 10.97g and 10.86g respectively. Tukey's multiple comparisons test showed that SWT had strong significant difference ($P<0.0001$) between Alupe (10.97±0.1389) and Oyani (3.62±0.06429), and between Kibos (10.86±0.05859) and Oyani (3.62±0.06429). There was great variation between SWT in GHI (9.42±0.1015) and B4 GHD (2.00±0.1670), where $P<0.0001$. The comparisons test showed that there was strong significant difference ($P<0.0001$) between all the field sites and the GHI (9.42±0.1015), and between all the field sites and the GHD (2.00±0.1670).

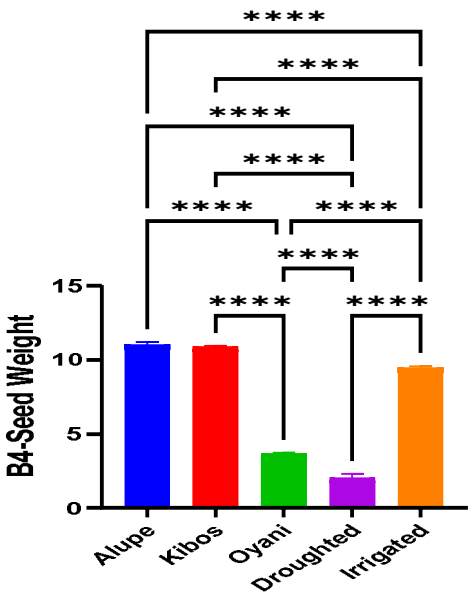


Figure 34: The Seed Weight per Plant (SWT g) of B4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of B4 seed weight per plant between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, $P \leq 0.05$) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.5.1.3 B1 Seed Weight per Plant (g)

In B1 accession, the comparisons test showed that SWT had significant difference ($P<0.05$) between all the three field sites. The significant difference ($P<0.0001$) was observed between Alupe (7.22±0.1852) and Kibos (13.02±0.2869), and between Kibos (13.02±0.2869) and Oyani (5.480±0.06028). Between Alupe (7.22±0.1852) and Oyani (5.48±0.06028), $P=0.0011$. In the greenhouse, Tukey's multiple comparisons test showed that there was significant difference ($P<0.0001$) in SWT between B1 GHI (15.79±0.3068) and B1 GHD (4.60±0.0608). The comparisons test showed that there was strong significant difference ($P<0.0001$) between all the field sites and the GHI (15.79±0.3068). The B1 droughted plants (4.60±0.06083), however, had strong had significant difference ($P<0.0001$) with only B1 plants at Alupe (7.220±0.1852) and Kibos (13.02±0.2869) (Figure 35).

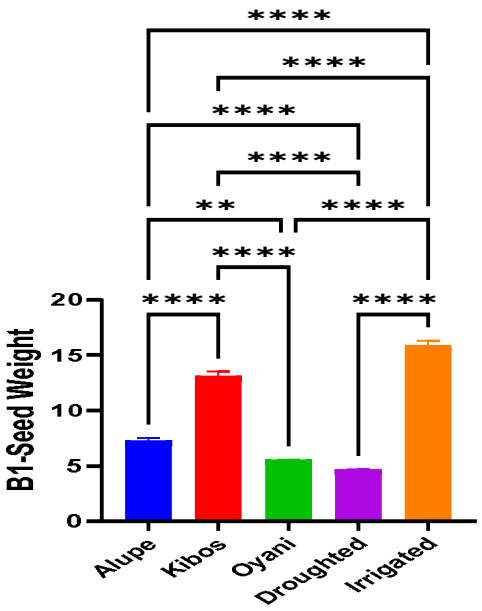


Figure 35: The Seed Weight per Plant (SWT g) of B1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of B1 seed weight per plant between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, $P \leq 0.05$) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.5.1.4 K1 Seed Weight per Plant

In K1 accession SWT across the three field zones and in the greenhouse is presented in Figure 36. Kibos recorded the highest seed weight per plant, followed by GHI at 9.92g and 9.22g respectively. The comparisons test showed that SWT had strong significant difference ($P<0.0001$) between Alupe (4.51±0.1929) and Kibos (9.92±0.6070), and between Kibos (9.92±0.6070) and Oyani (3.69±0.1159). Variation in SWT was also observed between K1 GHI (9.22±0.1595) and K1 GHD (4.72±0.1767), where $P<0.0001$. The irrigated K1 plants (9.22±0.1595) showed strong significant difference ($P<0.0001$) with Alupe (4.51±0.1929) and Oyani (3.69±0.1159) K1 plants. The droughted K1 plants (4.72±0.1767) had strong significant difference ($P<0.0001$) with only Kibos (9.92±0.6070) K1 plants.

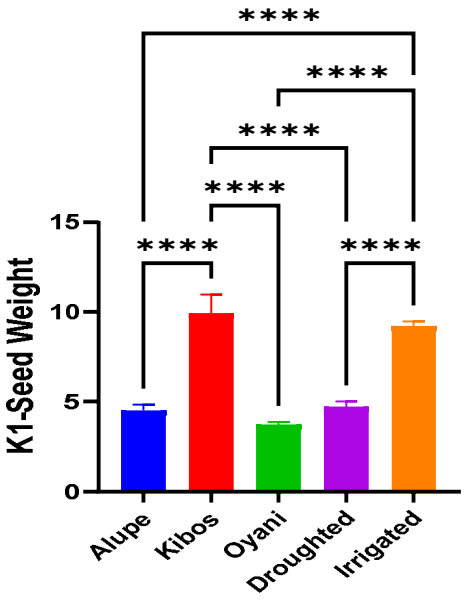


Figure 36: The Seed Weight per Plant (SWT g) of K1 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of K1 seed weight per plant between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, *P*≤0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.5.1.5 M4 Seed Weight per Plant

In M4 BGA, Tukey's multiple comparisons test showed that SWT had significant difference (P<0.05) between all the field sites. The strong significant difference (P<0.0001) was observed between Alupe (5.40±0.2237) and Kibos (11.29±0.0433), and between Kibos (11.29±0.0433) and Oyani (6.45±0.1153). However, the significant difference between Alupe (5.40±0.2237) and Oyani (6.45±0.1153) was P=0.0006. In the greenhouse, Tukey's multiple comparisons test showed that there was strong significant difference (P<0.0001) in SWT between M4 GHI (11.12±0.0413) and M4 GHD (4.26±0.0173). Amongst the field sites, only Alupe (5.40±0.2237) and Oyani (6.45±0.1153) had strong significant difference (P<0.0001) with the GHI (11.12±0.0416). The droughted M4 accession (4.26±0.0173) had strong significant difference (P<0.0001) with Kibos (11.29±0.0433) and Oyani (6.45±0.1153) M4 plants. However, the significant difference of P=0.0003 was observed between Alupe (5.40±0.2237) and the GHD (4.26±0.0173) (Figure 37).

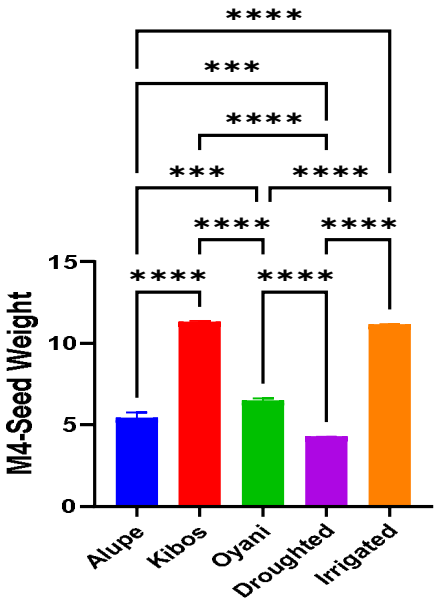


Figure 37: The Seed Weight per Plant (SWT g) of M4 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of M4 seed weight per plant between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, $P \leq 0.05$) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.5.1.6 M2 Seed Weight per Plant

Amongst the M2 accession, Kibos had the highest seed weight per plant, followed by GHI at 13.55g and 12.08g respectively. The comparisons test showed that SWT had strong significant difference ($P<0.0001$) between Alupe (7.00±0.2107) and Kibos (13.55±0.07638), and between Kibos (13.55±0.07638) and Oyani (6.23±0.1457). The significant difference (P=0.0152) was however observed between Alupe (7.00±0.2107) and Oyani (6.23±0.1457). In the greenhouse, great variation in SWT was observed between M2 GHI (12.08±0.1350) and M2 GHD (0.71±0.01155) at $P<0.0001$. The M2 SWT had significant difference ($P \leq 0.05$) between all the field sites and GHI (12.08±0.1350). The strong significant difference ($P<0.0001$) was also observed between all the three field sites and GHD (0.71±0.01155) (Figure 38).

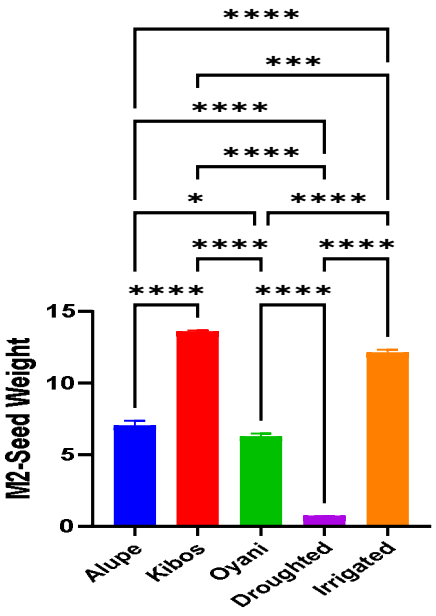


Figure 38: The Seed Weight per Plant (SWT g) of M2 accession grown in three agro-ecological zones (Alupe, Kibos and Oyani), and in greenhouse (Irrigated and Droughted pots). Comparisons of M2 seed weight per plant between the zones and in greenhouse based on Tukeys multiple comparison test (one-way ANOVA, P≤0.05) are graphically presented. Bars represent Mean±Standard Error of Mean (SEM). Asteriks (*) show level of significance. The combinations with no level of significance indicated, imply that there were no significant differences.

4.4.5.2 Seed Weight per Plant (SWT g) Intra-Sites Comparisons

4.4.5.2.1 Seed Weight per plant (SWT g) Intra-Site Comparisons in Alupe

In Alupe, B4 had the highest seed weight per plant (10.97±0.1389), followed by B1 (7.22±0.1852) and then M2 (7.00±0.2107). On the contrary K1 had the lowest seed weight per plant at a mean of 4.51±0.1929. The analysis (one-way ANOVA, $P \leq 0.05$, Tukey's multiple comparisons test) showed that there were significant differences ($P \leq 0.05$) in majority of the accessions comparisons except between K2 (5.75±0.37690) and M4 (5.40±0.2237), B1 (7.22±0.1852) and M2 (7.00±0.2107), and between K1 (4.51±0.1929) and M4 (5.40±0.2237), where ($P > 0.05$) (Table 17a).

4.4.5.2.2 Seed Weight per plant (SWT g) Intra-Site Comparisons in Kibos

The M2 accession had the highest seed weight per plant (13.55±0.07638), followed by B1 (13.02±0.2869) and then M4 (11.29±0.04333). The K1 accession recorded the lowest seed weight per plant at a mean of 9.92±0.6070. The analysis (one-way ANOVA, P≤0.05, Tukey's

multiple comparisons test) showed that there was significant difference ($P \leq 0.05$) in majority of the accessions comparisons (Table 17b).

4.4.5.2.3 Seed Weight per plant (SWT g) Intra-Site Comparisons in Oyani

The M4 accession had the highest seed weight per plant (6.45±0.1153), followed by M2 (6.23±0.1457) and then B1 (5.48±0.06028). The K2 accession had the least seed weight per plant at a mean of 2.77±0.1873. The analysis (one-way ANOVA, $P \leq 0.05$, Tukey's multiple comparisons test) showed that there were significant differences ($P \leq 0.05$) in majority of the accessions comparisons except between B4 (3.62±0.06429) and K1 (3.69±0.1159), and between M4 (6.45±0.1153) and M2 (6.23±0.1457), where $P > 0.05$ (Table 17c).

4.4.5.2.4 Seed Weight per plant (SWT g) Intra-Greenhouse Irrigated (GHI) Comparisons

Amongst the irrigated plants, B1 had the highest seed weight per plant (15.79±0.3068), followed by M2 (12.08±0.1350) and then K2 (11.91±0.1485). The K1 accession recorded the lowest SWT at a mean of 9.22±0.1595. The analysis (one-way ANOVA, $P \leq 0.05$, Tukey's multiple comparisons test) showed that there was strong significant difference ($P < 0.0001$) in majority of the accessions comparisons except between K2 (11.91±0.1485) and M4 (11.12±0.04163), between K2 (11.91±0.1485) and M2 (12.08±0.1350), and between B4 (9.42±0.1015) and K1 (9.22±0.1595) where $P > 0.05$ (Table 17d).

4.4.5.2.5 Seed Weight per plant (SWT g) Intra-Greenhouse Droughted (GHD Comparisons

Amongst the droughted plants, K1 had the highest seed weight per plant (4.72±0.1767), followed by B1 (4.60±0.0608) and then M4 (4.26±0.0173). On the contrary K2 had the least seed weight per plant at a mean of 0.45±0.0764, followed by M2 (0.71±0.0116). The analysis (one-way ANOVA, $P \leq 0.05$, Tukey's multiple comparisons test) showed that there was strong significant difference ($P < 0.0001$) in all the accessions comparisons except between K2 (0.45±0.0764) and M2 (0.71±0.0116), between B1 (4.60±0.0608) and K1 (4.72±0.1767), between B1 (4.60±0.0608) and M4 (4.26±0.0173), and between K1 (4.72±0.1767) and M4 (4.26±0.0173) where $P > 0.05$ (Table 17e).

Table 17a: Seed Weight per plant (SWT g) Intra-Site Comparisons in Alupe

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Alupe	5.75± 0.3769	10.97± 0.1389	7.22± 0.1852	4.51± 0.1929	5.40± 0.2237	7.00± 0.2107	B4 vs. B1 B4 vs. K1 B1 vs. K1 K1 vs. M2 B4 vs. M2	<0.0001 <0.0001 <0.0001 <0.0001 <0.0001

Acc accession. Apart from three comparisons (K2 vs. M4, B1 vs. M2 and K1 vs. M4) which had no significant differences ($P>0.05$), all other comparisons were strongly significant at $P<0.0001$ except K2 vs. B1 where $P\leq0.0080$, K2 vs. K1 where $P\leq0.0257$, K2 vs. M2 where $P\leq0.0244$ and B1 vs. M4 where $P\leq0.0014$. Seed weight per plant expressed as Mean±Standard Error of Mean (SEM).

Table 17b: Seed Weight per plant (SWT g) Intra-Site Comparisons in Kibos

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Kibos	10.24± 0.1480	10.86± 0.0586	13.02± 0.2869	9.92± 0.6070	11.29± 0.0433	13.55± 0.0764	K2 vs. B1 K2 vs. M2 B1 vs. M4 K1 vs. M2 M4 vs. M2 B4 vs. B1 B4 vs. M2 B1 vs. K1 K1 vs. M4	0.0002 <0.0001 0.0102 <0.0001 0.0012 0.0018 0.0002 <0.0001 0.0451

Acc accession. Nine comparisons tabulated were statistically significant ($P\leq0.05$), whereas six comparisons (K2 vs. B4, K2 vs. K1, K2 vs. M4, B4 vs. K1, B4 vs. M4 and B1 vs. M2) had no significant differences ($P>0.05$). Seed weight per plant expressed as Mean±Standard Error of Mean (SEM).

Table 17c: Seed Weight per plant (SWT g) Intra-Site Comparisons in Oyani

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
Oyani	2.77± 0.1873	3.62± 0.0643	5.48± 0.0603	3.69± 0.1159	6.45± 0.1153	6.23± 0.1457	K2 vs. M4 B4 vs. M4 B1 vs. K1 K1 vs. M4 K1 vs. M2	<0.0001 <0.0001 <0.0001 <0.0001 <0.0001

Acc accession. Apart from two comparisons (B4 vs. K1 and M4 vs. M2) which had no significant differences (P>0.05), all other comparisons were strongly significant at $P<0.0001$ except K2 vs. B4 where $P\leq0.0039$, K2 vs. K1 where $P\leq0.0020$, B1 vs. M4 where $P\leq0.0013$ and B1 vs. M2 where $P\leq0.0101$. Seed weight per plant expressed as Mean±Standard Error of Mean (SEM).

Table 17d: Seed Weight per plant (SWT g) Intra-Greenhouse Irrigated (GHI) Comparisons

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
GHI	11.91± 0.1485	9.42± 0.1015	15.79± 0.3068	9.22± 0.1595	11.12± 0.0416	12.08± 0.1350	K2 vs. B1 B1 vs. K1 B1 vs. M4 K1 vs. M4 K1 vs. M2	<0.0001 <0.0001 <0.0001 <0.0001 <0.0001

Acc accession. Apart from three comparisons (K2 vs. M4, K2 vs. M2 and B4 vs. K1) which had no significant differences ($P>0.05$), all other comparisons were strongly significant at $P<0.0001$ except B4 vs. M4 where $P\leq0.0001$ and M4 vs. M2 where $P\leq0.0167$. Seed weight per plant expressed as Mean±Standard Error of Mean (SEM).

Table 17e: Seed Weight per plant (SWT g) Intra-Greenhouse Droughted (GHD) Comparisons.

ACC / ZONES	K2	B4	B1	K1	M4	M2	Tukey's multiple comparisons test	Adjusted *p* value
GHD	0.45± 0.0764	2.00± 0.1670	4.60± 0.0608	4.72± 0.1767	4.26± 0.0173	0.71± 0.0116	K2 vs. B4 K2 vs. K1 B4 vs. M4 B1 vs. M2 K1 vs. M2	<0.0001 <0.0001 <0.0001 <0.0001 <0.0001

Acc accession. All comparisons were strongly significant at $P<0.0001$ except K2 vs. M2 where $P\leq0.5487$, B1 vs. K1where $P\leq0.9639$, B1 vs. M4 where $P\leq0.2885$ and K1 vs. M4 where $P\leq0.0861$. Seed weight per plant expressed as Mean±Standard Error of Mean (SEM).

4.5 Principle Component Analysis (PCA) for the Seven Quantitative Traits

In the analysis, the first principal component (PC1) accounted for more proportion of variation (72.7%) than PC2 which only accounted for 14.5%. Below, the loading plot displays the correlations graphically using the first and second components for X and Y axis respectively.

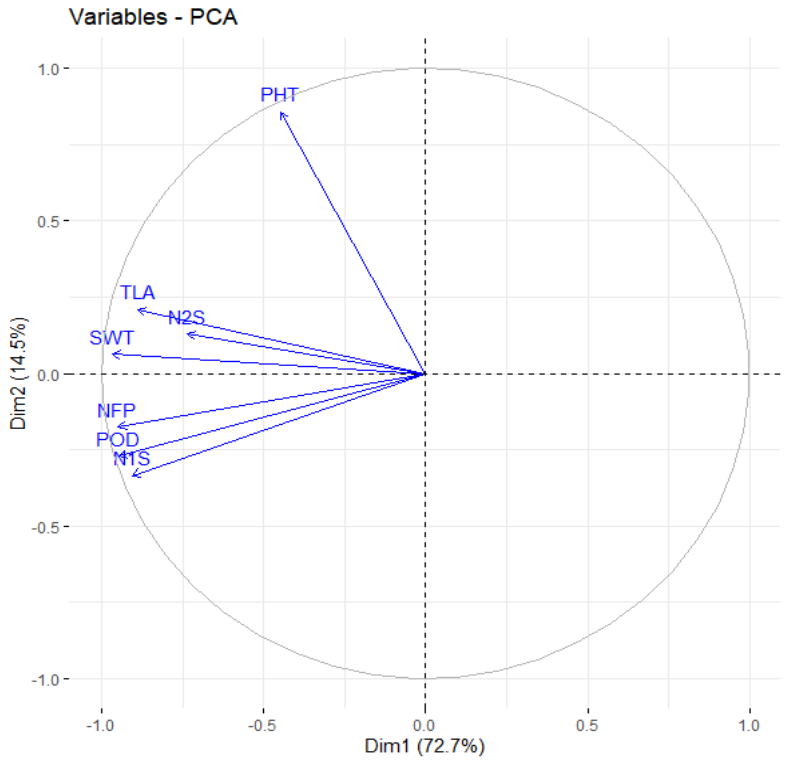


Figure 39: Principal Component Analysis (PCA) of quantitative trait scores from three zones and greenhouse. N1S: Number of pods with one seed, N2S: Number of pods with two seeds, POD: Total number of pods per plant, NFP: Number of flowers per plant, TLA: Terminal Leaflet Area, PHT: Plant height per plant, SWT: Seed weight per plant

The above loading plot shows how strongly each trait influences a principal component. All traits are skewed towards PC2 apart from plant height. Hence, N1S, POD, NFP, SWT, N2S and TLA account for the 14.5% variations observed within the accessions evaluated.

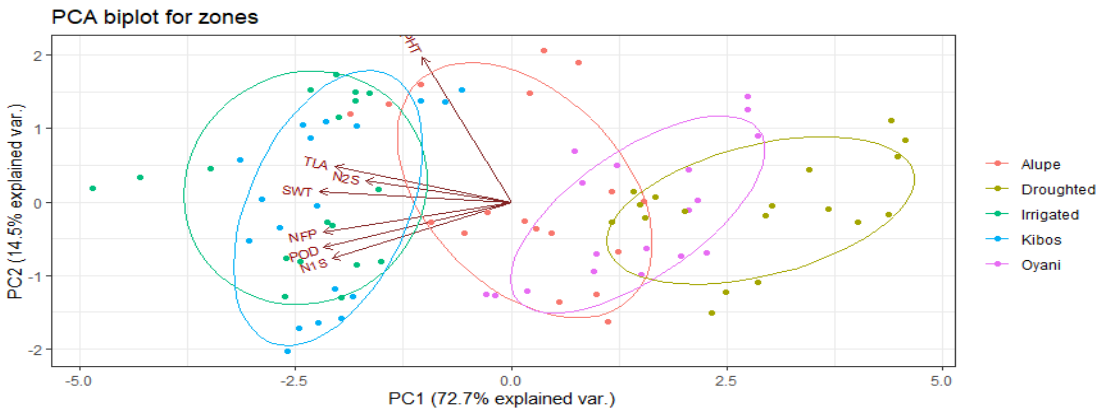


Figure 40A: PCA Biplot for Zones

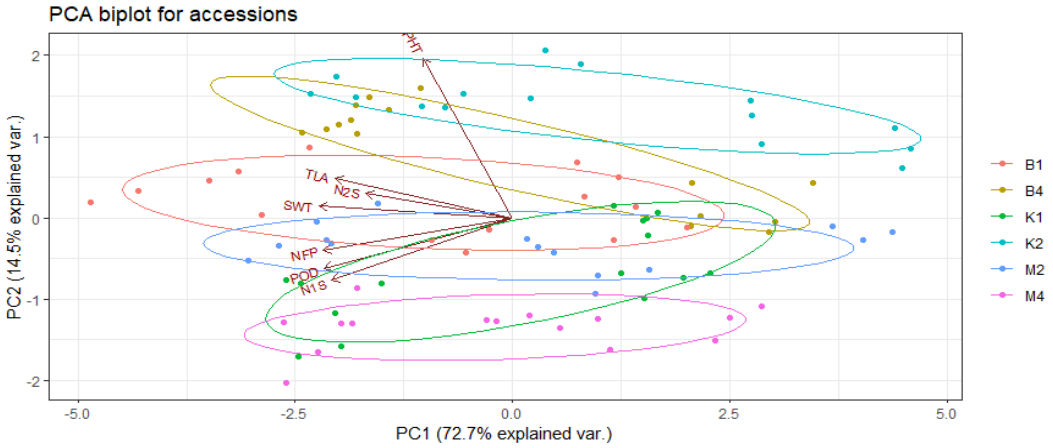


Figure 40B: PCA Biplot for Accessions

Figure 40A and 40B showing the PCA biplot for zones and accessions respectively. N1S: Number of pods with one seed, N2S: Number of pods with two seeds, POD: Total number of pods per plant, NFP: Number of flowers per plant, TLA: Terminal Leaflet Area, PHT: Plant height per plant, SWT: Seed weight per plant.

In PCA biplot, traits are superimposed on the plot as vectors. Biplot represents the association among different traits and independent variables such as accessions and zones, and the length of the vectors show the contribution of each trait to the observed variations. The PCA biplot loading both dependent variables (traits) and independent variables (accession or zones) show how strongly each trait influences a PC and how they are correlated to each other. The lesser the angle (approaching 0^0) between two vectors (Figure 39) the higher, the stronger and positive the correlation is (e.g N1S and POD, NFP). An angle of 90^0 implies no correlation, when more than 90^0 it indicates negative correlation (Khan *et al*., 2021b). PHT had a weak positive correlation with all other traits. The loading plots therefore strongly influence the correlations of most of the traits in this study apart from PHT, towards PC2 where variation is 14.5%. The second PC has high loadings on TLA, N2S and SWT (loadings between 0 and 1). Hence, PC2 is strongly associated with these three variables. The three traits hence tend to group together with moderately large positive loadings for the PC2. NFP, POD and N1S have loadings ranging between -0.9 and 0. These three traits also tend to group together with weak negative loadings for the PC2.

The zones exhibit a lot of influence on the clustering of these seven dependent variables. From the biplot 40A, the irrigated and the droughted zones clustered independently, with no overlap. Oyani zone on one hand, with Irrigated and Kibos zones on the other hand, also cluster independently with no overlap. The overlapping clustering is observed between irrigated and Kibos zones, and also between droughted and the Oyani zone. Alupe zone had partial overlap clustering with all the other zones.

