

**GENETIC DIVERSITY STUDIES IN TWENTY ACCESSIONS OF HOT
PEPPER (*Capsicum* spp L.) IN GHANA.**

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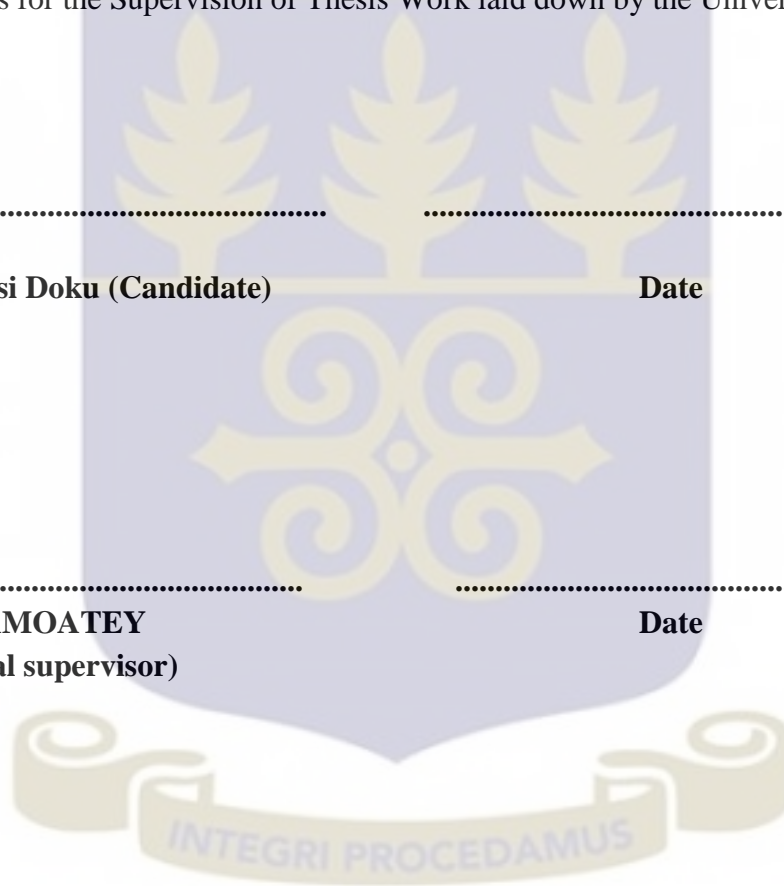
DECLARATION

I hereby declare that the thesis herein, with the exception of references to other people's work which have duly been acknowledged, is the result of my own work and, neither in whole nor in part, has it been presented for another degree in this University or elsewhere. I also declare that this project was supervised in accordance with the Guidelines for the Supervision of Thesis Work laid down by the University of Ghana.

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DEDICATION

This thesis is dedicated to my husband, Mr Augustus Djabatey for being very supportive and helpful towards the completion of this programme and also to my children Gilbert and Kevin.



ACKNOWLEDGEMENT

TO GOD BE THE GLORY FOR EVER AND EVER, AMEN.

My immense gratitude goes to my supervisors, Dr. H.M. Amoatey and Dr Samuel Amiteye for their support and guidance throughout this research. Also to Mr Emmanuel Quartey, Mr John Ahiakpa and Mr Henry Doku for their contributions, encouragement and support towards the completion of this research.

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Finally to all friends and relatives for their prayers, support and encouragement. God Bless you.

