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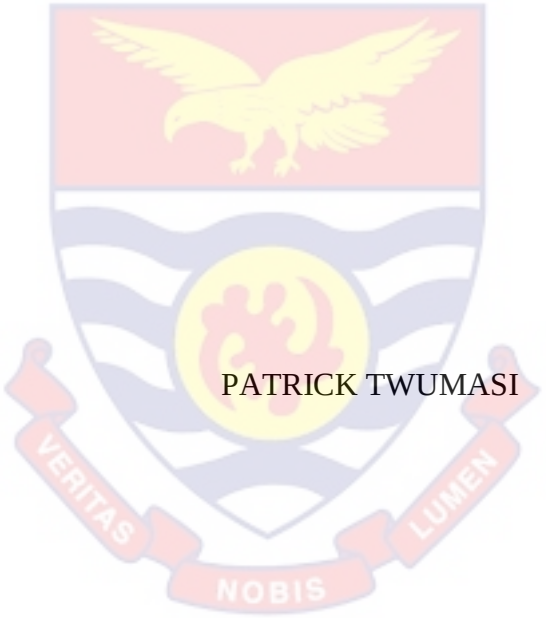
CHARACTERISATION AND WIDENING OF THE GENETIC BASE OF TIGER NUT (*Cyperus esculentus* L.) BY CHEMICAL MUTAGENESIS



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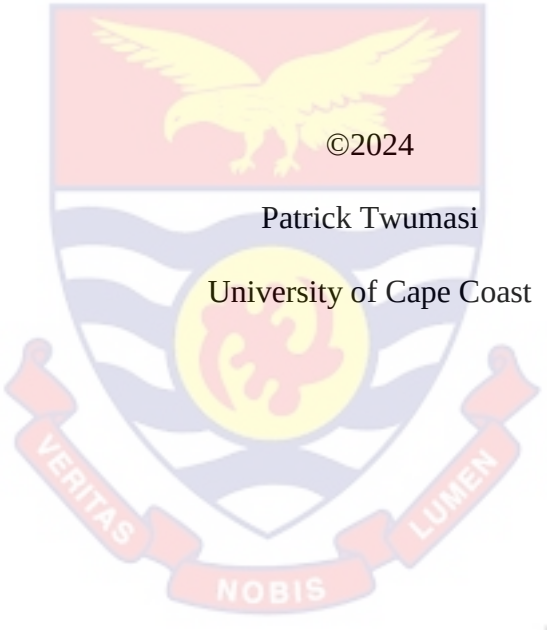

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CHARACTERISATION AND WIDENING OF THE GENETIC BASE OF TIGER NUT (*Cyperus esculentus* L.) BY CHEMICAL MUTAGENESIS

BY

PATRICK TWUMASI

Thesis submitted to the Department of Crop Science of the School of Agriculture, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirement for the award of Doctor of Philosophy degree in Crop Science (Plant Breeding)

June, 2024

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature ..... Date 02-06-2024

Name: Patrick Twumasi

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature ..... Date 06/06/2024

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2nd Co-Supervisor's Signature ..... Date: June 3, 2024

Name: Dr. Godwin Amenorpe

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ABSTRACT

Limited information exists regarding the diversity of tiger nuts (*Cyperus esculentus* L.) in Ghana, hindering potential improvements. To address this gap, an assessment of genetic diversity was conducted using 11 morpho-descriptors and nine simple sequence repeat (SSR) markers to explore variability among and within the accessions to provide valuable insights for further breeding through mutagenesis. A total of 42 accessions were collected from major tiger nut growing areas in Ghana and these were planted in the field using a randomized complete block design (RCBD) with 3 replications. The results from the field characterization showed significant differences among the accessions for some traits studied. The use of SSR markers demonstrated high mean polymorphism of 0.78.

UPGMA cluster analyses of both morphological and molecular data indicated low genetic variability within the accessions. Four high-yielding accessions were identified for potential improvement through mutagenesis. Subsequently, 400 tubers of the four genotypes (two brown and two black tuber-producing) underwent treatment with ethyl methanesulfonate (EMS) and colchicine at various concentrations. Analysis of quantitative data revealed LD_{50} and RD_{50} values for mass treatmeant of tiger nut tubers with the mutagens.

Subsequently, 600 tubers of the four selected genotypes were mass-treated with EMS and colchicine mutagens following the LD_{50} and RD_{50} values determined, and were field-planted for M1V1 to M1V4 generations. Twenty-one mutants have beeen identified for breeding enhancement and possible varietal release.

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KEY WORDS

Tiger nut

Accessions

Colchicine

Ethyl methanesulfonate (EMS)

Genetic diversity

Genotype

LD50

Morphological

Molecular

Morpho-descriptors

Mutagens

Mutation

RD50

Simple sequence repeat markers

Variability

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ACKNOWLEDGEMENTS

In His time, He makes all things beautiful. I am most grateful to all mighty God for His mercies and strength bestowed on me for the success of this work. His faithfulness endures forever.

I remain very appreciative and thankful to my suppervisors, Prof. Paul Agu Asare, Dr. Emmanuel Afutu, and Dr. Godwin Amenorpe for their professeional guidiance, hospitality, and unflintching support offered me for every bit of this work.

I am also indebted to Dr. Sylvester Nii T. T. Addy of the Council for Scientific and Industrial Rsearch- Crop Research Institute (CSIR-CRI), Fumesua for his imense contributions in advice, material and technical support. Special thanks again go to the team of the Department of Legumes of CSIR-CRI, Fumesua especially Patrick Boakye, Mark Kwabia, Paul Manu, and Franklin Bosompem in their effort contributed in the field works and data collection. The team of the Biochemistry Department most importantly Dr. Ruth Prempeh and Mr. Francis Amoako are not left out. Without them, the molecuclar evaluation would not have been completed. I also extend my gratitude to Mr. Clifford Asiedu for his contribution to this work on the mutagenesis aspect.

I would like to thank all lecturers and the working Staff of the School of Agriculture (UCC), for their interest, support, and constructive critisisms throughout this study.

My last and most profound gratitude goes to my wife, Gifty Osei Bonsu and my children (Malvin, Keren, Alvin and Andrew) for their prayers, financial support, and encouragement in difficult times of the study. God bless you all.

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DEDICATION

This work is dedicated to my wife, Gifty Osei Bonsu, and my children, Malvin Dompreh Twumasi, Keren Antwiwaa Twumasi, Alvin Obeng Twumasi, and Andrew Osei Twumasi.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
CAPS	Cleaved amplified polymorphic sequence
CSIR, CRI	Council for Scientific and Industrial Research, Crop Research Institute
CTAB	Cetyltrimethylammonium bromide
EDTA	Ethylenediamine tetraacetic acid
EMS	Ethyl methanesulfonate
F1	First Filia generation
FAO	Food and Agriculture Organaization
He	Eexpected heterozygosity
Ho	Observed heterozygosity
ISSR	Inter Simple Sequence Repeat
LD50	Lethal dose killing 50 % of the population of target organism
M1V1, M1V2, M1V3, M1V4	Mutant generations one, two, three, and four.
M1V1	Mutation one generation and population one
MAS	Marker Assisted Selection
MDS	Multidimesional Scaling

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MNU	N-methyl N-nitro-N-ntrose guanidinine
Na	Allele frequency
Ne	Allele number
NFSW	Nuclease free sterile water
NTSYS	Numerical taxonomy system software
PAST	Paleontological Statistics
PC	Principal component
PCA	Principal component analysis
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PIC	Polymorphic information content
QTLs	Quantitative trait loci
RAPD	Random amplified polymorphism
RCBD	Randomized complete block design
RD50	Reduction dose reducing 50 % plant growth characteristics
RFLP	Restriction fragment length polymorphism
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
UPGMA	Unweighted pair group method with arithmatic average

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USA	United States of America
USD	United States Dollar
UV	Ultraviolet

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LIST OF PUBLICATIONS MADE FROM THIS WORK

1. Genetic diversity of tiger nuts (*Cyperus esculentus* L.) grown in Ghana based on morpho-descriptors and SSR markers.
2. Chemosensitivity analysis of tiger nuts (*Cyperus esculentus* L.) using ethyl methanesulfonate (EMS) and colchicine mutagens.

The Journal of Agricultural Sciences - Sri Lanka
Vol. 19, No 2, May 2024. Pp 316-335
https://doi.org/10.4038/jas.v19i2.10091

Scopus

Genetic Diversity of Tiger Nuts (*Cyperus esculentus* L.) Grown in Ghana Based on Morpho-Descriptors and SSR Markers

P. Twumasi[1*], P. A. Asare[1], E. Afutu[1], G. Amenorpe[2], S. N. T. T. Addy[3], R. Prempeh[3], and E. F. Donkor[4]

Received: 29th December 2022 / Accepted: 14th March 2024

ABSTRACT

Purpose: *Limited information is available on the diversity of tiger nuts in Ghana for improvement. Therefore, evaluating the genetic diversity via the use of morphological descriptors and molecular markers (SSR) should generate substantial evidence to reveal the variability among and within the crop.*

Research Method: *Forty-two (42) accessions of Cyperus esculentus Lativum were collected from major tiger nut cultivation areas (phytogeographic zones) in Ghana and evaluated using 11 morpho-descriptors and 9 SSR markers to assess variability among and within the tiger nut accessions and underlined causes.*

Findings: *Significant differences were observed among the accessions in terms of percentage inflorescence ($p \leq 0.01$), distance of the last tiller from the main plant ($p \leq 0.05$) and the number of tubers per stand ($p \leq 0.05$). Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis grouped the accessions into seven clusters including one large heterogenous cluster at 0.94 Euclidean distance, and genetic distance ranged from 0.8 to 1.00. The SSR profiling revealed 141 alleles across 41 of the accessions used for the molecular study with a mean PIC value of 0.78 ± 0.15 and mean observed heterozygosity of 0.12 ± 0.16 coupled with four heterotic UPGMA cluster groupings including one large heterogenous cluster at a similarity coefficient of 0.88. The high variability among the accessions for some of the traits, and the low genetic variability within the accessions in cluster groupings for both morphological and SSR evaluations suggested some level of admixture of genes over years of cultivation.*

Originality/ Value: *The current study has identified some accessions for improvement. Also, it has contributed to knowing some SSR markers that would amplify tiger nuts for future molecular study for breeding enhancement.*

Keywords: *Accession, Cyperus esculentus, Genetic diversity, Morpho-descriptors, PIC, SSR markers*

INTRODUCTION

Tiger nut (*Cyperus esculentus* L.) is a perennial weed in the sedge family (*Cyperaceae*). The crop is known to many as chufa, nutgrass, earth almond, water grass, rush nut, yellow nutsedge and northern nutgrass (Shilenko *et al.*, 1979). The origin of *Cyperus esculentus* is generally uncertain.

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Heliyon 9 (2023) e22619

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Chemosensitivity analysis of tiger nuts (*Cyperus esculentus* L.) using ethyl methanesulfonate (EMS) and colchicine mutagens

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ARTICLE INFO

Keywords:
Genotype
EMS
Colchicine
LD_{50}
RD_{50}
Concentration levels
Plant architecture
Mutagens

ABSTRACT

Four hundred tubers of four genotypes, two brown and two black tiger nuts were subjected to Ethyl Methanesulfonate (EMS) and Colchicine treatments at concentrations of 0 %, 0.1 %, 0.25 %, 0.5 % and 1.0 % for 24 h. Each genotype had twenty tubers treated with each of the five different concentrations and were planted using Complete Randomized Design (CRD) in a greenhouse. Quantitative data was collected and LD_{50} and RD_{50} were analysed using Excell 2016 and Genstat 11.2. A general decreasing trend in percentage germination and plant height was observed with increasing concentrations of mutagens applied. An EMS treatment had LD_{50} and RD_{50} values of 0.97 % and 1.49 % for black and 0.63 % and 1.63 % for brown genotypes.

Similarly, the percentage colchicine treatment had LD_{50} and RD_{50} values of 1.65 % and 19.51 % concentrations for black and 0.91 % and 1.71 % concentrations for brown genotypes.

1. Introduction

Tiger nut (*Cyperus esculentus* L.), commonly called 'chufa', 'atadwe', nut grass, earth almond, water grass, rush nut, yellow nut sedge and northern nut grass [1,2], is a root tuber crop belonging to the sedge family. The origin of the crop to date is uncertain. While some believe that it is a native of Africa and tropical Asia [3–5] others are of the view that it originated from Europe and North America [6,7].

The tuber (nut as affectionately called) is a source of feed, food, medicine and perfumes [8,9]. It can be eaten raw, roasted, dried, baked or made into a refreshing beverage called Horchata De Chuf (in Spain), 'kunnu aya' (in northern Nigeria) and 'atadwe milk' (in Ghana).

The tuber is highly valued for its protein (7–8%) [10], fibre (8–10 %) [11], vitamins (C and E), and rich minerals (Sodium, Calcium, Potassium Magnesium, Zinc and traces of Copper) [12]. It contains almost all the functional nutrient components for a balanced diet. Approximately, the tuber contains 26–30 % starch and 21–25 % fat, providing about 400–450 kcal 100 g^{-1} energy [13].

Medically, by potency, the crop is an aphrodisiac, has carminative and diuretic effects, and is used as a stimulant and tonic. This moderates the incidence of colon cancer, coronary heart diseases, obesity, diabetes, excessive thirst, gastrointestinal disorders [13–15] constipation, high blood pressure, and diarrhoea [16]. Economically, the crop provides a source of foreign exchange for its high export

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https://doi.org/10.1016/j.heliyon.2023.e22619
Received 7 March 2023; Received in revised form 10 November 2023; Accepted 15 November 2023
Available online 19 November 2023
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CHAPTER ONE

INTRODUCTION

Background of the Study

Tiger nut (*Cyperus esculentus* L.) is a valuable subterranean crop renowned for its exceptional nutrition, belonging to the perennial sedge family. Despite being relatively underutilized in Ghana; its popularity is gradually on the rise due to its numerous advantages. Tiger nuts are known by various names such as 'chufa', 'atadwe', nutgrass, earth almond, water grass, rush nut, yellow nutsedge, and northern nutgrass (Shilenko *et al*., 1979; Bazine & Arslanoğlu, 2020). The origin of *Cyperus esculentus* is somewhat uncertain. While some propose that it is indigenous to Africa and tropical Asia (Acevedo-Rodríguez & Strong, 2012; DAISIE, 2014), others suggest its presence in Europe and North America (Govaerts, 2014; USDA-ARS, 2014), followed by its global dispersal (Holm *et al*., 1977).

In Ghana and other African nations like Nigeria and Sierra Leone, tiger nuts are available in fresh, semi-dried, and dried forms in local markets. These forms are consumed without cooking. *Cyperus esculentus* is primarily cultivated for both its dietary and feed value.

This robust plant, characterized by erect growth from perennial tuber-bearing rhizomes, produces fibrous roots and reaches heights of 30-90 cm. It generates seeds and extends runners above the ground through slender rhizomes (Walia, 2014). The underground stems give rise to tubers, which come in black, brown, and yellow hues, holding significant economic importance. These tubers (nuts) serve as sources of nourishment, sustenance, medicinal applications, and

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fragrances (De Vries, 1991; Gambo & Da'u, 2014). They can be consumed dried, raw, roasted, baked, or transformed into refreshing beverages like '*Horchata De Chufa*' (in Spain), '*kunnu aya*' (in northern Nigeria), and '*atadwe milk*' (in Ghana) (Ankomah, 2022; Twumasi *et al*., 2023).

Rich in nutritional content, the tiger nut tuber has abundant starch, dietary fiber, digestible carbohydrates (monosaccharides, disaccharides, and polysaccharides), protein (7–8%), fiber (8–10%), vitamins (C and E), and essential minerals (sodium, calcium, potassium, magnesium, zinc, and traces of copper) (Sanful, 2009). These components make it a nearly complete nutritional package. Approximately, the tuber contains 26–30% starch and 21–25% fat, contributing around 400–450 kcal per 100 g (Sánchez- Zapata *et al*., 2012).

Medicinally, the tiger nut tuber functions as an aphrodisiac, a carminative, a diuretic, a stimulant, and a tonic (Bazine & Arslanoğlu, 2020; Kouame *et al*., 2022). It contributes to the prevention of conditions such as colon cancer, coronary heart disease, obesity, diabetes, excessive thirst, and gastrointestinal disorders (Chukwuma *et al*., 2010; Sánchez- Zapata *et al*., 2012; Adebayo-Oyetoro *et al*., 2017). Furthermore, it addresses issues like constipation, high blood pressure, and diarrhea (Oladele & Aina, 2007).

Despite being an underutilized and relatively unexplored crop, tiger nuts have substantial export potential (Tetteh & Ofori, 1998). Thus, proper investment in its production could lead to significant foreign exchange earnings for Ghana. Ghana predominantly ships a significant portion of its tiger nuts to the United States, United Kingdom, and the Netherlands, and is the second-largest global exporter of the crop according to the Ghana Export Trade Data (GETD) (GETD,

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2023). The export statistics reveal a consistent upward trend in both the volume and value of tiger nuts over the past two years, with Ghana exporting 1,800 tons valued at US$2,200,000 in 2022, and 2,100 tons valued at US$2,600,000 in 2023 (Tridge, 2023). These figures underscore the promising potential for profitable investments in the production of tiger nuts in Ghana, as the demand for this product continues to grow in key international markets.

Tiger nut cultivation in Ghana is concentrated in select regions, such as Northern, Bono Ahafo, Bono East, Eastern, Central, and Volta, where it provides approximately 85% of jobs for the youth and women (Tetteh & Ofori, 1998; Donkor *et al*., 2019).

Notwithstanding its nutritional, medicinal, and economic advantages, tiger nut remains relatively neglected (Donkor *et al*., 2019), partly due to its invasive and troublesome weed characteristics. Additionally, limited scientific and technological studies have been conducted to fully exploit its potential.

Unlike other crops such as maize and cowpea, tiger nut cultivation has received limited attention for agronomic and breeding improvements. Few studies, especially in Ghana, have explored its production. In the Kwahu South District of Ghana, Tetteh and Ofori (1998) carried out a baseline survey to investigate tiger nut production. In a another study, Donkor *et al*. (2019) focused on characterizing local tiger nut accessions for the purpose of conservation and utilization. Additionally, Asare *et al*. (2020) conducted research on the phenotypic traits of tiger nuts from various significant growing regions in Ghana.

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The findings from Donkor *et al*. (2019) demonstrated extensive diversity among 24 genotypes for agronomic and yield-related traits. In contrast, Asare *et al*. (2020) indicated limited genetic variability among 64 genotypes for yield and phenotypic traits, suggesting that farmer-held accessions (landraces) remain unimproved. These studies have predominantly focused on morphological or phenotypic aspects, indicating the need for molecular profiling to validate and enhance breeding efforts (Akabassi *et al*., 2021). Presently, there is no germplasm repository or varietal release for tiger nuts in Ghana, leaving local accessions vulnerable to extinction over time.

Effective germplasm collection, characterization, and preservation are vital for crop improvement, particularly for underutilized ones like tiger nuts (Ahmed *et al*., 2016). Landraces, in particular, offer significant genetic reservoirs, hosting traits like stress resistance, disease resistance, and beneficial agronomic characteristics. Preserving landraces is crucial to developing modern varieties suited to changing environments. Hence, concerted efforts should be made to collect and conserve landraces for future breeding endeavours.

To harness genetic diversity effectively, understanding the genetic variations and relationships within and between germplasms is essential. This characterization aids in assessing breeding potential and promoting the optimal use of available genotypes (Sikdar *et al*., 2010; Asare *et al*., 2020). Employing a combination of morphological trait evaluation and molecular genotyping is necessary to uncover genetic variability among tiger nut accessions.

Morphological characterization relies on observable and heritable descriptors. Although straightforward and cost-effective, it has limitations, including time

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and labor intensiveness, and susceptibility to environmental influences. In contrast, molecular markers like simple sequence repeats (SSR) , amplified fragment length polymorphisms (AFLP) , restriction fragment length polymorphisms (RFLP) , and single nucleotide polymorphisms (SNP) have proven robust in distinguishing accessions (Kumar *et al*., 2009). SSRs, in particular, are widely used for diversity studies in crops due to their high polymorphism, repeatability, and cost-effectiveness (Guler & Imamoglu, 2023; Kaur *et al*., 2015).

Enhancing on yield and other traits for variability within or among accessions such as landraces of which local tiger nut germplasms are not of exception, necessitates mutation breeding for a worthy genetic diversity. Induced (artificial) mutation breeding involves using physical and chemical mutagens to create genetic variation for selection and breeding (Novak & Brunner, 1992). These mutations offer unique germplasm for plant breeders, expanding the genetic pool beyond what is naturally available. Induced mutagenesis, both through physical means like gamma rays, X-rays, UV light, and particle radiation, and through chemical agents such as ethyl methanesulfonate (EMS) or base analogues, have been utilized since the early 20th century to introduce beneficial variations (van Harten, 1998). Gamma irradiation, a widely employed physical mutagen, has proven effective in inducing genetic diversity in numerous crops (Horn & Shimelis, 2013).

Chemical mutagens offer advantages like higher mutation rates, specificity, and ease of application. Ethyl methanesulfonate (EMS) is a frequently used chemical mutagen, known for its efficiency and selectivity in creating mutations (van Harten, 1998; Girija & Dhanavel, 2009; Mba *et al*., 2010). Chemical

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mutagenesis is a cost-effective and practical method, with fewer potential damaging effects compared to radiation-based mutagenesis (Poehlman, 1987).

The introduction of mutations via mutagenesis plays a pivotal role in crop breeding, offering traits that may not naturally occur or that have been lost over time (Novak & Brunner, 1992). These induced mutations serve as a valuable source of genetic novelty, paving the way for improved varieties.

Tiger nuts are an under-appreciated yet promising crop with remarkable nutritional, medinal, and economic potential. Despite its benefits, the crop remains relatively neglected and lacks in-depth research and development efforts. Utilizing both morphological characterization and molecular genotyping can unveil the extent of genetic variability and relationships within tiger nut germplasms. Mutation breeding, facilitated through induced mutagenesis, presents an avenue to enhance and diversify the genetic pool of tiger nuts, leading to the development of improved varieties with desirable traits. This approach could contribute significantly to maximizing the crop's potential, addressing food security, and promoting sustainable agriculture in Ghana and beyond.

Statement of the Problem

The cultivation of tiger nuts has been marginalized, resulting in the crop being largely understudied and underutilized in Africa, particularly, in Ghana. Despite being rich in nutritional, medicinal, and economic benefits, little attention has been dedicated to its breeding efforts. This lack of investment has led to a limited genetic foundation, impeding its potential for genetic progress. The current trajectory raises concern about the preservation of local germplasm

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under the care of farmers, as the absence of germplasm resources and varietal releases contributes to their vulnerability over time.

In contrast to other crops such as maize, rice, and cassava, tiger nuts have received minimal attention in terms of agronomic and breeding research in Ghana. Existing studies focused primarily on morphological or phenotypic characterization, and a survey for its production even in only one district, Kwahu South District conducted in 1998 by Tetteh and Ofori. Moreover, the size of the tubers in tiger nuts is notably small, further underscoring the need for decisive action. Again, the conventional approach of hybridizing tiger nuts presents unique challenges compared to other sexually propagated crops, primarily due to their restricted genetic variability, infrequent flowering behaviour, and dependence on tubers for reproduction (Ubi & Brisibe, 2021).

To address these challenges, a proactive approach is required. There is a pressing necessity to undertake mutation breeding to enhance the genetic diversity of tiger nut genotypes, specifically aiming to achieve larger nut sizes. This endeavor is vital for bolstering production and facilitating improvement, thereby fostering a more robust and sustainable tiger nut industry.

Research Objectives

Main Objective

The main objective was to creat genetic diversity that would contribute to the development and release of improved tiger nut for high yield and nutritional content using chemical mutagenesis.

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Specific Objectives

1. To assess the diversity among tiger nut accessions using yield and morpho descriptors.
2. To determine the genetic diversity among tiger nuts using SSR markers.
3. To establish the LD_{50} and RD_{50} values for Ethyl methanesulfonate (EMS) and colchicine among tiger nut.
4. To select potential EMS and colchicine mutants with favorable traits for the tiger nut improvement.

Hypothesis

The following hypotheses were tested:

1. There is no significant difference in genetic diversity among tiger nut accessions based on yield and morphological descriptors.
2. There is no significant genetic variability and diversity among tiger nuts as revealed by SSR profiling.
3. There is no significant difference in the LD_{50} and RD_{50} values for Ethyl methanesulfonate (EMS) and colchicine-inducing genetic diversity among tiger nut tubers.
4. There is no significant difference (in yield, nut size, maturity, tuber integrity, and nutritional content) between the mutants and the original tiger nut accessions.

Significance of the Study

Tiger nut accessions cultivated in Ghana consist of landraces without any established varietal releases or germplasm repositories. To facilitate research

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aimed at enhancing breeding improvements for these underutilized accessions, particularly within the Ghanaian context, it is imperative to actively collect, characterize, and induce mutations in these accessions to widen the genetic base. Conducting a thorough characterization study employing a blend of morphological descriptors and molecular genotyping has proven essential in uncovering and confirming the true extent of genetic variation and diversity present within the studied crop. This knowledge serves as a foundation for subsequent genetic enhancements.

Presently, there exists a notable gap in terms of both information and practical application on molecular studies and mutagenesis within the realm of tiger nut breeding. This stands in contrast to other crops like rice, maize, cowpea, and cassava, wherein these methodologies have led to the development of multiple varieties, substantially ameliorating the economic attributes of these crops.

The utilization of mutagenesis has emerged as a potent tool, particularly when coupled with advanced molecular biology techniques like molecular genotyping. This synergy holds immense potential for elevating the process of crop breeding, a prospect that holds even greater significance in the contemporary era marked by the looming challenge of global climate change (Jain, 2010).

Delimitation

This study examined the tiger nut accessions held by farmers in the major cultivation regions of Ghana, specifically the Northern, Upper East, Upper West, Bono East, Eastern, and Central regions, while excluding the Volta region. The Volta region was not covered due to the unavailability of planting

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materials in the communities visited during the collection period, which coincided with the off-planting season.

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CHAPTER TWO

LITERATURE REVIEW

Origin and Distribution

There are four distinct wild varieties of tiger nuts, namely *Cyperus esculentus var. esculentus*, *var. heermannii* (Buckley) Britton, *var. leptostachyus* Boeckeler, and *var. macrostachyus* Boeckeler, originating from different geographic regions. *Cyperus esculentus var. esculentus* is indigenous to the southern Europe, Africa, and Asia (Schippers *et al*., 1995). *Cyperus esculentus var. heermannii* is limited to the southern USA but has also extended to the Netherlands since 1970 (Ter Borg & Schippers, 1992). *Cyperus esculentus var. leptostachyus* is found in both northern and southern America, with records in Europe since 1947 (Ter Borg & Schippers, 1992). *Cyperus esculentus var. macrostachys* is prevalent across Central America to the southern USA (Schippers *et al*., 1995).

The origins of the cultivated variant, *Cyperus esculentus var. sativus* Boeckeler, remain uncertain. Some suggest it originates from Africa and tropical Asia—while others propose tropical and subtropical regions spanning Africa, Asia, North America, and Europe (Acevedo-Rodríguez & Strong, 2012; Govaerts, 2014). Historically, it is believed to have been cultivated for its tubers since Ancient Egypt, ranking as the third-oldest domesticated food crop (Defelice, 2002).

Tiger nut cultivation has gained global presence due to dispersal during the Arabian expansion (Holm *et al*., 1977). It thrives across continents, notably in Europe, the Americas, Asia, and Africa (Udeozor & Awonorin, 2014; Maduka

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& Ire, 2018), including African nations like Nigeria, Burkina Faso, Cote d'Ivoire, and Ghana (Pascual *et al*., 2000). Tiger nuts have become sought-after for their nutritional and economic values, since they are used in various ways including preparation of snacks and beverages like 'Horchata De Chufa' and 'atadwe milk' (Sánchez- Zapata *et al*., 2012).

The adaptability of tiger nuts to diverse environments and their potential for sustainable cropping systems make them valuable in the face of climate change (Fahmy *et al*., 2014). Collaborative efforts can unlock their potential for food security and economic growth (Ezeh *et al*., 2014).

In in a nutshell, tiger nuts have evolved from historical origins to global cultivation, contributing to agriculture and nutrition. Embracing their genetic diversity, coupled with advancements in breeding and biotechnology, holds promise for enhancing their potential amid current challenges (Rebezov *et al*., 2021). The resilient and versatile tiger nut has a vital role to play in a more nourished and resilient future.

Production and Economic Importance of Tiger Nut

Tiger nut, a lesser-known and minimally researched cash crop, boasts a global presence, flourishing in both temperate and tropical regions. Its adaptability allows it to thrive in diverse soil and climatic conditions, contributing to its widespread cultivation across continents. The crop's economic significance resides primarily in its tubers, which are a storehouse of nutritional riches, including protein, carbohydrates, fiber, fatty acids, amino acids, minerals, and vitamins (Alogo & Ogbogo, 2007; Oladele & Aina, 2007).

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Beyond its nutritional value, tiger nut tubers possess medicinal attributes, offering relief from ailments like indigestion, dysentery, diarrhea, bloating, and excessive thirst (Chevalier, 1996). Moreover, these tubers exhibit aphrodisiac properties, function as stimulants, and act as tonics, contributing to the mitigation of conditions such as colon cancer, coronary heart diseases, obesity, and diabetes (Chukwuma *et al*., 2010; Sánchez- Zapata *et al*., 2012).

The nutritional potency of these tubers renders them not only a source of sustenance for humans and fodder for animals but also a means of generating foreign exchange through export, thereby bolstering a nation's economy.

Tiger Nuts Production in the World

The cultivation of tiger nuts spans across the entirety of the globe, with significant production occurring in various regions. Notably, tiger nut cultivation is prominent in Asia, East Africa, the Arabian Peninsula, and certain parts of Europe, particularly Spain (AbdelKader *et al*., 2017). The focal points of tiger nut production encompass the Mediterranean region, parts of Africa, India, North America, Mexico, Peru, and other locales (Rebezov *et al*., 2021). These distribution areas encompass regions such as Africa, (including, Nigeria, Ghana, Benin and Madagascar), Southern Europe, the Middle East and the Indian subcontinent (Sánchez- Zapata *et al*., 2012; Ezeh *et al*., 2014; Bazine & Arslanoğlu, 2020).

Tiger Nuts Production in Africa

The historical cultivation of tiger nuts traces back to North Africa, notably in Egypt, where it has been practiced since around 5000 BC (Allouh *et al*., 2015; Oyedele *et al*., 2015). Over time, this agricultural practice expanded its reach to

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various corners of the globe. Presently, tiger nut cultivation is well-established in countries such as Ghana, Nigeria, Benin, Cameroon, Niger, and Sierra Leone (Sánchez- Zapata *et al*., 2012) primarily to utilize its tubers for both domestic consumption and export purposes.

The tiger nut crop exhibits three distinct colour variations in its tubers: black, brown, and yellow. These colour types are present in the countries engaged in production. However, the specific types that are predominantly produced and marketed often depend on the preferences of the local consumers. For instance, in Nigeria, both the brown and yellow tuber types are readily available in the market year-round. Conversely, in Ghana, the primary focus lies on the production and marketing of brown and black tuber varieties (Asante *et al*., 2014). This nuanced approach to cultivation reflects the diverse consumer demands and preferences across different regions.

Tiger Nuts Production in Ghana

Tiger nut cultivation in Ghana has taken on a commercial aspect, albeit limited to certain regions. The scale of commercial cultivation remains relatively small, typically ranging from 0.06 to 0.4 hectares. This is influenced by the labor-intensive nature of tiger nut farming and its high yield potential, which can reach up to 11 tons per hectare (Tetteh & Ofori, 1998). Notably, the cultivation landscape is predominantly managed by women, as men have shifted their focus towards the production of crops like maize, rice, yam, and cassava.

While tiger nuts thrive well as a monocrop, it is a common practice in Ghana to intercrop them with cassava, cocoyam, or maize (Tetteh & Ofori, 1998). Agricultural practices, including weeding and irrigation, are undertaken as

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needed. Fertilization is sparingly employed due to consumer preferences; fertilized tubers are often rejected due to altered taste. In less fertile soils, occasional fertilizer application occurs (Atawodi *et al*., 2017; Ezeh *et al*., 2014). Harvesting takes place at maturity, usually around 2.5 to 3 months after planting, contingent on soil fertility and moisture levels. The harvested tubers are categorized into seed nuts for future planting and ware nuts for consumption (Tetteh & Ofori, 1998). Both types undergo thorough drying for preservation. Ware nuts undergo meticulous washing prior to drying for either sale or storage when a market is not immediately available.

Farmers typically sell their produce, the tubers, to intermediaries who further distribute them within the supply chain, encompassing retailers, processing companies, and ultimately, consumers. The demand for tiger nuts is robust both domestically and internationally, contributing to its relatively high market value (Tetteh & Ofori, 1998). Ghana's tiger nut exports reach various countries, including the Netherlands, Germany, United Arab Emirates, Austria, Canada, United Kingdom, United States, and France. Table 2.1 provides insight into the trends of tiger nut exports from Ghana between 2014 and 2021, detailing quantities and corresponding monetary values (Tridge, 2022).

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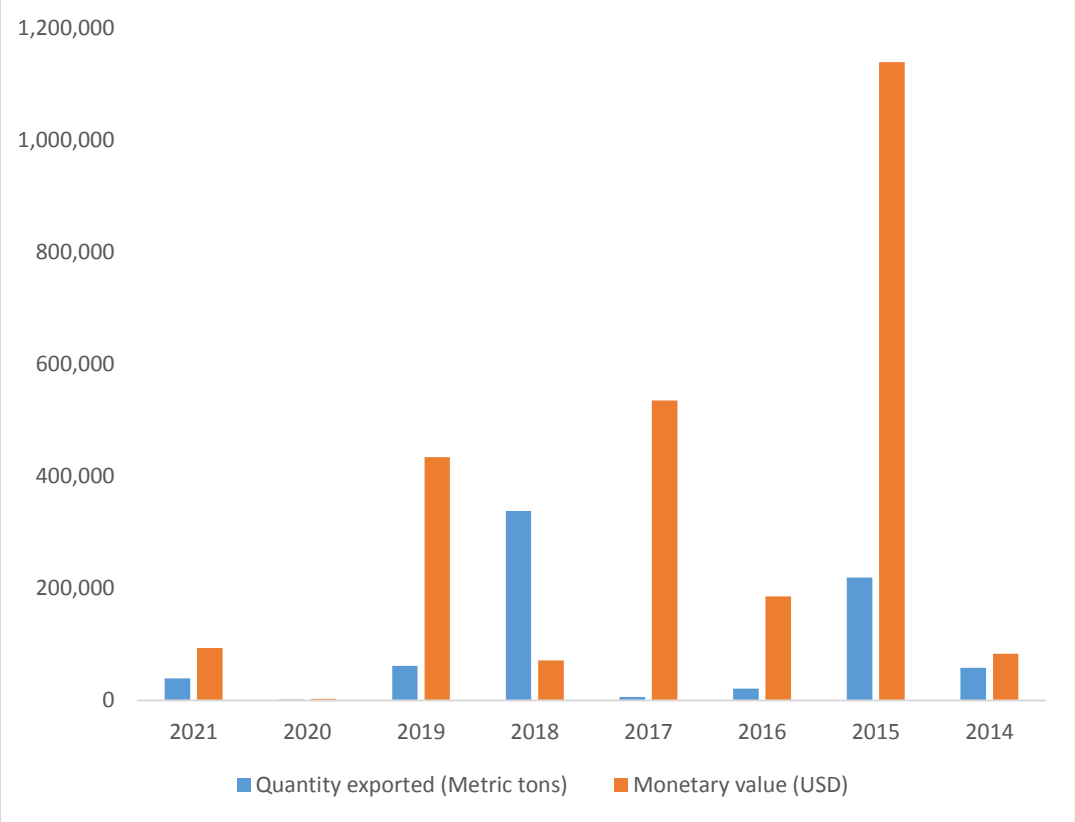

Figuare 2.1: Export trends of tiger nuts tubers by Ghana from 2014 to 2021.

Source: Tridge (2022)

Cultivation of Tiger nut

Tiger nut cultivation thrives in regions characterized by an annual rainfall of 50 to 100 mm, temperatures ranging from 22°C to 38°C, and relative humidity between 75% and 90%. Optimal soil conditions for cultivation involve sandy, moist soil rich in organic matter, with a pH range of 5 to 7 (Odubo *et al*., 2024). To ensure successful tuberization, it is essential to avoid poorly drained soils saturated with water and soil with high salinity levels (Bazine & Arslanoğlu, 2020).

The planting process involves preparing loosened soil through ploughing and harrowing, often forming ridges or mounds. Planting is carried out with a

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spacing of 30 cm between rows and 15 cm within rows (Ayeni, 2022). Before planting, tubers undergo hydro-priming, a process where they are soaked overnight or for 24 to 72 hours to expedite germination. In the case of a 3-day soak, daily water changes are essential. This soaking method not only enhances germination rates but also aids in separating viable tubers from unviable ones, as unviable tubers tend to float in water. Emergence of the crop typically occurs within 5 to 14 days after planting (Asare *et al*., 2020).

