CHAPTER ONE

1.0 INTRODUCTION

Pepper (*Capsicum* sp) is an economically important crop belonging to the family Solanaceae. It originated from South and Central America where it is still under cultivation (Pickersgill, 1997). The major centre of diversity is Brazil where representatives at all cited levels are found (Costa *et al.,* 2009). Peppers are considered the first spice to have been used by human beings and there is archaeological evidence of pepper and other fossil foods from as early as 6000 years ago (Hill *et al*., 2013). The genus *Capsicum* has five domesticated species (*C. annuum, C. frutescens, C. chinense, C. pubescens and C. baccatum*) of which *C. annuum* is the most widely cultivated species worldwide (Andrews, 1984). Pepper was introduced into Europe by Columbus and other early new explorers in the sixteenth century and cultivation spread throughout the world (Greenleaf, 1986). It is a small perennial shrub characterized by white or greenish-white corolla, one or more pedicels at a node with varying fruit sizes and shapes (Norman, 1992). The crop can also be distinguished by its pungency which varies with cultivar but generally higher in smaller fruit types than larger thick-fleshed types. Pepper grows relatively quick with a maturity period of 3-4 months. In Ghana, it is grown in home gardens and convenient sites near settlements often as intercrop but it is now grown as a monocrop on large scale by both peasant and commercial farmers. Norman (1992) has stated the derived savanna and northern savanna agro-ecologies are best suited for hot pepper production with an annual rainfall of 600-1250 mm. Major chilli pepper producing countries include China, Mexico, Turkey, which produce about 70% of the total worldwide production (MiDA, 2010). Ghana was ranked the 11th largest producer of pepper in the world and the 2nd largest producer in Africa with an estimated total production of 88,000 metric tons in 2011 which accounted for $96,397 (FAOSTAT, 2011).

Pepper is a vital commercial crop, cultivated for vegetable, spice, and value-added processed products (Kumar and Rai, 2005). It is an important constituent of many foods, adding flavour, colour, vitamins A and C and pungency and is, therefore, indispensable to Ghana and world food industries. It can be used medically for the treatment of fevers, colds, indigestion, constipation and pain killing (Dagnoko *et al*., 2013). It is also used by the security agencies in the preparation of tear gas. The crop is not only being cultivated for local consumption but it is also exported to Europe and has thus become a foreign exchange earner for Ghana (Norman, 1992). MiDA (2010) has reported that Ghana is the 5th largest exporter of chilli peppers to the European Union (EU) with an annual export increase of 17 per cent since the year 2000. Pepper exports to the European Union between 2005 and 2007 ranged from 26,000 to 41,000 metric tons. This was about 60% rise in the export of chilli pepper to the EU between 2005 and 2007. This increase in export of chilli pepper was as a result of the introduction of a new variety (Legon 18) and training of farmers in good cultural practices (MiDA, 2010).

Pepper production in Ghana is mainly under rain-fed conditions resulting in a drop in production and availability of fresh pepper during the dry season. The consequence of this shortage in the supply of pepper is an increase in the market price of both fresh and dried pepper. It is estimated that pepper growers in Ghana are producing about 50% of the attainable yields (MiDA, 2010). The low production may be attributed to low soil fertility, pests and diseases pressure, unavailability and high cost of irrigation systems, inadequate knowledge of improved technologies coupled with the use of unimproved varieties (MiDA, 2010). Most of the pepper varieties farmers cultivate are unimproved varieties that are low yielding.

Even though pepper is very popular in all the agro-ecological zones of Ghana, very little has been achieved in the improvement of the indigenous cultivars probably because of the limited information on the genetic diversity within the species. It has been observed that farmers select and give out seeds of elite genotypes to their colleagues which are later cultivated under different local names. These materials are named based on several criteria, such as the origin of the genotype, pungency, uses, size and shape of fruits. This phenomenon has resulted in the treatment of some genotypes as different cultivars in different localities. For this reason, estimation of the genetic diversity among cultivated genotypes has become the fundamental requirement of the crop industry, purposely, for identification and crop improvement (Tam *et al*., 2005).

Phenotypic characters such as fruit weight, flower colour, fruit shape, plant height etc., have been used to distinguish between pepper genotypes and classify them into groups (Fonseca *et al*., 2008; Weerakoon and Somaratne, 2010). The use of phenotypic characters in describing and classifying germplasm is the fundamental step in any characterization programme (Smith and Smith, 1989). However, studies have shown that morphological characterization in pepper, though a simple method of detecting differences in genotypes, is highly influenced by environmental factors and may not be able to distinguish between individuals that are closely related (Gilbert *et al*., 1999; Geleta *et al*., 2005). It has, therefore, become inevitable to back morphological characterization with molecular DNA marker analyses which have been proven to be very objective and independent of environmental factors (Se-Jong *et al*., 2012). These molecular markers are powerful tools in complementing phenotypic characterization in detecting additional sources of genetic diversity present within the gene pool.

DNA markers, such as isozymes, Restriction Fragment Length Polymorphisms (RFLP), Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphisms (AFLP) and Simple Sequence Repeats (SSR), have been used in studying genetic diversity in *Capsicum* species (Tam *et al.*, 2005). The knowledge of genetic variability estimated from isozymes, RAPD, AFLP, RFLP and SSRs markers provide plant breeders with different levels of information that would cater for germplasm management and crop improvement programmes (Tam *et al.*, 2005). These molecular markers are large in number and useful in determining genetic variability through the construction of linkage maps (Gupta *et al.*, 1996).

Of the molecular markers developed, SSR, markers stand out as exceptional in genetic diversity studies, because they are highly polymorphic and widely distributed in the pepper genome (Mimura *et al.*, 2012). They have been widely used for genetic diversity assessment of germplasm because of their ability to detect multi-allelic forms of variation and are reproducible. SSR markers, being co-dominant, are able to distinguish genetic relationships between genotypes based on specific traits and are more effective for inbred lines and breeding materials with special attributes (Tam *et al.*, 2005).

The extent of genetic variability within a species is vital for its continued existence and adaptation in different agro-ecologies. The more diverse the population is the better for the breeder in developing elite cultivars through careful selection of superior parents. Therefore, an understanding of the genetic variability of a population, through the use of both morphological and molecular markers, is of critical importance in developing effective strategies for germplasm conservation and breeding purposes (Se-Jong *et al.*, 2012).

The main objective of the work was to study the genetic diversity of some Ghanaian pepper genotypes in order to select desirable genotypes as parents for breeding in the Guinea Savanna zone of Ghana.

The specific objectives were to:

I. determine genetic variation among pepper genotypes using phenotypic characters;
II. detect differences between pepper genotypes based on simple sequence repeat (SSR) markers; and
III. classify the genotypes based on their phenotypic and molecular attributes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Classification and taxonomy of pepper

Capsicum (chilies and other peppers) belong to family Solanaceae (tribe Solaneae, subtribe Capsicinae), which also includes other economically important crops such as tomato, potato and tobacco (Dias *et al*., 2013). They consist of annual or perennial herbs or shrubs and are native to South and Central America and the Galapagos (Walsh and Hoot, 2001). They are predominantly diploid (2n=24, infrequently 2n=26), except for a few (Moscone *et al*., 2003). The genus *Capsicum* can be grouped into different categories based on the ability of members to successfully interbreed. These include *Annuum,* made up of the species *C. annuum* (varieties *glabriusculum* and *annuum*), *C. frutescens, C. chinense, C. chacoense* and *C.galapagoensis*; the *baccatum* group which consists of the species *C. baccatum* (varieties *baccatum*, *pendulum* and *praetermissum*) and finally *C. tovari*, and the *pubescens* group which is also made up of the species *C. cardenasii*, *C. eximium* and *C. pubescens* (Pickersgill, 1997). The genus has five major domesticated species of which *C. annuum* is the most widely cultivated species worldwide (Andrews, 1984). Pepper, though a self pollinated crop has been considered as a cross-pollinated crop as a result of its high rate of out crossing which ranges from 7 to 90% (Allard, 1960). Natural inter-specific crosses among *Capsicum* species are very high, resulting in intermediary forms which are complex to categorize (Allard, 1960). As a result, C *annuum,* C *chinense* and C *frutescens* have been considered as one species (C *annuun* L.) with four variety classes (Nsabiyera *et al*., 2013). These are the West Indies chilli (*chinense* group), bird chilli (*frutescens* group), hot chilli (*annuum* group) and sweet pepper group.

2.2 Morphology and growth of pepper

Capsicum is a highly heterogeneous plant which exhibits considerable morphological variation, especially in fruit shape, colour, and size (Walsh and Hoot, 2001). Pubescence of leaves and stems range from glabrous to very pubescent. Pepper produces bisexual flowers which are borne at the intersection between the stem and leaves at points where the stem splits into a fork. The inflorescences may vary from solitary to seven flowers at one node (Berke, 2000). The calyx may range from long, green sepals to truncate sepals to spine-like projections. The pedicel length varies among cultivars, ranging from 3 to 8 cm (Berke, 2000). In the species *C. annuum* the petals are usually white with five to seven individual stamens which vary in colour from pale blue to purple anthers. Shaw and Khan (1928) observed greenish-white corolla in *C. frutescens* and added that corolla colour is one of the most consistent features of distinguishing *Capsicum* species.

The pistil is made up of an ovary, which contains two to four carpels or locules, and a stigma borne at the tip of a slender style (Berke, 2000). The length of the style and relative position of the stigma and the anthers vary among genotypes, and it is an important factor determining the level of natural cross pollinations of the flowers. The flower colour, shape, length and relative positions of the styles also vary with different species and cultivars. The fruits are, botanically, classified as berries with different varieties of shapes, colours and sizes that vary among cultivars. Seeds are cream coloured, except for *C. pubescens* which has black seeds (Berke, 2000).

2.3 Importance of pepper

Pepper is a vital commercial crop, cultivated for vegetable, spice, and value-added processed products (Kumar and Rai, 2005). Besides vitamins A and C, the fruits contain mixtures of antioxidants notably carotenoids, ascorbic acid, flavanoids and polyphenols (Nadeem *et al.*, 2011). This makes it a very important constituent of many foods, adding flavour, colour and pungency and, hence, an important source of nutrition for humans. Peppers can be used whole, chopped or in various processed forms such as fresh, dried and ground into powder (with or without the seeds), or as an extract. In most advanced countries, the fresh fruits can be processed into paste and bottled for sale in supermarkets. In Ghana, a popular pepper sauce, *shito* is widely used by students, campers and even for export. Pepper can also be used medically for the treatment of fevers and colds (Norman, 1992). Bell pepper, being a very rich source of vitamins A, C, B6, folic acid and beta-carotene, provides excellent nutrition for humans (Nadeem *et al.*, 2011). Antioxidant compounds present in the different colours (green, yellow, orange, and red) in sweet bell peppers give them an antioxidative potential which helps protect the body from oxidative damage induced by free radicals when consumed (Simmone *et al.*, 1997). This reduces the risk of cardiovascular diseases, asthma, sore throat, headache and diabetes. Red pepper on the other hand contains lycopene which is believed to possess anti-cancer properties (Simmone *et al.*, 1997). It is also used by the security agencies in the preparation of tear gas for crowd control.

As a commercial crop, pepper was ranked as the second valuable vegetable crop ahead of popular vegetables like okra and egg plant with an estimated total production of 88,000 metric tons in 2011 which was valued at $96,397 (FAOSTAT, 2011). Agronomically, different pepper genotypes have been found to show differential responses to Egyptian broomrape, a chlorophyll-lacking root-parasite in Egypt. Hence, the crop is used as a catch/trap crop to reduce field infestation of the parasite (Hershenhorn *et al.*, 1996).

Notwithstanding the numerous advantages, the crop still remains a neglected crop that is of rare national priority in terms of agricultural development in many countries (FAO, 2010).

2.4 Pepper breeding

Pepper is traditionally a cross-pollinated crop with its bisexual flowers borne at the intersection between the stem and leaves at points where the stem splits into a fork (Greenleaf, 1986). The length of the style and relative position of the stigma and the anthers are important factors determining the level of natural cross-pollination of the flowers (Berke, 2000). Pepper breeding, depending on the objectives, involves selection for traits such as high yield, pungency, fruit colour, fruit size and shape as well as disease resistance (Liu *et al.*, 2009). These traits require simple traditional breeding methods with few cases of incompatibility. It involves intra-specific hybridisation between different cultivars to transfer simple phenotypic characters. However, limited genetic resources for breeding and increasing demand for better pepper varieties require new tools for pepper breeding. Wild relatives or distantly related species also serve as excellent sources of useful genes. In such cases, inter-specific hybridization has to be embarked upon (Hajjar and Hodgkin, 2007). Inter-specific hybridization has proven to be a useful tool for the transfer of genes for disease and pest resistance (Pickersgill, 1997), particularly, anthracnose resistant genes from *Capsicum baccatum* to cultivated pepper, C. *annuum* (Yoon *et al.*, 2006). Conventional inter-specific hybridization between two species can sometimes result in embryo abscission due to post-fertilization genetic barrier. The endosperm degenerates resulting in total or partial sterility of hybrid plants. These barriers have prevented the use of wild species which carry important genes that may be absent in the cultivated species (Monteiro *et al.*, 2011).

However in some partially cross-compatible species, embryo rescue techniques are used to save such crosses (Monteiro *et al*., 2011).

One major economically sound breeding approach is the production of hybrid seeds. Hybrid vegetable seeds which produce good quality high yielding plants are obtained through hand emasculation which is costly (Payakhapaab *et al*., 2012). In some cultivars of pepper, there is within species incompatibility which can be exploited to ensure cross-pollination (Greenleaf, 1986). Using such genotypes as females, the pollen grains from the males of choice can then be used to pollinate the desired females in the breeding programme. This within species incompatibility, often referred to as male sterility, has been employed to prevent self-fertilization in several species in the production of hybrid seeds (Berke, 2000). In a study involving several cases on natural sterile male pollens, Shifriss (1997) found and concluded that male sterility in pepper was controlled by both the genes in the cytoplasm and the nucleus as well as the interaction between them.

2.5 Germplasm collection

The first stage of every breeding programme is germplasm assembling, evaluation and selection (Dixon *et al*., 1992). Germplasm collection forms an important pool of genetic diversity of agriculturally important crops. The materials may be obtained either from local or foreign sources. The main objective of assembling germplasm is to acquire, preserve and make available as much genetic variation within a given gene pool to plant breeders and other users (Ramanatha *et al*., 1998). Broad-based germplasm resources are necessary for sound and successful crop improvement programmes. For effective breeding, the genetic diversity of the test materials needs to be maintained in order to minimize the weaknesses inherent in growing uniform and closely related cultivars on large scale (Chang, 1991). The degree of success in

improving any cultivar depends on the amount of diversity expressed by both improved and local cultivars as well as their wild relatives and weedy forms. These resources form invaluable sources of parental lines for developing improved cultivars (Agueguia, 1995).