ABSTRACT

Twenty (20) accessions of hot pepper (*Capsicum* spp L.) were collected from eight geographical regions of Ghana for genetic diversity studies. The objective was to assess genetic relationship among them using phenotypic and molecular traits and to evaluate their elemental composition. A replicated field experiment was conducted to assess their genetic diversity based on 13 quantitative traits and 22 qualitative traits using the IBPGR descriptor list for *Capsicum*. Confirmation of their identities was done using 10 SSR markers. The accessions were also evaluated for macro, micro and trace elements in their fresh fruits using the Instrumental Neutron Activation Analysis (INAA). Five essential macro elements (Ca, Cl, K, Mg and Na), two micro elements (Al and Mn) and one trace element (Br) were detected by INAA. Results from the agromorphological study revealed that accession Wes 01 had the widest stem width, matured leaf width, high fruit set but late maturing. Nor 03 was early maturing and had high fruit set, but also possessed the highest number of seeds per fruit. Fruit weight, fruit width, fruit length and plant canopy width, recorded the highest variabilities with 66.191; 53.24; 49.32; and 32.42 coefficients of variation (CVs), respectively. Few traits such as plant canopy width, plant height, fruit length, mature leaf length and number of seeds per fruit contributed substantially to total genetic variance as revealed by the principal component analysis (PCA). A dendrogram generated using morphological traits grouped accessions into cultivated and wild genotypes of pepper and all the accessions were identified as separate entities with no duplications. Strong correlation was recorded between plant canopy width and plant height, mature leaf length and mature leaf width, and also fruit weight and fruit width and fruit length. Negative correlation was however, observed between

fruit length and days to 50% fruiting and flowering. All three accessions from the Northern Region, Nor-01, Nor-02 and Nor-03, as well as Vol-01 from the Volta Region recorded high amount of Sodium in their fruit samples. Genetic analysis of the structure of the population showed close resemblance among the accessions with a high genetic identity coefficient of 0.975 between populations 1 and 2. A rather short genetic distance was recorded evidenced by high outcrossing rate of 0.72. Accessions Wes 01 and Nor 03 may be recommended for improvement for the export market.



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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background to the Study

Hot pepper (*Capsicum* spp L.), widely considered the first spice to have been used by humans, belong to the family Solanaceae and genus *Capsicum*. It ranks third in importance among vegetables after peas and tomatoes (Ali, 2006; Ochoa-Alej and Ramirez-Malagon, 2001) and is the world's second most important solanaceae vegetable after tomatoes (Yoon et al., 1989). It is an indispensable constituent in the diets of many people all over the world, and in the food industry. It is used for flavouring and colouring, and well cherished for its pungency.

Peppers are usually classified based on fruit characteristics-pungency, colour, shape, flavour and use (Bosland, 1992). Classification based on the pungency puts peppers into two groups, hot pepper and sweet pepper. Pungency is an indication of the heat sensation in pepper. It is caused by capsaicin- a colourless, odourless, oily chemical found in the fruit. The more capsaicin present in the fruit, the hotter that particular specie. Most of the capsaicin in pepper is contained in light coloured veins around the seed, an adaptation that prevents fungi and animals that would destroy the seed from eating the pepper (Bosland and Votava, 2000). While the seeds are not the source of pungency, they occasionally absorb capsaicin due to their proximity to the placenta. No other plant part produces capsaicinoids.

Pungency of pepper is expressed in Scoville Heat Units (Scouville, 1912). The scoville heat index ranks peppers in order from mild to hottest. It starts from zero being mild to 1,000,000 indicating the hottest. Capsaicin in hot pepper has been known to increase metabolism, relieve headache and pain as well as increase blood circulation (Dognoko *et al.*, 2013). Pepper seeds germinate between three to four weeks after nursing and fruits ripen between 14-15 weeks after transplanting (WAT). The fruits come in all shapes, sizes and colours.

In Ghana, peppers are grown in gardens or often intercropped at convenient sites near settlements but recently grown as a monocrop on a large scale by both peasant and commercial farmers. They are cultivated for local consumption as well as for export to Europe and have become a foreign exchange earner for Ghana (Normal, 1992). It is estimated that Ghanaian pepper farmers are producing only 50% of attainable yield. The production volume remain relatively constant for about 10years. It increased slightly from 270,000 metric tons in 2000 to 277,000 in 2006 and 279,000 in 2008 (MiDA, 2011). Ghana ranks 11th as major producer of pepper in the world and the second largest in Africa and the fifth largest exporter to the European market. Turkey, China, and Mexico contribute about 70% of the total world pepper production (MiDA, 2012). The derived Savanna agro-ecologies with an annual rainfall of 600-1250 mm are best for pepper production in Ghana (Norman, 1992).