The crop reaches maturity within a span of 2.5 to 4 months after planting, encompassing a cultivation period of 10 months, usually spanning from March to December (Olagunju & Oyewumi, 2019). Physiological maturity becomes apparent as the leaves undergo browning and eventually dry out. Harvesting is carried out through excavation or digging, followed by the separation of tubers from the plant (Zhang *et al*., 2022).

Constraints in Tiger Nut Production

Cyperus esculentus contends with limited attention and cultivation on small land plots. The growth of tiger nut crops is hindered by inadequate application of mineral fertilizers, which, if used, results in tubers that fail to meet consumer preferences due to perceived unfavorable taste (Haoua *et al*., 2018).

The process of cultivating tiger nuts, from planting to harvesting, is labour-intensive and demanding (Haoua *et al*., 2018; Sakatai *et al*., 2020). The labour-intensive nature becomes particularly pronounced during the manual harvesting phase, where the laborious extraction of numerous tubers from the soil and their careful separation from the plant is required (Qu *et al*., 2023). This manual harvesting relies on rudimentary tools like hoes, making the operation

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challenging. Moreover, the risk of tuber detachment during harvesting further complicates the process, demanding meticulous effort and time to identify and collect dislodged tubers (Qi *et al*., 2022).

Furthermore, mature tubers are susceptible to termite infestations, leading to reduced yields and posing a significant threat to tiger nut cultivation. The destructive impact of earthworms on tubers also poses a notable constraint for farmers in Benin (Adjahossou *et al*., 2021).

The available tiger nut accessions for cultivation predominantly consist of locally unimproved landraces with no established varieties, resulting in issues such as low yields, cumbersome harvesting procedures, and limited marketability (Haoua *et al*., 2018). To address these challenges, focused breeding efforts are required to develop robust stolon structures that prevent easy detachment of tubers during harvesting (Tetteh & Ofori, 1998).

Biology of Tiger Nut

Morphology of tiger nut

The fundamental chromosome number of tiger nut is denoted as n = 54, resulting in a diploid number of 108 (Hicks, 1929; Fedorov, 1969; Roalson, 2008). This plant is characterized by its resilient, erect growth pattern, featuring fibrous roots and lacking branching (Figure 2.1). It attains a height ranging from 30 to 90 cm.

Tiger nut employs various modes of growth and reproduction, including seeds, tubers, and rhizomes (Mohdaly, 2019; Edo *et al*., 2023). The basal bulb and tubers serve as vital organs for vegetative propagation, supported by short-lived rhizomes. While these rhizomes may exhibit occasional branching, they do not

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develop viable buds at their nodes and typically die out by the end of the growing season. Delicate runners are formed above ground from these slender rhizomes (Atawodi *et al*., 2017), leading to the production of tillers.

The tubers emerge from the tip of the rhizome within the soil. The central stem of the plant which grows from the tuber is upright and has a tristicate protective sheafereth of leaves. The leaves mostly collate at the plant's base, arranged alternately and numbering around 9 to 16 per plant. They externally develop on the stem showing a bright green pigmentation with a waxy surface (Bamishaiye & Bamishaiye, 2011; Maduka & Ire, 2018). The leaf length ranges from 20 to 90 cm growing pointedly to the tip with a basal width of 0.4 to 0.9 cm (Bazine & Arslanoğlu, 2020). The leaf sheaths are initially whitish-green and hairless, transitioning to a pale-red around the plant's base with aging, and eventually turning yellowish-brown shade at full maturity (Tetteh & Ofori, 1998).

Seeds develop within the inflorescence situated on the peduncle at the terminal part of the plant. They are often yellowish, small and lighter in weight with three sided achenes and are usually not viable particularly under unfavorable conditions (Wilma & Chester, 1986).

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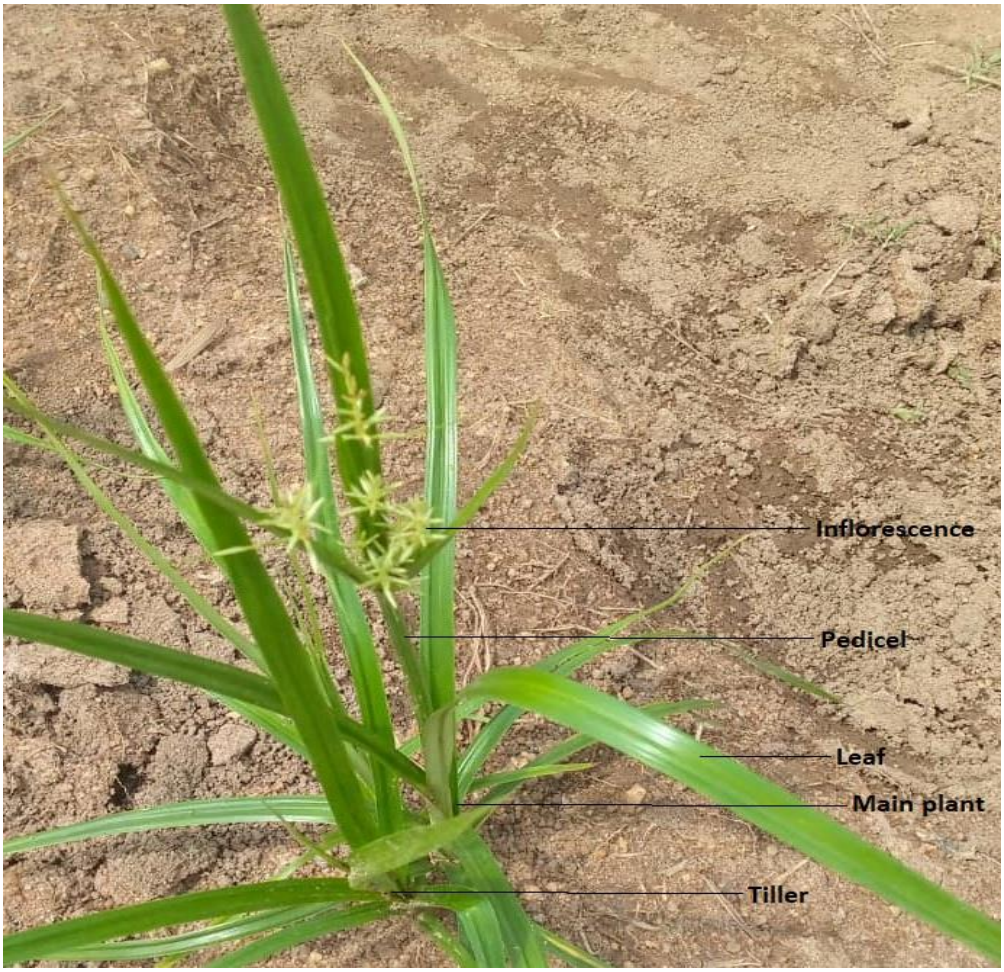

Figure 2.2: Morphological structure of tiger nut plant of the current research.

Inflorescence and pollination in tiger nut

Inflorescences emerge along the shoot, signifying the transition from the vegetative growth phase which is characterized by abundant foliage. At this, the central stem ends apical growth and gives rise to a compound umbel (inflorescence) consisting of 5 to 10 heterostalks floral spikes. The inflorescence bears terminal spikes with pinnate spiklets having golden brown bisexual flower heads containing the seeds (Bazine & Arslanoğlu, 2020; Bezerra *et al*., 2023). Beneath each umbel or compound umbel of spikelets, several leafy bracts (3-9) of differing lengths are present (Wills, 1987), with the largest bract usually surpassing the length of the inflorescence. Each floret

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showcases a white tripartite style characterized by a curly tip, complemented by yellowish-brown anthers situated on three stamens (Holm *et al*., 1977).

Wind serves as the primary pollination mechanism for the tiger nut plant (Bamishaiye & Bamishaiye, 2011; Bazine & Arslanoğlu, 2020). As florets undergo pollination, they transform into diminutive achenes, typically measuring 1.2 to 1.5 mm in length (Wilma & Chester, 1986), featuring an oblongoid or oblongoid-obovoid shape, and appearing flattened.

Tuber formation

Tiger nut, despite its literal name suggesting a nut, is actually a tuber crop. Tubers form from the apical ends of thin rhizomes. The process of tuberization typically begins around 40-50 days after emergence (Yang *et al*., 2022). During the early stages of development, these immature tubers have a whitish appearance and contain a milky-like liquid. Upon reaching maturity, the tuber transforms into a solid, creamy white structure. Brown and yellow tubers feature a yellow or light brown outer membrane and finally turn dark brown at maturity, while black tubers exhibit a light-reddish outer membrane and finally dark or black tuber colour at maturity (Wang *et al*., 2021).

These tubers exhibit diverse shapes, including oblong, ovoid, and oval forms (Asare *et al*., 2020). They display encircling leaf scars, often encompassing several rings, typically numbering from 4 to 7 per tuber. As noted by Bazine and Arslanoğlu in 2020, the length of these tubers ranges from 6 to 10mm. With proper care, a single plant has the potential to produce hundreds of tubers (Bamishaiye & Bamishaiye, 2010).

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Types of Tiger Nut Tubers

Tiger nut tubers exhibit size-based categorization into three types: micro tubers (6-7mm), standard tubers (8-11mm), and larger tubers (12-16mm) (Microbiology *et al*., 2021). The tubers also demonstrate varying shapes, including oval (< 1.3mm), ovoid (1.3-1.8mm), and oblong (>1.8mm) forms (Asare *et al*., 2020). Moreover, these tubers display a spectrum of colours, encompassing yellow, brown, and black variations (Figure 2.3).

A. Black tubers B. Brown tubers C. Yellow tubers

Figure 2.3: Colour types of tiger nut tubers

Uses and Nutritional Composition of Tiger Nut Tubers

Cyperus esculentus holds significant value in Africa and various regions around the globe, including Europe, Asia, and America. Its tubers find diverse culinary applications – from being consumed raw as a snack to being fried, roasted, baked, or transformed into refreshing beverages like "horchata de chufa" in Spain, "Aya" in Nigeria, and "atadwe milk" in Ghana. Beyond their culinary appeal, these tubers serve as a repository of essential functional compounds, contributing to a balanced diet (Chukwuma *et al*., 2010; Manek *et al*., 2012; Jing *et al*., 2013).

Tiger nut tubers are notably rich in starch (295g/kg), digestible carbohydrates (monosaccharides, disaccharides, and polysaccharides), dietary fiber, protein (7-8%), and fat (25.5%) (Mokady & Dolev, 1970; Oderinde & Tairu, 1988;

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Facciola, 1990). They also contain a range of minerals such as sodium, calcium, potassium, magnesium, zinc, and traces of copper, as well as vitamins including B1, C, D, and E (Achoribo & Ong, 2017; Sanful, 2009). Beyond their nutritional significance, tiger nut tubers serve as valuable sources of edible oil, cake and biscuit flour, animal feed, and ingredients for products like soap and cosmetics (Oladele & Aina, 2007; Sánchez‐Zapata *et al*., 2012; Al-Shaikh *et al*., 2013; Akabassi *et al*., 2022).

Due to its notable vitamin B1 content, tiger nut tubers play a vital role in supporting the immune system and tissue preservation (Rosello-Soto *et al*., 2018). Additionally, they have been recognized for their effectiveness in preventing and treating various conditions, including colon cancer, coronary heart diseases, gastrointestinal disorders, obesity, diabetes (Achoribo & Ong, 2017), and constipation disorders. Furthermore, tiger nut tubers are noted for their aphrodisiac, carminative, diuretic, stimulant, and tonic properties (Bazine & Arslanoğlu, 2020).

Tiger Nut Research in Ghana

Tiger nut cultivation is concentrated in select regions of Ghana (Tetteh & Ofori, 1998). Notable among these regions are Upper East, Upper West, Northern, Bono East, Eastern, Central, and Volta. The current practice involves utilizing local accessions (landraces) held by farmers as the primary planting materials for cultivating Cyperus esculentus. However, the knowledge base pertaining to tiger nuts remains deficient in terms of cropping systems, agronomic techniques, and production practices (Asare *et al*., 2020), setting it apart from more extensively studied crops like maize, rice, cowpea, cassava, and yam.

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At present, there exists no officially released tiger nut variety. The existing research indicates that the tiger nut accessions available in Ghana lack comprehensive collection, characterization, and conservation efforts (Donkor *et al*., 2019; Asare *et al*., 2020). This scenario implies that the genetic resources in the form of landraces can be susceptible to gradual erosion over time (Tsegaye & Berg, 2007).

Genetic Diversity in Tiger nut

Genetic diversity encompasses the variations in nucleotides, genes, chromosomes, or entire genomes within or among populations of organisms (Frankham *et al*., 2002). In simpler terms, it signifies the range of heritable traits present in a population of the same species (Swingland, 2001). The study of genetic diversity in crops such as tiger nuts constitutes a crucial resource for identifying alleles that can enhance yield and nutritional content, thereby addressing food security challenges and combating hidden hunger and malnutrition, particularly in regions like Africa that grapple with heightened levels of poverty (Handley, 2009).

This concept holds significant importance in the evaluation of the extent, pattern, and connections among breeding populations (Akintunde *et al*., 2019). Insight into the degree and nature of genetic diversity within germplasm facilitates the arrangement of populations into core subsets for utilization by breeders and researchers (Warburton *et al*., 2002). It aids in the preservation and management of germplasm, the identification of heterotic groups (Ajmone Marsan *et al*., 1998), and serves as guidance for (Akintunde *et al*., 2019) selecting testers in hybrid combination trials during breeding programmes (Enoki *et al*., 2002). Furthermore, genetic diversity has proven valuable in weed

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management (Dodet *et al*., 2008) and in the enhancement of nutritional aspects, such as carotenoid content in maize (Akintunde *et al*., 2019).

Additionally, investigations into the levels and distribution of genetic variation within and among plant populations of a crop species unveil the historical and evolutionary processes that govern genetic diversity (Zada *et al*., 2013). A significant level of genetic variability within a population is essential for the success of long-term plant breeding initiatives. It is noteworthy that genetic variability within and among accessions facilitates the early identification and exploitation of desirable traits, such as high yield and early maturity, contributing to yield improvement and other potential advantages of the crop (Makinde & Ariyo, 2013).

To uphold enduring genetic advancements in favorable traits and to ensure a broad genetic foundation in breeding gene pools, the conservation and enhancement of genetic diversity become imperative (Hallauer & Miranda, 1988; Smith *et al*., 2005). Nonetheless, comprehensive reports on global tiger nut genetic diversity are scarce, with Africa receiving limited attention. For instance, previous studies on the genetic diversity of this crop in Ghana have focused on morphological characterization (Donkor *et al*., 2019) and phenotypic analysis (Asare *et al*., 2020). To date, the available tiger nut accessions in Ghana lack molecular survey studies. These accessions, which are landraces, represent a valuable source of genetic variability. Regrettably, they remain underappreciated, underutilized, and unexplored, with their genetic makeup poorly understood (Molin *et al*., 2013).

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Methods of Estimation of Genetic Diversity

The existence of variability in an organism is a focal point of the living world (Longo *et al*., 2015). Variability in species fundamentally stages the process of adaptation and survival on which evolutionary success revolves for advancement in an organism. Population or individual variations are expressed at the phenotypic level or in a hidden form called polymorphism (Abdou, 2014; Saidou *et al*., 2014; Haoua *et al*., 2023).

Variability within organisms stands as a central theme in the realm of life (Longo *et al*., 2015). Such variability, pivotal for species, underpins the process of adaptation and survival, driving evolutionary success and organismal advancement. Population or individual differences manifest visibly at the phenotypic level or more subtly as polymorphism (Abdou, 2014; Saidou *et al*., 2014; Haoua *et al*., 2023).

Four primary methodologies are employed to explore genetic diversity and relationships among different germplasm classes, including landraces, inbred lines, hybrids, synthetics, and populations. These approaches encompass agro-morphological assessment using descriptors, pedigree analysis involving coancestry coefficient estimation (Malécot, 1948), biochemical profiling such as isozyme or storage protein analysis, and DNA-based molecular techniques (Pejic et al., 1998; Mohammadi & Prasanna, 2003). The agro-morphological and pedigree analyses operate at the phenotypic level, while biochemical and DNA-based molecular techniques function within the polymorphism domain. The selection of a specific method hinges on experiment objectives, resource availability, desired resolution, genome coverage, time, and labor constraints (Karp *et al*., 1997). Each approach possesses merits and limitations; therefore,

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their complementary utilization is recommended to enhance the resolving capacity of genetic diversity analyses (Singh *et al*., 1991).

Agromorphological traits evaluation

Advancements in field phenomics have been evident, yet phenotyping remains a more specialized and labour-intensive process compared to genotypic selection (Cobb *et al*., 2013; Fiorani & Schurr, 2013). The agromorphological or morphological characterization of germplasm holds a crucial position in the advancement of plant breeding (M. S. Lin, 1991), playing an essential role in the management of genetic resources (Smith & Smith, 1989). Morphological evaluation involves both qualitative and quantitative descriptors, encompassing attributes like color, size, shape, growth habits, and performance under diverse experimental conditions or treatments (Bhandari *et al*., 2017; Khadka *et al*., 2020). This approach of data collection and analysis proves cost-effective and relatively straightforward, not necessitating sophisticated technology as required by other genetic diversity estimation methods (Darling *et al*., 2017; Korir *et al*., 2013).

However, morphological traits are often susceptible to phenotypic plasticity, influenced by environmental changes, and characterized by limited heritability, thereby restricting the scope of morphological evaluations (Anumalla *et al*., 2015; Xiao *et al*., 2022) . These evaluations are time-consuming, labour-intensive, and demand substantial plant population sizes (Botha & Venter, 2000) as well as extensive land resources (Osawaru *et al*., 2015). Nonetheless, morphological characterization unveils the expression of highly heritable traits, encompassing morphological and agronomic features (Osawaru *et al*., 2015),

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thus ensuring optimal utilization of germplasm collections for end-users (Engels & Visser, 2003).

Various researchers have employed morphological characterization techniques in their studies. For instance, Osawaru *et al*. (2015) utilized this method in their investigation of West African Okro (*Abelmoschus cailei*). Donkor *et al*. (2019) reported a wide spectrum of genetic diversity among 24 tiger nut landraces from six major regions where the crop is cultivated, utilizing morphological descriptors. Based on phenotypic and yield trait evaluations, Asare *et al*. (2020) observed limited genetic diversity among 64 tiger nut accessions from four growing regions in Ghana.

Estimation of diversity by coancestry coefficients

Assessing diversity through coancestry coefficients relies on pedigree information of genotypes. This technique doesn't necessitate advanced technology; instead, it relies on precise pedigree records. However, it is incapable of gauging the impacts of selection and gene drift (Messmer *et al*., 1993). Genetic diversity however, arises from a combination of genetic factors and environmental influences, often shaped by their interactions.

Frequently, the extent of variability stemming from genotypic factors, including heritability, alongside environmental components of diversity, can be extracted through robust statistical analyses (Govindaraj *et al*., 2015).

Isozyme and storage protein analysis

Biochemical profiling entails the identification of isozymes through electrophoresis, with a primary focus on analyzing seed storage proteins and isozymes. Isozyme markers, which manifest co-dominance and straightforward

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inheritance, facilitate the detection of polymorphisms at the functional gene level, providing a faithful representation of genetic diversity (Pagnotta *et al*., 2009). With allozymes, the allelic variants of enzymes, serve as valuable tools for estimating gene and genotypic frequencies within and between populations. The utilization of isozymes offers advantages such as affordability (Osawaru *et al*., 2015) and the requirement of only a small amount of plant material for detection. Biochemical markers are adept at evaluating co-dominance, free from epistatic and pleiotropic effects, and are user-friendly (Osawaru *et al*., 2015). However, the scarcity of enzyme markers covering the entire genome restricts the resolution of genetic diversity (Govindaraj *et al*., 2015). Notably, isozyme and storage protein analysis fall short in identifying variations in nucleotide sequences that remain silent in terms of resultant amino acid sequences due to the degeneracy of the genetic code (Cheng *et al*., 2014; Ramesh *et al*., 2020). Moreover, this technique presents challenges when comparing samples from different species, loci, and laboratories, often influenced by extraction methods, plant tissues, and plant age (Park *et al*., 2009).

Assessment of genetic diversity using molecular markers

Organisms exhibit distinct genomic DNA sequences that set them apart genotypically. These unique dissimilarities are unveiled through the utilization of DNA molecular markers (Nadeem *et al*., 2018).

A molecular marker is a segment of DNA pinpointing the site on a chromosome where differences in DNA sequences manifest among members of the same species. It unveils DNA-level polymorphisms by identifying specific genes or alleles across a genome through probing. These markers differentiate chromosomal traits by associating with complementary gene pairs and adjacent

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chromosomal regions at the 5' and 3' ends (Barcaccia *et al*., 2000). DNA molecular markers exhibit diverse genetic attributes and can take the form of dominance or co-dominance. They have the ability to amplify anonymous or characterized loci and may encompass expressed or non-expressed sequences (Pagnotta *et al*., 2009).

Various DNA-based molecular techniques utilize molecular markers such as amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), simple sequence repeats (SSR) or microsatellites, random amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSRs), and single nucleotide polymorphisms (SNPs), among others, for the purpose of identifying genotype polymorphisms. These molecular markers are widely employed in ecological, evolutionary, taxonomical, phylogenetic, and plant genetic studies (Osawaru et al., 2015).

DNA-based molecular markers, widely dispersed throughout an organism's genome, stand as preferred tools for genetic diversity investigations (Adhikari *et al*., 2017). Their role involves identifying differences or polymorphisms within nucleic sequences among individuals, manifesting as insertions, deletions, translocations, point mutations, and/or duplications within a species' nucleic sequence (Banday *et al*., 2022; Ramesh *et al*., 2020).

Molecular markers hold a superior position over morphological, pedigree, and biochemical data because they are more efficient and sensitive in detecting distinct differences that arise from mutations among genotypes at the DNA level (Melchinger et al., 1991). Additionally, DNA-based molecular markers offer various advantages, such as being unaffected by environmental influences, being neutral in terms of pleiotropic and epistatic effects, and the ability to

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compare genotypes across generations and populations (Smith, 1988), which contributes to germplasm characterization. They also play a crucial role in tracing the genetic origins of crop plants and constructing genome maps. However, the disadvantages of molecular markers include their high cost and the requirement for sophisticated equipment (Nadeem et al., 2018; Vieira et al., 2016).

The utilization of primer methodologies, such as SSRs (Warburton et al., 2002), AFLP (Beyene et al., 2006), RFLP (Dubreuil et al., 1999), and SNPs (Yu et al., 2011), offers a more effective strategy for estimating gene flow, categorizing genotypes, and evaluating genetic diversity. These methodologies evaluate either multi-locus or single-locus markers. Multi-locus primers, known as dominant markers, enable the simultaneous examination of numerous genomic loci (A Sharma et al., 2008), although they may encounter challenges in distinguishing between heterozygotes and homozygotes for the same allele. Prominent examples include AFLPs and RAPDs (McGregor *et al*., 2000). On the other hand, single-locus markers, referred to as co-dominant markers, target specific genomic loci, enabling differentiation between heterozygotes and homozygotes (Panchariya *et al*., 2024; Undal & Ahir, 2023). Examples of co-dominant markers comprise RFLP, CAPS, SNPs, and SSRs (Amiteye, 2021; Naeem, 2014).

Microsatellite (Single Sequence Repeats) Markers

SSR markers are composed of short repetitive sequences of 2 to 6 base pairs that are found in varying numbers within the non-coding regions of the genome (Litt & Luty, 1989). These markers are highly polymorphic and can be found throughout the genome of crop plants. They are especially useful for studying

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population structure and the history of cultivated species because of their abundant allelic diversity (Vieira *et al*., 2016). In fact, SSR markers are more effective in revealing diversity patterns compared to other types of markers such as RFLP, AFLP, or SNP loci (Powell et al., 1996; McGregor et al., 2000).

The SSR technique employs a straightforward polymerase chain reaction (PCR) approach, which is easily detectable and reproducible (Bousba *et al*., 2013). Amplified fragments resulting from SSR PCR can be resolved using both polyacrylamide and high-quality agarose gels (Senior *et al*., 1998). Notably, SSR loci have been identified as sources of high polymorphism in maize (Senior & Heun, 1993). Additionally, when investigating Niger tiger nut ecotypes through microsatellite SSR markers, Haoua and colleagues observed substantial genetic diversity within the examined populations (Haoua *et al*., 2023).

Advantages of using SSR markers

SSR markers constitute a straightforward PCR-based approach, demonstrating co-dominant inheritance and locus-specificity (Boopathi & Boopathi, 2020a; Jiang, 2013). They exhibit a high degree of polymorphism and do not necessitate specialized equipment. These markers offer a cost-effective solution and facilitate high-throughput genotyping (Azizi *et al*., 2021; Hasan *et al*., 2021). SSR assays can be conducted using minimal DNA samples (as little as 100 mg per individual), and the sharing of SSR DNA markers between laboratories is easily achievable. Moreover, these markers can be multiplexed through PCR (Zalapa *et al*., 2012).

Drawbacks associated with using SSR markers

The advancement of SSRs demands a laborious and intensive process, contributing to elevated costs. Automated methods for SSR assays also entail

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high startup expenses (Distefano *et al*., 2012; Taheri *et al*., 2018). Furthermore, the creation of PCR multiplexes presents challenges that can be both intricate and costly (Appleby *et al*., 2009). Certain markers may prove unsuitable for multiplexing. Additionally, SSR markers find limited applicability beyond plant species (Zane *et al*., 2002).

Measurement of Genetic Diversity

The evaluation of genetic diversity involves the examination of variation and connections within and between populations and/or individuals. This analysis relies on quantitative traits obtained from agro-morphological data, binary data, fragment size, or allele frequencies of molecular markers. A variety of methods are available to measure and describe variability, depending on the type of population (inbred lines, hybrids, landraces, etc.), the population's adherence to Hardy-Weinberg equilibrium, and the characteristics of the data (Mohammadi & Prasanna, 2003). Commonly employed measures for the assessment of genetic variation include the frequency of polymorphism, average number of alleles per locus, proportion of rare alleles, gene diversity within populations for dominant loci, average polymorphic information content or expected heterozygosity for co-dominant loci, effective number of alleles, and genetic distance (Singh *et al*., 2022).

Rate of polymorphism (*Pj*)

According to Hartl et al. (1997), polymorphic loci are characterized by allele frequencies that are either equal to or lower than 0.95 or 0.99. The choice of this allele frequency threshold is arbitrary and is commonly set at 0.95 or 0.99. This threshold serves the purpose of pinpointing genes characterized by prevalent allelic variation (Cavalli-Sforza, 1981). This measurement of intra-population

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genetic diversity serves as an indicator of variation within a gene. Its calculation involves dividing the number of polymorphic loci by the total number of loci (both polymorphic and monomorphic), as depicted by Equation (2.1).

$$P = \frac{n_p}{n_{total}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2.1)$$

where n_p = number of polymorphic loci; n_{total} = total number of loci, both monomorphic and polymorphic.

Average number of alleles per locus (A_P)

When utilizing codominant markers that allow for the detection of alleles on gels, the mean number of alleles per locus is determined for polymorphic loci by dividing the total number of alleles across all loci by the total number of polymorphic loci. This calculation is represented by the following formula:

$$A_p = \frac{1}{k}\sum_{i=1}^{k} n_i \quad \ldots\ldots\ldots\ldots\ldots\ldots (2.2)$$

where i = any locus; k = number of polymorphic loci; n_i = number of alleles detected per polymorphic locus.

Rare allele

Alleles that are considered rare are those that have frequencies lower than 0.005. These alleles are not commonly found within a population and are therefore classified as rare due to their infrequent occurrence.

Average polymorphic information content (PIC)

PIC serves as an indicator of allele variety and distribution within accessions, aiding in the identification of markers that are most informative in distinguishing between different genotypes. The PIC, as defined by Botstein et

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al. (1980) and also known as expected heterozygosity (Nei, 1987), is computed for each SSR locus as:

$$\text{PIC} = 1 - \Sigma(P_i)^2 \quad \ldots\ldots\ldots\ldots\ldots (2.3)$$

where P_i is the proportion of the population carrying the *i*th allele.

Effective number of alleles (A_e)

The effective number of alleles quantifies the number of alleles that can exist within a population. It assesses the number of equally common alleles required to attain a specific level of gene diversity (Weir, 1990). This metric enables the comparison of populations with significant variations in the number and patterns of alleles. The calculation for the effective number of alleles is as follows:

$$A_e = \frac{1}{1-h} = \frac{1}{\sum P_i^2} \quad \ldots\ldots\ldots\ldots\ldots.. (2.4)$$

where P_i is the frequency of the i^{th} allele in a locus, and $h = 1\text{-}\sum P_i^2$ is the heterozygosity at a locus.

Determination of Relationships among Genotypes

Genetic distance

Genetic distance serves as a quantifiable gauge of genetic dissimilarities (or similarities) existing at the allelic level among individuals attributed to identity-by-state or identity-by-descent due to historical mutation events from a common ancestor (Beaumont *et al.*, 1998). This metric quantifies the degree of evolutionary alterations that have occurred since two populations separated from their common ancestral breeding population (Templeton, 2013). The

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genetic divergence scale ranges from 0 to 1, where lower similarity coefficients signify significant genetic differences. Conversely, reduced dissimilarity values indicate a closer genetic relationship.

(Zhang *et al*., 2024).

Multiple methods exist for estimating genetic distance, with the selection contingent upon the type of data being used. Specifically, interval data from morphological assessments, isozymes or DNA amplification products, and presence or absence data require unique methodologies for analysis (Osawaru *et al*., 2015). When dealing with morphological data, the Euclidean distance is a commonly used metric, representing a straight-line measure between two individuals A and B based on their morphological measures denoted by x1, x2, ..., xp and y1, y2..., and yp (Nikita & Nikitas, 2024; Zelditch *et al*., 2012). The Euclidean distance is calculated as the square root of the sum of squared pairwise differences, as outlined in Equation 2.5.

$$d_{AB} = [(x_1 - y_1)^2 + (x_2 - y_2)^2 + ... + (x_p - y_p)^2]^{\frac{1}{2}} \quad \text{..... (2.5)}$$

Various distance measures are utilized in genetic analysis, such as the correlation coefficient, Roger's distance (Rogers, 1986), Cavalli-Sforza & Edwards (1967) distance, and Nei (1972) distance. The correlation distance measure is particularly useful in estimating genetic distance by standardizing metric trait data with different units to ensure an unbiased comparison among genotypes (Fufa *et al*., 2005; Joly *et al*., 2015).

For microsatellite data analysis at the molecular level, where alleles are represented by repeat amplification products, allele frequency variation can be

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estimated, or bands can be scored as either present or absent to generate binary data. Roger's distance, Cavalli-Sforza & Edwards (1967) distance, and Nei (1972) distance are common distance measures that utilize allele frequencies in such analyses.

Rogers' distance, d_{ij} is given by the equation:

$$d_{ij} = \frac{1}{2l}\sum_k \sqrt{\sum(x_{ki} - x_{kj})^2} \quad \text{..... (2.6)}$$

where l is the number of loci, $x_{ki,}$ and x_{kj} are frequencies of allele k for the entities i and j.

Four commonly used measures of genetic distance for binary data matrices are the Modified Roger's distance GDMR (Wright, 1978) (Equation 2.7), the DICE coefficient (GDNL) developed by Nei and Li (1979) (Equation 2.8), the Jaccard coefficient (GDJ) (Equation 2.9), and the simple matching coefficient (GDSM) proposed by Sokal and Michener (1958) (Equation 2.9).

In these equations, X_{01} represents the number of bands or alleles present in individual j only, X_{10} represents the number of bands or alleles present in individual i only, X_{11} represents the number of bands or alleles present in both individuals, and X_{00} represents the number of bands or alleles absent in both individuals. It is worth noting that the simple matching coefficient and the Modified Roger's distance are examples of Euclidean distance measures. These measures provide valuable insights into the genetic distance between individuals based on their binary data matrix.

$$GD_{MR} = \sqrt{\frac{X_{10} + X_{01}}{2l}} \quad \text{..... (2.7)}$$

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$$GD_{NL} = 1 - \left(\frac{2X_{11}}{2X_{11} + X_{10} + X_{01}}\right) \quad \text{..... (2.8)}$$

$$GD_{J} = 1 - \left(\frac{X_{11}}{X_{11} + X_{10} + X_{01}}\right) \quad \text{..... (2.9)}$$

$$GD_{SM} = 1 - \left(\frac{X_{11} + X_{00}}{X_{11} + X_{10} + X_{01} + X_{00}}\right) \quad \text{..... (2.10)}$$

The concept of a similarity measure between two individuals is primarily based on the proportion of observed bands in a banding pattern that they have in common. Consequently, when developing a similarity measure, it is anticipated that equal significance would be given to both shared presences (1s) and shared absences (0s). Nevertheless, it is worth noting that the simple matching coefficient places value on shared absences (Galván *et al*., 2001; Laurentin, 2009), as demonstrated in Equation 2.10.

Multivariate Techniques for Interpretation of Genetic Distance

Irrespective of the size of the population, utilizing multivariate statistical methods enhances the representation of genetic distance among accessions. These techniques employ various measurements taken from individual operational taxonomic units to group accessions into clusters, thereby analyzing the interrelationships stemming from their genetic distances. Common multivariate methods include cluster analysis, principal components analysis (PCA), principal coordinate analysis (PCoA) and multidimensional scaling (MDS) as discussed in previous studies (Melchinger, 1993; Johns *et al*., 1997; Thompson *et al*., 1998; Brown-Guedira *et al*., 2000).

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Cluster analysis

Cluster analysis, arranges accessions into groups based on the similarity or dissimilarity of their attributes, ensuring homogeneity within clusters and heterogeneity across them (Hair *et al*., 1995). Commonly utilized cluster techniques include hierarchical and nonhierarchical methods, utilizing distance measurement techniques introduced by Johnson and Wichern (2002), as well as the robust maximum likelihood estimation and Bayesian approaches developed by Pritchard *et al*. (2000) to overcome the constraints of distance-based methodologies.

The hierarchical technique is agglomerative in nature, progressively grouping individuals and subsequently merging them based on shared similarities. Noteworthy cluster methods include single linkage, complete linkage, and Unweighted Pair Group with Arithmetic Mean (Panchen, 1992; Sneath & Sokal, 1973), which transform distance measures into graphical representations.

Principal components analysis (PCA) of morphological data

One method for uncovering patterns in data based on similarities and differences (Lindsay, 2002) is the use of principal components analysis (PCA). This technique, initially devised by Pearson (1901) and subsequently refined by Hotelling (1933), is a multivariate approach that employs the arrangement of multivariate data presented in matrix form to unveil inherent patterns or relationships when projected onto a two-dimensional space, emphasizing similarities and differences. The foundational principles of PCA can be found in works such as (Johnson & Wichern, 2002) and (Jolliffe & Jolliffe, 1986). In this two-dimensional space, similar variables manifest proximity, while

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dissimilar ones exhibit greater separation, thus exposing only the key data patterns.

PCA is a powerful technique used to reduce the dimensionality of multivariate data by creating a series of linear combinations of orthogonal variables called principal components (PCs) (Demšar *et al*., 2013; Mishra *et al*., 2017). These PCs are ordered in a way that each one explains a certain amount of variance in the data, with the first PC explaining the most variance and subsequent PCs explaining less. When all PCs are considered together, they collectively explain 100% of the variance in the data, making PCA a valuable tool for data analysis (Khattree & Naik, 2000). The eigenvectors associated with the PCs provide information about the direction and magnitude of the variance in the data (Mishra *et al*., 2017).

The significance of PCA can be assessed by examining a scree plot, which helps in identifying the point where there is a noticeable change in the curve, resembling an elbow (Saranya & Poonguzhali, 2024; Zhou, 2017; Zhu & Ghodsi, 2006). This point indicates the number of significant principal components to consider for analysis. Additionally, North *et al*. (1982) highlighted the importance of sampling error in PCA, showing that the gap between adjacent PCs in the formula (Equation 2.11) can help determine the significance of the eigenvalues. If the errors exceed this gap, it suggests that the corresponding eigenvalue is statistically significant and should be considered in the analysis.

$$\text{Sampling error} = \Delta\lambda - \lambda_i\left(\sqrt{\frac{2}{n}}\right) \quad \ldots\ldots\ldots\ldots\ldots \quad (2.11)$$

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where λ_i = a PC; $\Delta\lambda$ = change between neighbouring PCs; n= number of samples.

Principal coordinate analysis (PCoA)

Principal coordinate analysis, as described by Khayyam Nikoyie *et al*. (2009), is a method utilized in multivariate analysis to depict the variance relationships present within a population. This technique focuses on maximizing the linear correlation between sample distances, showcasing variations in multidimensional patterns for enhanced interpretation.

The relative variance of each coordinate in PCoA signifies the genetic distances either within or among populations, shedding light on the significance of the related coordinate in terms of total variance. This value is typically expressed as a percentage, providing valuable insights into the genetic diversity and structure of the studied populations.