From the biplot in Figure 40B, the accessions also influenced the clustering patterns of the seven dependent variables. The accession K2 clustered independently from other accessions apart from B4. The accession M4 clustered independently from other accessions except K1. The accession B1 is overlapping with all the other accessions apart from K2 and M4. The biplot (Figure 40B) is

revalidated using the hierarchical clustering analysis. It can be inferred that B1 shares some traits with K1, B4 and M2. M4 shares some traits with K1, and K2 accession shares some traits with B4.

The hierarchical clustering analysis produced a dendrogram categorizing the six accessions into two main groups based on their morphological quantitative traits (Figure 41). Cluster 1 and cluster 2 comprised of three accessions each. Cluster 1 consisted of accession M4, K1 and B1. Cluster 2 consisted of accession K2, B4 and M2. The cluster 1 accessions are distinguished by the high mean values for N1S, NFP and POD, although the B1 accession which stands alone in this cluster, is characterized by having the overall highest mean values for N1S, N2S, POD, TLA and SWT. The cluster 2 accessions are distinguished by the high mean values for PHT. These accessions are also characterized by slightly low mean values in their N1S, NFP and POD numbers.

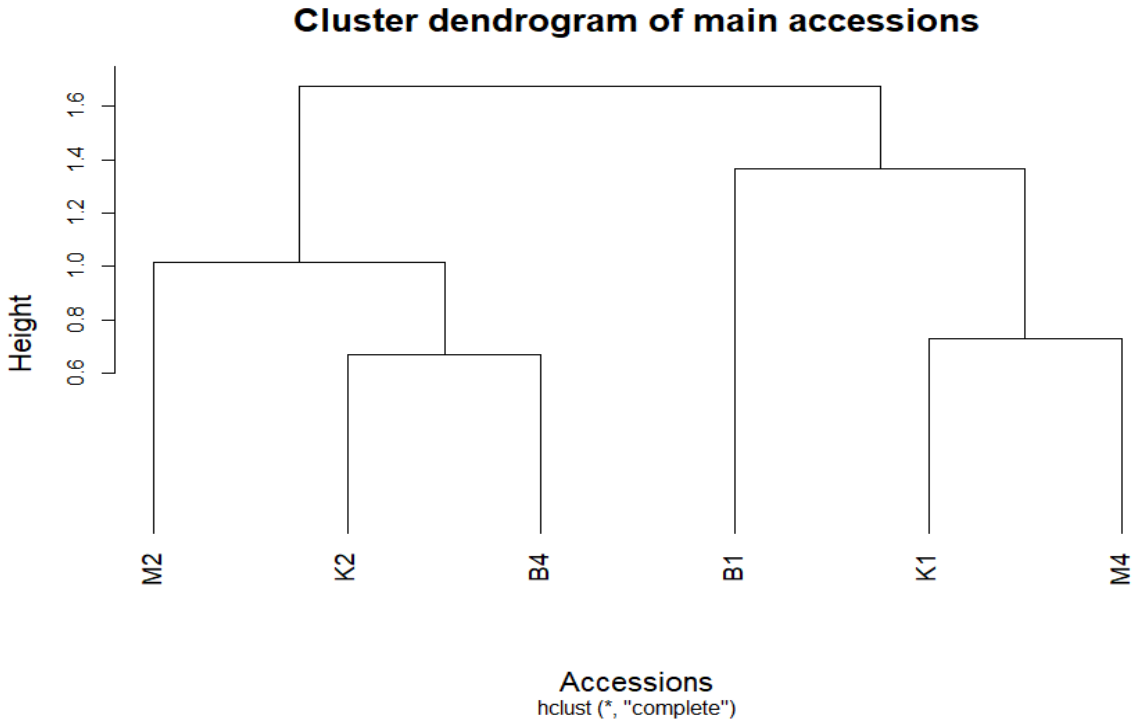


Figure 41: Hierarchical clustering dendrogram showing the relationship among the six Bambara accessions based on their quantitative traits scored in the three field sites and in greenhouse. The clustering is based on their similarity and dissimilarity distances.

4.6 Correlation Analysis of Traits

The correlation coefficients for seven quantitative morphological traits of Bambara are presented in Table18. There were significant differences among the correlations of the seven quantitative variables. According to the scale in Figure 42, the significant differences ($P \leq 0.05$) are shown with blue rounds which represent positive correlations. The more intense the colours of the relationships, the stronger the correlations. Basically, there were positive correlations among all the traits evaluated. However, PHT had weak positive correlation with all the traits (N2S (r=0.34), NFP (r=0.29), N1S (r=0.18), POD (r=0.22) and Seed weight (r=0.49) apart from TLA(r=0.52) (Table 9), an observation supported by the Principal Component analysis loading plot (Figure 39). The number of flowers per plant had a very strong, positive, and highly significant correlation with N1S (r=0.91), POD (r=0.93), TLA (r=0.79) and seed weight (r=0.91). The N1S also had a very strong, positive, and highly significant correlation with POD (r=0.99), TLA =0.70) and seed weight (0.86). Equally the POD had highly positive correlations with TLA (r=0.75) and seed weight (r=0.89). However, there were moderate correlations between N2S and NFP, N1S, POD, TLA and SWT, with correlation coefficients of r=0.64, 0.51, 0.64, 0.63 and 0.64 respectively.

Table 18: Pearson's Correlation Matrix (r) for seven quantitative traits of Bambara accessions grown across three environments, and among the greenhouse drought-stressed and irrigated plants.

Correlation is significant at 0.01 level. N1S: Number of pods with one seed, N2S: Number of pods with two seeds, POD: Total number of pods per plant, NFP: Number of flowers per plant, TLA: Terminal Leaflet Area, PHT: Plant height per plant, SWT: Seed weight per plant.

	PHT	N2S	NFP	N1S	POD	TLA	SWT
PHT	1.00	0.34	0.29	0.18	0.22	0.52	0.49
N2S		1.00	0.64	0.51	0.64	0.63	0.64
NFP			1.00	0.91	0.93	0.79	0.91
N1S				1.00	0.99	0.70	0.86
POD					1.00	0.75	0.89
TLA						1.00	0.87
SWT							1.00



Figure 42: Correlations among the quantitative morphological traits; Pearson's rank correlation matrix indicating the correlation among variables of Bambara accessions grown across three environments, and in the greenhouse drought-stressed and irrigated plants.

The strong positive correlation of seed weight per plant to a number of traits in all the zones is an indication of potential traits to target for Bambara yield improvement in the Lake Victoria Basin region of Kenya.

4.7 Yield

4.7.1 Yield inter-site comparisons

4.7.1.1 K2 yield (ton/ha)

The K2 accession yield (ton/ha) mean values for various zones are shown in Table 19a and Table 20. The highest grain yield was produced by the greenhouse irrigated crops followed by Kibos, while the least yield was from greenhouse droughted crops. Yield of K2 accession across the zones ranged from 0.11 to 2.90 ton/ha. The yield was compared in three different agro ecological zones (Alupe, Kibos and Oyani) and in the greenhouse. Tukey's multiple comparisons test showed that K2 yield (ton/ha) had strong significant difference (P<0.0001) between all the three

sites. Alupe and Oyani had high coefficients of variation of 11.3% and 11.7% respectively. Kibos however had a low (2.5%) coefficient of variation.

In the greenhouse, Tukey's multiple comparisons test showed that there was strong significant difference (P<0.0001) in yield between K2 GHI (2.90±0.036) and K2 GHD (0.11±0.019) BGA. The droughted plants had a high (29.6%) coefficients of variation as compared to the irrigated crops which had 2.2%. The comparisons test showed that Alupe (1.40±0.091) and Oyani (0.67±0.045) K2 accessions had strong significant difference (P<0.0001) with GHI K2 accessions (2.90±0.036).The significant difference between Kibos (2.49±0.036) and GHI (2.90±0.036) was however P=0.0017 (Table 19a). The strong significant difference (P<0.0001) was also observed between GHD (0.11±0.019) and Alupe (1.40±0.091), and between GHD (0.11±0.019) and Kibos (2.49±0.036). However, between Oyani (0.67±0.045) and GHD (0.11±0.019), the significant difference was (P=0.0001) (Table 19a).

4.7.1.2 B4 yield (ton/ha)

Table 19b and Table 20indicate means for B4 accession in various zones. Alupe (2.67 ton/ha), then Kibos (2.64 ton/ha) and GHI (2.29 ton/ha) had the highest yields while GHD (0.49 tons/ha) had the lowest yields. The B4 accession yield ranged from 0.49 to 2.67 ton/ha. The yield was compared in three different agro ecological zones (Alupe, Kibos and Oyani) and in the greenhouse. The accession showed significant differences for yield in the zones. Tukey's multiple comparisons test showed that B4 yield (ton/ha) had strong significant difference (P<0.0001) between Alupe and Oyani, and between Kibos and Oyani. However, there was no variation in B4 accession yield performance in Alupe and Kibos. The GHD had high coefficient of variation of 14.4%. All the other zones had low coefficient of variation of ≤3.1% (Table 19 b). In the greenhouse, Tukey's multiple comparisons test showed that there was strong significant difference (P<0.0001) in yield between B4 GHI (2.29±0.025) and B4 GHD (0.49±0.041) BGA. The comparisons test indicated that B4 yield had strong significant difference (P<0.0001) between GHI (2.29±0.025) and all the other three field sites (Table 19b). The strong significant difference (P<0.0001) was also observed between GHD (0.49±0.041) and all the other three field sites.

4.7.1.3 B1 yield (ton/ha)

The B1 accession yield (ton/ha) mean values for various zones are shown in Table 19c. The highest grain yield was produced by the greenhouse irrigated (GHI) crops followed by Kibos, while the lowest yield was from GHD followed by Oyani. Yield of B1 accession across the zones ranged from 1.12 to 3.84 tons/ha. This yield from GHI is quite high considering that highest yield per genotype has been obtained in these studies. The yield was compared in three different agro ecological zones (Alupe, Kibos and Oyani) and in the greenhouse. Tukey's multiple comparisons test showed that B1 yield (ton/ha) had strong significant difference (P<0.0001) between Alupe (1.75±0.045) and Kibos (3.16±0.070) and between Kibos (3.16±0.070) and Oyani (1.33±0.015). Between Alupe and Oyani, the significant difference was (P=0.0011). All the zones had low coefficients of variation of ≤.4.5%.

In the greenhouse, Tukey's multiple comparisons test showed that there was strong significant difference (P<0.0001) in yield between B1 GHI (3.84±0.075) and B1 GHD (1.12±0.015) BGA. Tukey's multiple comparisons test showed that B1 yield had strong significant difference (P<0.0001) between GHI (3.84±0.075) and all the other three field sites (Table 19c). The strong significant difference (P<0.0001) was also observed between GHD (1.12±0.015) and Alupe, and between GHD (1.12±0.015) and Kibos. However, there was no significant difference (P=0.0808) between Oyani (1.33±0.015) and droughted (1.12±0.015).

4.7.1.4 K1 yield (ton/ha)

In K1 accession, Kibos (2.41±0.147) had significantly higher yields than other zones except GHI (2.24±0.039) (Table 19d). Average yield was 1.87 ton/ha, and ranged from 1.10 in Alupe to 2.41 ton/ha at Kibos. Tukey's multiple comparisons test showed that K1 yield (ton/ha) had strong significant difference (P<0.0001) between Alupe (1.10±0.047) and Kibos (2.41±0.147) and between Kibos (2.41±0.147) and Oyani (0.90±0.028). However, there was no significant difference (P=0.3873) between Alupe (1.10±0.047) and Oyani (0.90±0.028). In the greenhouse, Tukey's multiple comparisons test showed that there was strong significant difference (P<0.0001) in yield between K1 GHI (2.24±0.039) and K1 GHD (1.15±0.043) BGA. Amongst the field sites, only Alupe and Oyani K1 accessions had strong significant differences (P<0.0001) with GHI (2.24±0.039) (Table 19d). The strong significant difference (P<0.0001) was also observed between GHD (1.15±0.043) and K1 yield at Kibos. However, there was no

significant difference ($P \geq 0.05$) between the K1 droughted plants and the K1 plants grown at Alupe (1.10±0.047) and Oyani (0.90±0.028). Kibos had high coefficient of variation (10.6%), followed by Alupe (7.4%), then GHD (6.5%). GHI had the lowest (3.00%) coefficient of variation.

4.7.1.5 M4 yield (ton/ha)

Table 19e summarizes the mean performance of M4 accession across the zones and in the greenhouse. Kibos had the highest mean yield (2.74 ton/ha) followed closely by the GHI (2.70 ton/ha). The GHD recorded the least yield at 1.04 ton/ha. Strong significant difference ($P<0.0001$) in yield was observed between Alupe (1.31±0.054) and Kibos (2.74±0.010), and between Kibos (2.74±0.010) and Oyani (1.57±0.028). Between Alupe and Oyani, the significant difference was $P=0.0006$. In the greenhouse, Tukey's multiple comparisons test showed that there was strong significant difference ($P<0.0001$) in yield between M4 GHI (2.70±0.010) and M4 GHD (1.04±0.004) BGA. Amongst the field sites, only Alupe (1.31±0.054) and Oyani (1.57±0.028) M4 accessions had strong significant differences ($P<0.0001$) in yield with GHI (2.70±0.010) M4 accessions. There was no significant difference ($P=0.8243$) in yield between GHI (2.70±0.010) and Kibos (2.74±0.010). The strong significant difference ($P<0.0001$) in yield was also observed between GHD (1.04±0.004) and Kibos (2.74±0.010), and between GHD (1.04±0.004) and Oyani (1.57±0.028). However, between Alupe and GHD, the significant difference was $P \leq 0.0003$ (Table 19e). Alupe had the highest (7.2%) coefficient of variation, followed by Oyani (3.1%). All the other zones had coefficient of variation of ≤ 1.0%.

4.7.1.6 M2 yield (ton/ha)

The averages of yield of M2 accession across the three zones, and in the greenhouse are presented in Table 19f. Kibos zone recorded the highest yield followed by the GHI at 3.29 ton/ha and 2.94 ton/ha respectively. The GHD had the lowest average M2 yield of 0.17 ton/ha. Strong significant difference ($P<0.0001$) in yield was observed between Alupe (1.70±0.051) and Kibos (3.29±0.019), and between Kibos (3.29±0.019) and Oyani (1.51±0.036). The significant difference between Alupe and Oyani was $P \leq 0.0152$. In the greenhouse, Tukey's multiple comparisons test showed that there was strong significant difference ($P<0.0001$) in yield between M2 GHI (2.94±0.033) and M2 GHD (0.17±0.003) BGA. The comparisons showed that there was strong significant difference ($P<0.0001$) in yield between GHI (2.94±0.033) and all the field

sites. Similarly, there was strong significant difference (P<0.0001) between the GHD (0.17±0.003) yield and all the zonal yields. Alupe had the highest coefficient of variation (5.2%). All the other sites recorded low coefficient of variation of ≤ 4.1%.

Table 19a: Descriptive statistics of K2 Accession Inter-Site Yield (ton/ha) Comparisons

Acc.	Zones	YIELD		Mean	STD	SEM	CV	Tukey's multiple comparisons test	Adjusted *P* Value
		Min	Max						
K2	Alupe	1.22	1.50	1.40	0.158	0.071	11.3%	Alupe vs. Kibos	**<0.0001**
	Kibos	2.43	2.55	2.49	0.062	0.028	2.5%	Alupe vs. Oyani	**<0.0001**
	Oyani	0.59	0.74	0.67	0.079	0.035	11.7%	Kibos vs. Oyani	**<0.0001**
	GHD	0.09	0.15	0.11	0.032	0.014	29.1%	Irrigated vs droughted	**<0.0001**
	GHI	2.82	2.94	2.90	0.063	0.028	2.2%	Alupe vs. Irrigated	**<0.0001**

Acc accession *Min* minimum *Max* maximum *STD* standard deviation *SEM* standard error of mean *CV* coefficient of variation. All comparisons were highly significant at $P \leq 0.0001$ except Kibos vs. Irrigated which was at $P \leq 0.0017$.

Table 19 b: Descriptive statistics of B4 accession inter-site yield (ton/ha) comparisons

Acc.	Zones	YIELD		Mean	STD	SEM	CV		
		Min	Max					Tukey's multiple comparisons test	Adjusted *P* Value
B4	Alupe	2.61	2.73	2.67	0.059	0.026	2.2%	Alupe vs. Kibos	0.9602
	Kibos	2.61	2.66	2.64	0.025	0.011	0.9%	Alupe vs. Oyani	**<0.0001**
	Oyani	0.85	0.90	0.88	0.027	0.012	3.1%	Kibos vs. Oyani	**<0.0001**
	GHD	0.44	0.57	0.49	0.070	0.031	14.3%	Alupe vs. droughted	**<0.0001**
	GHI	2.26	2.34	2.29	0.043	0.019	1.9%	Kibos vs. Irrigated	**<0.0001**

Acc accession *Min* minimum *Max* maximum *STD* standard deviation *SEM* standard error of mean *CV* coefficient of variation. All comparisons were highly significant at $P \leq 0.0001$ except Alupe vs. Kibos where there was no significant difference.

Table 19 c: Descriptive statistics of B1 accession inter-site yield (ton/ha) comparisons

Acc.	Zones	YIELD		Mean	STD	SEM	CV	Tukey's multiple comparisons test	Adjusted *P* Value
		Min	Max						
B1	Alupe	1.67	1.82	1.75	0.078	0.035	4.5%	Alupe vs. Kibos	**<0.0001**
	Kibos	3.04	3.28	3.16	0.120	0.054	3.8%	Alupe vs. Oyani	**0.0011**
	Oyani	1.32	1.36	1.33	0.025	0.011	1.9%	Kibos vs. Oyani	**<0.0001**
	GHD	1.09	1.14	1.12	0.026	0.012	2.32%	Kibos vs. droughted	**<0.0001**
	GHI	3.70	3.95	3.84	0.129	0.058	3.37%	Oyani vs. irrigated	**<0.0001**

Acc accession *Min* minimum *Max* maximum *STD* standard deviation *SEM* standard error of mean *CV* coefficient of variation. Apart from Oyani vs. droughted where there was no significant difference, all comparisons were highly significant at $P \leq 0.0001$ except Alupe and Oyani where $P \leq 0.0011$.

Table 19 d. Descriptive statistics of K1 accession inter-site yield (ton/ha) comparisons

Acc.	Zones	YIELD		Mean	STD	SEM	CV	Tukey's multiple comparisons test	Adjusted *P* Value
		Min	Max						
K1	Alupe	1.02	1.18	1.10	0.081	0.036	7.4%	Alupe vs. Kibos	**<0.0001**
	Kibos	2.16	2.67	2.41	0.255	0.114	10.6%	Alupe vs. Oyani	0.3873
	Oyani	0.85	0.95	0.90	0.049	0.022	5.4%	Kibos vs. Oyani	**<0.0001**
	GHD	1.07	1.22	1.15	0.074	0.033	6.4%	Droughted vs. Irrigated	**<0.0001**
	GHI	2.17	2.29	2.24	0.067	0.030	3.0%	Alupe vs. Irrigated	**<0.0001**

Acc accession *Min* minimum *Max* maximum *STD* standard deviation *SEM* standard error of mean *CV* coefficient of variation. All comparisons were highly significant at $P \leq 0.0001$ except between Alupe vs. Oyani, Alupe vs. droughted, Oyani vs. droughted and Kibos vs. Irrigated where there were no significant differences ($P \geq 0.05$).

Table 19e: Descriptive statistics of M4 accession inter-site yield (ton/ha) comparisons

Acc.	Zones	YIELD		Mean	STD	SEM	CV		
		Min	Max					Tukey's multiple comparisons test	Adjusted *P* Value
M4	Alupe	1.24	1.42	1.31	0.094	0.042	7.2%	Alupe vs. Kibos	**<0.0001**
	Kibos	2.73	2.77	2.74	0.018	0.008	0.7%	Alupe vs. Oyani	**0.0006**
	Oyani	1.51	1.61	1.57	0.049	0.022	3.1%	Kibos vs. Oyani	**<0.0001**
	GHD	1.03	1.04	1.04	0.007	0.003	0.7%	Kibos vs. Droughted	**<0.0001**
	GHI	2.69	2.72	2.70	0.018	0.008	0.7%	Droughted vs. Irrigated	**<0.0001**

Acc accession *Min* minimum *Max* maximum *STD* standard deviation *SEM* standard error of mean *CV* coefficient of variation. Apart from Kibos vs. Irrigated where there was no significant difference, all comparisons were highly significant at $P \leq 0.0001$ except Alupe vs. Oyani where $P \leq 0.0006$ and Alupe vs droughted where $P \leq 0.0003$.

Table 19f: Descriptive statistics of M2 accession inter-site yield (ton/ha) comparisons

Acc.	Zones	YIELD		Mean	STD	SEM	CV	Tukey's multiple comparisons test	Adjusted *P* Value
		Min	Max						
M2	Alupe	1.64	1.80	1.70	0.089	0.040	5.2%	Alupe vs. Kibos	**<0.0001**
	Kibos	3.27	3.33	3.29	0.032	0.014	1.0%	Alupe vs. Oyani	**0.0152**
	Oyani	1.46	1.58	1.51	0.062	0.028	4.1%	Kibos vs. Oyani	**<0.0001**
	GHD	0.17	0.18	0.17	0.005	0.002	2.9%	Kibos vs. droughted	**<0.0001**
	GHI	2.87	2.97	2.94	0.057	0.025	1.9%	droughted vs. Irrigated	**<0.0001**

Acc accession *Min* minimum *Max* maximum *STD* standard deviation *SEM* standard error of mean *CV* coefficient of variation. All comparisons were highly significant at $P \leq 0.0001$ except between Alupe vs. Oyani, where $P \leq 0.0152$.

Table 20: Yield (ton/ha) of various accessions in different agro-ecological zones

Accession	Alupe	Kibos	Oyani	Droughted	Irrigated
K2	1.40	2.49	0.67	0.11	2.89
B4	**2.67**	2.64	0.88	0.49	2.29
B1	1.75	3.16	1.33	1.12	**3.84**
K1	1.10	2.41	0.90	**1.15**	2.24
M4	1.31	2.74	**1.57**	1.04	2.70
M2	1.70	**3.29**	1.51	0.17	2.94
Zonal means	1.65	2.79	1.14	0.68	2.82

4.7.2 Yield Intra-Site Comparisons

4.7.2.1 Alupe Zone

Table 21a summarizes the mean performance in yield of the landraces grown at Alupe, in Busia County. The yield means for the accessions in this zone ranged from 1.10to 2.67 ton/ha. The zone had an average yield of 1.65 ton/ha. B4 and B1 accessions had the highest grain yield of 2.67ton/ha and 1.75 ton/ha respectively, and differed significantly ($P<0.05$) with the lowest yielding accessions which were K1 and M4 with a yield of 1.10 ton/ha and 1.31ton/ha respectively. Tukey's multiple comparisons test indicated that majority of the accessions comparisons had significant differences ($P<0.05$) in this zone (Table 21a). K2 accession had the highest coefficient of variation of 10.1%, whereas B4 had the lowest coefficient of variation of 2.0%.

4.7.2.2 Kibos Zone

M2 accession recorded significantly ($P<0.05$) higher grain yields than all other accessions in this zone except B1 accession which ranked second in this zone (Table 21b). The average grain yield in this zone was 2.79 ton/ha and varied from 2.41 to 3.29 ton/ha.There was no variation in yield between accessions K2, B4, K1 and M4 (Table 21b). Similarly, no variation was observed between accessions B1 and M2. K1 had the highest coefficient of variation at 9.5%, whereas M4 had the lowest coefficient of variation at 0.6%.

4.7.2.3 Oyani Zone

In this zone, M4 accession had the highest mean grain yield of 1.57 ton/ha followed closely by M2 accession which had grain yield of 1.51 ton/ha (Table 21c). B4 accession had the lowest grain yield of 0.88 ton/ha, and varied significantly ($P<0.05$) with all other accessions except K1, where there was no variation (Table 21c). Basically, the zone had an average yield of 1.14 ton/ha which is low considering the average performance of these accessions in other Lake Victoria basin zones have been obtained. M4 accession also varied significantly ($P<0.05$) with all other accessions except M2, where there was no variation (Table 21c). K2 had the highest (10.5%) coefficient of variation, whereas B1 had the lowest (1.7%) coefficient of variation.

4.7.2.4 Greenhouse Droughted (GHD) Zone

Table 21d summarises the mean yield performance of six accessions grown and exposed to water stress in the greenhouse. Among the droughted plants in the greenhouse, K1 and B1 accessions had highest grain yields of 1.15 and 1.12 ton/ha respectively. These yields are moderately high considering the conditions under which the crops grew. K2 and M2 had the lowest grain yields of 0.11 ton/ha and 0.17 ton/ha respectively. The average grain yield in this zone was 0.68 ton/ha. Tukey's multiple comparisons test showed that there was no variation in yield observed between K1, M4 and B1 (Table 21d). There was also no variation observed between K2 and M2 accessions. K2 had the highest (26.4%) coefficient of variation, whereas M4 had the lowest (0.6%) coefficient of variation among the landraces evaluated.

4.7.2.5 Greenhouse Irrigated (GHI) Zone

Among the greenhouse irrigated crops, the yield means for the accessions ranged from 2.24 to 3.84 ton/ha (Table 21e). The zone had an average yield of 2.82 ton/ha, which was the highest among the zones evaluated. B1 and M2 accessions had the highest grain yield of 3.84ton/ha and 2.94ton/ha respectively, and differed significantly ($P<0.05$) with the lowest yielding accessions which were K1 and B4 with a yield of 2.24 ton/ha and 2.29ton/ha respectively. Tukey's multiple comparisons test indicated that majority of the accessions comparisons had significant differences ($P<0.05$) in this zone (Table 21e). All the accessions had low coefficient of variations of ≤3.0%.