Peppers are very popular in all the agro-ecological zones of Ghana but very little has been done to improve the indigenous cultivars. Most of the pepper varieties grown by farmers are unimproved and yield poorly. The low production may be due to low soil

fertility, pest and disease incidences, unavailability and high cost of irrigation systems, inadequate knowledge of improved technology as well as unimproved varieties (MiDA, 2011).

Ghana has been exporting pepper for the past fifteen years, However significant growth in the production of chili pepper for export is relatively a recent phenomenon. This increase in export volume has been attributed to the release of Legon 18 by the University of Ghana research station. This variety has largely been successful due to its adaptation to local soil and agro-climatic conditions (MiDA, 2011). Currently, two varieties are exported by Ghana and these are Legon 18 and Bird's eye pepper which account for about 1% of the European market share (MiDA, 2011).

Worldwide, many scientists have characterised pepper using morphological (Medina *et al.*, 2006; Portis *et al.*, 2006; Zewdie and Zeeven,1997), biochemical (Tahan *et al.*, 2013; Aneta *et al.*, 2011) and molecular (Pacheco-Olivera *et al.*, 2012;Stagel *et al.*, 2009)properties. However, indigenous species as well as cultivars appear not to have been fully exploited as most investigations focused on morphological characterisation only (Bonsu, 2013; Nkansah *et al.*, 2011).

The use of phenotypic characters in describing and classifying germplasm is basic in any characterisation programme (Smith and Smith, 1989). However, studies have shown that morphological characterisation 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 (Geleta *et al.*, 2005; Gilbert *et al.*, 1999).

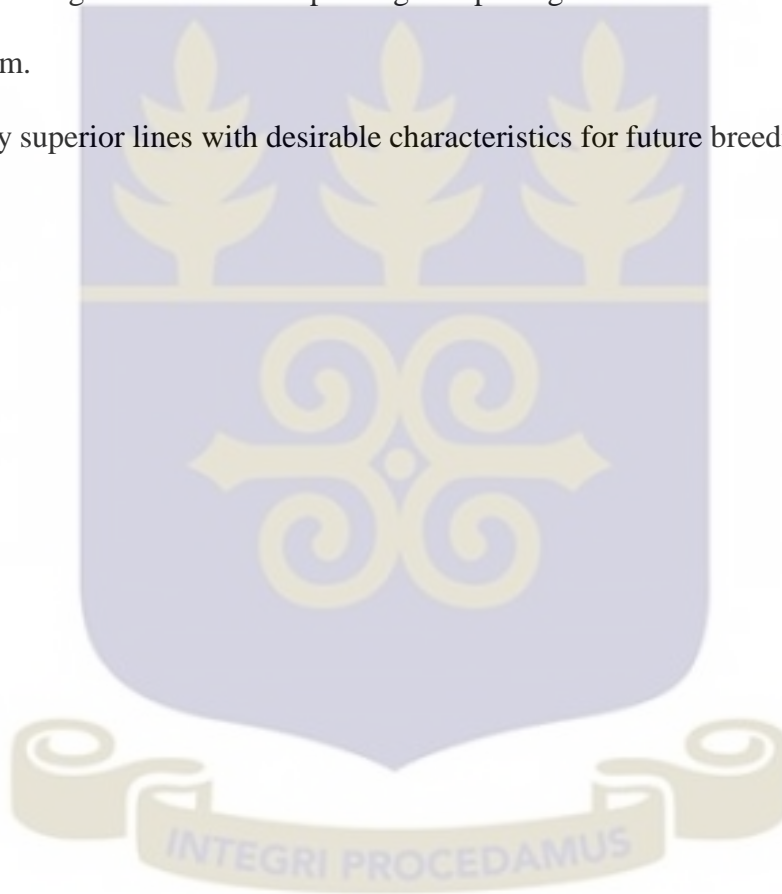
Molecular characterisation could differentiate between individuals which are closely related with accurate results due to its ability to recognise specific DNA sequences in organisms (Rocha *et al.*, 2010a). Hence, the need to also carry out biochemical and molecular characterisation, which would fully describe the available germplasm of indigenous pepper so as to determine their potential use in future breeding work.