2.6 Germplasm characterisation, conservation, evaluation and its significance in crop improvement

After assembling the germplasm, characterization of the materials is carried out to eliminate duplicates as well as closely related individuals to obtain a core collection of genetically distinct individuals (Yada *et al*., 2010). Removal of duplicates from closely related genotypes ensures that only genetically distinct genotypes are maintained helping to save space and funds in germplasm conservation and maintenance (Yada *et al*., 2010). Morphological differences between the accessions are catalogued based on IPGRI descriptors (IPGRI *et al*., 1995). Germplasm resources kept in gene banks can be more detailed and reliable if biochemical and molecular markers are associated with the morphological traits of agronomic importance. The use of biochemical characters, such as dry matter, β-carotene, capsaicin, sugar, ash, oleoresin and ascorbic acid content, provide useful information for detecting differences among pepper genotypes (Ilic *et al*., 2013). Employing molecular markers allows the detection of more expressive genetic differences among closely related genotypes in contrast to morphological agronomic descriptors (Rimoldi *et al*., 2010).

The objective of germplasm conservation is the maintenance of high viability among the assembled materials for a long time (Chang, 1985). It is also aimed at preserving genetic resources for use in cultivar development to boost agricultural productivity and meet the needs of the future (AVRDC, 1993). Proper germplasm characterization and conservation

either through *in-situ* (conservation in natural habitats) or *ex-situ* (gene banks and tissue culture) is very essential for the continued survival of these useful germplasm (Se-Jong *et al*., 2012). Vegetable species, such as pepper, tomato and egg plant, are maintained using seed preservation. This is the most effective method of conserving large numbers of accessions and making them available for distribution through seed multiplication. The genetic integrity of the accessions is maintained and their longevity is maximized in stores with least cost (Engle, 1994). The longevity of seeds in stores is affected by the condition under which the seeds were harvested and the storage conditions (Engle, 1994). According to Ellis *et al*. (1991) high seed viability, as a result of good processing, handling and storage practices, not only promote the establishment of healthy seedlings but also reduce the threat of genetic erosion by conserving the genetic diversity acquired through germplasm assembling.

Germplasm evaluation is vital in variability studies as it links conservation to crop improvement (Chang, 1985). It begins with characterization of the materials using standardized morpho-agronomic descriptors developed for specific crops. Evaluation of the genotypes for traits of interest (high yield, adaptation to varying environments, resistance to pests and diseases, and nutritional improvement) is carried out and selections are made based on their performance (Baht, 1970). The superior genotypes are selected as parents for hybridization purposes. Although genetic evaluation is costly and tedious, successful characterization will result in the maximization of the benefits obtained from germplasm assembling and conservation.

The structure of a population as described by its genetic characteristics is as a result of the interaction of genetic drift, gene flow and natural selection. In endangered species, estimation of genetic variability can help in developing appropriate strategies for maintaining and

conserving the genetic integrity of these species. Ishwaran and Erdelen (2005) have indicated that genetic diversity assessment, though expensive and time consuming, is very essential for human advancement. Maintaining biodiversity is important as these wild species are indicators of functional ecosystems and also provide products and services essential to human welfare (Ishwaran and Erdelen 2005). Studies of genetic diversity have led to several scientific breakthroughs (Wilcove and Master, 2008).

Characterization of conserved germplasm helps to identify and detect better genotypes and as well remove duplicates in breeding programmes, thereby improving the knowledge about these genotypes (Dias *et al*., 2013). In-depth understanding of the extent and magnitude of genetic variation within and between a breeding population is required to develop mechanisms for detecting purity and authenticity of parents and hybrids in commercial plant breeding programmes (Se-Jong *et al*., 2012). Information generated from germplasm characterization provides data on the potential usefulness of the accessions and the right identity during regeneration (Engle, 1994).

2.7 Genetic erosion

The existence of diversity in the ecosystem is of great importance to humans however, the actions of humans pose a threat to maintenance of biodiversity. Efforts in germplasm collection and conservation need to be stepped up especially in developing countries because of the danger posed by genetic erosion (Sastrapradja and Kartawinata, 1975).

Orobiyi *et al*. (2013) in studying varietal diversity in Benin reported that the mean rate of genetic erosion in pepper was 23.53% per community. They attributed this loss in genetic resources to abscission of plant parts (leaves, flowers and fruits), low yield, smaller fruit sizes, susceptibility to insect pests and diseases, lack of seed, introduction of improved varieties and poor post-harvest handling. Activities of humans such as deforestation, bush

burning, industrialization and land development, and robust marketing of improved cultivars are some of the reasons resulting in the speedy extinction of indigenous cultivars which may possess useful genetic characters that may not be present in the improved cultivars. Engle and Chang (1991) have indicated that loss of seed viability through improper handling before and during storage is one major contributing factor to genetic erosion. Genetic erosion is permanent so measures should be kept in place to prevent such germplasm from being lost.

2.8 Gains from genetic improvement in pepper

Genetic diversity within a population is important in its development as it serves as the raw material upon which diverse genetic combinations are generated to stand the test of climate change, new diseases and pests' resurgence (AVRDC, 1993). Domestication and diversity studies in pepper have resulted in careful selection of useful traits that have ensured the continued survival of the crop throughout the world. Pepper breeding has focused on addressing consumer needs, such as degree of hotness, colour, taste, fruit shape and thickness of wall and ability to dry (powdered pepper) (Bosland and Votava, 2000). Paran and van der Knaap (2007) further indicate that diversity studies have led to the selection of cultivars with larger fruits that are less pungent. Significant advances have also been made in the development of commercial cultivars resistant and or tolerant to tobacco mosaic virus (TMV), cucumber mosaic virus (CMV) and *Verticillium albo-atrum* (Mijatovic *et al.*, 2005).

2.9 Morphological characterisation

Knowledge of the phenotype given by morphological descriptors is important in giving correct species identification (Dias *et al.*, 2013). Morphological markers are readily available and very easy to identify and in most cases do not require special skills. They offer simple

and straight-forward approaches to distinguishing different genotypes even at the farm level compared to molecular markers which in most cases require sophisticated laboratories. Morphological characterization is the only means by which plants can be differentiated based on their physical appearance. It is very essential in bringing to light traits of agronomic importance especially quantitative traits for crop improvement (Geleta *et al.*, 2005). Even though morphological characterization is important in variety identification, its application is influenced by prevailing environmental factors (Gepts, 1993; Geleta *et al.*, 2005) and, as such, make its use limited. It also falls short in its ability to detect differences between closely related individuals.

Lack of polymorphism, environmental interference, dependence on the state of crop growth and masking of recessive characters, limit the effectiveness of phenotypic characters though they can be effective in some cases (Costa *et al.*, 2009). The use of DNA-based molecular markers provides a high throughput method for assessing genetic heterogeneity among genotypes (Moreira *et al.*, 2013).

2.10 DNA-based molecular techniques

Molecular characterization which is based on the ability to recognize specific DNA sequences in organisms is very important in distinguishing between even closely related species with accurate results (Rocha *et al.*, 2010). Kwon *et al.* (2002) report that molecular markers distinguish differences in nucleotide sequences which are independent of growth stage, time, place and agronomic practices. Molecular techniques are useful in identifying quantitative trait loci which are of agricultural importance (Rocha *et al.*, 2010). Phenotypic markers varying from flower to fruit characters are not many and their effects are usually masked by other markers (Geleta *et al.*, 2005; Rodriguez *et al.*, 1999). Genotypic characterization based

solely on morphological descriptors can also be frequently subject to errors that may arise from variations in environmental conditions, especially when dealing with genotypes of similar origin or in situations where some agronomic characteristics are not specific (Rimoldi *et al*., 2010). On the other hand, molecular markers are large in number, independent of environmental factors and are the best in evaluating genetic variability (Minamiyama *et al*., 2006; Oyama *et al*., 2006; Portis *et al*., 2004; Park *et al*., 2009).

DNA markers have been used significantly in crop improvement programmes (Legesse *et al*., 2007). Tam *et al*. (2005) argue that knowledge of genetic variability assessed from different DNA marker technologies should offer plant breeders different degrees of information to address different needs of crop improvement programmes and germplasm resources conservation.

These molecular markers include Isozymes, Restriction fragment length polymorphism (RFLP) (Kang *et al*., 2001), Random amplified polymorphic DNA (RAPDs) (Baral and Bosland, 2002; da Costa *et al*., 2006), Amplified fragment length polymorphism (AFLP) (Aktas *et al*., 2009), Simple sequence repeats (SSR) (Se-Jong *et al*., 2012) and Single nucleotide polymorphisms (SNPs) (Choi *et al*., 2007). DNA markers based on polymerase chain reaction (PCR) technology are efficient in genetic differentiation and varietal authenticity in crop plants and is simple and easy to use (Powell *et al*., 1996; Lal *et al*., 2010). These molecular markers differ in their purpose, time requirements, ease of application, cost and ability to detect variability.

RFLP markers have been developed and used in studying diversity in pepper but their use has been restricted, because it is cumbersome and involves the use of radioactive probes (Nahm *et al*., 1997; Kim *et al*., 2004). Similarly, RAPD and AFLP markers have been found to be dominant in nature (detecting only dominant alleles), show differences in band intensity and limited degrees of variability in some domesticated species (Weising *et al*., 2005).

Due to the many merits of SSR markers over the other PCR-based markers in genetic diversity studies, it was used in this study. SSR markers are locus-specific and co-dominant in nature and offer better resolution than the other PCR-based markers (Soni *et al*., 2010). Other advantages include the huge extent of allelic diversity (polymorphic information contents) making it possible to reveal variation among closely related individuals, ease of amplification, high reproducibility and abundance and even distribution throughout the genome (Powell *et al*., 1996; Weising *et al*., 2005). The only serious challenge with the use of SSR markers is the sequence information required for primer design, but this has now been managed with computer software's for designing primers based on conserved flanking regions (Weising *et al*., 2005)

SSR markers are important in genetic evaluation of a segregating population, genome mapping, parentage analysis and population genetic studies (Scott *et al*., 2000; Slavov *et al*., 2005; Soni *et al*., 2010). They are also useful in association analysis, gene function characterization and quantitative trait loci (QTL) analysis (Ronning *et al*., 2003; Crossa *et al*., 2007; Zeng *et al*., 2009).

As a result of the numerous advantages and usefulness of SSR markers, the technique is constantly being used by several researchers in population and genetic diversity studies in many agriculturally important crops. Asare *et al*. (2011) also studied genetic diversity in cassava using SSR markers and reported that SSR primers were more effective in classifying cassava genotypes than morphological descriptors. SSR markers were also employed by Doku *et al*. (2013) in assessing the genetic divergence of rice cultivars. They concluded that SSR markers were able to identify the cultivars as unique individuals with no duplication. Nawaz *et al*. (2009) also reported the effective discriminating power of SSR markers as they studied genetic diversity in wheat using SSR markers. SSR markers are more effective in detecting useful genetic differences

among closely related species with satisfactory results than morphological markers. Kwon *et al*. (2005) and Se-Jong *et al*. (2012) used SSR markers to evaluate genetic diversity in pepper and indicated that the amount of genetic variation within the genotypes is essential for their continued survival.

This experiment sought to study the genetic diversity of some Ghanaian pepper genotypes (*Capsicum* spp.) in order to select desirable genotypes as parents for breeding in the Guinea Savanna zone of Ghana.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of experiment

The experiment consisted of a field work and laboratory work. The field work was conducted at the research field of the Savanna Agricultural Research Institute (SARI), Nyankpala in the Guinea Savanna zone of Ghana. The zone has an average annual rainfall of about 800-1200 mm. The field had a gentle slope belonging to the Kumayili series and commonly classified as Ferric Luvisols. The soil was a well drained sandy-loam.

3.1.1 Field experiment

The field experiment consisted of morphological characterization of local pepper genotypes using standard descriptors for *Capsicum* sp developed by IPGRI (1995) with slight modifications by Asian Vegetable Research and Development Center-Genetic Resources and Seed Unit (AVRDC-GRSU).

3.2 Pepper genotypes used for the experiment

Forty-eight (48) local pepper genotypes supplied by GIZ and two released varieties, Legon 18 and Shito Adope were used for the characterization work (Table 1).

Table 1. Background of pepper genotypes used for the experiment

Genotype	Source	Region	Obtained from	Type
VR HOE 11	Market, Hohoe	Volta	Mrs. Nkansa	Long Cayenne
VR KPV 1	Market, Kpeve	Volta	Mrs. M Dei	Long Cayenne
VR KTS 2	kpogen	Volta	Pastor Justin	Long Cayenne
BA TAN 3	Hianmunchend, Tain	Brong Ahafo	Mr. Owusu	Long Cayenne
VR HOE 1	Ve-Koloenu, Hohoe	Volta	Mr. Anyamesem	Long Cayenne
VR KTS 13	Nogokpo, Ketu South	Volta	Mr. Kejuga	Long Cayenne
BA TAN 12	Kyekuewere, Tain	Brong Ahafo	Diana Nketiah	Long Cayenne
ER UMK 2	Market, Asesewa	Eastern	Mad A. Koryo	Long Cayenne
BA JMS 3	Babiania, Jaman South	Brong Ahafo	Atta Panyin	Long Cayenne
UE KNW 7	Paganiani Garden	Upper East	Mr. Dramani	Long Cayenne
UE TND 1	Talensi-Nabdam	Upper East	Mr. Bukari	Bird Eye
UE BOM 2	Bolgatanga	Upper East	Mr. Issifu	Bird Eye
NR TAM 4	Tamale	Northern	Mr. Mohammed	Bird Eye
UE BAW 2	Bawku	Upper East	Kofi	Bird Eye
UE BAW 7 a	Bawku	Upper East	Hajia Tene	Bird Eye
UE BAW 7 b	Bawku	Upper East	Mr. Abass	Bird Eye
NR WMP 4	Walewale	Northern	Mr. Musah	Bird Eye
GA ACC 1	Madina market	Greater Accra	Ajobenstu	Scotch bonnet
VR KTS 8	Kpogen	Volta	Pastor Justin	Scotch bonnet
UE KNW 3	Paganiani Garden	Upper East	Kojo Akombo	Scotch bonnet
VR KTS 9	Kpogen	Volta	Pastor Justin	Scotch bonnet
ER UMK 3	Asesewa	Eastern	Owusu	Scotch bonnet
BA SYW 8	Ayakomaso, Sunyani	Brong Ahafo	Mr. Osman	Scotch bonnet
VR HOE 8f	Market, Gbi Atabu, Hohoe	Volta	Mrs. J Kpeme	Scotch bonnet
UE KNE 4	Goo dam, Paga	Upper East	Mr. J Chaporo	Scotch bonnet
NR TKB 9	Cheschegu, Tolon	Northern	Mr. A Abdulai	Scotch bonnet
VR KPD 2	Kpando market	Volta	Mr. Zigah	Scotch bonnet
NR TKB 3	Nwogu	Northern	Mr. Iddrisu	Short cayenne
BA TAN 11A	Nsawkaw, Tain	Brong Ahafo	Mr. O Nfoigie	Short cayenne
BA TAN 11B	Nawkaw, Tain	Brong Ahafo	Mr. O Nfoigie	Short cayenne

Table 1. Cont'd

Genotype	Source	Region	Obtained from	Type
NR WMP 6	Diany, West Mamprusi	Northern	Mr. A Sulemana	Short cayenne
UE BNG 1	Bongo	Upper East	Atia Asaa	Short cayenne
UE KNE 8	Precast zone J	Upper East	Robert	Short cayenne
UE KNW 13	Market, Navrongo	Upper East	Pinda	Bullet type
NR TKB 6	Nwogu, Tolon	Northern	Mr. I Alhassan	Bullet type
GA DWW 7	Shai hills, Naglaosis	Greater Accra	Mr. Laryea	Bullet type
GA ACC 5	Madina market	Greater Accra	Naa Ayele	S bonnet big
UE KNW 8	Banyono, Navrongo	Upper East	Mr. Akowpiay	S bonnet big
UE KNW 9	Banyono, Navrongo	Upper East	Mr. Akowpiay	S bonnet big
UE KNE 2	Nangali Kenia	Upper East	James Chaporo	S bonnet big
VR HOE 10 B	Hohoe market	Volta	Mrs. Dewortor	S bonnet big
BA SYW 6	Ayakomaso	Brong Ahafo	Mr. Osman	S bonnet big
NR TAM 6	Tampelekukuo	Northern	Monica	S bonnet big
VR HOE 4	Ur Avegpo	Volta	Mr. Nkansah	Cherry type
NR SVN 4	Libga	Northern	Seblim	Cherry type
BA JMS 4	Babiania	Brong Ahafo	Atta Panyin	Cherry type
UE KNE 6	Bonia	Upper East	Mr. Kagri	Cherry type
ER UMK 1	Asesewa market	Eastern	Mad Akosua	Cherry type
Legon 18	Savanna Agric Res. Inst	Northern	Vegetable section	Long cayene
Shito Adope	Savanna Agric Res. Inst	Northern	Vegetable section	Long cayene

3.3 Nursery and nursery management practices

The seeds were nursed in seed boxes on 17th May, 2012 using potting mix sterilized by steam sterilization method. The potting mix consisted of two parts of top soil to one part of well decomposed cow dung. Nursery management practices, such as shading, forking, thinning, watering and hardening off, were carried out appropriately to ensure that healthy seedlings were produced.

