

**UNIVERSITY FOR DEVELOPMENT STUDIES**

**CHARACTERISATION OF CHILLI PEPPER (*Capsicum annuum* L.) ACCESSIONS IN  
NORTHERN REGION OF GHANA USING AGRO-MORPHOLOGICAL TRAITS AND  
MOLECULAR MARKERS**

**PAUL YAO ANANI**

**2026**



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MOLECULAR MARKERS**

**BY**

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FOR THE AWARD OF DOCTOR OF PHYLOSOPHY IN HORTICULTURE**

**JANUARY, 2026**



**DECLARATION**

Student

I hereby declare that this dissertation/thesis is the result of my original work and no part of it has been presented for another degree in this University or elsewhere:

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## ABSTRACT

Ghana ranks among the top five pepper producers in Africa; however, there is inadequate research regarding farmer practices, morphological and genetic enhancement initiatives for this important crop. Understanding of variabilities in pepper (*Capsicum species*) will contribute to effective breeding efforts, especially in northern Ghana, where more than 60% of smallholder farmer income is from pepper. The focus of this study was to assess farmers' knowledge and management practices in pepper cultivation, and to evaluate morphological and genetic variations among *Capsicum annuum* landraces cultivated in Northern region of Ghana. A cross-sectional survey was carried out using structured questionnaires and field observations to evaluate farmers' knowledge and *Capsicum annuum* management practices; morphological characters at the seedling, vegetative, inflorescence and fruiting stages were evaluated using standard descriptors outline by IPGRI, AVRDC & CATIE under rainfed and irrigation conditions. Distinct genetic populations within a collection of 40 *Capsicum annuum* accessions using 24 simple sequence repeat (SSR) markers. The average acres allocated to pepper farming is 1.04 acres with majority of the farmers using farmer-saved seeds (67.3%). Generally, the farmer rating of improved pepper seeds was low, indicating that the local pepper varieties used in the study were comparable to the improved pepper varieties. A strong positive correlation was detected between days to 50% flowering and days to 50% fruiting for both the rainfed ( $r = 0.87$ ) and irrigated ( $r = 0.73$ ) conditions. In general, high performance of the genotypes at the vegetative and inflorescence stages was observed in the dry season, with low fruiting performance (number of fruits per plant, fresh weight, and dry weight). Multivariate analysis revealed that 60.54% (rainy season) and 54.63% (dry season) variations were explained by the first three principal components, respectively. Three main clusters were identified, with linear relationships and no distinct separation pattern based on the sample source. The SSR markers exhibited a polymorphic information content (PIC) below 0.50, with an average value of 0.11. A modest level of genetic diversity among the accessions ( $HE = 0.063$ ) was observed, with marked genetic differentiation ( $F_{ST} = 0.57$ ). Employing the admixture model-based population structure analysis, set at a threshold of 60%, 29 pure genotypes from the 40 accessions used were identified. A phylogenetic analysis categorized the 40 *Capsicum* accessions into three primary clusters: Cluster I and Cluster II contained two individuals from the *Capsicum annuum* accession, while Cluster III was further subdivided into two subclusters. Overall, the study demonstrates that pepper production in northern Ghana is dominated by farmer-saved landraces



with modest but structured genetic diversity, underscoring the need for location-specific management strategies and targeted breeding programmes to sustainably improve productivity and resilience



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## DEDICATION

I dedicate this thesis to God Almighty for His mercy and grace that took me through this work.

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## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 Background of Study

The pepper genus, *Capsicum*, contains many important domesticated and wild species with highly diverse genetic and phenotypic properties. The domesticated *Capsicum* species usually bear larger, hanging, or bell-shaped, persistent fruits, while the wild type of the species typically bears small, erect and deciduous fruit (Scaldaferro et al., 2018). Of the 87 known *Capsicum* species (USDA-ARS, 2023), five taxa including *Capsicum annuum*, *C. frutescens*, *C. baccatum*, *C. chinense*, and *C. pubescens* have been domesticated. *Capsicum* is one of the principal crops in the *Solanaceae* family grown worldwide and has diverse industrial applications.

The crop is one of the most important groups of vegetable and spice crops known in the *Solanaceae* family. The crop has played an integral role in human civilization, including human nutrition, and medicinal values with cultural significance (Del Burgo-Gutiérrez et al., 2022; Luna-Romero et al., 2022; Yasin et al., 2023). Within the *Capsicum* genus, there are morphologically diverse fruits and leaves in both domesticated and wild types that have been explored for their ornamental value (Barchenger et al., 2019; Gilman et al., 2014; Stummel & Bosland, 2007; Zhang et al., 2020), resulting in increased demand for culinary and ornamental varieties of *Capsicum* species.

Global *Capsicum* species play an important role in vegetables, spices, and medicinal formulas (Haq et al., 2022). These species contain appreciable levels of essential nutrients such as pro-vitamin A (carotene) and E (a-tocopherol), vitamin C as well as phytochemicals that greatly improve human health (Del Burgo-Gutiérrez et al., 2022; Tuckeldoe et al., 2023). The fruits can be used as pesticide and in the pharmaceutical industries as natural colouring and repellent agents



(Luna-Romero et al., 2022; Yasin et al., 2023). Besides this, *Capsicum* extract has long been patented and applied as a biopesticide (Neumann & Carlos, 2003).

Economically, *Capsicum* production is a viable agricultural business, supporting several economies and smallholder farmers in Africa. Africa's 21.2% contribution to production of dry *Capsicum* is markedly higher than that of both Europe (2.7%) and America (5.8%), while Europe, Africa, and America contribute almost the same proportion to the total world fresh pepper production (Tripodi & Kumar, 2019). Although the export value of *Capsicum* has declined modestly, it is the leading vegetable exported from Ghana, mainly Legon 18, with main markets in the UK, USA, Canada, Australia, and the Netherlands (GEPA, 2017). Two-thirds of Ghana's pepper production is from the northern part of Ghana, and accounts for about 60-70% of farm income of smallholder farmers in this region (DAI, 2014).

Despite the economic, cultural, and morphological significance of *Capsicum* species, no major efforts have been made to identify morphologically diversified *Capsicum* species grown in northern Ghana for use in breeding programmes. The local *Capsicum* genotypes grown in northern Ghana have lower yield potential (Nkansah et al., 2011). Traditionally, about 70% of pepper is cultivated under rain-fed conditions, which does not support rapid growth, development, and all-year-round production. There are complications and challenges involved in pepper production in northern Ghana, including a huge research gap (Development Alternatives Incorporated (DAI, 2014)). Also, it has been recommended that irrigation should be used to supplement rainy season pepper production in the Guinea Savannah zone of Ghana (Adekaldy et al., 2021).



## 1.2 Problem Statement

Ghana, a country with numerous native ethnic groups, has a large variety of vulnerable traditional farming practices (Awaworyi Churchill & Danquah, 2022), including limited or no irrigation systems, high use of farmer saved seeds, limited drying platforms, repeated misuse of chemical fertilizer and technical inefficiencies (Adekaldy et al., 2021; Jacob et al., 2016). Recently, it has been shown that drying chilies (pepper) on bare floors significantly results in high aflatoxin levels in dried chilies (Sahar et al., 2022). Sarku (2023) found increased water requirements for pepper farmers due to erratic rainfall. Furthermore, the increasing participation of young people in agriculture has dramatically changed the traditional farming systems in Ghana (Sumberg et al., 2017). Pepper mineral composition and sensory profile were also found to be affected by agricultural management (Flores et al., 2009).

The selection of *Capsicum* species and their associated management practices have been widely explained from various perspectives, including social perspectives (Zhang et al., 2020), expert perspectives (Martínez-Ainsworth et al., 2023), and traditional perspectives (Rinaldi et al., 2021). However, these perspectives and management practices have implied consequences on yield and quality (Hamadani et al., 2021; Sumberg et al., 2017). While experts in agriculture have made considerable efforts to promote the capacity of smallholder farmers to adapt to modern farming techniques, Halbrendt et al. (2014) and Adimassu et al. (2019) indicated that these techniques when adopted and applied universally decreased crop yields and quality in certain geographical areas, and therefore should not be applied universally. Therefore, to improve the global food security index, it is necessary to evaluate various farming practices and apply targeted training to specific geographical locations.





Furthermore, It is now well established that diversity in pepper germplasm can be rapidly and effectively assessed using multivariate morphological traits and analytical methods. These morphological traits allow identification of duplicate accessions in the germplasm (Bharath et al., 2014), while the analytical methods allow good decision-making concerning germplasm conservation, and further research on accessions within the collection. However, there is limited morphological information on pepper germplasm from the northern part of Ghana.

Genetic diversity and population structure assessment are essential for improving *Capsicum* species' breeding, conservation, and evolution research. The genomic profile of *Capsicum* is complex (Scaldaferro & Moscone, 2019; Shirasawa et al., 2022), with huge variations in both molecular and morphological properties. These variations are influenced by geographical positions, evolutionary transition, breeding practices, agricultural practices, and environmental stress factors ( Kim et al., 2021; Li et al., 2022). Despite these wide variations in *Capsicum* germplasm, few studies have been conducted to better characterise current accessions of pepper diversity for breeding and large-scale population genetics analysis using advanced molecular genotyping technologies in Ghana (Agyare et al., 2016). Furthermore, there is a persistent reported decline in local pepper accession genetic diversity due to the selection and re-utilization of already adapted germplasm (Bedjaoui, , et al., 2022; Mahpara et al., 2023; Wassie et al., 2023). The discovery and development of DNA-based molecular markers for genetic and population studies have gained widespread attention mainly due to the increasing cost and labour-intensive process of next-generation sequencing technology (Lemmon et al., 2023). As such, molecular marker polymorphism offers quick, easy, reliable, and cost-effective methods for genetic diversity studies. Typically, the northern part of Ghana is a major contributor to Ghana's commercial pepper industry, however, pepper productivity in these areas is far lower (8t/ha) than the national

achievable yield (32.3mt/ha) (DAI, 2014). Limited research has been done to evaluate farmers' perception and management practices of pepper in the Northern region of Ghana.

### 1.3 Justification of the Study

Although considerable efforts have been made by agricultural experts to promote improved farming technologies, evidence suggests that the universal application of such technologies can be counterproductive in certain geographical contexts. This highlights the need for location-specific assessments of farming practices, farmer perceptions, and agro-ecological conditions to inform targeted interventions. In northern Ghana, where erratic rainfall and increasing youth participation in agriculture are reshaping traditional farming systems, there is limited empirical research that integrates farmer management practices with scientific evaluation of pepper performance. Addressing this gap is essential for designing context-appropriate extension services and capacity-building programmes that enhance productivity without compromising crop quality.

In addition, the sustainability of pepper production in Ghana is threatened by a documented decline in local genetic diversity, driven largely by repeated selection and re-use of already adapted germplasm. Genetic erosion reduces the adaptive potential of pepper populations to environmental stresses, pests, diseases, and climate variability. While morphological and molecular approaches have proven effective for assessing germplasm diversity and guiding conservation and breeding strategies, there is a notable lack of comprehensive morphological and genetic characterisation of pepper accessions from northern Ghana. This gap limits the availability of reliable baseline data for breeding programmes, conservation planning, and large-scale population genetics studies.

Given the complexity of the *Capsicum* genome and the influence of environmental and management factors on both molecular and morphological traits, the use of cost-effective DNA-



based molecular markers alongside multivariate morphological analyses offers a robust approach for evaluating genetic diversity and population structure. Such an approach is particularly justified in resource-limited contexts, where next-generation sequencing may be financially and logistically prohibitive. Generating detailed morphological and genetic information on pepper accessions from northern Ghana will support informed decision-making in germplasm conservation, reduce duplication, and identify valuable traits for breeding and improvement programmes.

Therefore, this study is justified by the need to (i) evaluate farmers' perceptions and management practices of pepper production in northern Ghana, (ii) assess the morphological and genetic diversity of locally cultivated pepper accessions, and (iii) provide scientifically grounded evidence to support targeted training, conservation strategies, and breeding initiatives. Ultimately, the findings of this study will contribute to improved pepper productivity, enhanced crop quality, strengthened food security, and the long-term sustainability of Ghana's pepper industry.

#### **1.4 Objective**

The main objective of the research was to characterize hot pepper (*Capsicum annum*) cultivars in northern region of Ghana using agro-morphological traits and molecular markers

The specific objectives were to:

1. Conduct a survey to identify how farmers handle pepper seeds before, during and after the planting season.
2. Characterize the 40 hot pepper cultivars in Northern Region by morphological traits.
3. Determine genetic diversity among the 40 hot pepper cultivars in Northern Region using molecular marker techniques.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Chilli Pepper (*Capsicum annuum*)

According to Paramata *et al.*, (2023), chilli pepper (*Capsicum annuum*) exhibits a considerable degree of genetic variation. As stated by Amde, (2022), this crop displays noteworthy genetic diversity in terms of various fruit and morphology characteristics. Kena *et al.*, (2022) asserted that a positive correlation exists among certain traits, such as plant height, fruit length, the number of fruits per plant, and yield. The study conducted by Barik *et al.*, (2022) revealed a substantial level of diversity in these attributes, suggesting that selecting for them could potentially enhance yield. Moreover, the bioactive components derived from hot pepper fruits may have applications in the cosmeceutical and pharmaceutical industries (Bal *et al.*, 2022; Barik *et al.*, 2022). Therefore, it is imperative to identify the molecular markers associated with these features and to have access to the appropriate genetic resources in order to produce varieties or hybrids that are abundant in these substances (Benchimol-Reis, 2023).

The cultivation of hot peppers involves the careful selection of cultivars that are compatible with the prevailing climatic conditions (Soh, 2022). Factors such as fruit quantity, fresh weight, dimensions (length and width), fresh weight per fruit, dry weight, color, capsaicin content, sugar content, organic acid content, soluble solid-acid ratio, preference for dried red fruits, and disease resistance are all taken into consideration during the selection process (Chewaka *et al.*, 2022). Additionally, employing superior cultivars, appropriate combinations of potting media, and optimal utilization of fertilizers can all contribute to augmenting both crop yield and quality (Handayati *et al.*, 2021). Through the implementation of techniques such as red-to-far-red ratios, manipulation of photoperiods, and maintenance of optimal light intensities, the growth and



development of hot peppers can be expedited through the practice of speed breeding (Liu *et al.*, 2022). Extensive research has been conducted to determine the most suitable plant spacing and mechanical harvesting techniques, which not only mitigate the risk of musculoskeletal injuries but also enhance harvesting efficiency (Kang *et al.*, 2021). In general, the cultivation of hot peppers necessitates the judicious selection of appropriate cultivars, the optimization of growth environments, and the effective implementation of harvesting methods.

## 2.2 Historical Background of Hot Pepper Cultivation

Pepper, also known as the chili or *Capsicum*, is a widely cultivated vegetable crop that exhibits significant genetic variation (Deresá *et al.*, 2023). With a global identification of over 400 distinct types, chilies have established themselves as a prominent commercial crop (Madala and Nutakki, 2020). The genesis of chilies can be traced back to Mexico, where they have become a valuable cash crop for small-scale farmers in developing nations. While there has been a recent decline in production, *Capsicum chinense* Jacq. remains a crucial cash crop, playing a significant role in the local cuisines of the African and Caribbean (Mohan Rao and Anilkumar, 2020). The hot pepper industry faces two critical challenges, namely high production costs and low profitability (Ramjattan and Umaharan, 2021). Genetic diversity has been observed to impact the yield and its constituent components in hot peppers. Noteworthy traits contributing to yield include fruit quantity, average fruit weight, and the number of clusters per plant (Deresá *et al.*, 2023; Ramjattan and Umaharan, 2021). To enhance the efficacy of genetic enhancement in chilies, various breeding techniques such as genetic engineering and marker-assisted selection are currently under investigation (Mohan Rao and Anilkumar, 2020).

### 2.2.1 Cultural Significance and Traditional Uses

Chilli peppers have numerous traditional and cultural applications. They are employed as natural colorants, flavouring agents, food vegetables, and in traditional remedies, finding culinary use in various regions across the globe (Hernández-Pérez *et al.*, 2020). Their popularity in hot areas can be attributed to their ability to induce gustatory perspiration, which aids in reducing body temperature and acts as a protective mechanism (Abdel-Salam, 2016). In traditional medicine, hot pepper has been used to address gastrointestinal issues, psychiatric and ocular ailments, as well as surgical conditions. Capsaicinoids, bioactive components found in hot peppers, are believed to possess therapeutic properties and have been associated with potential health benefits, including weight loss, anti-obesity effects, antioxidant properties, antibacterial properties, anticancer properties, and analgesic properties (Szolcsányi, 2005). Numerous empirical medical studies have documented the usage of hot peppers in traditional medicine, particularly during the late Chosun period in Korea ( Oh *et al.*, 2012).

### 2.2.2 Changes and Developments over Time

Over the course of time, there have been advancements and modifications in the realm of hot peppers. Initially, the activities involving capsaicin were not particularly captivating, but in the late 1970s, research on capsaicin as a tool for investigation gained significant popularity. The identification of the capsaicin receptor TRPV1 in 1997 further accelerated research on capsaicin (Pintér *et al.*, 2023). To address various pests and diseases, while maintaining productivity, an integrated pest management (IPM) system was developed for the cultivation of hot peppers, thereby reducing the reliance on pesticides. Additionally, a specialized hot pepper harvester was created to enhance the efficiency of harvesting. This harvester possesses notable features such as a buffer foam cushion for ease and safety of operation, as well as a hydraulic telescoping rod.

Furthermore, the aging process of hot pepper mash has been the subject of research, revealing that increasing the salt level or aging the mixture in oak barrels could delay the breakdown of pectic compounds. Consequently, this could lead to the creation of a more stable hot pepper sauce (Kim and Yun, 2013).

## **2.3 Agro-morphological Traits in Hot Pepper**

### **2.3.1 Definition and Importance of Agro-morphological Traits**

The attributes of plants that pertain to their physical characteristics, growth patterns, and developmental processes are commonly referred to as agro-morphological attributes. These attributes play a crucial role in the identification and selection of plants with desirable qualities, making them indispensable for plant breeding and initiatives aimed at enhancing agricultural practices. By evaluating agro-morphological attributes, breeders will be able to identify potential contributors for breeding programs, enabling them to assess the genetic diversity and variability within a particular crop species (Hussain *et al.*, 2022).. Examples of these attributes include plant height, blooming colour, seed size, seed shape, and pod length (Behera *et al.*, 2023; Kumar *et al.*, 2023). By examining these qualities, breeders can identify plants with desired characteristics such as early maturity, high production potential, and resistance to pests or diseases (Jawarkar *et al.*, 2023; Petrova, 2023). To address the pressing needs of agricultural sustainability and food security, it is imperative to develop improved crop varieties through a comprehensive understanding and characterization of agro-morphological attributes (Itoh and Sato, 2023).