Table 21a: Descriptive statistics of Alupe zone intra-site yield (ton/ha) comparisons of accessions K2, B4, B1, K1, M4 and M2.

Zones	Acc.	YIELD		Mean	STD	SEM	CV	Tukey's multiple comparisons test	Adjusted *P* Value
		Min	Max						
Alupe	K2	1.22	1.50	1.40	0.142	0.058	10.1%	K2 vs. B4	**<0.0001**
	B4	2.61	2.73	2.67	0.052	0.021	2.0%	K2 vs. B1	**<0.0001**
	B1	1.67	1.82	1.75	0.070	0.029	4.0%	K2 vs. K1	**<0.0001**
	K1	1.02	1.18	1.10	0.072	0.030	6.6%	K2 vs. M2	**<0.0001**
	M4	1.24	1.42	1.31	0.084	0.034	6.4%	B4 vs. B1	**<0.0001**
	M2	1.64	1.80	1.70	0.079	0.032	4.7%	B4 vs. K1	**<0.0001**

Acc accession *Min* minimum *Max* maximum *STD* standard deviation *SEM* standard error of Mean *CV* coefficient of variation. All comparisons were highly significant at $P \leq 0.0001$ except K1 vs. M4 which was at $P \leq 0.0023$.

Table 21b: Descriptive statistics of Kibos zone intra-site yield (ton/ha) comparisons of accessions K2, B4, B1, K1, M4 and M2.

Zones	Acc.	YIELD		Mean	STD	SEM	CV	Tukey's multiple comparisons test	Adjusted *P* Value
		Min	Max						
Kibos	K2	2.43	2.55	2.49	0.056	0.023	2.2%	K2 vs. B1	**<0.0001**
	B4	2.61	2.66	2.64	0.022	0.009	0.8%	K2 vs. M4	**0.0031**
	B1	3.04	3.28	3.16	0.108	0.044	3.4%	K2 vs. M2	**<0.0001**
	K1	2.16	2.67	2.41	0.228	0.093	9.5%	B4 vs. B1	**<0.0001**
	M4	2.73	2.77	2.74	0.016	0.007	0.6%	B4 vs. K1	**0.0101**
	M2	3.27	3.33	3.29	0.029	0.012	0.9%	B4 vs. M2	**<0.0001**

Acc accession *Min minimum Max* maximum *STD* standard deviation *SEM* standard error of mean *CV* coefficient of variation. All comparisons were highly significant at $P \leq 0.0001$ except K2 vs. M4 which was at $P \leq 0.0031$ and B4 vs. K1 which was at $P \leq 0.0101$.

Table 21c: Descriptive statistics of Oyani zone intra-site yield (ton/ha) comparisons of accessions K2, B4, B1, K1, M4 and M2.

Zones	Acc.	YIELD		Mean	STD	SEM	CV	Tukey's multiple comparisons test	Adjusted *P* Value
		Min	Max						
Oyani	K2	0.59	0.74	0.67	0.070	0.029	10.5%	K2 vs. B4	**<0.0001**
	B4	0.85	0.90	0.88	0.024	0.010	2.7%	K2 vs. B1	**<0.0001**
	B1	1.32	1.36	1.33	0.023	0.009	1.7%	K2 vs. K1	**<0.0001**
	K1	0.85	0.95	0.90	0.044	0.018	4.9%	K2 vs. M4	**<0.0001**
	M4	1.51	1.61	1.57	0.044	0.018	2.8%	K2 vs. M2	**<0.0001**
	M2	1.46	1.58	1.51	0.055	0.023	3.6%	B4 vs. B1	**<0.0001**

Acc accession *Min* minimum *Max* maximum *STD* standard deviation *SEM* standard error of mean *CV* coefficient of variation. All comparisons were highly significant at $P \leq 0.0001$.

Table 21d: Descriptive statistics of GHD intra-site yield (ton/ha) comparisons of accessions K2, B4, B1, K1, M4 and M2.

Zones	Acc.	YIELD		Mean	STD	SEM	CV	Tukey's multiple comparisons test	Adjusted *P* Value
		Min	Max						
GHD	K2	0.09	0.15	0.11	0.029	0.012	26.4%	K2 vs. B4	**<0.0001**
	B4	0.44	0.57	0.49	0.063	0.026	12.9%	K2 vs. B1	**<0.0001**
	B1	1.10	1.14	1.12	0.023	0.009	2.1%	K2 vs. K1	**<0.0001**
	K1	1.07	1.22	1.15	0.066	0.027	5.8%	K2 vs. M4	**<0.0001**
	M4	1.03	1.04	1.04	0.006	0.003	0.6%	B1 vs. M2	**<0.0001**
	M2	0.17	0.18	0.17	0.006	0.002	2.3%	M4 vs. M2	**<0.0001**

Acc accession *Min* minimum *Max* maximum *STD* standard deviation *SEM* standard error of mean *CV* coefficient of variation. There was no significant difference ($P \geq 0.05$) observed between B1 vs. K1, and K2 vs.M2. All the other comparisons were highly significant at $P \leq 0.0001$ except B1 vs.M4 which was at $P \leq 0.0149$.

Table 21e: Descriptive statistics of GHI intra-site yield (ton/ha) comparisons of accessions K2, B4, B1, K1, M4 and M2.

Zones	Acc.	YIELD		Mean	STD	SEM	CV	Tukey's multiple comparisons test	Adjusted *P* Value
		Min	Max						
GHI	K2	2.82	2.94	2.90	0.056	0.023	1.9%	K2 vs. B4	**<0.0001**
	B4	2.26	2.34	2.29	0.039	0.016	1.7%	K2 vs. B1	**<0.0001**
	B1	3.70	3.95	3.84	0.116	0.047	3.0%	K1 vs. M4	**<0.0001**
	K1	2.17	2.29	2.24	0.060	0.025	2.7%	M2 vs. M4	**<0.0001**
	M4	2.69	2.72	2.70	0.016	0.006	0.6%	K1 vs. M2	**<0.0001**
	M2	2.87	2.97	2.94	0.051	0.021	1.7%	B4 vs. B1	**<0.0001**

Acc accession *Min* minimum *Max* maximum *STD* standard deviation *SEM* standard error of mean *CV* coefficient of variation. There was no significant difference (P≥0.05) observed between K2 vs. M2 and B4 vs. K1. All the other comparisons were highly significant at $P \leq 0.0001$ except K2 vs.M4 which was at $P \leq 0.0002$.

4.7.3 Correlation Coefficients of Yield and Yield Components

The Pearson's correlation coefficients of all quantitative traits were estimated to establish which traits had a positive or negative association with yield in each zone. The results are presented in Table 22a-22e. At Alupe, all the traits had positive correlations with yield apart from N2S (r= -0.12, *p*= 0.6388). PHT, N1S, POD, TLA, NFP and SWT had a strong and positive correlations with yield (r=0.59, *p*=0.0093), (r=0.57, *p*=0.0128), (r=0.63, *p*=0.0053), (r=0.75, *p*=0.0003), (r=0.61, *p*=0.0071), (r=1.00, *p*=0.0000) respectively (Table 22a).

Table 22a: Pearson's correlation (r) coefficients for seven quantitative traits with yield of Bambara groundnut accessions in Alupe zone.

	PHT	N1S	N2S	POD	TLA	NFP	SWT
Yield (r)	0.59	0.57	-0.12	0.63	0.75	0.61	1.00
***P* value**	0.0093	0.0128	0.6388	0.0053	0.0003	0.0071	0.000

At Kibos all the traits had positive correlations with yield apart from N2S (r= -0.12, *p*=0.6265). PHT, N1S, POD and TLA had a moderate and positive correlations with yield (r=0.23, *p*=0.3500), (r=0.41, *p*=0.0893), (r=0.39, *p*=0.1116), (r=0.32, *p*=0.1896) respectively. NFP had a weak and positive correlation with yield (r=0.13, *p*=0.5996). However, SWT had a perfect, positive significant correlation with yield (r=1.00, *p*=0.0000) (Table 22b).

Table 22b: Pearson's correlation (r) coefficients for seven quantitative traits with yield of Bambara groundnut accessions in Kibos zone.

	PHT	N1S	N2S	POD	TLA	NFP	SWT
Yield (r)	0.23	0.41	-0.12	0.39	0.32	0.13	1.00
***P* value**	0.3500	0.0893	0.6265	0.1116	0.1896	0.5996	0.000

At Oyani, PHT and N2S showed a negative association with yield (r= -0.48, *p*= 0.0544) and (r= -0.13, *p*= 0.6074) respectively. All the other traits had positive correlations with yield. Yield had very strong positive and very highly significant correlation with N1S (r=0.82, *p*=0.0000), POD (r=0.81, *p*=0.0000), TLA (r=0.92, *p*=0.0000), and NFP (r=0.87, *p*=0.0000). However, SWT had a perfect, positive significant correlation with yield (r=1.00, *p*=0.0000) (Table 22c).

Table 22c: Pearson's correlation (r) coefficients for seven quantitative traits with yield of Bambara groundnut accessions in Oyani zone.

	PHT	N1S	N2S	POD	TLA	NFP	SWT
Yield (r)	-0.48	0.82	-0.13	0.81	0.92	0.87	1.00
***P* value**	0.0544	0.0000	0.6074	0.0000	0.0000	0.0000	0.0000

In the GHD, all the traits had positive correlations with yield. PHT had a weak and positive correlation with yield (r=0.16, *p*= 0.3018). N2S had a moderate and positive correlation with yield (r=0.39, *p*= 0.1074). N1S, POD, TLA and NFP (r=0.88, *p*=0.000), (r=0.90, *p*=0.000), (r=0.70, *p*= 0.0012), (r=0.80, *p*=0.000) respectively had a strong and positive significant correlations with yield. However, SWT had a perfect, positive significant correlation with yield (r=1.00, *p*=0.0000) (Table 22d).

Table 22d: Pearson's correlation (r) coefficients for seven quantitative traits with yield of Bambara groundnut accessions in GHD.

	PHT	N1S	N2S	POD	TLA	NFP	SWT
Yield (r)	0.16	0.88	0.39	0.90	0.70	0.80	1.00
***P* value**	0.3018	0.0000	0.1074	0.0000	0.0012	0.0000	0.0000

In the GHI, all the traits had positive correlations with yield apart from N2S (r= -0.01, *p*= 0.9730) that showed negative association with yield. PHT had a moderate and positive correlation with yield (r=0.23, *p*= 0.3538). Strong and positive correlations of yield with N1S (r=0.57, *p*=0.0128) POD (r=0.48, *p*=0.0427) TLA (r=0.84, *p*=0.000) and NFP (r=0.51, *p*=0.0323) were observed. However, SWT had a perfect, positive significant correlation with yield (r=1.00, *p*=0.0000) (Table 22e).

Table 22e: Pearson's correlation (r) coefficients for seven quantitative traits with yield of Bambara groundnut accessions in GHI

	PHT	N1S	N2S	POD	TLA	NFP	SWT
Yield (r)	0.23	0.57	-0.01	0.48	0.84	0.51	1.00
***P* value**	0.3538	0.0128	0.9730	0.0427	0.0000	0.0323	0.0000

4.7.4 Selection of high yielding BGN accession per zone based on Rank Summation Index (RSI)

Yield is a polygenic trait influenced by its component traits. All the qualitative traits evaluated had a direct impact on yield. To identify the highest-yielding accession per zone, Pearson's correlation coefficients were estimated to determine the traits positively correlated with yield. The high-yielding accession in each zone was then identified by calculating the Rank Summation Index (RSI) based on these positively correlated traits. Accessions were first ranked according to the mean values of the correlated traits (with 1 representing the highest and 6 the lowest), following the RSI method proposed by Mulamba and Mock (1978). The ranking values across traits were summed to determine the overall performance of each accession. In this method, the accession with the lowest RSI value is considered the most adaptable and highest yielding within a zone. Table 23a–e presents the RSI rankings of accessions based on traits positively associated with yield.

Table 23a: Mean values of yield-related traits and Rank Summation Index (RSI) of six BGN accessions at Alupe, Busia County.

Acc	PHT	rsi	N1S	rsi	POD	rsi	TLA	rsi	NFP	rsi	SWT	rsi	RSI =Σrsi	Yld Ton/ha
B4	21.29	1	23.00	1	25.67	1	26.05	1	58.17	2	10.97	1	7	2.67
B1	15.14	4	19.33	2	23.67	2	24.01	3	62.00	1	7.22	2	14	1.75
M4	12.27	6	19.33	2	22.67	3	17.79	5	51.67	3	5.40	5	24	1.31
M2	14.57	5	18.67	5	21.67	4	24.00	4	50.00	4	7.00	3	25	1.70
K2	19.08	2	12.50	6	17.33	6	24.28	2	45.00	6	5.75	4	26	1.40
K1	16.40	3	18.93	4	21.27	5	15.85	6	47.00	5	4.51	6	29	1.10

PHT plant height, *N1S* number of pods with one seed, *POD* total number of pods per plant, *TLA* terminal leaflet area, *NFP* number of flowers per plant, *SWT* seed weight(g) per plant, *RSI* rank summation index, *Yld* yield ton/ha.

Table 23b: Mean values of yield-related traits and Rank Summation Index (RSI) of six BGN accessions at Kibos, Kisumu County.

Acc	PHT	rsi	N1S	rsi	POD	rsi	TLA	rsi	NFP	rsi	SWT	rsi	RSI =Σrsi	Yld Ton/ha
M2	19.62	1	27.33	1	31.67	2	26.21	2	73.37	2	13.55	1	9	3.29
B1	19.45	2	26.33	4	30.67	4	25.04	4	69.17	3	13.02	2	19	3.16
M4	13.30	6	26.67	3	31.33	3	23.26	5	74.87	1	11.29	3	21	2.74
B4	19.35	3	21.67	5	27.33	5	26.41	1	59.67	5	10.86	4	23	2.64
K1	14.03	5	27.00	2	32.00	1	22.16	6	66.73	4	9.92	6	24	2.41
K2	17.27	4	17.67	6	21.33	6	25.82	3	56.33	6	10.24	5	30	2.49

PHT plant height, *N1S* number of pods with one seed, *POD* total number of pods per plant, *TLA* terminal leaflet area, *NFP* number of flowers per plant, *SWT* seed weight(g) per plant, *RSI* rank summation index, *Yld* yield ton/ha.

Table 23c: Mean values of yield-related traits and Rank Summation Index (RSI) of six BGN accessions at Oyani, Migori County.

Acc	N1S	rsi	POD	rsi	TLA	rsi	NFP	rsi	SWT	rsi	RSI =Σrsi	Yld Ton/ha
M4	21.00	1	24.33	1	25.76	1	53.17	1	6.45	1	5	1.57
M2	18.33	2	20.00	2	22.28	2	45.63	2	6.23	2	10	1.51
B1	17.00	3	20.00	2	20.52	3	44.03	3	5.48	3	14	1.33
K1	17.00	3	19.00	4	18.78	4	41.00	4	3.69	4	19	0.90
B4	14.00	5	17.33	5	18.08	5	31.67	6	3.62	5	26	0.88
K2	9.33	6	12.33	6	16.78	6	33.30	5	2.77	6	29	0.67

N1S number of pods with one seed, *POD* total number of pods per plant, *TLA* terminal leaflet area, *NFP* number of flowers per plant, *SWT* seed weight(g) per plant, *RSI* rank summation index, *Yld* yield ton/ha.

Table 23d: Mean values of yield-related traits and Rank Summation Index (RSI) of six BGN accessions under greenhouse drought stress (GHD).

Acc	PHT	rsi	N1S	rsi	N2S	rsi	POD	rsi	TLA	rsi	NFP	rsi	SWT	rsi	RSI =Σrsi	Yld Ton/ha
K1	14.80	1	15.00	3	2.667	2	17.67	3	20.26	2	43.50	1	4.72	1	13	1.15
B1	13.97	4	15.33	2	3.667	1	19.00	1	21.86	1	30.00	3	4.60	2	14	1.12
M4	12.11	6	16.33	1	2.333	3	18.67	2	12.61	5	35.00	2	4.26	3	22	1.04
B4	14.53	3	12.00	4	2.333	3	14.33	4	15.63	3	28.00	4	2.00	4	25	0.49
M2	13.17	5	9.00	5	2.333	3	11.33	5	12.94	4	26.33	5	0.71	5	32	0.17
K2	14.62	2	6.00	6	2.333	3	8.33	6	12.33	6	20.33	6	0.45	6	35	0.11

PHT plant height, *N1S* number of pods with one seed, *N2S* number of pods with two seeds *POD* total number of pods per plant, *TLA* terminal leaflet area, *NFP* number of flowers per plant, *SWT* seed weight(g) per plant, *RSI* rank summation index, *Yld* yield ton/ha.

Table 23e: Mean values of yield-related traits and Rank Summation Index (RSI) of six BGN accessions under greenhouse irrigation (GHI).

Acc	PHT	rsi	N1S	rsi	POD	rsi	TLA	rsi	NFP	rsi	SWT	rsi	RSI =Σrsi	Yld Ton/ha
B1	18.57	3	28.00	1	33.67	1	31.57	1	75.00	1	15.79	1	8	3.84
K2	20.87	1	21.33	5	25.00	6	29.04	2	64.00	4	11.91	3	21	2.89
M2	15.87	4	22.67	4	27.00	4	28.23	3	62.27	5	12.08	2	22	2.94
M4	13.73	6	24.67	2	29.00	3	27.48	5	72.30	2	11.12	4	22	2.70
K1	14.97	5	24.67	2	30.33	2	25.04	6	70.00	3	9.22	6	24	2.24
B4	19.57	2	20.33	6	26.00	5	27.89	4	55.80	6	9.42	5	28	2.29

PHT plant height, *N1S* number of pods with one seed, *POD* total number of pods per plant, *TLA* terminal leaflet area, *NFP* number of flowers per plant, *SWT* seed weight(g) per plant, *RSI* rank summation index, *Yld* yield ton/ha.

From Tables 23a–e, B4 had the lowest RSI (7) at Alupe, M2 had the lowest RSI (9) at Kibos, and M4 had the lowest RSI (4) at Oyani. Among the greenhouse drought-stressed plants, K1 recorded the lowest RSI (13), while among the irrigated plants, B1 had the lowest RSI (8). These accessions, exhibiting the highest yielding abilities in their respective zones, were selected for protein analysis.

4.8 Protein Analysis

4.8.1 Total Protein Concentrations in the Accessions

The total protein concentration of each accession was determined using Bradford assay, and M2 accession had the highest total mean protein concentrations (0.123±0.0006), followed by B1 accession (0.104±0.0007). M4 accession had third highest total mean protein concentrations (0.097±0.0012). On the contrary, K1 accession had the least total protein concentrations (0.075±0.0004), followed by B4 which had 0.081±0.0012. Tukey's multiple comparison test (one-way ANOVA, $p \leq 0.05$) showed that all the accessions comparisons differed significantly (Table 24). All the accessions K1, B4, B1, M4 and M2 recorded low coefficients of variations of 0.9%, 2.5%, 1.1%, 2.2% and 0.9% respectively.

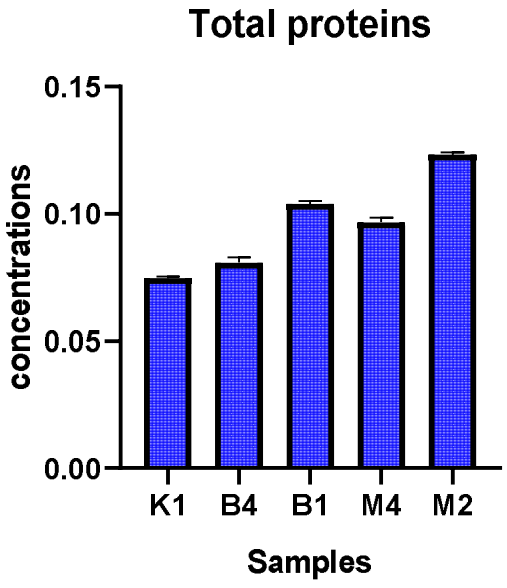


Figure 43: The five accessions (K1, B4, B1, M4 and M2) total protein concentrations. Comparisons of the five accessions total protein concentrations based on Tukey's multiple

comparison test (one-way ANOVA, *P*<0.05) are graphically presented. Bars represent mean±SEM of samples.

4.8.2 Protein Fractions

4.8.2.1 Protein Fractions Concentrations Intra-Accession Sample Comparisons

4.8.2.1.1 K1 Sample

The protein fractions concentration in K1 sample was determined and globulins had the highest concentration (0.034±0.0005), followed by albumins (0.025±0.0005). Prolamins had the lowest concentrations (0.004±0.0007) followed by glutelins (0.006±0.0006) (Figure 44). Tukey's multiple comparisons test (one-way ANOVA, P<0.0001) showed that all the protein fractions comparisons had significant differences apart from between glutelins and prolamins where there was no significant difference (*P*=0.2643). The protein fractions albumins, globulins, glutelins recorded low coefficients of variation of < 20% apart from prolamins which recorded a high coefficient of variation of 28.39%.

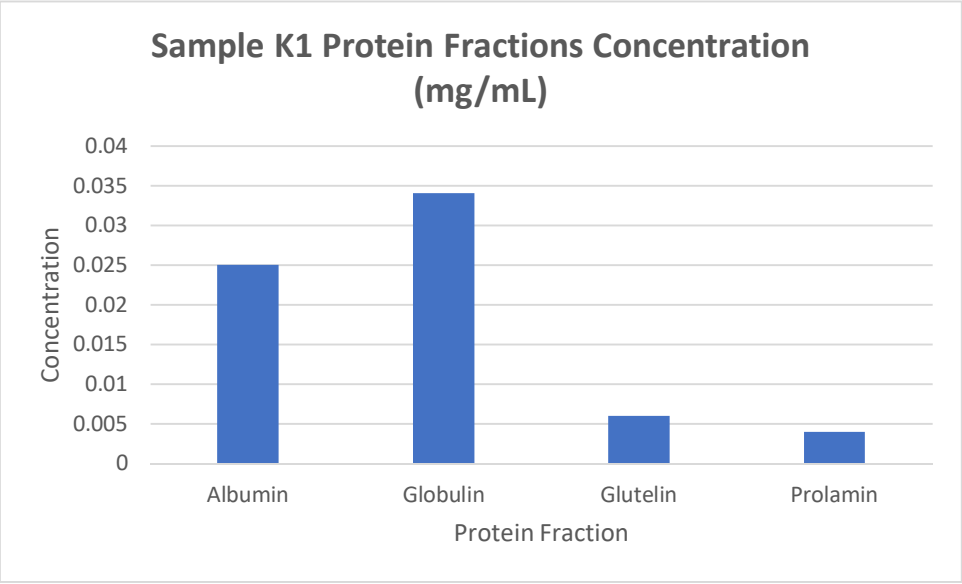


Figure 44: The four protein fractions (albumins, globulins, glutelins and prolamins) concentrations in K1 sample. Comparisons of the four protein fractions concentrations based on Tukey's multiple comparison test (one-way ANOVA, *P*<0.05) are graphically presented. Bars represent means of samples.

4.8.2.1.2 B4 Sample

In this sample, albumins had the highest concentration (0.050± 0.0006). Globulins had the second highest concentration (0.023±0.0007) in this sample, while glutelins and prolamins

recorded the least concentrations of 0.004±0.0005 and 0.002±0.0002 respectively (Figure 45). Tukey's multiple comparisons test (one-way ANOVA, $P<0.0001$) showed that all the protein fractions comparisons had significant differences apart from between glutelins and prolamins where there was no significant difference ($P=0.1426$). The protein fractions albumins, globulins, recorded low coefficients of variation of <10% apart from glutelins and prolamins which recorded high coefficients of variation of 24.67% and 22.04% respectively.

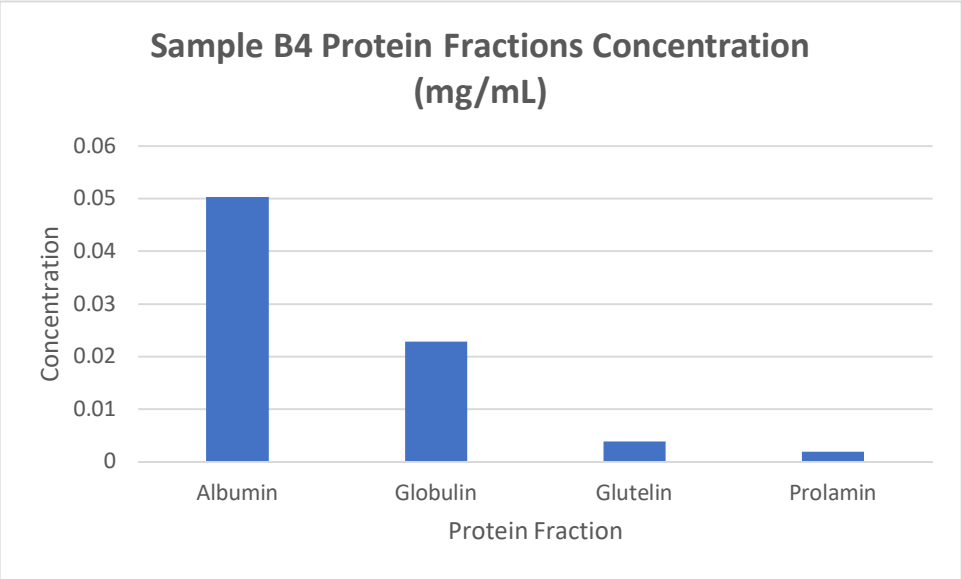


Figure 45: The four protein fractions (albumins, globulins, glutelins and prolamins) concentrations in B4 sample. Comparisons of the four protein fractions concentrations based on Tukey's multiple comparison test (one-way ANOVA, $P<0.05$) are graphically presented. Bars represent means of samples.