Correlation

Correlation stands as a bivariate statistical method that gauges the intensity of the connection between two variables while determining the direction of this association. The correlation coefficient, which ranges between +1 and -1, reflects the degree of strength in the relationship. A +1 value signifies an impeccable level of linkage between the two considered variables, while the correlation coefficient approaches zero (0), the connection between the variables weakens (Akoglu, 2018; Schober *et al*., 2018). The sign of the coefficient, denoted as "r," unveils the direction of the relationship: a positive correlation is denoted by a + sign, whereas a negative correlation is indicated by a – sign (Akoglu, 2018). The widely employed approach for assessing the

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extent of association between two variables is the Pearson r correlation coefficient, r, which can be calculated using the following formula:

$$r_{xy} = \frac{n\sum x_i y_i - \sum x_i \sum y_i}{\sqrt{n\sum x_i^2 - (\sum x_i)^2}\sqrt{n\sum y_i^2 - (\sum y_i)^2}} \quad \text{..... (2.12)}$$

Where;

r_{xy} = Pearson correlation coefficient between the variables x and y

n = number of observations

xi = value of x (for the ith observation)

yi = value of y (for the ith observation)

Breeding Methods (Techniques/ Strategies)

The generation of variability is crucial in the genetic improvement of crops. In plant breeding several methods are available in inducing genetic variability within and among crops. These methods can be grouped into conventional (classical/traditional) breeding and unconventional (contemporary/modern) breeding. Conventional breeding of crops makes use of selection and hybridization or crossing. While contemporary breeding involves techniques such as mutation, biotechnology, and marker-assisted selection (Lamichhane & Thapa, 2022).

Selection

Selection is the oldest plant breeding technique for a crop improvement process of choosing and maintaining plants with useful and desirable characteristics. In

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this method, plants with undesirable traits are discarded and often neglected (Acquaah, 2009). The basis for selection is in reliance on the phenotypic traits of species. Generally, the effectiveness of selection is dependent on the degree to which the phenotype reflects the genotype (Lin *et al*., 2010). Two main types of selection exist; mass selection and pure line selection (Begna, 2021).

Mass selection

Mass selection involves selecting a significant number of plants that share similar phenotypic traits, and then blending their seeds to create a new variety. The resulting population from this method typically exhibits greater uniformity compared to their original parental population (Acquaah, 2015).

This method of selection involves gathering seeds from superior individuals phenotypically from a population. The seeds are further mixed planted in the subsequent generation. Superior individuals are preserved while undesirable genotypes are disposed of (Khaeim, 2013).

Pure line selection

A pure line is derived from a solitary homozygous plant that undergoes self-fertilization (Degefa, 2019). Through pure line selection, the offspring of self-pollinated or fertilized crops inherit the same genetic traits as their parents, making these traits heritable (Hussain *et al*., 2021). However, any variation observed within a population is solely influenced by environmental factors and is therefore not passed on to future generations. The technique of pure line selection has proven effective in enhancing self-pollinated crops (Begna, 2021).

In asexually propagated crops such as tiger nuts, the selection is done by visual observation of clonal characteristics. The clone (progeny from a single plant

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through asexual reproduction) of vegetatively propagated plants is considered for selection more importantly by the qualitative characteristics rather than quantitative traits (Acquaah, 2015; Taji *et al*., 2024). Imposing selection on quantitative traits with vegetatively propagated crops will be misleading and unreliable as such traits are influenced by the environment (Grattapaglia, 2017). Therefore, the selection procedure involves; first, picking the desired clones from a large mixture of clones or bulked or mass clones in the first season. This is followed in the second season by selecting superior clones from individually grown selected plants (Acquaah, 2015). Preliminary yield, disease resistance and other quality trials with standard checks is/ are done in the third season and a few outstanding clones are selected (Micke & Donini, 1993). Multilocation yield trials with standard checks are carried out in the fourth to sixth seasons and the best clones are identified for release as new varieties. Finally, seed (propagule) multiplication of best clones is done in the seventh season for distribution (Bisognin, 2011).

Clonal hybridization

Clonal hybridization is the process of crossing two genetically unrelated lines or plants. The primary aim of hybridization is to introduce genetic variation (Seehausen, 2004). Through this approach, clonal crops are enhanced by combining two or more desirable clones (Wetzstein *et al*., 2018). In tropical agriculture, cassava is one of the most important crop clonally propagated (Duputié *et al*., 2007). The selection of clones for hybridization process begins with the first filial generation (F1) progeny and continues through subsequent clonal generations. Once the F1 generation is established, the clonal selection process is implemented (Grüneberg *et al*., 2009).

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Marker-assisted selection (MAS)

Marker-assisted selection (MAS) entails utilizing morphological, biochemical, and DNA molecular markers as an indirect approach in selecting desirable traits for crop breeding programmes, thereby expediting the development of improved varieties (Ashraf *et al*., 2012; Henkrar & UDUPA, 2020). This innovative plant breeding technology accelerates the creation of enhanced cultivars. Breeders employ molecular markers to trace specific gene or chromosome segments suspected of influencing the desired phenotype within individuals or populations of interest (Boopathi & Boopathi, 2020b; Williams, 2005).

MAS has been extensively explored across various crops and traits. DNA-based markers such as RFLPs, AFLPs, RAPDs, SNPs, and SSRs have been harnessed to construct gene maps, confer disease resistance, enhance drought tolerance, and facilitate the identification and transfer of quantitative trait loci (QTLs) to enhance crop characteristics (Aswini *et al*., 2023; Rahul Kumar *et al*., 2024). For instance, Chen *et al*. (2016) highlighted the discovery of the QTL qGW4.05, associated with kernel weight and size in maize. Similarly, Körber *et al*. (2016) dissected agronomic and quality traits of *Brassica napus* using GWAS6K SNP arrays.

In Indonesia, the implementation of MAS led to the development of two bacterial leaf blight-resistant rice varieties, 'Angke' and 'Conde' (Bustamam *et al*., 2002). Additionally, MAS has played a significant role in the improvement of wheat by enhancing GPC-B1 (84-60) and strengthening barley by introducing a thermostable β-amylase gene (Xu *et al*., 2018) as well as a scald (*Rhynchosporium commune* L.) resistance gene (Sayed & Baum, 2018). This

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dynamic approach showcases its efficacy in driving advancements in crop breeding and promoting agricultural innovation.

Biotechnology technique

Biotechnology, as defined by Persley (1992), is the breeding methodology that employs living organisms, developed microorganisms, or components derived from living organisms to modify or create products aimed at enhancing plants or animals for human requirements.

Plant biotechnology encompasses the utilization of techniques such as tissue culture, plant molecular markers, and genetic engineering to generate genetically modified plants exhibiting novel or improved desirable traits. This powerful tool facilitates the creation of new plant characteristics and varieties, contributing to advancements in crop breeding (Kumar *et al*., 2015).

Tissue culture, involving the induction of callus formation from plant parts, has found widespread application in Africa across crucial food crops like yam, cassava, sweet potato, banana, and other vegetatively propagated plants (Brink *et al*., 1998). In vitro mass propagation through tissue culture has proven effective in producing disease-free plants and establishing regeneration systems for plant transformation.

At the core of crop plant enhancement lies the concept of gene transfer, which forms the basis of "Transforming Principles" (Avery *et al*., 1999). This understanding paved the way for genetic engineering, a comprehensive approach encompassing DNA isolation, restructuring, and insertion into diverse cellular contexts (Scowcroft, 1977).

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Genetic engineering, yields transgenic plants through processes such as gene isolation and cloning, gene insertion or transformation, generation of transgenic plants, validation of successful transformation, and assessment of gene functionality (Brink *et al*., 1998). This technique involves modifying an organism using recombinant DNA technology and employs laboratory tools and specific enzymes to manipulate, insert, or alter segments of DNA containing desired genes of interest (James, 2014), thereby contributing to crop improvement endeavors.

Mutation

Initiating improvements in any crop necessitates the establishment of variability for subsequent selection. The presence of heritable variation is paramount for genetic enhancement efforts in crops. In situations where natural variation is inadequate, the introduction of variation can be achieved through either random or targeted means (Jankowicz-Cieslak *et al*., 2017). Mutation stands as the ultimate wellspring of variability, offering distinct germplasm to plant breeders (van Harten, 1998).

Mutation is defined as a sudden, inheritable alteration in the genotype of an organism (De Vries, 1991). It can transpire naturally over the course of several years or be induced artificially, a particularly promising approach within a shorter time frame. In either scenario, plant DNA mutation leads to equivalent effects on the phenotype. Notably, induced mutation has demonstrated success in enhancing the genetics of numerous crop genotypes. Plant breeders have employed this technique since the 1920s to instigate genetic diversity (Stadler, 1928; van Harten, 1998; Tambe & Apparao, 2009; Ahloowalia *et al*., 2004).

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Artificial or induced mutation is typically achieved through the application of physical or chemical mutagens (Jain, 2002; Mba *et al*., 2007).

Different types of physical mutagens include electromagnetic radiations like gamma rays, X-rays, UV light, and particle radiation such as beta and alpha particles. On the other hand, chemical mutagens often comprise alkylating agents like ethyl methane sulfonate (EMS), ethidium bromide, and base analogues such as bromouracil (van Harten, 1998; Girija & Dhanavel, 2009; Mba *et al*., 2010).

The selection of a specific mutagen for mutation breeding typically relies on past achievements observed in the species, alongside considerations like mutagen availability, cost, and infrastructure (Mba, 2013; Bado *et al*., 2015).

The process of mutation breeding involves three key steps: (a) inducing mutations, (b) screening for potential mutant candidates, and (c) evaluating and officially releasing mutants for use (Jankowicz-Cieslak *et al*., 2017).

Inducing mutationn and mutagenesis

Human-driven selection for novel alleles of structural or regulatory genes has resulted in the emergence of numerous vital plant phenotypes that play a crucial role in domestication and improvement. This multitude of essential phenotypes holds significant importance in the field of plant genetics and has been extensively studied (Olsen & Wendel, 2013). Plant breeders have been instigating genetic diversity in crops since the 1920s through the utilization of physical and chemical mutagenesis (Stadler, 1928).

Induced mutations were initially conducted in plants using physical mutagens and have contributed to the majority (approximately 77%) of varieties listed in the FAO/IAEA Mutant Varieties Database (Maluszynski, 2001). Physical

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mutagenesis primarily consists of ionizing radiation generated either directly by gamma rays and X-rays or indirectly through fast neutron bombardment (Roychowdhury & Tah, 2013), as well as alpha and beta particles. The mutagenic effectiveness of physical mutagens is determined by both the type of physical agent used and the particular genotype of the organism (Ramanathan, 1979). Notably, physical mutagens, particularly x-rays, have yielded successful outcomes in various crops, including groundnut (Gunasekaran & Pavadai, 2015), cowpea (Horn & Shimelis, 2013), lentil seed (Roy *et al*., 2019), sesame (Diouf *et al*., 2010) and pigeon pea (Ariraman *et al*., 2014).

Chemical mutagens for mutagenesis gained prominence following their discovery in the 1940s, marked by the treatment of *Drosophila melanogaster* with mustard gas (Auerbach & Robson, 1946; Auerbach, 1946). Chemical mutagens, unlike radiations, have the ability to cause gene mutations and single-nucleotide polymorphisms (SNPs) rather than chromosomal mutations (Khan *et al*., 2009). Some commonly used chemical mutagens include ethyl methane sulfonate (EMS), N-methyl-nitrosourea (MNU), and sodium azide (Az) (Sikora *et al*., 2011). EMS is particularly known for its strong mutagenic properties, causing random mutations in genetic material through nucleotide substitution, primarily guanine alkylation. Typically, EMS results in point mutations within the genome (Joya-Dávila & Gutiérrez-Miceli, 2020; Yan *et al*., 2021). Colchicine, on the other hand, is an alkaloid mutagen that is used to induce mutations, specifically polyploidy, in plant breeding (Blasco *et al*., 2015; Manzoor *et al*., 2019).

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Chemosensitivity and determination of the right dose of EMS and Colchicine

Determining the appropriate dosage of a mutagen for genotypes in mutagenesis is a crucial and fundamental step towards the success of mutation breeding. The impact of a mutagen on a genotype's response can either be stimulating or inhibitory, contingent on the quantity employed (Nura *et al*., 2011; El-Nashar & Ammar, 2016; Singh *et al*., 2020). Higher dosages of mutagens can completely halt seed germination (Spencer-Lopes *et al*., 2018b), while very low concentrations exhibit a stimulating effect, enhancing plant architectural traits (ZakyZayed *et al*., 2014; Twumasi *et al*., 2023). Hence, it becomes imperative to optimize a mutagen dosage that achieves 50% seedling emergence by lethally affecting half the population (LD_{50}), and also a 50% reduction in seedling growth (RD_{50}) effect. Mutagen dosages surpassing the LD_{50} lead to a higher frequency of mutations in the M1V1 population, whereas dosages below the LD_{50} result in a lower mutation frequency (Brown & Caligari, 2011; Szarejko *et al*., 2017).

Finding the appropriate dosage can be challenging, yet meticulous experimentation can unveil it (Acquaah, 2009). The ideal (optimal) dosage is the one that maximizes mutation frequency while minimizing unintended damage (Mba *et al*., 2010). According to van Harten (1998), the optimal dosage varies across plants, even within the same species, based on factors like the exposed planting materials, mutagen types, and physiological condition of the propagules.

Numerous factors influence the successful outcome of chemical mutagenesis. These factors encompass the attributes of the target plant material, the mutagen

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dosage applied, the physicochemical attributes of the chemical mutagen, characteristics of the mutagenic solution (such as pH), the laboratory's environmental conditions (e.g., temperature), as well as the growth conditions (greenhouse, nursery, field, in vitro, etc.) of plant seeds and/or propagules before and after mutagenic treatment (Spencer-Lopes *et al*., 2018). Therefore, it is recommended that a wide range of dosage concentrations (e.g., 0.0, 0.1, 0.25, 0.5, 0.75, 1.0 percent) be initially applied to a substantial population to ascertain the optimal LD_{50} dosage before implementing mass treatment of genotypes for the first mutation population (M1 for seed/pollen mutagenesis and M1V1 for vegetative organs) (Bado *et al*., 2015).

Screening for putative mutant candidates

The M1V1 population usually gets affected from physiological disorders and genetic injury as a result of the mutagen treatment. To enhance the survival and successful development of potential mutants for future generations, it is crucial to plant mutagenized propagules in an environment free from stress factors. This ensures that the plants have the best chance of thriving and allows for effective screening of the putative mutants (Bado *et al*., 2015). The phenotypic selection at the M1V1 for mutation is inappropriate as detection of homohistant mutants becomes impossible due to chimerism, which needs to dissolve in subsequent generations (Spencer-Lopes *et al*., 2018). Harvest from M1V1 can be bulked and planted for generation two (M1V2). Chimeric structures continually surface in M1V2 and therefore careful and close monitoring in identifying ‘deviants’ from standard normality must be observed. Visual selection and measurements are required to identify putative mutants for desired plant architectural traits such as plant growth habits, branching type, number of tillers, plant girth among

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others. Selected candidates are propagated for observation and confirmation of characteristics and assessment of uniformity and stability at M1V3 generation (Drake *et al*., 1998). In M1V3, the preliminary evaluation process begins by evaluating solid mutants and determining their level of uniformity. Non-uniform putative mutants' clones are subjected to further propagation generations to reach uniformity (Gunjaca *et al*., 2008; Vélez & Ibáñez, 2012). Evaluation for main desired traits such as earliness, yield, mineral content, fruit size, weight, resistance or tolerance to diseases and, biotic stresses, can be taken or delayed until further generations (Spencer-Lopes *et al*., 2018). The M1V3 to M1V8 generations are distinguished by the ongoing process of selecting, confirming genetically, multiplying, and stabilizing the field performance of mutant lines (Zakir, 2018). In the M1V4 to M1V9 stages, uniform clones can be reproduced and planted in experimental trials to assess their performance in terms of desired traits (Spencer-Lopes *et al*., 2018). Replicated trials of chosen mutants are conducted as early as M1V4, using either parental or local lines as checks.

Mutant testing and official release

After the emergence of a mutant line displaying a favorable trait, the subsequent step involves seed multiplication to facilitate extensive field and multi-locational trials, ultimately leading to potential varietal release (Zakir, 2018). During this phase, the mutant line, alongside the parent cultivar and other existing varieties, undergo thorough testing. Before its introduction as a commercial cultivar, the potential mutant is evaluated comprehensively for various attributes such as growth habits, structural characteristics, and yield components. These evaluations are conducted across a diverse range of

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environments, considering factors like water availability, plant density, sowing dates, and other relevant parameters (Roychowdhury & Tah, 2013).

The final assessment is executed in subsequent generations, typically M1V9 and M1V10, tailored to the specific plant species. Upon successful evaluations, the selected mutant clone(s) can be released as novel and improved mutant varieties, poised for cultivation and utilization (Spencer-Lopes *et al*., 2018).

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CHAPTER THREE

GENETIC DIVERSITY OF TIGER NUTS (*Cyperus esculentus* L.) GROWN IN GHANA BASED ON MORPHO-DESCRIPTORS AND SSR MARKERS

INTRODUCTION

Tiger nut (*Cyperus esculentus* L.) is a perennial weed in the sedge family (*Cyperaceae*). The crop is known to many as chufa, nutgrass, earth almond, water grass, rush nut, yellow nutsedge and northern nutgrass (Shilenko *et al*., 1979). The origin of *Cyperus esculentus* is generally uncertain. While some believe that tiger nut is a native to Africa and Asia (DAISIE, 2014), others are of the view that it originated from Europe and North America (Govaerts, 2014; USDA-ARS, 2014).

The plant is tough, erect, growing from a perennial tuber-bearing rhizome, and has fibrous roots. It grows to a height of 30-90 cm tall, producing seeds with many slender rhizomes forming runners above the ground. The tips of the underground stems produce tubers which are of economic importance that appear in three different colours; black, brown, and yellow (Edo *et al*., 2024).

The tuber, affectionately called nut, is a source of feed, food, medicine and perfumes (De Vries, 1991; Gambo and Da'u, 2014). It can be eaten dried raw, roasted, baked, or made into a refreshing beverage called *Horchata De Chuf* (in Spain), '*kunnu aya*' (in northern Nigeria), and '*atadwe milk*' (in Ghana).

Though tiger nut is an underutilised and largely unexploited crop, it has high export potential (Tetteh and Ofori, 1998) and if adequately resourced, would provide Ghana with foreign exchange. For example, in the year 2021, Ghana

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exported 39,560 mt worth US$ 93,960 (Tridge, 2022). Ghana exports her tiger nuts to many countries in Europe, Asia and United States of America (Novor & Donkor, 2024).

Despite the nutritional, medical, and economic benefits of tiger nut, the crop remains an orphan (Donkor *et al*., 2019), probably, due to its invasiveness and obnoxious weed character coupled with minor scientific and technological studies to promote its enormous potential for use.

Unlike other crops such as maize and cowpea, tiger nut cultivation has received little agronomic and breeding attention for improvement. Very few studies had been conducted on its production globally. Research available had been focused on its morphological studies and nutritional value with scanty information on its molecular survey. Limited studies in Ghana had focused only on the morphological characterization of Ghana accessions (Asare *et al*., 2020; Donkor *et al*., 2019). The use of these locally unimproved cultivars over the years accumulates pests and diseases and, consequently low yields. The use of morphological or phenotypic characterization for diversity studies presents challenges including time consuming, labour intensive and the interferance of the environment leading to phenotypic plasticity (Anumalla *et al*., 2015; Xiao *et al*., 2022). A combined methodology utilizing both morphological descriptors and molecular markers offer a robust approach to uncovering the diversity within population (Sharma *et al*., 2022; Verma *et al*., 2024). Consequently, it is essential to investigate the diversity present in the local tiger nuts accessions available for parental selection aimed at improvements in Ghana. The objective of this study was to assess the diversity among the 42 tiger nut accessions in

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Ghana using 11 morpho descriptors and 9 simple sequence repeats (SSR) markers.

Materials and Methods

Collection of germplasm

The studied accessions consisted of landrace varieties cultivated by farmers in Ghana. These germplasms were collected from major cultivation regions within the country. A total of 42 accessions, comprising 24 brown, 12 black, and 6 yellow varieties, were sourced directly from farmers in key growing communities across the Northern, Upper East, Upper West, Bono East, Eastern, and Central regions of Ghana. The geographical locations from which these accessions were gathered are depicted in Figure 3.1.

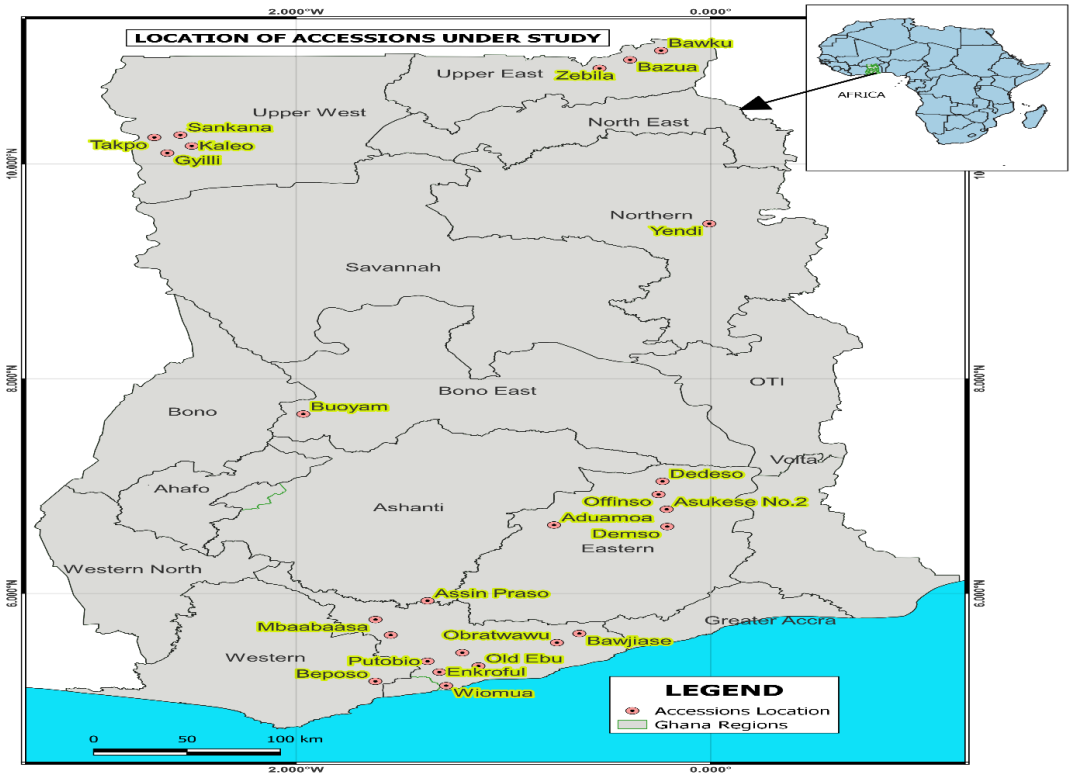

Figure 3.1: Map of Ghana showing geographical locations where accessions were collected.

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Accessions lacking passport data were assigned names based on the initial three letters of the corresponding town or community name where the collection took place, followed by the tuber colour. To distinguish accessions of the same colour from the same town, numerical identifiers were added. For instance, YEN-b1, YEN-b2, and YEN-b3 denote Yendi brown 1, Yendi brown 2, and Yendi brown 3, respectively (see Table 1). The tubers were securely packaged and tagged using zip-locked polythene bags before being transported to the University of Cape Coast Teaching and Research Farm.

Table 3.1: Accessions, place of collection and colour of the tuber

S/N	*Accession*	*Colour*	*Place/ Area of collection*	*Region*
1	ADU- b	*Brown*	*Aduamoa*	*Eastern*
2	ADU- B	*Black*	*Aduamoa*	*Eastern*
3	ADU- Y	*Yellow*	*Aduamoa*	*Eastern*
4	APR- b	*Brown*	*Assin Praso*	*Central*
5	ASU- b	*Brown*	Asukese No.2	*Eastern*
6	BAJ- b	*Brown*	Bawjiase	*Central*
7	BAJ- B	*Black*	Bawjiase	*Central*
8	BAJ- Y	*Yellow*	Bawjiase	*Central*
9	BAW- b	*Brown*	Bawku	*Upper East*
10	BEP- B	*Black*	Beposo	*Central*
11	BEP- Y	*Yellow*	Beposo	*Central*
12	BUO- b	*Brown*	Buoyam	*Bono East*
13	BUO- B	*Black*	Buoyam	*Bono East*
14	DED- b	*Brown*	Dedeso	*Eastern*
15	DEM- b	*Brown*	Demso	*Eastern*
16	ENK- b	*Brown*	Enkroful	*Central*
17	ENK- B	*Black*	Enkroful	*Central*

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18	GYI- b	*Brown*	Gyilli	*Upper East*
19	KAL- b	*Brown*	Kaleo	*Upper West*
20	KAL- B	*Black*	Kaleo	*Upper West*
21	MBA- b	*Brown*	Mbaabaasa	*Central*
22	MBA- B	*Black*	Mbaabaasa	*Central*
23	NEB- b	*Brown*	New Ebu	*Central*
24	OBR- b	*Brown*	Obratwawu	*Central*
25	OBR- B	*Black*	Obratwawu	*Central*
26	OBR- Y	*Yellow*	Obratwawu	*Central*
27	OEB- b	*Brown*	Old Ebu	*Central*
28	OEB- B	*Black*	Old Ebu	*Central*
29	OFF- b	*Brown*	Offinso	*Eastern*
30	PUT- b	*Brown*	Putobio	*Central*
31	PUT- B	*Black*	Putobio	*Central*
32	SAN- b	*Brown*	Sankana	*Upper West*
33	TAK- b	*Brown*	Takpo	*Upper West*
34	TAK- B	*Black*	Takpo	*Upper West*
35	TWI- b	*Brown*	Twifo Praso	*Central*
36	TWI- Y	*Yellow*	Twifo Praso	*Central*
37	WIO- b	*Brown*	Wiomua	*Central*
38	WIO- B	*Black*	Wiomua	*Central*
39	YEN- b1	*Brown*	Yendi	*Northern*
40	YEN- b2	*Brown*	Yendi	*Northern*
41	YEN- b3	*Brown*	Yendi	*Northern*
42	ZEB- Y	*Yellow*	Zebila	*Upper West*

Experimental site and culture

The experiment was conducted at the Teaching and Research Farm of the University of Cape Coast during the major growing season, spanning from May

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to September 2021. The experimental site is situated between 5° 6′ N and 1° 15′ W, experiencing a bimodal rainfall pattern with an annual rainfall amount of 773.7 mm. The primary rainy season typically occurs from April to June, followed by a minor season from September to October, while dry spells (harmattan) are prevalent from November to February. The average temperature at the site was 25.8°Cwith an average relative humidity of 86.3%. The land, previously utilized for eggplant cultivation, was ploughed and harrowed to a depth of approximately 30 cm. The soil type was identified as sandy loam with a pH of 6.1.

An 85 m^2 area (5 m x 17 m) of land was prepared and subdivided it into plots. A total of 126 plots were established, each measuring 0.6 m x 1 m, following a randomized complete block design with 3 replications. Each replication consisted of 42 plots, and within each plot, 10 tubers were planted per genotype at a spacing of 15 cm x 20 cm. Before planting, the genotypes underwent hydro-priming, involving a 6-hour water soaking in disposable Styrofoam cups measuring 12 oz (355 ml). This step aimed to eliminate non-viable tubers (those that floated) and promote sprouting. Planting commenced after priming, and the harvesting was conducted 98 days after planting (DAP), although certain genotypes exhibited early signs of maturity at 85/86 DAP. Routine weeding and irrigation were carried out as necessary.

Germplasm for molecular profiling

The 42 accessions were cultivated in pots within a screen house located at the Biotechnology Laboratory of the CSIR-Crops Research Institute in Fumesua, Ashanti Region of Ghana. These accessions were established using tubers collected from the field at the Teaching and Research Farm of the University of

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Cape Coast. Forty-one (41) were viable for DNA extraction, while accession ADU-b did not germinate (as shown in Table 3.2).

Table 3.2: Accessions used for the molecular study.

S/N	Genotype	S/N	Genotype	S/N	Genotype	S/N	Genotype
1	APR-b	12	OBR-Y	23	DED-b	34	OEB-b
2	BUO-b	13	NEB-b	24	SAN-b	35	TWI-b
3	ZEB-Y	14	OBR-b	25	OFF-b	36	ENK-B
4	ASU-b	15	BAJ-Y	26	TWI-Y	37	WIO-B
5	MBA-b	16	BEP-Y	27	YEN-b1	38	TAK-B
6	GYI-b	17	PUT-B	28	ENK-b	39	MBA-B
7	TAK-b	18	WIO-b	29	DEM-b	40	BAJ-B
8	YEN-b3	19	ADU-Y	30	OEB-B	41	ADU-B
9	BAW-b	20	PUT-b	31	BUO-B		
10	YEN-b2	21	BEP-B	32	KAL-B		
11	BAJ-b	22	KAL-b	33	OBR-B		

DNA extraction

Fresh young leaves, weighing approximately 200 mg, were carefully excised from two-week-old plants using forceps and placed immediately into 2 ml Eppendorf tubes containing liquid nitrogen. To isolate DNA, the samples were homogenized with liquid nitrogen within the Eppendorf tubes.

The DNA extraction process followed the Cetyltrimethylammonium bromide (CTAB) protocol established by Doyle and Doyle (1990). Firstly, 1 ml of freshly prepared CTAB extraction buffer (composed of 20 mM Tris HCl, 50 mM EDTA, 2 M NaCl, 2% CTAB, and 3% β-mercaptoethanol) was added to

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the homogenized leaf samples in 2 ml Eppendorf tubes. The mixture was vortexed for one minute and then incubated in a water bath at 65 °C for 25 minutes with periodic inversion to ensure proper mixing.

After cooling for five minutes, 800 µl of phenol chloroform isoamyl alcohol (in a ratio of 25:24:1) was gently added and mixed by inversion until the solution became milky. The tubes were then centrifuged at 13,000 rpm for 10 minutes using a GenFuge 24D centrifuge. Next, 800 µl of the supernatant was carefully transferred to new 2 ml labeled tubes without disturbing the middle layer.

To the transferred supernatant, ice-cold absolute ethanol (900 µl) and 30 µl of 3 M sodium acetate were added. The mixture was mixed and then incubated at -20 °C overnight. Following overnight incubation, the samples were again centrifuged at 13,000 rpm for 10 minutes, and the supernatant was discarded. The resulting DNA pellet was washed with 80% ethanol for 30 minutes.

Subsequently, the pellets were dissolved in 500 µl of low salt buffer (consisting of Tris HCL, 8.0 – 1mM; EDTA, 8.0 – 0.1mM and Nuclease free water), and 10 µl of RNase A (20 mg/ml) was added, followed by incubation at 37 °C for 45 minutes. After this step, 250 µl of 7.5 M ammonium acetate was mixed with the samples, followed by a five-minute ice incubation. The mixture was then centrifuged at 13,000 rpm for 10 minutes, and the resulting supernatant was transferred to newly labeled 1.5ml Eppendorf tubes.

To the transferred supernatant, isopropanol (700 µl) was added, mixed by inversion, and then incubated at -20 °C for 30 minutes. After this incubation, the samples were centrifuged at 13,000 rpm for 10 minutes. The supernatant was discarded, and the DNA pellets were washed with 1 ml of 80 % ethanol.

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The washed DNA pellets were air-dried at room temperature and subsequently dissolved in 100 µl of low salt buffer. The extracted genomic DNA was quantified using a Nanodrop 2000C Spectrophotometer (Thermo Scientific, USA), and its quality was assessed using a 0.8 % agarose gel.

SSR markers and Polymerase Chain Reaction (PCR) amplification

From the collection of primers used for *Cyperus rotundus* marker transferability investigations (Arias et al., 2011), a total of twenty SSR primers were initially chosen. A primer validation process for bands applification was executed, resulting in the final selection of nine primers for this study (Table 3.3). PCR was performed using the OneTaq Quickload master mix PCR kit. The PCR amplification reaction volume was 10 µl was composing 6 µl of 2 X OneTaq Quickload master mix, 0.5 µl each of forward and reverse primers, 1 µl of 50 ng DNA template, and 2 µl of nuclease-free sterile water (NFSW).

PCR amplification was executed using a 96-well PCR thermal cycler (Veriti$^{(R)}$ AB Biosystems). The PCR cycling profile involved an initial denaturation at 95°C for 1 minute, followed by 60°C for 1 minute (for 2 cycles), then 27 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, extension at 68°C for 30 seconds, a final extension at 68°C for 4 minutes, and a hold at 4°C. For visualization, the PCR products were combined with 6X bromophenol blue dye (2 µl).

The amplified products were then separated on a 6% polyacrylamide gel in Tris Boric acid EDTA (TBE) buffer, stained with Ethidium bromide, and subsequently captured using an Alphaimager HP (Protein Simple, USA) imaging system.

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Table 3.3: SSR primers, sequences and expected product size used for the study.

Primer Name	Repeat Motifs	Primer Sequence	Expected band
StvCyR_1a	(AG)	GCATTTCGTCACCTTCCATTAAAC (F) TTACTTTGTTTGCAGTTGCAGAGG (R)	193
StvCyR_27b	(TGG)	GAGTGAGGGAGTGAGAGAGGGAC (F) TATAGCAAAGTCAGCAGCGCAC (R)	95–444
StvCyR_64a	(GA)	GTACAAACCACAAACCGTAGACCC (F) ACTCTCCTCCTCCATCGTAAGCTC (R)	114–138
StvCyR_126a	(GA)	GGCCTCCGTAAGAAGAAGAATGAC (F) AAGTTCAAGGCAGACGTTAAGCAC (R)	165-166
StvCyR_142a	(GA)	CACGGTAAATTAAACATCACACGG (F) TTGATCTAACACTTGTACTGCGCC (R)	112
StvCyR_181a	(GATA)	TCAATAGAAGAATCCCACTCAGCC (F) GTGGAGGTAAAGATCAGCAACCAG (R)	135-157
StvCyR_156a	(AT)	TGCCCAGCTTTACATCTTAATTGC (F) CTGAACAACTGGCACATACAGAGC (R)	182-191
StvCyR_254a	(TA)	AATCATGAAGGTGATTGGACAAGG (F) CATCCATCCTCTTCTTTGTTCTCG (R)	104-149
StvCyR_483a	(GA)	TGTGTTGTGTGGAAGAGAGGAGAG (F) AACAAACCTCAGAACTCAACTGCC (R)	117-455

Source: Arias *et al*. (2011)

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Data Collection and Analysis

Agro-morphological data

A total of eleven (11) morphological traits were meticulously documented, encompassing one qualitative and ten quantitatives. The collected data encompassed the following traits: percentage germination (PG), number of tillers per stand (NT), distance from the last tiller to the mother plant (DTP), plant height (PH), percentage inflorescence (PI), number of tubers per stand (NTS), number of rings per tuber (NRT), length per tuber (LT), width per tuber (WP), tuber shape (TS), and tuber size represented by 100-tuber weight per genotype (WT).

For each genotype within a plot, data were extracted from five randomly selected plants. The measurements of NT, DTP, PH, and PI were taken during the flowering stage (inflorescence). The determination of NTS was carried out at harvest, while measurements for NRT, LT, WP, TS, and WT were conducted four days after harvesting.

PG, NT, PI, NTS, and NRT were determined through direct counting. Measurements of DTP and PH were acquired using a meter rule. LT and WP measurements were captured using a pair of Vernier calipers. Tuber shape was assessed based on the length-to-width ratio (L/W) (Figures 3.2A & 3.2B), categorizing tubers as oval (<1.3), ovoid (1.3-1.8), or oblong (>1.8) following the classification by Pascual et al. (2000). Tuber weight was obtained by weighing 100 randomly selected tubers per genotype using Shpunchun.en 1000g Electronic Precision Analytical Lab Balance from China.

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Figure 3.2A: Width measurement of tuber

Figure 3.2B: Length measurement of tuber

Univariate analysis, involving Analysis of Variance (ANOVA), as well as descriptive statistics such as mean, minimum, maximum, mean square, standard error, standard deviation, and coefficient of variation, were conducted using Genstat version 11.1 and Minitab version 21.1.1.0 statistical packages.

For multivariate analysis encompassing clustering and principal component analysis, the Genstat, Minitab, and PAST version 4.03 statistical packages were employed.

Assessment of the Relationship between Accessions for Morphological Studies

Euclidean distance measurement

Given the variations in measurement occuring in different units, the Pearson correlation coefficient was chosen as the suitable metric for genetic distance, as it not only standardizes the data but also mitigates the impact of scale differences. In this context, the squared correlation was adopted as the genetic distance, following the approach outlined by Edwards (Edwards 1976).

The computation of minimum, maximum, and mean genetic distances within the population was accomplished using the diagonal pairwise distance matrix. All calculations and analyses were executed employing the Minitab version 21.1.1.0 statistical software.

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Cluster analysis

The Unweighted Pair Group Method with Arithmetic Average (UPGMA) cluster analysis was utilized to examine the correlation distance matrix, aiming to discern clusters of similar morphological traits. This technique helps delineate both homogenous and potentially heterogeneous groups (Franco *et al*., 2001), shedding light on the relationships among descriptors. The dendrograms produced were a result of the cluster analysis conducted. The level of agreement between the distance matrix and the dendrogram was evaluated using the cophenetic correlation coefficient (Sokal & Rohlf, 1962), utilizing the Genstat statistical package version 11.1.

Principal component analysis

The correlation matrix was utilized to perform principal component analysis (PCA) in order to reveal non-hierarchical relationships among the genotypes and ascertain the traits contributing most significantly to the differentiation of accessions.

Utilizing singular value decomposition, the Eigen programme was employed to calculate correlation coefficients, eigenvalues, and eigenvectors, as well as the relative and cumulative proportions of total variance attributed to each character. Two-dimensional biplots were generated from the principal components using PAST version 4.03, allowing for the visualization of interrelationships between traits. The computations for Eigen analysis were conducted using the Minitab version 21.1.1.0 statistical package.