Agro-morphological attributes are of utmost importance in the field of plant breeding and development. Throughout history, crop breeding efforts have largely focused on these attributes, resulting in the creation of high-yielding cultivars (Itoh and Sato, 2023). The development of crops necessitates the selection of key attributes based on comprehensive data and an understanding of





the correlations between yield and yield-related components (Biswas *et al.*, 2023). Functional attributes of plants include disease resistance, tolerance to lodging, and the ability to intercept radiation (Noshita *et al.*, 2022). Morpho-physiological traits such as seed weight, pod characteristics, days to flowering, and plant height directly impact yield and productivity (Katoch, 2020). It is crucial to comprehend and characterize these attributes in order to enhance agricultural and crop output and select desired features.

### **2.3.2 Key Traits Used in Characterization**

The process of characterizing hot peppers involves the evaluation of several characteristics. These characteristics encompass fruit qualities, yield statistics, physical pod properties, as well as quantitative and qualitative morphological aspects. Quantitative features that were assessed include fruit width, fruit index, fruit weight, number of locules, placenta length, pedicel length, anther length, leaf width, number of flowers per axil, and the ratio of plant height to stem length (Bedjaoui, *et al.*, 2022). On the other hand, qualitative aspects that were evaluated include plant height, growth habit, bloom location, corolla form, fruit shape at pedicel attachment and blossom end, and calyx margin (Kaur *et al.*, 2016). Titratable acidity, dry matter content, total soluble solids, and ascorbic acid concentration are also significant characteristics that are taken into consideration. Additionally, fruit length, fruit diameter, fruit pericarp thickness, and average fruit weight are utilized as indicators of improved hot pepper output (Asiimwe *et al.*, 2021).

## **2.4 Molecular Markers in Plant Characterization**

### **2.4.1 Introduction to Molecular Markers**

In plant breeding programs, molecular markers have a pivotal role as they aid in identifying candidate genes and detecting genetic architecture (Benchimol-Reis, 2023). They have

revolutionized crop breeding by providing a faster, more precise, and more efficient alternative to conventional breeding techniques (Kumari *et al.*, 2023). Various types of molecular markers have been developed and commonly used in crop breeding, including Restriction Fragment Length Polymorphism (RFLP), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), and Single Nucleotide Polymorphism (SNP) (Sinha *et al.*, 2023). These markers are capable of measuring genetic diversity and identifying changes resulting from different DNA mutations (Bhat *et al.*, 2023). DNA markers have become a widely used method in mutant characterization, providing information about the type and extent of mutations (Badwei, 2023). Moreover, molecular markers have shown potential as diagnostic and prognostic tools for hepatocellular carcinoma, which could contribute to early diagnosis and improved patient outcomes (Badwei, 2023). Overall, molecular markers have made a significant contribution to advancements in various fields such as plant breeding, mutant analysis, and the diagnosis and prognosis of cancer.

#### **2.4.2 Applications of Molecular Markers in Plant Characterization**

The utilization of molecular markers has revolutionized crop breeding and is extensively employed in the characterization of plants. They offer significant advantages in terms of repeatability, precision, and efficiency. When studying the genetic diversity of common food plants like potato, maize, and tomato, various types of molecular markers, including SSRs, SNPs, and Sequence Characterized Amplified Regions (SCARs), are commonly used (Aslanbay *et al.*, 2023). These markers provide comprehensive information on genomic mapping, population dynamics, disease resistance, and polymorphisms. Moreover, markers have the capacity to identify candidate genes that play crucial roles in plant breeding programmes and the genetic architecture of phenotypes (Benchimol-Reis, 2023). SSRs, SNPs, and transposable elements are a few examples of DNA



markers that are frequently employed in various plant systems for molecular breeding, trait mapping, and mutant characterization (Bhat *et al.*, 2023). The availability of a wide range of molecular markers such as RFLP, RAPD, AFLP, SSR, SNP, and others, has considerably facilitated the evaluation of genetic diversity in plants. These markers have also proven to be indispensable in crop development (Kumari *et al.*, 2023; Sinha *et al.*, 2023).

### **2.4.3 Review of Molecular Marker Studies Related to *Capsicum annuum***

In order to assess genetic diversity and population structure, studies incorporating molecular markers have been conducted on *Capsicum annuum*. ISSR and SSR markers were used to identify the genotypes of 26 *Capsicum* spp. and observed genotype diversity (Haq *et al.*, 2023). Molla *et al.*, (2022) evaluated the genetic composition and population structure of 48 *Capsicum* accessions using enzyme-based EST-SSR markers. Their findings revealed a significant level of genetic diversity and differentiation within the population. Another study employed morphological descriptors and SSR markers to examine the genetic diversity among 10 genotypes of six *Capsicum* species, including *C. desi*. This analysis identified three main clusters and observed variations in fruit weight and pericarp thickness (Jindal *et al.*, 2021). Haq *et al.*, (2023) developed 240 SSR primers and screened them against cultivars, subsequently utilizing next-generation sequencing to identify SSR markers in the *C. annuum* genome. This approach enabled the discovery of 33 highly polymorphic markers and the classification of accessions into seven groups. These investigations have provided valuable insights into the genetic diversity and population dynamics of *Capsicum annuum*.

### **2.5 Characterization of Hot Pepper Accessions in Ghana**

Using agro-morphological markers, the investigation of hot pepper accessions in Ghana has been conducted. The assessment of genetic diversity and relatedness among pepper accessions involved

the utilization of 35 agro-morphological variables, encompassing both quantitative and qualitative aspects. The wide range of fruit characteristics was manifested through the identification of six distinct fruit forms and four diverse fruit colours. Fruit weight and yield components such as fruit length and fruit breadth, exhibited significant and positive associations (Agyare et al., 2016a). The utilization of morphological markers proved to be valuable for the examination of genetic diversity in *Capsicum* species, and the identified genotype diversity in peppers could be potentially harnessed for the enhancement of peppers through hybridization and selection. Moreover, an assessment of eight pepper genotypes, consisting of six foreign and two native varieties, was conducted to evaluate their agronomic performance in rain-fed environments in Ghana. The exotic hybrid cultivars displayed higher fruit yield, superior fruit weight, fruit length, and fruit maturity, as well as earlier ripening. However, the two local landrace types yielded the greatest number of undamaged fruits (Quartey *et al.*, 2014).

### **2.5.1 Findings and Trends in Existing Hot Pepper Accessions Characterization Studies**

Numerous studies have been conducted to characterize hot pepper accessions, revealing substantial variation in plant height, fruit diameter and length, locule count, and other morphological and agronomical characteristics. Notably, the A33, A27, G1, and A1 chilli pepper accessions have demonstrated superior performance in terms of plant growth and fruit quality. Furthermore, investigations have explored the chemical and biological variability of hot pepper fruits such as capsaicinoids and antioxidants, in relation to drying temperature and maturity stage. These studies have also classified hot pepper accessions into distinct categories based on qualitative characteristics, indicating notable distinctions among them. Collectively, these investigations provide valuable insights into the selection and cultivation of hot pepper cultivars with desirable traits (Akaza *et al.*, 2022; Mo *et al.*, 2015; Virga *et al.*, 2020).

### **2.5.2 Gaps and Limitations on Hot Pepper Accessions Characterization**

Despite the progress made in understanding the genetic history and structure of pepper populations, there are still gaps and limitations that need to be addressed (Aquino et al., 2022; Bedjaoui, *et al.*, 2022; Lee *et al.*, 2022). These include the need for more comprehensive agro-morphological characterization, exploration and quantification of genetic diversity, and a deeper understanding of hot pepper accessions' characteristics (Aquino et al., 2022; Bedjaoui, *et al.*, 2022; Lee *et al.*, 2022). Additionally, the extent of significant structural variations, such as presence-absence variants, inversions, and copy-number variants, in the pepper genome remains unknown (Tripodi *et al.*, 2021).. A more thorough characterization of the genetic diversity and traits of hot pepper accessions is crucial, including the identification of loci under selection and marker-trait correlations (Lima *et al.*, 2017). Furthermore, further investigation is required to determine the genotypes suitable for breeding programmes and to enhance our understanding of viral resistance mechanisms in hot pepper accessions.

### **2.6 Integration of Agro-morphological and Molecular Approaches**

The combination of molecular and agro-morphological approaches is indispensable in several fields, including plant disease control, conservation, and taxonomy. The identification of *Imparfinis* specimens was established through an integrated approach that involved a comprehensive morphological examination and molecular phylogenetic investigations (Joshi *et al.*, 2023). In order to support restoration and conservation endeavours, molecular tools and methodologies are employed to comprehend the characteristics and composition of agro-biodiversity at the molecular level (Schneider, 2023). The management of plant diseases has greatly benefited from the characterization of resistance genes using bioinformatics techniques and molecular tools (Singh Saharan *et al.*, 2023). To elucidate taxonomic structures within plant

species complexes, it is imperative to integrate genetic and morphological data (Nielsen *et al.*, 2023). In general, the combination of agro-morphological and molecular methodologies enables a more comprehensive understanding of plant species, genetic diversity, and disease control techniques (González-Toral and Cires, 2022).

### **2.6.1 Studies Combining Agro-morphological and Molecular Approaches**

Research that employs both molecular and agro-morphological methods has been conducted on a diverse range of plant species. For example, Jabbar and Al-Fatlawi (2023) utilized RAPD markers and traits such as plant height, grain weight, and grain density to investigate the correlation between molecular and morphological indicators in bread wheat. Mores *et al.*, (2021) examined various approaches to enhance crop resistance to diseases, including genomic technology and markers. Nogueira *et al.*, (2021) employed amplified fragment length polymorphism markers in conjunction with morpho-agronomic parameters to evaluate the genetic diversity of common bean accessions. Zhidkin *et al.*, (2022) combined molecular genetics and bioinformatic techniques to propose a comprehensive method for analysing the allelic status of a gene in plants belonging to the *Vaccinium* genus. Singh *et al.*, (2020) utilized morphological and molecular studies, such as SSR markers and Mahalanobis' D analysis to explore the genetic diversity of rice genotypes. These findings underscore the critical importance of integrating molecular and agro-morphological methods in order to comprehend genetic diversity, disease resistance, and breeding practices in diverse plant species.

### **2.6.2 Advantages and Limitations of Integrating These Approaches**

The combination of molecular and agro-morphological methods offers numerous advantages in the investigation of plant genetic variation. Morphological characterization serves as a foundation for future research by facilitating the assessment of genetic variability based on individual



phenotypic variations (Mondal *et al.*, 2022). However, morphological markers have limitations that have necessitated the development of molecular markers. In comparison to traditional phenotypic characterization, molecular marker techniques such as DNA fingerprinting and marker-assisted selection, provide several benefits, including enhanced accuracy and the ability to identify naturally occurring polymorphisms in DNA sequences (Anuragi *et al.*, 2022; Dalla Costa *et al.*, 2022). These molecular techniques have been instrumental in conserving and restoring significant crop species by providing insights into the molecular composition and nature of agrobiodiversity (Hammer *et al.*, 2016). By integrating agro-morphological and molecular methods, researchers can gain a more comprehensive understanding of plant genetic diversity and make informed decisions regarding the management of agricultural genetic resources (Bekele and Bekele, 2014).

### **2.6.3 Significance in the Context of Hot Pepper Characterization**

Characterizing hot peppers holds significant value due to several reasons. First, as stated by Bedjaoui *et al.*, (2022) and In *et al.*, (2020), it enables the identification and preservation of genetic diversity within and across different hot pepper varieties. This is crucial for the conservation of endangered indigenous landraces and the enhancement of pepper populations in the region (Hou *et al.*, 2018). Secondly, studies focused on characterisation provide valuable insights into the quantitative and qualitative morphological characteristics of hot peppers, along with other important agro-morphological aspects (Kaur *et al.*, 2016). Understanding such properties facilitates the selection and breeding of hot pepper cultivars with desired traits such as disease resistance and high productivity (Lima *et al.*, 2017). Additionally, characterisation studies shed light on the molecular and genetic attributes of hot peppers, aiding in the identification of genes and regulatory components involved in key processes like fruit ripening (Lee *et al.*, 2022). All in

all, the characterisation of hot peppers plays a vital role in the conservation, improvement, and knowledge advancement of this significant vegetable crop.

## 2.7 Methodologies in Characterization Studies

Studies examining the characterisation of hot peppers employ diverse methodologies to explore different aspects of the plant. For instance, one study utilized transcriptome analysis and promoter deletion analysis to uncover crucial regulatory elements associated with the genetic control of sesquiterpene phytoalexin production in hot peppers (In *et al.*, 2020). In a related investigation, the volatile chemicals present in various species of chili peppers were identified using solid-phase microextraction (SPME) in combination with gas chromatography-mass spectrometry (GC-MS). This information can be utilized for traceability and differentiation purposes (Traverso and Schiavo, 2020). Another study focused on the chemical changes that occur in hot pepper mash during aging, analysing factors such as pH, salt concentration, and pectic compounds. Furthermore, a study on hot pepper fruit ripening explored ethylene emission, ABA levels, and gene expression to elucidate the roles of ethylene and abscisic acid (ABA) (Hou *et al.*, 2018). Lastly, a research endeavour employed genotyping by sequencing to investigate the variation in morpho-agronomic characteristics and phytochemical components among different hot pepper varieties (Tripodi *et al.*, 2018).

### 2.7.1 Common Methodologies Used in Characterizing Hot Pepper Accessions

Typical approaches employed for the characterization of hot pepper accessions comprise the following:





1. The agro-morphological characterization involves the assessment of the morphological and agronomic traits of pepper accessions, encompassing plant height, fruit size, colour, shape, and leaf morphology (Virga *et al.*, 2020).
2. Molecular characterization: This methodology investigates the genetic diversity and population structure of pepper accessions by utilizing molecular markers such as microsatellites or single nucleotide polymorphisms (SNPs) (Hill *et al.*, 2013).
3. Phytochemical analysis: This approach entails the scrutiny of the chemical composition of pepper fruits, with particular attention given to their contents of antioxidants and health-related components (Virga *et al.*, 2020).
4. Analysis of heat maps: This technique visualizes the associations between diverse pepper accessions based on their morphological and agronomic features (Virga *et al.*, 2020).
5. Dendrogram analysis: To unveil the relationships between various pepper accessions on the basis of their morphological and agronomic properties, a dendrogram, resembling a tree-like diagram, must be constructed (Virga *et al.*, 2020).
6. Statistical analysis: This stage involves the utilization of statistical techniques to analyse the data obtained from the agro-morphological and molecular characterization of pepper accessions (Virga *et al.*, 2020).

These techniques collectively contribute to a wide ranging comprehension of the morphological traits, phytochemical composition, and genetic diversity of hot pepper accessions, all of which are advantageous for breeding endeavours and the development of novel pepper varieties.

### 2.7.2 Methodological Approaches Used in Characterizing Hot Pepper Accessions

Hot pepper accessions have been subjected to characterization using a plethora of methodological techniques, encompassing statistical analysis, heat map analysis, dendrogram analysis, phytochemical analysis, and agro-morphological analysis. The molecular characterization of pepper accessions involves the utilization of molecular markers to scrutinize the genetic diversity and population structure of said accessions, while agro-morphological characterization evaluates the morphological and agronomic properties of these accessions (Virga *et al.*, 2020). The examination of the chemical composition of pepper fruits, taking into consideration their antioxidant and health-related component contents, constitutes the realm of phytochemical study (Virga *et al.*, 2020). To portray the relationships between different pepper accessions, heat map and dendrogram analyses are employed based on their morphological and agronomic characteristics (Virga *et al.*, 2020). The information obtained from the agro-morphological and molecular characterization of pepper accessions is subjected to analysis using statistical methods (Virga *et al.*, 2020). Numerous studies have documented the evaluation of these methodological techniques such as the agro-morphological characterization of ornamental Sicilian chilli pepper accessions (Virga *et al.*, 2020). Furthermore, the utilization of SSR markers for the agro-morphological characterization of chilli pepper accessions (Rabuma *et al.*, 2020a) and the characterization of diverse pepper germplasms based on agro-morphological features and phytochemical contents (Moon *et al.*, 2023) have been investigated. The genetic diversity, physical characteristics, and phytochemical composition of hot pepper accessions have been extensively documented in these investigations.





### 2.7.3 Challenges Encountered in Implementing These Methodologies

The challenges encountered in the implementation of methodologies for hot pepper characterization include the impact of environmental factors on agro-morphological traits, the necessity for standardized descriptors to assess genetic diversity and population structure, as well as the influence of regional varieties and environmental conditions on phytochemical content. In an examination of the agro-morphological characterization of Sicilian chilli pepper accessions, the impact of environmental effects on agronomic traits, health-related compounds, and antioxidant properties was underscored (Virga *et al.*, 2020). This study highlights the significance of considering these factors during characterization studies. Moreover, the significance of accounting for climatic conditions and geographical variations when analysing phytochemical data was demonstrated by the characterization of various *Capsicum* types in a study conducted by González-Zamora *et al.*, (2013). Furthermore, a study on the characterization of genetic diversity in *Capsicum annuum* emphasized the importance of employing standardized descriptors for genetic diversity and population structure, thereby stressing the need for precise characterization through standardized terminology (Hill *et al.*, 2013). These challenges underscore the crucial nature of characterizing hot pepper accessions, while considering standardization, regional disparities, and environmental factors.

### 2.8 Comparative Analysis of Hot Pepper Characterization Studies

Studies on the characterisation of hot peppers have been carried out to assess the amount of capsaicinoids, pungency, and impact of high temperature on various pepper types (González-Zamora *et al.*, 2013). Five species,—known as bell, chilli, jalapeño, cayenne, and cherry varieties, respectively,—make up the genus *Capsicum*: *C. annuum*, *C. chinense*, *C. frutescens*, *C. pubescens*, and *C. baccatum* (Hou *et al.*, 2018). Plant hormones, particularly ethylene during climacteric



ripening and abscisic acid (ABA) during non-climacteric ripening, regulate the ripening of fleshy fruits, including spicy peppers (Hou *et al.*, 2018). A comparison examination of multi-element and metabolomic profiles between two types of chilli peppers has also been undertaken (Mi *et al.*, 2020). Ninety of the over one hundred varieties of native American chilli peppers have AsA content, according to a study done on their nutritional content (Kantar *et al.*, 2016). In a different research, two domesticated cultivars of the species *Capsicum annuum* and *C. chinense* were compared to a wild pepper (*Capsicum chacoense*) (Comparini *et al.*, 2021).