3.4 Land preparation

The field was ploughed, harrowed and ridged at a spacing of 75 cm between ridges. After this the trial was laid out and transplanted. Transplanting was done in the cooler period of the day (evening) after which watering was done to reduce transplanting shock.

3.5 Experimental design

The experimental design used was the augmented design with single rows of each genotype. The length of each row was 5 m long with inter and intra row spacing of 75 cm and 50 cm respectively. Legon 18 and Shito Adope were repeated after every eight (8) genotypes as required by the design. The genotypes were randomly assigned to plots by means of drawing lots to avoid bias.

3.6 Transplanting and fertilization

The genotypes were transplanted six (6) weeks after nursing on 28th June, 2012. Watering was done immediately after transplanting and, at two weeks after transplanting, NPK 15-15-15 was applied at 100 kg/ac as a split dose. The fertilizer application method was side placement which was done at about 5 cm away from the plants. Top dressing was done using sulphate of ammonia at 50 kg/ac.

3.7 Other agronomic practices

Weeds were manually controlled using hand hoe at the second and sixth weeks after transplanting was done. Reshaping of ridges was carried out at the fourth and eighth weeks

after transplanting with hand hoe. Insect pests and diseases were managed by spraying *PAWA* (20 g/l Lamda cyhalothrin) and *Topsin M* (70 WP), respectively.

3.8 Morphological data collection

3.8.1 Qualitative parameters

Data was collected at seedling, vegetative, inflorescence and fruiting stages using standard AVRDC-GRSU characterization descriptors for *Capsicum* species. Data collection was done by scoring for each parameter as described in Table 2.

Table 2. AVRDC-GRSU descriptors for some of the qualitative traits

Trait	Description	Score
Hypocotyl pubescence	Glabrous	0
	Sparse	3
	Intermediate	5
	Abundant	7
	Mixture	X
Cotyledon leaf shape	Deltoid	3
	Ovate	5
	Lanceolate	7
	Elong-deltoid	9
	Mixture	X
Stem colour	Green	1
	Green with few purple strips	2
	Green with many purple strips	3
	Purple	4
	Other	5
	Mixture	X
Plant growth habit	Prostrate	3
	Compact	5
	Erect	7
	Mixture	X

Table 2. Cont'd

Trait	Description	Score
Leaf colour	Yellow	1
	Light green	2
	Green	3
	Dark green	4
	Other	5
	Mixture	X
Pedicel position at anthesis	Pendant	3
	Intermediate	5
	Erect	7
	Mixture	X
Anther colour	Yellow	1
	Pale blue	2
	Blue	3
	Purple	4
	Other	5
	Mixture	X
Fruit shape	Elongate	1
	Oblate	2
	Round	3
	Conical	4
	Campanulate	5
	Bell or blocky	6
	Other	7
	Mixture	X
Fruit cross-sectional corrugation	Smooth	0
	Slightly corrugated	3
	Intermediate	5
	Corrugated	7
	Mixture	X

3.8.2 Quantitative parameters

3.8.2.1 Cotyledon leaf length

The lengths of ten cotyledon leaves were measured with a calliper and the average length used to denote each pepper genotype. Measurement was taken in centimetres (cm) and data recorded accordingly.

3.8.2.2 Cotyledon leaf width

The widths of ten cotyledon leaves were measured with a calliper and the average width used to denote each pepper genotype. Measurement was taken in centimetres (cm) and data recorded accordingly.

3.8.2.3 Plant height

The height of each pepper genotype was measured at 50% flowering. A metre rule was used to measure the height of the plants from the surface of the soil to the tip of the apical meristem. The height of ten plants was taken and the average used to represent that genotype. The measurements were taken in centimetres (cm) and the data was recorded accordingly.

3.8.2.4 Stem diameter

The stem diameter of each genotype was measured at 50% flowering. This was done by fitting a calliper to the stem. An average from ten plants was used to represent each pepper genotype. The measurement was taken in centimetres (cm).

3.8.2.5 Days to 50% flowering

Days to 50% flowering was estimated by counting from the day transplanting was done to the day half or 50% of the population of each genotype flowered.

3.8.2.6 Days to 50% fruiting

Days to 50% fruiting was determined by counting from the day of 50% flowering to the day 50% of each genotype fruited.

3.8.2.7 Fruit pedicel length

The lengths of ten fruit pedicels were measured with a calliper and the average used to denote that genotype. The lengths were measured in centimetres (cm).

3.8.2.8 Fruit pedicel width

Fruit pedicel width of each genotype was measured with a calliper. An average width from ten fruit pedicels was used to represent that genotype. The measurement was taken in centimetres (cm).

3.8.2.9 Fruit length

Fruit length was taken with a vernier calliper. The lengths of ten fruits per genotype were measured from the pedicel attachment to its apex and the average was used to represent fruit length of that genotype. Fruit length was measured in centimetres (cm).

3.8.2.10 Fruit width

The widths of the fruits were taken with a vernier calliper at the maximum width. Ten fruits were measured and the average was used to represent the fruit width of that genotype. The measurement was taken in centimetres (cm).

3.8.2.11 Fruit weight

The fruits were weighed with an electronic weighing scale. The weight of ten fruits of each genotype was measured in grams (g).

3.9 Laboratory experiment

The laboratory experiment consisted of molecular characterization of the genotypes using simple sequence repeat (SSR) primers to detect polymorphism between the genotypes under study. This experiment was also carried out at the Biotechnology Laboratory of the Savanna Agricultural Research Institute (SARI), Nyankpala.

3.9.1 Genomic DNA extraction

Genomic DNA was extracted from the 50 pepper genotypes using CTAB method described by Doyle and Doyle (1990) with slight modifications. Fully opened middle aged leaves were taken from the pepper genotypes into an ice chest and sent to the laboratory for DNA extraction.

Twenty (20) milligrams of fully photosynthesizing leave samples were ground in 2.0 ml Eppendorf tubes into fine powder with liquid nitrogen. 800 µl of 2% CTAB and 0.5 µl of 0.1% mercaptoethanol were added. The samples were incubated in a sand bath at 65°C for 30 minutes with intermittent vortexing. The samples were then cooled at room temperature and an equal volume (800 µl) of chloroform: isoamyl-alcohol (24:1) was added. The tube was inverted several times to ensure that a thorough mixture was obtained. The samples were centrifuged at 14000 rpm for 15 minutes. The aqueous phase was transferred into clean 1.5 ml Eppendorf tubes. Equal volume of chloroform: isoamyl-alcohol solution was added and centrifuged at 14000 rpm for another 15 minutes. Nucleic acids were precipitated by adding two thirds volume of ice cold isopropanol (400 µl) and shaking gently. Precipitation was enhanced by storing the samples at -20°C overnight in a freezer. Nucleic acids were pelleted by centrifuging at 14000 rpm for 5 minutes. The isopropanol was decanted and the pellet was

washed with 500 µl of washing buffer. The washing buffer was decanted and the pellet was washed in 400 µl of ethanol (80%) and then centrifuged at 6000 rpm for 4 minutes. The ethanol was decanted and the pellet was dried until the smell of ethanol was no longer detected. The DNA was suspended in 100 µl of TE buffer and centrifuged at high speed for 30 seconds and stored in the fridge at 4°C. DNA of each accession was confirmed by electrophoresis on 2% agarose gel.

3.9.2 SSR (microsatellite) markers and PCR amplification

Simple sequence repeats primers (SSR) used to detect polymorphism between the pepper genotypes are presented in Table 3. These 10 SSR primers are highly polymorphic and widely distributed in the pepper genome (Mimura *et al.*, 2012). They were procured from Metabion International AG (Germany). PCR reactions were carried out in a Techne Thermalcycler (TC-412) in a 10 µl reaction mixture in 96-well plates. PCR kits (KAPA 2G Fast ReadyMix with dye) procured from KAPA Biosystems (Pty) Ltd (South Africa) was used for the amplification. The kits composed of 2X PCR master mix containing KAPA2G Fast DNA Polymerase (0.2 U per 10 µl reaction), KAPA2 Fast PCR buffer, dNTPs (0.2 mM each at 1X), $MgCl_2$ (1.5 mM at 1X), stabilizers and loading dye. 1 µl genomic DNA and 0.5 µl each of forward and reverse primers were added to the PCR kits for DNA amplification.

PCR was subjected to initial denaturation at 95°C for 3 min, followed by cycles of 95°C for 10 sec, 52°C for 10 sec and 72°C for 10 sec. The reaction was repeated for 35 cycles and a final extension at 72°C for 10 minutes was carried out. The reactions were then held at 4°C until electrophoresis.

Table 3. SSR primers and their sequences

Primer name	Sequence (5'-3')	Motif	Chromoso-me	Expected product size (bp)	
				CW	LS
CAMS032	F: TGCCACATAGGTTGGCTTTC R: CAAAGCCAATGCACATAATCA	(gt)13	7	233	245
CAMS066	F: AAAAACATGCACCAGTCCTT R: CAACCGCCTGAATTTTCTCT	(ac)11	7	157	153
CAMS212	F: TTCCCTTTCCCAACATGGTA R: ACACCCGAAGATGGGTTAGA	(tg)10	9	154	150
CAMS228	F: GAGGGCTAAGCAAAGCAGAA R: TGCATGTTTCCCTTAGTTTCC	(ta)5(tg)13	4	241	239
CAMS396	F: GTCGGCCGTCATTCACTATT R: AGCTTGATGCACCTGGTCTT	(ag)12	6	240	244
CAMS406	F: TAAAAATCGCGGAAAGTTGC R: GTCGTTCTATGCGGCATTTT	(ga)8	4	184	182
CAMS476	F: TTTTCCCTTTCCAGTTGTTCA R: ATGGGTGAAGTGTGAAAAGAA	(tc)5	11	156	164
CAMS493	F: TCGATGACGAAAAAGTGTGAA R: AGGGCAAAAGACCCATTCTT	(ag)6	8	225	223
CAMS823	F: TCCTCCTCCTTCTCGTGTTC R: AAAGAAGCAGCAGGTGAAGA	(ctt)5	5	225	228
CAMS871	F: ACAAAGCATCGGCTGAAAAT R: GCGACCAAGTACCAACAGGT	(gaa)14	10	-	150

(Mimura *et al.*, 2012)

3.9.3 Gel electrophoresis

PCR products obtained were electrophoresed in Horizontal Polyacrylamide (6%) and ethidium bromide gel. The tracking dye in the PCR premix (KAPA 2G) made visual tracking

of the PCR products through the gel easier. Approximately 10 µl of the amplified products and a 50 bp and 100 bp molecular ladder (Ladder Plus) obtained from NBS Biologicals Ltd, Cambridge, UK were electrophoresed at 120 V for 150 minutes using Galileo Bioscience (81-2325) horizontal tank. The molecular ladder was loaded into lane one and the DNA of the pepper genotypes were loaded in the adjacent lanes. The gels were stained in 100 ml 1X TE buffer with ethidium bromide (3 µl) for 30 minutes and visualized by illumination on Benchtop UV transilluminator. The gels were photographed under UV light.

3.10 Data analysis

The data collected was analysed using Genstat statistical package (9th edition). Frequency distribution was used to classify the genotypes into groups based on the qualitative traits. For the quantitative traits mean, standard deviation, standard error, range and coefficient of variation were calculated using Microsoft Excel. Principal component analysis (PCA) was employed to examine the percentage contribution of each parameter to total genetic variability. Cluster analysis based on euclidean similarity matrix was used to generate a dendrogram for the morphological characters. For molecular characterization, DNA bands were scored as either present (1) or absent (0) for each of the genotypes by visual inspection. Bands with clear and good characteristics were considered and recorded. Loci were considered polymorphic if more than one allele was detected. Cluster analysis of the molecular data was carried out using NTSYS statistical package (version 4) to generate dendrograms based on genetic similarity matrix using unweighted pair group method with arithmetic mean (UPGMA) method. PowerMarker version 3.25 (Liu and Muse, 2005) was used to generate allele frequency, allele number, gene diversity, heterozygosity and polymorphism information content (PIC).

CHAPTER FOUR

4.0 RESULTS

4.1 Genetic diversity in phenotypic traits

4.1.1 Phenotypic evaluation of qualitative traits

The accessions exhibited a wide variation for several morphological characters studied. The frequency percentage of each parameter is presented in Table 4. Most of the accessions had abundant pubescence on the hypocotyl. Cotyledon leaf colour ranged from light green to green with ovate to elong-deltoid shape. Majority of the accessions had sparse stem pubescence with green stem colour. Two modes of plant growth were observed. Leaf shape varied between deltoid and lanceolate with sparse pubescence characterizing majority of them. Most of the accessions had intermediate plant size with abundant branches and dark green leaves. Yellow-green corolla (Figure 1) was common among the accessions with filament colour mostly white. Anther colour varied from pale blue to purple (Figure 2).

Fruit colour at mature stage ranged from red to orange. The accessions could be grouped into six different morphological classes based on fruit shape (Figure 3). Fruit shape at pedicel attachment ranged from acute to cordate with or without neck at base of fruit. Fruit shape at blossom end varied from pointed to sunken. Fruit cross-section ranged from smooth to corrugate with two or three locules per fruit (Figure 4).