Germplasm are considered strategic resources essential to both national and global food security (IBPGR, 1991). Characterisation is very important in plant breeding programme. It helps breeders in selecting suitable parents for hybridisation towards developing new cultivars (Frankel, 1975). It makes available essential information on genetic diversity within and among closely related genotypes for breeders to use these resources meaningfully. Pepper breeding has focused on addressing consumer needs such as degree of pungency, colour, taste, fruit shape, fruit wall thickness and ability to dry (Bosland and Votava, 2000). The extent of the genetic relationships among diversity expressed by improved and local cultivars as well as their wild relatives and weedy forms determines the degree of success in crop improvement. This study would bring out the genetic relations among these local cultivars as well as their wild relatives.

Selection of genotypes with traits of interest (high yield, high pungency, better taste) for hybridisation would enhance future breeding work to improve the crop. The biochemical analysis would bring out hidden qualities and nutritional potential of the local cultivars to facilitate commercial exploitation.

The primary objective of this study was to determine the genetic diversity in twenty accessions of hot pepper using morphological, molecular and biochemical properties. The research focused on the following specific objectives:

- i. To assess the phenotypic and molecular diversities among 20 accessions of pepper.
- ii. To determine variations in biochemical contents of the fresh fruits.
- iii. To generate a genetic relationship using morphological and molecular data through a dendrogram.
- iv. To identify superior lines with desirable characteristics for future breeding work.



CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and Distribution

Peppers (*Capsicum* spp L.) belong to the family Solanaceae, which also includes tomatoes, potatoes, tobacco, and the deadly nightshade (Ryan and Pearce, 2003; Lovell, 1993). Peppers were domesticated 10,000 to 12,000 years ago by the Aztecs, Mayas and the Incas. Grown for over 7000 years in the humid and tropic zones of Central and South America, Brazil became the major centre of diversity where representatives are all found (Costa *et al.*, 2009). Fragments of dried pepper fruits have been found in ancient Peruvian ruin, and a Mexican cave has yielded pieces of dried red peppers over 9,000 years old (Fett, 2003). Columbus, in the fifteenth century, introduced peppers to Europe and subsequently to Asia and Africa and later to India, China and Japan through the spice trade (Anon., 2014).

It is estimated that more than 3 million hectares of peppers are grown annually around the world. Asia is the largest producer, followed by Africa and Europe. Pepper production is found from the humid tropics to the dry deserts, as well as the cool temperate climates. The ability of pepper to thrive under this range of climatic conditions has rendered it a common crop worldwide (Ombrello, 2014).

2.2 Taxonomy and Classification

Pepper consist of annual or perennial herbs or shrubs and are predominantly diploid ($2n = 24$; infrequently, $2n = 26$), except a few (Moscone *et al.*, 2003). The nomenclature and classification of peppers has been fraught with confusion and change over the years (Andrews, 1993; DeWitt and Bosland, 1993). Christopher Columbus was the first to describe pepper and subsequently introduced it into Europe in the fifteenth century and other early explorers disseminated it through post voyage route to Africa, India, and Middle East. Cultivation has spread throughout the world (Mcmullan and Livsey, 2013; Greenleaf, 1986).

Terms like pepper, chili, chile, chilli, paprika, aji and *Capsicum* are frequently used interchangeably (DeWitt and Bosland, 2009). The genus *Capsicum* can be classified into different categories based on the ability of members to successfully interbreed. Currently, the *Capsicum* genus comprises five well-described domesticated species and at least 20 wild species, as well as many hybrids and cultivars (DeWitt and Bosland, 1993).

The most common of these is *Capsicum annum*. A herbaceous annual plant originally from the central part of South America. It is the most important species with sweet and spicy fruits. It is the type most often cultivated in Italy. The most common varieties include *Capsicum abbreviatum* (with small cone shaped fruits that do not grow larger than 5 cm); *Capsicum acuminatum* (with long, thin cone- shaped fruit that are slightly curved); *Capsicum fasciculatum* (with erect fruit, thin and grouped together), *Capsicum cerasiforme* (with small round fruit similar to cherries) and *Capsicum bicolor* (with two toned very small fruit, that's purple, orange and red) (Baral and Bosland, 2002).