### 2.8.1 Comparative Assessment of Different Studies

The following is a comparison of various research on the characterisation of hot peppers:

Zhani *et al.*, (2015) conducted a morphological analysis of Tunisian chilli pepper (*Capsicum frutescens L.*) accessions. In order to assess the variety and similarity between Tunisian accessions of the chilli pepper (*Capsicum frutescens L.*), morphological traits were evaluated in this study.

Comparing the Nutritional Content and Volatile Compounds of Fruit from Wild and Domesticated Hot Peppers (Comparini *et al.*, 2021). The purpose of this study was to compare two domesticated cultivars of the species *Capsicum annuum* and *C. chinense* with a wild pepper, *Capsicum chacoense*. Analysing the fruits' volatile chemicals and nutritional makeup was the goal.

Multi-Element and Metabolomic Profiles for the Characterization and Discrimination of Chilli Peppers (Mi *et al.*, 2020): The multi-element and metabolomic profiles of two types of chilli peppers were compared in this study. The purpose of the study was to evaluate and differentiate the chilli peppers according to their metabolomic profiles and nutritional makeup.

An examination of the nutritional content of more than 100 varieties of native American chilli peppers (Kantar *et al.*, 2016): This study examined the nutritional value of more than a hundred

varieties of native American chilli peppers. For the goals of breeding, selection, and quality control, these investigations offer insightful data on the morphological, volatile, nutritional, and metabolomic properties of several hot pepper types.

### 2.8.2 Identification of Patterns or Discrepancies in Findings

The findings of numerous investigations regarding the characterization of hot peppers revealed a variety of patterns and distinctions:

1. The primary study, titled "Metabolomic Characterization of Hot Pepper (*Capsicum annuum*) During Fruit Development 1" (Jang *et al.*, 2015), examined the constituents present in hot pepper (*Capsicum annuum*) during the process of fruit development, encompassing carotenoids, capsaicinoids, ascorbic acid, and other substances.
2. Another research endeavour, "Morphological and Gene Expression Characterization of Maf-1, a Floral Chilli Pepper Mutant" (Tanaka *et al.*, 2022), sought to comprehend the progression of pungency in *Capsicum* species by investigating the genome sequence of the hot pepper.
3. Rasekh *et al.*, (2022) conducted an initial study on non-destructive sorting techniques for pepper. In order to classify sweet and spicy peppers, this study utilized Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA), Support Vector Machine (SVM), and Artificial Neural Network (ANN) techniques to evaluate the Padrón (*Capsicum annuum* L.) variety.
4. The purpose of this study was to scrutinize two varieties of chili peppers through a comparative analysis of multi-element and metabolomic profiles. The study titled "Characterization and Discrimination of Chilli Peppers Based on Multi-Element and Metabolomic Profiles" (Mi *et al.*, 2020) aimed to achieve this objective.



5. In an effort to gauge the diversity and similarity among Tunisian accessions of Chilli Pepper (*Capsicum frutescens L.*), the study titled "Comparative Analysis of Morphological Features of Tunisian Chilli Pepper (*Capsicum frutescens L.*) Accessions" (Zhani *et al.*, 2015) compared their morphological attributes.

The focus on several aspects of hot pepper characterization, which include metabolomic, morphological, and gene expression analyses, represents one of the trends observed in the results. The multitude of pepper types and the techniques employed for categorization and analysis contribute to the disparities in the obtained outcomes.

### 2.8.3 Insights Gained from Comparative Analysis

The following are some conclusions drawn from the comparison of several research on the characterisation of hot peppers:

#### *Analysis of the Metabolism of Hot Peppers (Capsicum annuum) Throughout Fruit Development:*

In this work, the ascorbic acid, carotenoids, capsaicinoids, and other chemicals present in hot peppers (*Capsicum annuum*) throughout fruit development were identified and quantified.

Understanding the manufacture of pungent chemicals and other secondary metabolites can be aided by the study's insights into the changes in the metabolomic profile of hot peppers throughout fruit development (Jang *et al.*, 2015).

#### *Macromorphological and Gene Expression Analysis of a Floral Chilli Pepper Mutant:*

In order to learn more about the evolution of pungency in *Capsicum* species, this study examined the genomic sequence of the hot pepper. According to Jang *et al.*, (2015), the study shed light on the genetic foundation of hot pepper pungency as well as the possible function of gene expression in the synthesis of pungent chemicals.



*Initial Research on Non-Destructive Pepper Sorting Methods:*

In order to categorise sweet and spicy peppers, this study employed PCA, LDA, SVM, and ANN techniques to assess the Padrón (*Capsicum annuum* L.) variety. According to their degree of pungency, the study's findings may lead to non-destructive pepper sorting methods (Jang *et al.*, 2015).

The objective of this work was to conduct a comparative analysis of multi-element and metabolomic profiles in order to characterize and distinguish between two types of chilli peppers. For the goals of breeding and selection, the study shed light on how the two kinds differed in terms of their metabolomic profiles and nutritional content (Jang *et al.*, 2015).

*Comparative Analysis of Tunisian Accessions' Morphological Characteristics (Capsicum frutescens L.):*

In order to assess the variety and similarity between Tunisian accessions of the chilli pepper (*Capsicum frutescens* L.), morphological traits were evaluated in this study. According to Jang *et al.*, (2015), the study shed light on the genetic variety of hot pepper types as well as the possibility of breeding and selection based on physical traits.

Overall, a thorough understanding of the metabolomic, morphological, and gene expression characteristics of hot peppers has been gained by the comparative study of several research on hot pepper characterization. The hot pepper sector may benefit from these insights for the purposes of breeding, selection, and quality control.





## 2.9 Current Trends and Emerging Technologies

### 2.9.1 Advancements in Agro-morphologic and Molecular Characterization Technologies

The latest advancements in agro-morphologic and molecular characterisation technologies have greatly enhanced our understanding of genetic diversity and plant growth (Adetunji *et al.*, 2022; Sinha *et al.*, 2023). These cutting-edge technologies encompass a wide range of molecular markers such as RFLP, RAPDs, AFLP, ISSR, SSR, SCAR, STS, CAPSs, ESTs, SNP, Diversity Arrays Technology (DArT), SCoT, and GBS, which enable the evaluation of genetic diversity in plants (Fayed *et al.*, 2020). These markers are not influenced by developmental phases or environmental factors, making them reliable tools for genetic research (Dalla Costa *et al.*, 2022). Furthermore, the application of molecular technology in plant breeding and gene mapping processes can lead to improvements in agricultural productivity, food safety, and sustainability (De Assis Leite and Gabiatti, 2022). According to Islam *et al.*, (2023), these technologies can also be utilized to enhance production, increase tolerance to biotic and abiotic stressors, and improve end-user quality attributes. The development of agro-morphologic and molecular characterisation technologies has opened up new avenues for crop enhancement, which can contribute to global food security (Mahajan and Kapoor, 2023; Patel *et al.*, 2023).

### 2.9.2 Implications for the Future of Hot Pepper Characterization Studies

The study of hot peppers and their characterisation has significant implications for the future. When examining the genetic variability and correlation between yield and yield-related factors in hot peppers, researchers have discovered significant variations in genotypes for different morphological and fruit characteristics (Deresa *et al.*, 2023). It has been found that the promoter pCaD, which controls the *sesquiterpene phytoalexin capsidiol*, has bidirectional action in hot peppers, suggesting its potential usefulness in the development of disease-resistant crops (In *et al.*,

2020). The ripening process of hot pepper fruit is regulated by both ethylene and abscisic acid (ABA); ethylene positively affects fruit pigmentation, while ABA aids in de-greening (Hou *et al.*, 2018). Hot tasting spices such as hot pepper, with their strong antioxidant and antibacterial properties, have the potential to be utilized as food preservatives and nutraceuticals (Bhattacharya *et al.*, 2022). A comparative proteome study of hot pepper mutants induced by space has provided insights into mutagenesis pathways and could assist in future breeding efforts (Lb *et al.*, 2017). These findings underscore the importance of characterisation research on hot peppers in order to understand their genetic diversity, disease resistance, ripening process, and their potential applications in the food and pharmaceutical industries.

### **2.9.3 Areas of Innovation and Potential Research Directions**

The characterisation studies conducted on hot peppers have revealed novel areas of investigation and potential research directions. For instance, there is a need for phytochemical and pharmacological screening of hot-tasting spices to explore their potential use in nutraceuticals and food preservation, as well as their health benefits (Bhattacharya *et al.*, 2022). De Sá Mendes *et al.*, (2019) have reported that through characterisation, the morphological, chemical, and metabolomic features of *Capsicum baccatum* fruits have been uncovered, indicating their potential as a food component with functional and technical qualities. Non-targeted metabolomic research on hot peppers has demonstrated biochemical changes that occur at different stages of growth, highlighting the interactions between metabolites and genes and variations in antioxidant activity (Jang *et al.*, 2015). These studies highlight the importance of characterisation research on hot peppers for understanding their genetic diversity, disease resistance, ripening process, and their potential applications in the food and pharmaceutical industries. In order to further augment the properties of chilli, the implementation of Diallel mating design has identified hybrid varieties that



possess desirable traits such as high yield, quality, and economic features, thereby evaluating the heterosis in chilli hybrids (Rohini and Lakshmanan, 2017). Recent studies have shown that the inclusion of hot pepper seed powder in seasonings leads to an elevation in their colour values, antioxidant activity, and total phenolics content. This implies that seasonings have the potential to enhance their physicochemical characteristics and antioxidant properties (Jang *et al.*, 2017).



## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 Description of the study area

The study was carried out in the Northern region of Ghana during the rainy and dry seasons. The rain-fed experiment was conducted in 2022 (July to December) at Savannah Agriculture Research Institute's experimental field in Nyankpala (latitude: 9.388°, longitude: -0.999°), while the dry season experiment was undertaken under irrigation from January to March (2023) at Golinga (latitude: 9.352°, longitude: -0.948°). The region is located within the Guinea-savannah belt of Ghana, at latitude 8 30" and 10 30" N. The climate is semi-arid with minimum and maximum mean monthly temperatures of 21.9°C and 34.1°C. With its unimodal rainfall pattern spanning from March to November, the region receives about 4953 mm of precipitation and has an average annual rainfall of 90.73 days. The region was selected for this study because it is one of the major chili pepper-producing areas in Ghana. The five districts (Kumbungu, Savelugu, Nanton, Tolon and Tamale) included in the study were selected based on recommendations from the DFID Market Development (MADE) in Northern Ghana program (DAI, 2014).

#### 3.2 Experimental design

A total of 40 *C. annuum* landraces obtained from local farmers, in five districts (Kumbungu, Savelugu, Nanton, Tolon and Tamale) in the Northern regions of Ghana were evaluated together with a check variety using multi-seasonal (rainy and dry seasons) experiments with three replications at each season using Randomized Complete Block Design (RCBD). Seeds were nursed in seed trays and kept in a greenhouse to obtain pepper seedlings.



### 3.3 Soil and Weather Data

### 3.4 Survey

#### 3.4.1 Procedure and participant

A cross-sectional survey was carried out using structured questionnaires and field observations. The ethnic background was not considered as long as the farmer was familiar with the management of pepper farms. A total of 52 respondents were selected using a snowball sampling techniques. Participating farmers were selected through systematic random sampling and interviews were conducted on every 15 houses. The questionnaire consisted of three sections: the sociodemographic profile of participants, agronomic and management practices used to produce pepper, and farmers' perception of 11 collative descriptor statements evaluated on a 5-point Likert scale (1 = strongly disagree, 5 = strongly agree). To understand and resolve problems of participants misunderstanding the survey instrument, the questionnaire was tested in a pilot study using 12 respondents from the study area.

A back-translation technique was employed to translate the questions into farmers' indigenous language (Dagbani) (Fibri & Frøst, 2019). Thus, two bilingual-trained translators translated all the questions from English to Dagbani and administrated the questionnaire separately to the same farmers. Thereafter, the translators transcribed the Dagbani version into English. The translated versions were then compared against each other and the necessary adjustments were effected to obtain contextual and linguistic equivalence (Fibri & Frøst, 2019). Cronbach's alpha test was used to calculate the reliability of the survey instrument (Taber, 2018) and redundant statements were removed.



### 3.4.2 Data analysis - Survey

All data analysis was carried out in SPSS 24 for Windows™ (IBM SPSS Inc., version 26). Descriptive statistics were used to compute frequencies and percentages for socio-demographic characteristics, agricultural land, and farm management practices. ANOVA was used to observe the relationship between perception scores (Likert scale question) of categorical variables and farming experiences.

## 3.5 Morphological

### 3.5.1 Nursery establishment, transplanting and field management

*Capsicum* seeds were nursed in seed trays containing top soil with biochar (1:2) in a screen house. The nursed seeds were watered regularly and other nursery management practices were carried out to ensure the production of healthy seedlings. The seedlings stayed in the nursery for six weeks after which 15 healthy seedlings of each accession were transplanted. Each experimental plot consisted of 1.5 m<sup>2</sup> containing 15 plants with 60 × 40 cm inter-and intra-row spacings. Watering was done immediately after transplanting and, at two weeks after transplanting to ensure establishment of seedlings.

YaraMila (15 N – 9 P – K 20 + 2MgO +2S +TE) was applied at 100 kg per acre as a split dose after four weeks of transplanting. At the flowering stage, Yara nitrabor (15.4 N + 26 CaO + 0.3 B) at 100 kg per acre was applied. Weeds were manually controlled when necessary to ensure the trial is devoid of weed. No insect infestation was observed hence no insecticide was used.

### 3.5.2 Morphological descriptor measurement

Ten competitive plants were selected at random from each plot in each replication and phenotypic data were recorded for eleven morphological characters at the seedling, vegetative, inflorescence

and fruiting stages using the *Capsicum* species standard described by IPGRI *et al.* (1959). Quantitative characters were measured and recorded using a measuring tape, ruler, and digital vernier caliper while qualitative parameters were obtained by description. The morphological traits were reduced to 13 based on the findings and suggestions of Bharath *et al.* (2014).

#### **3.5.4 Vegetative descriptors**

Cotyledon leaf length and width were measured at full maturity of *Capsicum* landraces (i.e., 4 weeks after transplanting) using a measuring rule. Cotyledon leaf length was measured from the petiole to the apex, while cotyledon leaf width was taken at the widest area.

#### **3.5.5 Plant descriptors**

Plant height (mm) was measured when 50% of the plants had their first ripe fruits using measuring tape, and stem diameter (mm) was taken in the middle part to the first bifurcation immediately after the first harvest using a Venier caliper.

#### **3.5.6 Inflorescence and fruit characteristics**

Days to 50% flowering and 50% fruiting were counted from the transplanting day to the day that 50% had at least one open flower and 50% of the plants bore mature fruits at the first and second bifurcation, respectively. Fruit length (mm), fruit width (mm), fruit pedicel length (mm), fruit pedicel width and fruit wall thickness (mm) were measured on the second harvest. Fruits were weighed with an electronic weighing scale (g) to obtain the fruit weight from four plants of each accession.

#### **3.5.7 Data Analysis of Morphological traits**

Descriptive statistics (minimum, maximum, mean, standard deviation) were analysed in GenStat (Payne *et al.*, 2015). Two-way analysis of variance (ANOVA) was performed to evaluate the



combined effects of genotype and season (rainy and dry) on morphological descriptors using GenStat Statistical package (version 12.1). Duncan multiple comparison was used to compare differences between means at a significance level of  $P < 0.05$ .

Correlation, principal component and clustering analysis were all computed using SPSS (IBM Corp, 2020). Two separate Pearson's correlation analyses were performed to study the correlation among the traits under rainfed and irrigation. Dissimilarity levels among genotypes were determined using agglomerative hierarchical clustering, employing Ward's method.

### **3.6 Molecular**

#### **3.6.1 DNA extraction and purification**

Six weeks old healthy young leaves were harvested into deep well sample collection plates and sent to the microbiology laboratory located at the University for Development Studies for DNA extraction. Genomic DNA was extracted from 40 *Capsicum* accessions using the Tiangen Plant Genomic DNA Kit (Tiangen Biotech Co., Ltd, Beijing, China) following the manufacturer's instructions. The extracted DNA quality was checked using NanoDrop Lite (Thermo Fisher Scientific), and all samples with an absorbance ratio (A260/A280) greater than 1.7 and were used for preparing the libraries. A working stock of 50 ng  $\mu\text{l}^{-1}$  of the genomic DNA was prepared and used in PCR amplification.

#### **3.6.2 Microsatellite genotyping**

In this study, a total of 24 simple sequence repeat (SSR) microsatellite markers, as previously developed by Nagy *et al.* (2007) (comprising 17 loci) and by Lee *et al.* (2004) (comprising seven loci) for *Capsicum annuum*, were carefully selected and used (Table 3.1). The PCR amplification reactions were conducted in 10  $\mu\text{l}$  reaction volumes, consisting of 1  $\mu\text{l}$  of DNA, 10  $\mu\text{mol}$  each of



forward and reverse primers, and a premix PCR standard buffer [pH 8.9] 1X tartrazine, 1X xylene cyanol, 0.05% Tween® 20, 0.06% IGEPAL® CA-630, 5% glycerol, 20 mM Tris-HCl, 22 mM KCl, 22 mM NH<sub>4</sub>Cl, 1.8 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, and 0.625 U of One Taq® DNA polymerase (New England Biolabs). The thermal cycling conditions for the PCR amplification were set as follows: an initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing (vary per marker, Table 3.1) for 30 s, extension at 72 °C for 30 s, and a final extension step at 72 °C for 7 min in the peqSTAR 96x standard thermal (PEQLAB, Germany). Subsequently, the resulting PCR amplicons were separated and visualized on a 1.5% agarose gel that had been stained with ethidium bromide (Supplementary Figure 3.1). The analysis of PCR product patterns for each primer pair across the *Capsicum* accessions was carried out using PyElph 1.4 software (Pavel & Vasile, 2012).