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Statistical Analysis of Molecular Data

Bands were ascribed a value of '1' to denote presence and '0' to indicate absence, based on the expected band sizes (as shown in Figure 3). Subsequently, a cluster analysis was conducted on the binary data using the Simple Matching Coefficient method and the Unweighted Pair Group Method with Arithmetic Average (UPGMA) in NTSYS Ver. 2.2.0.

To assess genetic diversity, several parameters were calculated using the method proposed by Nei et al., (1983), utilizing Power Marker Software Ver. 3.25. These genetic diversity parameters included major allele frequency (Na), allele number (Ne), gene diversity or expected heterozygosity (He), observed heterozygosity (Ho), and polymorphic information content (PIC).

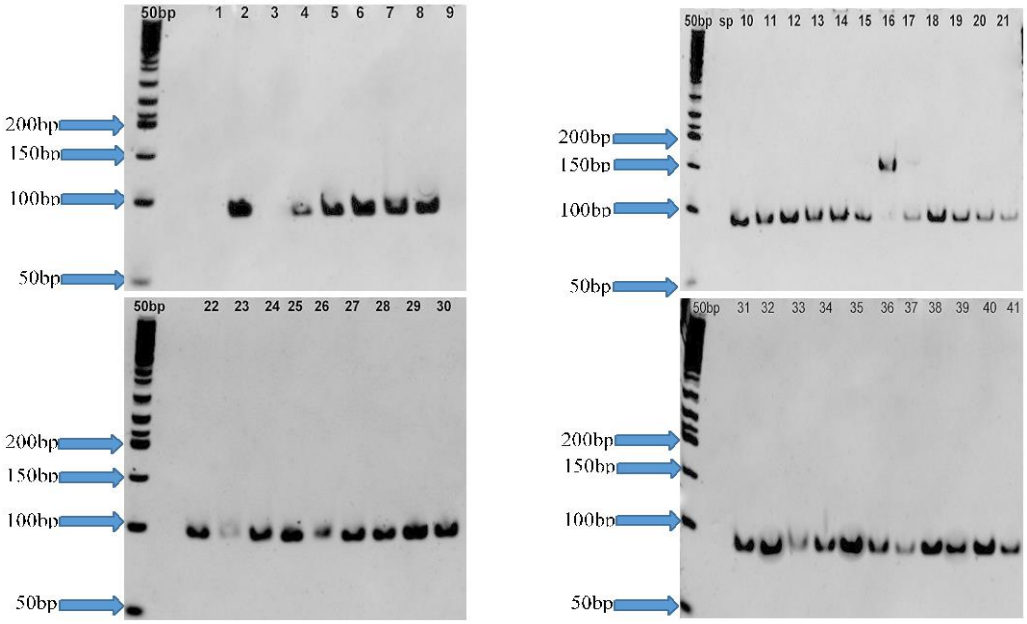

Figure 3.3: PAGE gel image of PCR products of the SSR marker 64A.

50bp= Marker, Sample 1= APR-b, 2= BUO-b, 3= ZEB-Y, 4= ASU-b, 5= MBA-b, 6= GYI-b, 7=TAK-b, 8= YEN-b3, 9= BAW-b, 10= YEN-b2, 11= BAJ-b, 12= OBR-Y, 13= NEB-b, 14= OBR-b, 15= BAJ-Y, 16= BEP-Y, 17= PUT-B, 18= WIO-b, 19= ADU-Y, 20= PUT-b, 21= BEP-B, 22=KAL-b, 23= DED-b, 24= SAN-b, 25= OFF-b, 26= TWI-Y, 27= YEN-b1, 28= ENK-b, 29= DEM-b, 30= OEB-B, 31= BUO-B, 32= KAL-B, 33= OBR-B, 34= OEB-b, 35= TWI-b, 36= ENK-B, 37= WIO-B, 38= TAK-B, 39= MBA-B, 40= BAJ-B, 41= ADU-B.

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Results and Discussion

Variability in Agro-morphological Traits

In all, percentage germination ranged from 3.33% to 100% with a mean of 70.04. The least observed germination percentage was 3±7.7 and was recorded for the accession, OEB-b, a central region brown accession. The mean squares were significantly different for all traits except for the number of tubers per stand (NTS) which on average recorded 39.00 and ranged from 8.20 to 123.60 per stand. A very large coefficient of variation (260.27%) indicating large phenotypic variation was observed for percentage inflorescence (PI). Among the 42 accessions, only seventeen (17) produced inflorescences. The presence of low genetic diversity within the accessions of the population accounted for the very few having inflorescence wheras majority did not flower. This is not surprising as tiger nuts hardly flower (Defelice, 2002) especially with such local accessions which are unimproved coupled with the vegetative propagated nature of the crop that rarely hybridizes naturally. The flowers observed were whitish-green (pale green) for all the accessions that flowered irrespective of the differences in the colours of the tubers. Inflorescence was less frequently observed in the black-tuber genotypes (3) and yellow-tuber genotypes (3) as compared to the brown-tuber genotypes (11). This is in agreement with earlier reports that not all tiger nut accessions do produce flowers (Yang *et al*., 2022) and even those which produce flowers rarely do so on the field (De Vries, 1991). Therefore the assertion that tiger nuts produce copious seeds for propagation (Stoller and Sweet, 1987; Larridon *et al*., 2013; De Castro *et al*., 2015) may not apply to all accessions.

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Yield (HTW) and yield components (LT, WT, TS) also varied widely as indicated by a high coefficient of variation values and highly significant ($p < 0.001$) mean squares (Table 3.4). This is an indication that selecting accessions with higher number and weight of tubers per stand and inducing mutation in them is very crucial to addressing the issue of lower yield among this gene pool for more diversity (Roychowdhury & Tah, 2013; Oladosu *et al*., 2016).Among the 42 accessions, the highest mean weight (HTW) of 201.7g± 0.4 was produced by in OFF-b from the Eastern Region. This was followed by another brown accession, APR-b from the Central Region with 186.0g ± 0.1. BUO-B from the Bono East Region ranked 7th with HTW of 175.4g± 0.4 and was the highest yield among the black accessions. Next to this was ENK-B from the Central Region, another black accession which produced a HTW of 174.4g±0.4 (Table 3.5).

Twenty- five (25) accessions out of the 42 studied were oval. Majority of these were accessions collected from the Central Region of Ghana and possessed all three tuber colours (black, brown and yellow). Ten were ovoid where the majority were brown and were also Central Region accessions. The remaining seven accessions had oblong and brown tubers with the majority (99%) made up of Eastern Region collection (Table 3.1). This affirms an earlier observation made that a greater number of brown oblong tiger nut accessions in Ghana are of Eastern Region origin (Asare *et al*., 2020).

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Table 3.4: Means, standard errors, mean squares, standard deviations, coefficient of variations and range of 11 morpho-descriptors of the 42 tiger nut genotypes used for the study and levels of significance differences.

Variable	Mean	SE	MS	SD	CV	Min	Max	Sig. Level (p-value)
PG	70.04	2.47	1323.50	27.70	39.54	3.33	100.00	***
NT	13.09	0.51	53.72	5.752	43.93	1.00	33.00	***
DTP	9.45	0.24	9.53	2.673	28.27	1.40	17.20	*
PH	91.77	1.97	806.10	22.10	24.08	15.80	132.40	***
PI	0.33	0.08	1.02	0.85	260.27	0.00	5.00	**
NTS	39.00	1.82	504.70	20.46	52.45	8.20	123.60	Ns
NRT	5.75	0.05	0.62	0.50	8.71	5.00	7.00	***
LT	19.46	0.53	76.12	5.95	30.58	8.45	38.70	***
WT	14.22	0.21	11.33	2.38	16.71	6.75	19.75	***
TS	1.38	0.04	0.36	0.40	29.32	0.92	2.79	***
HTW	133.46	3.44	4554.42	38.65	28.96	30.98	201.87	***

PG= percentage germination; NT= number of tillers per plant, DTP= distance of the last tiller from the main plant, PH= plant height, PI= percentage inflorescence, NTS= number of tubers per stand, NRT= number of rings per tuber, LT= length of tuber, WT= width of tuber, TS= tuber shape; HTW= hundred tuber weight.

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Table 3.5: Mean, minimum, maximum and standard deviations of morpho-descriptors and yield traits of the 42 tiger nut genotypes.

Genotype	PG (%)	NT	DTP (cm)	PH (cm)	PI (%)	NTS	NRT	LT (mm)	WT (mm)	TS	HTW (g)
ADU- b	86.7 (60-100) 23.1	14.9 (11-20) 4.4	11.5 (10-15) 2.8	103.6 (93-117) 12.5	0.0 (0-0) 0.0	28.7 (11-46) 17.1	6.0 (6-6) 0.0	26.5 (22-34) 6.8	14.0 (12-16) 2.1	1.9 (2-2) 0.3	183.0 (183-183) 0.1
ADU- B	94.4 (87-100) 6.9	10.1 (8-13) 2.9	8.4 (7-10) 1.3	101.6 (91-114) 11.83	0.0 (0-0) 0.0	42.1 (29-64) 18.9	6.0 (6-6) 0.0	18.8 (17-20) 1.4	16.7 (15-19) 1.9	1.1 (1-1) 0.1	132.0 (132-132) 0.0
ADU- Y	91.1 (80-100) 10.2	8.1 (7-10) 1.2	9.6 (9-10) 0.3	95.1 (86-111) 13.8	0.3 (0-1) 0.6	12.9 (11-15) 2.0	6.0 (6-6) 0.0	22.5 (19-25) 3.0	13.1 (12-15) 1.2	1.7 (2-2) 0.3	113.4 (113-114) 0.4
APR- b	74.4 (63-83) 10.2	22.7 (12-29) 9.2	9.9 (8-12) 2.0	95.5 (90-101) 5.5	0.3 (0-1) 0.6	27.9 (11-48) 18.9	5.0 (5-5) 0.0	23.3 (17-32) 7.8	13.4 (11-15) 2.1	1.7 (1-2) 0.3	186.0 (186-186) 0.1
ASU- b	57.8 (40-83) 22.7	18.2 (14-26) 6.6	11.5 (10-13) 2.9	104.2 (93-112) 9.6	0.3 (0-1) 0.6	42.1 (42-43) 0.6	6.3 (6-7) 0.6	26.5 (23-32) 5.1	14.6 (14-16) 0.9	1.8 (2-2) 0.2	185.2 (185-185) 0.2
BAJ- b	41.1 (20-63) 21.7	12.0 (5-18) 6.7	10.0 (5-13) 4.5	96.6 (34-108) 38.1	0.3 (0-1) 0.6	34.1 (16-48) 16.3	5.3 (5-6) 0.6	18.3 (15-22) 3.7	16.1 (15-17) 0.9	1.1 (1-1) 0.2	114.1 (114-114) 0.1
BAJ- B	85.6 (73-100) 13.5	9.7 (6-12) 3.4	9.6 (9-10) 0.7	83.3 (81-85) 1.6	0.3 (0-1) 0.6	21.9 (12-32) 10.0	6.0 (6-6) 0.0	16.5 (14-19) 2.1	15.3 (13-17) 2.0	1.1 (1-1) 0.0	131.2 (131-132) 0.4
BAJ- Y	22.2 (7-40) 16.8	10.7 (6-18) 6.8	4.2 (1-8) 3.1	49.3 (26-81) 28.0	0.0 (0-0) 0.0	47.9 (25-87) 33.8	5.0 (5-5) 0.0	14.4 (13-16) 1.5	13.4 (12-16) 1.9	1.1 (1-1) 0.0	78.4 (78-79) 0.4
BAW- b	85.6 (60-100) 22.2	24.2 (14-33) 9.6	8.7 (8-10) 1.1	104.8 (97-118) 11.6	1.3 (0-4) 2.3	61.7 (15-113) 49.0	5.0 (5-5) 0.0	8.8 (8-9) 0.4	8.7 (13-14) 2.0	1.0 (1-1) 0.1	177.2 (177-177) 0.2
BEP- B	62.2 (37-80) 22.7	22.2 (16-29) 6.6	11.4 (10-13) 1.4	88.6 (83-94) 5.2	0.0 (0-0) 0.0	39.1 (33-45) 6.1	6.0 (6-6) 0.0	18.0 (16-20) 1.7	13.7 (13-14) 1.1	1.3 (1-1) 0.1	143.3 (143-144) 0.4
BEP- Y	47.8 (37-57) 10.2	21.7 (14-33) 10.0	10.5 (10-12) 1.2	82.6 (81-85) 2.5	0.7 (0-2) 1.2	20.6 (8-28) 10.8	5.0 (5-5) 0.0	15.5 (14-17) 1.5	13.4 (12-16) 2.3	1.2 (1-1) 0.1	86.1 (86-86) 0.1
BUO- b	88.9 (77-97) 10.7	13.6 (11-18) 3.8	8.5 (7-11) 2.2	113.3 (96-126) 15.8	0.0 (0-0) 0.0	43.1 (40-46) 3.0	6.0 (6-6) 0.0	30.8 (27-34) 3.3	15.4 (15-16) 0.6	2.0 (2-2) 0.2	126.0 (126-126) 0.1
BUO- B	74.4 (50-100) 25.0	16.6 (10-22) 5.6	9.2 (7-11) 2.0	100.1 (92-104) 6.6	0.0 (0-0) 0.0	45.9 (29-71) 22.4	6.0 (6-6) 0.0	19.9 (18-23) 2.6	17.2 (16-20) 2.2	1.2 (1-1) 0.0	175.4 (175-176) 0.4
DED- b	77.8 (73-83) 5.1	14.3 (4-19) 5.0	10.9 (9-14) 2.7	99.3 (90-108) 9.1	0.0 (0-0) 0.0	38.5 (12-54) 23.2	6.0 (6-6) 0.0	30.3 (25-39) 7.3	12.4 (10-14) 1.8	2.4 (2-3) 0.4	136.0 (136-136) 0.1
DEM- b	63.3 (40-100) 32.1	10.1 (8-12) 1.8	8.9 (8-10) 1.2	94.6 (84-108) 12.5	0.0 (0-0) 0.0	51.2 (36-69) 17.1	5.3 (5-6) 0.6	25.5 (21-28) 3.8	13.6 (12-16) 2.2	1.9 (2-2) 0.4	180.1 (180-180) 0.2
ENK- b	45.6 (30-67) 19.0	13.5 (9-19) 5.1	10.5 (10-11) 0.7	93.3 (79-107) 13.6	0.0 (0-0) 0.0	38.7 (17-61) 22.3	6.0 (6-6) 0.0	19.7 (16-22) 3.0	14.9 (14-17) 1.7	1.3 (1-1) 0.1	133.3 (133-133) 0.3
ENK- B	87.8 (77-100) 11.7	17.7 (13-23) 4.8	12.6 (11-14) 1.4	97.9 (84-107) 12.4	0.0 (0-0) 0.0	58.9 (53-64) 5.2	6.0 (6-6) 0.0	18.2 (17-20) 1.7	15.8 (13-18) 2.3	1.2 (1-1) 0.1	174.4 (174-175) 0.4
GYI- b	57.8 (43-67) 12.6	14.3 (8-19) 5.3	10.1 (5-15) 4.8	82.1 (69-104) 19.1	0.0 (0-0) 0.0	24.3 (10-37) 13.6	6.0 (6-6) 0.0	15.2 (13-18) 2.6	14.8 (13-17) 1.8	1.0 (1-1) 0.1	100.7 (100-101) 0.6
KAL- b	82.2 (47-100) 30.8	11.8 (10-15) 3.0	9.8 (9-11) 1.2	95.5 (77-109) 17.0	0.0 (0-0) 0.0	35.3 (17-51) 17.1	5.0 (5-5) 0.0	16.7 (14-20) 3.3	13.3 (12-15) 1.1	1.2 (1-1) 0.1	78.0 (78-78) 0.0
KAL- B	98.9 (97-100) 1.9	12.0 (10-14) 2.0	9.7 (8-11) 1.4	97.7 (90-102) 7.0	0.3 (0-1) 0.6	33.3 (21-42) 10.9	6.0 (6-6) 0.0	17.6 (17-20) 1.6	15.8 (14-18) 2.1	1.1 (1-1) 0.1	105.6 (105-106) 0.6
MBA- b	61.1 (17-90) 39.1	10.8 (7-15) 4.1	9.5 (6-12) 3.1	84.1 (42-119) 39.0	0.0 (0-0) 0.0	41.1 (21-59) 19.0	6.0 (6-6) 0.0	24.3 (20-30) 5.3	14.8 (12-17) 2.3	1.6 (2-2) 0.1	162.0 (162-163) 0.4
MBA- B	60.0 (27-90) 31.8	13.1 (10-15) 2.8	9.9 (8-11) 1.4	90.5 (78-105) 13.5	0.0 (0-0) 0.0	57.8 (52-64) 6.0	6.0 (6-6) 0.0	17.8 (17-18) 1.0	16.4 (16-18) 1.0	1.1 (1-1) 0.1	174.1 (174-174) 0.3

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Table 3.1 Continued

NEB- b	82.2 (53-100) 25.2	9.9 (8-12) 2.1	7.4 (6-9) 1.6	105.5 (75-122) 26.1	1.0 (0-2) 1.0	20.0 (14-26) 6.0	6.0 (6-6) 0.0	18.0 (14-23) 4.8	12.5 (11-17) 1.0	1.4 (1-2) 0.3	119.0 0.1	(119-119)
OBR- b	90 (77-100) 12.0	16.1 (16-16) 0.1	10.5 (7-13) 3.1	104.0 (99-107) 4.2	0.7 (0-2) 1.2	33.1 (17-42) 14.2	5.0 (5-5) 0.0	23.5 (16-32) 7.8	13.3 (12-14) 1.0	1.7 (1-2) 0.4	169.5 0.5	(169-170)
OBR- B	66.7 (47-90) 21.9	14.7 (9-18) 4.8	11.3 (8-13) 2.7	86.4 (82-89) 3.6	0.0 (0-0) 0.0	49.7 (32-77) 23.9	6.0 (6-6) 0.0	23.0 (17-30) 6.7	14.4 (11-16) 2.6	1.7 (1-3) 0.9	138.2 0.3	(138-138)
OBR- Y	95.6 (87-100) 7.7	11.7 (10-14) 2.3	9.9 (7-11) 2.2	110.3 (104-121) 9.2	1.0 (0-2) 1.0	33.3 (30-35) 2.7	5.3 (5-6) 0.6	18.2 (15-23) 4.5	15.5 (15-17) 0.5	1.1 (1-1) 0.2	118.1 0.3	(118-118)
OEB- b	12.2 (3-17) 7.7	2.6 (1-3) 1.4	3.4 (2-4) 1.6	27.8 (16-34) 10.4	0.0 (0-0) 0.0	13.0 (12-14) 1.0	6.0 (6-6) 0.0	12.6 (12-13) 0.2	9.9 (10-10) 0.2	1.3 (1-1) 0.0	43.4 (43-44) 0.4	
OEB- B	48.9 (27-83) 30.0	11.5 (10-14) 2.5	10.3 (8-12) 1.9	77.0 (67-82) 8.8	0.0 (0-0) 0.0	46.9 (43-53) 5.1	6.3 (6-3) 0.6	19.4 (15-24) 4.8	16.3 (14-20) 3.0	1.2 (1-1) 0.1	163.0 0.0	(163-163)
OFF- b	76.7 (43-100) 29.6	11.6 (8-15) 3.3	8.9 (7-10) 1.4	112.8 (99-132) 17.4	3.0 (1-5) 2.0	33.6 (22-49) 13.9	6.0 (6-6) 0.0	28.7 (27-31) 2.0	14.4 (11-18) 3.2	2.1 (2-3) 0.6	201.7 0.4	(201-202)
PUT- b	63.3 (40-97) 29.6	14.7 (11-17) 3.3	12.3 (10-14) 1.7	83.4 (82-86) 2.3	0.0 (0-0) 0.0	41.0 (37-43) 3.6	6.7 (6-7) 0.6	23.4 (21-28) 3.7	16.0 (15-17) 1.2	1.5 (1-2) 0.1	159.1 0.2	(159-159)
PUT- B	85.6 (70-97) 13.9	11.5 (10-12) 1.5	9.9 (7-11) 1.2	104.1 (93-117) 12.3	0.0 (0-0) 0.0	38.6 (24-52) 13.8	6.0 (6-6) 0.0	17.3 (17-18) 0.9	16.2 (16-17) 0.9	1.1 (1-1) 0.1	171.2 0.2	(171-171)
SAN- b	76.7 (67-97) 17.3	8.7 (7-11) 2.0	8.8 (8-10) 1.3	101.7 (92-109) 8.6	0.0 (0-0) 0.0	30.4 (23-43) 11.1	6.0 (6-6) 0.0	19.5 (18-21) 1.3	14.8 (14-16) 1.3	1.3 (1-1) 0.1	113.1 0.3	(113-114)
TAK- b	81.1 (63-93) 15.8	12.0 (8-16) 3.8	10.5 (8-13) 2.7	96.1 (83-105) 11.2	1.0 (0-3) 1.7	28.7 (22-41) 11.0	6.0 (6-6) 0.0	19.7 (15-24) 4.4	15.5 (15-17) 1.3	1.3 (1-1) 0.2	135.1 0.3	(135-135)
TAK- B	71.1 (50-87) 19.0	12.5 (9-18) 4.5	10.1 (8-12) 2.1	92,6 (89-99) 5.4	0.3 (0-1) 0.6	47.7 (26-63) 19.0	6.0 (6-6) 0.0	18.6 (16-20) 2.2	15.8 (15-17) 0.8	1.2 (1-1) 0.1	119.4 0.4	(119-120)
TWI- b	41.1 (27-57) 15.0	11.7 (8-17) 4.5	8 (5-10) 2.5	92.7 (62-108) 26.4	0.3 (0-1) 0.6	54.3 (35-91) 32.0	5.3 (5-6) 0.6	25.7 (17-36) 9.8	14.6 (13-17) 2.2	1.7 (1-2) 0.4	167.1 0.5	(167-168)
TWI- Y	73.3 (20-100) 46.2	10.5 (8-14) 2.9	9.3 (8-12) 2.2	86.9 (50-109) 32.1	0.0 (0-0) 0.0	51.3 (32-80) 25.2	5.7 (5-6) 0.6	14.0 (13-15) 1.1	13.5 (13-14) 0.9	1.0 (1-1) 0.1	119.0 0.1	(119-119)
WIO- b	36.3 (13-50) 20.0	11.6 (7-14) 3.8	7.3 (4-9) 2.5	81.7 (48-99) 29.5	0.0 (0-0) 0.0	44.5 (21-67) 23.2	5.0 (5-5) 0.0	19.6 (19-21) 1.0	13.7 (13-15) 1.1	1.4 (1-1) 0.0	129.4 0.5	(129-130)
WIO- B	43.3 (10-77) 33.3	14.4 (6-21) 7.3	7.9 (3-11) 4.1	60.6 (16-85) 38.5	0.0 (0-0) 0.0	40.4 (18-57) 20.2	6.0 (6-6) 0.0	16.1 (15-18) 1.7	14.5 (14-16) 1.0	1.1 (1-1) 0.0	120.3 0.3	(120-121)
YEN- b1	100 (100-100) 0.0	10.3 (8-14) 2.9	10.1 (7-12) 2.7	111.7 (105-118) 6.2	0.0 (0-0) 0.0	37.9 (29-54) 14.0	5.0 (5-5) 0.0	15.5 (14-17) 1.5	12.5 (12-13) 0.5	1.2 (1-1) 0.2	93.4 (93-94) 0.4	
YEN- b2	85.6 (60-100) 22.2	9.2 (5-12) 4.0	8.0 (6-10) 2.1	99.3 (94-109) 8.1	1.3 (0-2) 1.2	27.4 (25-31) 3.5	5.3 (5-6) 0.6	16.0 (14-20) 3.4	14.2 (14-15) 0.6	1.1 (1-1) 0.2	97.4 (97-98) 0.4	
YEN- b3	76.7 (30-100) 40.4	14.1 (6-20) 7.1	7.7 (5-10) 2.1	89.2 (52-110) 32.0	1.0 (0-2) 1.7	75.7 (14-124) 55.8	6.0 (6-6) 0.0	9.8 (9-11) 1.1	8.0 (7-9) 1.1	1.2 (1-1) 0.1	31.0 (31-31) 0.1	
ZEB- Y	88.9 (67-100) 19.2	8.3 (6-11) 2.3	10.4 (7-17) 5.9	97.1 (93-129) 28.8	0.0 (0-0) 0.0	44.5 (33-51) 9.8	6.0 (6-6) 0.0	14.5 (13-17) 2.5	14.9 (13-18) 3.1	1.0 (1-1) 0.1	120.5(120-121) 0.2	

PG= percentage germination; NT= number of tillers per plant, DTP= distance of the last tiller from the main plant, PH= plant height, PI= percentage inflorescence, NTS= number of tubers per stand, NRT= number of rings per tuber, LT= length of tuber, WT= width of tuber, TS= tuber shape; HTW= hundred tuber weight.

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Cluster analysis of morpho-descriptors

The hierarchical cluster analysis based on complete linkage technique and Euclidean distance similarity matrix grouped the accessions into seven major clusters at a similarity distance measure of 0.94 within a range of 0.8 to 1.0 (Figure 3.5). The optimum number of clusters were generated following the elbow method (Thorndike, 1953). With this method, the nuber of clusters were chosen at the point on the screw plot where the curve bends (Figure 3.4). The high-ranged similarity index indicated low genetic variability within the genotypes as indicated by Asare and co-workers (Asare *et al*., 2020). A similar observation was made when twenty-four local tiger nut accessions from Ghana were categorized into six main clusters as per the agro-morphological traits (Donkor *et al*., 2019).

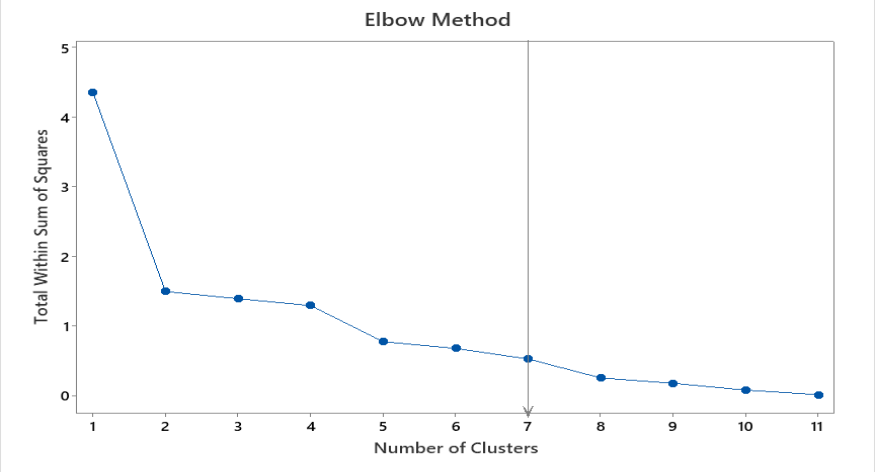

Figure 3.4. Srew plot showing the optimal number of clusters determined by the elbow method.

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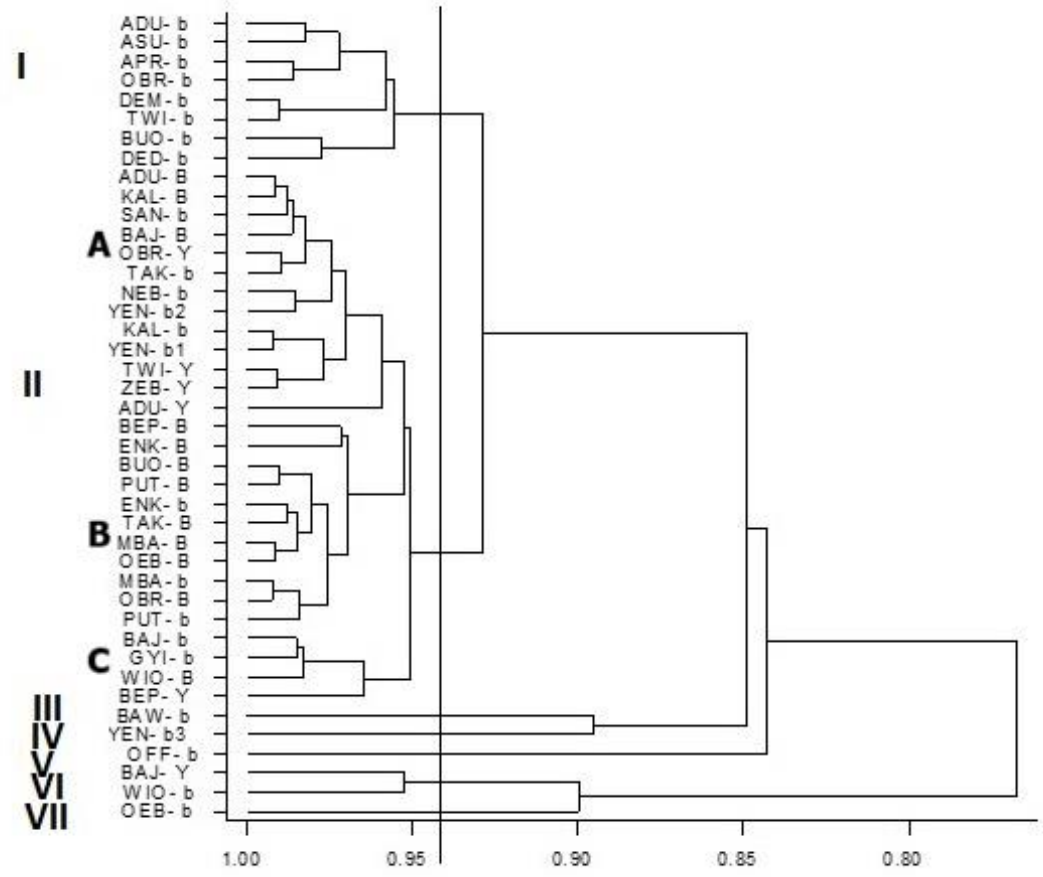

Figure 3.5: Relationship among the 42 tiger nut genotypes based on 11 morpho-descriptors. Clusters were generated using the Euclidean similarity matrix and UPGMA cluster method.

Cluster I was made up of 8 accessions (19.0% of the total accessions). These accessions were clustered on the premise of similarity in yield and its related characteristics as the length of tuber, tuber shape and hundred tuber weight (Figure 3.6). Their tubers were oblong longer and characterized by high tuber weight (hundred tuber weight) (Table 3.5). The accessions were all brown-coloured with four from the Eastern Region, three from Central Region and one from Bono East Region.

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Cluster II was the largest and heterogeneously consisted of 28 accessions with three sub-clusters. It contained 67% of the accessions being a mixture of brown, yellow and black-coloured accessions and the tubers were oval or ovoid. Accessions here were grouped based on percentage germination, number of tillers, distance of the last tiller from the main plant, plant height, number of rings per tuber and tuber width. Sub-cluster A had 13 of the accessions, which were characterized by tall plant height, high number of rings per tuber and production of inflorescences. The tubers were mostly oval with few ovoid shapes and from Upper East, Upper West, Northern, Eastern and Central Regions. Sub-cluster B had 11 accessions that were characterized by very high numbers of tiller numbers (Table 3.5). They were dominated by accessions with black-coloured tubers; and made up of about 90% of Central Region collections, except for TAK-B which was collected from Takpo in the Upper West Region of Ghana. These findings agree with earlier observations made that black tiger nuts in Ghana are mostly found in the Central Region (Asare *et al*., 2020). TAK-B might have found itself in the Upper West Region probably due to migration by traders. Sub-cluster C had four accessions consisting of two browns, one black and one yellow accession which produced oval-shaped tubers. These accessions were similar in traits such as having moderately low germination percentages and producing medium numbers of tillers (Table 3.5). However, three accessions (BAJ-b, WIO-B, BEP-Y) were Central Region collections with the remaining one (GYI-b) from Upper West.

Clusters III and IV comprised of only one accession each (BAW-b and YEN-b3) which produced brown-coloured and oval in shaped tubers, and from the Upper East and Nothern regions respectively. YEN-b3 produced the highest

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number of tubers and was followed by BAW-b. Though YEN-b3 produced the highest number of tubers, it had the least in hundred-tuber-weight among all the accessions; thus, the smallest tubers were produced by it. Cluster V was made up of only one accession (OFF-b), an Eastern Region collection. It produced oblong-brown tubers, the highest number of inflorescence and the highest hundred-tuber-weight (Table 3.5). Cluster VI had two accessions from the Central Region, which produced oval-type, yellow and brown tubers (Figure 3.5). They were grouped because they had low percentage germination and were characterized by short plant height (Table 3.5). Cluster VII was made up of only one oval-brown accession which hailed from the Central Region of Ghana. It had the lowest germination percentage and was the shortest in height with very short tiller distances (Table 3.5).

Principal component analysis of morphological studies

Variability among the 42 tiger nut accessions for the traits (both qualitative and quantitative) by the PCA biplot revealed a scattered distribution of the accessions for all the traits indicating large variations. However, the biplot topography exhibited clustered coverage for many accessions with only three of them largely dispersed from the others inferring less genetic diversity among the accessions. Accessions within tight angles of $< 90^0$ and angles $> 270^0$ are closely related while those 180^0 apart are uncorrelated and could be considered in heterotic groups for future improvement. The three accessions that separated from the others were (18: GYI-b), (28: OEB-B) and (30: PUT-b) (Figure 3.6.).

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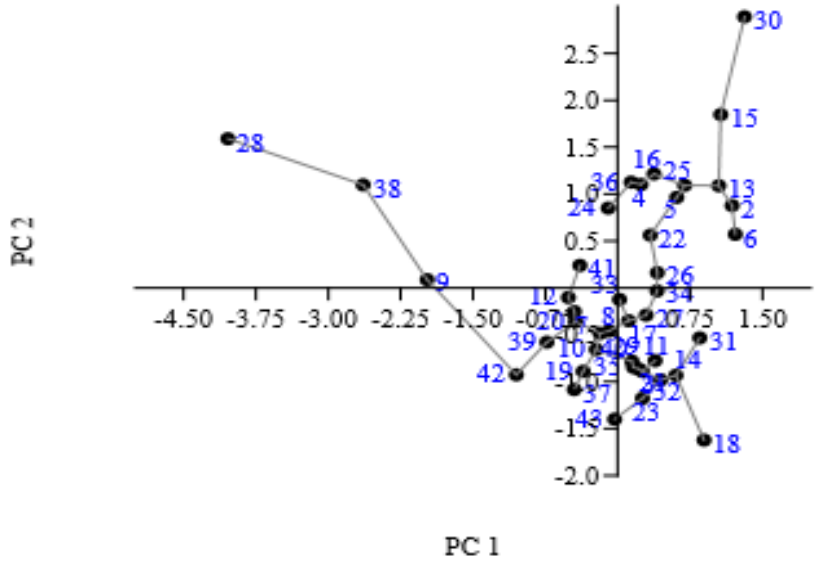

Figure 3.6: PCA biplot showing groupings of the 42 tiger nut accessions based on 11 morpho-descriptors evaluated on the Eigenvalue scale.

The first five PCs accounted for a total of 84.55% variability among the 42 genotypes (Table 3.6). PC1 accounted for 39.629% of the variability with an Eigenvalue of 4.359 (Tables 3.6. & 3.7.). Traits that made major contribution to the variability included percentage germination, plant height, number of tillers, the distance of the last tiller from the main plant, number of rings per tuber and width of the tuber (Table 3.6.). PC 2 contributed to the variation by the length of the tuber, tuber shape and hundred tuber weight (Figure 3.7.).

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Table 3.6: Eigenvalues of and contribution to the variance by the first five principal components of morphological trait evaluated.

Variable	PC1	PC2	PC3	PC4	PC5
PG	0.307	-0.157	0.465	0.261	-0.183
NT	0.227	-0.153	0.108	-0.562	0.536
DTP	0.399	-0.265	-0.024	0.001	0.273
PH	0.403	-0.040	0.357	0.068	-0.153
PI	0.070	0.303	0.623	-0.061	0.064
NTS	0.051	-0.306	-0.037	-0.607	-0.677
NRT	0.338	-0.255	-0.136	0.199	-0.202
LT	0.354	0.447	-0.297	0.049	-0.107
WT	0.307	-0.280	-0.313	0.310	0.089
TS	0.291	0.577	-0.114	-0.090	-0.186
HTW	0.327	0.138	-0.193	-0.304	0.154
Eigenvalue	4.359	1.492	1.389	1.291	0.770
% Variance	39.63	13.56	12.63	11.73	7.00
% Cumulative	39.63	53.19	65.82	77.55	84.55

PG= percentage germination; NT= number of tillers per plant, DTP= distance of the last tiller from the main plant, PH= plant height, PI= percentage inflorescence, NTS= number of tubers per stand, NRT= number of rings per tuber, LT= length of tuber, WT= width of tuber, TS= tuber shape; HTW= hundred tuber weight.

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Table 3.7: Principal component analysis using 11 morphological traits evaluated among the 42 tiger nut accessions cultivated in Ghana based on correlation coefficient similarity matrix.