4.8.2.1.3 B1 Sample

The protein fractions concentration in B1 sample was determined and albumins had the highest concentration (0.042± 0.0005), followed by glutelins (0.029± 0.0007). Prolamins had the lowest concentrations (0.003±0.0003) followed by globulin (0.022±0.0003) (Figure 46). Tukey's multiple comparisons test (one-way ANOVA, $P<0.0001$) showed that all the protein fractions comparisons had significant differences. All the protein fractions albumins, globulins and glutelins recorded low coefficients of variation of <5%, whereas prolamins recorded a high coefficient of variation of 23.48%.

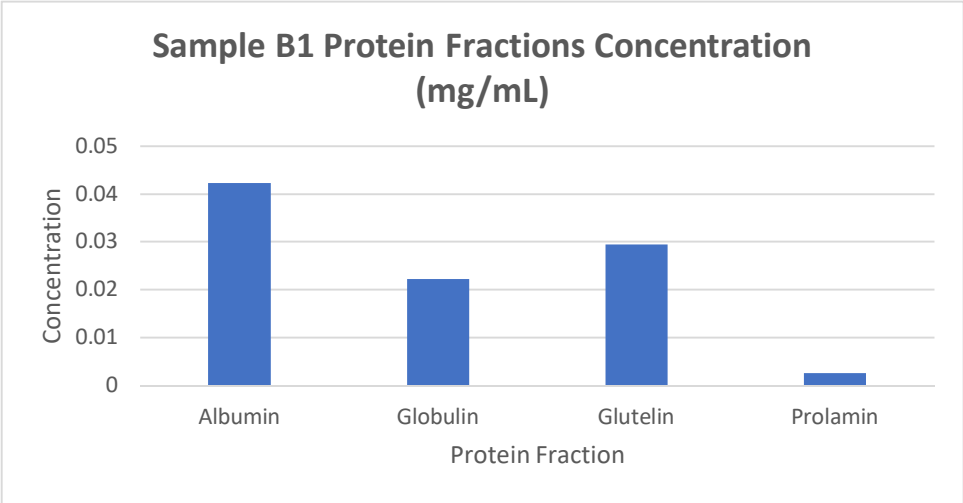


Figure 46: The four protein fractions (albumins, globulins, glutelins and prolamins) concentrations in B1 sample. Comparisons of the four protein fractions concentrations based on Tukey's multiple comparison test (one-way ANOVA, *P*<0.0001) are graphically presented. Bars represent means of samples.

4.8.2.1.4 M2 Sample

The protein fractions concentration in M2 sample was determined and albumins had the highest concentration (0.065±0.0004), followed by globulins (0.041±0.0008). Glutelins had the third highest concentrations (0.012±0.0008). Prolamins recorded the least concentration (0.002± 0.0003) (Figure 47). Tukey's multiple comparisons test (one-way ANOVA, *P*<0.0001) showed that all the protein fractions comparisons were statistically significant. The protein fractions albumins and globulins recorded low coefficients of variation of <5%. The glutelins and prolamins recorded high coefficients of variation of 10.86% and 24.16% respectively.

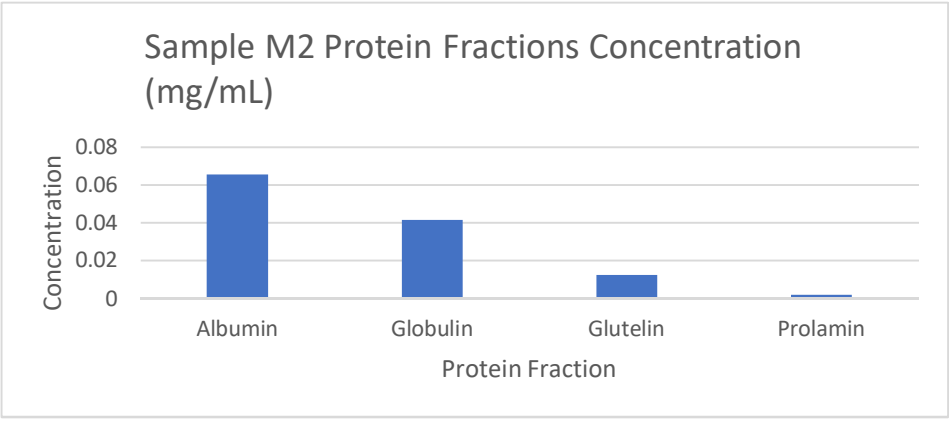


Figure 47: The four protein fractions (albumins, globulins, glutelins and prolamins) concentrations in M2 sample. Comparisons of the four protein fractions concentrations based on Tukey's multiple comparison test (one-way ANOVA, *P*<0.0001) are graphically presented. Bars represent means of samples.

4.8.2.1.5 M4 Sample

In this sample, albumins had the highest concentration (0.050± 0.0007). Globulins had the second highest concentration (0.031±0.0005) in this sample, while glutelins and prolamins recorded the least concentrations of 0.012±0.0004 and 0.002±0.0004 respectively (Figure 48). Tukey's multiple comparisons test (one-way ANOVA, $P<0.0001$) showed that all the protein fractions comparisons had significant differences. All the protein fractions albumins, globulins, and glutelins recordedlowcoefficients of variation of <10%, while and prolamins recorded a high coefficients of variation of 36.83%.

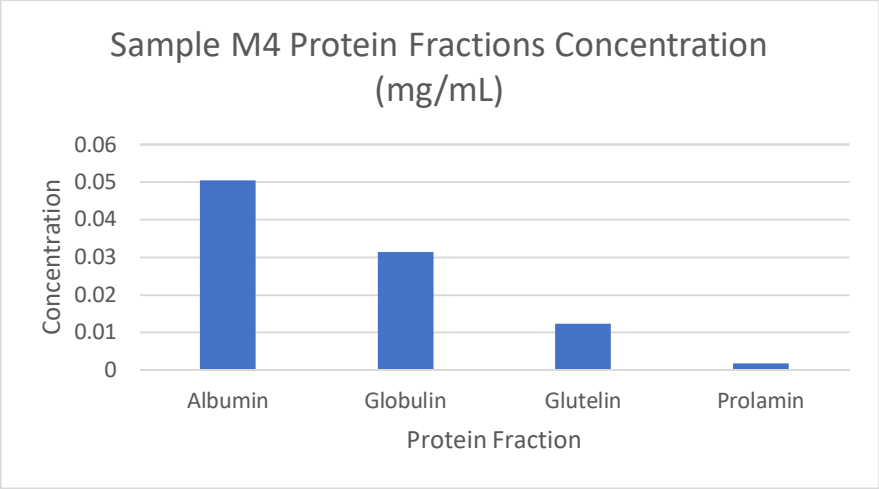


Figure 48: The four protein fractions (albumins, globulins, glutelins and prolamins) concentrations in M4 sample. Comparisons of the four protein fractions concentrations based on Tukey's multiple comparison test (one-way ANOVA, $P\leq0.0001$) are graphically presented. Bars represent means of samples.

4.8.2.2 Protein Fraction Concentrations Inter-Accessions Sample Comparisons

4.8.2.2.1 Albumins

Amongst the accessions samples, M2 had the highest albumins concentration (0.065±0.0005), followed by M4 (0.050±0.0007) and B4 (0.050±0.0006) (Figure 49a). K1 recorded the lowest albumin concentration (0.025±0.0005) followed by B1 (0.042±0.0004). The analysis (one-way ANOVA, P≤0.05, Tukey's multiple comparisons test) showed that there was significant difference (P≤0.0001) in all the accessions comparisons, such as between K1 (0.025±0.0005) and B4 (0.050±0.0006), between M2 (0.065±0.0005) and M4 (0.050±0.0007), except between B4 (0.050±0.0006) and M4 (0.050±0.0007) where there was no significant difference (P=0.9987) (Table 24). All the accessions recorded low coefficients of variation of <5%.

4.8.2.2.2 Globulins

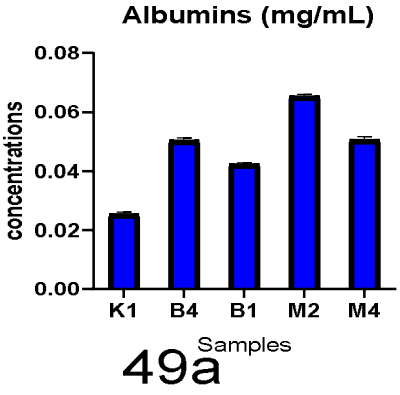
The globulins concentrations were measured in the five samples, and M2 recorded the highest concentration (0.041±0.0004), followed by K1 (0.034±0.0004) and M4 (0.031±0.0003) (Figure 49b). The sample B1 had the least globulins concentration (0.022±0.0003), closely followed by B4 sample, which had a concentration of 0.023±0.0004. The analysis (one-way ANOVA, $P \leq 0.05$, Tukey's multiple comparisons test) revealed that there was significant difference ($P < 0.05$) in all the accessions comparisons, except between B4 (0.023±0.0004) and B1 (0.022±0.0003) (Table 24) where there was no significant difference ($P = 0.8080$) (Figure 49b). All the accessions recorded low coefficients of variation of <3%.

4.8.2.2.3 Glutelins

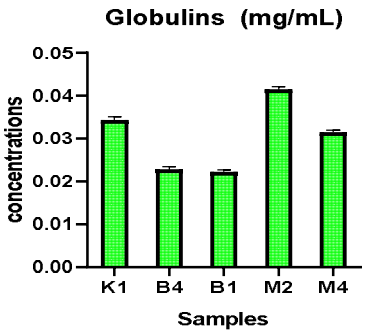
The B1 sample had the highest glutelins concentration (0.029±0.0005). On the contrary, B4 sample recorded the least glutelin concentration (0.004±0.0003) followed by K1 (0.006±0.0004) (Figure 49c). The analysis (one-way ANOVA, $P \leq 0.05$, Tukey's multiple comparisons test) showed that there was statistical difference ($P < 0.05$) in all the accessions comparisons, except between M2 (0.012±0.0004) and M4 (0.012±0.0004) concentrations where there was no statistical difference ($P > 0.9999$) (Table 24). All the accessions recorded low coefficients of variation of <20%.

4.8.2.2.4 Prolamins

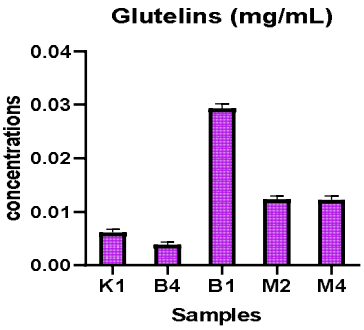
In this protein fraction, K1 sample had the highest concentration (0.004±0.0004), followed by B1 (0.003±0.0004) (Figure 49d). However, the accessions M2 (0.002±0.0003), B4 (0.002±0.0002), M4 (0.002±0.0002) and B1 (0.003±0.0004) concentrations did not differ significantly ($P \geq 0.05$) (Table 24). The analysis (one-way ANOVA, $P \leq 0.05$, Tukey's multiple comparisons test) showed that there was significant difference ($P \leq 0.05$) in all the accessions comparisons, except between M2, M4, B1 and B4 accessions. The accessions B1, B4 an M2 had high coefficients of variation of >20%.



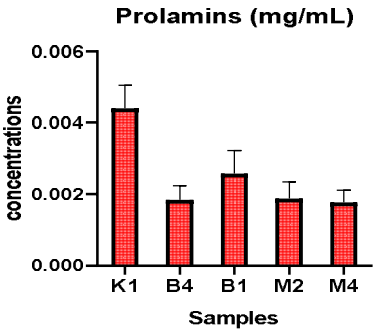
49a



49b



49c



49d

Figure 49a: The protein fraction albumins in the five accessions (K1, B4, B1, M2 and M4) samples. Figure 49b. Globulins in the five accessions (K1, B4, B1, M2 and M4) samples. Figure 49c. Glutelins in the five accessions (K1, B4, B1, M2 and M4) samples. Figure 49d. Prolamins in the five accessions (K1, B4, B1, M2 and M4) samples. From the analysis (one-way ANOVA, *P*≤0.05, and Tukeys multiple comparisons test) most of the protein fraction inter-accessions sample comparisons had significant differences (P≤0.0001). Bars represent mean±SEM.

Table 24: Protein concentrations (mg/mL) of five Bambara groundnut accessions

Acc.	Total proteins	Albumins	Globulins	Glutelins	Prolamins
K1	0.075^{a}±0.0004	0.025^{a}±0.0005	0.034^{c}±0.0004	0.006^{b}±0.0004	0.004^{b}±0.0004
B4	0.081^{b}±0.0012	0.050^{c}±0.0006	0.023^{a}±0.0004	0.004^{a}±0.0003	0.002^{a}±0.0002
B1	0.104^{d}±0.0007	0.042^{b}±0.0004	0.022^{a}±0.0003	0.029^{d}±0.0005	0.003^{a}±0.0004
M4	0.097^{c}±0.0012	0.050^{c}±0.0007	0.031^{b}±0.0003	0.012^{c}±0.0004	0.002^{a}±0.0002
M2	0.123^{e}±0.0006	0.065^{d}±0.0005	0.041^{d}±0.0004	0.012^{c}±0.0004	0.002^{a}±0.0003

Proteins concentrations (mg/mL) expressed as means±SEM. Means with different superscripts down the columns are significantly different ($p \leq 0.05$).

4.8.3 Electrophoretic Banding Patterns

4.8.3.1 SDS-PAGE gel: Comparative Protein Peptides Profile in five BGN Accession Samples.

The protein peptides in kilo Daltons (kDa) present in each accession sample was determined using Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis (SDS-PAGE). Thirteen detectable peptides of seed storage proteins were resolved in SDS-PAGE. The molecular weights of detectable peptides ranged from 21kDa to >250kDa (Table 25). The electrophoretic banding pattern of total seed storage protein was as shown below in Figure 50.

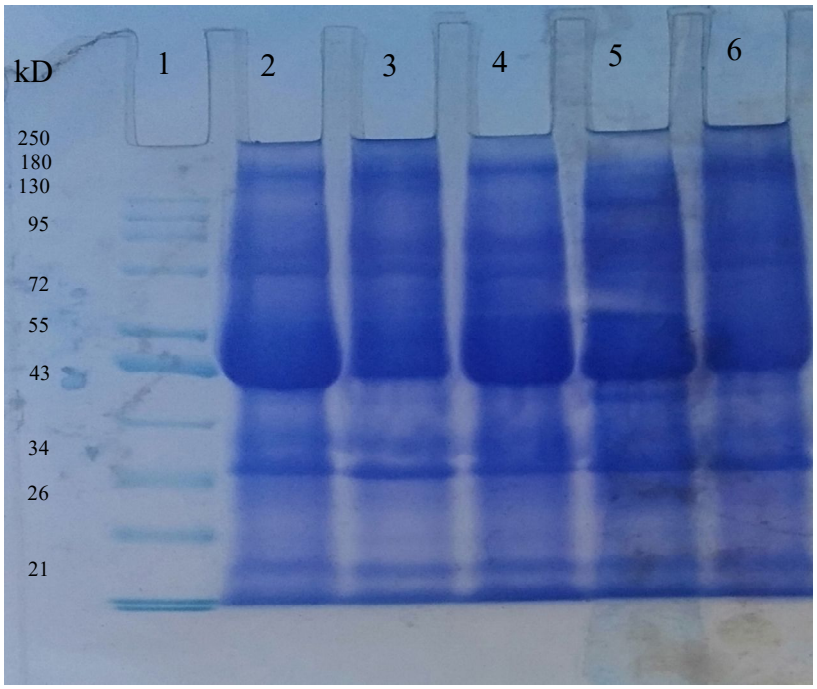


Figure 50: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of Bambara groundnut isolates of total proteins: Lane 1=Protein ladder (11-250 kDa), Lane 2= Sample K1, Lane 3= Sample B1, Lane 4= Sample B4, Lane 5= Sample M2, Lane 6= Sample M4.

The electrophoretic banding pattern of total seed protein revealed similarities between the studied accessions, though the number of detected peptide bands differed in various accessions (Table 25). All the five landraces had seven bands in common, corresponding to 21kDa, 22kDa, 35kDA, 36kDa, 43kDa, 55kDa and 110kDa.

Table 25: The protein peptides (kDa) present in each Bambara groundnut sample.

accessions / kDa	K1	B1	B4	M2	M4
21	+	+	+	+	+
22	+	+	+	+	+
35	+	+	+	+	+
36	+	+	+	+	+
40	+	+	-	+	+
43	+	+	+	+	+
52	-	-	-	+	+
55	+	+	+	+	+
97	+	+	+	-	+
110	+	+	+	+	+
112	-	+	+	-	-
180	-	-	-	+	-
>250	+	+	+	-	+
Total number of bands	10	11	10	10	11

Key: (+) -peptide size present, (-) -peptide size absent.

Jaccard index was computed for five BGN isolates to establish the dissimilarity index in their protein peptide composition.

Table 26: The dissimilarity indices (r^2 values) of protein peptides based on Jaccard index

Accessions	K1	B1	B4	M2	M4
K1	0.000				
B1	0.072	0.000			
B4	0.098	0.044	0.000		
M2	0.367	0.380	0.400	0.000	
M4	0.123	0.055	0.097	0.356	0.000

The dissimilarity index generated by pairing and comparing each of the landraces with one another. The r^2 values range from 0-1, and can be expressed as decimals or as percentages. The lower the index value, the higher the degree of overlap, hence higher the similarity, and the higher the index value, the lower the degree of overlap, hence higher the dissimilarity.

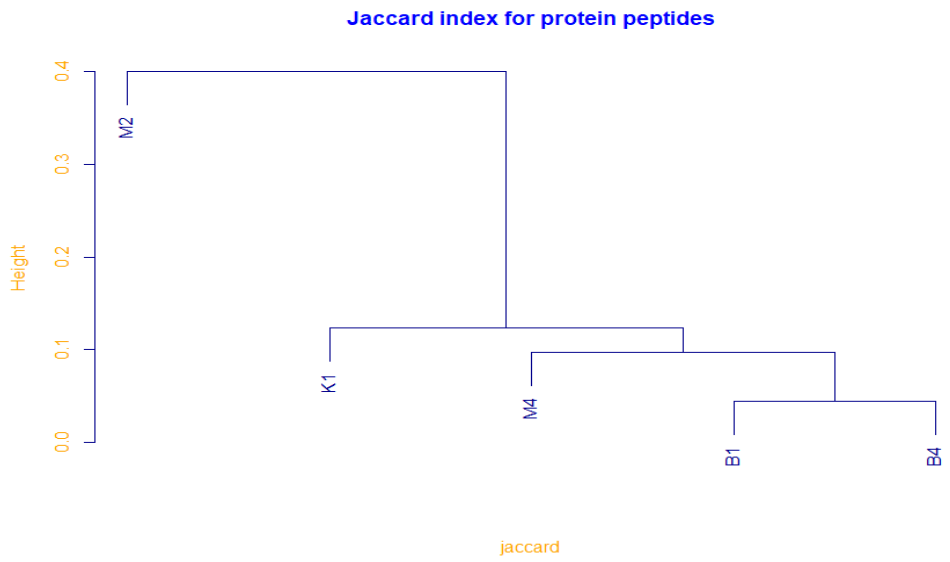


Figure 51: Hierarchical clustering dendrogram showing the relationship amongst the five Bambara groundnut accessions based on their protein peptides profiles, generated by Jaccard indices using R statistical software version 3.3.1 (R Development Core Team 2016).

The clustering is based on their dissimilarity distances (height). The dissimilarity index conversion to similarity index was done using the formula: similarity=1-dissimilarity. Based on the similarity indices, percentage homologies were computed from the dendrograms.

The dendrogram based on the electrophoretic data revealed two main clusters for the protein peptides present in the five accessions (Figure 51). The M2 accession was on its own cluster, whereas M4, K1, B1 and B4 were in another cluster at about 60% homology, the latter cluster having 2 sub-clusters consisting of K1 alone and M4, B1 and B4 in another sub-cluster at about 87% homology. The accessions in latter sub-cluster were further divided into two sub–clusters comprising of M4 on one node, and B1and B4 on another node at about 91% homology. The B4 genotype was closest to B1 genotype, at about 96% homology, and only varied with B1, by having protein peptide of 40kDa. This protein marker (protein peptide with molecular weight 40kDa) can therefore be used to differentiate genotype B1 from B4, germplasms which are farmer-held in Busia County.

As much as M4 is closely related to B1 and B4 at a homology of 91%, this germplasm has a unique protein marker (protein peptide with molecular weight 52kDa) which differentiates it from B1 and B4. This protein peptide with molecular weight 52kDa, is an important protein

marker present in only M2 and M4 germplasms, genotypes which are farmer-held in Migori County. This protein marker is lacking in other three accessions.

Basically, the seed storage protein profiling for genetic diversity assessment showed that accession B4 and M2 were varied from other accessions. In all the accessions, protein peptide of molecular weight 40kDa was detected apart from in accession B4, where it was not detected. This protein marker (protein peptide with molecular weight 40kDa) can therefore be used to differentiate genotype B4 from all these accessions. Maximum variability was however, observed in accession M2, where, unlike other accessions, protein peptides with 97kDa and >250kDa were not detected. M2 accession is the only accession that had protein peptide of 180kDa. This accession was therefore more divergent from other germplasm, and can be differentiated from the other genotypes based on the presence of protein marker with molecular weight of 180kDa.

4.8.3.2 Electrophoretic Banding Patterns of Protein Fractions in Accessions Isolates

The electrophoretic banding pattern of seed storage protein fractions in each accession isolates was determined. The banding patterns are illustrated as below:

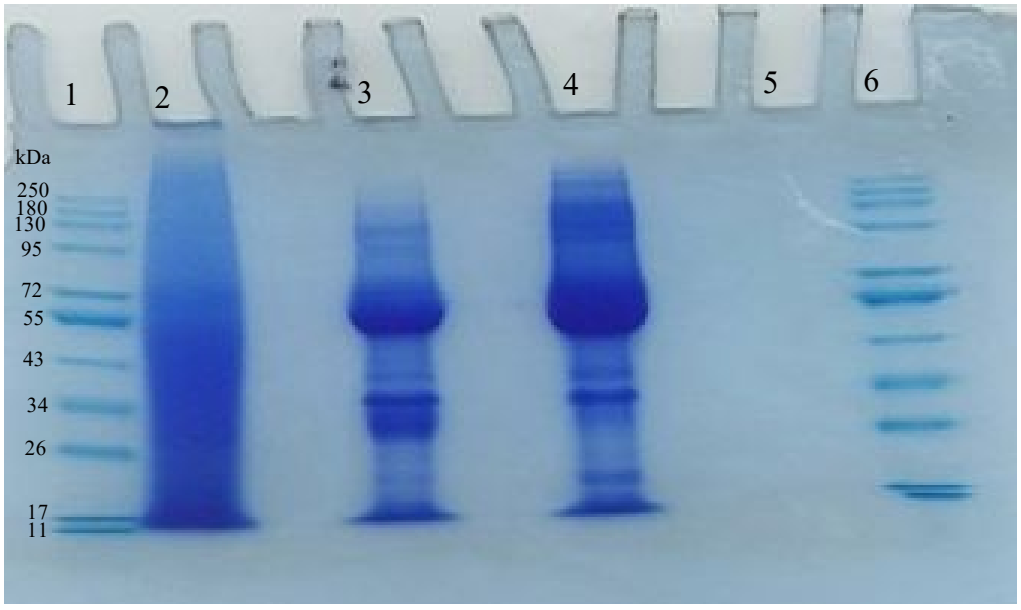


Figure 52: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) banding patterns of protein fractions of K1 isolates. Lane 1= Protein ladder (11-250 kDa), Lane 2= Prolamins, Lane 3= Globulins, Lane 4= Albumins, Lane 5= Glutelins, Lane 6= Protein ladder.