1 **Table 3.0.1: Primers used in this study**

S/No.	Marker name	Chromosome number	Type	Sequences	Ta °C	N <sub>N</sub>	Allele size	Reference
1	EPMS397	P1	Forward	GCACCCTCCCAATACAAATC	55	20	102-117	(Nagy et al., 2007)
			Reverse	GATCACGGAGAAAGCAAAGG				
2	GPMS169	P2	Forward	TCGAACAAATGGGTCATGTG	57	20	176-220	(Nagy et al., 2007)
			Reverse	GATGAGGGTCCTGTGCTACC				
3	GPMS6	P2	Forward	CAGAGCACTTGACATGCCTT	54	20	122-172	(Nagy et al., 2007)
			Reverse	GATCTTTATAGTAGCTCATCAATA				
4	EPMS-755	P2	Forward	CGCTCGCTACCCTTTCATTA	53	20	141	(Portis et al., 2007)
			Reverse	AATTTTCGGAAGGGCAAAGAT				
5	GPMS100	P2	Forward	TCCATACGGTTGGAGGAGAG	57	20	141-169	(Nagy et al., 2007)
			Reverse	ACTATGCTCTGCTGTGCCCT				
6	EPMS386	P3	Forward	ACGCCAAGAAAATCATCTCC	53	20	122-170	(Nagy et al., 2007)
			Reverse	CCATTGCTGAAGAAAATGGG				
7	HPMSE008	P3	Forward	CCCCTTAACTTTTAATTCTAGATCTGC	58	27	230	(Yi et al., 2006)
			Reverse	TCGTTGTTCCCTCCATCACCTCA				
8	GPMS93	P3	Forward	ATCCTTGGCGTATTTTGCAC	53	20	202-268	(Nagy et al., 2007)
			Reverse	TTCACTTTGCACACAGGCTT				
9	GPMS165	P5	Forward	TGAACAATAATAATTGACAGGACAG	55	25	242-317	(Nagy et al., 2007)
			Reverse	AGCCTCGCAGTTTGTCTTAC				
10	HPMS1-5	P6	Forward	CCAAACGAACCGATGAACACTC	58	22	311	(Yi et al., 2006)
			Reverse	GACAATGTTGAAAAAGGTGGAAGAC				
11	HPMSE088	P6	Forward	GCAAATGGTTCCCTAAACTGCTT	57	23	199	(Yi et al., 2006)
			Reverse	GCTCTCCGTTTCCGATGTGATT				
12	GPMS161	P7	Forward	CGAAATCCAATAAACGAGTGAAG	55	23	184-259	(Nagy et al., 2007)
			Reverse	CCTGTGTGAACAAGTTTTCAGG				
13	EPMS310	P8	Forward	TGGGAAGAGAAATTGTGAAAGC	54	22	140-172	(Nagy et al., 2007)

S/No.	Marker name	Chromosome number	Type	Sequences	Ta °C	N <sub>N</sub>	Allele size	Reference
14	EPMS342	P8	Reverse	AGGAAACATGGTTCAATGCC	56	20	323-343	(Nagy et al., 2007)
			Forward	CTGGTAGTTGCAAGAGTAGATCG		23		
15	EPMS419	P9	Reverse	ATGATCTTTGACGACGAGGG	56	20	224-248	(Nagy et al., 2007)
			Forward	TTCAGGTGCAGGTATCATCG		20		
16	HPMS2-24	P9	Reverse	GGGTACTTGTCATTATCCAG	57	22	208	(Yi et al., 2006)
			Forward	TCGTATTGGCTTGTGATTACCG		23		
17	HPMSE013	P10	Reverse	TTGAATCGAATACCCGCAGGAG	57	22	256	(Yi et al., 2006)
			Forward	GCGCCAAGTGAGTTGAATTGAT		22		
18	EPMS331	P11	Reverse	CACCAATCCGCTTGCTGTTGTA	54	20	92-107	(Nagy et al., 2007)
			Forward	AACCCAATCCCCTTATCCAC		20		
19	GPMS29	P11	Reverse	GCATTAGCAGAAGCCATTTG	54	18	238-255	(Nagy et al., 2007)
			Forward	CAGGCAATACGGAGCATC		20		
20	GPMS101	P11	Reverse	TGTGTTGCTTCTTGACGAC	56	20	176-211	(Nagy et al., 2007)
			Forward	CCTATCACCTCTTTGAGCC		20		
21	EPMS391	P11	Reverse	TAAAGACCAGCCCTGGATGA	53	20	177-187	(Nagy et al., 2007)
			Forward	TTTCTTCTCTGGCCCTTTTG		20		
22	HPMSE064	P12	Reverse	ACGCCTATTGCGAATTCAG	57	23	221	(Yi et al., 2006)
			Forward	CCCTCCTTTTACCTCGTCAAAAA		22		
23	HPMSE128	P12	Reverse	ATGCCAAGGAGCAATGAGAACC	57	23	226	(Yi et al., 2006)
			Forward	TGGATCCCAAAGACTCAGAACA		22		
24	GPMS197	P12	Reverse	TATTTCCCTCAGTCGAGGTCGT	52	22	272-344	(Nagy et al., 2007)
			Forward	GCAGAGAAAATAAAATTCTCGG		20		
			Reverse	CAATGGAAATTCATCGACG				

2 Ta: Annealing temperature, N<sub>N</sub>: Number of nucleotides.

### 3.6.3 Data scoring and analysis

Genetic diversity statistics, including allele frequency, observed heterozygosity, gene diversity, and Polymorphic Information Content (PIC) for the designated microsatellite markers were determined. The analysis was carried out using PowerMarker v3.25 software (<http://powermarkr.net>). To assess the genetic relationships among the *Capsicum* accessions, an unrooted neighbour-joining tree was constructed. The genetic distances among these accessions were estimated using PowerMarker v3.25 software, as outlined by Liu and Muse (Liu & Muse, 2005). For an in-depth examination of the population structure of the 40 *Capsicum* accessions, Bayesian clustering algorithm within Structure v2.3.4, as proposed by Pritchard *et al.* (2000) was used. During this analysis, a range of presumed populations (K) from 3 to 6, and the procedure was repeated five times in the test. A burn-in period of 50,000 steps, utilizing a Monte Carlo Markov Chain (MCMC) of 100,000 was integrated. The model incorporated features such as admixture and correlated allele frequency, in accordance with the method outlined by Falush *et al.* Accessions with a membership coefficient (Q) greater than or equal to 0.60 were assigned to a specific genetic group, while those with Q values below 0.60 were categorized as admixture. To determine the most probable genetic population grouping for the *Capsicum* accessions, Structure Harvester tool (<http://taylor0.biology.ucla.edu>) was deployed.



## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 Survey

##### 4.1.1 Socio-demographic profile of farmers participating in the study

As presented in Table 4.1, of the 52 respondents who participated in the study, 40.4% were younger than 30 years old, 90.4% were male participants, 21.2% had secondary education, 78.8% were married and 57.7% had more than five years' experience in pepper farming. Pepper farmers in the study area have cultivate the pepper as their secondary crop. The average acres allocated to pepper farming is 1.04 acres. Participants characteristic of more than 60% being younger than 40 years old, and married with more than 5 years of experience in pepper farming were considered good samples.

**Table 4.1: Socio-demographic profile of farmers participating in the study**

Characteristic	Category	Frequency (%)
Age (n = 52)	21-30	21 (40.4)
	31-40	18 (34.6)
	41-50	9 (17.3)
	51-60	3 (5.8)
	>60	1 (1.9)
Gender (n = 52)	Male	47 (90.4)
	Female	5 (9.6)
Education	Secondary	11 (21.2)
	Tertiary	0 (0)
	Non-formal	41 (78.8)
Marital status (n = 52)	Single	11 (21.2)
	Married	41 (78.8)
Experience in farming pepper (n = 52)	<5	22 (42.3)
	5-10	18 (34.6)
	>10	12 (23.1)
Role of pepper in income	Sole income	0



	Secondary income	49 (94.2)
	Hobby	3 (5.8)
Mean total farm size (acres)		5.98 (1-30)
Mean acres allocated for pepper		1.04 (0.5-2)

#### 4.1.2 Agricultural land, seed properties and cropping systems

Although agriculture was the main occupation for 95.6% of the participants with 73.1% farming on less than 6 acres of farmland, none of the participants had more than two acres of pepper farm. About 19.2% (Table 4.2) of farmers intercropped pepper with other crops such as maize, okra, sorghum, and tomato.

Most of the participants used farmer-saved seeds (67.3%), while 25.0% and 7.7% purchased certified pepper seeds and seedlings respectively (Table 4.3). As expected, the majority of the farmers (76.9%) used produce from the previous year's harvest as seed leading to the observed different pepper varieties (65.4%).

**Table 4.2: Varieties, type and source of planting materials**

Characteristic	Category	Frequency (%)
Type of seed	Farmer saved seed	35 (67.3)
	Certified seeds	13 (25.0)
	Seedlings	4 (7.7)
Source of planting material	Last year harvest	40 (76.9)
	Friends	4 (7.7)
	Market	7 (13.5)
	MoFA	1 (1.9)
Varieties	Single variety	18 (34.6)
	Multiple varieties	34 (65.4)



### 4.1.3 Management practices

Within the study area, pepper is mostly grown on ridges (82.7%). Most farmers indicated that weed control is carried out about 3-5 times alongside tillage, which is enough for optimum growth and development of pepper plants (Table 4.3). About 94.2% used fertilizer with 81.6% of the farmers applying inorganic fertilizer mostly at one dose (Basal application). Pepper harvesting is done manually, and dried with pedicle mostly on the bare floor (44.2%). Dried pepper fruits are mostly stored in nylon sacks (98.1%).

**Table 4.3: Pepper management practices**

Characteristic	Category	Frequency (%)
Cropping system (n = 52)	Monocropping	42 (80.8)
	Intercropping	10 (19.2)
Crops intercropped with pepper (n = 10)	Maize	4
	Okra	2
	Sorghum	2
	Tomato	2
Planting on ridges	Yes	43 (82.7)
	No	9 (17.3)
Earthing up of soil	< 3	4 (7.7)
	3-5	45 (86.5)
	>5	3 (5.8)
Fertilizer application (n = 52)	No	3 (5.8)
	Yes	49 (94.2)
Fertilizer type (n = 49)	Organic fertilizer	9 (18.4)
	Inorganic fertilizer	40 (81.6)
Fertilizer application rate (n = 40)	Basal	33 (82.5)
	Top dressing	7 (17.5)
Harvesting methods	Hand harvesting	52 (100)
	Mechanical harvesting	0 (0)
Dry with Pedicle	Harvest with pedicle	52 (100)



	Harvest without pedicles	0 (0)
Drying platform	Bare floor	23 (44.2)
	Black polythene sheet	9 (17.3)
	Tarpaulin	14 (26.9)
	Cemented surface	6 (11.5)
Storage sacks	Jute sack	1 (1.9)
	Nylon sack	51 (98.1)

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Generally, the farmer rating of improved pepper seeds was low, indicating that the local pepper varieties used in the study were comparable to the improved pepper varieties (Table 4.4). Although most farmers disagreed that improved pepper seeds are more resistant to climate change variabilities (2.19), farmers acknowledge that improved seeds are moderately responsive to nutrient application, and require more nutrients. Improved pepper seeds scored 3.67 for yield



**Table 4.4: Responses to the Likert scale statements**

Item	How well do you agree or disagree with this statement?	Pepper farming experience			p-value
		< 5 (n = 22)	5-10 (n = 18)	> 10 (n = 12)	
1	Ridges help increase yield	4.05 ± 0.95 <sup>a</sup>	3.61 ± 0.69 <sup>a</sup>	4.92 ± 0.29 <sup>b</sup>	0.000
2	Earthing up helps increase yield	3.09 ± 1.07	3.17 ± 0.98	3.67 ± 0.65	0.232
3	Organic fertilizers support pepper productivity more than inorganic	2.5 ± 1.14)	3.28 ± 0.95 <sup>ab</sup>	4.08 ± 1.31 <sup>b</sup>	0.001
4	Basal application is enough for pepper growth	2.73 ± 1.24	3.11 ± 1.23	2.83 ± 1.27	0.619
5	Drying on a bare floor facilitates fruit rot	2.73 ± 1.45	3.44 ± 1.38	3.67 ± 1.30	0.121
6	Selling in weight is more profitable than in volume	3.73 ± 0.77 <sup>a</sup>	2.78 ± 1.00 <sup>a</sup>	2.17 ± 0.94 <sup>b</sup>	0.000
7	Agronomic practices affect pepper flavour	2.95 ± 1.13 <sup>a</sup>	3.17 ± 1.04 <sup>ab</sup>	3.92 ± 1.08 <sup>b</sup>	0.054
8	Agronomic practices affect the hotness of pepper	2.59 ± 1.18 <sup>a</sup>	2.83 ± 1.29 <sup>a</sup>	4.00 ± 0.43 <sup>b</sup>	0.003
9	Agronomic practices affect the antioxidant properties of pepper	3.18 ± 0.79	2.78 ± 0.73	3.42 ± 0.67	0.065
10	Inorganic fertilizer increases fruit rot more than organic	2.82 ± 1.14	3.5 ± 1.04	3.50 ± 1.24	0.111
11	Improved seeds are more responsive to nutrients than farmer-saved seeds	3.5 ± 1.47) <sup>b</sup>	2.56 ± 1.29 <sup>a</sup>	3.83 ± 1.40 <sup>b</sup>	0.034
12	Improved seeds are more resistant to pests and diseases than FSS	3.32 ± 1.21 <sup>a</sup>	2.33 ± 1.08 <sup>b</sup>	2.42 ± 1.24 <sup>c</sup>	0.021
13	Improved seeds give more yield than farmer-saved seeds	3.78 ± 0.53 <sup>a</sup>	3.39 ± 0.78 <sup>b</sup>	2.33 ± 1.15 <sup>c</sup>	0.000
14	Improved seeds require more nutrients than farmer-saved seeds	3.95 ± 0.49	3.94 ± 0.54	3.67 ± 0.49	0.245
15	Improved seeds are more resistant to climate variabilities than Farmer-saved seeds	1.86 ± 1.13	1.78 ± 0.81	1.67 ± (0.78)	0.846

Mean ± (SD) scores show responses from respondents separated by farming experiences (< 5; n=22, 5-10; n=18, > 10; n = 12). Mean scores within a row with unlike superscript letters are significantly different (p < .05).



## 4.2 Morphological

### 4.2.1 Descriptive statistics of the agro-morphological traits

Of the 13 morphological traits evaluated in this study, the mean squares were significant ( $P < 0.001$ ) for five traits (plant height, fruit pedicle width, total number of flowers, total flesh weight, and dry weight) for season and 12 traits (except CLW) for the interaction between the genotypes and season (Table 4.5).

The variability (minimum, maximum mean and standard deviations) among the pepper genotypes for both rainfed and irrigated is presented in Table 4.6. Higher mean variations were observed for TNF, TFW and DW under rainfed conditions, while irrigation supported higher variations among the pepper genotypes for CLL, CW, PH, SD, DF, DFF, FPL, FPL, FL and FW (Table 4.6).

**Table 4.5: Combined mean squares from analysis of variance for agro-morphological traits across two seasons**

Trait	Genotypes	Season	Genotype * Season	Residual
CLL	1.27***	0.00ns	0.41***	0.08
CLW	0.31***	0.01ns	0.08ns	0.07
PH	62.28***	4646.88***	55.54***	18.31
SD	2.29***	1.56ns	1.91***	0.41
DF	48.38***	3.50ns	40.19***	11.77
DFF	91.50***	82.84ns	49.16***	16.68
FPL	36.94***	20.03ns	32.47***	9.16
FPW	0.43***	0.98*	0.45***	0.19
FL	479.50***	133.52ns	426.10***	92.11
FW	12.73***	5.15ns	7.16***	2.63
TNF	29775.00***	1012441.00***	36282.00***	11867
TFW	0.05***	23.09***	0.03***	0.01
DW	0.06***	5.63***	0.04***	0.00

**CLL**; Cotyledon leaf length, **CW**; Cotyledon width, **PH**; Plant height, **SD**; Stem diameter, **DF**; Days to 50% flowering, **DFF**; Days to 50% fruiting, **FPL**; Fruit pedicle length, **FPW**; Fruit pedicle width, **FL**; Fruit length, **FW**; Fruit width, **TNF**; Total number of fruits, **TFW**; Fresh weight, **DW**; Dry weight. \*, \*\*, \*\*\* and ns represent significant differences at  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  and not significant, respectively.

**Table 4.6: Descriptive statistics of the agro-morphological traits**

Trait	Rainfed				Irrigation			
	Minimum	Maximum	Mean $\pm$ SEM	Standard deviation	Minimum	Maximum	Mean $\pm$ SEM	Standard deviation
CLL	2.08	4.54	3.03 $\pm$ 0.04	0.49	1.90	4.50	3.04 $\pm$ 0.06	0.65
CLW	0.80	2.16	1.25 $\pm$ 0.02	0.24	0.60	3.60	1.26 $\pm$ 0.04	0.40
PH	22.60	43.20	33.30 $\pm$ 0.40	4.34	19.30	55.13	42.10 $\pm$ 0.61	6.67
SD	3.22	8.39	6.15 $\pm$ 0.07	0.79	3.90	8.90	5.99 $\pm$ 0.10	1.15
DF	26.00	54.00	35.51 $\pm$ 0.45	4.93	22.00	54.00	35.27 $\pm$ 0.41	4.53
DFF	32.00	64.00	42.98 $\pm$ 0.52	5.65	32.00	64.00	44.16 $\pm$ 0.55	6.05
FPL	20.55	37.24	25.95 $\pm$ 0.27	2.97	13.50	39.71	26.53 $\pm$ 0.47	5.12
FPW	1.21	3.34	2.46 $\pm$ 0.04	0.42	0.99	3.98	2.33 $\pm$ 0.06	0.61
FL	33.95	97.57	70.09 $\pm$ 1.20	13.18	43.65	129.85	71.58 $\pm$ 1.43	15.71
FW	5.05	14.43	8.87 $\pm$ 0.16	1.72	4.33	17.11	9.16 $\pm$ 0.24	2.66
TNF	83.00	977.00	351.58 $\pm$ 15.47	169.47	46.00	596.00	221.68 $\pm$ 8.60	94.26
TF	0.30	1.50	0.71 $\pm$ 0.02	0.21	0.04	0.18	0.08 $\pm$ 0.00	0.03
DW	0.10	1.10	0.34 $\pm$ 0.02	0.20	0.01	0.12	0.04 $\pm$ 0.00	0.02

**CLL**; Cotyledon leaf length, **CW**; Cotyledon width, **PH**; Plant height, **SD**; Stem diameter, **DF**; Days to 50% Flower, **DFF**; Days to 50% fruiting, **FPL**; Fruit pedicle length, **FPW**; Fruit pedicle width, **FL**; Fruit length, **FW**; Fruit width, **TNF**; Total number of fruits, **TFW**; Fresh weight, **DW**; Dry weight.

There were significant variations among the morphological traits in the rainy season (Table 4.7). The average cotyledon leaf width per plant was 3.04 mm, with a range from 2.26 (M46) to 4.22 (K10) cm (Table 4.7). The lowest cotyledon leaf length was recorded for K09 (0.96) while S40 (4.27) recorded the highest cotyledon leaf width, with an average mean of 1.25 cm. Plant height varied significantly from 27.31 cm (T27) to 43.44 cm (S43), with an average height of 37.70 cm. Genotype K08 had the lowest stem diameter (4.99), while genotype (T27) had the widest stem diameter (7.30 mm), with an average stem diameter of 6.07 mm.