Capsicum frutescens, another domesticated species originating from South America - spread first in Africa and later in Europe - is a perennial with spicy fruits. The best-known varieties are Diende de pezzo (Columbia), Tabasco (Louisiana, Mexico) and Malagueta (Brazil) (Fett, 2003). The domesticated *Capsicum chinense* (originally from the Amazon) first spread in Africa. It includes the hottest varieties in the world. Scotch Bonnet (Jamaica), Congo Pepper (Trinidad–Tobago), Chile Habanero Yellow (Mexico–Belize), Chocolate Habanero (West Indies), Charapita (Peru), Azr (India) and Red Squash (Puerto Rico) are some well-known examples (Costa *et al.*, 2009). *Capsicum pubescens* was introduced and cultivated in the Peruvian Andes. Today, it is widespread in upper Bolivia, Peru and Ecuador. The best-known varieties are Rocoto, Caballo, Canario (Costa Rica, Mexico, and Guatemala) and Rocoto Manzano (Peru) (Monteiro *et al.*, 2011). The last domesticated, *Capsicum baccatum*, comes from the dry regions of Bolivia and Peru. The best-known varieties are the Christmas Bell (South America), Aji Amarilli (Peru, Andes), Dedo Do Moca (Brazil) and Aji Serranito (Peru) (Baral and Bosland, 2002).

2.3 Morphological Description

Though some *Capsicum* species may share similar morphological features which makes identification difficult at their vegetative states, most are highly heterogenous—exhibiting considerable morphological variation especially in fruit shape, colour and size (Walsh and Hoot, 2001). They can be herbaceous or shrub-like but are generally branching with greenish brown stems and simple oval leaves. The plant produces flowers with five petals

which are usually white in colour and can reach heights in the range of 0.5-1.5 m. Pubescence of leaves and stems range from glabrous to very pubescent.

Pepper produces bisexual flowers usually borne at the intersection between the stem and leaves at points where the stem splits into a fork (Greenleaf, 1986). The inflorescences may vary from solitary to seven flowers at one node. The calyx may range from long, green sepals to truncate sepals to spine-like projections (Berke, 2000). The pedicel length varies among cultivars, ranging from 3-8 cm. The pistil is composed of an ovary containing 2-4 carpels or locules, a style that is 3.5-6.5 mm long and a stigma with a diameter slightly greater than the style (Berke, 2000). In the species, *C. annuum*, the petals are usually white with 5-7 individual stamens which vary in colour from pale blue-purple anthers. DeWitt and Bosland (1993) observed greenish-white corolla in *C. frutescens* and added that corolla colour is one of the most consistent features of distinguishing *Capsicum* species.

Pepper is a self-pollinated crop but cross-pollination is widespread and occurs when insects move from one flower to another to feed. It has a high rate of outcrossing which ranges from 70-90%. Natural inter-specific crosses among *Capsicum* species are very high, resulting in intermediary forms which are complex to categorise (Andersson *et al.*, 2007; Allard, 1960).

In the tropics, *Capsicum* can be cultivated from sea level to elevations of over 6,000 feet. In their native habitat, most Red Peppers live for several years and grow as shrubs with heights of 6-8 feet and “woody” trunks over 3 inches in diameter. The plants are capable

of fruiting in the first year from seed and are grown as annuals in temperate regions, not developing “woody” tissue before being killed by frost (Fett, 2003). Fruits vary in shape, size and colour as well as pungency and duration of maturity (Fig 2.1)



Figure 2.1: Mature fruits of assorted hot pepper varieties (Source: Fett, 2003).

2.4 Economic Importance

Pepper is a vital commercial crop, cultivated in the tropical and temperate zones of the world for vegetable, pest control, spice, and value-added processed products (Kumar and Rai, 2005). It can be used whole, chopped or processed into various forms; fresh, dried and ground into powder or as an extract (Ortiz *et al.*, 2010). In Ghana, a popular pepper

sauce, 'shito' is widely used by students, campers and even for export. In herbal medicine, *Capsicum* are used internally to aid circulation, relieve gas and colic, aid digestion, and prevent infection. External uses include local analgesia and a remedy for cold feet (Dagnoko *et al.*, 2013). They are also used by the security agencies in the preparation of tear gas (Kumar and Rai, 2005).