Table 4. Frequency percentage of qualitative traits in pepper accessions

No.	Descriptor	Descriptor state (frequency percentage)			
1	Hypocotyl pubescence	Sparse (6%)	Intermediate (16%)	Abundant (78%)	
2	Cotyledon leaf shape	Ovate (6%)	Lanceolate (82%)	Elong-deltoid (10%)	Mixture (2%)
3	Cotyledon leaf colour	Light green (18%)	Green (82%)		
4	Stem colour	Green (72%)	Green with few purple strips (8%)	Green with many purple strips (20%)	
5	Plant growth habit	Compact (62%)	Erect (38%)		
6	Stem pubescence	Glabrous (4%)	Sparse (66%)	Intermediate (26%)	Abundant (4%)
7	Leaf pubescence	Glabrous (16%)	Sparse (58%)	Intermediate (16%)	Mixture (10%)
8	Leaf shape	Deltoid (20%)	Ovate (54%)	Lanceolate (22%)	Mixture (4%)
9	Leaf colour	Light green (8%)	Green (36%)	Dark green (50%)	Mixture (6%)

Table 4 Cont'd. Frequency percentage of qualitative traits in pepper accessions

No	Descriptor	Descriptor state (frequency percentage)				
10	Branching habit	Sparse (2%)	Intermediate (28%)	Abundant (62%)	Mixture (8%)	
11	Plant size	Small (14%)	Intermediate (70%)	Large (16%)		
12	Filament colour	White (76%)	Light purple (12%)	Purple (2%)	Mixture (10%)	
13	Calyx margin shape	Intermediate (74%)	Dentate (26%)			
14	Calyx annular constriction	Absent (2%)	Not clear (16%)	Clear (22%)	Distinct and uniform in whole plant (58%)	Mixture (2%)
15	Fruit position	Declining (44%)	Erect (14%)	Mixture (42%)		
16	Fruit colour at mature stage	Green (4%)	Yellow (2%)	Orange (2%)	Red (86%)	Mixture (6%)
17	Fruit shape at pedicel attachment	Acute (6%)	Obtuse (44%)	Truncate (44%)	Cordate (6%)	
18	Neck at base of fruit	Absent (74%)	Present (26%)			
19	Fruit shape at blossom end	Pointed (60%)	Blunt (22%)	Sunken (16%)	Mixture (2%)	
20	Fruit cross-sectional corrugation	Smooth (6%)	Slightly corrugated (40%)	Intermediate (24%)	Corrugated (30%)	

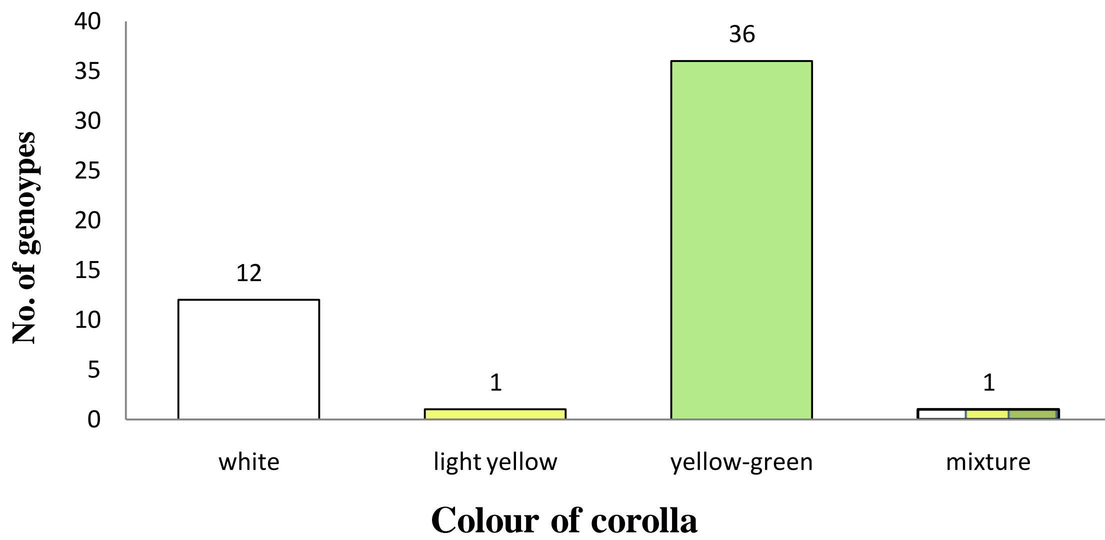

Fig 1. Frequency of corolla colour among pepper genotypes

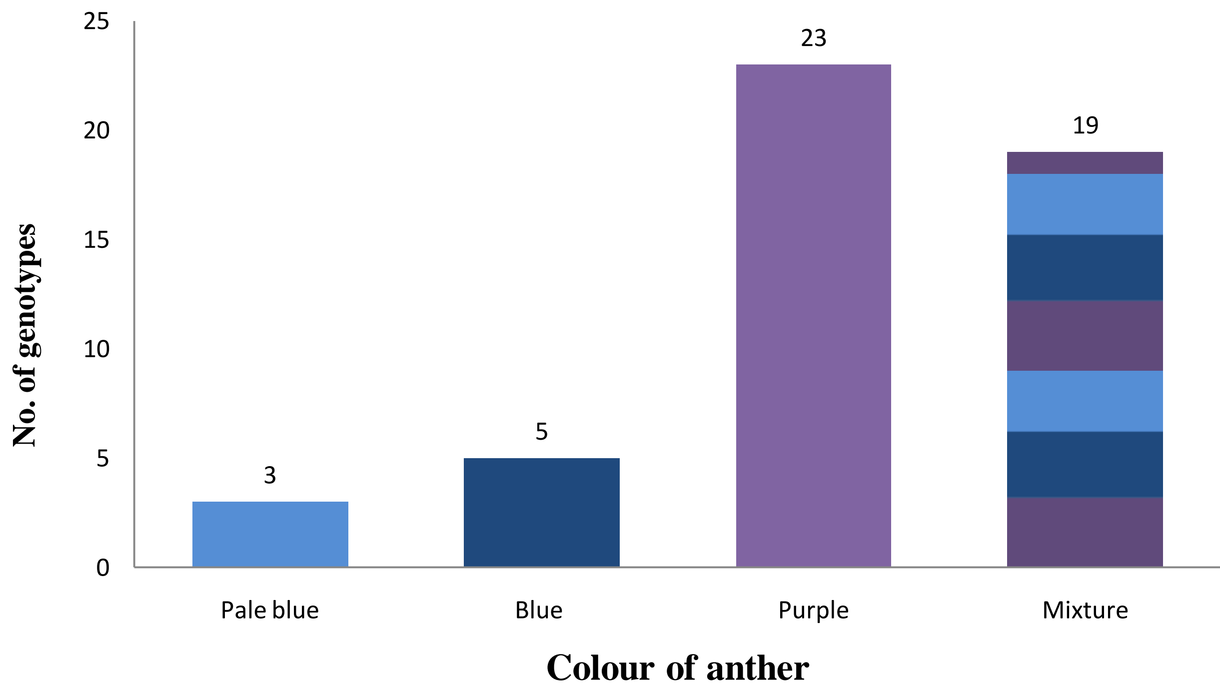

Fig 2. Frequency of anther colour among pepper genotypes

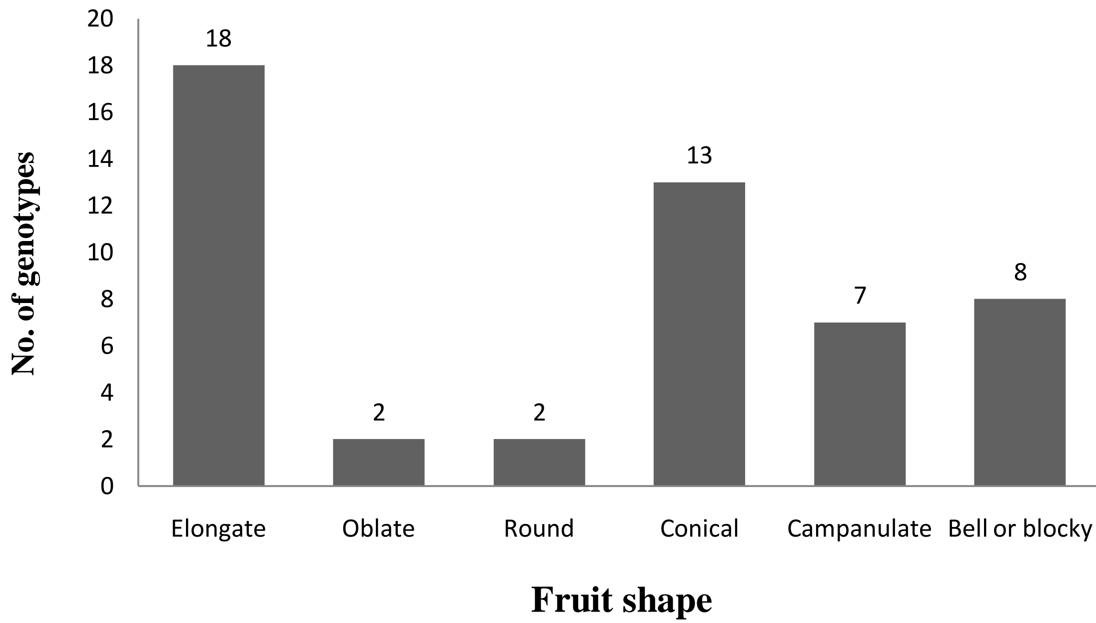

Fig 3. Frequency of fruit shape among pepper genotypes

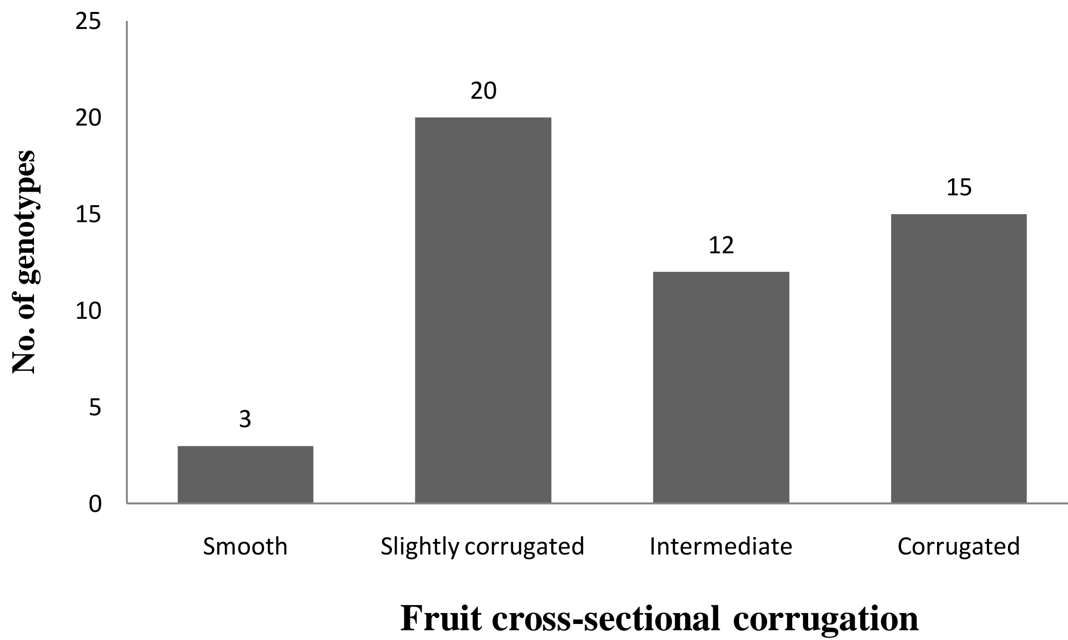

Fig 4. Frequency of fruit cross-sectional corrugation among pepper genotypes

4.1.2. Phenotypic analysis of quantitative traits

Table 5 shows the descriptive statistics for all the quantitative traits measured. Cotyledon leaf length ranged from 1.24 cm in BA JMS 4 to 2.36 cm in VR HOE 11 with a mean of 1.76 cm. Cotyledon leaf had widths ranging from 0.45-0.87 cm. The heights of the pepper genotypes ranged from 31.00-91.70 cm with an average height of 49.61 cm. The diameters of the stems were from 0.60-1.73 cm. The average genotype used about 41 days for 50% of its population to flower. The days to 50% flowering ranged from 22-58 days after transplanting was done. Days to 50% fruiting ranged from 26-29 days after days to 50% flowering was achieved. Fruit pedicel length and width ranged from 1.49-3.21 cm and 0.09-0.39 cm, respectively. The mean fruit length was 4.44 cm with BA TAN 3 and VR KTS 2 having the shortest and longest fruit lengths of 0.70 cm and 10.46 cm, respectively. Fruit width also ranged from 0.63-3.90 cm with an average of 1.58 cm. Fruit weight which is the most economic trait ranged from 4.50-131.50 g with genotypes UE TND 1 and GA ACC 5 having the smallest and greatest fruit weight, respectively. These results are also presented in appendices B1 and B2.

Table 5. Descriptive statistics for the quantitative parameters

Parameter	Mean ± SE	Range	SD	CV (%)
Cotyledon leaf length (cm)	1.76± 0.03	1.24-2.36	0.24	13.37
Cotyledon leaf width (cm)	0.69± 0.01	0.45-0.87	0.09	13.30
Plant height (cm)	49.61± 1.66	31.00-91.70	11.71	23.60
Stem diameter (cm)	1.07± 0.03	0.60-1.73	0.22	20.52
Days to 50% flowering	40.78± 1.34	22.00-58.00	9.46	23.19
Days to 50% fruiting	28.46± 0.17	26.00-29.00	1.16	4.09
Fruit pedicel length (cm)	2.30±0.06	1.49-3.21	0.41	17.70
Fruit pedicel width (cm)	0.17± 0.01	0.09-0.39	0.06	32.11
Fruit length (cm)	4.44±0.34	0.70-10.46	2.39	53.78
Fruit width (cm)	1.58±0.10	0.63-3.90	0.68	42.60
Fruit weight (g)	35.55±3.72	4.50-131.50	26.33	74.08

4.2 Principal component analysis of quantitative traits

The first four principal components contributed 23.32%, 18.79%, 17.5% and 12.83%, respectively, with a cumulative variation of 72.44% (Table 6). The relative discriminating power of the principal axes as indicated by the eigen values was high for axis 1 (2.57) and low for axis 4 (1.41). From PC1, fruit length and days to 50% fruiting showed 49% and 46% variation, respectively. Fruit length and days to 50% fruiting were the most important traits explaining the diversity within the pepper genotypes. PC2 revealed plant height (58%) and fruit pedicel length (48%) as the key traits which significantly contributed to total genetic variance. Fruit width (63%) and fruit weight (46%) were considered as the most important

parameters that contributed to the total genetic variance as revealed by PC3. PC4 showed that cotyledon leaf width (71%) and cotyledon leaf length (48%) were the traits which made substantial contribution to total variation among the genotypes.