Genotype M44 had the least days to 50% flowering, while genotype L18 had the most days to 50% flowering. Genotype M44 exhibited the least days to 50% fruiting and genotype L18 had the most days to 50% fruiting. The fruit pedicle length ranged from 22.50 mm in genotype N20 to 32.34 mm in genotype S40, while the fruit pedicle width ranged from 1.90 (S43) to 3.12 (T04).

The average fruit length and width were 70.84 mm and 9.02 mm, respectively. Genotype T26 had the lowest fruit length (54.79 cm) and fruit width (7.04), and genotype T27 (97.67 mm) and genotype T28 (12.89) had the highest fruit length and width, respectively. The total number of fruits (average = 286.6) produced per plant was the lowest in genotypes K13 (143.3) and the highest in N21 (453). The average fresh weight was 0.39 kg, with a range of 0.26 kg (N24) to 0.69 kg (S43). The fruit dry weight varied significantly from 0.09 kg (K16) to 0.54 kg (S43) with an average of 0.04 kg.

**Table 4.7: Mean performance of the 40 pepper genotypes tested under rainfed conditions**

ACC	CLL	CW	PH	SD	DF	DFE	FPL	FPW	FL	FW	TNF	TFW	DW
K08	2.95 <sup>f-o</sup>	1.28 <sup>a-f</sup>	35.05 <sup>b-g</sup>	4.99 <sup>a</sup>	36.33 <sup>d-k</sup>	45.83 <sup>f-m</sup>	23.27 <sup>a-d</sup>	2.134 <sup>a-e</sup>	64.15 <sup>a-e</sup>	8.86 <sup>a-h</sup>	279.7 <sup>a-g</sup>	0.29 <sup>ab</sup>	0.17 <sup>a-j</sup>
K09	2.87 <sup>e-m</sup>	0.96 <sup>a</sup>	40.83 <sup>ghi</sup>	4.99 <sup>lm</sup>	33.33 <sup>a-g</sup>	40.67 <sup>a-f</sup>	31.78 <sup>jk</sup>	2.279 <sup>a-f</sup>	70.26 <sup>b-j</sup>	7.19 <sup>ab</sup>	299.2 <sup>a-g</sup>	0.41 <sup>a-f</sup>	0.23 <sup>ijk</sup>
K10	4.22 <sup>x</sup>	1.88 <sup>gh</sup>	38.98 <sup>d-l</sup>	4.99 <sup>g-m</sup>	31.00 <sup>ab</sup>	44.67 <sup>d-m</sup>	27.3 <sup>b-i</sup>	2.261 <sup>a-f</sup>	57.58 <sup>abc</sup>	7.57 <sup>a-d</sup>	345.3 <sup>c-i</sup>	0.35 <sup>a-e</sup>	0.11 <sup>abc</sup>
K11	3.26 <sup>n-r</sup>	1.47 <sup>b-f</sup>	40.01 <sup>f-i</sup>	4.99 <sup>g-m</sup>	34.33 <sup>a-h</sup>	40.33 <sup>a-f</sup>	29.49 <sup>g-k</sup>	2.455 <sup>a-f</sup>	70.27 <sup>b-j</sup>	7.36 <sup>ab</sup>	295.3 <sup>a-g</sup>	0.39 <sup>a-e</sup>	0.19 <sup>c-j</sup>
K12	3.24 <sup>m-r</sup>	1.11 <sup>a-c</sup>	34.62 <sup>b-f</sup>	5.34 <sup>a-d</sup>	40.00 <sup>jk</sup>	48.5 <sup>j-m</sup>	26.87 <sup>b-i</sup>	2.83 <sup>fg</sup>	70.02 <sup>b-i</sup>	10.18 <sup>e-k</sup>	201.3 <sup>abc</sup>	0.31 <sup>abc</sup>	0.22 <sup>g-k</sup>
K13	3.11 <sup>j-p</sup>	1.62 <sup>fg</sup>	33.27 <sup>b-d</sup>	5.07 <sup>ab</sup>	34.83 <sup>a-i</sup>	44.33 <sup>d-m</sup>	27.84 <sup>e-j</sup>	1.98 <sup>ab</sup>	70.98 <sup>c-j</sup>	8.63 <sup>a-h</sup>	143.3 <sup>a</sup>	0.28 <sup>ab</sup>	0.12 <sup>a-f</sup>
K14	3.29 <sup>o-u</sup>	1.29 <sup>a-f</sup>	38.75 <sup>d-l</sup>	6.65 <sup>klm</sup>	36.5 <sup>e-k</sup>	43.67 <sup>c-k</sup>	26.72 <sup>a-i</sup>	2.46 <sup>a-f</sup>	64.71 <sup>a-f</sup>	8.26 <sup>a-g</sup>	402 <sup>f-i</sup>	0.37 <sup>a-e</sup>	0.12 <sup>a-f</sup>
K15	3.09 <sup>j-p</sup>	1.18 <sup>a-d</sup>	33.39 <sup>b-e</sup>	5.73 <sup>a-i</sup>	37.33 <sup>e-k</sup>	44 <sup>c-l</sup>	27.04 <sup>b-i</sup>	2.19 <sup>a-f</sup>	72.56 <sup>d-k</sup>	8.56 <sup>a-h</sup>	244 <sup>a-e</sup>	0.37 <sup>a-e</sup>	0.22 <sup>h-k</sup>
K16	3.65 <sup>s-w</sup>	1.42 <sup>b-f</sup>	38.35 <sup>c-i</sup>	5.69 <sup>a-i</sup>	36.00 <sup>c-j</sup>	45 <sup>d-m</sup>	27.36 <sup>c-i</sup>	2.48 <sup>a-f</sup>	73.02 <sup>d-k</sup>	8.56 <sup>a-h</sup>	151.3 <sup>ab</sup>	0.32 <sup>abc</sup>	0.08 <sup>a</sup>
L18	2.79 <sup>c-k</sup>	1.15 <sup>a-d</sup>	34.34 <sup>b-f</sup>	5.95 <sup>b-k</sup>	44.5 <sup>l</sup>	56.67 <sup>n</sup>	24.54 <sup>a-e</sup>	2.29 <sup>a-f</sup>	74.36 <sup>d-k</sup>	10.63 <sup>h-k</sup>	236.5 <sup>a-d</sup>	0.32 <sup>abc</sup>	0.12 <sup>a-f</sup>
M44	3.23 <sup>m-r</sup>	1.43 <sup>c-f</sup>	34.28 <sup>b-f</sup>	6.63 <sup>j-m</sup>	30.33 <sup>a</sup>	36.67 <sup>a</sup>	25.71 <sup>a-g</sup>	2.46 <sup>a-f</sup>	71.50 <sup>d-j</sup>	9.71 <sup>c-j</sup>	299.3 <sup>a-g</sup>	0.37 <sup>a-e</sup>	0.10 <sup>ab</sup>
M45	2.90 <sup>e-n</sup>	1.11 <sup>abc</sup>	38.83 <sup>d-l</sup>	5.23 <sup>a-d</sup>	34.50 <sup>a-i</sup>	46 <sup>f-m</sup>	23.61 <sup>a-e</sup>	1.99 <sup>ab</sup>	64.87 <sup>a-f</sup>	7.40 <sup>abc</sup>	281.7 <sup>a-g</sup>	0.30 <sup>ab</sup>	0.10 <sup>ab</sup>
M46	2.26 <sup>a</sup>	1.09 <sup>abc</sup>	37.70 <sup>b-i</sup>	6.58 <sup>i-m</sup>	37.83 <sup>f-k</sup>	49.17 <sup>klm</sup>	30.57 <sup>h-k</sup>	2.40 <sup>a-f</sup>	82.37 <sup>ijk</sup>	7.67 <sup>a-d</sup>	294.8 <sup>a-g</sup>	0.49 <sup>c-f</sup>	0.16 <sup>a-i</sup>
M47	2.36 <sup>ab</sup>	1.17 <sup>a-d</sup>	37.96 <sup>c-i</sup>	5.70 <sup>a-i</sup>	37.83 <sup>f-k</sup>	45.33 <sup>e-m</sup>	26.07 <sup>a-g</sup>	2.35 <sup>a-f</sup>	80.53 <sup>h-k</sup>	9.71 <sup>c-j</sup>	198.3 <sup>abc</sup>	0.48 <sup>c-f</sup>	0.34 <sup>l</sup>
N17	2.46 <sup>a-d</sup>	1.20 <sup>a-e</sup>	38.11 <sup>c-i</sup>	5.45 <sup>a-e</sup>	35.17 <sup>a-i</sup>	42.5 <sup>b-i</sup>	26.67 <sup>a-i</sup>	2.33 <sup>a-f</sup>	82.69 <sup>ijk</sup>	9.77 <sup>d-j</sup>	297.2 <sup>a-g</sup>	0.29 <sup>ab</sup>	0.11 <sup>ab</sup>
N18	2.78 <sup>c-k</sup>	1.23 <sup>a-e</sup>	32.6 <sup>bc</sup>	6.12 <sup>d-k</sup>	31.33 <sup>abc</sup>	38.5 <sup>abc</sup>	25.52 <sup>a-g</sup>	2.19 <sup>a-f</sup>	78.3 <sup>f-k</sup>	7.10 <sup>ab</sup>	398.3 <sup>e-i</sup>	0.53 <sup>ef</sup>	0.34 <sup>l</sup>
N19	2.83 <sup>d-l</sup>	1.12 <sup>abc</sup>	39.35 <sup>d-l</sup>	5.63 <sup>a-g</sup>	35.67 <sup>b-j</sup>	41.83 <sup>a-i</sup>	24.12 <sup>a-e</sup>	2.66 <sup>c-g</sup>	76.58 <sup>e-k</sup>	10.46 <sup>g-k</sup>	255.5 <sup>a-f</sup>	0.43 <sup>a-f</sup>	0.18 <sup>b-j</sup>
N20	3.20 <sup>l-r</sup>	1.07 <sup>abc</sup>	39.14 <sup>d-l</sup>	6.35 <sup>f-l</sup>	35.17 <sup>a-i</sup>	46 <sup>f-m</sup>	22.5 <sup>a</sup>	2.51 <sup>a-f</sup>	63.25 <sup>a-e</sup>	8.9 <sup>a-h</sup>	293.2 <sup>a-g</sup>	0.39 <sup>a-e</sup>	0.22 <sup>g-k</sup>
N21	2.57 <sup>a-h</sup>	1.22 <sup>a-e</sup>	41.82 <sup>hi</sup>	5.99 <sup>c-k</sup>	37.50 <sup>e-k</sup>	45 <sup>d-m</sup>	30.66 <sup>ijk</sup>	2.06 <sup>a-d</sup>	60.98 <sup>a-d</sup>	7.18 <sup>ab</sup>	453 <sup>i</sup>	0.48 <sup>c-f</sup>	0.36 <sup>l</sup>
N22	2.54 <sup>a-e</sup>	1.02 <sup>a</sup>	38.94 <sup>d-l</sup>	5.59 <sup>a-f</sup>	34.17 <sup>a-h</sup>	44 <sup>c-l</sup>	29.6 <sup>g-k</sup>	2.35 <sup>a-f</sup>	64.17 <sup>a-e</sup>	8.64 <sup>a-h</sup>	243.7 <sup>a-e</sup>	0.35 <sup>a-e</sup>	0.11 <sup>a-d</sup>
N23	3.28 <sup>n-t</sup>	1.25 <sup>a-e</sup>	41.23 <sup>hi</sup>	7.04 <sup>lm</sup>	33.5 <sup>a-h</sup>	40.17 <sup>a-f</sup>	25.47 <sup>a-g</sup>	2.18 <sup>a-e</sup>	67.22 <sup>a-h</sup>	8.39 <sup>a-h</sup>	295.7 <sup>a-g</sup>	0.50 <sup>d<sup>ef</sup></sup>	0.33 <sup>l</sup>
N24	2.82 <sup>c-l</sup>	1.13 <sup>a-d</sup>	36.13 <sup>b-h</sup>	6.11 <sup>c-k</sup>	33.17 <sup>a-g</sup>	40.67 <sup>a-f</sup>	25.58 <sup>a-g</sup>	2.47 <sup>a-f</sup>	66.57 <sup>a-g</sup>	8.21 <sup>a-g</sup>	311 <sup>c-i</sup>	0.26 <sup>a</sup>	0.09 <sup>ab</sup>
S35	3.17 <sup>k-q</sup>	1.44 <sup>c-f</sup>	39.82 <sup>f-i</sup>	6.54 <sup>h-m</sup>	38.33 <sup>h-k</sup>	49.83 <sup>m</sup>	23.04 <sup>abc</sup>	2.45 <sup>a-f</sup>	66.67 <sup>a-g</sup>	10.29 <sup>f-k</sup>	196.8 <sup>abc</sup>	0.29 <sup>ab</sup>	0.17 <sup>a-j</sup>
S36	2.73 <sup>b-j</sup>	1.13 <sup>a-d</sup>	36.79 <sup>b-h</sup>	5.73 <sup>a-j</sup>	34.17 <sup>a-h</sup>	40.5 <sup>a-f</sup>	25.37 <sup>a-g</sup>	2.58 <sup>b-g</sup>	81.77 <sup>ijk</sup>	10.34 <sup>g-k</sup>	291 <sup>a-g</sup>	0.39 <sup>a-e</sup>	0.13 <sup>a-f</sup>
S38	2.44 <sup>abc</sup>	0.97 <sup>a</sup>	36.84 <sup>b-h</sup>	5.43 <sup>a-e</sup>	32.67 <sup>a-e</sup>	42.83 <sup>c-j</sup>	25.79 <sup>a-g</sup>	2.00 <sup>ab</sup>	62.15 <sup>a-d</sup>	8.90 <sup>a-h</sup>	415.3 <sup>ghi</sup>	0.37 <sup>a-e</sup>	0.12 <sup>a-e</sup>
S40	3.82 <sup>w</sup>	1.96 <sup>h</sup>	39.43 <sup>e-i</sup>	5.66 <sup>a-h</sup>	38.00 <sup>g-k</sup>	45.17 <sup>e-m</sup>	32.34 <sup>k</sup>	2.01 <sup>abc</sup>	56.74 <sup>ab</sup>	7.44 <sup>abc</sup>	328 <sup>c-i</sup>	0.35 <sup>a-e</sup>	0.11 <sup>abc</sup>

ACC	CLL	CW	PH	SD	DF	DFE	FPL	FPW	FL	FW	TNF	TFW	DW
S42	3.37 <sup>p-v</sup>	1.40 <sup>b-f</sup>	40.08 <sup>f-i</sup>	5.59 <sup>a-f</sup>	34.50 <sup>a-i</sup>	39.67 <sup>a-e</sup>	22.96 <sup>ab</sup>	2.36 <sup>a-f</sup>	57.48 <sup>abc</sup>	7.98 <sup>a-f</sup>	272 <sup>a-g</sup>	0.42 <sup>a-f</sup>	0.22 <sup>g-k</sup>
S43	2.73 <sup>b-j</sup>	1.08 <sup>abc</sup>	43.44 <sup>i</sup>	6.49 <sup>f-m</sup>	34.00 <sup>a-h</sup>	41.83 <sup>a-i</sup>	26.06 <sup>a-g</sup>	1.90 <sup>a</sup>	64.22 <sup>a-e</sup>	8.93 <sup>a-h</sup>	219.5 <sup>a-d</sup>	0.69 <sup>g</sup>	0.54 <sup>n</sup>
T01	3.67 <sup>v-w</sup>	1.40 <sup>b-f</sup>	35.91 <sup>b-h</sup>	5.24 <sup>a-d</sup>	35.83 <sup>b-j</sup>	41.67 <sup>a-i</sup>	25.85 <sup>a-g</sup>	2.42 <sup>a-f</sup>	65.09 <sup>a-f</sup>	8.18 <sup>a-g</sup>	235.2 <sup>a-d</sup>	0.37 <sup>a-e</sup>	0.11 <sup>a-d</sup>
T03	2.56 <sup>a-f</sup>	1.01 <sup>a</sup>	37.59 <sup>b-i</sup>	6.25 <sup>e-l</sup>	33.00 <sup>a-f</sup>	41.33 <sup>a-g</sup>	27.02 <sup>b-i</sup>	2.16 <sup>a-e</sup>	69.13 <sup>b-i</sup>	8.84 <sup>a-h</sup>	275.5 <sup>a-g</sup>	0.33 <sup>a-d</sup>	0.15 <sup>a-g</sup>
T04	2.78 <sup>c-k</sup>	0.98 <sup>a</sup>	41.78 <sup>hi</sup>	6.26 <sup>e-l</sup>	33.83 <sup>a-h</sup>	40.5 <sup>a-f</sup>	23.94 <sup>a-e</sup>	3.12 <sup>g</sup>	79.03 <sup>g-k</sup>	9.41 <sup>b-i</sup>	284.8 <sup>a-g</sup>	0.52 <sup>ef</sup>	0.29 <sup>kl</sup>
T05	3.01 <sup>i-p</sup>	1.28 <sup>a-f</sup>	39.27 <sup>d-l</sup>	5.63 <sup>a-g</sup>	36.17 <sup>c-j</sup>	47.17 <sup>g-m</sup>	26.42 <sup>a-h</sup>	2.64 <sup>c-g</sup>	73.92 <sup>d-k</sup>	11.78 <sup>ijkl</sup>	263 <sup>a-g</sup>	0.37 <sup>a-e</sup>	0.16 <sup>a-h</sup>
T06	3.51 <sup>q-w</sup>	1.49 <sup>def</sup>	42.00 <sup>hi</sup>	6.38 <sup>f-l</sup>	31.5 <sup>a-d</sup>	36.83 <sup>ab</sup>	27.53 <sup>d-l</sup>	2.71 <sup>efg</sup>	83.62 <sup>jk</sup>	9.85 <sup>d-j</sup>	367.3 <sup>d-l</sup>	0.44 <sup>b-f</sup>	0.24 <sup>jk</sup>
T07	2.56 <sup>a-g</sup>	1.14 <sup>a-d</sup>	39.89 <sup>f-i</sup>	5.21 <sup>abc</sup>	39.33 <sup>ijk</sup>	49.67 <sup>lm</sup>	28.95 <sup>f-k</sup>	2.25 <sup>a-f</sup>	73.85 <sup>d-k</sup>	7.936 <sup>a-e</sup>	267.5 <sup>a-g</sup>	0.41 <sup>a-f</sup>	0.17 <sup>a-j</sup>
T25	2.53 <sup>a-e</sup>	1.02 <sup>a</sup>	36.88 <sup>b-h</sup>	6.10 <sup>c-k</sup>	35.67 <sup>b-j</sup>	43.5 <sup>c-k</sup>	24.97 <sup>a-f</sup>	2.75 <sup>efg</sup>	85.06 <sup>k</sup>	11.41 <sup>i-l</sup>	302.3 <sup>b-h</sup>	0.56 <sup>fg</sup>	0.44 <sup>m</sup>
T26	2.71 <sup>b-i</sup>	1.05 <sup>ab</sup>	39.78 <sup>f-i</sup>	6.85 <sup>klm</sup>	36.33 <sup>d-k</sup>	44.00 <sup>c-l</sup>	23.2 <sup>a-d</sup>	2.48 <sup>a-f</sup>	54.79 <sup>a</sup>	7.04 <sup>a</sup>	449.2 <sup>hi</sup>	0.31 <sup>abc</sup>	0.20 <sup>f-j</sup>
T27	3.54 <sup>r-w</sup>	1.33 <sup>a-f</sup>	27.31 <sup>a</sup>	7.30 <sup>m</sup>	41.00 <sup>kl</sup>	47 <sup>g-m</sup>	23.41 <sup>a-d</sup>	2.70 <sup>efg</sup>	97.67 <sup>l</sup>	12.32 <sup>kl</sup>	237.3 <sup>a-d</sup>	0.35 <sup>a-e</sup>	0.11 <sup>a-d</sup>
T28	3.58 <sup>r-w</sup>	1.28 <sup>a-f</sup>	31.95 <sup>ab</sup>	6.82 <sup>klm</sup>	32.83 <sup>a-e</sup>	39.17 <sup>a-d</sup>	24.06 <sup>a-e</sup>	2.66 <sup>d-g</sup>	71.67 <sup>d-k</sup>	12.89 <sup>l</sup>	232.3 <sup>a-d</sup>	0.35 <sup>a-e</sup>	0.11 <sup>a-e</sup>
T29	3.28 <sup>n-s</sup>	1.28 <sup>a-f</sup>	39.15 <sup>d-l</sup>	7.05 <sup>lm</sup>	33.83 <sup>a-h</sup>	41.33 <sup>a-h</sup>	23.78 <sup>a-e</sup>	2.53 <sup>a-g</sup>	69.22 <sup>b-i</sup>	8.58 <sup>a-h</sup>	293.2 <sup>a-g</sup>	0.52 <sup>ef</sup>	0.15 <sup>a-h</sup>
T30	3.84 <sup>w</sup>	1.55 <sup>ef</sup>	36.33 <sup>b-h</sup>	6.52 <sup>g-m</sup>	34.17 <sup>a-h</sup>	41.33 <sup>a-h</sup>	26.52 <sup>a-i</sup>	2.75 <sup>efg</sup>	74.5 <sup>d-k</sup>	9.39 <sup>b-i</sup>	315.3 <sup>c-i</sup>	0.35 <sup>a-e</sup>	0.10 <sup>ab</sup>
<b>CV%</b>	<b>9.3</b>	<b>20.6</b>	<b>11.4</b>	<b>10.6</b>	<b>9.7</b>	<b>9.4</b>	<b>11.5</b>	<b>18.6</b>	<b>13.5</b>	<b>18</b>	<b>38</b>	<b>30.3</b>	<b>30.9</b>
<b>Mean</b>	<b>3.04</b>	<b>1.25</b>	<b>37.7</b>	<b>6.07</b>	<b>35.39</b>	<b>43.57</b>	<b>26.24</b>	<b>2.39</b>	<b>70.84</b>	<b>9.02</b>	<b>286.6</b>	<b>0.39</b>	<b>0.19</b>
<b>SE</b>	<b>0.28</b>	<b>0.26</b>	<b>4.28</b>	<b>0.64</b>	<b>3.43</b>	<b>4.08</b>	<b>3.03</b>	<b>0.44</b>	<b>9.59</b>	<b>1.62</b>	<b>108.94</b>	<b>0.119</b>	<b>0.06</b>
<b>LSD</b>	<b>0.23</b>	<b>0.295</b>	<b>6.9</b>	<b>1.036</b>	<b>5.532</b>	<b>6.585</b>	<b>4.88</b>	<b>0.719</b>	<b>15.476</b>	<b>2.616</b>	<b>175.66</b>	<b>0.193</b>	<b>0.10</b>
<b>Sig</b>	<b>***</b>	<b>***</b>	<b>***</b>	<b>***</b>	<b>***</b>	<b>***</b>	<b>***</b>	<b>***</b>	<b>***</b>	<b>***</b>	<b>***</b>	<b>***</b>	<b>***</b>