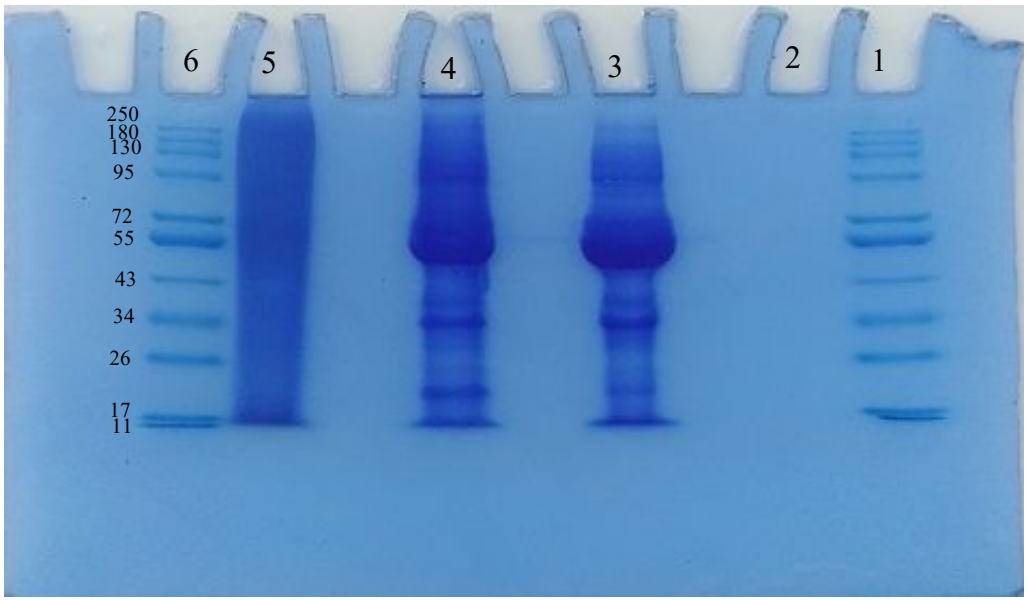


Figure 53: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) banding patterns of protein fractions of B1 isolates. Lane 6= Protein ladder(11-250kDa), Lane 5= Prolamins, Lane 4= Albumins, Lane 3= Globulins, Lane 2= Glutelins Lane 1= Protein ladder

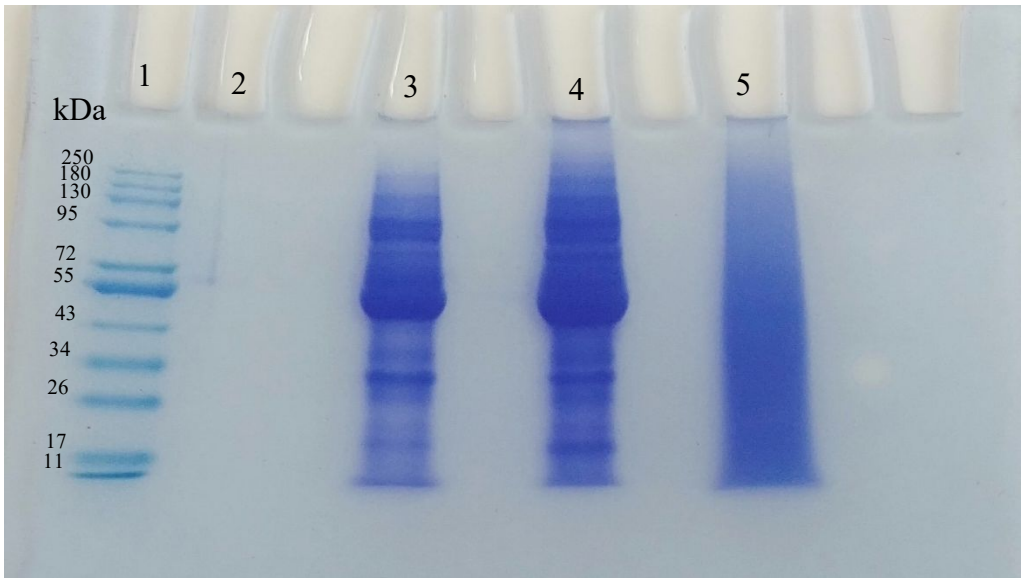


Figure 54: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) banding patterns of protein fractions of B4 isolates. Lane 1= Protein ladder (11-250 kDa), Lane 2= Glutelins, Lane 3= Globulins, Lane 4= Albumins, Lane 5= Prolamins

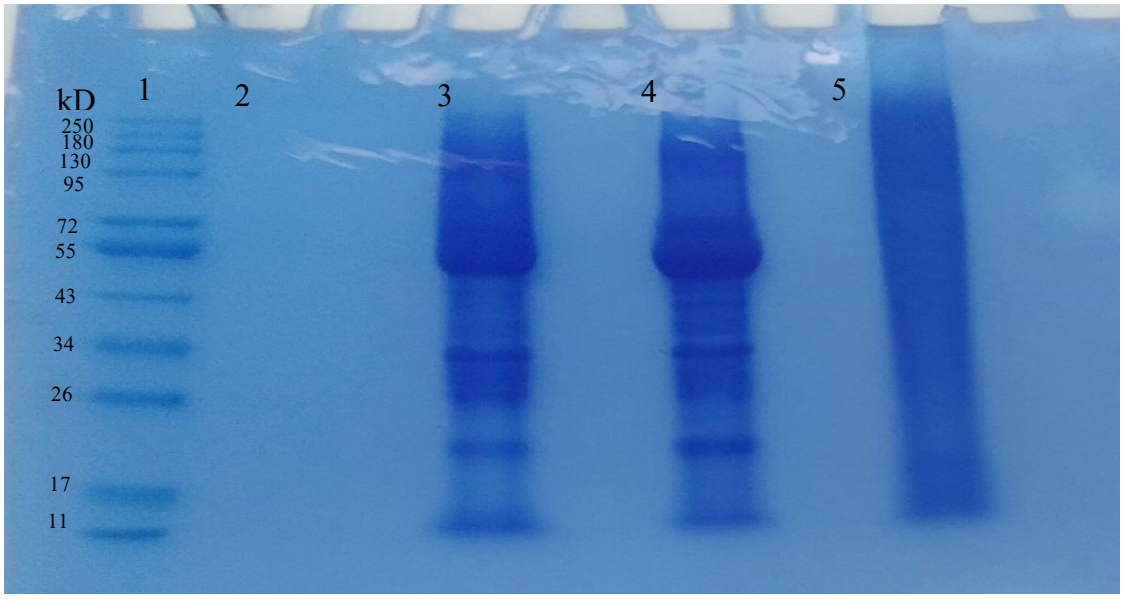


Figure 55: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) banding patterns of protein fractions of M2 isolates. Lane 1= Protein ladder (11-250 kDa), Lane 2 = Glutelins, Lane 3= Globulins, Lane 4= Albumins, Lane 5= Prolamins

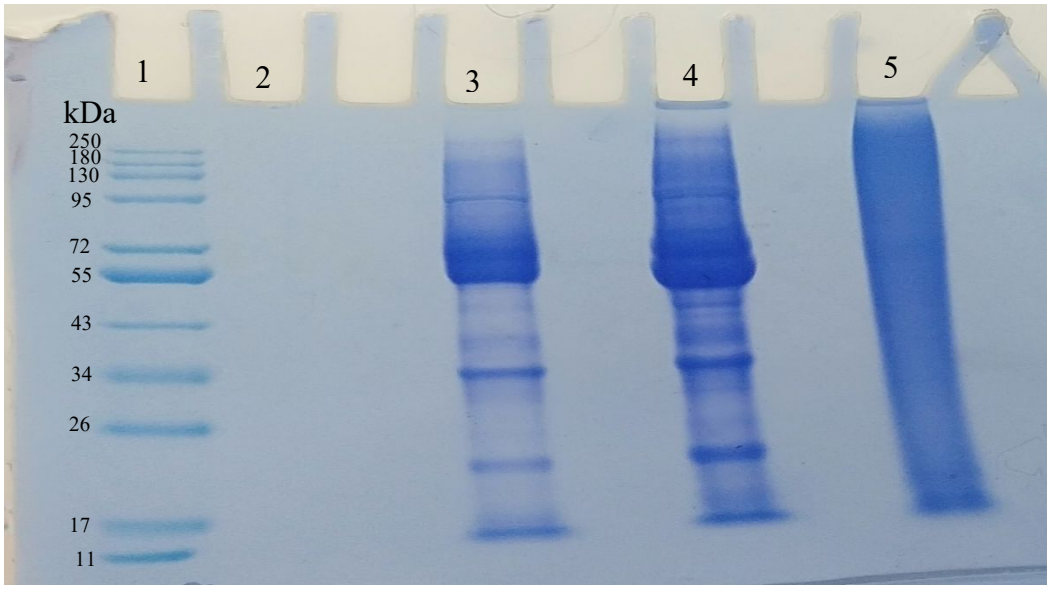


Figure 56: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) banding patterns of protein fractions of M4 isolates. Lane 1 = Protein marker (11-250 kDa), Lane 2 = Glutelins, Lane 3 = Globulins, Lane 4 = Albumins, Lane 5= Prolamins

The electrophoretic banding patterns of glutelins and Prolamins fractions in accessions isolates could not be visualized probably because the gel thickness in terms of concentration did not allow them to settle.

4.8.3.2.1 Electrophoretic Banding Patterns of Albumins in Accessions Isolates

Table 27: Molecular weights of Albumins fractions in various BGN accession samples.

Accessions / kDa	K1	B1	B4	M2	M4
21	+	+	+	+	+
34	-	-	+	+	+
37	+	+	+	+	-
40	+	-	+	-	+
43	-	-	-	+	+
55	+	+	+	+	+
72	-	-	-	+	+
97	+	+	+	-	+
130	+	-	+	-	-
250	+	-	-	-	+
Total number of bands	**7**	**4**	**7**	**6**	**8**

(+)-Present, (-)-Absent

Jaccard index was computed to establish the dissimilarity indices of Albumin fractions in various BGN accessions isolates.

Table 28: The dissimilarity indices (r^2 values) of albumin concentrations based on Jaccard index

Accessions	K1	B1	B4	M2	M4
K1	0.000				
B1	0.438	0.000			
B4	0.343	0.206	0.000		
M2	0.521	0.234	0.309	0.000	
M4	0.228	0.399	0.375	0.437	0.000

The dissimilarity index generated by pairing and comparing each of the landraces with one another.

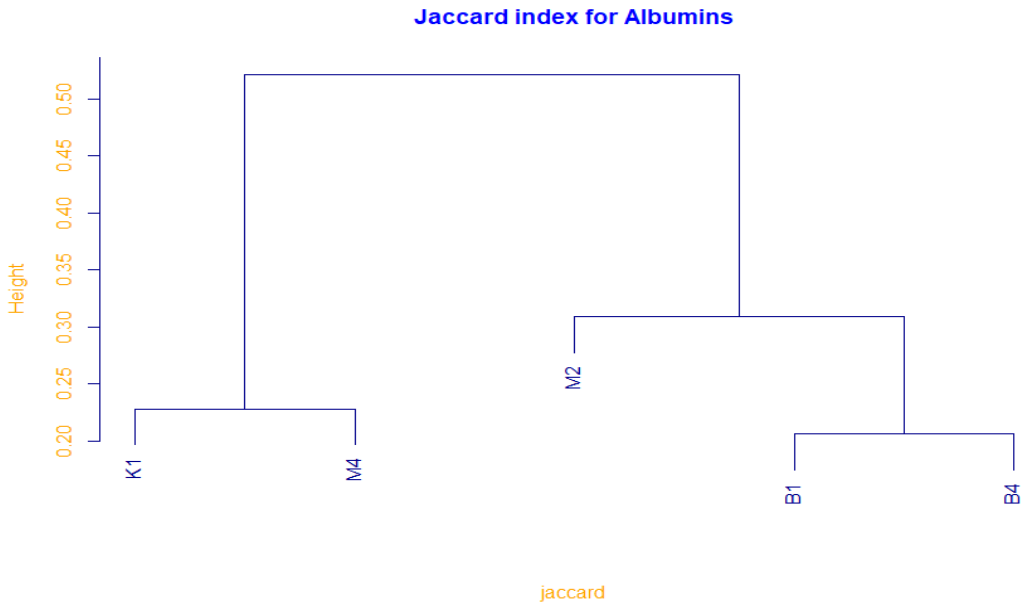


Figure 57: Hierarchical clustering dendrogram showing the relationship amongst the five Bambara groundnut accessions based on their albumins profiles, generated by Jaccard distance indices using R statistical software 3.3.1. The clustering is based on their dissimilarity indices (height).

Ten albumin peptide bands were observed in the five BGN landraces studied, and only albumin protein peptide 21kDa and 55kDa were similar in all the landraces studied.The dendrogram revealed two main clusters for the albumins present in the five accessions (Figure57). The cluster 1 comprised of K1 and M4 accessions, whereas cluster 2 comprised of M2 , B1 and B4 accessions, at about 45% homology, the latter cluster having 2 sub-clusters consisting M2 at about 68% homology with B1 and B4. The B1 genotype was closest to B4 genotype, at about 79% homology. The B4 genotype had peptide bands corresponding with 34kDa, 40kDa and 130kDa, which distinguished it from B1 landrace. All the accessions under study had protein marker (albumin protein peptide with molecular weight 37kDa) apart from in M4 accession where it was not detected. This albumin protein marker can therefore be used to distinguish M4 from the other farmer-held landraces in the LVB, Kenya. The M2 accession was characterized with albumins with low molecular weight peptides (≤72kDa).

Basically, the albumin peptides profiling for genetic diversity assessment showed that accession K1 and M4 were varied from other accessions. They were the only accessions that had albumins peptides with high molecular weight of 250kDa. This protein marker (albumin protein peptide

250kDa) can hence be used to distinguish these two accessions (K1 and M4) from the rest of the farmer-held landraces in LVB, Kenya.

4.8.3.2.2 Electrophoretic banding patterns of globulins in accessions isolates

Table 29: Molecular weights of different fractions in Globulins in various Bambara groundnut accessions samples

Accessions / kDa	K1	B1	B4	M2	M4
21	+	+	+	+	+
26	-	-	-	+	-
34	+	+	+	+	+
37	+	+	+	-	+
43	+	-	-	-	+
55	+	+	+	+	+
72	-	-	-	-	+
95	+	+	+	-	+
130	+	-	-	-	-
180	-	-	+	-	-
250	-	+	-	-	-
Total number of bands	**7**	**6**	**6**	**4**	**7**

(+)-Present, (-)-Absent

Jaccard index was computed to establish the dissimilarity indices of different fractions in globulins in various Bambara groundnut accessions isolates.

Table 30: The dissimilarity indices (r^2 values) of globulins concentrations based on Jaccard index

Accessions	K1	B1	B4	M2	M4
K1	0.000				
B1	0.324	0.000			
B4	0.240	0.353	0.000		
M2	0.464	0.587	0.490	0.000	
M4	0.142	0.249	0.175	0.450	0.000

The dissimilarity index generated by pairing and comparing each of the landraces with one another.

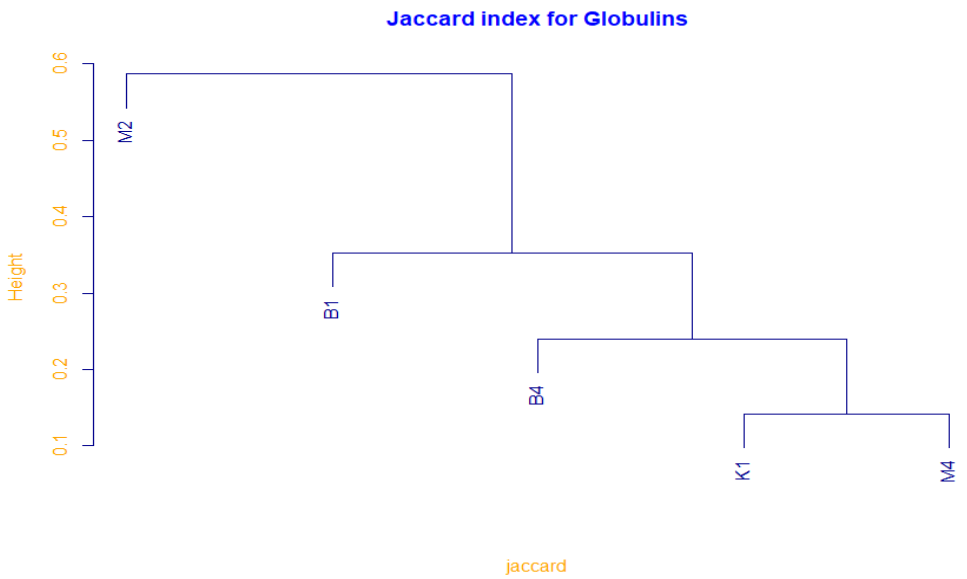


Figure 58: Hierarchical clustering dendrogram showing the relationship amongst the five Bambara groundnut accessions based on their globulins profiles, generated by Jaccard indices using R statistical software version 3.3.1. The clustering is based on their dissimilarity indices (height).

Eleven globulin peptide bands were observed in the five BGN landraces studied, and only globulin protein peptide bands with molecular weight 21kDa, 34kDa and 55kDa were similar in all the landraces studied.

The dendrogram based on the globulins electrophoretic data revealed two main clusters for the globulins peptides present in the five accessions (Figure 58). The M2 landrace was on its own cluster, whereas B1, B4, K1 and M4 were in another cluster at about 41% homology, the latter cluster having 2 sub-clusters consisting of B1 alone and B4, K1 and M4 in another sub-cluster at about 65% homology. This second sub-cluster was further divided into two sub–clusters comprising of B4 on one node, and K1and M4 on another node at about 76% homology. The M4 genotype was closest to K1 genotype, at about 86% homology, and only varied with K1, having protein peptide of 72kDa. This protein marker (globulin protein peptide with molecular weight 72kDa) was unique to accession M4, and can therefore be used to differentiate genotype M4 from all the other landraces.

In all the isolates, the glutelin sample seem to escape in the running buffer, whereas the prolamins only show a smear in the gel. Hence, the electrophoretic bands of the two protein fractions could not be observed.

CHAPTER FIVE
DISCUSSION

5.1 Qualitative morphological characters.

5.1.1 Frequency distribution of qualitative morphological characters

In this study, the similarity of Bambara groundnut qualitative traits across all zones and in the greenhouse (Figure 7) suggests minimal environmental influence on these traits. This aligns with previous findings indicating that qualitative traits are largely genetically controlled (Molosiwa *et al*., 2015). Morphological characterization is essential in germplasm evaluation, as it provides insights into phenotypic variability, a critical factor in breeding programs.

Despite this similarity, substantial variation was observed among the six accessions in qualitative traits (Table 7). The most common leaflet shape was oval (50%), followed by elliptic (33.3%), while lanceolate leaves were least frequent (16.7%). The dominance of oval and elliptic leaflets may have agronomic advantages, as their larger surface area enhances light interception and photosynthetic efficiency (Kidner and Umbreen, 2010). This is consistent with findings by Valombola *et al*. (2021), where farmers in Namibia preferred Bambara groundnut plants with larger leaves, associating them with higher yields due to improved photosynthetic capacity. Conversely, the presence of lanceolate leaves, though less frequent, and suggests potential adaptation to semi-arid environments, as previously reported by Bamshaiye *et al*. (2011).

These variations in morphological traits highlight the potential for targeted breeding strategies to enhance productivity and adaptation. Selecting for larger leaf sizes could improve yield potential, while maintaining diversity in leaf morphology may support resilience under variable climatic conditions.

The findings (Table 7) indicate that brown pod colour was predominant (66.7%), while black and yellowish-brown were less common (16.7% each). Pod texture also varied, with little-grooved and much-grooved textures each at 33.3%, whereas smooth and much-folded textures were least frequent (16.7%). Pod colour and texture are critical traits influencing marketability and consumer preference. As noted by Valombola *et al*. (2022), brown pods are often associated with freshness, which may explain their dominance in the study. Similar findings have been reported by Coulibaly *et al*., (2020), who highlighted that consumer preference for pod colour can drive

selection in breeding programs in kerstings groundnut. Similarly, pod texture affects processing, with smoother pods potentially offering advantages in dehulling, while grooved textures may enhance durability and resistance to post-harvest losses (Aviara *et al*., 2013).

These variations highlight the importance of considering consumer demand and post-harvest efficiency in breeding programs. Prioritizing brown pod colour and an optimal texture could enhance market acceptance, while maintaining genetic diversity could support resilience and future breeding efforts.

Bambara groundnut seed testa colour is an important determinant of pest resistance, yield, nutrient composition, and serves as a selection criterion in planting and breeding programs. In this study, 50% of the landraces had cream-coloured seed coats, while dark brown, light red, and black testa each accounted for 16.7% (Table 7). Dark-coloured seed coats have been associated with greater resistance to beetle infestation during storage, as they attract fewer pests for oviposition (Baidoo *et al*., 2015), suggesting that cultivating varieties with darker testa may reduce post-harvest losses.

In Zimbabwe, Goli (1995) reported that red- and cream-coloured landraces showed greater seed yield stability compared to black landraces. Bamshaiye *et al*. (2011) observed that red seeds were particularly rich in iron, containing nearly twice as much iron as cream seeds, highlighting their potential for addressing iron deficiency. In Namibia, Valombola *et al*. (2021) found that 45% of farmers considered seed colour an important selection criterion, with cream (36%) and tan (33%) being the most preferred colours.

According to Mabhaudhi *et al*. (2013), Bambara groundnut plants derived from dark-coloured seeds exhibit greater drought tolerance, likely due to higher levels of polyphenols that act as antioxidants under water stress. The predominance of cream-coloured testa observed in this study is consistent with findings by Mohammed *et al*. (2016a), and Esan *et al*. (2023a), who also reported cream testa as the dominant colour in their studies in Nigeria. This suggests that the cream seed coat may confer certain agronomic or consumer-preferred advantages, such as yield stability and other yet-to-be-identified beneficial traits, contributing to its widespread cultivation.

In this study, fifty percent of the landraces had no eye pattern (plain). This observation is in agreement with what Mohammed *et al*. (2016a) observed in their study, where more germplasm from Zambian collection had plain eye. The variations in seed eye patterns displayed by the landraces in this study are useful to differentiate between the germplasm in a program of genetic improvement of Bambara (Mohammed *et al*., 2016a).

In this study, the six accessions had only two pod shape types, with shape ending in a point, round on other side (Figure 7) accounting for 66.7% (Table 7) while 33.3% of the accessions had pods ending in a point, with a nook on the other side (Table 7). Variations in pod shape facilitate the classification of Bambara landraces. For instance, a study identified landraces with pointed and round pod shapes, aiding in the systematic categorization of these varieties (Mohammed *et al*., 2016b). Pod shape also serves as a key descriptor in distinguishing different landraces. For instance a study on Malaysian landraces found that 60% of the landraces had nook with ending point on both side, 26.67% of the landraces had round with ending point on one side, 13.33% of the landraces had nook with ending point on one side (Khan *et al*., 2021a).

In this study majority (66.7%) of landraces seeds had no testa pattern, whereas seeds with black marbled spots on cream background with grey butterfly like eyes and seeds with black marbled spots on brown background without eye, each had 16.7%. The seed testa pattern exhibits significant variation among accessions. This diversity is valuable for germplasm characterization and breeding programs aimed at enhancing desirable traits (Onwubiko, 2020).

5.1.2 The Qualitative Morphological Traits Diversity Indices

In this study, the Shannon-Weiner diversity (H') indices for the Bambara groundnut qualitative traits assessed were quite high (Table 8). Terminal leaflet shape (2.0708), seed eye pattern (2.0708) and pod texture (2.0620) exhibit the highest morphological diversity among the observed traits. Pod shape (1.8136) and seed testa colour (1.9356) traits have a relatively moderate level of diversity. However, the pod colour (1.6784) and seed testa pattern (1.6501) traits show the least diversity among the qualitative morphological traits studied.

Amongst these traits, terminal leaflet shape and seed eye pattern have the highest diversity, suggesting high variability in these characteristics within the population. Pod texture is also highly diverse, which may indicate environmental or genetic factors influencing pod texture

variability. Seed testa pattern has the lowest diversity, meaning that most accessions share similar patterns in their seed testa.

A study by Valombola *et al*. (2022) observed low diversity in seed testa colour among 25 Bambara accessions, reporting an H' value of 0.75. However, their study found significant diversity in seed eye patterns, with an H' value of 1.22. The same study also identified notable diversity in pod colour, with an H' value of 1.49 among the accessions evaluated. Additionally, diversity in pod shape was highlighted, with an H' value of 0.59, indicating variability among accessions. Valombola *et al*. (2022) further reported significant diversity in terminal leaflet shape, with an H' value of 0.88. Olukolu *et al*. (2012) reported H' Index of nineteen qualitative traits (0.1 to 0.15) in Bambara.

The high H' values across various traits in this study suggest substantial genetic diversity within Bambara populations. This diversity is likely due to traditional farming practices, where farmers maintain and cultivate diverse landraces (Olukolu *et al*., 2012), and seed management methods such as recycling, sorting, exchange and new introductions (Bonny *et al*., 2019)

The diversity in traits could also be explained by the phenotypic plasticity (Valombola *et al*., 2022) which results from adaptability of accessions to different agro-ecological environments with different soil types and nutrient compositions. The study encompass accessions from different geographical locations, which may account for some differences in diversity indices. These factors are crucial and may contribute to significant qualitative trait diversity by creating or maintaining polymorphism as observed in this study. Therefore, (H') can be useful in identifying the traits that need to be improved by breeders. Even though the diversity of the crop remains largely unexploited (Mohammed *et al*., 2016b) in Africa.

The knowledge of variation of characters is important in classification of these germplasm as they can be used in determining relationships and in distinguishing taxa from all others at the next level of the hierarchy (Stuessy, 1990). Knowledge of variation of characters is also important in crop improvement programs, as plant breeders should know which population is more varied for which characters.

5.1.3 Utilization of Qualitative Morphological Taxonomic Markers in Classification

Qualitative morphological characters are important in Taxonomy and can be utilized as taxonomic markers in classification. The Cluster analysis revealed the importance of qualitative morphological markers to differentiate and classify the farmer-held accessions in the Lake Victoria Basin. Two major clusters were observed based on their similarities. The dendrogram, therefore, showed how varied the accessions were for the traits studied. The similarity in most qualitative traits amongst some accessions as observed in the M4 and M2 accessions from Migori County, could be due to their adaptability to the same environment. This can be explained by the fact that since most farmers plant the germplasm within their possession year in and year out (Ngugi, 1995), germplasm recycling could have led to adaptability to the environment. Due to the proximity of Migori County to Tanzania, the two accessions most probably may have originated from Tanzania through seed exchange at the border (Bonny *et al*., 2019), hence their close similarity to each other. Massawe *et al*. (2003), observed that landraces were grouped based on their areas of origin and that this is an indication of the importance of environmental adaptation on the genetic variation in Bambara groundnut (Molosiwa, 2012).