PC	Eigenvalue	% Variance	Proportion	Cumulative
1	4.359	39.629	0.396	0.396
2	1.492	13.564	0.136	0.532
3	1.389	12.630	0.126	0.658
4	1.291	11.734	0.117	0.776
5	0.769	6.995	0.070	0.846
6	0.674	6.131	0.061	0.907
7	0.522	4.743	0.047	0.954
8	0.249	2.263	0.023	0.977
9	0.172	1.561	0.172	0.016
10	0.074	0.670	0.007	1.00

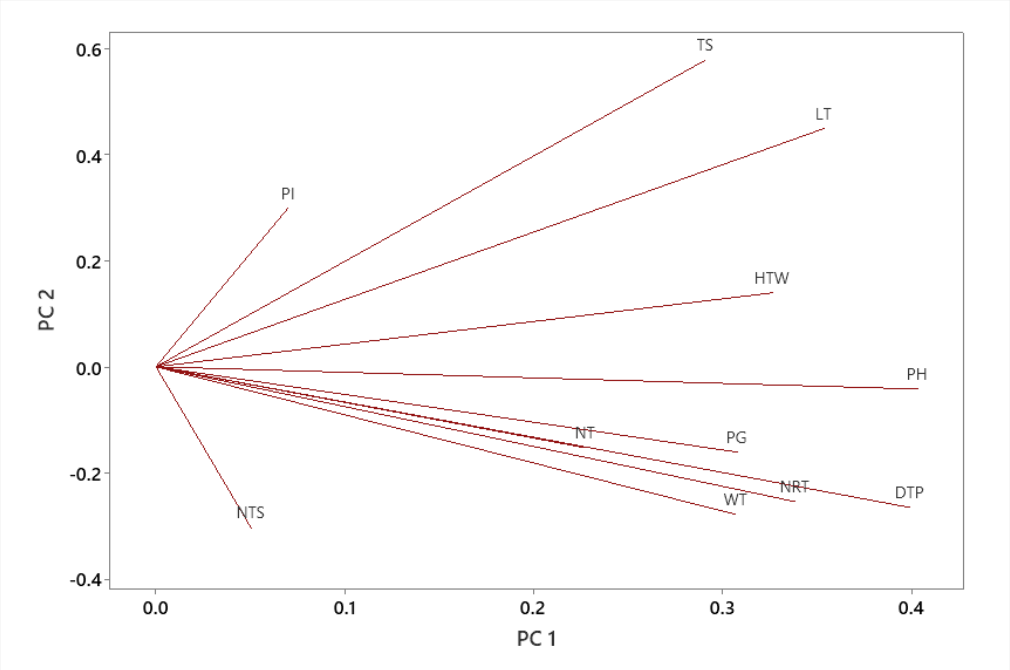

Figure 3.7: PC biplots of variables indicating the contributions of the various traits of the tiger nut accessions.

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Correlation among the morphological traits:

The highest significant positive correlation of 0.899 at $p<0.0001$ was observed between the tuber shape and length of the tuber. This was followed by the association between plant height and percentage germination recording 0.844 at $p<0.0001$. A strong significant positive correlation of 0.636 ($p<0.0001$) existed between the distance of the last tiller from the main plant and the number of rings per tuber on one side and also between the width of the tuber (0.584 at $p<0.0001$) on another (Figure 3.8). Tillering and its effects on yield and yield related traits in tiger nuts have not fully been exploited (Asare *et al*., 2020) as in in rice and wheat (Iftikhar et al., 2012; Y. Wang et al., 2017). However, the strong significant positive correlation of the current study is suggestive that, selecting a genotype for tuber characteristics such as length/width ratio (tuber shape) and number of rings per tuber will not be deleterious to the selection of tillering distance, as certain traits could be selected as proxies for others (Adu *et al*., 2018). Again, tillering distance (DTP) significantly positively correlated with percentage germination (0.543 at $p<0.0001$). On yield and its related traits, a strong positive correlation was observed between hundred-tuber-weight and length of tuber (0.593 at $p<0.0001$). On plant growth characteristics, positive correlations were recorded between hundred tuber weight and the number of tillers (0.407 at $p<0.001$), hundred-tuber-weight and distance of the last tiller from the main plant (0.474 at $p<0.001$) and hundred-tuber-weight and plant height (0.429 at $p<0.001$). Hence in tiger nut breeding, imposing selection of a genotype for tuber weight can be based on plant architectural characteristics including number of tillers, tillering distance and plant height (Asare *et al*., 2020).

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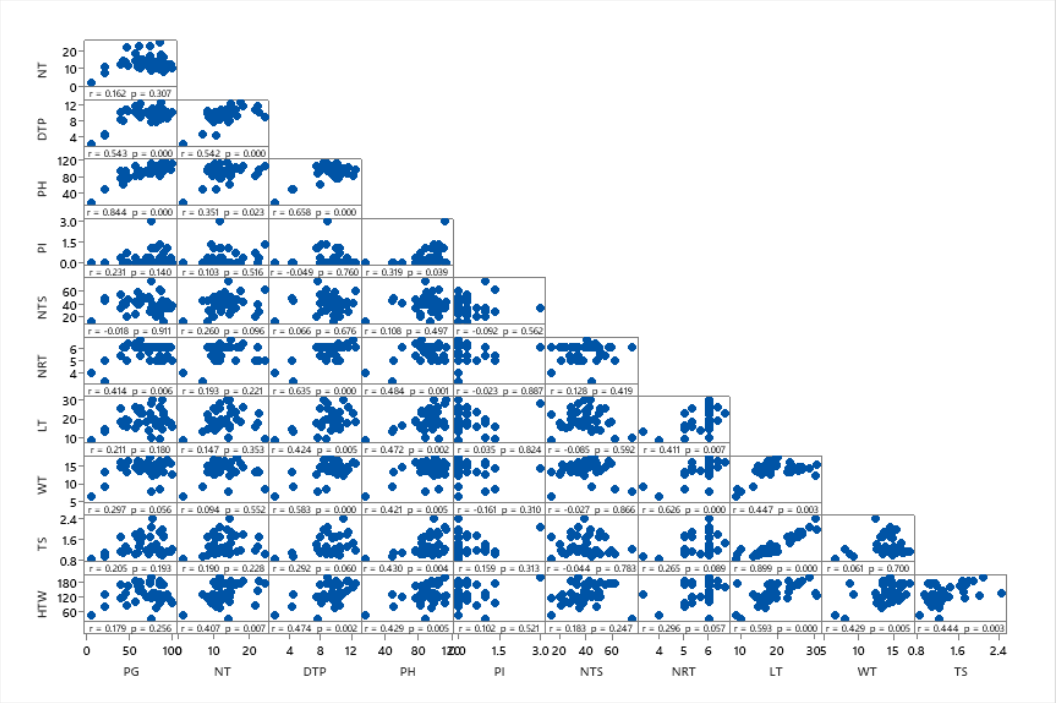

Figure 3.8: Pearson correlation matrix among the 11 morpho-descriptors of the 42 tiger nut accessions.

Diversity among 41 Ghanaian Tiger Nut Accessions using SSR Markers

A total of 141 alleles were generated across the nine SSR loci of the 41 tiger nut genotypes and the number of alleles per locus ranged from 9 to 22 with an average of 15.67 ± 3.97 per locus (Table 3.8.).

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Table 3.8: Number of alleles (Na), effective number of alleles (Ne), gene diversity or expected heterozygosity (He), observed heterozygosity (Ho), polymorphic information content (PIC) based on nine SSR loci

Marker	Na	Ne	He	Ho	PIC
StvCyR_1a	0.27	14.00	0.84	0.12	0.82
StvCyR_27b	0.59	19.00	0.65	0.10	0.64
StvCyR_64a	0.17	18.00	0.91	0.07	0.91
StvCyR_126a	0.24	14.00	0.87	0.07	0.86
StvCyR_142a	0.63	12.00	0.58	0.05	0.57
StvCyR_156a	0.12	18.00	0.92	0.02	0.92
StvCyR_181a	0.24	15.00	0.87	0.10	0.85
StvCyR_254a	0.15	22.00	0.93	0.54	0.93
StvCyR_483a	0.63	9.00	0.58	0.00	0.56
Total	3.04	141	7.15	1.07	7.06
Min	0.12	9	0.58	0	0.56
Max	0.63	22	0.93	0.54	0.93
Mean	0.34	15.67	0.79	0.12	0.78
SD	0.21	3.97	0.15	0.16	0.15
X^2cal				5.38	
X^2tab				15.5	
Probability				0.05	

The lowest and highest number of alleles were recorded for StvCyR_483a and StvCyR_254a loci respectively. Allele frequency ranged from 0.12 for StvCyR_156a to 0.63 for StvCyR_142a and StvCyR_483a with an average of 0.34 ± 0.21. The gene diversity or expected heterozygosity ranged from 0.58 to 0.93 with a mean of 0.79 ± 0.15 exceeding 0.50, indicating the existence of rich variability for the SSR loci assessed among the tiger nut accessions. The observed heterozygosity however, ranged from 0.00 to 0.54 with the mean of 0.12 ± 0.16, an indication of low variability among the genotypes evaluated.

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Again based on Chi square (X^2) value of 5.38 at 8 degrees of freedom, the H_o was not significantly different from the critical Chi square value of 15.5 at $P<0.05$, signifying low genetic diversity for the population. This is not surprising as vegetatively propagated crops such as tiger nuts are usually self-sterile (Nybom and Lācis, 2021) and rarely hybridize naturally. In contrast, the high values of H_e over H_o is indicative of admixture effect of genes among tiger nut populations in Ghana, genetic drift, and the high mutation reproducibility rate of SSR primers (Mukhopadhyay and Bhattacharjee, 2016). The PIC was of 0.78 ± 0.15, with StvCyR_483a having the lowest (0.56) and the highest (0.93) recorded for StvCyR_254a.

The expected heterozygosity values for all loci were greater than 0.50, with over 84% having PIC values greater than 0.60, which indicates a high discriminating ability of the SSR markers among the accessions. The most discriminative marker was StvCyR_254a with a PIC value of 0.93 followed by StvCyR_156a (0.92) and StvCyR_64a (0.91) (Table 3.9). Using 191 microsatellite markers, five of them were found that distinguished *Cyperus esculentus* from 12 accessions of *Cyperus rotundus* (Arias *et al*., 2011). However, the markers used in the current study were not part of the 191 microsatellites used by Arias *et al*. (2011). Okoli and others found a wide range of variability in purple and yellow nutsedge with RAPD (Okoli *et al*., 1997). High genetic variability in cultivated and wild yellow nutsedge was also detected using RAPD (Abad *et al*., 1998). On the contrary low genetic diversity was observed in *Cyperus esculentus* when AFLP markers were used (Dodet *et al*., 2008). They then suggested that the application of microsatellite genetic diversity studies could be more effective in determining genetic variability in a crop. Akabassi *et al*. (2021), in a systematic

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review, also suggested the use of a more robust tool, such as microsatellite markers to the study of the genetic diversity of *Cyperus esculentus* to elucidate the crop's diversity, especially in Africa, where studies had tailored more on the morphological characteristics and food use. The discriminatory power of SSR markers has been demonstrated in this study.

Cluster analysis using molecular data

The genetic relationship among the estimated 41 tiger nut genotypes using similarity coefficients ranged from 0.85 – 0.96. The pairwise associations among the genotypes at 0.96 were found between BAJ-B/ APR-b, PUT-B/ BUO-b and OEB-B/ KAL-b. The least similarity groupings (0.85) were between NEB-b/ TAK-b, BEP-Y/ BUO-b, BEP-Y/ GYI-b, ADU-Y/ GYI-b, PUT-b/ BUO-b, PUT-b/ GYI-b, and PUT-b/ NEB-b. The UPGMA analysis grouped the 41 accessions into four heterotic groups (clusters); A, B, C and D at a similarity coefficient of 0.88. Cluster A was distinct and was made up of only one genotype, PUT-b as an outlier. Cluster B was made up of three genotypes, all of which were Central Region collections; one brown (OBR-b), and two yellow (BAJ-Y and BEP-Y). Cluster C was the most heterogeneous cluster with 35 accessions of a mixture of accessions which produce brown, yellow and black coloured tubers and had three sub-clusters: I, II, and III. However, each intrasubcluster accessions had more genetic elements in common than the others, evidence of less genetic variability within the population. This is evident by the observation of one type of colour dominating the others in each subcluster. For example, subcluster III had the accessions dominated with black-coloured tubers while subcluster I was dominated with brown. Cluster D,

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however, was made up of two genotypes; APR-b and TAK-b (Figure 3.9), both brown but from different regions of cultivation in Ghana.

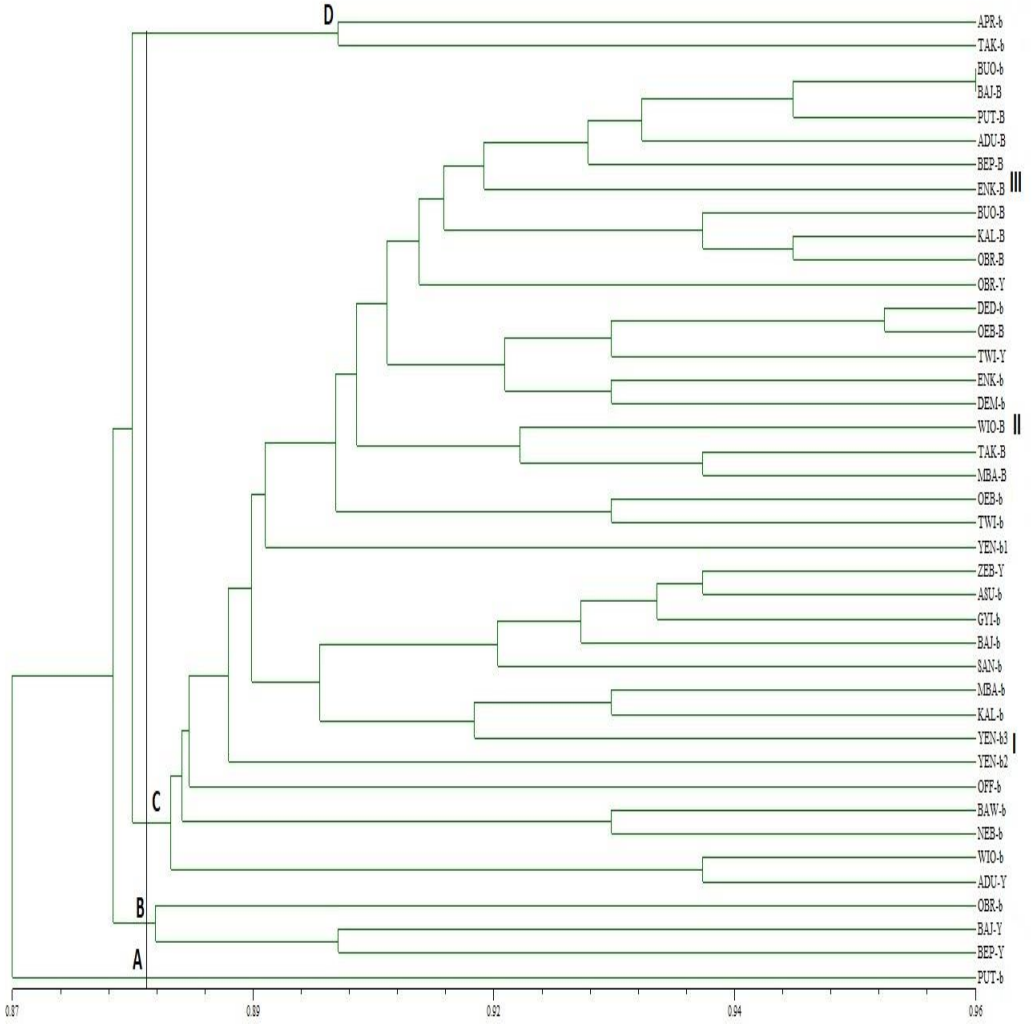

Figure 3.9: UPGMA cluster analysis (Nei *et al.*, 1983) showing relationship among the 41 tiger nut genotypes generated from NTSYS version 2.01.

Principal coordinate analysis of molecular studies

The principal coordinate analysis is a multivariate technique used to describe the variance relationship existing among or within individuals of a population (Khayyam *et al.*, 2009). The relative variance of each coordinate indicates the genetic distances within or among populations.

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All the nine SSR loci generated some level of genetic variability for the population on basic coordinate analysis using simple matching coefficient similarity matrix across the 41 tiger nuts accessions. Three populations were identified, indicating the presence of genetic diversity among the genotypes. Whiles majority of the accessions (brown and few yellow), were from Upper West (4), Upper East (2), Northen Region (2), and Central Region (6), with few of Eastern Region (1) accessions were skewed to one-half of the quadrants, the remaining accessions (black, yellow and few browns) from Upper West (2), Eastern (5), Central (16), Bono East (2) and Nothern (1) regions separated to the other side of the quadrant (Figure 3.10). The findings, therefore, confirm the existence of genetic variability among the accessions on one side but high genetic similarity within. This is in consonance with the observations made in the morphological characterisation of the current study, and also in support with earliar agro-morphological characterization of tiger nut accessions in Ghana by Donkor *et al*., (2019).

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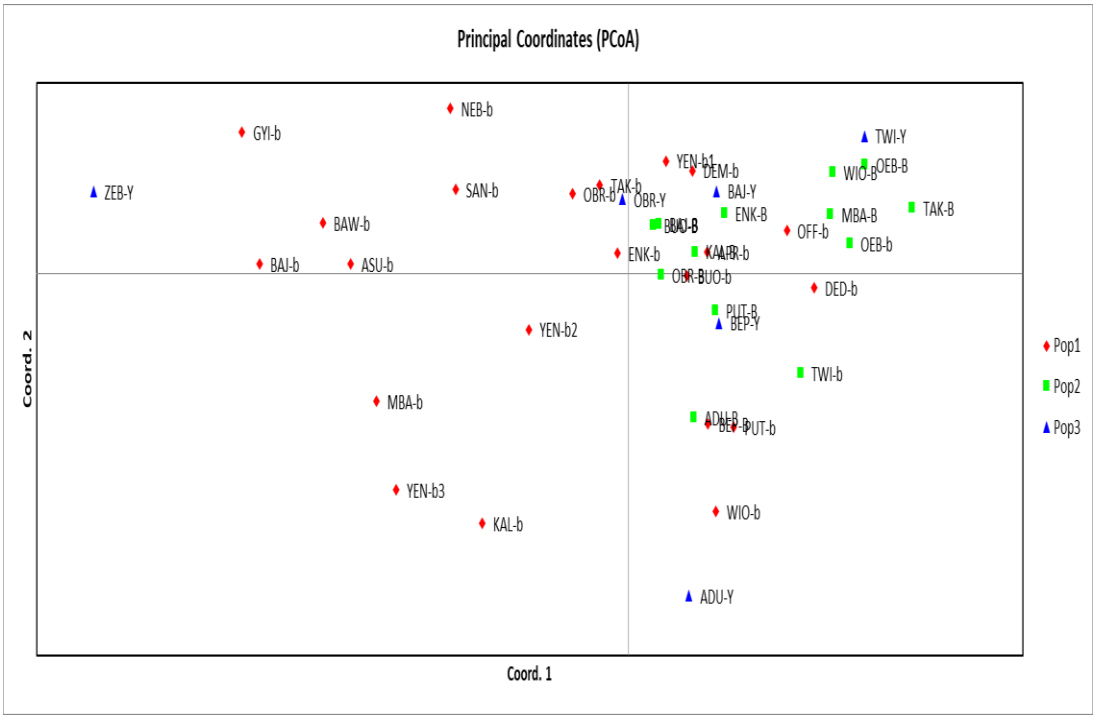

Figure 3.10: Groupings of 41 tiger nut accessions based on principal coordinate analysis of SSR data.

Conclusion

Cyperus esculentus is a cross-pollinated crop which is mainly propagated vegetatively using tubers. Vegetative propagation produces true-to-type offspring which result in low genetic diversity over generations. The 42 accessions collected in various locations in Ghana differed highly significantly in all 11 morphological characteristics. However, the morphological UPGMA cluster analysis grouped the accessions into seven main clusters with one large heterogenous cluster using the Euclidean similarity index of 0.94. The heterogeneous cluster contained more than half the study population (28 accessions) and had three sub-clusters, an indication of low genetic variability within the population. A total of 141 alleles were obtained for the nine SSR loci across 41 accessions with a mean of 15.67 ± 3.97 per loci and a mean PIC of

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0.78 ± 0.15, depicting high discriminating power of the markers. Nonetheless, molecular UPGMA cluster analysis partitioned the population into four heterotic groups with one large heterogenous cluster, suggesting evidence of low genetic variability within the accessions. This is coupled with a low mean heterozygosity value of 0.12 ± 0.16. The differences in variability among the studied accessions for some traits indicate some level of admixture of genes over generations. The exchange of planting material of tiger nut among farmers along the boundaries of the different geographical regions in the country and the subsequent selection of the accessions for planting could have also contributed to the narrow genetic base of the crop.

Therefore, enhancing the genetic diversity of tiger nut accessions for genetic improvement will demand mutation breeding as tiger nuts are seldom propagated sexually. Also, exotic accessions could be imported to support breeding and selection for improved varieties.

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CHAPTER FOUR

CHEMOSENSITIVITY ANALYSIS OF TIGER NUTS (*Cyperus esculentus* L.) USING ETHYL METHANESULFONATE (EMS) AND COLCHICINE MUTAGENS

INTRODUCTION

Tiger nut, which commonly referred to as 'chufa', 'atadwe', nut grass, earth almond, water grass, rush nut, yellow nut sedge, and northern nut grass (Shilenko *et al*., 1979; Bazine & Arslanoğlu, 2020) is known globally by its scientific name as *Cyperus esculentus* L. It is a root tuber crop that belongs to the sedge family as Cyperaceae. The exact origin of the crop is a topic of debate among researchers. Some are of the view that it is a native of Africa and tropical Asia (Acevedo-Rodríguez & Strong, 2012; DAISIE, 2014; Govaerts, 2014), while others suggest that it may have originated from Europe and North America (Holm *et al*., 1979; USDA-ARS, 2014).

Though the crop is inundated with a lot of nutritional, medical and economic benefits (De Vries, 1991; Gambo & Da'u, 2014), the inadequate attention invested in its breeding has resulted in a lower genetic base, limiting its genetic advancements. There is the risk of the local germplasm in the hands of farmers becoming endangered with time as there is a lack of germplasm resources for the accessions coupled with no varietal release. Studies so far have been on morphological characterization and have revealed low diversity within the populations studied (Donkor *et al*., 2019; Asare *et al*., 2020). Also, as a vegetatively propagated crop, it scarcely undergoes natural hybridization and therefore it has low genetic variability.

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There is little information on the crop's improvement by any method. Improving any crop, first demands creating variation in a population for selection. A mutation is the ultimate source of variability and provides unique germplasm for plant breeders (van Harten, 1998), especially in vegetatively propagated crops. This can occur by natural means, which takes several years, or by artificial (induced) mutation, which gives results in a relatively shorter period. Induced mutation has been used successfully in the genetic improvements of many crop genotypes. This technique has been used by plant breeders since the 1920s to create genetic variation (Stadler, 1928; van Harten, 1998; Ahloowalia *et al*., 2004; Tambe & Apparao, 2009).

Induced mutation has been functionally performed either physically or chemically. Physical mutagens are mostly electromagnetic radiations such as gamma rays, X-rays, UV light, and particle radiation (beta and alpha particles). Chemical mutagens are usually alkylating agents and include ethyl methanesulfonate (EMS), ethidium bromide, and base analogues such as bromouracil (van Harten, 1998; Girija & Dhanavel, 2009; Mba *et al*., 2010). Colchicine is another chemical mutagen, an alkaloid for mutation induction and more purposely for polyploidy evocation in plant breeding. EMS is also noted to be a powerful chemical mutagen producing random mutations in genetic material by nucleotide substitution; particularly by guanine alkylation. EMS generally produces only point mutations in a genome. The higher doses of mutagen completely arrest seed germination (Spencer-Lopes, *et al*., 2018). Hence understanding the optimal dosage of a mutagen on genotypes is essential for the success of mutation breeding. Therefore, the objective of this study was to establish the lethal dose that results in the death of 50% of the population

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(LD_{50}) and reduction dose that leads to a 50% decrease in plant height or traits (RD_{50}) of EMS and colchicine for effective mutagenesis in tiger nut tubers.

Materials and Methods

Experimental site for chemosensitivity

The research work was carried out at the Centre for Scientific and Industrial Research, Crop Research Institute (CSIR- CRI), Fumesua in the Ashanti Region of Ghana in late March 2022 in a greenhouse at the onset of the major rainy season.

Plant materials for chemosensitivity

The experimental set consisted of four distinct genotypes, comprising two distinct brown genotypes (denoted as OFF-b and APR-b) and two different black genotypes (labeled as BUO-B and ENK-B) of tiger nut tubers. These genotypes were derived from landraces and were selected from a collection of accessions obtained from previous studies on the morphological diversity of tiger nuts across major cultivation regions in Ghana. Specifically, BUO-B was obtained from Buoyam in the Bono East Region, ENK-B, and APR-b were collected from Enkroful and Assin Praso, respectively, both located in the Central Region of Ghana. Meanwhile, OFF-b originated from Offinso in the Eastern Region of Ghana. These genotypes were chosen based on their superior yield performance, representing the highest-yielding selections. Afterwards, the chosen genotypes were subjected to mutation following the schmatic process shown in Figure 4.1.

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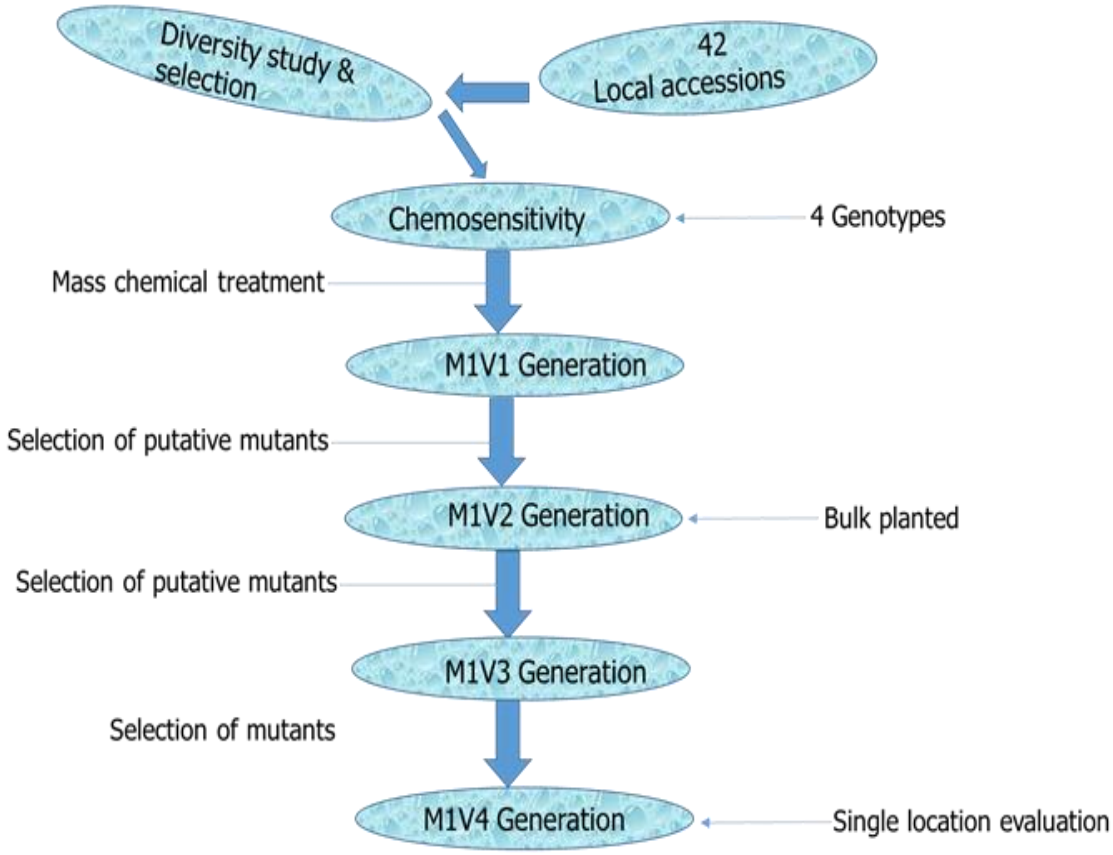

Figuare 4.1: Shematic mutation breeding process of the tiger nut genotypes employed in the study.

Treatment of tubers for chemosensitivity

Two groups of genotypes, consisting of black (BUO-B) and brown (OFF-b) type set, and another black (ENK-B) and brown (APR-b) set, were subjected to mutagenesis using different treatments. The genotypes (BUO-B and OFF-b) were treated with Ethyl Methanesulfonate (EMS) at five varying concentrations, while ENK-B and APR-b genotypes underwent treatment with Colchicine at five different concentrations. The concentrations used for each mutagen were 0%, 0.1%, 0.25%, 0.5%, and 1.0%.

In total, a set of four hundred tubers, comprising twenty tubers for each concentration, was employed for mutagenesis across the four genotypes. The

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mutagen solutions were prepared by mixing the respective chemical mutagens with distilled water.

Colchicine was obtained in powdered form from KEM Light Spechem Laboratory Private Limited, Mumbai, India. On the other hand, EMS was obtained as a liquid chemical product from CDH Private Limited, New Delhi, India.

Initially, a 1% stock solution (1 g of chemical mutagen per 100 ml of distilled water) was prepared and subsequently diluted to achieve the desired concentration levels. The control (0%) consisted solely of distilled water.

To facilitate efficient chemical inhibition, the dried tubers were soaked in each concentration of EMS or Colchicine for 24 hours. This soaking process, observed based on the method of Ye *et al*. (2020), helped soften the tubers and open their pores. Subsequently, the soaked tubers were removed from the solutions and thoroughly washed with distilled water in preparation for immediate planting.

Experimental design and planting culture for chemosensitivity test

Treated tubers and the controls were planted in polybags filled with steamed sterilized sandy-loam soil in a greenhouse in three replicates in a completely randomized design (CRD).

Irrigation was done regularly. No fertilization and insecticide treatments were performed as insects were not a problem as well as soil for the trial was intact with nutrients without any deficiency symptoms. Weeds were manually removed by handpicking when necessary.

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Data collection and analysis

Germination assessment was conducted on the seventh-day post-planting. Initially, germination was observed in some of the control groups, while sprouting in the treated group commenced from the eighth day onwards.

The collected data encompassed several parameters, including germination percentage, plant height, number of tillers, main plant diameter, and leaf count per plant. Germination count began concurrently the first day of the observation of germination and continued until the tenth day. The germination percentage was computed as the ratio of the number of germinated seeds to the total number of planted seeds, expressed as a percentage.

Plant architectural traits, such as plant height, were derived from the average measurements across the three replications. Plant height measurements were taken from the soil surface to the tip of the primary (terminal) leaf when the plants were approximately one month old, specifically four weeks from the day of plating. A measuring rule was utilized for this purpose. Figure 4.2 shows the plant growth stage in the third week after germination.

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Figure 4.2: Plants at 16 days of growth after germination (sprout).

Concurrently, within the same timeframe, observations were made on the number of tillers produced by the plants. The girth (diameter) of the main plant was determined using a digital Vernier calipers, with the measurement taken at the base of the plant just above the soil level, and the value expressed in millimeters. The leaves present on the main plant were counted and documented, along with the number of the produced tillers from the main plant. Chemosensitivity analyses were conducted using Excel 2016 and Genstat statistical software version 11.2 to determine LD_{50} (lethal dose resulting in a 50% population fatality rate) and RD_{50} (reduction dose causing a 50% decrease in plant height or traits). The LD_{50} and RD_{50} values for the genotypes were calculated by fitting a simple linear regression model with the equation $y = mx + c$, where y represents the response (dependent) variable (percentage germination or plant height), x denotes the independent variable (mutagen

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concentration), and m and c symbolize the slope and intercept of the linear equation, respectively.

Results and Discussion

In general, a gradual reduction in the percentage germination or sprouts of the tubers was observed with corresponding increasing levels of mutagen concentrations for both EMS and Colchicine (Figure 4.3). This is in agreement with the findings of Horn and others in cowpea (Horn *et al*., 2016) and Rangaswamy in sesame (Rangaswamy, 1973). However, each genotype responded differently to the different levels of mutagen concentrations in germination (Figure 4.3) and plant architectural traits (Table 4.2 and Table 4.3). Nonetheless, the percentage rate of germination was higher with the tiger nut accessions which produced black-coloured tubers than brown tuber-producing ones in both EMS and Colchicine (Figure 4.3). This indicates that the black tuber-producing accessions were hardier than their brown tuber-producing counterparts as the latter were more sensitive to the effects of the mutagens.

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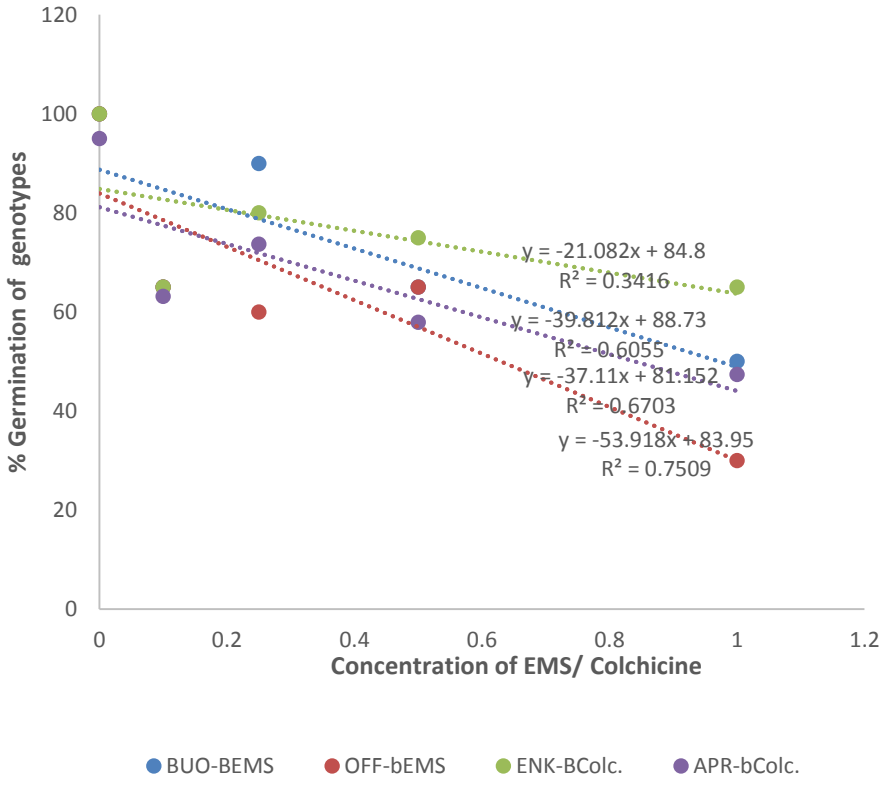

Figure 4.3: Regression percentage germination among the four tiger nut genotypes on the different concentrations of EMS and Colchicine treatments.

BUO-B_{EMS}= Black EMS-treated tiger nut genotype from Buoyam; OFF-b= Brown EMS-treated tiger nut genotype from Offinso; ENK-$B_{Colc.}$= Black Colchicine-treated tiger nut genotype from Enkroful and APR-b_{Colc}= Brown Colchicine-treated tiger nut genotype from Assin Praso.

For the black genotype's, there wass 100% germination under control conditions for BUO-B_{EMS} and a steady decline in germination thereafter increasing mutagen concentrations (Figure 4.3). Percentage germination was 90% at a concentration of 0.25%, whereas it was only 50% at a concentration of 1.0%. The population-wide EMS concentration dose to produce LD_{50} was calculated to be 0.97% (Table 4.1) for black tiger nuts.

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Table 4.1: LD50 and RD50 of EMS and Colchicine among the treated genotypes with their corresponding regression equations.

Genotype/ Mutagen	LD_{50} Regression Equation	% LD_{50} Calculated	RD_{50} Regression Equation	% RD_{50} Calculated
BUO-B_{EMS}	y = -39.812x + 88.73	0.97	y = 38.468x - 7.3965	1.49
OFF-b_{EMS}	y = -53.918x + 83.95	0.63	y = 37.013x - 10.5	1.63
ENK-$B_{Colc.}$	y = -21.082x + 84.8	1.65	y = 2.6686x - 2.0875	19.51
APR-$b_{Colc.}$	y = -37.11x + 81.152	0.91	y = 29.866x - 1.1461	1.71

BUO-B_{EMS}= Black EMS-treated tiger nut genotype from Buoyam; OFF-b= Brown EMS-treated tiger nut genotype from Offinso; ENK-$B_{Colc.}$= Black Colchicine-treated tiger nut genotype from Enkroful and APR-b_{Colc}= Brown Colchicine-treated tiger nut genotype from Assin Praso.

The regression graph (Figure 4.3) established an LD_{50} of 0.63% with the equation, y = -53.918x + 83.95 (Table 4.1) for the brown tiger nuts treated with EMS. The black genotypes are confirmed to be less sensitive to the mutagen at the different concentration levels than observed in the brown type resulting in the lower LD_{50} value descovered for the brown (0.63%) compared with the black (0.97%) (Table 4.1). Hence, the black genotype is seen to be more resilient to some environmental changes such as chemical mutagens than the brown genotype due to the hardier character of the nuts. Except for 0.1% concentration which drastically reduced percentage germination by 65%, the remaining concentration at the different levels of Colchicine used to treat ENK-B_{Colc} resulted in a proportional decrease in germination (Figure 4.3).