CLL; Cotyledon leaf length, CW; Cotyledon width, PH; Plant height, SD; Stem diameter, DF; Days to 50% flowering, DFE; Days to 50% fruiting, FPL; Fruit pedicle length, FPW; Fruit pedicle width, FL; Fruit length, FW; Fruit width, TNF; Total number of fruits, TFW; Fresh weight, DW; Dry weight. ACC; Accession, Sig; Significance \* and \*\*represent significant differences at  $p < 0.05$  and  $p < 0.01$ , respectively.

#### 4.2.2 Pearson's Correlation Analysis

There was a strong positive correlation between days to 50% flowering and days to 50% fruiting for the rainy season ( $r = 0.87$ ,  $P < 0.001$ ; Table 4.8). Similarly, total fresh weight correlated positively with total number of fruits for the rainy season ( $r = 0.37$ ,  $P < 0.001$ ). Total number of fruits negatively correlated with days to 50% flowering ( $r = -0.28$ ,  $P < 0.001$ ), days to 50% fruiting ( $r = -0.39$ ,  $P < 0.001$ ), fruit pedicle width ( $r = -0.29$ ,  $P < 0.001$ ), fruit length ( $r = -0.33$ ,  $P < 0.001$ ) and fruit width ( $r = -0.39$ ,  $P < 0.001$ ) during the rainy season.

There was a strong positive correlation between days to 50% flowering and days to 50% fruiting for the dry season ( $r = 0.73$ ,  $P < 0.001$ ; Table 4.9). Cotyledon length positively correlated with cotyledon width ( $r = 0.41$ ,  $P < 0.001$ ) and negatively correlated with fruit fresh weight ( $r = -0.33$ ,  $p < 0.01$ ) and fruit dry weight ( $r = -0.27$ ,  $p < 0.01$ ). The total number of fruits revealed a positive correlation with fruit dry weight ( $r = 0.74$ ).



**Table 4.8: Pearson correlation coefficient among plant and fruit quantitative traits of 40 pepper genotypes (rainfed)**

	<b>CLW</b>	<b>PH</b>	<b>SD</b>	<b>DF</b>	<b>DFE</b>	<b>FPL</b>	<b>FPW</b>	<b>FL</b>	<b>TFW</b>	<b>TNF</b>	<b>FW</b>	<b>DW</b>
<b>CLL</b>	0.880**	0.215*	0.044	-0.152	-0.167	0.12	0.015	-.200*	-0.032	0.115	-0.077	-.204*
<b>CLW</b>		0.162	0.013	-0.061	-0.091	0.228*	-0.018	-0.231*	0.054	0.031	-0.082	-.229*
<b>PH</b>			0.214*	-0.309**	-.372**	0.114	-0.083	-0.014	-0.098	.477**	0.428**	0.293**
<b>SD</b>				-0.002	-0.139	-0.019	-0.108	-0.038	-0.213*	0.190*	0.138	0.039
<b>DF</b>					0.871**	-0.018	-0.026	0.037	-0.075	-0.284**	-0.073	-0.06
<b>DFE</b>						-0.068	-0.022	0.026	-0.034	-0.389**	-0.153	-0.081
<b>FPL</b>							0.077	0.179	0.115	-0.055	0.082	-0.092
<b>FPW</b>								0.541**	0.430**	-0.285**	-0.097	0.001
<b>FL</b>									0.463**	-0.333**	0.128	-0.017
<b>TFW</b>										-0.383**	0.027	0.02
<b>TNF</b>											0.37**	0.293**
<b>FW</b>												0.738**

**CLL**; Cotyledon leaf length, **CLW**; Cotyledon leaf width, **PH**; Plant height, **SD**; Stem diameter, **DF**; Days to 50% flowering, **DFE**; Days to 50% fruiting, **FPL**; Fruit pedicle length, **FPW**; Fruit pedicle width, **FL**; Fruit length, **TFW**; Total fruit width, **TNF**; Total number of fruits, **FW**; Fresh weight, **DW**; Dry weight. \* and \*\* represent significant differences at  $p < 0.05$  and  $p < 0.01$ , respectively.

**Table 4.9. Pearson correlation coefficient among plant and fruit quantitative traits of 40 core collections of pepper (Irrigation)**

	<b>CLW</b>	<b>PH</b>	<b>SD</b>	<b>DF</b>	<b>DFE</b>	<b>FPL</b>	<b>FPW</b>	<b>FL</b>	<b>TFW</b>	<b>TNF</b>	<b>FW</b>	<b>DW</b>
<b>CLL</b>	0.41***	-0.20*	0.13ns	-0.07ns	-0.09ns	-0.16ns	0.21*	-0.04ns	0.20*	-0.02ns	-0.22**	-0.33**
<b>CLW</b>		-0.13ns	-0.08ns	-0.11ns	-0.03	0.07	-0.06	-0.05	0.02	0.07	-0.24**	-0.27**
<b>PH</b>			-0.04ns	0.01	0.06	0.22**	0.05	-0.24**	-0.14	-0.02	0.17	0.17
<b>SD</b>				-0.07	-0.14	-0.04	0.19	0.14	0.01	-0.06	0.21*	0.16
<b>DF</b>					0.73**	-0.01	0.16	0.18	0.10	-0.08	-0.03	0.06
<b>DFE</b>						0.05	0.02	0.01	0.03	-0.11	-0.12	-0.07
<b>FPL</b>							-0.01	0.02	-0.28	0.10	0.11	0.06
<b>FPW</b>								0.26**	0.27**	0.01	-0.02	-0.11
<b>FL</b>									0.38**	0.04	0.03	0.09
<b>TFW</b>										0.01	-0.11	-0.08
<b>TNF</b>											-0.23**	-0.23*
<b>FW</b>												0.74

**CLL**; Cotyledon leaf length, **CLW**; Cotyledons leaf width, **PH**; Plant height, **SD**; Stem diameter, **DF**; Days to 50%Flower, **DFE**; Days to 50% fruiting, **FPL**; Fruit pedicle length, **FPW**; Fruit pedicle width, **FL**; Fruit length, **TFW**; Total fruit width, **TNF**; Total number of fruits, **FW**; Fresh weight, **DW**; Dry weight. \*, \*\* and \*\*\* represent significant differences at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  respectively.



### 4.2.3 Principal component and clustering analysis

The Principal Component Analysis (PCA) identified five Principal Components (PCs) with eigenvalues greater than 1, which accounted for 80.92% (rainfed) and 73.46% (irrigation) of the total variation for the assessed phenotypic traits (Table 4.10). For rainfed conditions, PC1 accounted for 24.70% of the total variation and plant height, days to 50% fruiting, and total number of fruits accounted for discrimination along this axis. Fruit pedicle width, fruit length, and fruit width contributed the most to the total variance (22.23%) observed in PC2. PC3 was associated with cotyledon leaf length and width, and accounted for 13.61% of the total variation, while total fresh weight, dry weight, and fruit pedicle length contributed most to the PC4 and PC5 axes, respectively.

The first PCs explained the major portion of variance (54.63) in the pepper germplasm under irrigation. Factor loadings for the traits under consideration showed that CLL is the major contributor to PC1; SD and FW to PC2; DF and DFF to PC3 in the germplasm. The proportion of variance explained by individual PCs was much lower in the irrigation than in the rainfed dataset.

Three main clusters were identified (Figure 4.1, A and B) for both rainfed and irrigation conditions (Figure 4.1) with no consistent pattern of separation of the genotypes by location. Genotypes clustering in the same plane suggests a high correlation and similarity between them. Cluster I contained seven and two individuals of the pepper accessions for rainfed and irrigation conditions, respectively. Cluster II was further divided into two sub-clusters; subcluster C1 contained 27 and 23 *Capsicum* accessions for rainfed and irrigation conditions, respectively, with subcluster C2 containing the remaining 6 and 15 *Capsicum* accessions. Sixteen (rainfed) and eighteen (irrigation)

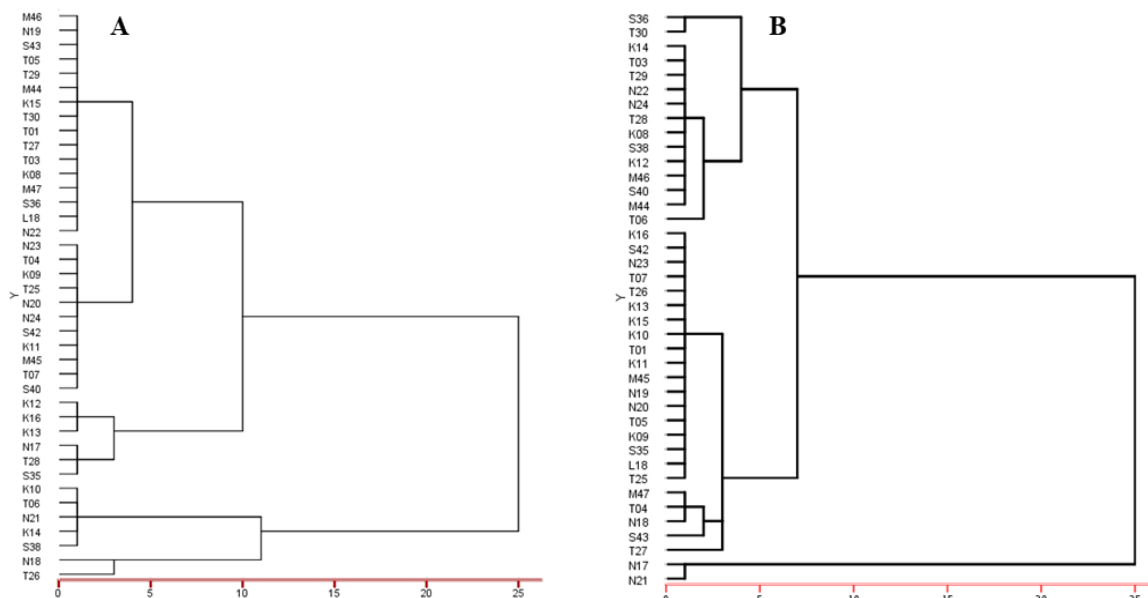
of the *Capsicum* accessions clustered with the improved variety Legon 18 (L18), indicating that these genotypes may be similar to L18.



**Table 4.10: Principal component analysis showing the contributions of each trait to the variation in the germplasm**

Traits	Rainfed					Irrigation				
	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5
CLL	0.36	0.57	0.69	-0.12	-0.21	0.60	0.01	-0.51	0.18	-0.13
CLW	0.33	0.58	0.70	-0.14	-0.20	0.41	-0.50	-0.27	0.43	-0.02
PH	0.79	-0.15	-0.11	0.02	0.29	-0.53	-0.14	0.22	-0.49	0.09
SD	0.37	-0.02	0.11	-0.18	0.49	0.03	0.64	-0.19	-0.43	-0.13
DF	-0.64	-0.15	0.46	0.50	0.07	0.28	0.18	0.85	0.10	-0.10
DFE	-0.70	-0.19	0.39	0.49	0.03	0.30	-0.06	0.87	0.08	-0.21
FPL	-0.05	0.22	0.36	-0.12	0.73	-0.31	-0.53	0.16	0.18	0.60
FPW	-0.01	0.79	-0.27	0.02	-0.20	0.29	0.56	-0.04	-0.19	0.31
FL	-0.21	0.79	-0.26	0.14	0.12	0.20	0.53	0.14	0.40	0.60
FW	-0.22	0.77	-0.29	0.02	0.11	0.47	0.69	0.01	0.01	0.13
TNF	0.71	-0.46	0.20	-0.02	-0.21	0.25	-0.34	-0.01	-0.50	0.48
TFW	0.63	0.21	0.00	0.68	0.09	-0.74	0.46	-0.17	0.22	0.04
DW	0.57	0.07	-0.18	0.73	-0.03	-0.78	0.34	0.07	0.35	-0.06
<b>Eigenvalue</b>	<b>3.21</b>	<b>2.89</b>	<b>1.77</b>	<b>1.59</b>	<b>1.06</b>	<b>2.62</b>	<b>2.50</b>	<b>1.97</b>	<b>1.29</b>	<b>1.16</b>
<b>Variance</b>	<b>24.70</b>	<b>22.23</b>	<b>13.61</b>	<b>12.21</b>	<b>8.17</b>	<b>20.18</b>	<b>19.26</b>	<b>15.19</b>	<b>9.94</b>	<b>8.90</b>
<b>Cumulative</b>	<b>24.70</b>	<b>46.93</b>	<b>60.54</b>	<b>72.75</b>	<b>80.92</b>	<b>20.18</b>	<b>39.43</b>	<b>54.62</b>	<b>64.56</b>	<b>73.46</b>

PC; Principal component, CLL; Cotyledon leaf length, CLW; Cotyledon leaf width, PH; Plant height, SD; Stem diameter, DF; Days to 50% flowering, DFE; Days to 50% fruiting, FPL; Fruit pedicle length, FPW; Fruit pedicle width, FL; Fruit length, TFW; Total fruit width, TNF; Total number of fruits, FW; Fresh weight, DW; Dry weight



**Figure 4.1: Hierarchical dendrogram average linkage clusters of the 13 agro-morphological traits. A; Rainfed, B; Irrigation**

### 4.3 Molecular

#### 4.3.1 Heterozygosity and informativeness of SSR markers

The genetic diversity statistics for the 24 SSR markers are presented in Table 4.11. The SSRs had substantial low variation in the number of alleles, which ranged from 1 to 3. Overall, 39 alleles at 24 loci were obtained with an average of 1.63 alleles per locus and about 58.3 % of the SSRs producing 1 allele at different loci (Table 4.11). All the 24 SSR markers had major allele frequency (MAF) above 50 %. Marker GPMS29 had the lowest MAF of 53 %, while EPMS397, GPMS169, GPMS6, EPMS-755, GPMS100, HPMSE008, GPMS93, GPMS161, EPMS342, EPMS419, HPMS2-24, HPMSE013, EPMS331, GPMS101, and EPMS391 had 100 % MAF. All the markers had PIC values  $<0.50$  with 20.8% having  $PIC \geq 0.30$ . HPMS1-5 had the maximum PIC value (0.40), and 14 of the markers had a PIC value of 0.00, with an average PIC value of 0.11. Except for marker HPMSE064, which had 0.10 heterozygosity, the calculated heterozygosity was 0.00 for all the genetic markers. Similarly, the gene diversity (expected heterozygosity) ranged from 0.00

(58 % of the SSR markers) to 0.51 for marker HPMS1-5, with a mean value of 0.13. All these indicate that hot pepper in the Northern region has a narrow genetic base.