Table 6. Principal component analysis of the contribution of quantitative traits to total variation among pepper genotypes using the first 4 principal components

Variable	PC 1	PC 2	PC 3	PC 4
Cotyledon leaf length	0.26	0.04	-0.23	0.48
Cotyledon leaf width	-0.09	0.02	0.05	0.71
Days to 50% flowering	-0.11	0.35	-0.13	-0.35
Days tof 50% fruiting	-0.46	0.13	0.29	0.13
Fruit weight	0.36	0.29	0.46	-0.09
Plant height	-0.14	0.58	-0.21	0.09
Stem diameter	-0.33	0.37	0.02	0.23
Fruit length	0.49	0.14	-0.11	-0.01
Fruit pedicel length	0.19	0.48	-0.31	-0.08
Fruit pedicel width	0.40	0.06	0.29	0.24
Fruit width	-0.01	0.22	0.63	-0.08
Eigen value	**2.57**	**2.07**	**1.93**	**1.41**
% Variance	**23.32**	**18.79**	**17.50**	**12.83**
Cumulative % variance	**23.32**	**42.11**	**59.61**	**72.44**

4.3 Genetic relationship among pepper accessions using morphological traits

Figure 5 shows a dendrogram generated using both qualitative and quantitative traits of the 50 pepper accessions. Genetic similarity ranged from 0.88-0.99. From the dendrogram, the accessions were grouped into 2 main clusters at 88.4% genetic similarity. Cluster A had only one genotype, BA TAN 3 while cluster B had 49 genotypes. Cluster B was further regrouped into 6 sub-clusters (I, II, III, IV, V and VI). Sub-cluster I consisted of genotype UE KNW 7. Sub-cluster II was made up of genotypes VR HOE 10B, BA TAN 11A and BA TAN 11B. Genotype GA DWW 7 alone was found in sub-cluster III. Sub-cluster IV was made up of genotype VR KPV 1. Sub-cluster V had only one genotype, BA JMS 4. Sub-cluster VI consisted of 42 genotypes which were further classified into 2 sub-sub-clusters (VI (A) and VI (B)).

Sub-sub-cluster VI (A) had 10 genotypes of which most of them belong to the long cayenne group. Sub-sub-cluster VI (B) was made up of 32 genotypes belonging to all the groups. Two genotypes UE BAW 7A and UE BAW 2 were 99.4% similar. From the figure it was clear that the most diverse genotypes were VR HOE 1 and BA TAN 3

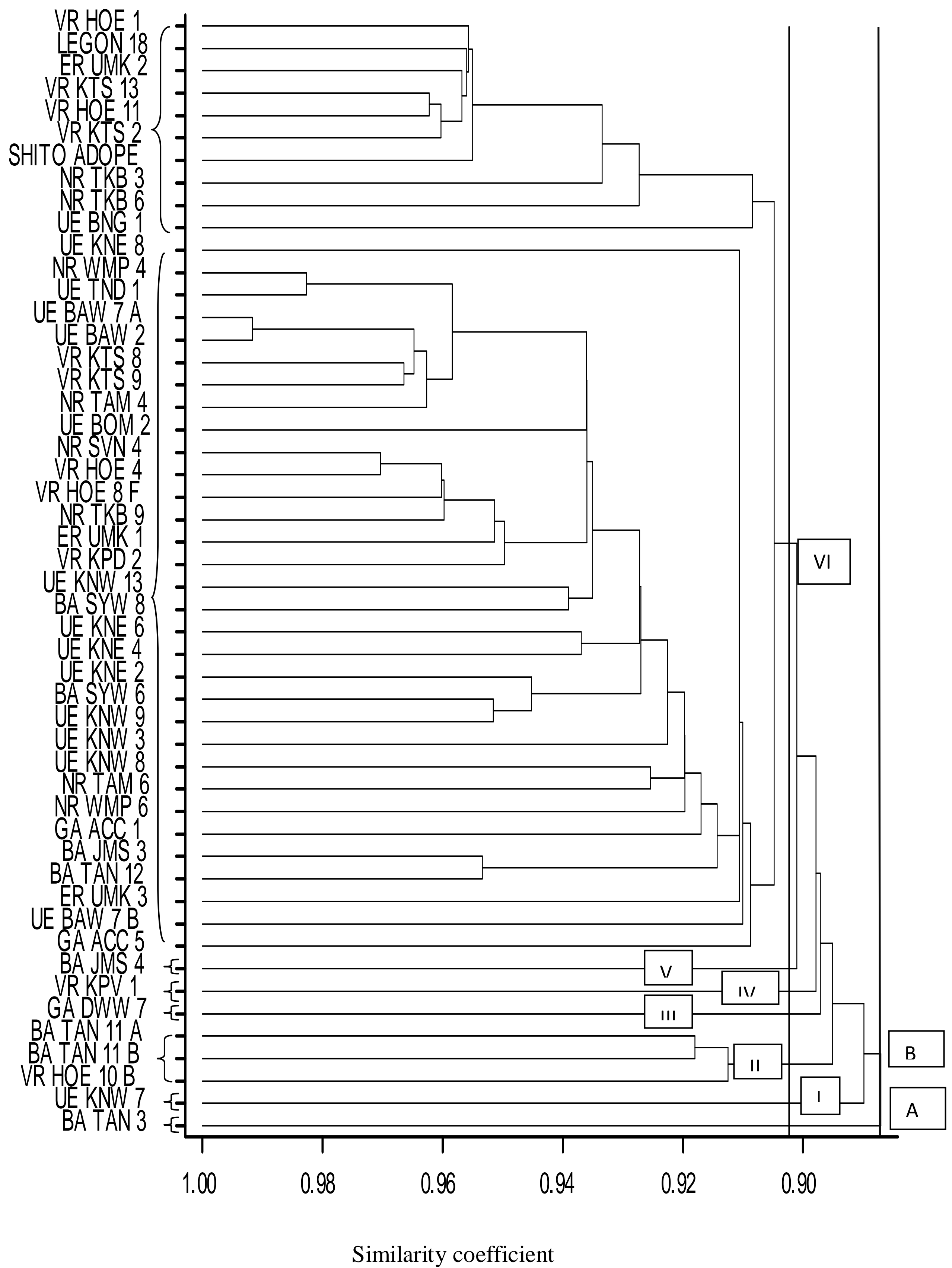

Fig 5 Genetic relationships among 50 pepper accessions based on morphological traits using coefficient of euclidean, single-linked similarity matrix

4.4 Genetic diversity assessment using microsatellite (SSR) markers

Although all 10 SSR primers which were used in the experiment produced clear bands for determination of variability among the pepper accessions, two of them were monomorphic while the other eight showed polymorphic bands. Figures 6 and 7 show the banding patterns of PCR products for CAMS 493 and CAMS 228. It is evident from the figure that the locus was polymorphic in nature.

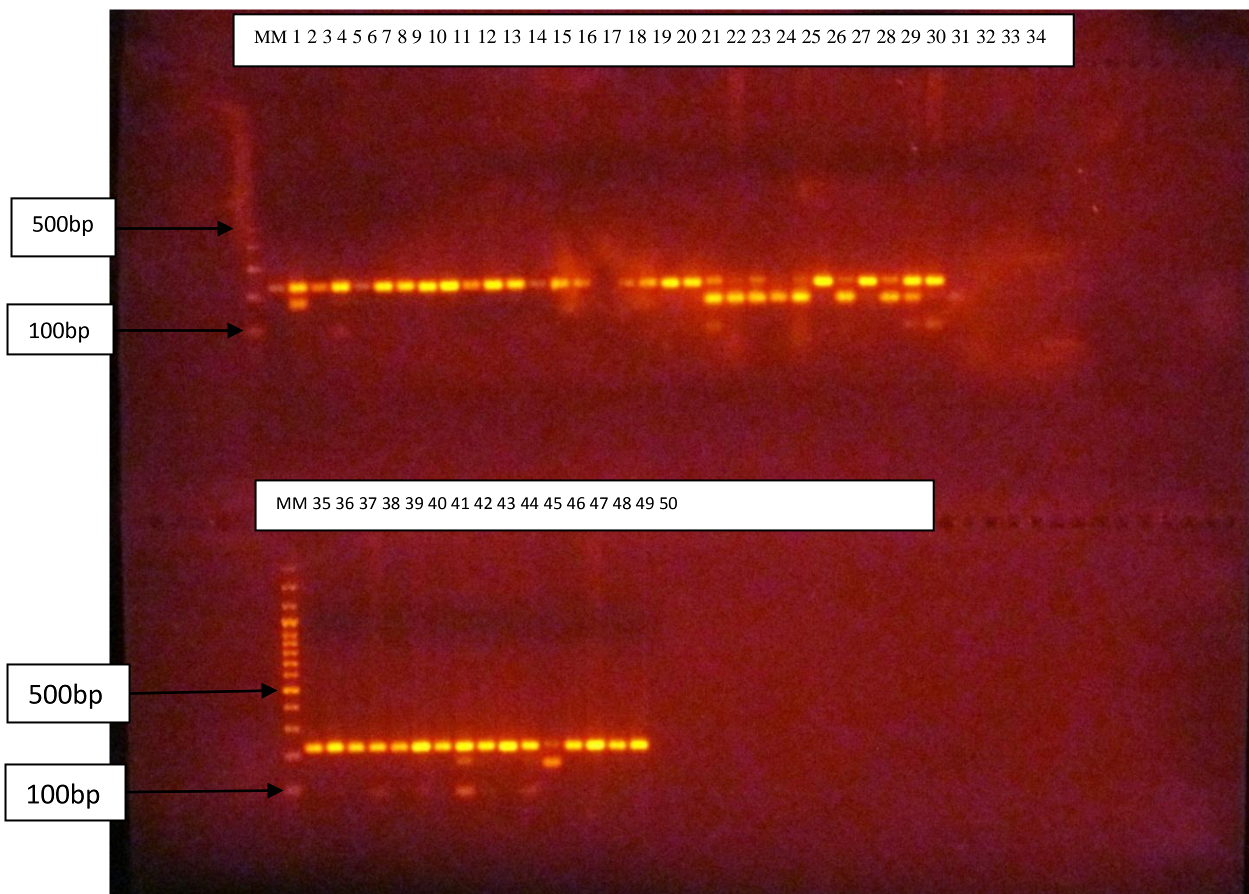

Fig 6: Banding pattern of PCR-amplified products of CAMS 493 among the 50 pepper accessions.

Lanes MM 100bp ladder, 1: Legon 18, 2: UE KNW 7, 3: NR TKB 9, 4:UE BAW 2, 5:NR WMP 4, 6: UE BAW 7A, 7:UE KNW 8, 8:GA ACC 5, 9: VR HOE 4, 10: BA TAN 3, 11: VR KTS 13, 12: UE BAW 7B, 13: NR WMP 6, 14: UE TND 1, 15: BA JMS 3, 16: ER UMK 2, 17: VR HOE 1, 18: VR HOE 11, 19 BA TAN 11A, 20: BA TAN 12, 21: BA JMS 4, 22: ER UMK 3, 23: VR KTS 9, 24: UE KNE 2, 25: VR HOE 10B, 26: VR KPV 1, 27: UE KNW 3, 28: VR KTS 2, 29: VR KTS 8, 30:UE BOM 2, 31: BA TAN 11B, 32: GA ACC 1, 33: UE KNW 9, 34: NR SVN 4, 35: NR TAM 4, 36: ADOPE, 37: UE KNE 4, 38: BA SYW 8, 39: BR TAM 6, 40: VR HOE 8F, 41: VR KPD 2, 42: BA SYW 6, 43: GA DWW 7, 44: UE KNW 13, 45: NR TKB 6, 46: NR TKB 3, 47: UMK 1, 48: UE KNE 6, 49: UE KNE 8 and 50: UE BNG 1

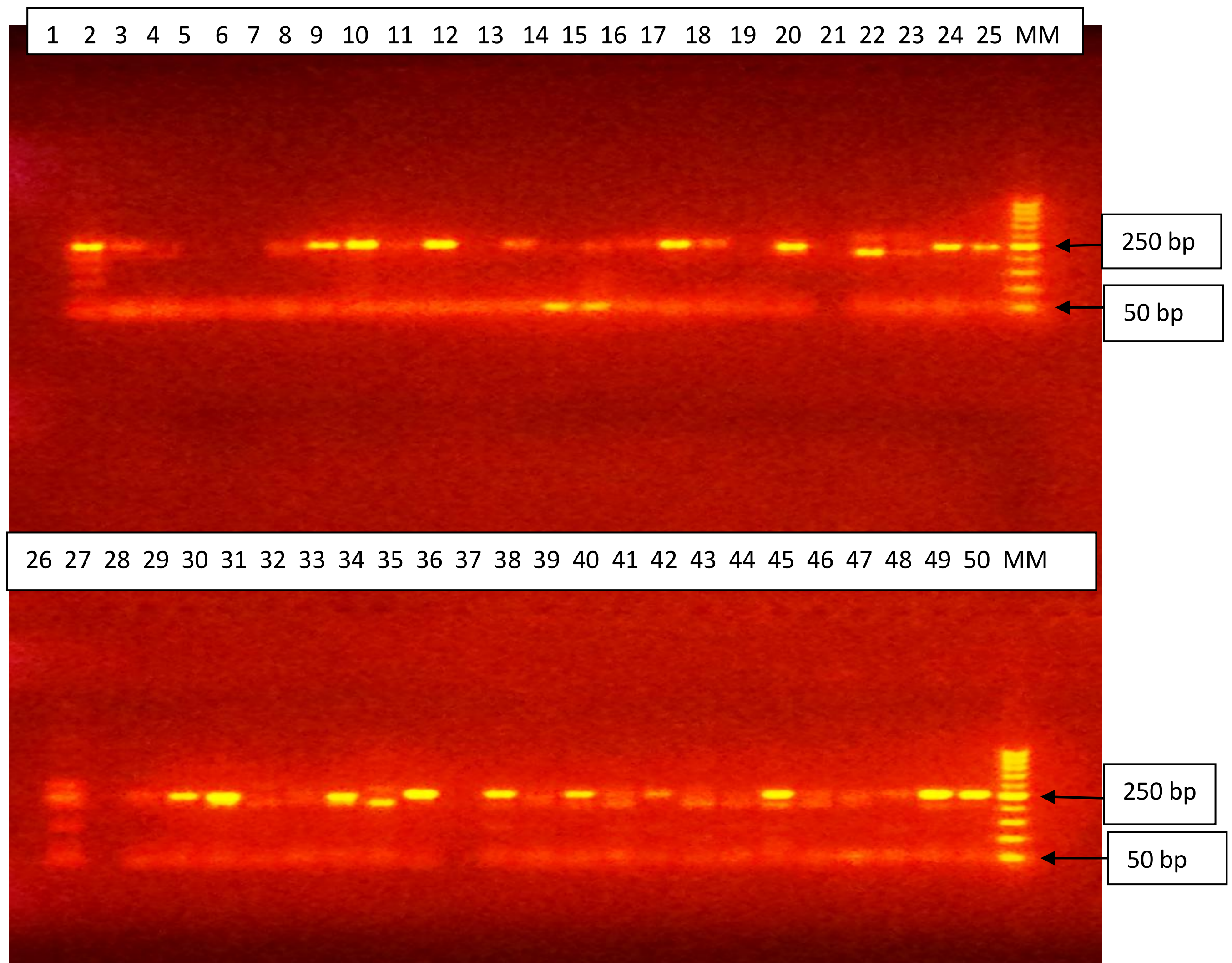

Fig 7: Banding pattern of PCR-amplified products of CAMS 228 among the 50 pepper accessions.