The regression equation, y = -21.082x + 84.8 suggets an LD_{50} value of 1.65% for the black colchicine treated tubers (ENK-B_{Col}.). In contrast, thhe brown

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genotypes (APR-b_{Colc}.) (Figure 4.3), consistently had a severe reduction in germination as colchicine concentration rose, with the exception of the 0.25% colchicine concentration, which resulted in a germination rate of 70%. The lethal dose effect as per the regression equation y = -37.11x + 81.152 (Figure 4.3) that would eliminate fifty percent of the population of the brown colchicine-treated tubers is determined to be 0.91% (Table 4.1).

Averagely, relatively low concentrations of both mutagens resulted in increasing plant architectural growth, such as plant height, leaf count, main plant diameter, and tiller count (Table 4.2). For example, plant height growth was positively redueced at high concentration doses of mutagens compared to the stimulatory growth response observed at low concentration doses (Figure 4.4). This is not surprising as mutagens such as colchicine have both stimulatory and inhibitory effects on mutants at different concentrations (Nura et al., 2011; El-Nashar & Ammar, 2016; Singh et al., 2020), and hence at lower concentrations such as 0.1%, an increase in plant architectural traits was observed (Table 4.2). Based on their means, these quantitative traits to some extent showed a steady increase with lower doses of mutagen concentrations up to a dose of 1.0% before eventually declined (Table 4.2 and Table 4.3). This is in line with the observed effects of RD_{50} for both mutagens, that to establish RD_{50}, the concentrations for both mutagens should be from 1.5% and above for the black and brown tiger nut genotypes (Figure 4.4). On the contrary, results from Figure 4.3 anticipate that at very low concentrations, below the control in the event where water loses its chemical composition properties (Sidorenko *et al*., 2024), plant height reduction is likely to be negatively affected.

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Similar findings about a decline in plant heights, when chemical mutagen concentrations rose in onions were reported (Joshi *et al*., 2011). For instance, in Table 4.2, the mean plant heights for the controls of the black and brown genotypes were 34.84 cm and 34.50 cm, respectively. At a concentration of 0.1% EMS, the height of the plants was 38.26 cm and 36.89 cm, respectively. This pattern persisted for EMS concentrations of 0.25% to 0.5%, and then plant height began to decline at a concentration of 1.00% of EMS being 23.66 cm and 22.28 cm in the black and brown genotypes, respectively.

Table 4.2. The mean, minimum, maximum and standard deviation of genotypes treated with EMS.

Genotype	Treatment in Concentration (%)	PHT (cm)	NLP	NTP	DMP (mm)
Black	Control (0)	34.84^{a} (33.43-36.80) 1.75	7.56^{ab} (7.00-8.33) 0.69	1.67^{bc} (1.33-2.00) 0.33	3.78^{a} (3.59-4.10) 0.26
	0.1	38.26^{a} (35.27-42.43) 3.73	7.89^{a} (7.33-8.33) 0.51	2.00^{abc} (1.67-2.33) 0.33	4.02^{a} (3.30-4.41) 0.62
	0.25	35.02^{a} (31.63-37.83) 3.14	7.67^{ab} (7.00-8.00) 0.51	2.22^{ab} (2.00-2.67) 0.39	3.83^{a} (3.48-4.16) 0.35
	0.5	30.53^{ab} (29.70-31.97) 1.25	7.78^{a} (7.00-9.00) 1.07	2.89^{a} (2.33-4.00) 0.96	4.49^{a} (3.85-5.09) 0.62
	1.0	23.66^{b} (12.47-30.50) 9.77	6.56^{ab} (4.33-8.67) 2.17	1.89^{abc} (1.67-2.33) 0.39	3.29^{a} (2.27-3.85) 0.88
Brown	Control (0)	34.50^{a} (30.60-36.47) 3.32	7.33^{ab} (7.00-7.67) 0.33	1.67^{bc} (1.33-2.00) 0.33	3.86^{a} (4.05-4.17) 0.44
	0.1	36.89^{a} (35.07-38.53) 1.74	7.33^{ab} (7.00-7.67) 0.33	2.78^{ab} (2.33-3.00) 0.39	3.85^{a} (3.56-4.02) 0.26
	0.25	35.96^{a} (29.97-42.67) 6.38	8.00^{a} (7.33-8.00) 0.39	2.22^{ab} (1.33-3.67) 1.26	3.99^{a} (3.78-4.29) 0.27
	0.5	37.37^{a} (34.80-40.90) 3.16	7.67^{ab} (7.33-8.00) 0.33	2.33^{ab} (1.33-3.33) 1.00	3.87^{a} (3.37-4.34) 0.50
	1.0	22.28^{b} (12.67-32.50) 9.93	5.44^{b} (2.67-9.00) 3.24	1.00^{c} (0.00-1.67) 0.88	3.14^{a} (1.08-5.43) 2.18

PHT: plant height, NLP: number of leaves per plant, NTP: number of tillers per plant, DMP: Diameter of the main plant

Means within the same column with the same letter(s) is/are not significantly different by Fisher's test at $P > 0.05$.

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On an individual plant basis, there was a wide range plant architectural traits measurements recorded for most genotypes treated with the mutagens than their control counterparts. For example; the minimum height for brown genotype treated with 0.5% cEMS (Table 4.2) was 34.80 cm and the maximum was 40.90 cm as compared with the control which recorded 30.60 cm for the minimum and 36.47 cm for the maximum. This is a suggestive evenidence of possible occurence of mutation by chemical mutagens as being distributed randomly across the genome (Greene *et al*., 2003; Till *et al*., 2003). Therefore, to effectively induce about 50% reduction in plant architectural traits for the population, an observation made for this study recommended the use of high doses of mutagen concentrations of both EMS and Colchicine for the mutagenesis of the genotypes.

Table 4.3: The mean, minimum, maximum and standard deviation of genotypes of Colchicine

Genotype	Treatment in Concentration (%)	PHT (cm)		NLP		NTP		DMP (mm)	
Black	Control (0)	33.73^{ab} 2.50	(31.97-36.60)	7.56^{ab} 0.19	(7.33-7.67)	2.44^{ab} 0.51	(2.00-3.00)	4.26^{a} 0.87	(3.47-5.19)
	0.1	34.24^{ab} 0.20	(34.10-34.47)	7.89^{a} 0.39	(7.67-8.33)	2.56^{a} 0.39	(2.33-3.00)	4.01^{ab} 0.55	(3.38-4.39)
	0.25	35.11^{ab} 0.05	(35.07-35.17)	7.89^{a} 0.51	(7.33-8.33)	1.89^{abc} 0.19	(1.67-2.00)	4.39^{a} 0.42	(3.99-4.83)
	0.5	34.23^{ab} 0.72	(33.47-34.90)	8.11^{a} 0.77	(7.67-9.00)	2.67^{a} 0.88	(1.67-3.33)	4.50^{a} 0.15	(4.33-4.58)
	1.0	33.20^{ab} 2.56	(30.33-35.27)	8.00^{a} 0.33	(7.67-8.33)	2.33^{ab} 0.00	(2.33-2.33)	4.37^{a} 0.19	(4.15-4.49)
Brown	Control (0)	35.97^{ab} 1.04	(35.37-37.17)	7.78^{a} 0.19	(7.67-8.00)	2.44^{ab} 0.39	(2.00-2.67)	3.91^{ab} 0.19	(3.80-4.13)
	0.1	30.42^{ab} 15.16	(13.00-40.57)	5.56^{bc} 2.59	(2.67-7.67)	1.56^{bc} 0.84	(0.67-2.33)	3.55^{ab} 1.84	(1.52-5.11)
	0.25	38.52^{a} 2.98	(35.30-41.17)	7.00^{abc} 0.33	(6.67-7.33)	1.56^{bc} 0.19	(1.33-1.67)	3.96^{ab} 0.12	(3.85-4.08)
	0.5	33.38^{ab} 10.25	(21.63-40.53)	6.78^{abc} 1.68	(5.00-8.33)	2.11^{ab} 0.39	(1.67-2.33)	3.84^{ab} 1.28	(2.56-5.11)
	1.0	23.73^{b} 14.38	(9.93-38.63)	4.89^{c} 2.17	(2.67-7.00)	1.11^{c} 0.69	(0.33-1.67)	2.65^{b} 1.56	(1.22-4.31)

PHT: plant height, NLP: number of leaves per plant, NTP: number of tillers per plant, DMP: Diameter of the main plant.

Means within the same column with the same letter(s) is/are not significantly different by Fisher's test at P > 0.05.

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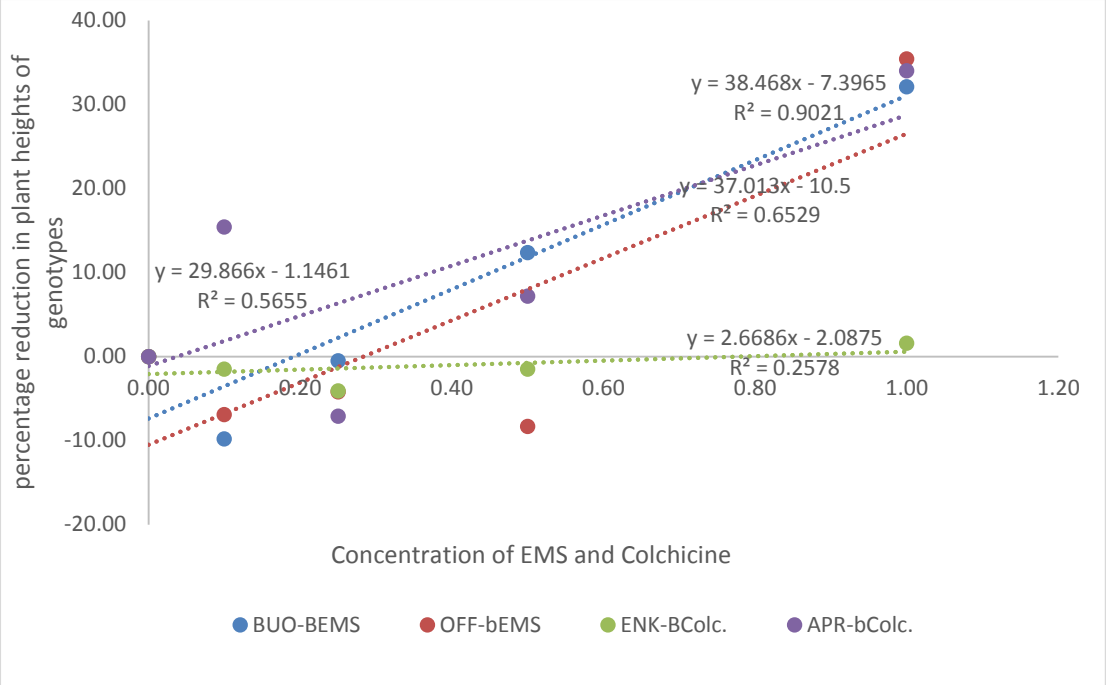

Figure 4.4: Regression graph showing the RD_{50} for plant heights for the four different tiger nut genotypes in the different concentrations of EMS and Colchicine treatments.

BUO-B_{EMS}= Black EMS-treated tiger nut genotype from Buoyam; OFF-b= Brown EMS-treated tiger nut genotype from Offinso; ENK-$B_{Colc.}$= Black Colchicine-treated tiger nut genotype from Enkroful and APR-b_{Colc}= Brown Colchicine-treated tiger nut genotype from Assin Praso.

As shown in Figure 4.3, the RD_{50} regression analysis for the genotypes based on plant height revealed varied regression coefficients. Additionally, RD_{50} values for various genotypes ranged from 1.49% to 19.51%. (Table 4.1). The RD_{50} for the black genotype treated with EMS was 1.49%, as opposed to 1.63% for the brown genotype (Table 4.1). The brown genotype (APR-b_{Col}) had an RD_{50} based on Colchicine treatment of 1.71% as opposed to the enormous value of 19.51% for black Colchicine treated. However, this was expected because the black Colchine-treated genotype outperformed its control in height. Overall,

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the genotypes treated with Colchicine had greater RD_{50} values than the genotypes treated with EMS (Table 4.1).

Conclusion

The results of the current investigation showed that EMS and Colchicine mutagens prolonged the time of germination in tiger nut tubers. The brown tiger nut was more sensitive to these chemical mutagens than the black genotypes. Additionally, it was seen that the percentage rate of germination gradually decreased when the concentration dose was increased. The effective dose (LD_{50} value) for the black genotypes was determined to be a high dose of 0.97% EMS concentration as opposed to 0.63% EMS concentration in the brown genotypes.

Genotypes responded to Colchicine treatment less sensitively compared to EMS. In comparison to a considerably lower concentration of EMS that could do the same task, a higher concentration of Colchicine—as high as 1.65% and 0.91%—was required to kill half the population of the black and brown genotypes, respectively.

As hormesis (a growth vigour simulative effect of mutagen at lower concentrations) progressed, a rise in the plant's architecture, involving plant height, diameter, number of leaves, and tillers, were also observed. The RD_{50} is established in addition to hormesis. For the black and brown genotypes treated with EMS, the ideal doses to reduce plant height were found to be 1.49% and 1.63%, respectively. Those that received Colchicine recorded high RD_{50} values of 1.71% and 19.51% for the brown and black genotypes respectively. To broaden the genetic scope of the tiger nut genotypes for breeding enhancement, an efficient mass treatments of the accessions with the right doses of these

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mutagens as per established, and further screening or selections for successive generations must be implemented.

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CHAPTER FIVE

MUTATION BREEDING FOR TIGER NUTS (*Ceperus esculentus* L.) IMPROVEMENT USING ETHYL METHANESULFONATE (EMS) AND COLCHICINE

INTRODUCTION

Currently, the world's human population is 8.2 billion and still experiencing a gradual increase every day (Lam, 2024). It is envisaged to be 9 billion by 2050 (Bongaarts, 2009; Zakir, 2018) and further peaks at 10.3 billion in 2084 (Lam, 2024). This surge in population presents a massive concern for a reliable and efficient food supply system to meet the nutritional demands of the rising population for assured food security. According to FAO 2020, 8.9% of the global human population suffers from hunger, 3 billion people face malnutrition problems, and 2 billion are food insecure (Arora & Mishra, 2022; Mohajan, 2022). This underscores the need for innovative crop improvement techniques by plant breeders to forestall the food and nutritional need challenges ahead of time.

As an underutilized and neglected food crop, tiger nut tubers are rich in all the food nutrients (Bamishaiye & Bamishaiye, 2011; Twumasi et al., 2023) providing quick access to a balanced and nutritious diet for healthy body growth and development. Once harvested, the tubers are ready for consumption without any tedious and sophisticated cooking process. They can be wash-dried, eaten raw, roasted, or milled into powder for consumption. Tiger nut tubers account for a great deal of fiber for digestive health, fats (monounsaturated and polyunsaturated) for a healthy heart, vitamins such as vitamin E providing

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antioxidants to protect cells from damage, and minerals such as calcium, magnesium, potassium, and iron (Roselló-Soto *et al*., 2018; Sabah *et al*., 2019). These minerals help in the mineralization of bones, act as cofactors to enzyme systems, assist in the sustenance of muscles and nerve excitation, and the maintenance of the oxygen-carrying capacity of the blood (Clarkson, 2013; Ghosh *et al*., 2016). Tiger nut is not only a source of food in the form of chewable tubers, processed oil and milk, but it is also beneficial as feed, medicine, and perfumes (De Vries, 1991; Gambo & Da'u, 2014), and provides great returns of foreign exchange.

For many decades, agricultural practices for crop improvement had been on the approach of conventional breeding which takes several years to create a diversity of gene pool for genetic advancement to meet breeder's interests. To improve crop plants, plant scientists including plant breeders have to rely on three methods: cross-breeding, mutation, and transformation (Bado *et al*., 2023). For a fast and reliable approach to creating genetic variation for trait improvement in crop plants, plant breeders since 1920 have exploited the use of induced mutation (van Harten, 1998; Ahloowalia *et al*., 2004; Tambe & Apparao, 2009). Mutation breeding for crop improvement utilizes the deliberate action of generating genetic variations among existing genotypes (Yadav *et al*., 2021). Mutation can spontaneously occur naturally taking several years or be artificially induced within a shorter space of time using mutagens. Induced mutation has been frequently applied in widening the genetic diversity of vegetatively propagated crops of which tiger nut is no exception. It has been successfully used in bananas, potatoes, cassava, and other crops (Jankowicz - Cieslak et al., 2012; Chepkoech et al., 2019). It makes use of either physical or

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chemical mutagens. Physical mutagens employ the use of ionizing radiations, such as alpha rays, beta rays, X-rays, gamma rays and cosmic rays, or non-ionizing radiations, including ultraviolet rays, infrared rays, microwaves, and laser among others. Ionizing radiations have been largely used in plant mutagenesis. They cause biological injuries in plants resulting in point mutation to deletion, single and double chromosomal strand break, and chromosomal rearrangement (Bado *et al*., 2023). However, they are costly compared to chemical mutagenesis. Commonly used chemical mutagens in plants include alkylating agents, for example; ethyl methane sulfonate (EMS), mustard gas, ethyleneimine (EI), dimethyl sulfide (DMS), sodium azide (SA) (Leitão, 2012) and Colchicine (Siregar *et al*., 2022). Chemical mutagens such as EMS cause point mutation and have relatively silent (missense) mutational effect (50%) with about 5% nonesense mutations (Viana *et al*., 2019). Colchicine on the other hand induces polyploidy by doubling or multipling the chromosome sets of plants resulting in changes in plant characteristics. Some polyploids blatantly produce bigger organs via flowers, seeds, leaves, stems, and roots (Sattler *et al*., 2016). Induced mutations are crucial for crops enhancement by creating genetic diversity, which if properly managed and screened would result in the creation of novel and superior crop varieties with promising traits fulfilling the breeder's objectives. Breeding by this technique proffers a substantial scope for promoting sustainable agricultural progress and food security (Mohan & Suprasanna, 2011; Mir *et al*., 2020). It increases crop yield while addressing the problems of pests and diseases, nutrition, and climate shocks (Jain & Maluszynski, 2004; Jain, 2010; Penna *et al*., 2023).

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The cultivation of tiger nuts has over the years witnessed little or no significant breeding improvements. Hence, the tiger nut accessions presently in the hands of African farmers constitute a warehouse of an unimproved natural gene pool coupled with no varietal release. Additionally, the vegetative nature of this crop does not promote natural hybridization over years of cultivation. Improving on such natural germplasm therefore could be done using induced mutation breeding techniques. The objective of this study was to use chemical mutagenesis to create diverse cultivars of tiger nuts which could be used to breed and select for increased productivity, superior nutritional value, early maturity, strong tuber attachment to plant on harvest, and resilience to environmental stressors.

Materials and methods

Experimental site and land preparation for putative mutants

All the experimental trials were conducted at the CSIR-Crops Research Institute, situated in Fumesua within the Ashanti Region (01^0 36'W; 06^0 43'N). This locale is characterized by an elevation of 186 metres above sea level, positioned within the semi-deciduous forest zone. The average temperature range is 21°C to 31°C.

The annual cumulative rainfall averages at 1727.2mm, exhibiting a bimodal distribution pattern. The primary rainy season extends from March to July, while the secondary rainfall phase spans from August to November. The mean relative humidity stands at 95% during mornings and decreases to 61% by noon.

For the initial (M1V1) and second (M1V2) mutation generations, field planting was executed. The soil type at the experimental site is identified as Ferric

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Acrisol, belonging to the Asuansi series, as per the classification provided by FAO/UNESCO (1988). It is characterized by a substantial layer depth, predominantly sandy loam in composition, and displays a gentle slope of 1-5%. The field had been previously utilized for cultivating groundnut and cowpea. To address the issue of termites in the field, mutation generation three (M1V3) was cultivated in sacks filled with soil. Consistently, sandy-loam soil was employed for all generations.

The experimental site was manually cleared-off vegetation and later ploughed and harrowed for M1V1 and M1V2. Row- ridges were subsequently made. For M3, sacks field with soil (sandy loam) were used. The soil was mixed with groundnut husk and packed into the sacks to enrich the soil fertility and also enhance soil porosity for good soil-water infiltration, plant root penetration, and tuber development. Prepared sacks filled with soil were sprayed with a solution of Dusban insecticide at a dose of 200 ml per 15 liters of water to control termites and other insects.

Planting materials and treatment

A total of six hundred tubers were planted for the M1V1 generation. These tubers comprised 50 BUO-B control, 100 BUO-B EMS-treated, 50 OFF-b control, 100 OFF-b EMS-treated, 50 ENK-B control, 100 ENK-B colchicine-treated, 50 APR-b control, and 100 APR-b colchicine-treated specimens. The EMS treatment for the black and brown genotypes involved solution concentrations of 0.97% and 0.63%, respectively. Meanwhile, the colchicine-treated black and colchicine treated brown genotypes were exposed to solution concentrations of 1.65% and 0.83%, respectively, as determined by their LD_{50} and RD_{50} computations in an earlier work (Twumasi *et al.*, 2023).

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For the subsequent M1V2 generation, a total of 1,168 tubers were screened from the M1V1 harvest and used for bulk planting. Among these, there were 73 BUO-B control tubers, 219 BUO-B EMS-treated tubers, 73 OFF-b control tubers, 219 OFF-b EMS-treated tubers, 73 ENK-B control tubers, 219 ENK-B colchicine-treated tubers, 73 APR-b control tubers, and 219 APR-b colchicine-treated tubers.

For the M1V3 generation, 100 tubers were allocated for planting. These included 3 BUO-B control tubers, 22 BUO-B EMS-treated tubers, 3 OFF-b control tubers, 22 OFF-b EMS-treated tubers, 3 ENK-B control tubers, 22 ENK-B colchicine-treated tubers, 3 APR-b control tubers, and 22 APR-b colchicine-treated tubers.

Experimental design, planting, and culture

The M1V1, M1V2, and M1V3 generations were field planted for screening and selection with no replications, as each distinct putative mutant line was exclusive and still undergoing segregation to achieve homozygosity.

Regular weeding by hoe was performed to control weeds when needed. Throughout each planting season of the M1V1-M1V3 generations, manual weed control was effected three times. The initial weeding took place 3 weeks after planting, followed by the second and third weeding at 8 weeks and 12 weeks after planting, respectively. Irrigation was carried out based on the water requirements of both the soil and the plants, with no fertilizer application. In the case of M3 plants, weeds in sacks were controlled by hand-picking.

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Screening and selection of putative mutants

From the M1V1 to M1V3, putative mutants were selected based on the assessment of yield parameters (overall tuber weight, hundred tuber count weight, size of tuber), earliness to maturity, flowering ability, and attachment of tubers to plants during harvesting.

Evaluation of selected mutant lines (M1V4)

Selected mutants were experimentally evaluated openly at the site of the Legumes Department, Crop Research Institute, Fumesua in the Ashanti Region (01^0 36'W; 06^0 43'N). Twenty- five tubers were selected from the M3 as planting materials for the M1V4 evaluation trial. The tubers consisted of 1 BUO-B control, 5 BUO-B EMS treated, 1 OFF-b control, 6 OFF-b EMS treated, 1 ENK-B control, 5 ENK-B colchicine treated, 1 APR-b control, and 5 APR-b colchicine treated. These genotypes were planted two each in a block and three in replications giving rise to 150 tubers.

Planting materials (mutant and non-mutated control lines) were planted in 150 sacks filled with sandy-loam soil in a randomized complete block design (RCBD) with three replications on 11th June 2023. The soil-filled sacks, each with a volume of 0.02160667 m^3 were treated with Dusban solution a day before planting against termites and were subsequently arranged on a cleared open field but covered with a black polythene sheet to control weed growth (Figure 5.1).

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Figure 5.1 Sacks filled with soil containing one week old plants arranged on a spread black polythene sheet.

As part of cultural practices, weeds in sacks were regularly controlled when they appeared by handpicking. Weeds on the ground however were suppressed in growth by the polythene sheet. Irrigation was done occasionally according to the water needs of the crops and soil, especially during periods of low rainfall. No fertilizer and pesticides were applied.

Data collection and analysis

Agro-morphological data

A total of 20 agro-morphological traits were meticulously gathered in this study. These traits encompassed days to germination (DG), number of tillers per stand (NT), distance from the last tiller to the mother plant (DTP), plant height (PH), girth of mother plant (GP), number of leaves (NL), leaf length (LL), leaf width (LW), days to flower/inflorescence set (DF), number of inflorescence (NI), days to physiological maturity (DPM), number of tubers attached (NTA), number of

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tubers detached (NTD), number of tubers per stand (NTS), number of rings per tuber (NRT), length per tuber (LT), width per tuber (WP), tuber shape (TS), hundred tuber weight per genotype (HWT), and overall tuber weight per plant (OTW).

Data were cautiously collected from each plant within all three replications. Specifically, NT, DTP, PH, and PI were assessed at the time of flowering (inflorescence). NTS and NTA were recorded by direct counting at harvest. NRT, LT), WP, TS, HWT, and OTW were measured in four days after harvest and subsequent drying.

For specific measurements, NT, NTS, and NRT were quantified. DTP and PH were determined using a meter rule. The PG and PI were observed and expressed as percentages by having the number of plants that germinated or flowered over the total planted and multiplied by 100%. DG and DF were counted from the day of planting. DM was noted at the period corresponding to the physiological maturity of the crop retrospective to planting. NTA, NTD, and NTS were counted and recorded at harvest. LT and WP were precisely measured using a vernier caliper. TS was computed using the length-to-width (L/W) ratio, categorized as oval (<1.3), ovoid (1.3-1.8), or oblong (>1.8) based on previous research (Pascual *et al.*, 2000). OTW and HWT were determined using an electronic balance. The latter was randomly selected from the composite of all three replications per genotype.

Proximate and mineral data

After mild sun-air drying for two weeks, the tubers were subjected to proximate and mineral analysis. The proximate analysis was carried out at the Department

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of Biological Science, Kwame Nkrumah University of Science and Technology (KNUST) following the protocol of the Association of Official Analytical Chemists (AOAC) to determine the protein, fat, ash, carbohydrate, moisture, and energy contents in triplicates of 13 tiger nut genotypes. Thirteen out of the 25 genotypes were selected for the nutrients analyses due to available resources during the course of the experiment.

Moisture content was determined using AOAC 930.15, crude fat (AOAC 2003.05), ash (AOAC 942.05), crude fibre (AOAC 978.10) and crude protein (AOAC 2001.11). The percentage of carbohydrates was calculated using the formula:

Carbohydrate (%) = 100 – (% moisture + % fat + % protein + % ash + % fibre).

For mineral analysis, six mineral elements (phosphorous, potassium, calcium, magnesium, sodium, and iron) were determined for the 13 genotypes in three replications at the Department of Crops and Soil Sciences, KNUST using the protocol of (Jones & Case, 1990) and (Motsara & Roy, 2008).

Data analysis

Data were analysed using analysis of variance (ANOVA) and descriptive statistics. The means, minimum and maximum values, mean squares, standard errors, standard deviations, and coefficient of variations were computed. Genstat version 11.1 and Minitab version 21.1.1.0 statistical softwares were used to analyse the data. Furthermore, multivariate analyses, incorporating clustering and principal component analysis were conducted using Minitab

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version 21.1.1.0. These multivariate analyses were conducted by employing the means derived from the recorded entries across the three replications.

Results and Discussion

Variation in plant shoot characteristics

Qualitatively, few phenotypic variations were observed among the putative mutants at M1 to M3 generations for plant architectural traits. Some of the EMS-treated genotypes showed chimeric features in the form of coloration of the leaves (Figure 5.2 B), and multiple-shoots for the main plant (Figure 5.2 C) among others at the M1V1 to M1V3 generations compared to the controls (Figure 5.2 A). These were much visible in the EMS brown-treated genotypes, suggestive of the occurrence of mutation. This is not surprising as the frequency of chlorophyll mutation is said to be frequent in populations treated with EMS (Singh & Singh, 2007). In practical mutation breeding, chimera formation is observed (Spencer-Lopes *et al*., 2018). For example, chimeras have been found in sweet potatoes (Shin *et al*., 2011; Somalraju *et al*., 2018), yam (Yalindua *et al*., 2014) and cowpeas (Singh *et al*., 2006).

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Control Chlorination of leaves Multiple shoots

Figure 5.2 Shoot characteristics of brown tiger nut type treated with EMS having the control (A) showing normal growth and putative mutant morphotypes showing leaf variegation (B) and multiple shoot formation of the main plant (C).

Morpho-physiological traits

Plant growth parameters such as plant height, stem girth, number of leaves, leaf length, and leaf width are essential morpho-physiological traits that contribute to increased biomass. Significant ($p < 0.000$) differences existed among the 25 genotypes for plant height. It ranged from 92.00 cm for CBC (colchicine black control) to 115.75 cm for Cb5 (colchicine brown mutant). The majority of the colchicine-treated genotypes were taller than their controls as well as the EMS-treated genotypes (Table 5.1). This is not surprising as colchicine is known to stimulate growth in plants due to the modification of the signalling pathway (Singh *et al*., 2020). According to Moore *et al*. (2001), the use of colchicine leads to a modification in the genetic makeup of plants. This modification

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occurs due to an accelerated rate of cellular division and growth of the meristematic zones.

Similarly, stem girth ranged from 10.8 mm (Eb22) to 13.5 mm (EB2) accross all the 25 genotypes including the 4 controls. Four colchicine mutants, Cb5 (13.3 mm), CB5 (13.0 mm), Cb19 (12.7 mm), and CB3 (12.6 mm) ranked second, third, fourth and fifth while the control (EbC) ranked sixth with a girth of 12.59 mm. However, the differences in stem girth among the genotypes were not significant ($P > 0.05$), suggesting that both EMS and colchicine mutants and their controls were much similar to each other in terms of stem girth when compared. However, on colchicine–tuber treated basis, colchicine had some level of influence on the stem girth expansion of most of the colchicine mutants relative to their controls (CbC, and CBC) (Table 5.1).

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Table 5.1: Mean values of 20 morpho-descriptors among tiger nut genotypes (21 mutants and 4 controls).

Genotype	DG	NT	DTP (cm)	PH (cm)	GP (mm)	NL	LL (cm)	LW (cm)	DF	NIP	DPM	NTA	NTD	TNT	OTW (g)	HTW (g)	TL (mm)	TW (mm)	TS (mm)	NRT
EBC	8.5^{a} bc	18.2 ab	9.9^{a}	92.8 c	11.0^{a}	16.2^{a}	51.3^{d} ef	1.4^{a}	24.7 a	1.8^{a}	85.2^{ab}	59.3^{abc} d	9.2^{cde} fg	68.5^{ab}	79.8^{ab}	126.3 ab	21.2 cdefg	17.4^{ab} c	1.2^{de}	6.7^{ab}
EB2	9.5^{a}	17.2 ab	9.9^{a}	97.5 abc	13.5^{a}	15.8^{a}	52.3^{c} def	1.5^{a}	17.5 a	0.7^{a}	81.3^{ab} cdef	75.7^{abc}	10.2^{b} cdefg	85.8^{a}	71^{ab}	110.0 ab	15.7^{g}	15.7^{bc} def	1.0^{e}	6.4^{abc}
EB5	8.3^{a} b	18.2 ab	10.0^{a}	96.5 abc	12.3^{a}	15.3^{a}	50.0^{e} f	1.4^{a}	24.5 a	1.3^{a}	83.3^{ab} cde	80.3^{a}	3.8^{fg}	84.2^{a}	73.5^{ab}	110.3 ab	17.2^{fg}	16.8^{ab} cd	1.0^{e}	6.2^{bc}
EB7	7.7^{a} bc	16.2 ab	8.8^{a}	98.3 abc	11.5^{a}	14.5^{a}	55.3^{a} bcdef	1.4^{a}	25.8 a	1.2^{a}	83.7^{ab} cd	79.5^{ab}	6.7^{cde} fg	86.2^{a}	74.8^{ab}	113.3 ab	17.2^{fg}	16.0^{ab} cde	1.1^{e}	6.2^{bc}
EB8	8.2^{a} b	17.5 ab	10.8^{a}	99.7 abc	12.1^{a}	14.8^{a}	56.3^{b} cdef	1.4^{a}	24.7 a	1.8^{a}	84.0^{ab} c	73.8^{abc} d	4.0^{efg}	77.8$_{ab}$	99.2^{a}	163.0 a	22.6 cdefg	18.2^{a}	1.2^{de}	6.8^{ab}
EB12	9.3^{a} b	13.2 b	8.2^{a}	99.4 abc	12.0^{a}	14.8^{a}	51.4^{d} ef	1.4^{a}	26.7 a	1.3^{a}	85.2^{ab}	65.7^{abc} d	4.7efg	70.3^{ab}	87.8^{ab}	151.7 ab	20.0^{de} fg	17.8^{ab}	1.1^{de}	6.8^{ab}
EbC	7.2^{a} bc	18.0 ab	12.7^{a}	95.0 bc	13.0^{a}	14.7^{a}	66.7^{a} b	1.5^{a}	19.3 a	1.0^{a}	77.5^{cd} ef	46.3^{bcd}	11.0^{b} cdefg	57.3^{ab}	67.7^{ab}	122.7 ab	22.9^{bc} def	13.1^{gh}	1.8^{abc}	6.8^{ab}
Eb1	6.8^{a} bc	18.7 ab	9.9^{a}	109.5^{abc}	11.0^{a}	14.8^{a}	59.5^{a} bcdef	1.5^{a}	25.0 a	1.5^{a}	76.3^{ef}	44.0^{cd}	2.8^{g}	46.8^{b}	45.0^{b}	88.3^{b}	28.3^{ab}	12.7^{h}	2.2^{a}	6.7^{ab}
Eb2	6.2^{c}	23.0 a	13.0^{a}	113.2^{abc}	12.0^{a}	14.8^{a}	59.7^{a} bcdef	1.5^{a}	26.2 a	1.3^{a}	86.2^{a}	72.7^{abc} d	7.8^{cde} dfg	80.5^{a}	98.3^{a}	166.0 a	31.9^{a}	15.5^{cd} ef	2.1^{ab}	7.3^{a}
Eb8	6.2^{c}	15.0 ab	12.2^{a}	104.4^{abc}	12.2^{a}	16.2^{a}	63.4^{a} bc	1.5^{a}	24.7 a	2.0^{a}	75.7^{f}	47.0^{bcd}	23.0^{a} bcd	70.0^{ab}	76.0^{ab}	129.3 ab	22.6^{bc} def	14.1^{ef} gh	1.6^{bcd}	6.0^{bc}
Eb12	5.8^{c}	20.4 ab	10.8^{a}	107.1^{abc}	10.9^{a}	14.8^{a}	66.0^{a} b	1.5^{a}	25.8 a	1.5^{a}	78.0^{cd} ef	59.8^{abc} d	5.8^{def} g	65.7^{ab}	66.5^{ab}	119.7 ab	26.5^{ab} cd	13.6^{fg} h	2.0^{ab}	6.3^{bc}
Eb14	7.0^{a} bc	20.5 ab	10.9^{a}	108.0^{abc}	11.9^{a}	16.2^{a}	66.2^{a} b	1.5^{a}	25.5 a	1.8^{a}	79.8^{ab} cdef	60.0^{abc} d	11.5^{b} cdefg	71.5^{ab}	78.8^{ab}	138.7 ab	27.2^{ab} c	13.5^{fg} h	2.0^{ab}	6.2^{bc}
Eb22	7.2^{a} bc	18.2 ab	11.8^{a}	109.0^{abc}	10.8^{a}	15.0^{a}	62.3^{a} bcd	1.4^{a}	24.2 a	2.3^{a}	80.2^{ab} cdef	54.2^{abc} d	20.0^{a} bcdefg	74.2^{ab}	85.7^{ab}	136.0 ab	20.6 cdefg	15.1^{de} fg	1.4^{cde}	5.7^{c}
CBC	7.7 abc	14.3 ab	11.0^{a}	92.0 c	11.7^{a}	15.5^{a}	49.0^{f}	1.4^{a}	24.8 a	1.0^{a}	81.5^{ab} cdef	50.3^{abc} d	11.5^{b} cdefg	61.8^{ab}	70.7^{ab}	117.0 ab	17.2^{fg}	16.9^{ab} cd	1.0^{e}	6.4^{abc}
CB2	7.8 abc	15.3 ab	10.0^{a}	93.3 c	11.8^{a}	15.7^{a}	51.5^{d} ef	1.5^{a}	22.5 a	1.5^{a}	80.3^{ab} cdef	55.8^{abc} d	5.7^{def} g	61.5^{ab}	77.8^{ab}	137.7 ab	18.4 efg	16.9^{ab} cd	1.1^{de}	6.6^{abc}
CB3	6.5^{b} c	13.5 b	10.5^{a}	97.0 abc	12.6^{a}	15.2^{a}	50.7^{e} f	1.5^{a}	22.8 a	1.5a	80.3^{ab} cdef	58.5^{abc} d	6.3^{def} g	64.8^{ab}	78.3^{ab}	129.3 ab	17.1^{fg}	16.9^{ab} cd	1.0^{e}	6.4^{abc}

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Table 5.1 Continued

CB5	7.8 abc	17.8 ab	10.8^{a}	93.5 bc	13.0^{a}	16.2^{a}	52.9^{c} def	1.5^{a}	25.5 a	1.5^{a}	79.7^{ab} cdef	62.2^{abc} d	3.5^{fg}	65.6^{ab}	70.8^{ab}	126.7 ab	18.3^{ef} g	16.6^{ab} cd	1.1^{de}	6.5^{abc}
CB5	7.8 abc	17.8 ab	10.8^{a}	93.5 bc	13.0^{a}	16.2^{a}	52.9^{c} def	1.5^{a}	25.5 a	1.5^{a}	79.7^{ab} cdef	62.2^{abc} d	3.5^{fg}	65.6^{ab}	70.8^{ab}	126.7 ab	18.3^{ef} g	16.6^{ab} cd	1.1^{de}	6.5^{abc}
CB7	7.5^{a} bc	14.5 ab	12.0^{a}	95.7 bc	12.0^{a}	15.0^{a}	48.8^{f}	1.5^{a}	25.0 a	1.5^{a}	80.2^{ab} cdef	55.2^{abc} d	4.5^{efg}	59.7^{ab}	73.8^{ab}	135.0 ab	20.3^{de} fg	17.0^{ab} cd	1.2^{de}	6.4^{abc}
CB16	8.2 abc	16.3 ab	11.6^{a}	94.2 bc	12.0^{a}	16.0^{a}	50.5^{e} f	1.4^{a}	27.5 a	1.2^{a}	80.8^{ab} cdef	73.0^{abc} d	7.2^{cde} fg	80.2^{a}	91.2^{a}	135.7 ab	16.1^{fg}	16.5^{ab} cd	1.0^{e}	6.2^{bc}
CbC	6.2^{c}	18.2 ab	12.5^{a}	109.2^{abc}	12.3^{a}	16.0^{a}	64.5^{a} b	1.5^{a}	25.0 a	2.3^{a}	79.3^{ab} cdef	45.2^{cd}	28.3^{a} b	73.5^{ab}	75.7^{ab}	132.0 ab	20.0^{de} fg	14.1^{ef} gh	1.4^{cde}	6.1^{bc}
Cb2	6.2^{c}	17.0 ab	11.8^{a}	109.2^{abc}	11.3^{a}	15.7^{a}	66.6^{a} b	1.5^{a}	25.5 a	2.0^{a}	76.7^{de} f	47.0^{bcd}	18.0^{a} bcdefg	65.0^{ab}	69.5^{ab}	123.7 ab	20.1 defg	14.7^{de} fgh	1.4^{cde}	6.1^{bc}
Cb5	6.0^{c}	17.3 ab	10.8^{a}	115.8^{a}	13.3^{a}	15.2^{a}	61.5^{a} bcde	1.4^{a}	26.0 a	2.2^{a}	76.7^{de} f	44.3^{cd}	22.2^{a} bcde	66.5^{ab}	66.8^{ab}	118.0 ab	24.8^{bc} de	14.0^{ef} gh	1.8^{abc}	6.1^{bc}
Cb9	6.2^{c}	15.0 ab	13.6^{a}	105.2^{abc}	12.5^{a}	14.8^{a}	69.2^{a}	1.5^{a}	26.8 a	1.7^{a}	76.3^{ef}	41.3^{d}	21.7^{a} bcdef	63.0^{ab}	75.3^{ab}	133.7 ab	21.7^{bc} defg	15.4^{cd} ef	1.4^{cde}	6.3^{bc}
Cb11	6.8^{a} bc	15.8 ab	13.3^{a}	105.0^{abc}	12.0^{a}	16^{a}	64.7^{a} b	1.5^{a}	26.5 a	2.2^{a}	77.0^{cd} ef	51.3^{abc} d	29.8^{a}	81.2^{a}	89.7^{ab}	140.3 ab	21.4^{cd} efg	15.5^{bc} def	1.4^{cde}	6.1^{bc}
Cb19	6.8^{a} bc	18.0 ab	14.9^{a}	101.7^{abc}	12.8^{a}	16.2^{a}	66.3^{a} b	1.5^{a}	17.2 a	1.3^{a}	79.0^{bc} def	51.7^{abc} d	24.7^{a} bc	76.3^{ab}	84.7^{ab}	138.0 ab	21.5 cdefg	15.4^{cd} ef	1.4^{cde}	6.4^{abc}

Values in the same column followed by the same lettter (s) do not significantly differ, by Tukey's test at P> 0.05.