**Table 4.11: Genetic diversity and polymorphism indexes among the SSR locus**

Marker	MAF	Genotype No	Na	GD	Heterozygosity	PIC
EPMS397	1.00	1.00	1.00	0.00	0.00	0.00
GPMS169	1.00	1.00	1.00	0.00	0.00	0.00
GPMS6	1.00	1.00	1.00	0.00	0.00	0.00
EPMS-755	1.00	1.00	1.00	0.00	0.00	0.00
GPMS100	1.00	1.00	1.00	0.00	0.00	0.00
EPMS386	0.78	3.00	3.00	0.36	0.00	0.31
HPMSE008	1.00	1.00	1.00	0.00	0.00	0.00
GPMS93	1.00	1.00	1.00	0.00	0.00	0.00
GPMS165	0.85	3.00	3.00	0.26	0.00	0.24
HPMS1-5	0.58	3.00	3.00	0.51	0.00	0.40
HPMSE088	0.95	3.00	3.00	0.10	0.00	0.09
GPMS161	1.00	1.00	1.00	0.00	0.00	0.00
EPMS310	0.60	2.00	2.00	0.48	0.00	0.36
EPMS342	0.75	2.00	2.00	0.38	0.00	0.30
EPMS419	1.00	1.00	1.00	0.00	0.00	0.00
HPMS2-24	1.00	1.00	1.00	0.00	0.00	0.00
HPMSE013	1.00	1.00	1.00	0.00	0.00	0.00
EPMS331	1.00	1.00	1.00	0.00	0.00	0.00
GPMS29	0.53	2.00	2.00	0.50	0.00	0.37
GPMS101	1.00	1.00	1.00	0.00	0.00	0.00
EPMS391	1.00	1.00	1.00	0.00	0.00	0.00
HPMSE064	0.98	2.00	2.00	0.05	0.00	0.05
HPMSE128	0.88	3.00	3.00	0.23	0.10	0.21
GPMS197	0.88	2.00	2.00	0.22	0.00	0.19
<b>Mean</b>	0.91	1.63	1.63	0.13	0.00	0.11

MAF; Major Allele Frequency, Na; Number of Allele, GD; Gene Diversity, PIC: Polymorphism Information Content.

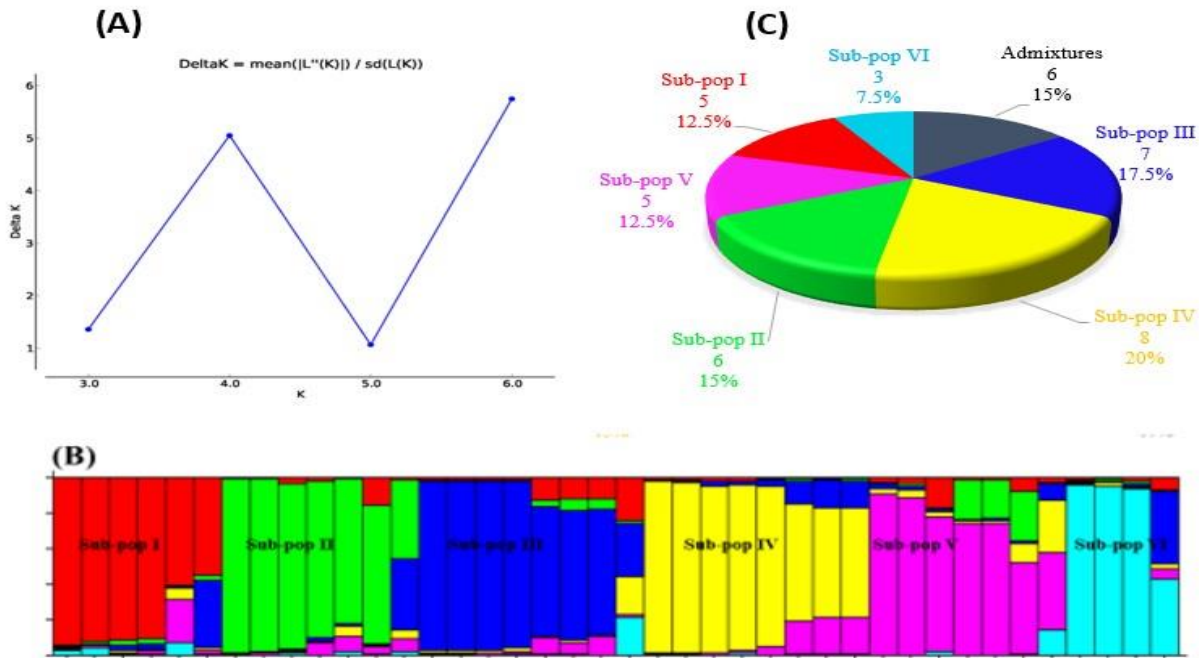
### 4.3.2 Diversity analysis

#### 4.3.2.1 Population Structure

Using the admixture model-based population structure analysis, the STRUCTURE software detected 6 genetic sub-populations clusters (K = 6) (Figure 4.2A-B), without a district clustering



pattern according to the location of the collection. Based on a membership threshold of 0.6, the sub-population (sub-pop) I consisted of five pure accessions (S42, S43, T03, T27, and T30), thus 12.5% of the total accessions used in this study (Figure 4.2 C; Supplementary Table 4.12). Sub-pop II comprised 15% accessions of the collection, largely from S and T (S35, S36, S38, T06 and T07, including N18). The sub-pop III had the greatest number of pure lines, thus 17.5% with K08, K09, M45, M47, N19, T05 and T29 accessions. The sub-pop IV had eight accessions (K10, K11, K12, K14, K15, K16, L18 and M44). The sub-pop V comprised four pure lines (N17, N21, N22, N23 and N24), while the sub-pop VI consisted of three pure lines (S40, T04, and T25) (Figure 4.2 C; Supplementary Table 1).



**Figure 4.2: Population structure analysis of the 40 *Capsicum* accessions.**

(A). Optimum delta K for the population structure obtained by the Evanno method was employed in STRUCTURE HARVESTER (Earl & vonHoldt, 2012). (B). Model based population structure



obtained from Structure software. The six colours (red, green, blue, yellow, purple, and sea blue) represent four inferred ancestral populations based on the membership coefficient ( $Q$ ) obtained from STRUCTURE software. The accessions with  $Q \geq 0.6$  were considered as pure lines, while those with  $Q < 0.6$  were regarded as admixture. (C). Pie chart of proportions of pure lines and admixtures.

#### 4.3.2.3 Analysis of Molecular Variance (AMOVA) and allelic patterns among subpopulations

The genetic differentiation between the sub-populations was very high ( $F_{ST} = 0.34-0.71$ ). The average mean expected heterozygosity and  $F_{ST}$  of all sub-populations were 0.06 and 0.57, respectively (Table 4.12), with sub-pop VI having the highest expected heterozygosity and lowest  $F_{ST}$  values (0.12 and 0.34, respectively). The mean alpha value for expected heterozygosity was 0.04.

**Table 4.12: Expected heterozygosity and  $F_{ST}$  among individuals in the same cluster**

Sub-populations	Expected heterozygosity	Fixation index
I	0.04	0.63
II	0.08	0.50
III	0.03	0.71
IV	0.05	0.63
V	0.06	0.61
VI	0.12	0.34
Mean	0.06	0.57
Alpha value	0.04	

Additionally, a pairwise sub-population matrix of the accessions was carried out to check the population distance among individuals in the sub-population. The results showed significant diversity among sub-pop I and subpopulation IV (Table 4.13). The average weighted net nucleotide distance value between the six population groups was 0.07. The highest net nucleotide distance

value was observed between sub-pop IV and sub-pop II (0.12) and the lowest between sub-pop VI and sub-pop I (0.05) (Table 4.13). Taken together, these results suggest high levels of similarity among even the pure lines identified in this study.

**Table 4.13: Allele frequency divergence among pepper populations (Net nucleotide distance), computed using point estimates of population**

	Sub-pop I	Sub-pop II	Sub-pop III	Sub-pop IV	Sub-pop V
Sub-pop II	0.07	-			
Sub-pop III	0.06	0.07	-		
Sub-pop IV	0.09	0.12	0.06	-	
Sub-pop V	0.07	0.06	0.05	0.05	-
Sub-pop VI	0.05	0.10	0.07	0.07	0.10

#### 4.3.2.4 Phylogenetic relationship among 40 accessions of hot pepper

The unrooted neighbour-joining tree was constructed using the 24 SSR markers. Based on the phylogenetic tree, three major clusters were identified from all the 40 *Capsicum* accessions (Figure 4.3). Accessions clustering in the same plane suggests a high correlation among the pepper accessions. Cluster I and Cluster II contained two individuals of the *Capsicum* accession. Cluster III was further divided into two sub clusters; subcluster C1 contained 10 *Capsicum* accessions with subcluster C2 containing the remaining 26 *Capsicum* accessions. Six of the *Capsicum* accessions (K10, K11, K12 K14, K15, and K16) samples from Kunbumgu and one (M44) clustered with the improved variety Legon 18 (L18), pinpointing that these accessions may be identical to L18. The groupings obtained from the evolutionary analysis are largely in conformity to the model-based population structure analysis (Figure 4.2B; Figure 4.3).



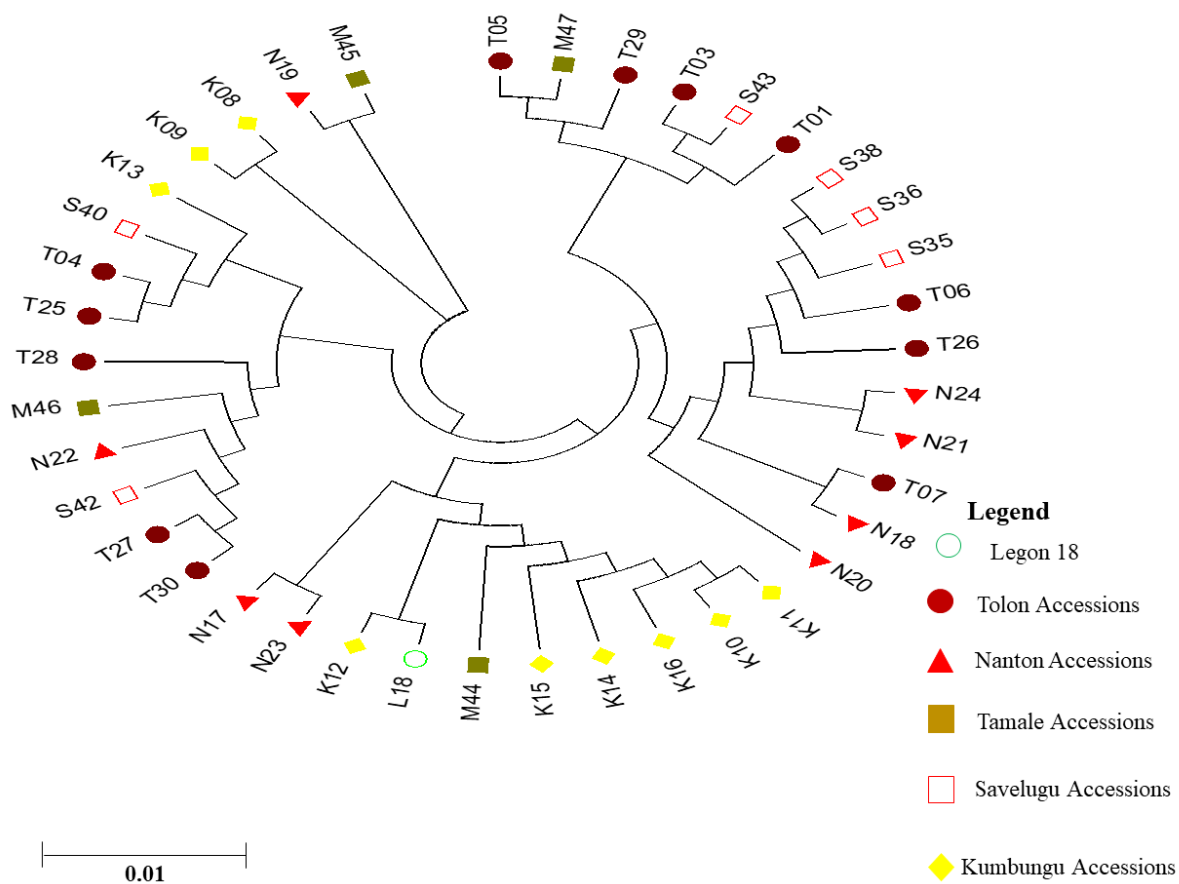


Figure 4.3: The neighbour-joining phylogenetic tree based on genetic distance matrix representing the grouping of 40 *C. annuum* accessions



## CHAPTER FIVE

### 5.0 DISCUSSION

#### 5.1 Survey

Northern Ghana is a major contributor to Ghana's chili pepper production volumes in Africa (Abate et al., 2019; DAI, 2014), making the region a good example for studying farming practices, knowledge, perception, and challenges faced by chili pepper farmers in Ghana. The results demonstrated very limited variations in farming practices and knowledge of improved farming methods in Ghana. Furthermore, farmers produce pepper as a side business despite the crop's potential to increase farmers' revenue in northern Ghana. Surprisingly, the mean area allocated for farming chilies (1.04 acres) was higher than the acres reported for Bangladesh, (Islam et al., 2020), Benin (Zohoungbogbo et al., 2024), Thailand, and Vietnam (Schreinemachers et al., 2015); however, it is lower (2.13 acres) than the allocated farm size for red pepper in Ethiopia (Hegena & Tigistu, 2022).

The use of farmer-saved seeds is widespread in developing countries, including Ghana (Gyasi et al., 2020). Similarly, in this study, only 25% of the sampled farmers used certified seeds. Previously in Ghana, (Gyasi et al., 2020) showed that 75% of pepper farmers in the Ashanti region used farmer-saved seeds, often extracted from rotten pepper fruits, in pepper production. This practice may have contributed to the observed high disease infestation and low yield reported by farmers in this study. In Benin, Zohoungbogbo *et al.* (2024) and Orobisi *et al.* (2017) reported that 67-80.7% of chili pepper farmers used farmer-saved seeds. In Bangladesh, 74% of pepper farmers used farmer saved seeds Islam *et al.* (2020). Schreinemachers et al. (2015), found that 47% and 80% of farmers in Thailand and Tamil Nadu, India respectively, use farmer-saved seeds as plant material for chili pepper production.





Although there is no consensus on how soil structure aids crop growth (Whitmore & Whalley, 2009), most of the farmers indicated that loosening up soil structure 3-5 times at the early stage of pepper growth enhances roots development and flowering initiation (Raper & Bergtold, 2007). Getahun et al. (2022) recently found that subsoil loosening resulted in significantly lower bulk density, increased porosity, plant height, and SPAD-index; however, the treatment resulted in negligible crop yield. Furthermore, about 82.7% of the farmers cultivated pepper on ridges. Zeb et al. (2018) found decreased root rot diseases with increasing ridge height in *Capsicum annuum*, possibly due to increased root zone temperature (Li et al., 2020). The high diseased burden reported by farmers in this study suggests that this may be particularly important for specific *Capsicum annuum* cultivars.

It is strongly established that Intercropping *Capsicum annuum* with other crops enhanced the stability and sustainable management of disease incidence in *Capsicum annuum* (Gao et al., 2021; Jaya et al., 2023; Ojeda et al., 2015; Oundo et al., 2023). Unfortunately, this improved practice is less practiced in the study area, thus about 96.4% of the farmers practice monocropping. Similarly, majority of Bangladesh (92%) and Nigeria (100%) pepper farmers practice monocropping (Ekenma et al., 2018; Islam et al., 2020). This practice was however moderate (monocropping = 45%) among Chili pepper farmers in Kenya (Waweru et al., 2020).

More recently, drying and processing methods used in processing pepper have attracted widespread attention due to their involvement in mycotoxin and their producers' levels in pepper. For instance, drying chilies on a bare floor is widely practised, including the finding of this study; and has been known as one of the leading causes of aflatoxin in red chilies (Sahar et al., 2022). It is worth mentioning that facilitation and training of farmers on dry methods could help reduce this

risk as 55.7% of the farmers are already using drying platforms to reduce aflatoxin levels. Similarly, chili peppers packed in jute bags were reported to be more susceptible to aflatoxin contamination than those packed in polyethylene bags (Iqbal *et al.*, 2011). Only one farmer used jute sacks for storing pepper.

## 5.2 Morphology

The use of quantitative morphological traits (particularly the fruit traits) has been useful in determining diverse *Capsicum* accessions for further breeding of traits of commercial and agronomic importance (Bharath *et al.*, 2014). However, morphological relationships of the *Capsicum annum* complex, which is the most widely commercialized species within the *Capsicum* genus (Tripodi & Kumar, 2019), are unresolved due to insufficient data on species sampling.

It is evident in this study that wide phenotypic variations exist within the *Capsicum* genotypes cultivated in Northern Ghana. This wide range of phenotypic variations is an indication of substantial variability in the studied pepper genotypes, and thus, can contribute to enhancing the gene pool for *Capsicum* breeding. Among functional morphological leaf traits, leaf size and width are linked to variations in plant growth and productivity (Westoby *et al.*, 2002). In *Capsicum* species, larger cotyledon length is associated with increased fruiting. The results showed a higher diversity among the pepper genotypes for cotyledon length (2.26-4.22 mm), and leaf width (0.96-1.96 mm) than those previously reported for *Capsicum annum* genotypes from the Northern region: cotyledon leaf length (1.24-2.36 mm) and width (0.45-0.87 mm) (Agyare *et al.*, 2016).

Additionally, plant height is one of the primary determinants of crop standability and has a direct measurable impact on crop lodging resistance (Boateng *et al.*, 2021). Thus, the crop improvement





programme is now moving toward short stature plants to reduced lodging and invariably fruit rot diseases. Consistent with this improvement programme, the pepper genotypes used in this study showed shorter plant height (27.31 - 43.44 cm) than those reported by Nkansah *et al.* (2011) and Agyare *et al.* (2016b). Therefore, the shorter plant height recorded in this study will make the *Capsicum* genotypes naturally resistant to lodging. The relatively high variations in fruit length, fruit width, total harvest (yield), total fresh weight and dry weight, can be especially useful for breeding purposes. Furthermore, fruit pedicle length (2.24-3.39 mm) exhibited by the genotypes used in this study were consistent with those (2.234-3.78 mm) reported earlier (Nkansah *et al.*, 2011).