The proximity of Kisumu and Busia Counties may explain their germplasm similarities. In Western Kenya, Busia County is the largest producer of Bambara and hosts the most diverse Bambara accessions (Odongo *et al*., 2015). Due to trade and market demands, Busia's germplasms likely spread to Kisumu County. When these ‘alien’ germplasms are cultivated in Kisumu’s unique environmental and edaphic conditions, they may adapt, leading to genetic similarity and clustering.

Bambara groundnut is both cleistogamous and autogamous (Ajilogba *et al*., 2022), meaning accessions within the same cluster likely share genetic similarities (Valombola *et al*., 2022). These findings align with Molosiwa (2012), who analyzed 34 morpho-agronomic traits in Botswana and observed diversity through cluster analysis. Using both qualitative and quantitative traits, Molosiwa identified three major clusters. Similarly, Khan *et al*. (2021b) used 14 qualitative traits to cluster 15 landraces in Malaysia, grouping them into five major clusters based on similarity.

In this study, seven qualitative traits were used to cluster six accessions, making it more comparable to Khan *et al*. (2021b), as both relied on qualitative traits. However, Khan's use of 14 traits compared to this study's seven may have resulted in a more detailed clustering. Nonetheless, this study provides valuable insights into the classification of farmer-held Bambara germplasm in the Lake Victoria region of Kenya.

5.2 Quantitative Morphological Traits

5.2.1 Plant Height

The plant height (PHT) of Bambara accessions varied significantly ($P<0.05$) across the three agro-ecological zones (Alupe, Kibos, and Oyani) and the greenhouse conditions. In the field sites, the findings revealed that Kibos recorded the highest plant heights across most accessions (K2, B1, M4 and M2) (Table 9b), followed by Alupe (B4 and K1) (Table 9a). Oyani recorded low plant heights (Table 9c) across all accessions. Greenhouse irrigated (GHI) plants exhibited significantly ($P<0.05$) greater heights (Figures 9,10,11,13 and14) compared to their droughted (GHD) counterparts, except in K1 accession where there was no significant difference ($P>0.05$) (Figure 12). Significant differences ($P<0.05$) were observed between field sites and greenhouse conditions (Figures 9-14) with droughted plants consistently exhibiting the lowest heights.

Plant height across agro-ecological zones indicated distinct differences influenced by environmental factors unique to each location. The rainfall amounts received during the growing season and the temperatures influenced the plant heights in each zone. Kibos (600.1 mm, 17.9 - 31.6°C) (Figure 6b) recorded the highest plant heights in majority of accessions, indicating that optimal rainfall and moderate temperatures provided optimal conditions for plant height development. The crop thrives in regions with rainfall range of between 600-750 mm (Amwenyo *et al*., 2025) and temperatures between 20°C and 28°C (Majola *et al*., 2021) during growing seasons. These favorable conditions likely contributed to the superior plant heights observed in Kibos. Alupe (882.5 mm, 21.0-30.5°C) (Figure 6a) had moderate plant heights apart from B4 and K1 accessions which recorded the highest heights (Table 9a), suggesting that higher rainfall above optimal and a wider temperature range may not contribute to higher PHT in most accessions in this zone. Berchie *et al*. (2012) investigated the growth, phenological, and yield responses of Bambara groundnut to varying water stress levels, showing that zones with optimal rainfall resulted in greater plant heights.

Oyani (480.6 mm, 20.7-29.6°C) (Figure 6c) recorded the lowest heights amongst the zones (Table 9c), possibly due to lower rainfall which could have restricted water availability for PHT development, suggesting a water-deficit impact. Obidiebube *et al*. (2019), in their study conducted in the rain forest agro-ecological in Nigeria, reported reduced plant height due to soil moisture deficits in Bambara.

The optimal PHT is associated with higher rainfall within the optimal range of 600-750 mm (Amwenyo *et al*., 2025). Studies indicate that insufficient rainfall below the optimal range results in moisture stress, inhibiting Bambara's growth potential (Muhammad *et al*., 2015). Similarly, Khan *et al*. (2020) studied Bambara's adaptability to Malaysian climatic conditions, highlighting morphological variations in plant height among landraces.

Well-drained loamy soils are ideal for Bambara cultivation (Olanrewaju *et al*., 2022). Alupe's loamy silt texture (Table 6) likely provided better drainage and aeration compared to the clay loam (Table 6) of Kibos and silty clay (Table 6) of Oyani, facilitating enhanced plant height. Nitrogen and phosphorus, essential for vegetative growth, were below optimal levels in all zones (Table 6) with Oyani showing the lowest post-planting nitrogen levels (0.2%) (Table 6). Bambara being nitrogen fixer (Gerrano *et al*., 2021), can fix approximately 4 -200 kg N ha $^{-1}$. The relatively higher post-planting nitrogen content in Kibos (0.3%) (Table 6) and Alupe (0.4%) (Table 6) may have supported better growth and higher plant heights.

Plant height is a crucial trait as it determines a plant's ability to compete for light, essential for photolysis during photosynthesis (Moles *et al*., 2009). According to Khan *et al*. (2021b) and Swanevelder (1998), the average PHT for most Bambara accessions ranges between 25 and 37 cm. In this study, the plant heights across zones ranged from 12.27 to 21.29 cm, recorded both at the Alupe zone (Table 9a). These ranges were below the reported range, likely due to genetic differences among accessions and environmental factors across agro-ecological zones. Despite being the same species, genetic diversity among accessions contributes to variability in plant height (Molosiwa *et al*., 2015).

Greenhouse conditions highlighted the critical role of water availability, with irrigated plants exhibiting significantly greater heights ($P < 0.05$) than drought-stressed plants (Figures 9, 10, 11,

12, and 14), underscoring the importance of adequate moisture for plant height (PHT) development. The significant differences observed between irrigated and droughted plants in the greenhouse align with studies by Chibarabada *et a*l. (2015) and Nautiyal *et al*. (2017) demonstrating the impact of water availability on Bambara plant height. Furthermore, Mabhaudhi *et al*. (2019) confirms that Bambara thrives under moderate drought but experiences stunted growth characterized by low plant heights under extreme moisture stress, which is evident from the reduced heights in droughted greenhouse plants. Similarly, research by Mabhaudhi and Modi (2013) indicated that water stress adversely affects the growth indices of Bambara, including plant height. These studies corroborate the current findings, emphasizing the importance of selecting suitable cultivation sites.

5.2.2 Number of Pods with One Seed (N1S)

The results indicate that the number of pods with one seed (N1S) varied significantly across the three agro-ecological zones: Alupe, Kibos, and Oyani. Among the zones, Kibos exhibited the highest N1S (Table 11a), whereas Oyani recorded the lowest (Table 12a). Accessions K2, B1, K1, M4 and M2 performed best in Kibos (Table 11a), while accession B4 performed best in Alupe (Table 10 a). The control greenhouse trials under irrigated conditions showed significantly ($P \leq 0.05$) higher N1S compared to droughted plants (Figures 15A-20A), indicating water availability as a key factor influencing this trait. The Bambara optimal water (between 600 mm - 750 mm) (Amwenyo *et al*., 2025) content leads to more production of one-seeded pods. Mabhaudhi *et al*. (2019) in their study observed that optimal water content is essential for maximizing pod production, and variations in water availability can influence seed set and pod formation. Amongst the irrigated plants (Table 13a) and the plants grown at Kibos (Table 11a) where soil water was within the expected range (600.1 mm) (Figure 6b), the numbers of pods with single seeds were many in number.

Optimal rainfall in Kibos likely contributed to the increased N1S by supporting pod development. Joubert *et al*. (2013) in their study on *Acacia mellifera* in an arid Namibian savanna found that seed production was highly correlated with annual rainfall, with en masse seed production occurring only in high rainfall seasons. This suggests that optimal rainfall supports pod development, leading to increased seed production. Rainfall has been previously linked to pod formation in Bambara, as shown in studies by Doku, (2008), where regions with

more consistent rainfall patterns exhibited increased N1S. Bambara is tolerant to heavy rainfall (Gerrano *et al*., 2021). Despite high rainfall (882.5 mm) (Figure 6a) received at Alupe, the zone recorded moderately high N1S (Table 10a), an indication that other environmental factors could have affected pod filling. The low N1S at Oyani (<20.00) apart from accession M4 that recorded twenty one (21) N1S (Table 12 a) could be attributed to suboptimal rainfall amounts (480.6 mm) (Figure 6c) and possible soil constraints. Water scarcity also leads to lower pod numbers according to Berchie *et al*. (2012).

Large temperature range (17.9^0C -31.6^0C) (Figure 6b) during the growing season in Kibos may have favored better seed formation within pods, while small temperature range (29.6^0C -20.7^0C) in Oyani potentially reduced pod fertility. According to a study by AlShareef *et al*. (2011), high temperatures (33°C and above) cause heat stress in Bambara, adversely affecting reproductive development, leading to reduced pod set and seed yield. Płażek *et al*. (2019), also reported that high temperatures led to a reduction in pod setting due to flower abortion in common buckwheat (*Fagopyrum esculentum* Moench).

Soil pH and nutrient levels, particularly phosphorus and nitrogen, have been associated with pod development (Hasan *et al*., 2018). In Kibos, clay loam soil texture and high fertility evidenced with relatively high pre-planting K^+, Ca^{2+} and Mg^{2+} (Table 6) content may have contributed to high N1S recorded in this zone, an observation similar to what Hasan *et al*. (2018) made that high fertility favours production of high pod numbers, although their study focused mostly on nitrogen and phosphorus. Although pre-planting nitrogen content was below deficiency level (<0.2 %) (Table 6) in Kibos, Bambara being a nitrogen fixer (Ajilogba *et al*., 2022) was able to fix adequate nitrogen, evidenced with higher post planting nitrogen content (0.3%) (Table 6). Khan *et al*. (2022b) evaluated 30 Bambara groundnut genotypes across four environments in tropical Malaysia, finding that environmental factors, including rainfall, played a more significant role than genetic factors in determining yield and yield components.The study emphasized the importance of selecting genotypes with broad adaptation to varying rainfall patterns to ensure stable pod production. Mabitsela *et al*. (2024) reported that drought conditions significantly reduced pod numbers, similar to the trends observed amongst greenhouse drought-stressed plants.

5.2.3 Number of Pods with Two Seeds (N2S)

The number of pods with two seeds (N2S) showed variability across the zones, with Kibos recording the highest N2S (Table 11b) and Oyani the lowest (Table 12b). The greenhouse experiment demonstrated a significantly higher N2S under irrigation than under drought conditions (Figures 13A, 14A, 16A, 17A and 18 A) apart from accession B1 where there was no significant difference ($P>0.05$) between the two controls. Across the zones, accession K2 had the highest N2S in Alupe (Table 10b), whereas accession M2 had the lowest N2S in Oyani (Table 12b). Accession B1 maintained moderate N2S across all zones (Tables 10b-12b), suggesting it possesses intermediate drought tolerance traits.

Adequate moisture in Kibos supported better seed filling, leading to more two-seeded pods. This is supported by research conducted by Massawe *et al*. (2005), who found that higher rainfall enhances the number of two-seeded pods in Bambara by improving overall plant vigor. Previous research by Atoyebi *et al*. (2021), found that higher soil fertility and consistent rainfall correlate with an increased number of two-seeded pods. However, in drought-prone areas, pod abortion and seed number per pod reduction were observed. This probably explains why Oyani, a zone characterized by below optimal rainfall (480.6 mm) (Figure 6c) recorded low N2S. Similarly, a study in South Africa (Mabhaudhi and Modi, 2013) confirmed that irrigation boosts higher pod formation, explaining why greenhouse irrigated plants performed better in this study.

Favourable temperatures in Kibos (17.9–31.6ºC) (Figure 6b) could have enhanced pollination success and subsequent pod development (Massawe *et al*., 2003). A study by Brink, (1997) found that optimal temperatures between 20.9°C to 26.2°C accelerated the rate of pod development.

The clay loam soil with balanced pre- planting K^+, Ca^{2+} and Mg^{2+} (Table 6**)** nutrient composition may have likely facilitated the formation of two-seeded pods in Kibos. This aligns with findings by Umeugokwe *et al*. (2021) who highlighted that loamy soils with balanced nutrient composition encourage better performance in Bambara.

All the accessions in all the zones had very few two-seeded pods. The production of the two-seeded pods could be influenced by intrinsic genetic factors amongst the germplasm. A study

assessing genetic diversity among Bambara groundnut landraces using morphological and molecular markers (Minaar-Ontong *et al*., 2021) revealed substantial variability in pod traits. Such diversity suggests that the number of seeds per pod is, at least in part, genetically determined.

5.2.4 Total Number of Pods (POD)

The total number of pods per plant (POD) in all accessions were highest in Kibos (Table 11c) and lowest in Oyani (Table 12c) apart from M4 accession which was lowest in Alupe (Table 10c). The greenhouse trials showed significant differences ($P \leq 0.05$) between irrigated and droughted plants, with irrigation favoring higher pod numbers (Figures 15c-20c). Across the zones, accession K1 exhibited the highest POD in Kibos (Table 11c), while accession K2 had the lowest in Oyani (Table 12c).

Higher total pod numbers in Kibos (Table 11c) were likely influenced by sufficient rainfall (600.1 mm) (Figure 6b), ensuring continuous flowering and pod filling (Ntundu *et al*., 2006). This aligns with observations by Massawe *et al*. (2005), who emphasized that adequate soil moisture, particularly during the reproductive phase**,** is critical for pod development in Bambara groundnut.

The loamy silt soil texture in Alupe may have played a role in water retention, affecting pod yield positively (Nandkangre *et al*., 2022) as Alupe recorded relatively high POD. In a study conducted by Olayinka *et al*. (2016), loamy soils enhanced yield components, including the number of pods per plant, confirming the results observed in Alupe. This suggests that loamy soils, which typically have better water and nutrient retention capacities, are more conducive to Bambara production.

According to Mwale *et al*. (2007), Bambara pod number per plant is significantly affected by soil soil moisture content. Similar trends have been noted in studies where sandy soils with low water retention resulted in reduced pod numbers. Additionally, research in South Africa (Mabitsela *et al*. (2024) found that drought-stressed conditions caused a sharp decline in total pod numbers, reinforcing the low pod numbers observed in Oyani where rainfall (480.6 mm) (Table 6) was below the optimum (600-750 mm) required by the Bambara. Ntundu *et al*. (2006) also observed

that drought stress reduces pod number in Bambara, confirming the results of drought-stressed plants in this study.

A cross-zone comparison of the three pod traits shows that accession B4 performed best in Alupe particularly for N1S (Table 10a), while accession K2 had lower performance in Oyani, particularly for POD (Table 12c) and N1S (Table 12a) indicating susceptibility to water stress. Accession M4 performed moderately across all zones (Tables 10c-12c), suggesting it has good adaptability traits, likely due to a balance between stress tolerance and yield potential.

Compared to previous studies, the results align with findings by Olanrewaju *et al*. (2021a), which highlight that significant genotype × environment interactions affect traits like number of pods in Bambara groundnut, emphasizing the importance of evaluating accessions across diverse environments for effective breeding programs. The superior POD performance of K1 in Kibos (Table 11c) suggests it could be a viable candidate for cultivation in high-rainfall zones , while the lower POD performance of K2 in Oyani (Table 12c) implies that it may not be suited for arid environments. These observations also suggests that environmental adaptability plays a crucial role in the expression of pod traits in Bambara.

5.2.5 Terminal Leaflet Area (TLA cm2)

The terminal leaflet area (TLA) of Bambara varied significantly ($P \leq 0.05$) across the three agro-ecological zones: Alupe, Kibos, and Oyani. Greenhouse controls provided valuable comparative data. The irrigated plants showed an increase in TLA compared to field-grown plants (Figures 21-26), indicating the impact of moisture availability. However**,** droughted conditions led to a significant reduction in TLA (Table 15e), emphasizing water stress's effect on leaf expansion.

The variations observed in TLA across the three agro-ecological zones can be attributed to differences in climatic and edaphic factors unique to each zone. The highest TLA values were recorded in Kibos, likely due to favorable soil nutrients. Nitrogen, phosphorus, and potassium are crucial for leaf development (Marschner, 2012). The soil in this zone was characterized with high Potassium amount (1.3 me %) (Table 6) which was within the range (>0.24 me %) (Table 6) required by the Bambara, supporting larger leaflet areas. The Phosphorus pre-planting level (27 ppm) was slightly below the expected minimum (30 ppm) and this could have impacted

negatively on leaf development. The pre-planting Nitrogen level in this zone was low (0.1%) (Table 6). However Bambara being a nitrogen fixer (Ajilogba *et al*., 2022), was able to meet its own nitrogen requirements, evidenced by the zone recording relatively higher post-planting nitrogen content of 0.3% (Table 6). Kibos experienced the most favorable rainfall amount (600.1 mm) (Figure 6b), which was within the optimal range of 600-750 mm (Amwenyo *et al*., 2025), contributing to higher TLA values. The zone also experienced optimal temperature ranges (17.9-31.6ºC) (Figure 6b) which favored maximum leaf area expansion. High temperatures beyond 35°C can cause stomatal closure, reducing leaf expansion and decreased photosynthesis due to disruption of cellular functions and enzyme activities (Wahid *et al*., 2007). The leaf is an important organ where plant's anabolic reactions occur. The biosynthesis of photosynthates occurs in the chloroplasts, found in the leaves. A larger leaf area thus implies a larger surface area for CO_2 assimilation and fixation in the leaves.

Alupe also recorded high TLA (>24.00 cm^2) (Table 15a) except in accessions K1 and M4 which attained low TLA (<20.00 cm^2) (Table 15a). This could be due to optimal conditions in terms of temperature which was within the range 21-30.5^{0}C (Figure 6a) during growing season and loamy silt soil which provided favorable balance of water retention and drainage, as well as good aeration, making them ideal for plant growth compared to the clay loam (Table 6) of Kibos and silty clay (Table 6) of Oyani, facilitating enhanced leaf area (Brady and Weil, 2016). The low terminal leaflet area of the two forementioned accessions can be attributed to their genetic factors. The high TLA in most accessions at Alupe could also be due to high rainfall amount (882.5 mm) (Figure 6a) received in the zone, as increased water availability enhances leaf area development (Mabhaudhi and Modi, 2013).

Bambara thrives in soils with pH 5.0-6.5 (Yao and Zoro, 2005). Both Alupe and Kibos had low pre-planting soil pH of 4.7 and 4.6 respectively (Table 6). The low pre-planting soil pH level could have impacted negatively on TLA, by reducing nutrient availability (Tenga *et al*., 2018) and restricting leaf expansion.

The lowest TLA values were observed in Oyani, which could be attributed to suboptimal soil conditions characterized by low nitrogen (0.1 %), phosphorus (25 ppm) and Magnesium (0.4 me %) (Table 6) and below optimal rainfall (480.6 mm) (Table 6c) during growing season. Lower

precipitation at Oyani restricted leaf development due to inadequate moisture. Studies indicate that inadequate rainfall can result in reduced leaf expansion as water stress limits cellular enlargement (Bertolino *et al*., 2019). The low TLA observed amongst the droughted plants and in Oyani confirms that reduced water use due to water stress (Blum, 2005) has a direct impact on leaf development. Berchie *et al*. (2012) observed that leaf area in Bambara was lower under drought conditions compared to irrigated conditions, an observation aligning with the results from this study, where drought-stressed plants had the least TLA (Table 15e) and the irrigated plants had the highest TLA (Table 15d), further supporting the positive relationship between water availability and leaf area expansion.

Across all the zones, B1, B4 and M2 landraces (Table 15a-15c) had higher terminal leaflet areas. This could be due to certain genetic factors in them that determine terminal leaflet size. Across all the zones, the irrigated plants had highest TLA (Table 15d), followed by plants at Kibos (Table 15b), then Alupe (Table 15a). This indicates that soil moisture content influences the TLA of this crop, as these are zones that had adequate water. Chibarabada *et al*. (2013) observed that under rain-fed conditions, Bambara exhibited lower leaf area index compared to irrigated conditions, implying that increased water availability may enhance leaf area development, aligning with results in this study. This study confirms that Bambara terminal leaflet area is highly influenced by agro-ecological variations, with Kibos performing best due to optimal rainfall, soil properties and temperature.

5.2.6 Number of Flowers (NFP)

The number of flowers per plant (NFP) of Bambara accessions varied significantly ($P<0.05$) across the three agro-ecological zones (Alupe, Kibos, and Oyani) and the greenhouse conditions. In the field sites, the findings revealed that Kibos recorded the highest number of flowers per plant in all the accessions evaluated (Table 16b). Alupe recorded moderate NFP (Table 16 a), whereas Oyani recorded low NFP (Table 16c) across all accessions. Greenhouse irrigated (GHI) plants exhibited significantly ($P<0.05$) higher NFP (Figures 27-32) compared to the droughted (GHD) plants. Significant differences ($P<0.05$) were observed between field sites and greenhouse conditions (Figures 27-32) with drought-stressed plants consistently exhibiting the lowest number of flowers per plant.

Flowers are important reproductive structures in plants, which directly determine seed yield as they form the fruits and seeds after fertilization. The prevailing environmental factors in each agro-ecological zones played crucial role in influencing the number of flowers produced in a given accession. The rainfall amounts received during growing season and the temperatures influenced the NFP in each zone. Werner (2006), observed that the amount of rainfall had a direct influence on the number of flowers per plant in Bambara. Seasons with low rainfall amounts, the Bambara recorded low NFP.

Kibos (600.1 mm, 17.9-31.6°C) (Figure 6b) recorded the highest NFP (Table 16b) in all accessions, indicating that optimal rainfall and moderate temperatures provided optimal conditions flower development compared to Oyani, where rainfall (480.6 mm) (Table 6) was below optimal range, hence affecting reproductive success. According to Amwenyo *et al*, (2025), Bambara performs well in regions with rainfall range of between 600-750 mm and temperatures between 20°C and 28°C (Majola *et al*., 2021) during growing seasons. These favorable conditions likely contributed to high NFP observed in Kibos. The high NFP in this zone can also be attributed to nutrient composition. Nitrogen is critical for vegetative growth and chlorophyll production, ensuring the plant produces sufficient energy for flower development (Havlin *et al*., 2014). Phosphorus is a key component of ATP and nucleic acids, promoting early flowering and root development (Marschner, 2012). Potassium is essential for enzyme activation and water regulation, improving flower retention and seed set (Hassan *et al*., 2017). Calcium strengthens cell walls, reducing flower abortion (Wdowiak *et al*., 2024) whereas Magnesium is component of chlorophyll, enhancing photosynthesis and energy transfer for flower formation (Ahmed *et al*., 2023).

All the three zones were characterized with low pre-planting Nitrogen levels (0.1%). However, the higher post-planting nitrogen levels confirms that Bambara being a nitrogen fixer (Ajilogba *et al*., 2022), was able to meet its own nitrogen requirement through Nitrogen fixation process. The soil in Kibos zone had Magnesium (1.1 me %), Calcium (2.1 me%) and Potasium (1.3 me%) which were within the expected range required by Bambara (Table 6). These may have promoted flowering, hence higher NFP.

The accessions grown in Alupe (882.5 mm, 21.0-30.5°C) (Figure 6a) had moderate NFP (Table 16a), suggesting that Alupe's favorable temperatures may have enhanced flower initiation. However, higher rainfall above optimal could have impacted negatively on flower retention (Akinmoladun and Aremu, 2021). Phosphorus is an essential nutrient for reproductive growth (Marschner, 2012). The P levels in Alupe (28 ppm) (Table 6) were not far below the required range (30 pmm) (Table 6), and may have promoted flowering. Loamy soils in Alupe may have provided better water retention and aeration, favoring flower production (Brady and Weil, 2016). Oyani (480.6 mm, 20.7-29.6°C) (Figure 6c) recorded the lowest NFP amongst the zones (Table 16c), possibly due to suboptimal rainfall and soil constraints, suggesting a water-deficit impact. Oyani's potential clay soils could have caused poor drainage, negatively impacting flower numbers (Brady and Weil, 2016).

In Botswana, Vurayai *et al*. (2011) observed that water stress are associated with reduced flower production, an observation similar to the findings from Oyani zone. Mabhaudhi and Modi (2013) investigated the responses of Bambara groundnut landraces to imposed water stress under field conditions. Their study demonstrated that irrigated conditions led to improved growth parameters, including increased flower production, compared to rain-fed conditions. Hence, controlled irrigation positively influences flower numbers, supporting the findings from greenhouse-controlled plants in this study. This underscores the positive influence of adequate water supply on the flowering of Bambara.

Basically, the accessions M4, B1 and M2 recorded high number of flowers per plant across all the zones (Tables 16a-c). However, K2 accession had the least number of flowers across the zones followed by B4 (Table 16a-c), accessions which were light cream coloured. This observation supports what Mabhaudhi and Modi (2013), reported that darker-coloured Bambara seeds may have better vigour compared to light-coloured Bambara seeds.

5.2.7 Seed Weight per Plant (SWT g)

The study conducted in Alupe, Kibos, and Oyani agro-ecological zones revealed significant ($P<0.05$) variations in Bambara seed weight per plant. Among the three zones, Kibos recorded the highest seed weight per plant (Table 17b), followed by Alupe (Table 17a), while Oyani exhibited the lowest seed weight per plant (Table 17c). The greenhouse control treatments under

both irrigated and droughted conditions further indicated that water availability significantly ($P<0.05$) influences seed weight per plant, with irrigated plants consistently producing higher seed weights (Table 17d) than drought-stressed ones (Table 17e).