Lanes 1: Legon 18, 2: UE KNW 7, 3: NR TKB 9, 4:UE BAW 2, 5:NR WMP 4, 6: UE BAW 7A, 7:UE KNW 8, 8:GA ACC 5, 9: VR HOE 4, 10: BA TAN 3, 11: VR KTS 13, 12: UE BAW 7B, 13: NR WMP 6, 14: UE TND 1, 15: BA JMS 3, 16: ER UMK 2, 17: VR HOE 1, 18: VR HOE 11, 19 BA TAN 11A, 20: BA TAN 12, 21: BA JMS 4, 22: ER UMK 3, 23: VR KTS 9, 24: UE KNE 2, 25: VR HOE 10B, 26: VR KPV 1, 27: UE KNW 3, 28: VR KTS 2, 29: VR KTS 8, 30:UE BOM 2, 31: BA TAN 11B, 32: GA ACC 1, 33: UE KNW 9, 34: NR SVN 4, 35: NR TAM 4, 36: ADOPE, 37: UE KNE 4, 38: BA SYW 8, 39: BR TAM 6, 40: VR HOE 8F, 41: VR KPD 2, 42: BA SYW 6, 43: GA DWW 7, 44: UE KNW 13, 45: NR TKB 6, 46: NR TKB 3, 47: UMK 1, 48: UE KNE 6, 49: UE KNE 8, 50: UE BNG 1 and MM 50bp ladder

In Table 7, a total of 35 alleles with a mean of 4.38 alleles per locus were obtained from 8 out of the 10 SSR primers. Primers CAMS 212 and CAMS 406 PCR products were monomorphic, therefore they were not used in the analysis. Number of alleles ranged from 3-7 per locus. Allele frequency ranged from 0.30 to 0.88 with an average of 0.68. The locus CAMS 032 had the highest level of polymorphism with PIC value of 0.74 and gene diversity value of 0.77. CAMS 228 had the highest heterozygosity of 0.45 followed by CAMS 823 (0.41) with the least heterozygosity of 0.07 in CAMS 476.

Table 7. Allele frequency, allele number, gene diversity, heterozygosity and Polymorphism Information Content (PIC) values generated from molecular data

Marker	**Allele Frequency**	**Allele Number**	**Gene Diversity**	**Heterozygosity**	**PIC**
CAMS 032	0.30	6.00	0.77	0.39	0.74
CAMS 066	0.88	3.00	0.22	0.17	0.20
CAMS 228	0.65	5.00	0.52	0.45	0.48
CAMS 396	0.75	4.00	0.41	0.17	0.38
CAMS 476	0.87	4.00	0.24	0.07	0.23
CAMS 493	0.79	3.00	0.33	0.23	0.29
CAMS 823	0.38	7.00	0.76	0.41	0.73
CAMS 871	0.83	3.00	0.29	0.17	0.27
Mean	0.68	4.38	0.44	0.26	0.42

4.5 Cluster analysis based on SSR primers

Figure 8 shows the genetic relationship revealed by 10 SSR primers using similarity coefficients based on unweighted pair group method with arithmetic mean (UPGMA). From the figure, the genotypes clustered into two major groups at a similarity coefficient of 0.26. Cluster 1 contained only genotype GA ACC 1 with the other 49 genotypes clustering into another group. Cluster 2 had 15 sub-clusters at a similarity coefficient of 0.46. Notable amongst them were cluster 2A containing 7 genotypes, cluster 2B containing 13 genotypes and cluster 2C with 8 genotypes. UE BAW 7A and UE KNW 8 in cluster 2A, showed the closest resemblance among the 50 accessions at a similarity coefficient of 88.8%. However the most diverse accessions were GA ACC 1 in cluster 1 and Legon 18 in cluster 2A. Although the morphological descriptors indicated that UE BAW 7A and UE BAW 2 were highly related at a similarity coefficient of 98%, the molecular markers revealed these were clearly different genotypes as they clustered separately. Similarly, Legon 18 and NR TKB 9 were placed in different sub-clusters in the morphological descriptors but the SSR data revealed that they were closely related at a similarity coefficient of 0.66.

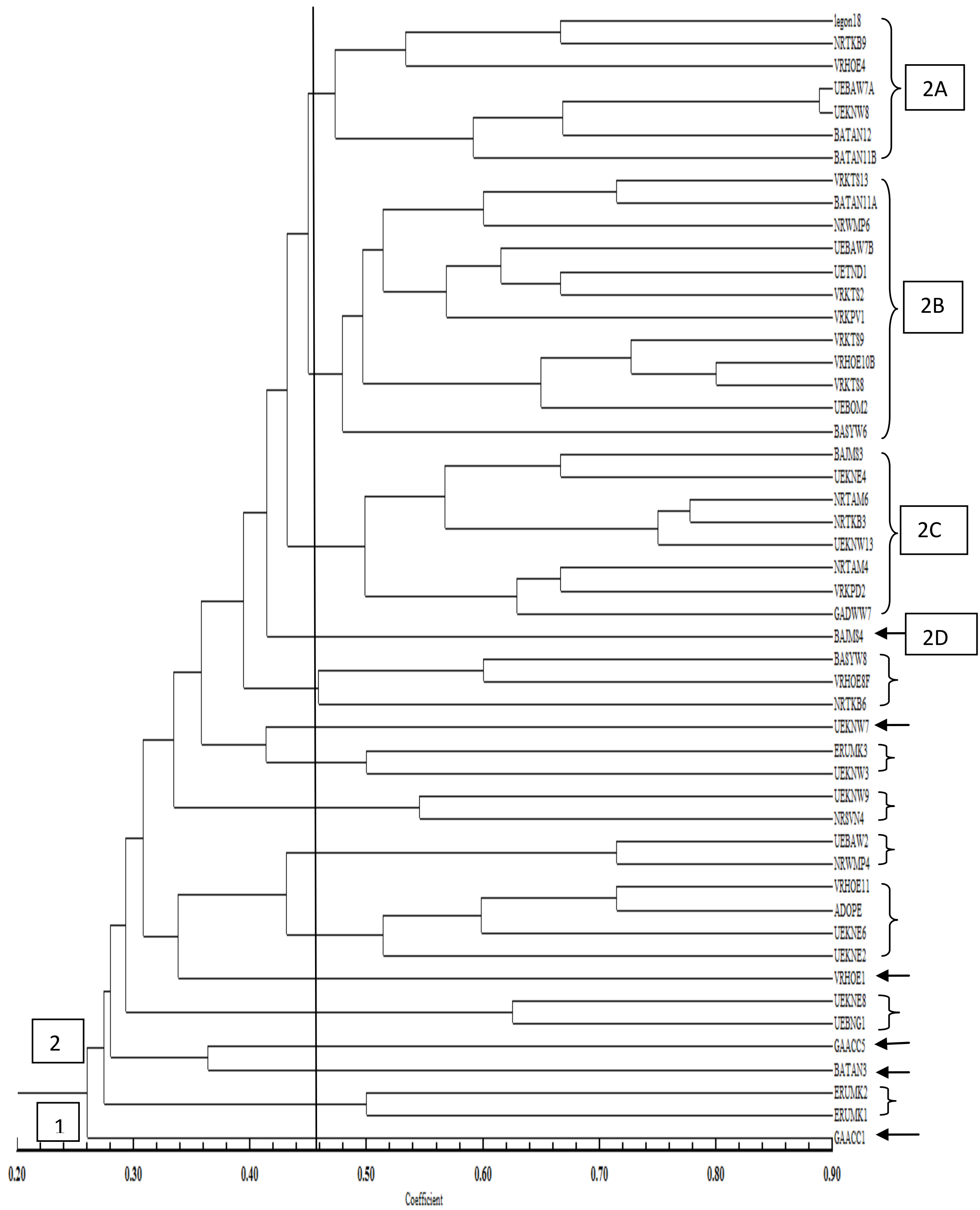

Fig 8 Genetic relationships among 50 pepper accessions based on similarity matrix using unweighted pair group method with arithmetic mean (UPGMA).

4.6 Combined analysis of morphological and molecular characterization

Figure 9 shows a dendrogram generated using a total of 35 phenotypic traits and 10 SSR primers based on unweighted pair group method with arithmetic mean (UPGMA). The accessions were grouped into two major clusters at a genetic similarity of 0.68. Group A was made up of 14 genotypes while group B consisted of 36 genotypes. At a genetic similarity of 0.72 the accessions were grouped into 7 sub-clusters. Group A consisted of 4 sub-clusters (A1, A2, A3 and A4). UE KNW 7, VR KPV 1 and GA DWW 7 were found in sub-cluster A1, A2 and A3, respectively. Sub-cluster A4 was made up of 11 genotypes, which was further grouped into 2 sub-sub-clusters. Legon 18 and Adope were the most closely related genotypes in group A at a genetic similarity coefficient of 0.91. Three main sub-clusters were identified in group B at similarity coefficient of 0.72. Sub-cluster B1 in this group was made up of genotypes UE KNW 9, GA ACC 5 and UE KNW 8. Sub-cluster B2 also consisted of 7 accessions. Sub-cluster B3 in this group contained 26 accessions of which several sub-sub-clusters could be identified. UE BAW 2 and UE BAW 7A were the most genetically related individuals in cluster B at a genetic similarity of 0.91. This combined analysis revealed UE KNW 9 and Legon 18/Adope to be the most genetically diverse genotypes among the accessions studied.

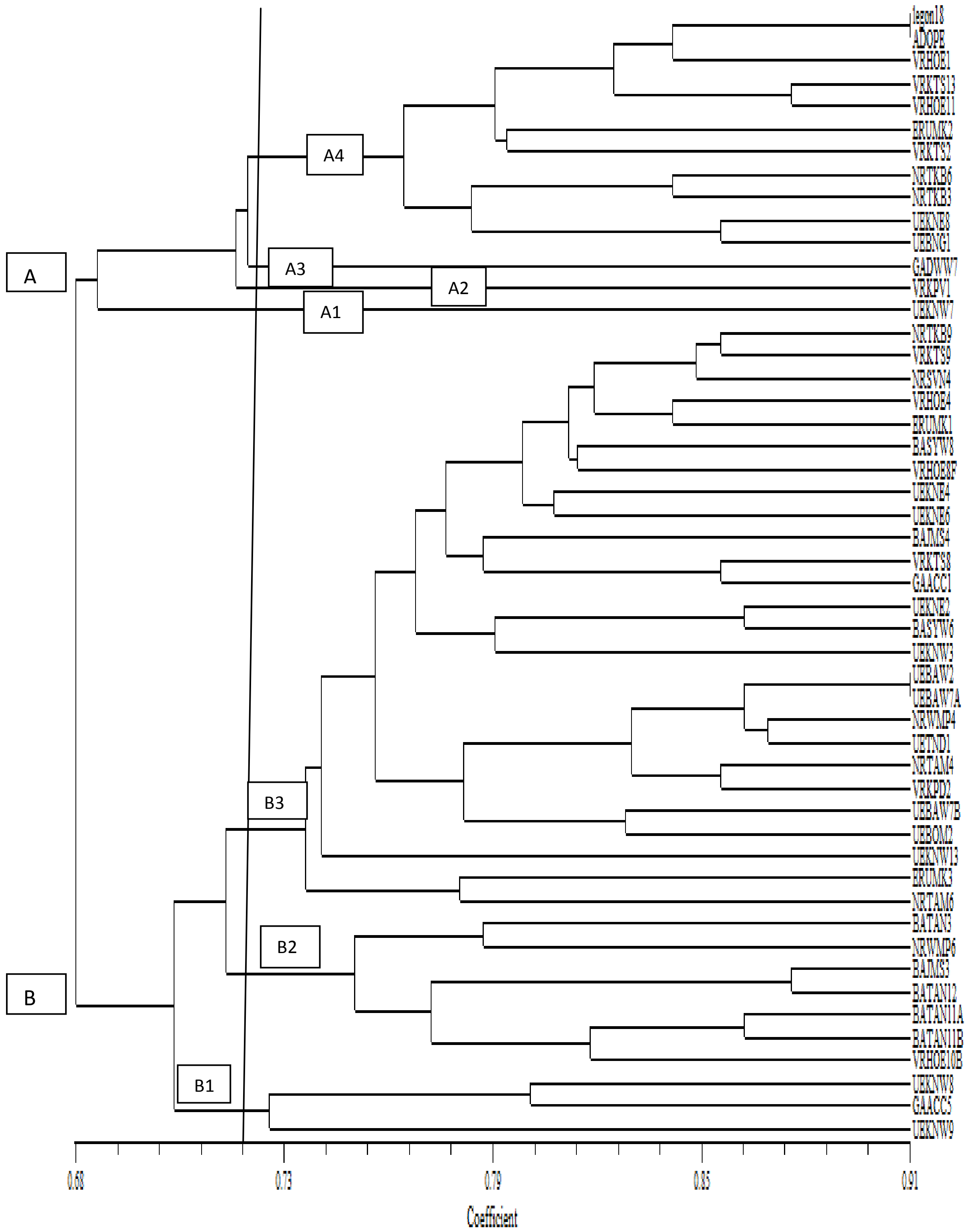

Fig 9 Genetic relationships among 50 pepper accessions based on similarity matrix using unweighted pair group method with arithmetic mean (UPGMA)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Variation in qualitative and quantitative traits

This research was conducted with the objective of studying and characterizing the genetic variability and relationships among pepper genotypes. It was also intended to provide information for the conservation and management of *Capsicum annum* germplasm resources in Ghana. In this study, 35 agro-morphological traits were used to examine the genetic diversity in 50 accessions of pepper (*Capsicum spp.*). The estimation of genetic diversity among accessions is beneficial for the conservation and maintenance of genetic resources. This is aimed at widening the genetic base of the cultivars and preventing genetic erosion (Yuzbapyoglu *et al*., 2006).

Morphological characters such as plant size, plant growth habit, branching habit, stem pubescence, leaf shape, fruit shape at peduncle attachment have been used to estimate genetic diversity in many important agricultural crops (Fonseca *et al*., 2008; Weerakoon and Somaratne, 2010; Asare *et al*., 2011). Manju and Sreelathakumary (2002) indicated that genetic characterization based on standard descriptors helps to easily describe the morphological features of a genotype and makes diversity assessment easier

From the study, these morphological markers revealed high genetic variation among the accessions. This result is in consonance with studies by Manju and Sreelathakumary (2002) who found significant variation in thirty-two (32) pepper accessions using 18 morphological parameters.

Similar studies by Se-Jong *et al*. (2012) also revealed high genetic variability among 61 pepper accessions from Bulgaria using 9 quantitative parameters.