Despite good performance of the genotypes at the vegetative and inflorescence stages under irrigation, better fruiting performance (i.e., number of fruits per plant, fresh fruit weight and dry weight) was recorded for pepper under rainfed conditions. This phenomenon was expected since *Capsicum annuum* L. is highly sensitive to temperature variation and high temperatures strongly influence flower drop and reduce fruit yield (Kim *et al.*, 2023; Polowick & Sawhney, 1985; Rosmaina *et al.*, 2022). It has been established that temperatures above 30°C reduce fruit development, including fruit set and fruit growth and enhances vegetative growth of *Capsicum annuum* L. (Oh & Koh, 2019; Rosmaina *et al.*, 2021). In the northern part of Ghana, day temperatures can rise above 35°C during the dry season.

PCA is a commonly used method of identifying morphological traits contributing to significant variations among pepper accessions. The contribution of the morphological traits loaded on the major PCs was relatively low (vector loading < 0.40) and similar, suggesting a low and linear relationship among the studied genotypes, most likely linked to limited number of genotypes (Pereira-Dias *et al.*, 2019). In previous studies in Ghana, morphological traits of pepper have been



shown to exhibit lower Eigenvectors, mostly below 0.40 (Adekaldu *et al.*, 2021; Agyare *et al.*, 2016b). This supports the findings of the current study and need for *Capsicum* germplasm improvement. The hierarchical cluster analysis supported this linear relationship and revealed no distinct separation based on location. This data offer an opportunity to identify duplicates in the *Capsicum* germplasm to facilitate proper rationalisation of the resources for conservation and evaluation (Bharath *et al.*, 2014).

### 5.3 Molecular

*Capsicum* is one of the most widely genus cultivated and consumed food spices in Ghana. With different morphologically distinct *Capsicum* species cultivated, Ghana is the fourth major producer of *Capsicum* in Africa. Although morphological characters such as fruit and flower colours are credible for evaluating variations in *Capsicum* species, these methods are subject to changing environmental conditions (dos Santos Pessoa *et al.*, 2018; Jha & Bhowmick, 2021). Hence, assessing genetic variations using molecular markers is more advantageous because the molecular marker is not regulated by environmental factors. Different molecular markers, including random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), simple sequence repeat (SSR), restriction fragment length polymorphism (RFLP), and amplified fragment length polymorphism (AFLP) are used for variability and diversity assessments of pepper at the molecular levels.

In Ghana, few studies have assessed the genetic diversity of *Capsicum* species primarily grown in Ghana using agro-morphological characteristics (Agyare *et al.*, 2016b). Although these morphological characters observed in various *Capsicum* landraces cultivated in northern Ghana present significant variations, they have not been developed through intentional breeding programmes. Modern breeding has resulted in the development of *Capsicum* varieties that are



more uniform with varieties of phytonutrients and adapted to different environments (Srivastava & Mangal, 2019). Therefore, the development of improved *Capsicum* varieties with higher yield and quality requires the availability of genetic information within the *Capsicum* species grown and consumed in Ghana. This study addressed this gap and provided baseline genetic information of 40 *Capsicum annuum* accessions cultivated in Northern region of Ghana utilizing 24 simple sequence repeat (SSR) markers.

Compared to the high gene diversity profile of the SSR markers reported by (Nagy et al., 2007) and (Lee et al., 2004), lower average gene diversity (0.13), heterozygosity (0.00), PIC (0.11) and a smaller number of average alleles (1.63) per locus in the 24 SSR markers used in this study were detected. These low gene diversity profile in SSR markers was also observed by Terefe et al. (2022) and Rabuma et al. (2020b) in Ethiopia.

Gene diversity values as low as the ones found here are not unusual for autogenous species such as pepper. Additionally, reports from several studies found lower heterozygosity values in cultivated *Capsicum* accessions than in wild types. A recent global study found reduced genetic diversity in more widely cultivated *Capsicum annuum* and *Capsicum chinense* (McCoy et al., 2023). This reduction in nucleotide diversity may be attributable to a limited number of parents (Pereira-Dias et al., 2019) or a low mutation rate, providing an empirical basis for improvement programs for pepper landraces in Africa. PIC values are standardized for evaluating the quality or informativeness of molecular markers: PIC > 0.5 are highly informative, PIC values between 0.25 and 0.5 are moderate and PIC < 0.25 gives low information (Dalimunthe et al., 2020). This suggests that all the SSR were considered moderately or low informative markers



The study population was divided into six groups including both major and minor groups (Figure 4.2), with no pattern of distribution relative to the sample location. (Agyare *et al.*, 2016) tested on 35 agro-morphological markers on 40 local pepper genotypes that were separated into six groups based on different agroecological zones in Ghana. This supports the findings of the current study. The admixture model-based structure analysis supported this interspecific grouping, suggesting that there was low genetic diversity among the pepper accessions. Thus, the markers used in this study did not perfectly separate the population structure of the *Capsicum* accessions. However, certain clusters were more frequently associated with a particular location. This information extracted from STRUCTURE analysis supports a strong correlation between geographic distance and genetic variation. Interestingly, 15% of the accessions, thus K13, M46, N20, T01, T26 and T28, were admixtures, suggesting that farmers handling of seed before and after planting might have contributed to this.

The average weighted  $F_{ST}$  value was high (0.57), indicating lower gene flow between the individuals in the same population (Frankham *et al.*, 2002; Holsinger & Weir, 2009). For a population of plants that belong to the same species, an  $F_{ST}$  value  $> 0.15$  is considered significant in differentiating two or more populations (Frankham *et al.*, 2002). Thus, a significant divergence was found within each of the *C. annuum* six subpopulations according to the  $F_{ST}$  values obtained from the STRUCTURE. However, the average weighted pairwise  $F_{ST}$  for the six determined populations was low (0.063), indicating a low genetic differentiation among these six subpopulations. Although one could argue that such a low pattern could be biased by the proximity of the study locations, it was found that this pattern is consistent with the area with the wild relative of domesticated peppers is grown (*C. annuum* var. *glabriusculum*).

## CHAPTER SIX

### 6.0 CONCLUSION AND RECOMMENDATION

#### 6.1 Conclusion

The first experiment (Survey) focused on pepper farmers' responses to pre- and post-harvest management practices using face-to-face interviews to determine variations in cultivation and processing practices used by farmers in the Northern region of Ghana. Their responses were evaluated alongside the reported impact on food and nutrition security, i.e., farmers' drying surfaces/platforms have been reported to facilitate aflatoxin levels in dried chili pepper. The findings in this study point to limited variations in pepper farming; the high use of farmer-saved seeds are associated with high disease burden. Almost all the farmers were storing their pepper in nylon sacks, which has been reported to decrease mycotoxin levels in their pepper. The results gained from this study provide a strong basis for a dramatic transformation of the management and practices used in the cultivation and processing of *Capsicum annuum* in the Northern region and Ghana as a whole. This will help improve some issues relative to food security and consumer health.

The second experiment has shown that pepper genotypes cultivated in the northern parts of Ghana have very narrow genetic base and low variability for genetic gains in a breeding program emphasizing the need for deliberate good conservation practices, and germplasm enhancement programmes aimed at removing duplicate genotypes. Genotypes exhibited significant variability among the 13 measured morphological traits, although they share similar genetic properties as expressed in the principal component and cluster analysis. Association analysis revealed that morphological traits like plant height, days to 50% flowering and days to 50% fruiting could be



targeted to improve total number of fruits per plant. Thus, this study will provide breeders with an understanding of superior morphological parents for use in development of improved cultivars.

Molecular markers offer advantages as they are not influenced by environmental factors. The population structure analysis divided the population into six groups, with no clear distribution pattern relative to the sample location. The high average weighted  $F_{ST}$  value (0.57) indicates limited gene flow among individuals in the same population. An  $F_{ST}$  value above 0.15 is significant for differentiating populations of the same species. Despite the high  $F_{ST}$  value within subpopulations, the low average weighted pairwise  $F_{ST}$  (0.073) suggests limited genetic differentiation between the six subpopulations. This pattern aligns with the area where wild relatives of domesticated peppers are found. It was found that the traditional agricultural methods in the study area have greatly contributed to preserving the genetic resources of *C. annuum* landraces.

## 6.2 Recommendations

Drying chilies on bare floors poses a significant risk of mycotoxin contamination. Farmers should be trained and supported to adopt improved drying platforms and hygienic postharvest handling practices. Additionally, awareness campaigns on safe storage materials, particularly the use of polyethylene bags over jute sacks, should be intensified to minimize aflatoxin contamination. Given the sensitivity of *Capsicum annuum* to high temperatures and the better fruiting performance observed under rainfed conditions, breeding efforts should prioritize genotypes that exhibit tolerance to heat stress and reduced flower drop under high temperature regimes typical of Northern Ghana. The low polymorphism information content (PIC) and gene diversity observed suggest the need for more informative markers. Future studies should incorporate a larger number of SSR markers or high-throughput markers such as SNPs to enhance resolution of genetic

diversity and population structure. For effective variety development, molecular data should be combined with agro-morphological and agronomic performance data. This integrated approach will facilitate the identification of superior genotypes for yield, quality, and resilience.



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## APPENDICES

### Research Pictures



















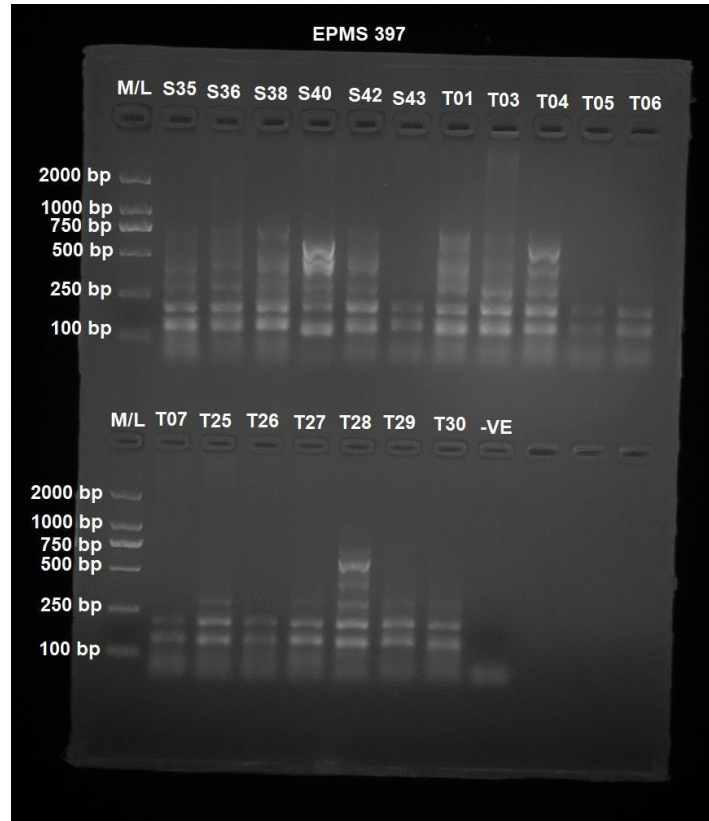


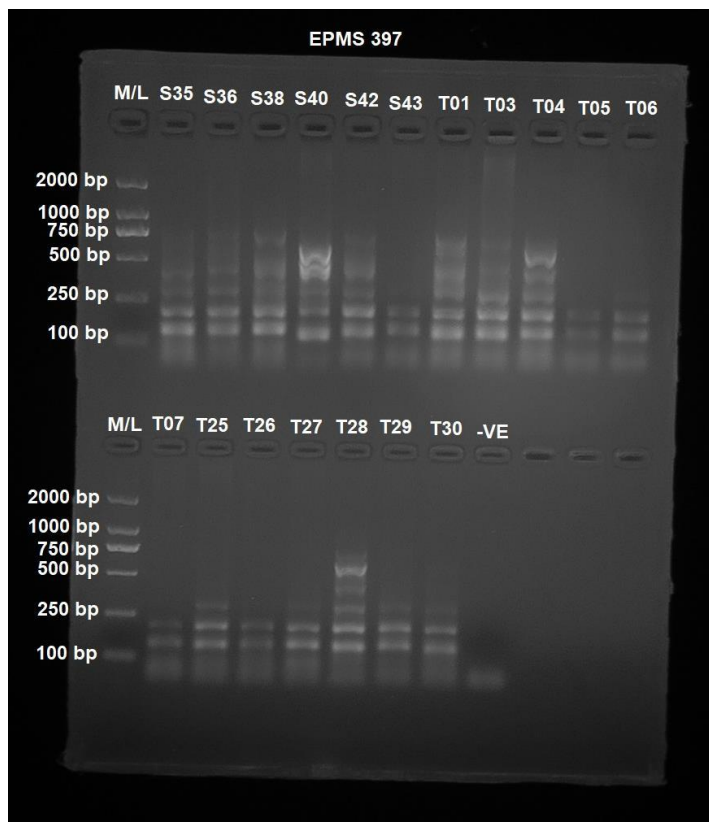






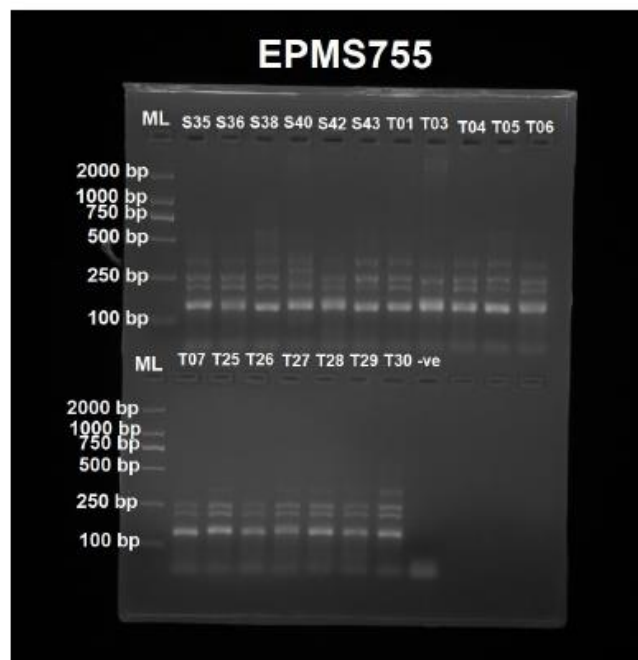
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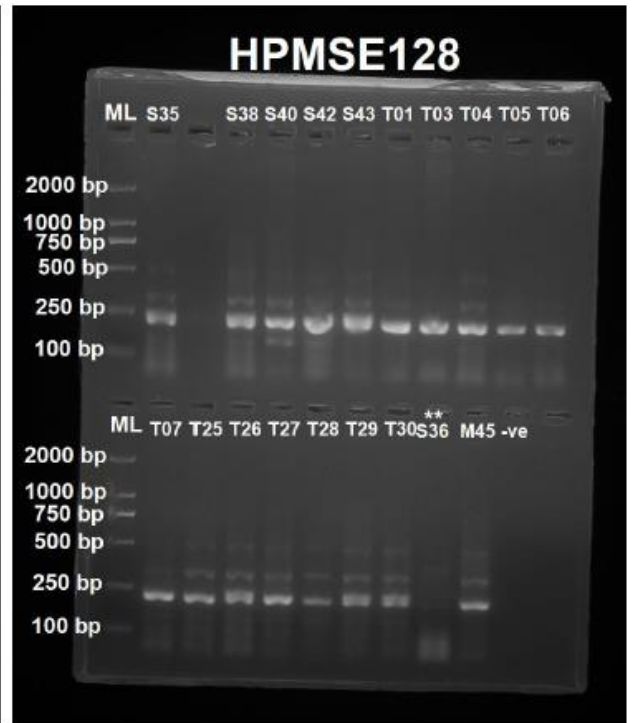
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**EPMS755**





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Research Paper

Exploring morphological variation and stability in hot pepper (*Capsicum annuum*) germplasm collection from the northern region of Ghana

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ABSTRACT

Understanding genetic variability in pepper (*Capsicum annuum* L.) will contribute to effective breeding efforts, especially in northern Ghana, where pepper contributes significantly to the smallholder farmer's income. This study evaluated 13 morphological traits including those related to vegetative, inflorescence, and fruit-related within a collection of 40 pepper genotypes from five different locations in the northern region of Ghana. Significant variations ( $p < 0.05$ ) were detected among the pepper genotypes for all measured traits. A strong positive correlation was detected between days to 50% flowering and days to 50% fruiting for both the rainfed ( $r = 0.87$ ) and irrigated ( $r = 0.73$ ) conditions. High performance of the genotypes at the vegetative and inflorescence stages was observed in the dry season, with low fruiting performance (number of fruits per plant, fresh weight, and dry weight). Multivariate analysis revealed that 50.54% and 54.63% variations were explained by the first three principal components, respectively. Three main clusters were identified, with linear relationships and no distinct separation pattern based on location, thus providing an opportunity to remove duplicate genotypes in the studied region. The results indicated that the selection of genotypes with better agronomic traits could be achieved from this pepper gene pool. The present findings may pave the way for better utilization and production of pepper, leading to improved adaptation in the region.

1. Introduction








Pepper (*Capsicum* spp) is one of the most important groups of vegetable and spice crops known in the Solanaceae family. The crop has played an integral role in human civilization, including human nutrition, and medicinal values with cultural significance (Del Burgo-Gutiérrez et al., 2022; Luna-Romero et al., 2022; Yasin et al., 2023). Within the *Capsicum* genus, there are morphologically diverse fruits and leaves in both domesticated and wild types that have been explored for their ornamental values (Stummel and Bosland, 2007; Gilman et al., 2014; Sarchenger et al., 2019; Zhang et al., 2020), resulting in increased demand for culinary and ornamental varieties of *Capsicum* species.

Morphological characterization of *Capsicum* species (pepper) germplasm is a prerequisite in a crop improvement program (Baba et al., 2016; Fasikaw et al., 2019; Nankar et al., 2020). Traditional phenotypic descriptors such as flower and fruit attributes, and growth habits have helped to identify intra and inter-specific variability in pepper germplasm for important biochemical and genetic traits (Ortiz et al., 2010). For instance, germplasm with yellow colour and pendant-oriented fruits could be important traits for high vitamin C levels (Moon et al., 2023). A recent study reported a positive relationship between vitamin C content and fruit diameter ( $r = 0.75$ ), fruit weight ( $r = 0.73$ ), and fruit wall thickness ( $r = 0.81$ ) (Moon et al., 2023).

Economically, pepper production is a viable agricultural business



## Genetic diversity and population structure analysis of *Capsicum annum* cultivated in the Northern region of Ghana using simple sequence repeat markers

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### ABSTRACT

Pepper (*Capsicum annum*) is an essential spicy crop in sub-Saharan African, however, limited genetic improvement initiatives have been undertaken to enhance its productivity. This study characterized the genetic diversity and population structure of 40 *C. annum* accessions using 24 simple sequence repeat (SSR) markers. Profiling *C. annum* accessions with 24 SSR markers revealed a total of 39 alleles, with an overall mean of 1.63 alleles per locus. The polymorphic information content and observed

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