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The mean number of leaves (NL) and leaf width (LW) also did not differ significantly (P> 0.05). Mean NL ranged from 14.7 (EB7) to 16.2 (CB5, Eb8, Cb11, Eb14, and EBC). Also, mean LW ranged from an average of 1.4 (EB8) to 1.5 (EbC, Cb11, Eb12, CB5, Cb19, Eb2) (Table 5.1).

Leaf length (LL) differed significantly (P<0.0001) among the genotypes. LL ranged from 48.8 cm in CB7 to 69.2 cm in Cb9. The majority of colchicine-treated brown mutants (Cb9, Cb2, Cb19, Cb11) outperformed their control (CbC) and many of the genotypes including their black colchicine-treated counterparts (Table 5.1). This was expected as colchicine resulted in increased chromosome number of organisms leading to increased plant growth and production of larger plants (Liu *et al*., 2007; Spencer-Lopes *et al*., 2018; Kumar *et al*., 2019). The results indicated that the influence of colchicine had a significantly greater effect on the growth characteristics of brown tiger nuts compared to the black genotypes. This aligns with previous fundings indicating that brown tiger nut accessions are more susceptible to the impacts of mutagens in plant growth traits than the black types (Twumasi *et al*., 2023).

Earliness to maturity

Ordinarily, tiger nuts take 90 to 110 days after planting to reach physiological maturity if properly managed (Bazine & Arslanoğlu, 2020). Physiological maturity involving signs of yellowing of leaves and the cessation of new inflorescence occurs when the plant is 75 to 90 days old (Tetteh & Ofori, 1998). Overall, all the 25 genotypes used in the current study physiologically matured in a space range of 75 to 86 days indicating general earliness of maturity for the mutants and their controls. This is not surprising as earliness was an important trait considered in the selection process of the genotypes for the successive

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generations from M1V1 to M1V4. Therefore, these genotypes present a choice of candidates for breeding for earliness. However, six mutants outperformed the so-early-to mature control (EbC), which physiologically matured at 78 days (Figure 5.3). The very-early-to mature mutant, Eb8, which initiated flower setting as early as 17 days (Table 5.1) had a mean of 75.7 days to reach physiological maturity. This was followed by the mutants Eb1, Cb9, Cb2, Cb5, and Cb11, which respectively, on average took 76.3, 76.3, 76.7, 76.7, and 77.0 days to reach physiological maturity (Figure 5.3).

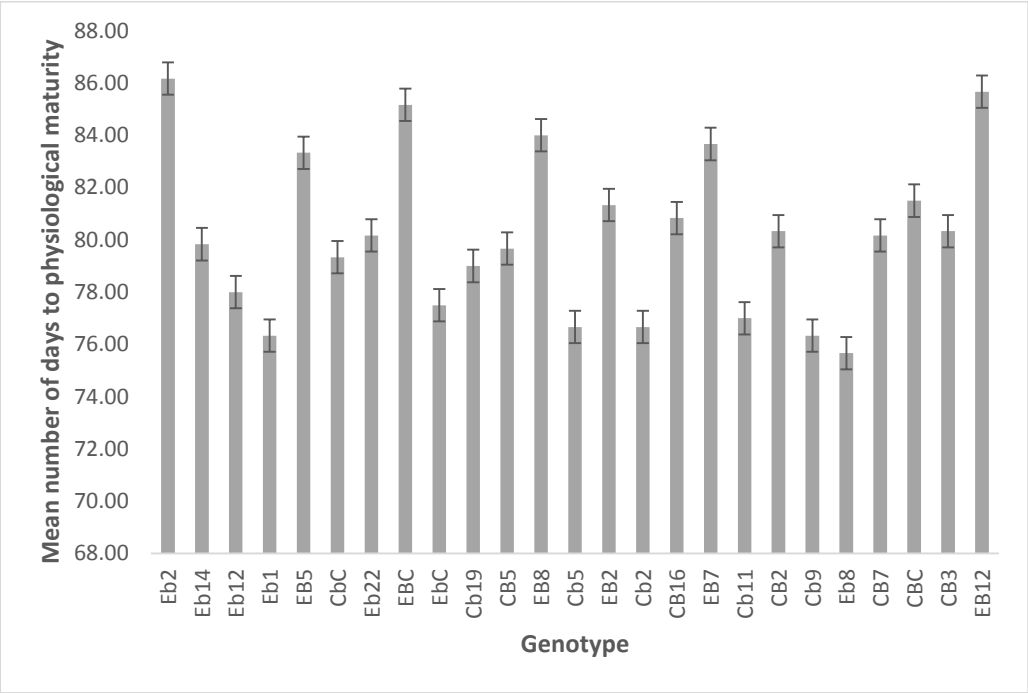

Figure 5.3: Effects of mutagens on days to physiological maturity among the 25 tiger nut genotypes.

All six mutants were brown with only two (Eb1, Eb8) EMS treated while the rest were colchicine treated, indicative of the probable influence of colchicine for early maturity in brown tiger nuts. Also, the brown tiger nuts were more responsive to the effects of the chemical mutagens. The very sensitive nature of

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brown tiger nuts to chemicals and other environmental stresses has been reported (Mu *et al*., 2022; Li *et al*., 2023). This findings of the brown tiger nut genotypes as highly sensitive to the chemical mutagens (EMS and colchicine) than their black counterparts were not evident only in earliness but also in days to germination (DG), number of tillers (NT), plant height (PH), and number of inflorescence per plant (NIP) among others (Table 5.1). The onset of flowering was early in the mutant Cb19 with a mean of 17.2 days while it was late in the mutant CB16 with a mean of 27.5 days. Flowering was 100% as all genotypes flowered with an average of 1.00 to 2.3 inflorescences per plant (Table 5.1). This is very promising for breeding advancement for the crop as tiger nuts scarcely flower. However, poor flowering was observed in an earlier work on the phenotypic characterization of tiger nut accessions in Ghana (Asare *et al*., 2020). The observation of flowering in the current study can be attributed to the effects of the mutagens, for example, EMS might have altered some genes in the genotypes for flower initiation whiles colchicine disrupted microtubles formation for polyploidy and altered flowering (Kumar *et al*., 2021; Kumari, 2024). The mean numbers of inflorescece per plant were higher in brown colchinine-treated genotypes than the brown EMS-treated ones, and also all the black tiger nut types for both EMS and colchicine-treated (Table 5.1). Additionally, the careful selection for inflorescence (flowering) of the genotypes for the successive generations might have been another cause of 100% formation of percentage inflorescence for all the 25 genotypes.

Yield

Generally, there was wide variability in yield among the 25 tiger nut genotypes in terms of mean hundred-tuber-weight per genotype (HTW) and mean overall

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tuber weight per plant (OTW). For instance, HTW differed significantly (P <0.01) among the genotypes (Figure 5.4). In all, 11 mutant genotypes made up of both EMS-treated and colchicine-treated brown and black tiger nuts performed better than the best control in HTW ranking. The highest HTW was observed in Eb2 with a remarkable HTW of 166.0 g compared to 132.0g in the control, CbC. The others included EB8 (163.0 g), EB12 (151.7 g), Cb11 (140.3 g), Eb14 (138.7 g), Cb19 (138.0 g), CB2 (137.7 g), Eb22 (136.0 g), CB16 (135.7 g), CB7 (135.0 g), and Cb9 (133.7 g) (Figure 5.4).

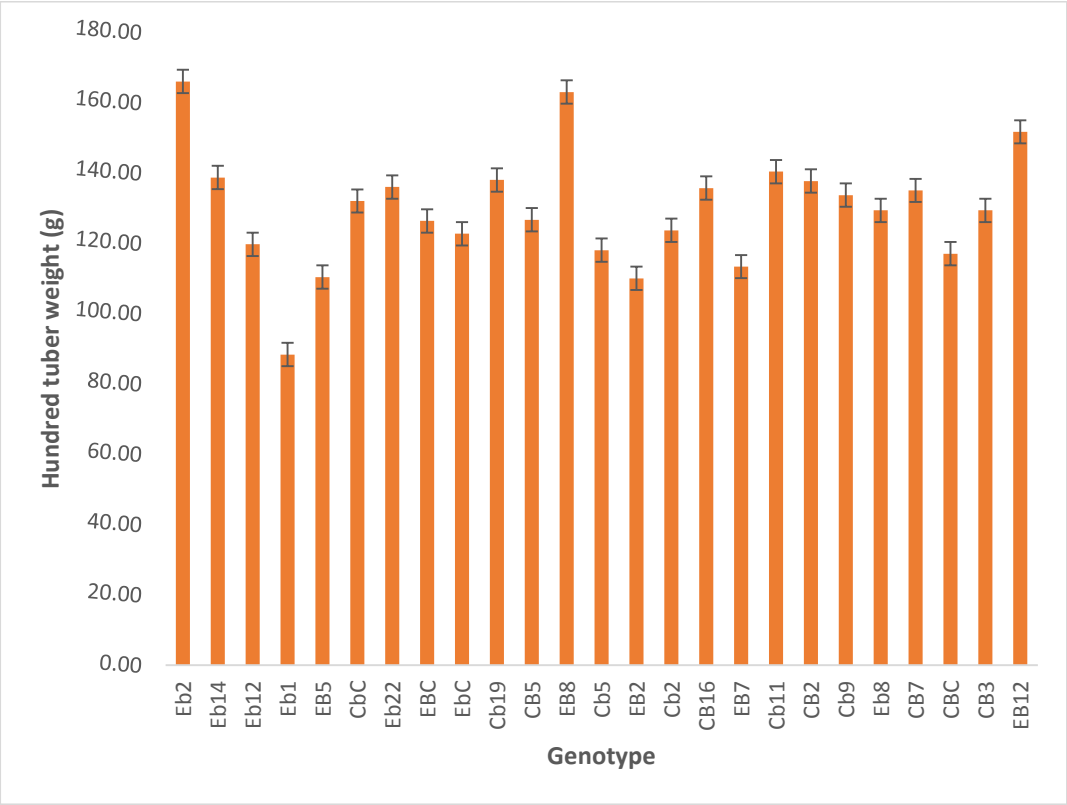

Figure 5.4: Effects of mutagens on hundred-tuber-weight among the genotypes.

The HTWs obtained in the current study are higher than those (99.28 g in 2019 and 102.85 g in 2020) recorded by Yang *et al*. (2022). Again, they outweighed the majority of the accessions evaluated in Ghana. For instance, in a morphological characterization of local tiger nut accessions, Donkor and her

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colleagues recorded 17.8- 132.3 g for HTW among 23 out of 24 accessions, with only one out of the 24 accessions producing a higher HTW of 295.3 g compared to that observed in the current study (Donkor *et al.*, 2019). Nonetheless, the mutants of the recent study, on average, are commendable for their mean values in HTW as compared to their controls, and hence present added reserve to tiger nut accessions in Ghana for breeding improvement. Seven of these mutants were the best-performing genotypes in mean tuber yield per stand (Figure 5.5). They outperformed the best control genotype EBC (119.8 g). The mutants and their differences in performacnce from the best control were EB8 (29.0 g), Eb2 (27.7 g), CB16 (17.0 g), Cb11(14.7 g), EB12 (12.0 g), Eb22 (8.7 g), and Cb19 (7.2 g).

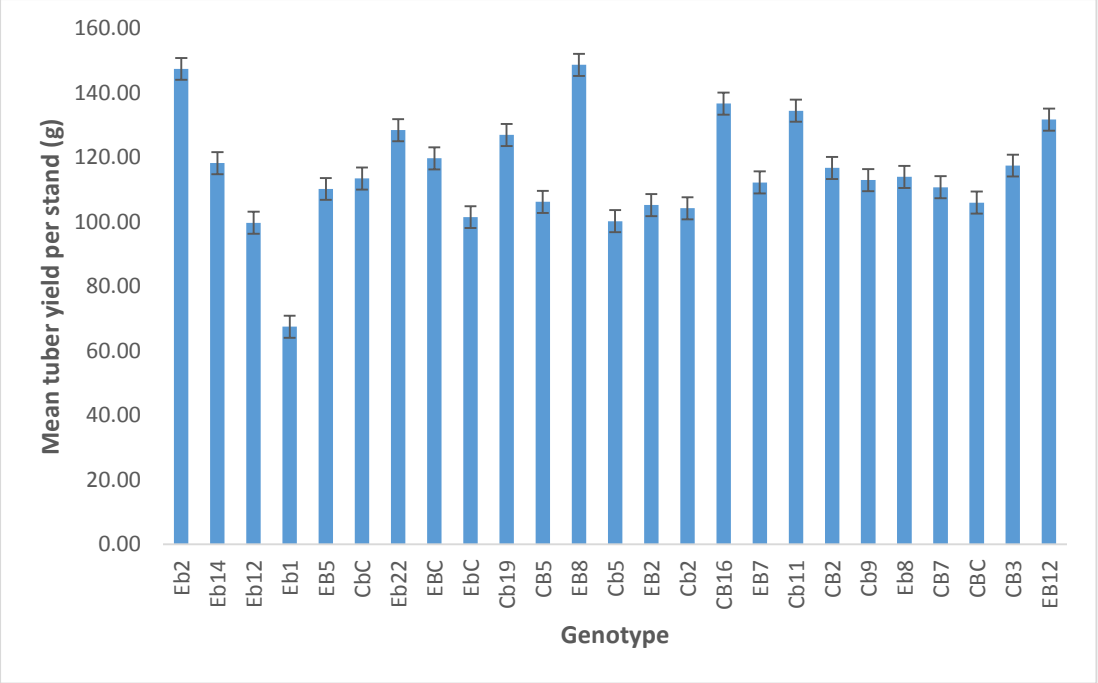

Figure 5.5: Effects of mutagens onmean tuber yield of tiger nut mutants and their controls.

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Mean tuber yield or overall tuber weight (OTW) ranged from 67.5 g for the mutant Eb1 to 148.8 g for the mutant EB8; and the differences were significant ($P<0.05$) among the accessions. Among the treated genotypes, only the colchicine-treated black mutants outperformed their control. The rest, however, had some mutants outperforming their control while the others' performance was below that of the control. For example, two of the EMS-treated black mutants, EB8 (148.8 g) and EB12 (131.8 g) outperformed their control EBC (119.8 g). Whilst EB7 (112.2 g), EB5 (110.2 g), and EB2 (105.3 g) performed below the control. Overall tuber weight is influenced by several factors involving genetics, environmental conditions, soil quality, water availability, and cultivational practices (Liliane & Charles, 2020). The differences in tuber yield may be attributed to genetics as the genotypes were cultivated under the same environmental conditions apart from the mutagen applied. According to Khursheed *et al*. (2021), the impact of a mutagen is species-specific and also affected by the type and dose of the mutagen. Hence given the optimum or recommended dose of mutagen as per the current study, it can be concluded that colchicine influenced the genotype of the black tiger nuts translating into an overall increased in tuber weight.

Tuber characteristics

In general, tuber coat colour among the genotypes for putative mutant and mutant lines remained unchanged under treatment with the mutagens (both EMS and Colchicine) from M1V1 to M1V4. EMS-treated black and colchicine-treated black tubers were black respectively upon successive generations. Similarly, the brown genotypes treated with EMS and colchicine showed no colour change in the tubers produced (Figure 5.6).

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Figure 5.6: Tuber characteristics of mutants and control tiger nut genotypes.

This indicates that coat colour is sparsely affected in vegetatively propagated crops when exposed to mutagens, unlike other sexually propagated crops. This findings of the current study is in agreement with Shin *et al*. (2011) and Somalraju *et al*. (2018) who observed no colour change in the tuber coat colour of sweet potato tubers mutagenized with gamm irradiation. However, with sexually propagated crops, the effect is clear and domineering with colour

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changes as reported in the seeds of cowpea exposed to mutagens (Horn *et al*., 2016; Opoku Gyamfi *et al*., 2022).

Another interesting observation made on tuber development was twined or multiple tuber formation among some of the mutants, EMS-treated brown genoypes (Figure 5.6). Four of the EMS-treated brown mutants had multiple tuber development, resulting in enhanced tuber sizes and various shapes. For example, the tuber lengths of Eb2 (47.9 mm), Eb1 (42.5 mm), Eb14 (40.8 mm) and Eb12 (39.8 mm) were longer than EbC (33.9 mm), the control (Table 5.1).

Effects of mutagens on tuber attachment to plant on harvest

overall, the mutant EB5 had the highest number of tuber attached per plant of 80.3 out of the total mean of 84.2 tubers underground (Table 5.1), representing 95.4% of tubers attached to the plant during harvesting. This was followed by EB7 (79.5), EB2 (75.7), and EB8 (73.8), all of which were black tiger nuts treated with EMS (Figure 5.6). Colchicine-treated mutant, CB16, another black tiger nut ranked 5th with a mean tuber count of 73.0 (Figure 5.6) accounting for 91% of nuts produced. Among the EMS-treated brown mutants, Eb2 had the highest mean number of tubers attached with 72.7 out of 80.5 tubers per stand representing 90.3% to rank 6th. The mutants; EB12, CB5, Eb14, and Eb12 ranked next in succession having the control, EBC occupying the 11th position with a mean number of 59.3 tuber attached (86.6%) out of 68.5 tubers harvested. on the basis of colchicine-treated brown genotypes, 3 mutants- Cb19 (51.7 tubers), Cb11 (51.3 tubers) and Cb2 (47.0 tubers) performed better than their control CbC which had an average of 45.2 tubers attached to the plant (Figure 5.7).

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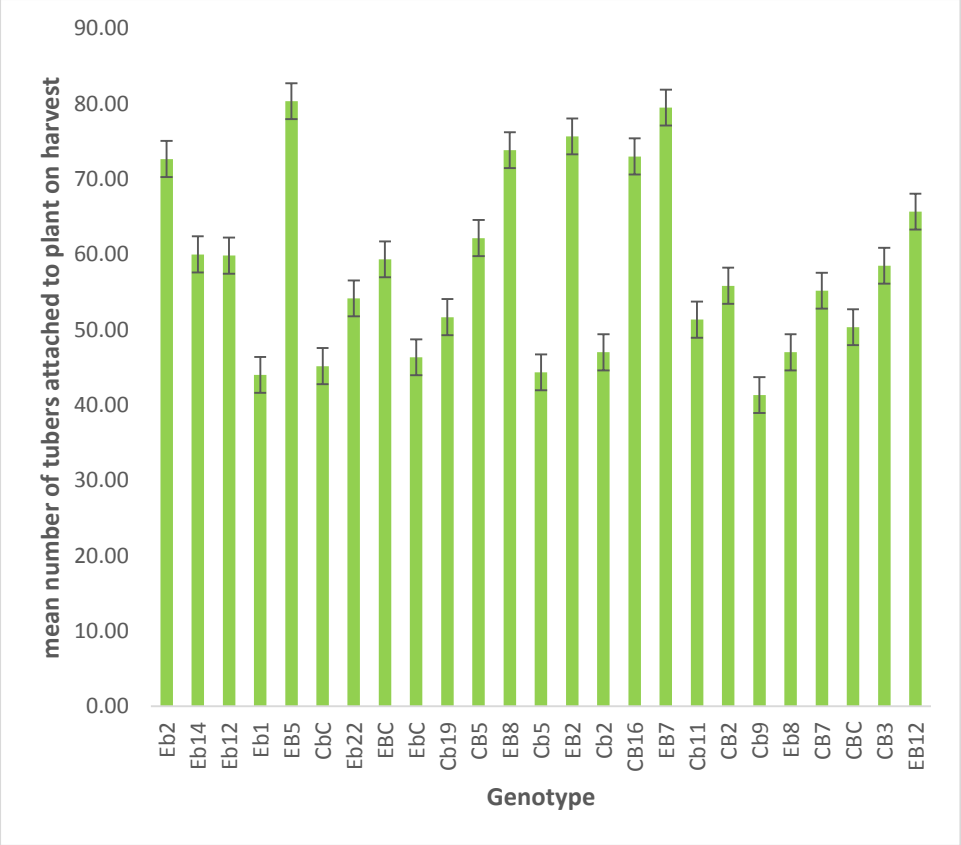

Figure 5.7: Effects of mutagens on mean number of tubers attached to genotypes on harvest.

In addressing the problem of difficulty in harvesting arising from detachment of the tubers in the soil at harvesting as lamented by farmers (Tetteh & Ofori, 1998; Akabassi *et al*., 2021), producers and plant breeders stand the chance of selecting these identified mutants with such resilient and robust runners for tuber attachment on plants during harvesting.

Genetic relationship of genotypes

The genetic similarity among 21 mutants and their 4 controls was estimated utilizing standardized variables using Euclidean distance measure on 20 morphological traits. A wide range of variations existed among the genotypes. The genotypes were grouped into nine clusters using the elbow method (Figure

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5.8), and at a Euclidean similarity distance of 48.76% (Figure 5.9). The elbow method illustrates a graph depicting the clustering performance (Within Cluster Sum of Squares) across varying number of clusters. The ideal number of clusters is identified at the point in the graph where it bends or forms a knee or elbow, with subsequent points showing a decrease in significance (Tan *et al*., 2019).

Three clusters (I, V and IX) were independently grouped from the rest. Clusters I and V were made up of only Eb2, and Eb1 mutants respectively. They were distantly apart from each other and their control (EbC) which was grouped with Cb19 in cluster VIII (Figure 5.9) on the premise of having a broader leaf width of 1.5mm. Cluster IX consisted of only one black EMS genotype (EB2) which varied distantly from its control (EBC) in another cluster (VI). This indicates high genetic variability and substantial genetic diversity in existence. Cluster II was made up of two genotypes consisting of the mutants Eb12, and Eb14. These mutants were all EMS-treated brown genotypes.

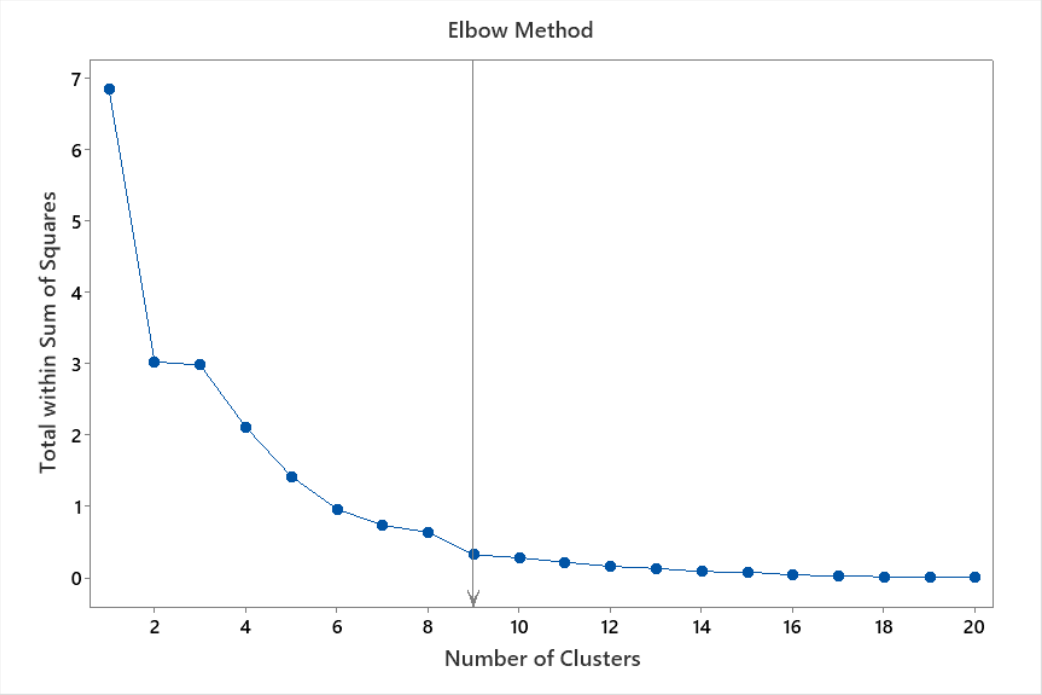

Figure 5.8: Elbow technique showing the optimum number of clusters.

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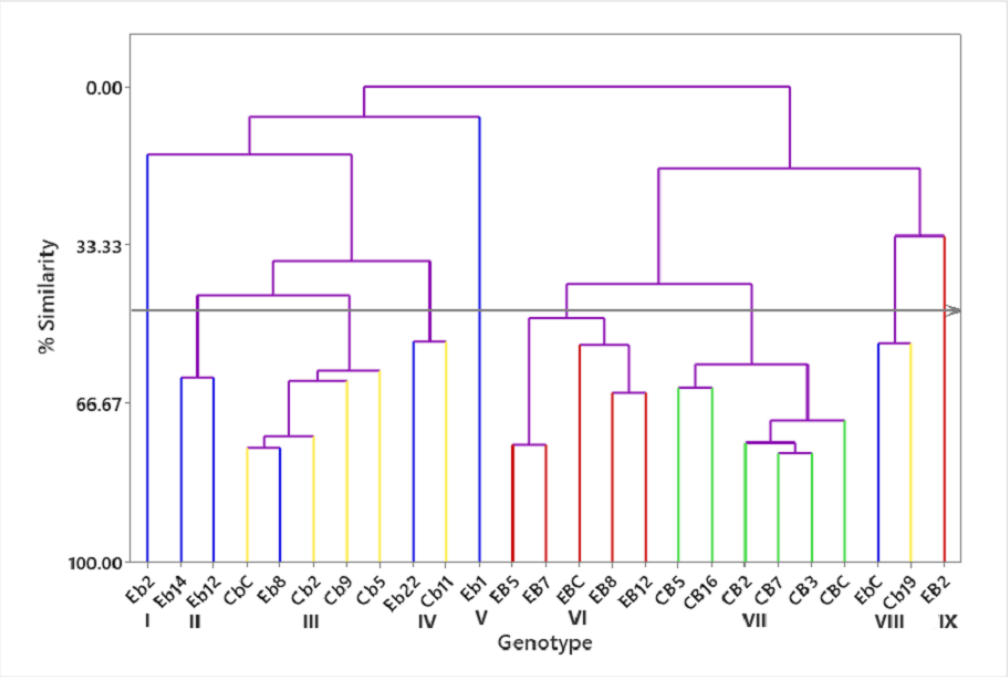

Figure 5.9: Dendrogram showing the diversity of mutants and control across twenty morpho descriptors using Euclidean measure similarity index.

The red colour indicates black genotypes treated with EMS; green colour shows black genotypes treated with colchicine; blue colour signifies brown genotypes treated with EMS; and yellow colour denotes brown genotypes treated with Colchicine.

Cluster III consisted of 5 genotypes. Four were colchicine-treated brown and the remainder was an EMS brown mutant (Eb8). The colchicine-treated brown genotypes were made up of the control (CbC) and 3 colchicine-treated brown mutants (Cb2, Cb5, and Cb9) (Figure 5.9; Figure 5.10A). The clustering was done according to various factors, including the height of the plants, the number of inflorescences per plant, the length of the leaves, the distance between the last tiller and the main plant, the number of tillers, the number of detached tubers, and the shape of the tubers (as shown in Figure 5.10B). They were tall in height, had 2 or more inflorescences, a large number of tubers detached in the soil, and oval-shaped tubers among others (Table 5.1).

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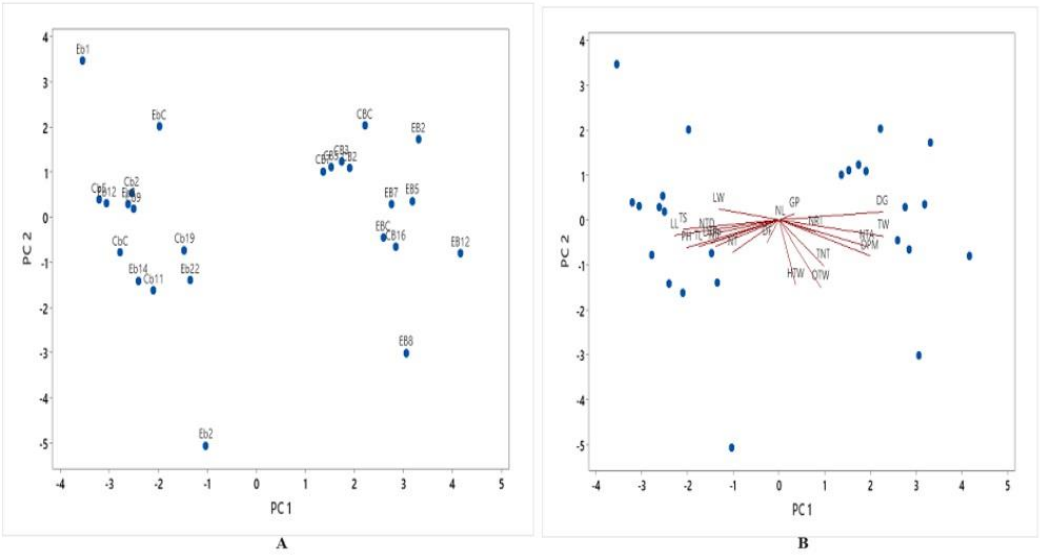

Figure 5.10: Principal components analysis using the correlation matrix of the characteristics among the 25 genotypes (mutants and control) of tiger nuts: A- PC score plot showing the distribution of genotypes. B- PC biplot showing the distribution of genotypes and morpho descriptors.

Members of cluster IV were made up of only two mutants (Cb11 and Eb22). Though they were treated differently, they were grouped together because they had more than 2 inflorescences (Figure 5.10B; Table 5.1).

Cluster VI is a group of 5 black tuber-producing genotypes. Four were EMS black mutants (EB5, EB7, EB8, EB12) clustered together with their control (EBC). They were clustered here because they share the characteristics of having similar days to physiological maturity (83-86 days) and higher number of tuber attached to the plant on harvesting among the genotypes. Breeders and farmers desire to have tiger nuts with tubers attached at physiological maturity to overcome the difficulty in harvesting, recover all tubers, and to save time and labour costs. Though the mutants clustered here with their control, the mutants had higher tuber numbers than their control.

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Similarly, cluster VII was composed of six colchicine-treated black genotypes, including the control (CBC) and 5 mutants (CB2, CB3, CB5, CB7, CB16) (Figure 5.9). They germinated between 7 and 8 days after sowing (Table 5.1).

Nutritional value of mutants

Increased consumption and utilization of tiger nuts, such as in the form of flour, milk or oil, is influenced by their nutritional composition. The tubers are rich in almost all functional food nutrients. However, the nutrient composition of the tuber is dependent on the type or variety of the tuber (yellow, brown, or black), the area of cultivation, planting seasons and the postharvest method used in the processing of the tubers (Adgidzi *et al*., 2011; Asante *et al*., 2014). The findings of the present study showed that the highest mean carbohydrate content of 52.6% was obtained for the mutant Cb19 with the least of 43.9% in EB8 (Table 5.2). EB12 ranked second highest with 50.6% followed by Eb22, Cb11 and CB2 with carbohydrate contents of 49.3%, 48.7% and 48.1%, respectively, all of which performed better in carbohydrates than the best-performing control, EBC. The carbohydrate content in the four controls EBC, CbC, CBC, and EbC, were 46.9%, 46.7%, 46.6% and 45.0%, respectively (Table 5.2). The carbohydrate contents in the controls are in conformity of previous findings of 45.73% (Sabah *et al*., 2019) and 46.99% for yellow-type tiger nut tubers (Nwaoguikpe, 2010). However, the current findings are higher than values to 43.3% (Sánchez-Zapata *et al*., 2012) and 41.22% for brown-type, and 65.66% of black-type (Nwaoguikpe, 2010), suggesting that the percentage nutrient content varies with the type of tiger nut tuber (Asante *et al*., 2014).

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Table 5.2: Proximate composition of tiger nut tubers in mutants and their controls.

Genotype	% Moisture	% Crude Ash	% Fat	% Crude Fibre	% Crude Protein	% Carbohydrate
EBC	16.6±0.18^{ef}	3.2±0.14^{bcd}	20.1±0.31^{a}	11.3±0.88^{def}	2.5±0.01^{d}	46.9±0.25^{cd}
EB8	16.9±0.31^{e}	3.5±0.24^{ab}	20.6±0.22^{a}	12.4±0.91^{bcd}	3.6±0.33^{bc}	43.9±1.09^{e}
EB12	16.3±0.19^{fg}	3.2±0.04^{bcd}	16.8±0.20^{d}	9.7±0.22^{fg}	3.7±0.09^{bc}	50.6±0.48^{ab}
EbC	20.6±0.10^{a}	3.4±0.12^{ab}	14.9±0.36^{e}	11.1±0.76^{def}	4.6±0.22^{a}	45.0±2.11^{de}
Eb2	17.8±0.14^{d}	3.7±0.22^{a}	17.1±0.01^{cd}	13.9±0.24^{ab}	3.3±0.15^{c}	44.5±0.25^{de}
Eb14	20.5±0.12^{a}	2.8±0.22^{def}	16.9±0.17^{cd}	13.8±1.00^{ab}	1.9±0.02^{e}	44.8±1.05^{de}
Eb22	19.9±0.01^{b}	2.6±0.0.04^{ef}	15.0±0.51^{e}	10.5±0.45^{efg}	3.3±0.16^{c}	49.3±0.82^{bc}
CBC	15.4±0.27^{hi}	3.4±0.10^{abc}	19.0±0.28^{b}	14.2±0.54^{a}	2.1±0.05^{de}	46.6±1.23^{cde}
CB2	14.8±0.51^{i}	3.7±0.07^{ab}	19.0±0.36^{b}	11.7±0.37^{cde}	3.5±0.26^{c}	48.1±0.55^{bc}
CB16	16.8±0.15^{ef}	2.6±0.31^{ef}	19.7±0.51^{ab}	13.5±0.12^{abc}	3.5±0.33^{c}	44.6±1.11^{de}
CbC	19.5±0.05^{bc}	3.1±0.12^{bcde}	17.8±0.56^{c}	11.2±0.84^{def}	2.5±0.02^{d}	46.7±1.00^{cde}
Cb11	19.1±0.01^{c}	2.5±0.08^{f}	16.9±0.32^{cd}	9.0±0.50^{gh}	4.1±0.14^{ab}	48.7±0.24^{bc}
Cb19	15.9±0.11^{gh}	2.9±0.22^{cdef}	17.5±0.13^{cd}	7.5±0.06^{h}	3.7±0.04^{bc}	52.6±0.23^{a}

Means and standard deviations for n =3. Means having the same letter(s) do not differ significantly differby Tukey's test at (P>0.05).