The genotypes in the present study could be grouped into three classes according to plant height. Genotypes with heights ranging between 30-50 cm were classified as short, 51-70 cm as medium height and above 71 cm as tall. In relation to plant size, few of the genotypes were small with sparse branches while majority were intermediate with abundant branches. The genotypes with intermediate plant size will be suitable for intercropping with maize or sorghum.

There was a wide variation among the genotypes in fruit weight. The lightest fruit was obtained in UE TND 1 (4.5 g) while the heaviest (131.5 g) was obtained in GA ACC 5. The widest fruit width was also obtained by genotype GA ACC 5 making it the best among all the genotypes in terms of fruit weight and width. In general, genotypes belonging to scotch bonnet big group had the widest fruit widths and greatest weights while those from the long cayenne group had the longest fruits. Quantitative traits are of major agronomic importance and therefore the variations found among the genotypes in the present study will be useful in variety identification and improvement.

The study also revealed six major classes of fruit shape ranging from elongate to blocky and four fruit colours at mature stage with red as the predominant fruit colour. These findings are in consonance with work done by Fonseca *et al*. (2008) who found four fruit shapes varying from elongate to bell and nine fruit colours when they characterized 38 pepper genotypes from Brazil. Wide genetic variability in fruit shapes and colours in *Capsicum* species have been reported worldwide (Carvalho *et al*., 2003; Lannes *et al*., 2007). These variations among the accessions for the traits studied could be used in future pepper improvement programmes.

5.2 Influence of agronomic traits on total genetic variance as revealed by Principal Component Analysis

In genetic diversity studies using morphological traits, the most important variables describing phenotypic variation are defined by principal component (PC) analysis. The PC analysis in this study showed that 72.44% of the total genetic variance encountered among the pepper accessions was accounted for by the first four principal components taking into account all the 11 quantitative traits studied. The PC scores of fruit length and days to 50% fruiting, plant height and fruit pedicel length, fruit width and fruit weight, cotyledon leaf width and cotyledon leaf length were mostly associated with the first, second, third and fourth principal components (PC1, PC2, PC3 and PC4), respectively.

Diversity studies using principal component analysis have been carried out in pepper by several authors to understand and prioritize the most essential traits which explain much of the variability among the studied accessions (Ravindran *et al*., 1997; Portis *et al.,* 2006; Bozokalfa *et al*., 2009; Rohami *et al*., 2010; Lahbib 2012; Ilic *et al*., 2013).

The total contribution of the first four principal component axes of the study was higher (72.44%) than what was observed by Bozokalfa *et al*. (2009) where the first six principal component axes contributed 54.29% to total genetic variance among 48 pepper accessions. Nsabiyera *et al*. (2013), working on 37 local and introduced hot pepper genotypes, indicated that 55.4% of the total genetic variance was explained by the first two principal components. Lahbib *et al*. (2012) also observed 87% of the total genetic variance taking into account the first three principal component axes. For cassava, Asare *et al*. (2011) observed that 72.7% of the total genetic variance in the accessions was accounted for by the first three principal components.

In the present study, it can be deduced that cotyledon leaf width, fruit width, plant height and fruit length were the most important traits which accounted for much of the variability among the pepper genotypes.

5.3 Genetic relationship based on morphological descriptors

Cluster analysis is very useful in revealing complex relationships among populations of diverse origins in a more simplified manner. It is also effective in indicating accessions with useful traits belonging to different clusters for hybridization. The 50 genotypes in the study were classified into two main clusters at a similarity coefficient of 0.88. Genotype BA TAN 3 was very unique and stood alone in one cluster while the rest of the genotypes were placed in another cluster. This may be as a result of its lateness to flower and smaller fruit size (shortest fruit length and narrowest width). Although the majority of the genotypes were grouped into the second cluster, there were wide variations among them resulting in further clustering and sub-clustering. Even though majority of the genotypes clustered into their various groups, clustering did not strictly follow the geographic location or group under which the genotypes were collected but was based on their morphological features. For example VR KPV 1 which was collected under long cayenne group clustered separately from most of the long cayenne genotypes. At a genetic similarity above 98%, NR WMP 4 and UE TND1 as well as UE BAW 7A and UE BAW 2 may be considered as possible duplicates, respectively. Similar results of possible duplicates have been observed by other researchers such as Andersson *et al*. (2007) and Karuri *et al*. (2010). Genetic similarity coefficients from the morphological characterization ranged from 0.88 to 0.99 indicating low genetic diversity among the accessions. Although the dendrogram shows high genetic relatedness, the diversity within the genotypes can be used for improving desirable traits through selection and hybridization.

Even though morphological descriptors are efficient in variety identification they are influenced by environmental factors (Gepts, 1993) and therefore need to be backed with molecular markers which are accurate and independent of environmental factors.

5.4 Genetic diversity using SSR markers

The study revealed high allelic polymorphism (80%) among the accessions using the 10 SSR primers. The number of alleles produced by the primers varied from 3 to7 with an average of 4.38 alleles per locus which is similar to Kong *et al.* (2012) as they observed 2 to 6 alleles with an average of 2.7 alleles per locus. A total of 35 alleles were observed which is an indication of the polymorphic nature of the primers. Expected Heterozygosity ranged from 0.07 to 0.45 with an average of 0.26. These results are congruent with Tam *et al.* (2005) who obtained 31 alleles with expected heterozygosity of 0.35 using 35 pepper genotypes and 13 SSR primers. From this study PIC ranged from 0.20 to 0.74 with an average of 0.42 which is similar to results by Se-Jong *et al.* (2012) who recorded a PIC range of 0.06 to 0.63 with an average of 0.33. Kwon *et al.* (2005) also made similar observations when they assessed the potential of 27 SSR markers for variety identification in 66 pepper varieties. They recorded PIC values ranging from 0.03 to 0.88 with an average of 0.53. Similar results have been obtained by several researchers working on crops such as cassava (Asare *et al*., 2011), sweetpotato (Yada *et al*., 2010), apple (Lacis *et al*., 2011) and *Cajanus cajan* (Lal *et al*., 2010).

The dendrogram shows that there is considerable amount of genetic variation among the accessions in relation to the SSR primers studied. Genetic dissimilarity generated by the dendrogram ranging from 0.26 to 0.90 is an indication of the wide variation among the accessions. This high variability detected by the SSR primers shows that molecular

characterization is more efficient in revealing genetic divergence than phenotypic traits. This is in agreement with Asare *et al*. (2011) as they observed that SSR primers were more effective in discriminating cassava genotypes than morphological descriptors.

The accessions were grouped into two main clusters at a genetic similarity of 0.26 but were however grouped into sixteen clusters at genetic similarity of 0.46 which indicates wide genetic variability among the accessions.

Although there were wide variations among the genotypes as revealed by the dendrogram, this was not consistent with the groupings by dendrogram generated from morphological descriptors. Several genotypes, which were morphologically classified as closely related individuals, were clearly distinguished as different genotypes. For example ER UMK 1 and ER UMK 2 which were grouped into different clusters based on morphological descpritors were identified by the molecular markers as possible duplicates. Similarly, VR HOE 10B and VR KTS 8 were regarded as almost identical by the SSR primers but were found in different clusters based on morphological descriptors. On the other hand, genotypes UE BAW 7A and UE BAW 2 were morphologically similar but were regarded as different genotypes by the molecular analysis. The SSR primers revealed high genetically relatedness in genotypes UE BAW 7 A and UE KNW 8.

5.5 Genetic diversity using morphological and molecular markers

Cluster analysis using both morphological and molecular markers generated a dendrogram with a genetic dissimilarity range of 0.68 to 0.91 (Figure 9). The genetic diversity range as indicated by the dendrogram reflects a considerable amount of total genetic variation among the genotypes studied. The accessions were grouped into two main clusters with most of them in cluster B. Majority of the accessions in cluster A had elongate fruit shape with short to medium fruit length. They also belonged to the cayenne group with few exceptions belonging to bullet type. Accessions in cluster B had fruit shapes ranging from oblate to blocky. The combined analysis gave a true picture of the genetic relatedness among the accessions as there were groupings different from what was observed based on either the morphological or molecular markers. For instance, genotypes UE KNW 8 and UE BAW 7A were the most genetically related individuals in the molecular characterization but the combined analysis revealed that they were genetically diverse as they clustered separately in different sub-clusters. Similarly, genotypes UE BAW 2 and UE BAW 7A were highly related in the morphological analysis and this genetic relatedness was confirmed by the combined analysis as they appeared highly related at a genetic similarity of 0.91. The study has also confirmed the close relationship between Legon 18 and Adope which are released varieties being cultivated by farmers in the country.

In general clustering followed the groups under which the genotypes were collected. For instance sub-cluster 1 of group B contained three genotypes which all belonged to the scotch bonnet big group. Similarly, the bird eye, cayenne and cherry type group also clustered together in most cases.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The use of both morphological and molecular markers in studying genetic diversity is imperative in providing useful information on the genetic variability within a species. Genetic diversity revealed through characterization helps in germplasm conservation and maintenance. It is also important as it provides the basis for successful breeding programmes.

The study was carried out using 35 morphological descriptors and 10 SSR markers to ascertain the genetic relationship among 50 pepper accessions from diverse origins in Ghana. From the results obtained, the following conclusions can be drawn:

The study revealed enough genetic variability among the 50 genotypes at both morphological and molecular levels.

Substantial variation existed among the accessions especially in fruit traits (shape, colour, weight, length and width).

The first four principal components accounted for 72.44% of the total genetic variance among the accessions. The larger part of variance was accounted for by cotyledon leaf width, fruit width, plant height and fruit length.

Morphological cluster analysis revealed genetic dissimilarity of 0.88-0.99. VR HOE 1 and BA TAN 3 showed the widest diversity while the highest degree of similarity existed between UE BAW 7A and UE BAW 2.

A total of 35 alleles with mean PIC of 0.42 obtained from the molecular analysis show the informative nature of SSR primers and their superiority in genetic diversity assessment.

SSR loci CAMS 032 and CAMS 823 were the most polymorphic with PIC values of 0.74 and 0.73 respectively.

The high amount of genetic variability established by the SSR primers is an indication of the high amount of additive genetic variance within the population. This implies that substantial progress can be made through hybridization.

The results have proven that both morphological and SSR markers are effective tools in studying genetic diversity in *Capsicum* species.

6.2 Recommendations

From the results of the experiment these recommendations can be made:

It was observed that the accessions used in this work were limited to few districts from six regions, therefore, future studies must widen the scope of germplasm collection to cover all regions and districts in the country.

The number of morphological parameters should be increased to cover all seedling, vegetative, inflorescence and fruit traits.

Further studies should consider using more SSR primers.

Biochemical parameters such as dry matter, total sugar, ascorbic acid, β-carotene should be taken into consideration in future studies.

A breeding programme should commence with the more distinct genotypes (Legon 18/Adope and UE KNW 9) as parental materials for further improvement.

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APPENDICES

Appendix A. Descriptive statistics

A1. Hypocotyl pubescence

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
5	Intermediate	8	16	8	16
7	abundant	42	84	50	100

A2. Cotyledon leaf shape

Value	description	Frequency	Percentage	Cumulative	Cumulative %
5	Ovate	3	6	3	6
7	Lanceolate	39	78	42	84
9	Elong-deltoid	7	14	49	98
x	Mixture	1	2	50	100

A3. Cotyledon leaf colour

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
3	Light green	7	14	7	14
5	Green	43	86	50	100

A4. Stem colour

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
1	Green	40	80	40	80
2	Green with few purple strips	2	4	42	84
3	Green with many purple strips	8	16	50	100

A5. Plant growth habit

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
5	compact	35	70	35	70
7	erect	15	30	50	100

A6. Stem pubescence

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
0	Glabrous	1	2	1	2
3	Sparse	36	72	37	74
5	Intermediate	11	22	48	96
7	Abundant	2	4	50	100

A7. Leaf pubescence density

Value	density	Frequency	Percentage	Cumulative	Cumulative %
0	Glabrous	6	12	6	12
3	Sparse	31	62	37	74
5	Intermediate	8	16	45	90
x	Mixture	5	10	50	100

A8. Leaf shape

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
3	Deltoid	15	30	15	30
5	Ovate	25	50	40	80
7	Lanceolate	8	16	48	96
x	Mixture	2	4	50	100

A9. Leaf colour

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
2	Light green	4	8	4	8
3	Green	18	36	22	44
4	Dark green	25	50	47	94
10	Mixture	3	6	50	100

A10. Branching habit

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
3	Sparse	1	2	1	2
5	Intermediate	14	28	15	30
7	Abundant	31	62	46	92
10	Mixture	4	8	50	100

A11. Plant size

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
3	Small	3	6	3	6
5	Intermediate	34	68	37	74
7	Large	13	26	50	100

A12. Corolla colour

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
1	White	8	16	8	16
2	Light yellow	1	2	9	18
4	Yellow-green	40	80	49	98
x	Mixture	1	2	50	100

A13. Anther colour

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
3	Green	3	6	3	6
4	Blue	7	14	10	20
5	Light purple	25	50	35	70
x	Mixture	15	30	50	100

A14. Filament colour

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
1	White	38	76	38	76
5	Light purple	6	12	44	88
6	Purple	1	2	45	90
10	Mixture	5	10	50	100

A15. Calyx margin shape

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
5	Intermediate	36	72	36	72
7	Dentate	14	28	50	100

A16. Calyx annular constriction

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
0	Absent	1	2	1	2
3	Not clear	7	14	8	16
5	Clear	8	16	16	32

7	Distinct and uniform in the whole plant	31	62	47	94
x	Mixture	3	6	50	100

A17. Fruit position

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
3	Declining	17	34	17	34
7	Erect	7	14	24	48
x	Mixture	26	52	50	100

A18. Fruit colour at mature stage

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
1	Green	2	4	2	4
2	Yellow	1	2	3	6
3	Orange	1	2	4	8
5	Red	43	86	47	94
x	Mixture	3	6	50	100

A19. Fruit shape

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
1	Elongate	19	38	19	38
2	Oblate	2	4	21	42
4	Conical	11	22	32	64
5	Campanulate	9	18	41	82
6	bell or blocky	9	18	50	100

A20. Fruit shape at pedicel attachment

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
1	Acute	4	8	4	8
3	Obtuse	19	38	23	46
5	Truncate	23	46	46	92
7	Cordate	4	8	50	100

A21. Neck at base of fruit

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
0	Absent	35	70	35	70
1	Present	15	30	50	100

A22. Fruit shape at blossom end

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
3	Pointed	32	64	32	64
5	Blunt	10	20	42	84
7	Sunken	8	16	50	100

A23. Fruit cross-sectional corrugation

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
0	Smooth	2	4	2	4
3	Slightly corrugated	19	38	21	42
5	Intermediate	13	26	34	68
7	Corrugated	16	32	50	100

A24. Number of locules

Value	Description	Frequency	Percentage	Cumulative	Cumulative %
2	Two	30	60	30	60
3	Three	20	40	50	100