The results indicate that environmental factors play a crucial role in Bambara seed weight development. Rainfall is a critical factor influencing seed weight per plant in Bambara as it affects soil moisture content (Laary *et al*., 2012). Optimal rainfall for Bambara growth ranges between 600-750 mm (Amwenyo *et al*., 2025) during the growing season. Kibos, receiving rainfall within this range (600.1 mm) (Figure 6b), provided the best conditions for optimal seed development. Sufficient moisture ensures proper seed filling and biomass accumulation, leading to higher seed weight per plant **(**Mabhaudhi and Modi, (2013). The temperature range within this zone during growing season did not deviate much from what Majola *et al*., (2021) reported (20°C and 28°C) to be the temperature range required by Bambara. Ideal temperatures promote enzymatic activity crucial for seed development, while extreme temperatures induce stress, lowering seed weight (Choukri *et al*., 2022). A study examining the effects of temperature and drought stress on Bambara growth and development found that plants grown at 33 °C produced pod yields that were significantly lower than those grown at 23 °C, suggesting that temperatures above the optimal range may impair seed development (Alshareef *et al.*, 2013).

Alupe, with above optimal rainfall (882.5 mm) (Figure 6a), and with optimal temperature range (21.0-30.5°C) (Figure 6a), exhibited moderate performance (Table 17a), implying that Bambara seed development does not do well in areas with high rainfall amounts (Majola *et al*., 2021), especially during harvest which leads to seed yield losses. Oyani, with significantly lower rainfall (480.6 mm) (Figure 6c), and with optimal temperature range (20.7-29.6ºC) (Figure 6c) struggled to support high seed weight development. Studies have shown that Bambara is drought-tolerant, but prolonged water stress affects seed filling, ultimately reducing seed weight per plant (Iheanacho *et al*., 2021).

Soil properties such as pH (Limon-Ortega and Martinez-Cruz, 2014), nutrient content and texture (Bashir *et al*., 2022) significantly affect seed weight per plant. The optimal pH for Bambara is 5.0-6.5 (Yao and Zoro, 2005)**.** Kibos and Alupe's pre-planting soil pH was slightly below this range (pH 4.6 and 4.7 respectively) (Table 6), affecting nutrient availability and uptake in these

two zones. The low pH in these two zones hence may have impacted negatively on seed weight development. Oyani's soil, being least acidic (pH 6.0) (Table 6), provided favourable soil pH for seed weight development, although other edaphic factors like soil nutrient composition and texture (Bashir *et al*., 2022), coupled with environmental factors like rainfall amounts (Iheanacho *et al*., 2021) could have contributed to low seed weights among plants grown in this zone.

Higher Potassium (1.3 me %) (Table 6), Calcium (2.1 me %) (Table 6), Magnesium (1.1 me %) (Table 6) levels in Kibos contributed to better vegetative and reproductive growth, increasing seed weight per plant. The higher post-planting nitrogen levels in all the zones imply that Bambara was able to meet its own nitrogen requirement through nitrogen fixation process (Ajilogba *et al*., 2022). According to Zoundji *et al*. (2022), Bambara requires between 80-150 kg N ha^{-1} during its growing period, yet it can fix approximately 4-200 kg N ha^{-1}. This contributed to better reproductive success. Nutrient availability influences photosynthesis efficiency and seed filling, with nitrogen and phosphorus playing primary roles (Savala *et al*., 2021). Alupe had moderate nutrient availability, characterized by only Magnesium (2.0 me %) (Table 6) and Potassium (1.5 me %) (Table 6) levels being above the minimum required by the Bambara, supporting average seed development. The average seed development at Alupe could also be as a result of loamy silt soils which had high water retention and aeration (Brady and Weil, 2016). Oyani, with its nutrient-poor soil (Table 6) coupled with its higher clay content (Table 6), suffered from poor drainage (Brady and Weil, 2016), negatively impacting seed development (Table 17c). Tenga *et al*., (2018) demonstrated that soil fertility significantly affects Bambara seed yield in different agro-ecological zones in Tanzania.

Mabhaudhi and Modi, (2013) found that seed weight per plant was highest amongst irrigated plants as compared to rain-fed Bambara plants, an observation supporting the findings in this study, where irrigated plants had the highest seed weights (Table 17d). Berchie *et al*. (2012) reported that water stress reduces seed yield in Bambara, aligning with the findings in Oyani and amongst drought-stressed plants. Ntundu *et al*. (2006) also observed that drought stress reduces seed weight in Bambara, further emphasizing the importance of soil moisture in seed weight development. According to Mwale *et al*., (2003), Bambara growing in water stress regions, develop drought tolerance mechanisms such as reduction in photosynthesis resulting from reduced CO_2 assimilation and fixation because of stomatal closure and reduced chlorophyll in

the leaves, adaptations meant to minimize water loss and enhance its water use efficiency under such conditions. The reduced CO_2 assimilation and fixation could have led to the low seed weights amongst drought-stressed plants, and plant grown at Oyani where rainfall amounts were low (480.6 mm) (Figure 6c). Additionally, a 2018 study by Chibarabada, Modi, and Mabhaudhi assessed the adaptation and productivity of various grain legumes, including Bambara, in contrasting environments of Kwa Zulu-Natal, South Africa. The research highlighted that the environment, particularly factors like rainfall, significantly influences yield and yield components. This underscores the importance of sufficient moisture in ensuring proper seed filling and biomass accumulation, leading to higher seed weight per plant.

Basically, the significant differences observed in intra-zonal comparisons of quantitative traits indicated that different landraces responded in different ways to the abiotic factors in the same zone. These traits also showed significant differences in inter-zonal comparisons. This indicated that same accession responded in different ways to the prevailing edaphic and other climatic factors in different zones, leading to phenotypic plasticity. Phenotypic plasticity is one of the causes of variation in Bambara (Valombola *et al*., 2022). Bambara exhibits significant variations both within and across accessions (Esan *et al*., 2023a), and their growth environments further amplify the variations in accessions (Esan *et al*., 2023b).

Generally, K1 landrace recorded low seed weights amongst the irrigated plants (Table 17d), at Alupe (Table 17a) and Kibos (Table 17b) zones, in comparison to other accessions in those zones. These zones were characterized with high soil water implying that K1 accession is not adapted to growing in soils with high soil moisture contents. K1, B1 and M4 landraces seem to have more drought tolerance mechanisms especially in their water use efficiency. This is evidenced by the fact that these three landraces produced appreciable quantities of seed yield under droughted conditions (Table 17e) despite facing intense water stress. Due to their greater drought tolerance adaptability, these landraces can therefore thrive in sub-Saharan Africa's arid and semi-arid regions, which are primarily marked by a variety of abiotic pressures, the primary one being erratic and low rainfall patterns (Majola *et al*., 2021). These three landraces attest to the Bambara groundnut's drought tolerance and ability to generate a reasonable yield even in situations of water stress (Harris and Azam-Ali, 1993).

Quantitative traits performed best at Alupe, Kibos, and among the irrigated plants in the greenhouse, where soil moisture levels were higher compared to the drought-stressed greenhouse plants and those at Oyani. In Oyani, water stress led to reduced plant height, number of pods, leaf area, number of flowers, and seed weight across the accessions. These results highlight water as a major factor influencing the performance of quantitative traits in Bambara across different zones. This finding is consistent with Mwale *et al*. (2003), who reported that drought reduced leaf area, dry matter accumulation, seed weight, and yield. Similarly, Berchie *et al*. (2012) observed reductions in leaf area index and plant height under water stress conditions.

5.3 Principal Component Analysis

From Figure 40A, the irrigated and the droughted zones cluster far apart, with no overlap. This implies that the two zones had very unique prevailing conditions under which the same landraces were growing. The edaphic factors such as the pH, soil texture, and the nutrient compositions of the soils were similar at the beginning of the trials (Table 6). Soil moisture contents in the irrigated and droughted soils was the main factor that determined the performance of the traits evaluated in the greenhouse. The irrigated soils had adequate soil moisture content for the plants, whereas the droughted plants suffered severe water stress. The soil moisture also determined the quantities of various nutrients available for uptake by plants since most nutrients are absorbed while in solution form (Leghari *et al*., 2016). Soil moisture hence was a limiting factor for the absorption of nutrients by the droughted plants. According to Laary *et al*., (2012), the knowledge of soil moisture conditions under which crops are grown is necessary for overall selection of good quality seeds. It has also been established that certain nutrient compositions in Bambara such as protein amounts in the seed depend on soil moisture content. Higher soil moisture contents imply bigger seed sizes and protein amounts (Laary *et al*., 2012).

Kibos and the GHI zones shared many similar edaphic and environmental factors (Table 6). The two zones had soil pH in the acid class. High K^+ levels (>0.24me %) were recorded in the two zones at the beginning of the trials. Given that the soil textures in the two zones were of clay loam, this supports the idea that clay soil texture has an impact on the soils' ability to retain available K^+. Due to the high soil moisture content in the two zones, exchangeable K^+ in the soil were dissolved and changed into water-soluble K^+, which was then available for plant uptake and

usage (Uchida, 2000). Because of this, the plants at Kibos and those under irrigation had more access to water-soluble K^+ in the soil.

It is therefore deduced that the uptake of K^+ in the Kibos and GHI soils were relatively high compared to the other sites as K^+ regulates plants use of water (Uchida, 2000). Just like in other zones, the pre-planting N levels in the two zones were low (Table 6) although BGN being a nitrogen fixer (Olanrewaju *et al*., 2021a), replenished the nutrient in the soils, evidenced by higher post-planting N levels (Table 6). Nitrogen was therefore not a limiting nutrient in the zones. These similar factors led to near similar performance in traits evaluated in these accessions, hence overlapping cluster pattern observed between irrigated and Kibos zones.

Oyani had an overlapping clustering with droughted zone. These two zones were characterized with low soil moisture contents which basically affected the performance of all traits evaluated, as evidenced by strong significant ($P \leq 0.05$) differences observed between the irrigated and droughted crop performance in traits evaluated. The droughted and Oyani zones had low phosphorus levels in the soils. According to Bechtaoui *et al*., (2021), this element is crucial for plants growth and development as it controls flowering and fruit production in plants. Low quantities of this element in the two zones therefore led to poor flowering and podding in the landraces under study. Goli, (1995), and Khan *et al*., (2020), observed that flowering in BGN could be influenced by temperature, soil conditions as well as genes of the accessions. The nitrogen fixing capacity of leguminous plants is also enhanced by the availability of P in the soil (Malhotra *et al*., 2018). Low quantities of this nutrient in the soil therefore to certain extent reduced the nitrogen fixing capacity of these plants in both zones. In addition, both the zones were characterized by acidic soil pH and low K^+ levels. Oyani soils had very minimal levels of Mg^{2+} (Table 6). Basically, low soil moisture contents in both zones, coupled with low quantities of nutrients in the soils led to poor performance in traits evaluated, hence the overlapping clustering observed between these two zones.

The non-overlap between some zones can be explained by the varied and distinct prevailing environmental conditions in the zones which affected the performance of traits being evaluated. According to Olanrewanju *et al*., (2021a), the environment is vital and dominant source of variation hence must be prioritized in plant breeding. The partial overlap observed between

Alupe and the other zones may be attributed to similarities in certain edaphic and climatic factors.

Genotype classification benefits significantly from the use of cluster analysis as a statistical method, as demonstrated by Nankar *et al*. (2020). In this statistical method, materials under study are grouped according to their affiliation and resemblance. In this study, the clustering was based on the mean performance of traits evaluated. Because each cluster has unique characteristics, it is best to choose breeding materials from a variety of clusters. According to Esan *et al*. (2023a), this could help maximize the desirable qualities of the recently developed variety in terms of performance.

The cluster analysis based on quantitative traits showed that key characteristics like the number of pods with one seed (N1S), the number of flowers per plant (NFP), and the total number of pods per plant (POD) can be used to differentiate Bambara groundnut plants in the Lake Victoria Basin (Figure 40B). This observation is consistent with that made by Molosiwa (2012), in Botswana and the United Kingdom, where it was found that the key characteristics that divided the genotypes into distinct clusters were the number of pods per plant and differences in shoot dry weight. The accession clustering pattern observed in Figure 40B could be attributed to certain genotypic factors unique to each accession, which interact with the environments to determine performance in traits. Goli, (1995) reported that certain genetic factors which are unique to the genotypes could influence performance of traits in different environments. Because this crop exhibits both autogamy and cleistogamy, there should be more inter-population variability within the clusters (Molosiwa, 2012). According to Massawe *et al.* (2005), the many overlaps seen between these accessions (Figure 40B) attest to the significance of establishing seed sources in Bambara.

According to Principal Component Analysis (PCA), all the quantitative traits (N1S, POD, NFP, SWT, N2S and TLA) were strongly and positively correlated apart from the PHT which was weakly and positively correlated with other traits. All the quantitative traits evaluated were skewed towards PC2. According to Greenacre *et al.,* (2022), PCA is a valuable technique for estimating the overall variation within a set of characters. Basically, all the traits apart from PHT were important in explaining the variation, with PC2 accounting for 14.5%. Esan *et al.*, (2023a),

observed that PCA assigns various traits into distinct groups, hence plays the same role as hierarchical cluster analysis.

From the biplot (Figure 40A), SWT, TLA and N2S are the main traits with positive loadings that influenced the clustering of Irrigated, Kibos and Alupe zones, hence their overlap. In these zones, the accessions had high SWT and TLA means, and moderately high N2S means. Alupe and Kibos zones were characterized by optimal rainfall amounts (Figures 6a and 6b respectively) for the BGN, thus it is deduced that production of high SWT, TLA and N2S in BGN requires moderate soil moisture content. Leaf area is vital in plant anabolic process like photosynthesis, as it determines amount of light a plant can absorb, and its carbon assimilation efficiency. A larger leaf area increases the plant's capacity to capture light and assimilate carbon, these enhance production of more sugars which are stored in various plant organs such as seeds. The leaf area therefore affects seed weight and yield. The over-lap between the droughted and Oyani zones could be due to their low soil moisture contents and low nutrient (PO_4^{3-} and K^+) which affected performance of traits, as no traits from the biplot (Figure 40A) directly influenced their clustering.

From the biplot (Figure 40B), SWT, TLA and N2S are the main traits that influenced the clustering of K2 and B4, whereas N1S, NFP and POD are the main traits that influenced the clustering of K1 and M4. These clusters have been confirmed by the hierarchical clustering dendrogram (Figure 41). Accessions B1 and M2 overlap around loading of 0, and all the traits apart from the PHT influence their clustering pattern. This indicates the importance of these traits in identifying various Bambara landraces (Molosiwa, 2012). From the biplot (Figure 40B), the B1 accession overlaps with B4, K1, and M2, while K2 clusters with B4, and M4 clusters with K1. This pattern suggests that B1 is likely the progenitor of the other five farmer-held accessions in the Lake Victoria Basin (LVB), as it exhibits the majority of traits found across the other accessions. This broad trait composition may contribute to its adaptability across all agro-ecological zones in the region. This is supported by both the quantitative dendrogram (Figure 41) and the rank summation indices (Table 23a-e) where it was ranked number two in Alupe and Kibos zones, and number three in Oyani zone. It however emerged as the most adaptable and high yielding amongst the greenhouse irrigated plants. This accession is characterized by high performance in qualitative traits evaluated and high yields. It is also deduced that the K2 and the

M4 accessions are the recently evolved accessions amongst these landraces. The clusters observed amongst these accessions could be due to intrinsic genetic factors in the clustered accessions, which determine the performance of these three traits irrespective of the zones, although plant genotypes tested in different locations are also affected by differences in climatic factors such as rainfall and temperature, and soil fertility (Olanrewaju *et al*., 2021a).

Multivariate analysis was employed by Zarei *et al*., (2019), to assess the genetic diversity and variability of Pyrus species. PCA analysis was performed by Khan *et al*. (2021b), to assess the genetic diversity and variability in BGN. Esan *et al*., (2023a), assessed genetic diversity and variability in BGN grown in multi-environment using PCA. According to Khan *et al*., (2021b), the first axis (PC1) in any PCA represents the greatest percentage of the total variation. Similar to earlier studies, we found out that PC1 (72.7%) accounted for the largest percentage of variance, whereas PC2 accounted for only 14.5% of total variation. Khan *et al*., (2021b) reported a total variation of 45.88% for PC1, whereas PC2 accounted for 10.63% of the total variation amongst the traits evaluated. Olanrewaju *et al.,* (2021a) found 24.67% for PC1 and 17.63% for PC2, whereas Esan *et al*., (2023a) found that PC1 accounted for 39.85% and the PC2 represented 16.31% of the total variation.

5.4 Correlation Analysis

All yield-related traits except PHT showed strong positive associations (Table 18). Such correlations were found between number of flowers and number of pods with one seed (r=0.91), number of flowers and total number of pods(r=0.93), number of flowers and terminal leaflet area (r=0.79) and between number of flowers and seed weight (r=0.91). The high r- values were also observed between the number of pods with one seed and total number of pods (r=0.99), number of pods with one seed and terminal leaflet area (r=0.70), and number of pods with one seed and seed weight (r=0.86). Similar observations were made between total number of pods and terminal leaflet area (r=0.75), and total number of pods and seed weight (r=0.89). Esan *et al*., (2023a) and Khan *et al*., (2021b), also observed strong positive correlations between yield-related traits.

The efficiency of light interception for photosynthesis by the plant is demonstrated by the strong and significant correlations found between the terminal leaflet area and the seed weight.

According to Helms, (1976), there is frequently little correlation between photosynthesis and yield in both field crops and forest trees. This result runs counter to these findings because all other yield components had strong, positive, and highly significant correlations with the TLA, a region where photosynthesis occurs, with the exception of PHT, which had a moderately positive correlation (r=0.52).

The plant height recorded low correlations with all other traits. The low correlations of PHT with other yield components reveal that within the diverse Lake Victoria region, Bambara plant height affect and determine the performance of other yield components only to a lesser extent. This could probably be due to the spreading accession types, with minimal overlapping of leaves. All the plant leaves are therefore exposed to light interception. Hence PHT is not solely the determinant of these accessions ability to trap and compete for sunlight for photosynthesis. However, the PHT positive correlation with other yield components indicates that it is inter-related with other vegetative and reproductive traits. Similar results were reported by Esan *et al*., (2023a), Khan *et al*., (2021b) and Olanrewaju *et al*., (2021a).

5.5 Yield

5.5.1 Coefficients of Variation of Yield

The intra-site yield comparisons showed that K2 accession in Alupe (Table 21a), Oyani (Table 21c) and amongst the droughted plants (Table 21d) had high (>10 %) Coefficients of Variation (CV). The inter-site comparisons indicate that in all the zones, the accessions had low CV (< 15%) apart from K2 accession which had high CV of 29.1% (Table 19a) amongst the droughted plants. This is an indication that environment has a direct influence on the variations observed amongst Bambara accessions (Olanrewaju *et al*., 2021a). In this study, the estimated Coefficients of Variation (CV) shown in yield of accessions is an indication of heterogeneous nature of BGN, an observation in concurrence with that made by Goli *et al*. (1997). Variation within a given species is crucial as selection criteria (Jonah *et al.,* 2012) in breeding programs. Moreover, the relative amount of variability in a population is best expressed based on the genotypic coefficient of variation as the variable is computed based on mean values of accessions and units of measurement (Jonah *et al.,* 2012). The low coefficient of variation (≤ 20%) in yield observed for all accessions both in intra and inter zonal yield comparisons, apart from the droughted intra-zonal comparison where K2 accession had coefficient of variation of 26.4% (Table 21d) and K2

inter-site comparisons, where droughted plants had high CV of 29.1% (Table 19a) are contrary to what Khan *et al*., (2020), reported for yield trait where all the accessions recorded coefficient of variation of ≥20%. Masindeni, (2006), also reported a coefficient of variation of 24.79% for grain yield. The differences in CV can be attributed to the accessions genotype and growth environment (Obidiebube *et al*., 2019).

5.5.2 Yield Intra and Inter-Zonal Comparisons

The yield of this crop varied considerably both in intra and inter-zonal comparisons. The strong significant differences (P<0.0001) observed in the same accession grown in different zones could be due to environmental variations. According to Obidiebube *et al*., (2019), variation in yield is normally due to environment, season and genotype. The droughted plants had low yields whereas the irrigated crops and those in Kibos zones recorded high yields, an observation attributed mainly to soil moisture content and edaphic factors such as soil nutrient composition. The significance of soil moisture content as a key determinant in yield performance in BGN can be shown by the strong significant (P<0.0001) difference between the GHD and GHI yield in all accessions. In intra-zonal yield comparisons, some accessions showed no significant differences (P≥0.05). This could be attributed to the ability of these accessions to partition the dry matter into pod filling at the same pace (Obidiebube *et al*., 2019). According to Stephen, (2009), pod filling is a crucial process in leguminous plants, a process which impacts directly on yield. Other accessions had significant differences (P≤0.05) in yield in same zones. These observations according to Collinson *et al*., (1996), can be attributed to individual accessions response to prevailing environmental conditions in the zone. This can allow for various accessions to be localized in various environments (Esan *et al*., 2023b).

The yields reported in this study ranged from 0.11ton/ha in the GHD, which was K2 accession yield, to 3.84ton/ha in the GHI, which was B1 accession yield. These yields are within the range of those recorded elsewhere. Khan *et al*., (2021b) recorded 0.588ton/ha to 2.991ton/ha, Gbaguidi *et al*., (2018) in Benin recorded 0.146 to 2.678ton/ha. Obidiebube *et al*., (2019), recorded 2.419 to 3.738ton/ha, even though these were achieved under different spacing. FAO (2017), however reports 0.703ton/ha as the average yield harvested by majority of farmers. The minimum yield recorded in this study was relatively low, likely due to the drought-induced water stress conditions.

5.5.3 Correlation Coefficients of Yield and Yield Components

All the quantitative traits that were assessed directly affected yield. According to Khan *et al*. (2022a), yield is a polygenic trait that does not express independently, but rather results from complex interactions with other factors. R-values were utilized in this study to ascertain the link between two dependent variables, yield being one of them. SWT showed a perfect positive significant association with yield in every zone that was studied. Because of its direct, perfect, and positive effect on yield ton per hectare, this trait can therefore be exploited in programs aimed at improving seed yield (Khan *et al*., 2022a). Crop yield at Oyani was negatively correlated with PHT. This could probably be due to the prevailing environmental conditions coupled with edaphic factors which could have affected PHT in this zone. In all the other zones PHT had a positive association with yield, an observation which is in agreement with what Onwubiko *et al*., (2019) observed in their research.

Across all the zones, N1S, POD, TLA, NFP and SWT had significant positive associations and contributions to yield. This indicates the important influence of these five traits on yield. These results corroborate those obtained by Esan *et al*., (2023a); Karikari and Tabona (2004) where yield showed a strong and positive correlation with POD, SWT, PHT and number of leaves per plant. In breeding programs, within the Lake Victoria basin, it is preferable to select them together since they all contribute positively to BGN seed yield improvement. More farmers in the area may choose to adopt the crop as a result of this crop enhancement strategy. These results are similar to those of Khan *et al*., (2022a), where yield was positively impacted by a number of characteristics, POD being among them. According to Iqbal *et al*., (2003), number of pods per plant (POD) is a significant feature in legumes that influences production; as such, it should be taken into consideration to increase and enhance BGN seed yield. Positive correlations between total number of pods per plant (POD) and yield were noted by Jonah *et al*., (2011), in their BGN study, and Kumari and Sasidharan (2020), in their groundnut study. The findings of Olanrewaju *et al*. (2021a), however, were inconsistent since they discovered a negative correlation between POD and yield but a positive correlation between SWT and POD. The negative correlation between POD and yield was probably due to the effect of genotype and environment interaction, which is reported, brought significant variation in various traits, number of pods and yield being some of them. The high ability of the plants to intercept light for

photosynthesis and prioritize seed filling was shown by the positive correlation between yield and terminal leaflet area (TLA).

5.5.4 Selection of High Yielding BGA per Zone based on Rank Summation Index (RSI)

According to RSI, the accessions B4, M2, M4, K1 and B1 emerged as the high yielding accessions in Alupe, Kibos, Oyani, GHD and GHI respectively. This shows that these accessions are the most adaptable in the zones. The results from RSI give valuable information in a breeding program for the selection of high yielding Bambara (Khan *et al*., 2021b). RSI is one of the commonly used accurate methods in the identification and selection of high yielding accessions (Onwubiko *et al*., 2019). The results from correlation analysis revealed traits that have positive correlation with yield (Tables 22a-e) in each zone. The accessions with the lowest 'rsi' scores were identified as the highest-yielding genotypes in each zone. Notably, these same accessions also recorded the highest yield (ton/ha) based on agronomic field evaluations in each zone and in the greenhouse. This observation is in agreement with that of Onwubiko *et al*., (2019). This observation proves the accuracy of 'rsi' as a reliable method of determining an accession with high yielding abilities and adaptable in a given agro-ecological area considering the fact that yield is a polygenic trait.

The precision of the results on identification and selection of B4, M2, M4, K1 and B1 as high yielding accessions in Alupe, Kibos, Oyani, GHD and GHI respectively are further supported by performance of quantitative traits in the field and greenhouse trials, traits which have been reported to have positive effect in improving seed yield in BGN (Ntundu *et al*., 2006; Jonah *et al*., 2011). These five high yielding accessions were used in molecular studies to establish their similarity indices, hence their putative relationship since in Taxonomy, morphological traits are unreliable as indicators of relationship because they are subject to environmental influences.