As a commercial crop, pepper was ranked as the second valuable vegetable crop ahead of popular vegetables like okra and eggplant with an estimated total production of 88,000 metric tons in 2011 which was valued at \$96, 397 (FAOSTAT, 2011). Agronomically, it has also been used as a trap crop to reduce field infestation of Egyptian broomrape, a chlorophyll lacking root parasite (Hershenhorn *et al.*, 1996).

2.5 Fruit Nutrition

The fruits contain vitamins A and C, also mixtures of antioxidants notably carotenoids, ascorbic acid, flavonoids and polyphenols (Nadeem *et al.*, 2011). Vitamin C is a potent water-soluble antioxidant which helps protect from scurvy, develop resistance against infectious agents (boosts immunity), and scavenge harmful, pro-inflammatory free radicals from the body. Vitamin-A and flavonoids like β -carotene, α -carotene, lutein, zeaxanthin, and cryptoxanthin help protect the body from injurious effects of free radicals generated during stress and disease conditions (Costa *et al.*, 2010). Pepper contains a good amount of minerals like potassium, manganese, iron and magnesium. Pepper is also a good source of the B-complex group of vitamins such as niacin, pyridoxine (vitamin B-6), riboflavin and thiamin (vitamin B-1) essential in the normal

development of the body. Hot pepper is undeniably the chief source of capsaicin—a dominant compound responsible for the pungency and heat (Fett, 2003).

2.5.1 Capsaicin

There are many varieties of carotenoids in pepper fruits and these have been noted to confer some antioxidant properties to it. The presence of these polyphenols is often attributed to the red, yellow or orange colour of pepper. Some authors have stated that lycopene – probably the world’s most potent antioxidant – is responsible for the red colour of some pepper varieties (Simmone *et al.*, 1997). In fact, they are attributed to the capsacinoids capsanthin (about 60%) and capsorubin, red carotenoids that give mature pepper fruits their red colour (Shin-Lin *et al.*, 2014; Francis *et al.*, 2008).

Capsaicin is a powerful and stable alkaloid seemingly unaffected by cold or heat, which retains its original potency despite time, cooking or freezing. The precise amount of capsaicin present in *Capsicum* spp . can only be measured by a specialised laboratory procedure known as high performance liquid chromatography (HPLC) (Shin-Lin *et al.*, 2014) as it has no flavour, colour or odour.

Although, it has no odour or flavour, it is one of the most pungent compounds known, detectable to the palate in dilutions of one to seventeen million. It is slightly soluble in water, but very soluble in alcohols, fats and oils. Capsaicin and its co-compounds are used for their astringent, counter-irritant and analgesic properties in the preparation of ointments, rub and tinctures. These formulations have been in use in the treatment of

arthritic pain, post-herpetic neuropathic pain, sore muscles, etc. Scientific studies on experimental mammals suggest that capsaicin has anti-bacterial, anti-carcinogenic, analgesic and anti-diabetic properties. It has also been found to reduce cholesterol levels in obese persons (Francis *et al.*, 2008).

2.6 Pepper Breeding

Pepper breeding 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. They have been used primarily to introgress genes that confer disease resistance into sweet blocky types (Caranta *et al.*, 1997; Daubeze *et al.*, 1995). Recently, the potential of wild species germplasm as a source of valuable yield and quality genes has been demonstrated in tomato (*Lycopersicon* sp.) and rice (*Oryza sativa*) through the use of molecular markers (Bernacchi *et al.*, 1998; Xiao *et al.*, 1996). These studies presented evidence that wild species, although inferior to modern cultivars, have favorable genes that are masked by other deleterious ones, but are capable of improving yield and quality of elite cultivars after being introgressed into them. In such cases, inter-specific hybridisation has to be embarked upon (Hajjar and Hodgkin, 2007). Inter-specific

hybridisation 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 hybridisation between two species can sometimes result in embryo abscission due to post-fertilisation 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. However, embryo rescue techniques are used to save partially cross-compatible species, during crosses (Monteiro *et al.*, 2011).