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On the part of crude protein content, eight mutants out of the 13 genotypes (9 mutants and 4 controls) outperformed 3 controls (EBC-2.51%, CbC-2.46%, CBC-2.12%). They included Cb11 (4.08%), Cb19 (3.74%), EB12 (3.74%), EB8 (3.6%), CB16 (3.51%), CB2 (3.47%), Eb2 (3.30%), and Eb22 (3.30%) (Table 5.2), However they scored low crude protein values to the contorl, EbC, which recorded the highest value of 4.60%. Nontheless, . many of these mutants performed well for the carbohydrates (Table 5.2), hence they have great potential to be used for food. The crude protein content of genotypes used in the current study was comparatively below 5.04% (Sánchez - Zapata *et al*., 2012), 7.15-9.20% (Oladele & Aina, 2007), and 7.15-12.00% (Nwaoguikpe, 2010). However, they are in support of the findings of Bado et al. (2015) (3.30-4.33 %), and also in agreement with the score range of 4.16- 5.56% of 24 Ghanaian accessions evaluated for proximate and mineral composition (Donkor *et al.*, 2021).

The percentage fat content ranged from a mean of 14.86% for EbC (control) to 20.61% for EB8. The EMS-treated black mutant, EB8 outperformed the EMS black control (EBC) which recorded 20.08%. Additionally, all three EMS-treated brown mutants—Eb2 (17.14%), Eb14 (16.94%), and Eb22 (15.03%)—exhibited superior fat values compared to their control, EbC (14.89%). Among the two colchicine-treated black mutants, CB16 (19.70%) outperformed the control CBC (18.98%), while CB2 recorded a similar level at 18.97%.

Conversely, neither of the two colchicine-treated brown mutants exceeded their control counterpart CbC (17.79%). Overall, the findings indicate that black tiger nut tubers possess a higher fat content than their brown counterparts.

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Specifically, five black genotypes, which included one EMS black mutant, two colchicine black mutants, one EMS black control, and one colchicine black control, ranked higher in fat content than the brown genotypes. This suggests that the black tiger nut accessions in Ghana are more suitable for oil production due to their higher crude fat levels compared to the brown and yellow varieties assessed for their proximate and mineral composition (Donkor *et al*., 2021). These results are however in contrast with previous studies that reported higher crude fat percentages in yellow and brown tiger nut tubers compared to black types (Oladele & Aina, 2007; Nwaoguikpe, 2010; Sánchez - Zapata *et al*., 2012; Bado *et al*., 2015). Furthermore, the nutritional composition of both brown and black tiger nut tubers is significantly influenced by the cultivation site and planting periods (Aremu *et al*., 2015).

Besides the chemical constituents of proximate composition, tiger nut tubers are also valued for their rich mineral deposits. Table 5.3 shows the high levels of phosphorous, potassium, and iron in the tubers with calcium, magnesium, and sodium being in low amounts.

In general, the mutants had increased phosphorous and potassium contents compared to the controls. Contrarily, the calcium and iron levels were lower in the mutants than in the control. Also, the effects of both EMS and colchicine on the richness of the mutants for magnesium, and sodium contents were insignificant to their controls (Table 5.3). This finding of the current study is suggestive that the EMS and the colchicine mutagens did not significantly affected the calcuim, iron, magnessium and sodium contents of tiger nuts as observed in tomato (Islam *et al*., 2024).

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Percentage phosphorous on the other hand, ranged from 0.49% (EB8) to 0.64% (Eb2). The mutant Eb2 outperformed all the accessions, including the four controls, EBC, CBC, EbC and CbC which recorded 0.59%, 0.57%, 0.50%, and 0.49% respectively (Table 5.3). The value of 0.64% far exceeds that of 0.141% (Sabah *et al*., 2019), 0.121% (Oladele & Aina, 2007), 0.229- 0.283% (Bado et al., 2015), and the majority of accessions evaluated in Ghana (Donkor *et al*., 2021). The EMS-treated brown mutants, Eb2 (0.64%) and Eb22 (0.59%) were richer in phosphorous than their control, Ebc (0.50%). Whiles none of the EMS-treated black mutatants outwitted their control, EBC (0.50%) in phosphorous content (Table 5.3). On the part of colchicine, the colchicine-treated brown mutants Cb11 (0.58%) and Cb19 (0.54) performed better than their control, CbC (0.49) in phosphorous. Wheras, only one colcichine-treated black mutant, CB2 (0.59%) was a little higher than the control, CBC (0.57%) in phosphorous.

Potassium content ranged from 1.28% for CbC (control) to 1.75% for Eb14 (mutant). Within the EMS based accessions, the black mutants, EB8 (1.53%) and EB12 (1.42%) were richer in potassium than their control, EBC (1.40%). Conversely, all the three EMS-treated brown mutants (Eb2-1.64%, Eb14-1.75% and Eb22-1.64%) were lower in potassium content to their control, EbC (1.65%) (Table 5.3). This narrative however was opposite with the colchicine based accessions, where all the two brown mutants Cb11 (1.40%) and Cb19 (1.55%) had high potassium values than their control, CbC (1.28%). While the colchicine-treated black mutant, CB2 (1.56) was richer in potassium than the control, CBC (1.55%). Eb14, overall, ranked the highest and was followed by the control, EbC with 1.65%. The high percentage of potassium in Eb14 exceeds that of 0.556-0.845% (Bado *et al*., 2015), and 0.216% (Oladele & Aina, 2007).

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Notwithstanding, the high potassium values including that of the controls for the current study as also identified by Donkor *et al*. (2021), are indicative that Ghanaian tiger nut accessions are endowed with a good amount of potassium for the normal functioning of the heart, regulation of muscular contraction, and the synthesis of protein and carbohydrates metabolism (Pohl *et al*., 2013). The mutant

Table 5.3: Mineral composition in tuber of tiger nut mutants and their controls.

Genotype	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Fe (%)
EBC	0.59±0.00^{b}	1.40±0.00^{e}	0.34±0.01^{c}	0.11±0.00^{cd}	0.03±0.00^{cd}	0.94±0.03^{a}
EB8	0.49±0.00^{h}	1.53±0.01^{d}	0.33±0.00^{cd}	0.09±0.00^{f}	0.03±0.00^{cd}	0.66±0.03^{e}
EB12	0.52±0.00^{e}	1.42±0.01^{e}	0.33±0.00^{d}	0.10±0.00^{de}	0.03±0.00^{ab}	0.83±0.02^{b}
EbC	0.50±0.00^{fg}	1.65±0.00^{e}	0.33±0.00^{cd}	0.11±0.00^{bc}	0.03±0.00^{ef}	0.68±0.01^{de}
Eb2	0.64±0.00^{a}	1.64±0.01^{b}	0.34±0.01^{c}	0.08±0.00^{f}	0.03±0.00^{de}	0.75±0.03^{cd}
Eb14	0.49±0.00^{gh}	1.75±0.01^{a}	0.34±0.00^{cd}	0.09±0.00^{ef}	0.03±0.00^{abc}	0.85±0.03^{b}
Eb22	0.59±0.01^{b}	1.64±0.01^{b}	0.29±0.00^{e}	0.09±0.00^{f}	0.03±0.00^{bc}	0.58±0.01^{f}
CBC	0.57±0.01^{c}	1.55±0.02^{cd}	0.34±0.00^{cd}	0.09±0.00^{ef}	0.03±0.00^{f}	0.78±0.01^{bc}
CB2	0.59±0.00^{b}	1.56±0.01^{c}	0.29±0.00^{e}	0.12±0.00^{b}	0.03±0.00^{de}	0.84±0.03^{b}
CB16	0.50±0.01^{ef}	1.41±0.01^{e}	0.37±0.00^{b}	0.14±0.00^{a}	0.03±0.00^{f}	0.67±0.02^{e}
CbC	0.49±0.01^{h}	1.28±0.01^{f}	0.41±0.01^{a}	0.14±0.00^{a}	0.02±0.00^{g}	0.74±0.04^{cd}
Cb11	0.58±0.00^{bc}	1.40±0.00^{e}	0.34±0.01^{cd}	0.09±0.00^{f}	0.03±0.00^{de}	0.81±0.03^{bc}
Cb19	0.54±0.01^{d}	1.55±0.01^{cd}	0.30±0.01^{e}	0.12±0.01^{bc}	0.03±0.00^{a}	0.76±0.03^{c}

Means and standard deviations for n =3. Means having the same letter(s) do not significantly differ by Tukey's mean separation test at P>0.05.

A combined proximate and mineral components analysis revealed a dendrogram of nine clusters using the elbow method (Figure 11) at 49.19% Euclidean similarity distance measure of standardized variables (Figure 5.12).

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The genotypes EB8, EbC, Eb14, Eb22, and Cb11 individually stood alone indicating the presence of variability, and also how distantly they differ from each other and the other groups. The mutant, CB2 was put in the same cluster with EBC (control) as having similar nutrient composition. The mutant, Eb2, though brown and EMS treated, was put in the same cluster with a control black genotype (CBC). Also, the black EMS mutant (EB12) was in the same cluster as a brown colchicine-treated mutant (Cb19). Another black colchicine mutant (CB16) was also put together with a colchicine brown control genotype (CbC) (Figure 5.12). The clustering pattern indicates that genotypes in the same cluster have similar nutrient composition but differ from one cluster to the other. Though genotypes in the same cluster may differ in phenotypic characteristics, such as tuber colour, they have similar nutrient composition. The dissimilarities observed in the phenotype can be explained by the diverse genetic makeups and environmental factors that individuals possess and exposed to (Dingemanse & Wolf, 2013). It is possible that the mutagens acted differently at the different loci of the accessions resulting in an effect relative to each genotype. Mutagenesis does not entirely change the genetic composition of a mutant from its control but modifies an aspect or some aspects of its characteristics bringing about speciation of organisms. This might be the reason for the results in Figure 5.12. Similar findings were reported by Campbell & Sederoff (1996) and Fehr (2007).

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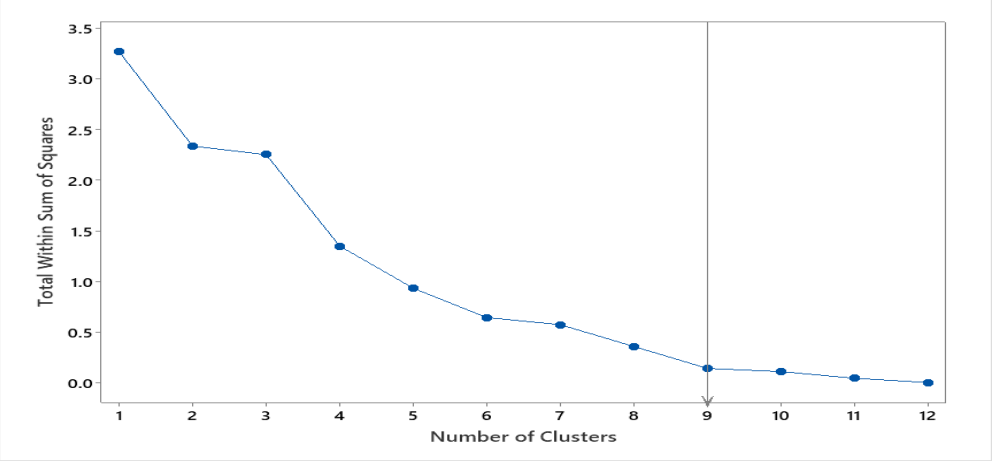

Figure 5.11: The optimal number of clusters determined using the elbow method.

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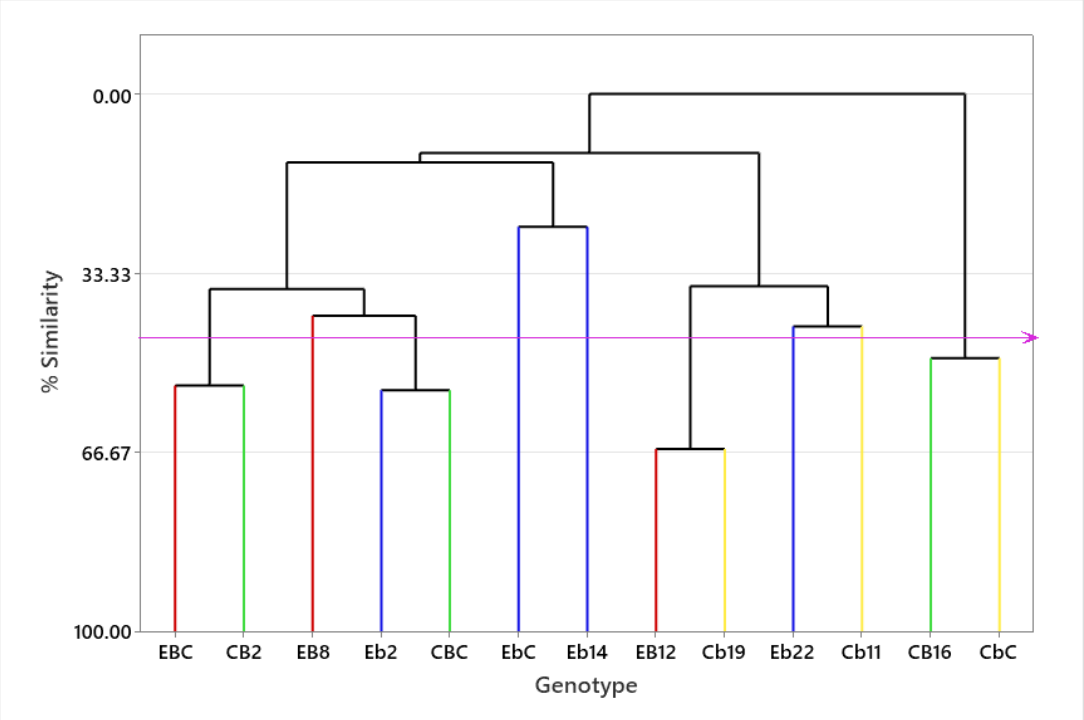

Figure 5.12: Relationships among the 13 tiger nut accessions (mutants and controls) based on their proximate and mineral compositions.

The red colour indicates black genotypes treated with EMS; green colour shows black genotypes treated with colchicine; blue colour signifies brown genotypes treated with EMS; and yellow colour denotes brown genotypes treated with colchicine.

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Conclusions

Mutagenesis in crop production holds the key to variation in plant breeding in unlocking variability for diversity among and within vegetatively propagated crops for improvement. Improving the yield, earliness, and nutritional contents of tiger nuts adds to plant breeders' quest to achieve food security, and also overcome the problem of hidden hunger in the 21st century.

The research was conducted at the Crop Research Institute of the Council for Scientific and Industrial Research, Fumesua. Six hundred tiger nut tubers (control, EMS-treated and colchicine-treated tubers) consisting of brown and black types were used in M1V1. One thousand one hundred and sixty-eight (1168) black and brown types (bulked tubers from M1V1 harvest) were used for the M1V2. Subjecting to further screening, 100 tubers were used for M1V3, and finally, 25 genotypes made up of 21 mutants and 4 controls were used for the M1V4 evaluation in a randomized complete block design.

The results indicated no effect by the EMS and colchicine mutagens on the tuber colour of the genotypes throughout the M1V1-M1V4 generations. However, there were changes in plant growth characteristics with significant differences among the 25 genotypes with the mutant Cb5 having the greatest mean plant height of 115.75 cm. Colchicine had a positive impact on influencing plant height, stem girth expansion, and leaf length. Additionally, colchicine controlled earliness by having four of its treated brown-mutants (Cb9, Cb2, Cb5, and Cb11) ranking with two EMS brown mutants (Eb8, and Eb1) maturing earlier (75-77 days) than the other genotypes including their controls.

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On yield, eleven mutants including 2 EMS black (EB8, EB12), 3 EMS brown (Eb2, Eb14, Eb22), 3 colchicine black (CB2, CB7, CB16), and 3 colchicine brown (Cb11, Cb16, Cb19) genotypes outperformed the best control based on hundred tuber weight. Four EMS-treated brown mutants (Eb2, Eb1, Eb12, and Eb14) had multiple tuber development and produced larger tuber sizes with various shapes. The tubers of the black mutants of both colchicine and EMS treated accessions showed strong attachment to the plant at harvesting leaving few tubers in the soil for retrieval. The highest tuber attachment was recorded in EB5. Among the brown genotypes, Eb2 had the highest number of tuber attached to the parent plant. Proximate and mineral analysis of the tubers revealed wide variability with nine cluster groupings at a Euclidean similarity measure of 49.19% among the 13 accessions evaluated. A similar number of cluster groupings was confirmed for all 25 genotypes across 20 morpho-descriptors, evidence of existence of wide genetic diversity among the mutants and the controls.

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CHAPTER SIX

SUMMARY, GENERAL CONCLUSIONS AND RECOMMENDATIONS

SUMMARY

The first aim of this study was to assess the genetic diversity among tiger nut accessions by examining both yield and morphological descriptors. Forty-two accessions were obtained from farmers in the Northern, Upper East, Upper West, Bono East, Eastern, and Central regions of Ghana, -. which constituted the phytogeographic zones or major tiger nuts growing regions in Ghana. The experiment was conducted at the Teaching and Research Farm of the University of Cape Coast, following a randomized complete block design with three replications. Results revealed the presence of variability among the accessions for some traits. However, there was low diversity among the accessions across the 11 morpho-descriptors.

This study also sought secondly to determine the genetic variability and diversity of tiger nuts using SSR markers. Forty-one out of the 42 accessions used in the previous experimental study planted in pots in a greenhouse, successfully germinated, and were analyzed in the Biochemistry Laboratory of CRI, Fumesua. A total of 141 alleles were obtained for nine SSR loci across the 41 accessions with a mean of 15.67 ± 3.97 per loci and a mean PIC value of 0.78 ± 0.15, indicating the high discriminating power of the markers. However, mean hererozygosity (0.12 ± 0.16) was low. UPGMA cluster analysis grouped the accessions into four at a similarity index of 0.88. These findings suggest

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substantial evidence of low genetic diversity characterized by some level of admixture of genes over generations.

The third aim was to establish the lethal dose that will kill 50 % of the population (LD_{50}) (and reduction dose that will reduce 50 % plant height or traits (RD_{50}) values for Ethyl methanesulfonate (EMS) and Colchicine for the mutagenesis of tiger nuts. Four best-yielding genotypes consisting of two distinct brown (OFF-b and APR-b) and two different black (BUO-B and ENK-B) genotypes were selected from the morphological studies. Four hundred tubers in all were treated with EMS and colchicine at the concentrations; 0%, 0.1%, 0.25%, 0.5%, and 1.0% for both mutagens and further planted in a greenhouse. For EMS, LD_{50} and RD_{50} values of 0.97 % and 1.49 % were obtained for the black, respectively, while the corresponding values for the brown genotypes were 0.63 % and 1.63 % respectively. Likewise, for the colchicine mutagen, LD_{50} and RD_{50} values of 1.65 % and 19.51 % were obtained for the black and 0.91 % and 1.71 % for the brown genotypes, respectively.

The final objective was to determine mutants with favorable traits (including high yield, larger nut size, early maturity, intact attachment of tubers to plant on harvesting, and high nutritional content) for potential enhancement in breeding programmes. Six hundred tubers of the four genotypes were mass treated with the EMS or colchicine mutagens using their recommended doses of LD_{50} and RD_{50} as established in the chemosensitivity study. Treated tubers were planted in the field as M1V1 at CRI, Fumesua. Tubers (1168) of the genotypes from the M1V1 harvest were bulked planted as M1V2. One hundred tubers from the M1V2 harvest were used for the M1V3. Finally, 25 genotypes, consisting of 21 mutants and 4 controls were used for the M1V4 evaluation in a randomized

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complete block design with three replications. Results showed that there was no impact of the mutagens on the tuber coat colour of tiger nuts. On plant growth characteristics, the colchicine mutant Cb5 was the tallest. Also, the effect of colchicine was evident in colchicine mutants for other plant growth characteristics such as stem girth expansion, leaf length, and earliness to maturity. Yield was remarkable for 11 mutants out of the 25 genotypes. Two EMS-treated black (EB8, EB12), 3 EMS-treated brown (Eb2, Eb14, Eb22), 3 colchicine-treated black (CB2, CB7, CB16), and 3 colchicine-treated brown (Cb11, Cb16, Cb19) genotypes outperformed the best control based on hundred tuber weight. Additionally, 4 EMS mutants (Eb2, Eb1, Eb12 and Eb14) had multiple tuber development resulting in larger tuber sizes and oblonged shapes. On tuber attachment at harvesting, the black mutants were stronger in tuber attachment than their brown counterparts. EMS black mutant, EB5 had the highest number of tubers attached to the main plant among the 25 genotypes at harvest. Overall, variability existed among the genotypes for proximate and mineral analyses, and also across 20 morpho-descriptors. This indicates that the mutants were significantly different from the controls. Hence, the mutants could serve as candidates for futher breeding evaluation and selection for release as varieties and also for tiger nut breeding improvement.

General Conclusion

Improving on any crop demands genetic diversity study of the available accessions, further creation of variability, and the selection of accessions with promising traits. The current study revealed the existence of high variability among the accessions for some of the traits studied morphologically. Both morphological descriptors and SSR molecular markers discovered the existence

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of some levels of variability among the 42 tiger nut accessions collected from major growing areas in Ghana. However, hierarchical cluster groupings using both morphological descriptors or SSR markers revealed low genetic variability within the accessions. Accessions from different locations clustered together with many characteristics in common, probably due to some level of admixture of genes over years of cultivation through the exchange of planting materials by farmers. Again, the overtime use of vegetative parts for planting material for an area, results in the repeatitive occurrence of plants with same or similar genetic attributes as vegetatively propagated crops poorly hybridize naturally.

To establish the LD_{50} and RD_{50} values for the mass treatment of tiger nuts for optimal germination and growth, chemosensitivity analysis of the current study recommends EMS dose concentrations of 0.63 % and 1.63 % for both LD_{50} and RD_{50} respectively for the brown tiger nut accessions.

While the black tiger nut accessions should be treated with EMS dose concentrations of 0.97 % and 1.49 %. However, for colchicine, the brown tiger nuts must be treated with the dose concentrations of 0.91 % and 1.71 % for LD_{50} and RD_{50} respectively. Whereas the dose concentrations of 1.65 % and 19.51 % must be used for the black genotype for both LD_{50} and RD_{50} respectively. The differences in dose concentrations of the mutagens were species or plant-type specific. The black tiger nut genotypes exhibited a higher tolerance to mutagens compared to their brown counterparts. Additionally, when subjected to colchicine treatment, the genotypes displayed a lesser degree of sensitivity in contrast to EMS.

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Following successive selections based on the breeding objectives of enhancing yield, earliness to maturity, intact attachment of tubers to plant on harvest, and improved nutrient content (from M1V1-M1V3), 25 genotypes made up of 21 mutants and 4 controls were finally evaluated at M1V4 generations. Both EMS and colchicine mutagens had no effect on coat or skin colour indicating that the chemical mutagens may not affect the skin colour formation of tubers in tiger nuts. TThe colchicine mutants, Cb5, CB5, Cb19, and CB3 had bigger stem girth and longer leaves compared to the others. However, they were shorter compared to the EMS mutant EB2 for plant height. Similarly, in earliness to maturity, the colchicine mutants, Cb9, Cb2, Cb5, and Cb11, and the the EMS mutants, Eb1 and Eb8 had a mean of 75-77 days to physiologically matured. Colchicine is recognized for its ability to induce an increase in ploidy levels in organisms, while EMS is associated with the occurrence of random mutations. Consequently, there is a potential for these effects manifested. The impact of colchicine on plant growth characteristics and earliness was noticeable than anticipated in the yield and tuber size development. The EMS brown mutant, Eb2 had the highest hundred tuber weight (HTW) in yield. Ten other mutants comprising 4 EMS mutants (EB8, EB12, Eb14, Eb22) and 6 colchicine mutants (Cb11, Cb19, CB2, CB16, CB7, Cb9) performed better in yield than the other genotypes including the controls in HTW. With tuber attachment to plant on harvest, the black-type tiger nuts, especially, the EMS black mutants were resilient to detachment. This might be due to the presence of developed thickened runners not easily prone to tear when tubers are pulled. On nutritional composition, the tubers of many mutants were richer in carbohydrates, phosphorous, and potassium than the controls. The combined proximate and

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mineral analysis generated a dendrogram of 9 clusters for 13 selected mutants across 12 nutrient elements showing wide variability of the genotypes with nutrient composition. An analogous trend was noted in the hierarchical cluster grouping of the mutants, where 9 clusters were identified for all 25 genotypes across 20 morpho-descriptors. The existence of significant diversity, both within and among the mutants and controls, suggests a substantial genetic variation in the genotypes. These are a valuable resource for inclusion in the breeding and selecting of tiger nuts for improvemennt of the crop and release of new varieties.

Recommendation

From the research, the following recommendations are made:

1. Molecular analysis should be conducted to examine the extent of genetic alterations that might have occurred with the chemical mutagenesis.
2. Multi- location trials must be carried out to determine the interaction of the genotypes of hypothetical mutants with different environments and for a varietal release.
3. Consumer preference tests should be conducted to find out the acceptability of the mutants generated.
4. The colchicine mutants should be examined for the occurrence of polyploidy.
5. Physical mutagenesis using gamma irradiations should be carried out to complement the findings of the current research.

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APPENDICES

Appendix A. GENETIC DIVERSITY OF TIGER NUTS (*Cyperus esculentus* L.) GROWN IN GHANA BASED ON MORPHO-DESCRIPTORS AND SSR MARKERS

1. Analysis of variance: Percentage germination

Source	DF	SS	MS	F-Value	P-Value
Genotype	41	54263	1323.5	2.67	0.000
Error	84	41615	495.4		
Total	125	95878			

2. Analysis of variance: Number of tillers

Source	DF	SS	MS	F-Value	P-Value
Genotype	41	2203	53.72	2.33	0.001
Error	84	1934	23.02		
Total	125	4136			

3. Analysis of variance: Distance of the last tiller from the main plant

Source	DF	SS	MS	F-Value	P-Value
Genotype	41	33049	806.1	2.42	0.000
Error	84	28002	333.4		
Total	125	61051			

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4. Analysis of variance: Plant height

Source	DF	SS	MS	F-Value	P-Value
Genotype	41	33049	806.1	2.42	0.000
Error	84	28002	333.4		
Total	125	61051			

5. Analysis of variance: Percentage inflorescence

Source	DF	SS	MS	F-Value	P-Value
Genotype	41	41.66	1.0161	1.78	0.013
Error	84	48.00	0.5714		
Total	125	89.66			

6. Analysis of variance: Number of tubers per stand

Source	DF	SS	MS	F-Value	P-Value
Genotype	41	20694	504.7	1.34	0.129
Error	84	31622	376.5		
Total	125	52316			

7. Analysis of variance: Number of rings per tuber

Source	DF	SS	MS	F-Value	P-Value
Genotype	41	25.373	0.61885	8.66	0.000
Error	84	6.000	0.07143		
Total	125	31.373			

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8. Analysis of variance: Length of tuber

Source	DF	SS	MS	F-Value	P-Value
Genotype	41	3121	76.12	4.90	0.000
Error	84	1305	15.53		
Total	125	4426			

9. Analysis of variance: Width of tuber

Source	DF	SS	MS	F-Value	P-Value
Genotype	41	464.5	11.328	3.95	0.000
Error	84	241.1	2.871		
Total	125	705.6			

10. Analysis of variance: Tuber shape

Source	DF	SS	MS	F-Value	P-Value
Genotype	41	14.922	0.36394	5.59	0.000
Error	84	5.473	0.06516		
Total	125	20.395			

11. Analysis of variance: Hundred tuber weight

Source	DF	SS	MS	F-Value	P-Value
Genotype	41	186731	4554.42	45735.14	0.000
Error	84	8	0.10		
Total	125	186740			

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12. Correlation matrix

	PG	NT	DTP	PH	PI	NTS	NRT	LT	WT	TS
NT	0.162									
DTP	0.543	0.543								
PH	0.844	0.351	0.657							
PI	0.231	0.103	-0.050	0.319						
NTS	-0.018	0.260	0.069	0.108	-0.092					
NRT	0.414	0.193	0.636	0.484	-0.023	0.128				
LT	0.211	0.147	0.426	0.472	0.035	-0.085	0.411			
WT	0.297	0.094	0.584	0.421	-0.161	-0.027	0.626	0.447		
TS	0.205	0.190	0.295	0.430	0.159	-0.044	0.265	0.899	0.061	
HTW	0.179	0.407	0.474	0.429	0.102	0.183	0.296	0.593	0.429	0.444

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Appendix B. CHEMOSENSITIVITY ANALYSIS OF TIGER NUTS (*Cyperus esculentus* L.) USING ETHYL METHANESULFONATE (EMS) AND COLCHICINE MUTAGENS

1. Fishers pairwise comparison at 95 % confidence on mean plant height per plant versus EMS concentrations of genotypes.

Conc. of genotype	N	Mean	Grouping	
BUO-BE0.1	3	38.26	A	
OFF-bE0.5	3	37.37	A	
OFF-bE0.1	3	36.89	A	
OFF-bE0.25	3	35.96	A	
BUO-BE0.25	3	35.02	A	
BUO-BE0	3	34.84	A	
OFF-bE0	3	34.50	A	
BUO-BE0.5	3	30.533	A	B
BUO-BE1	3	23.66		B
OFF-bE1	3	22.28		B

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2. Fishers pairwise comparison at 95 % confidence on mean number of leaves per plant versus EMS concentrations of genotypes.

Conc. of genotype	N	Mean	Grouping		
BUO-BE0.1	3	7.889	A		
OFF-bE0.25	3	7.778	A		
BUO-BE0.5	3	7.778	A		
OFF-bE0.5	3	7.667	A	B	
BUO-BE0.25	3	7.556	A	B	
BUO-BE0	3	7.556	A	B	
OFF-bE0.1	3	7.333	A	B	
OFF-bE0	3	7.333	A	B	
BUO-BE1	3	6.56	A	B	
OFF-bE1	3	5.44		B	

3. Fishers pairwise comparison at 95 % confidence on mean number of tillers per plant versus EMS concentrations of genotypes.

Conc. of genotype	N	Mean	Grouping		
BUO-BE0.5	3	2.889	A		
OFF-bE0.1	3	2.778	A	B	
OFF-bE0.5	3	2.333	A	B	
OFF-bE0.25	3	2.222	A	B	
BUO-BE0.25	3	2.222	A	B	
BUO-BE0.1	3	2.000	A	B	C
BUO-BE1	3	1.889	A	B	C
OFF-bE0	3	1.667		B	C
BUO-BE0	3	1.667		B	C
OFF-bE1	3	1.000			C

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4. Fishers pairwise comparison at 95 % confidence on mean diameter of main plant versus EMS concentrations of genotypes.

Conc. of genotype	N	Mean	Grouping
BUO-BE0.5	3	4.490	A
BUO-BE0.1	3	4.018	A
OFF-bE0.25	3	3.988	A
OFF-bE0.5	3	3.871	A
OFF-bE0	3	3.856	A
OFF-bE0.1	3	3.849	A
BUO-BE0.25	3	3.829	A
BUO-BE0	3	3.776	A
BUO-BE1	3	3.287	A
OFF-bE1	3	3.14	A

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5. Fishers pairwise comparison at 95 % confidence on mean plant height per plant versus colchicine concentrations of genotypes.

Conc. of genotype	N	Mean	Grouping		
APR-bC0.25	3	38.52	A		
APR-bC0	3	35.967	A	B	
ENK-BC0.25	3	35.1111	A	B	
ENK-BC0.1	3	34.244	A	B	
ENK-BC0.5	3	34.233	A	B	
ENK-BC0	3	33.73	A	B	
APR-bC0.5	3	33.38	A	B	
ENK-BC1	3	33.20	A	B	
APR-bC0.1	3	30.42	A	B	
APR-bC1	3	23.73		B	

6. Fishers pairwise comparison at 95 % confidence on mean number of leaves per plant versus colchicine concentrations of genotypes.

Conc. of genotype	N	Mean	Grouping		
ENK-BC0.5	3	8.111	A		
ENK-BC1	3	8.000	A		
ENK-BC0.1	3	7.889	A		
ENK-BC0.25	3	7.889	A		
APR-bC0	3	7.778	A		
ENK-BC0	3	7.556	A	B	
APR-bC0.25	3	7.000	A	B	C
APR-bC0.5	3	6.778	A	B	C
APR-bC0.1	3	5.56		B	C
APR-bC1	3	4.89			C

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7. Fishers pairwise comparison at 95 % confidence on mean number of tillers per plant versus colchicine concentrations of genotypes.

Conc. of genotype	N	Mean	Grouping		
ENK-BC0.5	3	2.667	A		
ENK-BC0.1	3	2.556	A		
ENK-BC0	3	2.444	A	B	
APR-bC0	3	2.444	A	B	
ENK-BC1	3	2.333	A	B	
APR-bC0.5	3	2.111	A	B	
ENK-BC0.25	3	1.889	A	B	C
APR-bC0.25	3	1.556		B	C
APR-bC0.1	3	1.556		B	C
APR-bC1	3	1.111			C

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8. Fishers pairwise comparison at 95 % confidence on mean diameter of main plant versus colchicine concentrations of genotypes.

Conc. of genotype	N	Mean	Grouping	
ENK-BC0.5	3	4.4978	A	
ENK-BC0.25	3	4.388	A	
ENK-BC1	3	4.372	A	
ENK-BC0	3	4.262	A	
ENK-BC0.1	3	4.009	A	B
APR-bC0.25	3	3.9633	A	B
APR-bC0	3	3.909	A	B
APR-bC0.5	3	3.836	A	B
APR-bC0.1	3	3.55	A	B
APR-bC1	3	2.654		B

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Appendix C. MUTATION BREEDING FOR TIGER NUTS (*Ceperus esculentus* L.) IMPROVEMENT USING ETHYL METHANESULFONATE (EMS) AND COLCHICINE

1. Analysis of variance: Days to physiological maturity

Source	DF	SS	MS	F-Value	P-Value
Genotype	24	690.7	28.780	5.75	0.000
Error	50	250.2	5.003		
Total	74	940.9			

2. Analysis of variance: Hundred tuber weight

Source	DF	SS	MS	F-Value	P-Value
Genotype	24	19821	825.9	2.03	0.017
Error	50	20327	406.5		
Total	74	40148			

3. Analysis of variance: Overall tuber weight

Source	DF	SS	MS	F-Value	P-Value
Genotype	24	9332	388.8	1.91	0.027
Error	50	10169	203.4		
Total	74	19501			

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4. Analysis of variance: Number of tubers attached to plant on harvest

Source	DF	SS	MS	F-Value	P-Value
Genotype	24	10269	427.9	3.87	0.000
Error	50	5523	110.5		
Total	74	15791			

5. Tukey pairwise comparison at 95 % confidence on mean percentages of carbohydrates of mutants and controls.

Genotype	N	Mean	Grouping				
Cb19	3	52.638	A				
EB12	3	50.638	A	B			
Eb22	3	49.256		B	C		
Cb11	3	48.719		B	C		
CB2	3	48.135		B	C		
EBC	3	46.909			C	D	
CbC	3	46.666			C	D	E
CBC	3	46.614			C	D	E
EbC	3	45.03				D	E
Eb14	3	44.816				D	E
CB16	3	44.589				D	E
Eb2	3	44.484				D	E
EB8	3	43.857					E

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6. Tukey pairwise comparison at 95 % confidence on mean percentages of phosphorous of mutants and controls.

Genotype	N	Mean	Grouping						
Eb2	3	0.63867	A						
CB2	3	0.59433		B					
EBC	3	0.58600		B					
Eb22	3	0.58500		B					
Cb11	3	0.58067		B	C				
CBC	3	0.56967			C				
Cb19	3	0.53767				D			
EB12	3	0.51833					E		
CB16	3	0.50367					E	F	
EbC	3	0.50267						F	G
Eb14	3	0.48833							G
CbC	3	0.48700							H
EB8	3	0.48600							H

7. Tukey pairwise comparison at 95 % confidence on mean percentages of potassium of mutants and controls.

Genotype	N	Mean	Grouping						
Eb14	3	1.75467	A						
EbC	3	1.65233		B					
Eb2	3	1.64433		B					
Eb22	3	1.63533		B					
CB2	3	1.56400			C				
Cb19	3	1.55067			C	D			
CBC	3	1.5453			C	D			
EB8	3	1.52567				D			
EB12	3	1.42267					E		
CB16	3	1.40767					E		
EBC	3	1.40167					E		
Cb11	3	1.39700					E		
CbC	3	1.28000							F

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