5.6 Proteins

5.6.1 Protein Concentrations

In this study, M2 accession had the highest total protein concentration which differed significantly ($P \leq 0.05$) with all the other accessions' total proteins, while K1 had the least concentrations (Figure 43). This is an indication that protein concentration in BGN accessions is not uniform and varies with accession types. The differences in protein contents could be an

indicator of genetic variation in the accessions of this species since proteins are direct products of gene expressions (Oludare *et al*., 2017). M2 accession also had the highest albumins (Figure 49a) and globulins (Figure 49b) concentrations amongst all the accessions evaluated. This was the accession with black seed coat (Plate 5) from Migori County. The high protein concentration in M2 accession could be due to the interaction of the genotype and the environmental growing conditions at Kibos where the accession performance indices were highest. Moreover, Valombola *et al*., (2022) reported that dark-coloured seeds have higher vigour compared to light-coloured seeds, probably explaining the M2 accessions higher protein concentrations. Saminu *et al*., (2021) also recorded high total proteins, as well as high albumins and globulins in the Yobe black Bambara accession in Nigeria. Traore-Barro *et al*., (2023), asserts that protein content in BGN varies with cultivar genetic constitution, environmental growing conditions, and possibly the analytical methods used. Kibos soils were characterized by adequate K^{+}, Ca^{2+} $Mg^{2+}$and although the pre-planting nitrogen level was low (0.1 %), BGN being a nitrogen fixer, met its nitrogen requiments through nitrogen fixation process (Ajilogba *et al*., 2022), evidenced by higher post-planting nitrogen levels (0.3 %) (Table 6). The pre-planting phosphate level in the Kibos soils was 27ppm (Table 6), concentration not so much below the critical minimum threshold of 30ppm. The acidic soil pH in this zone favoured phosphorus uptake by the Bambara (Malhotra *et al*., 2018). Kibos zone also received optimal rainfall (600.1mm) and optimal temperature range (17.9-31.6^{0}C) (Table 6) for BGN growth and developmnt during the growing season. These edaphic and weather conditions, combined with genetic factors, favored the synthesis of high protein content, including elevated levels of albumins and globulins in this Kibos-grown M2 accession (Massawe *et al*., 2005).

Amongst the accessions evaluated in this study, four (B4, B1, M2 and M4) out of five accessions had albumins as the dorminant protein fraction. The high albumins concentrations in these four accessions could probably be due to the edaphic and other prevailing environmental factors in the zones where they had high performance indices, an observation supported by Shewry and Halford, (2002) who assert that soil nutrients, temperature, and water availability influence protein accumulation in plant seeds. Moreover, Mamoudou and Mune, (2024), asserts that albumins is one of the major storage proteins in Bambara, and contribute significantly to the total protein content. The accessions B1, B4, M2 and M4 had high performance indices amongst the irrigated plants, Alupe, Kibos and Oyani respectively. These are zones that had adequate water,

apart from Oyani which had slightly low rainfall (480.6mm) below the optimum (600-750mm) required by this crop. The M2 and B4 accessions grown at Kibos and Alupe zones respectively, zones that had optimal and above optimal rainfalls respectively, recorded high albumin concentrations, while K1 accession grown in droughted soils had the least albumin content. It can therefore be deduced that soil moisture content is a major determinant factor in albumin synthesis. This could be tied to its water-solubility character. According to Mamoudou and Mune, (2024), the primary protein fraction within Bambara is albumin, which is water-soluble and constitutes a substantial portion of the total protein content. Traore-Barro *et al*., (2023), in their study observed that albumin fraction was the most dominant seed storage proteins in the eight Bambara accessions representing about 95.42% of the total soluble proteins.

The K1 accession had globulins as the dorminant protein fraction. Additionally, this accession had high quantitative traits performance indices and the highest yield indices amongst the droughted plants (Table 20). In this study, K1 accession is exemplified as a low water tolerant accession. Henriet *et al*., (2019), in their study, imposed a 9-day water stress to the pea plant, and observed the accumulation of S-rich globulins (11S) in seeds. This they attributed to a lower seed sink strength for nitrogen. However, in their study, they were evaluating the effect of water stress combined with sulfur deficiency in pea plant. Globulins hence could be having physiological anti-water stress mechanisms it confers on plants when soil moisture contents is low. According to Henriet *et al*., (2019), water stress may reduce seed sink strength for nitrogen, allowing a readjustment in the ratio of sulfur-poor (7S) to sulfur-rich (11S) globulins. This indicates that water stress can influence globulin synthesis favorably under certain nutrient-deficient conditions. This accession therefore should mostly be grown in areas of low soil moisture content. It can therefore be deduced that probably extreme water stress led to the synthesis of higher globulins in this accession in the droughted environment.

Amongst these accessions, albumins and globulins were the dominant proteins (Mamoudou and Mune, 2024). All the accessions evaluated had high concentration (>0.02mg/ml) of albumins and globulins. These two seed storage proteins are most abundant in the seeds of crops as evidenced in some studies (Alabi 2023; Saminu *et al*., 2021; Yagoub and Abdalla, 2007). In this study, albumins and globulins concentration ranged from 0.022-0.065 mg/mL. Saminu *et al*., (2021) reported albumins and globulins protein content in the same range (0.023-0.088 mg/mL).

Albumins are important seed proteins rich in glutamine and undergoes rapid degradation during the early stage of germination (Youle and Huang, 1978). These ubiquitous, water-soluble proteins (Youle and Huang, 1978) that are expressed as a 2S albumin storage protein in seeds (Rasheed *et al*., 2020) therefore play pivotal roles in germination.

In all the accessions evaluated, the glutelins and prolamins were present, albeit in quite low quantities, and with no significant differences ($P \geq 0.05$) in majority of the accessions. According to Rasheed *et al*., (2020), prolamins and glutelins have several similarities, including a high proportion of proline and glutamine, and a high proportion of repetitive sequences, with non-repetitive domains in their N- and C-terminals. The main difference between the two protein types is the formation of intra- and inter-molecular disulfide bonds in their native states. This difference in their pattern of disulfide bonding explains the differences in their solubilities and extractability. These two seed storage proteins are normally found in minimal quantities in legumes, but are the dorminant storage proteins in seeds of the grass family (Triticeae), such as rice where they account for up to 85% of total protein (Rasheed *et al*., 2020). In this study, the M2 and M4 accessions' glutelin concentrations were not significantly different. It can be deduced that the two accessions from Migori County, in terms of glutelin synthesis, responded in a similar way to the prevailing edaphic and other environmental factors at Kibos and Oyani zones respectively, where they were high yielding and with similar glutelin concentrations. In this study, the prolamins were found in very low quantities (≤0.003mg/mL) in all the accessions evaluated. This observation is in agreement with what Yagoub and Abdalla (2007), observed in their study where prolamins concentrations were the lowest (0.84%) amongst the seed storage proteins. Saminu *et al*., (2021), also recorded the least concentrations of prolamins in their study (≤0.004mg/mL). It can be deduced therefore, that prolamins concentration in BGN is low. In this study, there were no significant differences ($P \geq 0.05$) observed between prolamin concentrations of M2, M4, B4 and B1 accessions. This shows that the four accessions responded in a similar way to the abiotic factors at Kibos, Oyani, Alupe and amongst the green house irrigated plants respectively, with reference to synthesis of prolamin and its concentrations.

Basically, there were narrow ranges in protein contents in these accessions. The narrow ranges could be an indicator of genetic resemblances amongst these accessions. The estimated low coefficients of variations observed in the total proteins concentrations of the five accessions and

in the protein fractions is an indication of the low degree of heterogeneity in the BGN studied. This could be attributed to cultivar homogeinity or purity (Kumar *et al*., 2010). Prolamins, however, recorded high coefficients of variations (≥20%) in the accessions studied. This is an indication of the heterogeneous nature of this storage protein in BGN.

5.6.2 Protein Electrophoretic Banding Pattern

In this study, the protein peptides detected in the five accessions ranged from 21 kDa to >250 kDa. K1, B4 and M2 accession had ten bands each, whereas B1 and M4 accessions had eleven bands each (Table 25). From the electrophoretic banding patterns of proteins in accessions, a high homology (96%) was observed between B1 and B4 accessions, accessions from Busia County. These two accessions were characterized by having a unique protein peptide of 112 kDa, a peptide lacking in all the other accessions. The two accessions also lacked protein peptides of 180 kDa and 52 kDa. The presence of protein peptide 40 kDa in B1 accession distinguishes it from B4 accession. The dissimilarity indices (r^2 values) of protein peptides based on Jaccard index show that M2 accession had high r^2 values with other accessions (Table 26). This indicates lower degree of overlap, hence higher dissimilarity between this accession and the other accessions. The dissimilarity observed could be attributed to the existence of unique protein peptide 180 kDa, which was only present M2 accession. This protein marker can therefore be used to distinguish M2 from all the other farmer-held accessions under study. The dissimilarity could also be due to the absence of protein peptides with molecular weights 97 kDa and >250 kDa in this accession, protein peptides that were detected in all the other accessions. The dissimilarity is supported by the protein dendrogram (Figure 51) that clustered the M2 accession in its own cluster. Equally, M2 and M4, accessions from Migori County, both had a unique protein peptide of 52 kDa, which was lacking in all other accessions. The K1 accession lacked protein peptides of 52 kDa, 112 kDa and 180 kDa, and lacked unique protein peptide that could be used in its varietal identification (Iqbal *et al*., 2005). Cluster analysis put the five Bambara accessions in two main groups, M2 in one cluster, and K1, M4, B1 and B4 in another cluster. The grouping of four accessions into one single cluster reveals low genetic diversity among the studied landraces. This observation aligns with that of Saminu *et al*., (2021), where all the six Bambara under their study were clustered in one major group, signifying low genetic diversity amongst their studied accessions. Hence from this study, it can be deduced that the environment of origin, where the plant has been domesticated for years, to some extent could be having an

influence on the protein types expressed in the plants. These protein types could be conferring certain adaptive advantages to the plant to enable them survive in those environments, although Iqbal *et al*., (2005), reported that proteins are largely independent of environmental influences.

Saminu *et al*., (2021) analysed the seed storage protein in Bambara using SDS-PAGE and observed the electrophoretic banding patterns in the accessions under study. They observed that Niger cream and Yobe cream each had five bands 97.4 kDa, 45 kDa, 29 kDa, 20.1 kDa and 18 kDa, while each of Yobe black, Kano maroon and Kano brown had four bands 97.4 kDa, 45 kDa, 29 kDa and 18 kDa. Kano mottle had only 3 bands 97.4 kDa, 45 kDa and 18 kDa. A total of five peptide bands with molecular weights corresponding to 97.4 kDa, 45 kDa, 29kDa, 20.1 kDa and 18 kDa were observed among the different landraces.

All the protein isolates from the five accessions had major bands at 21-22 kDa, 35-36 kDa, 43 kDa, 55 kDa and 110 kDa. The presence of peptide bands of similar molecular weight across the landraces signifies that the genes encoding those proteins are ubiquitous and fixed in the BGN species (Alege, 2015). Moreover, the presence or absence of bands can be useful in the identification of landrace (Saminu *et al*., 2021). Adebowale *et al*., (2011), reported major bands at 35 kDa, 43 kDa and 112 kDa in their isolates. In this study, band 112 kDa was only reported in accessions B1 and B4. The diversity in major bands observed in these studies could be due to genetic variability (Adewumi *et al*., 2022) existing in the accessions. According to Iqbal *et al*., (2005), the genetic diversity of seed storage proteins based on SDS-PAGE can be used as a tool for varietal identification, phylogenetic relationship determination and characterization of germplasm. Electrophoretic banding pattern of proteins in various organisms is an important tool that can be utilized to evaluate inter and intra species genetic variability, since proteins are direct products of gene expressions (Oludare *et al*., 2017).

5.6.3 Albumin and Globulin Electrophoretic Banding Pattern

Glutelins and prolamins could not form distinct bands. Prolamins only showed a smear in the gel, as glutelins seemed to escape in the running buffer (Figures 52-56), hence no clarity in observation. Hence only albumin and globulin electrophoretic banding patterns were observed. Both the albumin and globulins peptides molecular weights ranged from 21 kDa to 250 kDa. Bambara landraces may have similar seed storage proteins, but with differences at finer levels

(Odeigah and Osanyinpeju, 1998). All the four accessions were characterized by having albumin sub units of both lower and higher molecular weights (≥97 kDa) apart from M2 accession where albumins with higher molecular weights (≥97 kDa) were not detected. Lack of albumins with higher molecular weights in M2 accessions further indicates the divergent nature of M2 accession at finer molecular levels.

The similarity indices of albumins indicate that the two accessions B1 and B4 have a homology of 79%, and are grouped together in their own sub-cluster in the dendrogram (Figure 57). The two accessions from Busia County also share albumin peptide bands of 21 kDa, 37 kDA, 55 kDa and 97 kDa. The similarity could be an indicator of their genetic relatedness. Alabi (2023) in his study identified albumin fractions in Bambara with molecular weights of approximately 18 kDa and 29 kDa. Saminu *et al*., (2021) in their study detected five peptide bands with molecular weights corresponding to 97.4 kDa, 45 kDa, 29 kDa, 20.1 kDa, and 18 kDa. Notably, the 29 kDa and 18 kDa bands were associated with albumin fractions. The slight differences in the albumin molecular weights could be attributed to the environmental influences (Traore-Barro *et al*., 2023). The similarity indices of albumins also indicate that K1 and M4 accessions are related at a homology of 77%, indicating that although phenotypically they posses different seed attributes, genetically they are close. The K1 and M4 accessions are the only accessions that possessed albumin peptides of 250 kDa. These two accessions though currently found in different regions, may have had a common environment of origin where they were domesticated, and evolved together, hence their protein resemblances to counter certain abiotic stresses in that environment (Zhou *et al*., 2023).

Most farmer-held accessions lacked globulins of higher molecular weight (≥180 kDa) apart from B1 and B4 accessions from Busia County. Seed storage proteins are important genetic markers whose variability in a species can be useful as a molecular tool in crop breeding programs (Ghafoor *et al*., 2002). The major globulin bands present in all accessions were 21 kDa, 34 kDa and 55 kDa. This observation aligns with that of Alabi, (2023) who observed that globulin had major protein bands at low molecular weights, including major broadband at around 50 kDa. The M2 accession had least (4) number of globulin bands (Table 29). This accession was highly dissimilar to other accessions, as evidenced by high Jaccard dissimilarity values (Table 30) which indicate lower degrees of overlap with other accessions. The globulin peptide of 95 kDa

was present in all accession apart from M2 accession. The M2 divergence from other accessions at the globulin sub-unit level is further supported by the dendrogram (Figure 58), where M2 is found in its own cluster, with a Jaccard dissimilarity index of 59% from other four accessions. The 95 kDa corresponded with globulin type legumin a storage protein common in certain leguminous crop seeds (Saminu *et al*., 2021). Bambara globulin contains some small proportion of legumin, which is stabilized by disulfide bonds (Alabi, 2023). At globulin level, the similarity between K1 and M4 accessions is further shown by their similarities at 86%. These two accessions were clustered together in the dendrogram (Figure 58). Although K1 was collected from Kisumu County and M4 collected from Migori County, the two accessions have many genetic similarities. Their original place of origin should be established. This will help establish their suitable agronomic conditions. The globulin peptides also indicated the close relationship between B1 and B4 accessions, with a homology of about 65%, indicating their genetic relatedness. The two accessions had globulin concentrations that were statistically insignificant.

The globulin band 55 kDa which was common to all the accessions corresponded with convicilin subunit (Alabi, 2023). This observation aligns with that of Arise *et al*., (2017) who reported globulin 55 kDa and 62 kDa as major fractions in Bambara storage proteins. In his BGN globulin fraction characterization studies, Alabi, (2023), obtained results nearly similar to the ones found in this study, though he did his protein analysis under non-reducing condition. In this study, globulins have shown indications as good taxonomic markers that can be used to delimit the BGN germplasm which are farmer-held in the LVB. Each of the five accessions studied has a unique globulin protein peptide. The globulins protein markers with molecular weights 26 kDa, 72 kDa, 130 kDa, 180 kDa, 250 kDa are unique to M2, M4, K1 B4 and B1 accessions respectively and can therefore be used taxonomically to delineate the accessions into different classification groups. Minnaar-Ontong, (2021), reported that the genetic diversity observed within groups of BGN can majorly be attributed to their adaptability to various environments.

5.6.4 Protein Banding Pattern and Phenetic Relationship of the Five Accessions

Phenetics and phylogenetic approaches are the main approaches used to delimit species in plant classification. In this study phenetic approach, based on Jaccard's coefficients has been used to access relationship that exists between the five accessions, based on their protein peptides. Seed storage proteins are important genetic markers and are valid components for species or sub-

species identification (Naworu, 2020). Species is the basic unit of classification, from which all other taxons are anchored (Manoko, 2018). All accessions of BGN are currently in one species, creating a taxonomic phenotypic dilemma on wether speciation is in progress or not. Proteins cellular markers have hence been used to unravel this disbute since they are direct products of gene expression (Oludare *et al*., 2017). In this study, the dendrogram (Figure 51) grouping of four accessions B1, B4, K1 and M4 in one major cluster based on their protein peptides reveals low genetic diversity amongst these studied accessions. Hence phenetically, based on overall similarity of their protein peptides, the four accessions are related. According to Peddakasim *et al*. (2015), homogeneity or purity in the accessions may be the cause of the low genetic diversity. Similar observations of low genetic diversity have been shown in the protein profile of Kabuli chickpea (Peddakasim *et al.*, 2015) and in chilli cultivars (Kumar *et al*., 2010). The studied accessions have provided electrophoretic banding pattern that shows some trends that can be used for taxonomic decision. The M2 accession is found in its own cluster (Figure 51). This is non-coicidental as this accession was characterized by having unique protein peptide of 180 kDa, a protein marker that could be used to distinguish it from all the other four accessions. The accession also lacks protein peptide 97 kDa and >250 kDa, which are present in all the other accessions. From the protein peptide profile, the M2 accession seem to be distantly related to the other four accessions at 60% homology. The protein peptide composition, of M2 accession is an indicator that it's slowly diverging from the other accessions, and speciation could be in progress, though more research needs to be done further to ascertain this with clarity. The divergent nature of M2 accession is also observed at finer levels in the globulin banding patterns, where the accession is found in its own cluster in the dendrogram (Figure 58) at about 41% homology with other accessions. According to Stuessy (1990), since all the operational taxonomic units (OTUs) in this study belong to only one species, M2 accession which is the most isolated accession may be regarded as a variety or sub-species. Manoko (2007), used the phenetic approach to delimit the African *Solanum* section solanum.

According to Moss (1979), these two classification approaches are not mutually exclusive. Assessment of similarity is key to the reconstruction of ancestry, likewisely OTUs are classified based on their derived character states which have been acquired in the course of evolution. It can therefore be deduced that the accessions B1, B4, K1 and M4 in one major cluster based on their protein peptides are also phylogenetically related, an observation in concurrence with what

Nisar *et al*., (2007), reported that seed storage proteins are important genetic markers used to establish phylogenetic relationships in accessions.

CHAPTER SIX
CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FUTURE RESEARCH

6.1 Conclusions and Outcomes

6.1.1 Outcome 1: The study revealed significant morphological variation among the selected Bambara landraces, both in qualitative and quantitative traits. Clustering based on qualitative traits grouped accessions by geographic origin, while quantitative traits resulted in a different grouping pattern, highlighting the influence of environmental conditions on morpho-traits. This inconsistency confirms that morphological traits alone are not stable or reliable indicators for classification due to environmental variability. However, these morphological traits are still useful in breeding programs for preliminary grouping and selection. Principal Component Analysis (PCA) indicated that B1 could be the ancestral accession, forming a baseline for genetic improvement. Overall, the morphological data offer a foundational understanding for future taxonomic refinement and breeding decisions.

6.1.2 Outcome 2: Significant yield variations were recorded among the landraces across zones and water treatments. Accessions B4, M2, M4, K1, and B1 showed superior adaptability and productivity based on Rank Summation Index (RSI) and yield indices. Kibos emerged as the most suitable site for BGN cultivation, with M2 achieving the highest yield of 3.3 t/ha. K1 demonstrated strong performance under drought conditions, indicating potential for drought-resilient breeding. Positive correlations were observed between yield and key traits such as number of pods with one seed (N1S), pod number (POD), total leaf area (TLA), number of flowers per plant (NFP), and seed weight (SWT), suggesting that these can serve as reliable selection criteria in yield improvement programs.

6.1.3 Outcome 3: Protein profiling through electrophoretic banding patterns demonstrated clear biochemical distinctions among accessions. Unique protein markers (e.g., 180 kDa in M2) and at finer levels globulin markers (e.g., 26 kDa in M2, 72 kDa in M4, 130 kDa in K1, 180 kDa in B4, 250 kDa in B1) enabled differentiation and classification of landraces, supporting their utility as stable taxonomic tools. M2 stood out as the most divergent accession in protein composition, showing evidence of potential speciation, as indicated by its unique clustering in both protein and globulin dendrograms. Kibos-grown M2 also recorded the highest total protein content,

further confirming its superior agronomic and nutritional value. These findings underscore the importance of using molecular protein markers as taxonomic tools in Bambara classification, as well as integrating them into selection and breeding strategies for high-protein, adaptable cultivars.

Thus, the findings of this study led to the rejection of the null hypotheses and acceptance of the alternative hypotheses for all the stated objectives.

6.2 Recommendations

Based on the findings of this study, it is recommended that:

6.2.1 Plant taxonomists should note that morphological traits in Bambara groundnut landraces exhibited variability across environments and were inconsistent between qualitative and quantitative assessments. This suggests environmental influence and limited reliability of morphology alone for taxonomic resolution. Taxonomists are advised to conduct multi-location morphological evaluations and use environmentally robust descriptors to improve accuracy in varietal identification and classification efforts.

6.2.2 Farmers should adopt high-performing accessions**,** where they prioritize growing accessions B4, M2, M4, K1, and B1 depending on their local agro-ecological zone. These have shown the highest adaptability and yield. They should choose location-specific accessions, for instance, M2 is best suited for Kibos, B4 for Alupe, and M4 for Oyani. This will help maximize yields based on local climate and soil conditions.

6.2.3 Agronomists should develop zone-specific cultivation protocols, where they use Rank Summation Index (RSI) and yield index data to formulate best practices for each accession in Alupe, Kibos, Oyani, and under drought/irrigated conditions. They should conduct further trials to validate findings across more seasons and micro-zones within the Lake Victoria Basin (LVB) to fine-tune recommendations for extension services.

Further, they should promote soil and water management, especially in Kibos, where growth conditions support high protein and yield performance of Bambara. They should also collaborate with breeders' inorder to provide field-based feedback on agronomic performance and farmer preferences to inform breeding decisions.

6.2.4 Taxonomists should incorporate molecular markers in classification. They should utilize stable and specific protein markers (e.g., globulin and albumin sub-units) to refine the Taxonomy of Bambara landraces. Taxonomists should also use these findings to monitor speciation trends. The distinctiveness of M2 suggests active divergence. They should study this further for evolutionary and conservation insights.

6.2.5 Crop Breeders should use protein markers in selection, hence should integrate stable protein markers (e.g., 180 kDa in M2, 250 kDa in B1) into breeding programs for efficient selection and trait stability. They should prioritize M2 for future cultivars. Given its divergence and high yield/protein, M2 may represent emerging speciation and offers excellent breeding potential.

The crop breeders should also target wide adaptation genotypes. They should develop cultivars adaptable across zones but especially optimize for Kibos, the most productive site. They should introduce improved cultivars**,** hence use this study as a foundation to release the first improved BGN cultivars in Kenya, addressing the current lack of such varieties.

6.3 Suggestions for Future Research

The outlined suggestions for future research are typically derived from the limitations encountered, gaps identified, and new insights which emerged during the course of the study. These suggestions for future research aim to guide subsequent investigations toward deepening understanding, enhancing methodologies, or exploring related areas that remain insufficiently addressed.

6.3.1 Investigation of Speciation in M2

Carry out whole-genome or transcriptome analysis of M2 to verify signs of speciation and identify the genetic basis for its morphological divergence.

6.3.2 Origin and Genetic Background of B1

Analyze the genetic origin of B1 to understand its evolutionary position and the environmental conditions favoring its protein profile and performance. This can be achieved through phylogeographic studies and ancestral population structure analysis.

6.3.3 Drought Tolerance Mechanisms in K1

Investigate physiological and molecular mechanisms responsible for K1's superior drought tolerance to support development of climate-resilient cultivars. Its agronomic performance in the droughted environment was the highest, but produced the least yields amongst the irrigated plants, in Alupe and Kibos zones where water was optimal for the growth of Bambara.

6.3.4 Yield-Related Trait improvement

Focus on improving key traits positively correlated with yield components using heritability studies and Quantitative Trait Loci (QTL) mapping.

6.3.5 Refined Protein Separation Techniques

Apply two-dimensional electrophoresis (2-DE) to accessions with overlapping banding patterns (e.g., B1 vs. B4) for finer protein resolution and explore alternative protocols or buffers to successfully resolve glutelins and prolamins which failed to form distinct bands in SDS-PAGE, perhaps by adjusting pH or ionic strength of extraction solutions.

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APPENDICES

Appendix 1: Soil sample collection at Alupe KALRO research farms, Busia County.



Appendix 2: Randomization table

8-17-2 SPRAY SHEETS

Page 1

LSU Rice Research Station, Crowley, LA

Morphological Characterization, Yield Determination and Proteomic Analysis of Selected Bambara Groundnut Landraces in Lake Victoria Basin, Kenya

Project Code: SELINE By Richard Dunand

Location ALUPE, KIBOS AND OYANI Co-operator: PROF. J.C. ONYANGO

6 BGA

All rates are specified as

Trt No	Treatment Name		Rate	Growth Stg.	ml Trt	or g/ mix	Plot	No.	By	Rep	
							1	2	3		
1	K1						104	201	304		
2	K2						101	206	306		
3	M2						106	204	301		
4	M4						105	202	303		
5	B1						103	203	305		
6	B4						102	205	302		