The production of a hybrid seed is one major economically sound breeding approach. Hybrid vegetable seeds which produce good quality high yielding plants are obtained through hand emasculation which is usually 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 avert self-fertilisation in several species in the production of hybrid seeds (Berke, 2000). Much breeding work has been performed on sweet pepper in temperate regions. Many cultivars, at present mostly F₁ hybrids, are commercially available for glasshouse and field production. *Capsicum* shows rather strong heterosis effects for plant growth traits and yield. The use of molecular markers and doubled haploids is quite common (Park *et al.*, 2009).

2.7 Gains from Pepper Improvement

2.7.1 Pepper Domestication and Varietal Improvement

Domestication is defined as the selection of wild plants and animals for adaptation to cultivation and human use, involving usually the selection of beneficial alleles at a collection of loci underlying yield and quality of the cultivated plant compared to its wild relative (Paran and van der Knaap, 2007). Domestication as well as diversity studies in pepper have brought about careful selection of useful traits that have ensured the continued existence of the crop throughout the world. Pepper breeding has focused on addressing consumer needs, such as degree of pungency, colour, taste, fruit shape and thickness of wall and ability to dry (powdered pepper) (Bosland and Votava, 2000). Different genetic combinations could be created within a diverse population to serve as raw materials upon which diverse genetic combinations are generated to stand the test of climate change, new diseases and pests resurgence (AVRDC, 1993).

Hot chile pepper was one of the first plants domesticated in the Americas. In the early days of cultivation, chile was used mainly for seasoning and as a medicinal plant whose effect was attributed to the pungency or hotness of the fruit. Today, peppers are consumed fresh or processed as vegetables and spice. The wild progenitor of *C. annuum* is thought to be the bird pepper, whose domestication occurred in Mexico (Eshbaugh, 1993). The fruit of wild bird pepper is small (about 1 cm in length), erect, red-coloured, pungent (hot), deciduous (falls off the plant when ripe), and soft-fleshed. These traits contribute to good adaptation for seed dispersal by birds (Paran and van der Knaap, 2007). Moreover, capsaicin, the secondary metabolite responsible for pungency in chile

pepper, has been shown to discourage herbivores, but has no repelling effect on more beneficial seed dispersers such as birds (Paran and van der Knaap, 2007; Tewksbury and Nabhan, 2001).

Two of the key traits that were selected during domestication of pepper were non-deciduous fruit that remained on the plant until harvest and the change in position from erect to pendant fruit. This latter change may be associated with an increase in fruit size, better protection from sun exposure and predation by birds. Other changes associated with domestication and varietal improvement were fruit appearance and reduced pungency. While wild peppers can be found in several basic shapes including oval, spherical, or elongated, continued selection resulted in a large increase of shape variation and increases in fruit mass. Selection also resulted in yellow, orange and brown fruit colours in addition to the wild-type red, which occurs in all cultivated pepper species. Lastly, another important selection was that of non-pungent fruits (Allard, 1960). Today, fresh non-pungent peppers, generally of the bell-types, are economically the most important peppers and are part of the human diet throughout the world (Bosland and Votava, 2000). Non-pungent peppers mostly belong to *C. annuum*. However, sources of non-pungency can be found in the other pepper species (Votava and Bosland, 2002; Bosland and Votava, 2000)

2.7.2 Fruit Weight

The progenitor species of pepper and tomato bear fruit of much smaller size compared with the cultivated one and thus, increased fruit size was a major selection criterion in

both species. Fruit weight is a quantitatively inherited character and is controlled by many genetic loci, some with a large effect and others with a minor effect (Paran and van der Knaap, 2007). The quantitative inheritance of fruit weight has made it challenging to identify the underlying genes, despite extensive studies into the genetics of the trait in tomato and pepper as well as other Solanaceous fruit crops (Ben-Chaim *et al.*, 2006; Doganlar *et al.*, 2002; Grandillo *et al.*, 1999). Several fruit weight QTLs were detected in crosses of large blocky cultivars with small-fruited accessions (Ben-Chaim *et al.*, 2006).