Appendix B

B1. Means for quantitative traits

Accession no	Cotyl leaf length	Cotyl leaf width	Plant height	Stem diameter	Days of flowering	Days of fruiting
LEGON 18	2.17	0.7	43	0.7	42	26
UE KNW 7	1.78	0.56	42.3	1.06	32	29
NR TKB 9	1.77	0.7	47	1.13	37	29
UE BAW 2	1.87	0.7	60	1	50	29
NR WMP 4	1.67	0.6	43.7	1.03	35	29
UE BAW 7 A	1.7	0.67	50	0.87	48	29
UE KNW 8	1.4	0.47	60.3	1.03	52	29
GA ACC 5	1.44	0.68	55.3	1.03	53	29
VR HOE 4	1.86	0.64	48.7	1.27	49	29
BA TAN 3	1.74	0.62	57.3	0.93	56	26
VR KTS 13	1.98	0.74	39	0.87	44	26
UE BAW 7 B	1.47	0.73	62.3	0.93	42	29
NR WMP 6	1.7	0.78	67	1.47	47	29
UE TND 1	1.77	0.73	46.3	0.93	31	29
BA JMS 3	2.12	0.7	91.7	1.2	53	29
ER UMK 2	2.06	0.68	53.7	1.03	34	26
VR HOE 1	1.9	0.54	45.3	0.97	47	26

VR HOE 11	2.36	0.72	49	0.83	44	26
BA TAN 11 A	1.58	0.86	57	1	47	29
BA TAN 12	1.64	0.66	73.7	1.6	52	29
BA JMS 4	1.24	0.78	48.3	1.1	48	29
ER UMK 3	1.97	0.8	77	1.47	36	29
VR KTS 9	1.9	0.73	44.3	1	35	29
UE KNE 2	1.72	0.64	43.7	1	42	29
VR HOE 10 B	1.48	0.64	56	1.13	47	29
VR KPV 1	2.0	0.58	41	0.77	25	26
UE KNW 3	2.07	0.73	47.3	1.03	58	29
VR KTS 2	1.56	0.7	41.3	0.77	33	26
VR KTS 8	2.13	0.77	46.7	0.97	34	29
UE BOM 2	1.47	0.7	42	0.97	28	29
BA TAN 11 B	1.33	0.45	52	1.13	47	29
GA ACC 1	1.83	0.87	32	1.07	30	29
UE KNW 9	1.48	0.55	52.5	1.1	27	29
NR SVN 4	1.66	0.68	47	1.13	35	29
NR TAM 4	1.67	0.67	53.7	1.73	33	29
ADOPE	1.8	0.61	31	0.6	46	26
UE KNE 4	1.4	0.73	42.3	1.07	44	29
BA SYW 8	1.87	0.73	53.7	1.1	48	29
NR TAM 6	1.92	0.76	65	1.43	42	29
VR HOE 8 F	1.83	0.67	38.7	1.47	33	29
VR KPD 2	1.8	0.65	45	1.1	33	29
BA SYW 6	1.8	0.66	42.3	0.83	52	29
GA DWW 7	1.84	0.78	52	1.1	42	29
UE KNW 13	2.14	0.84	60.3	1.13	48	29
NR TKB 6	1.8	0.74	42.3	1.1	26	29
NR TKB 3	1.82	0.84	47.3	1.23	29	29
ER UMK 1	1.54	0.6	39	1.17	53	29
UE KNE 6	1.7	0.68	34.7	0.93	45	29
UE KNE 8	1.63	0.6	32.3	0.93	23	29
UE BNG 1	1.72	0.82	37.3	0.93	22	29
Grand Mean	**1.76**	**0.69**	**49.61**	**1.07**	**40.78**	**28.46**
SE	**0.03**	**0.01**	**1.66**	**0.03**	**1.34**	**0.17**
CV (%)	**13.37**	**13.30**	**23.60**	**20.52**	**23.19**	**4.09**

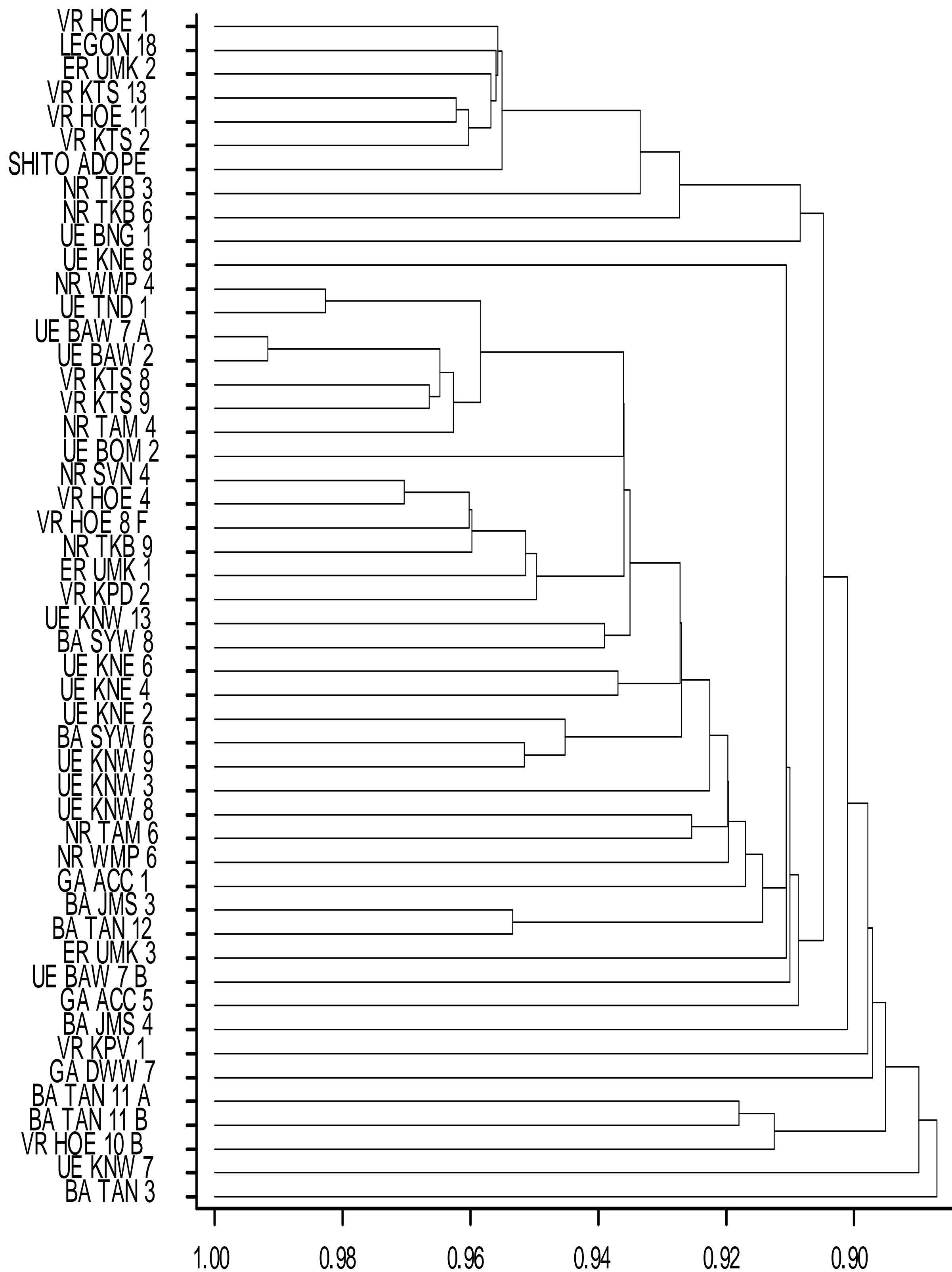
B2. Means for quantitative traits continuation

Accession no	Fruit-pedicel width	Fruit-pedicel length	Fruit length	Fruit width	Fruit weight
LEGON 18	0.13	2.36	4.74	0.85	39
UE KNW 7	0.19	2.27	9.39	1.22	59
NR TKB 9	0.14	1.67	3.67	2.71	75.5
UE BAW 2	0.11	2.39	3.83	0.88	10.5
NR WMP 4	0.11	2.24	2.84	0.9	9
UE BAW 7 A	0.14	2.36	2.56	0.93	6.5
UE KNW 8	0.2	3.03	4.26	2.83	88.5
GA ACC 5	0.24	2.82	5.15	3.9	131.5
VR HOE 4	0.15	2.34	1.89	1.86	24
BA TAN 3	0.17	2.83	0.7	0.63	10.5
VR KTS 13	0.21	2.43	5.97	1.5	41.5
UE BAW 7 B	0.12	2.39	2.53	1.04	7.5
NR WMP 6	0.18	3.17	4.25	0.95	11
UE TND 1	0.11	2.53	2.9	0.62	4.5
BA JMS 3	0.14	3.09	6.28	1.11	22.5
ER UMK 2	0.18	2.43	7.95	1.03	32
VR HOE 1	0.15	2.86	9.41	0.86	32.5
VR HOE 11	0.24	2.5	6.97	1.28	47.5
BA TAN 11 A	0.13	2.11	4.84	1.18	18.5
BA TAN 12	0.13	2.93	7.47	1.25	28
BA JMS 4	0.19	1.91	1.44	1.57	15.5
ER UMK 3	0.16	2.39	3.62	1.77	37
VR KTS 9	0.14	2.36	2.71	2.34	40.5
UE KNE 2	0.18	1.94	4.3	1.7	40
VR HOE 10 B	0.19	2.06	4.4	2.14	33.5
VR KPV 1	0.39	2.56	9.92	1.91	91.3
UE KNW 3	0.14	2.58	2.99	1.78	15
VR KTS 2	0.19	2.42	10.46	1.2	56
VR KTS 8	0.15	1.56	2.68	1.92	24
UE BOM 2	0.09	2.25	1.83	2.09	5
BA TAN 11 B	0.15	2.1	5.81	1.21	26.5
GA ACC 1	0.2	1.87	3.55	2.11	43.5
UE KNW 9	0.18	2.35	5.08	1.84	60
NR SVN 4	0.2	1.61	1.87	1.47	13.5
NR TAM 4	0.11	2.45	2.57	0.9	8
ADOPE	0.12	1.93	5.35	0.95	30.5
UE KNE 4	0.12	1.71	2.07	2.94	54.5
BA SYW 8	0.14	1.97	3.47	1.84	40
NR TAM 6	0.19	2.25	3.69	2.58	74
VR HOE 8 F	0.13	2.03	1.52	1.61	17.5
VR KPD 2	0.18	2.15	2.57	1.78	23.5
BA SYW 6	0.16	2.06	4.04	2.18	38.5

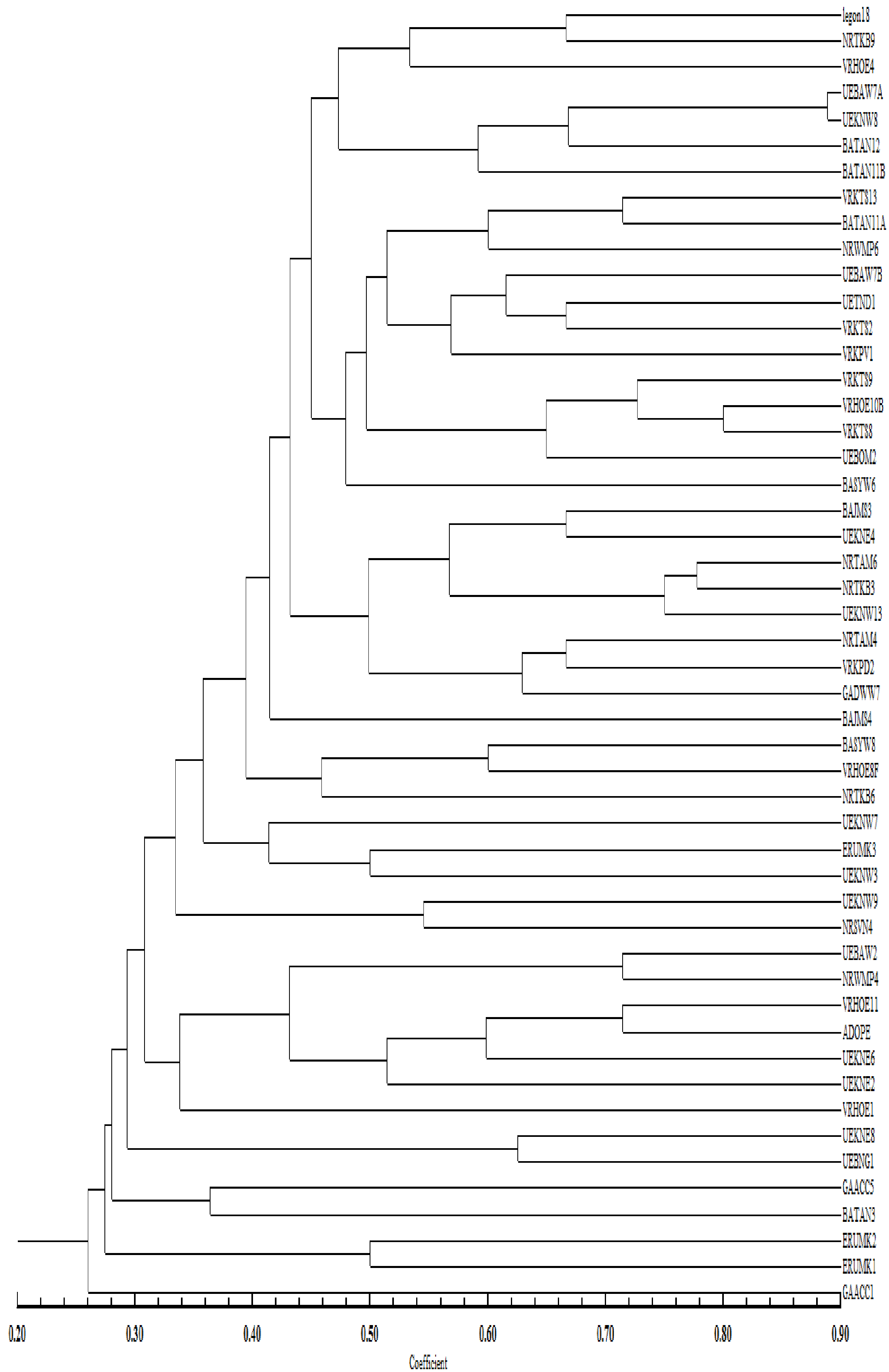
GA DWW 7	0.24	3.21	7.99	1.58	69
UE KNW 13	0.29	2.19	5.3	2.2	74
NR TKB 6	0.23	2.14	4.02	1.42	39
NR TKB 3	0.22	2.48	6.55	1.3	33
ER UMK 1	0.18	1.49	1.15	1.41	11.5
UE KNE 6	0.22	2.03	2.2	2.17	30.5
UE KNE 8	0.16	2.22	6.44	0.98	21
UE BNG 1	0.31	1.89	4.86	0.8	12
Grand Mean	**2.30**	**0.17**	**4.44**	**1.58**	**35.55**
SE	**0.06**	**0.01**	**0.34**	**0.10**	**3.72**
CV (%)	**17.70**	**32.11**	**53.78**	**42.60**	**74.08**

Appendix C

C1. Dendrogram for morphological characterization of 50 pepper accessions



C2. Dendrogram for molecular characterization of 50 pepper accessions



C3. Dendrogram for combined analysis using morphological and molecular data