To date, none of the genes underlying fruit size in pepper have been identified. However, the use of molecular markers that are shared between tomato and pepper allowed comparison of QTL locations in these two species (Ben-Chaim *et al.*, 2006; Zygier *et al.*, 2005). On pepper chromosome 2, a single major QTL, *fw2.1*, maps in the syntenic region as *fw2.1* of tomato (Ben-Chaim *et al.*, 2006). By contrast, the locus syntenic to *fw2.2* does not have an effect on fruit weight variation in pepper, albeit minor. On pepper chromosome 4, two fruit-weight QTLs, *fw4.1* and *fw4.2*, are present. The latter QTL, *fw4.2* maps to the syntenic location of *fw4.2b* in tomato (Monforte *et al.*, 2001).

2.7.3 Fruit Shape

Like fruit size, shape is also a quantitative inherited character. However, whereas a major fruit weight QTL such as *fw2.2* can contribute up to 30% of the variance in certain populations, major shape QTL can contribute as much as 67% of the variance (Brewer *et al.*, 2007), which is particularly helpful when the map-based cloning of the underlying gene is concerned. The genetic analyses of elongated fruit shape in pepper identify

several QTLs that control this trait. Two major QTLs, *fs3.1* and *fs10.1* that account for up to 67% and 44% of the phenotypic variation, respectively, are detected in multiple populations (Ben Chaim *et al.*, 2001). Unlike the high level of conservation of QTLs controlling fruit weight in tomato and pepper, only one pepper elongated fruit shape QTL, *fs8.1*, was found in common genomic positions in both species (Ben Chaim *et al.*, 2006). The lack of common loci that control fruit shape in pepper and tomato may reflect differences in organ structure and development in the two species.

In tomato fruit, the seeds are surrounded by a gelatinous and juicy matrix (gel) whereas in pepper fruit, the seeds are in a hollow and dry area of the fruit (Ben Chaim *et al.*, 2003). In addition, the placenta to which the seeds are attached forms a central column in the tomato fruit (the septum), whereas in pepper, the placenta is attached to the pericarp or valves of the fruit and a central column is lacking (Paran and van der Knaap, 2007). An alternative explanation is that the shape of the fruit can be perturbed by many genes, only a few of which were selected in each crop species. It is known that shape features are more pronounced in larger fruit (Van der Knaap and Tanksley, 2003). Therefore, selection for interesting and novel shapes could only occur after alleles conferring larger fruits were fixed in the population. Thus, this could mean that the shape features exhibited by both species are the result of different trajectories during the last several hundred years of crop improvement (Paran and van der Knaap, 2007).

2.7.4 Fruit Colour

To a large extent, the variation in colour of pepper fruit is controlled by mutations in the enzymes of the carotenoid biosynthetic pathway. These mutations give rise to easy scorable phenotypes which greatly facilitates the identification of the underlying genes (Wann, 1997). Moreover, unlike fruit shape and size, for which the biochemical pathways leading to the trait variation are largely unknown, carotenoid biosynthetic proteins can often be predicted based on biochemical studies (Blum *et al.*, 2003). The wild-type red colour of the mature fruit of pepper results from the accumulation of carotenoid pigments. Green unripe fruits contain chlorophyll and carotenoid pigments such as lutein, b-carotene, and violaxanthin, which are also present in leaves (Rao and Paran, 2003). Upon ripening, the chloroplasts are converted into chromoplasts giving rise to the red colour of the ripening fruit. While the red tomato colour is due to the accumulation of lycopene, the red pepper colour results from the accumulation of the xanthophylls capsanthin and capsorubin (Paran and van der Knaap, 2007). These xanthophylls are products that are downstream of lycopene. Consequently, the differences in the carotenoid biosynthesis pathway in chromoplast-containing tissues resulted in the selection of different genes controlling colour variation in pepper and tomato (Paran and van der Knaap, 2007).

Whereas in tomato the yellow colour is thought to result from low amounts of yellow carotenoids such as lutein (which are normally found in green tissues, and from flavonoids in the skin), the yellow pepper fruit colour is the result of a mutation in an entirely different enzyme in the carotenoid biosynthesis pathway (Paran and van der

Knaap, 2007). The yellow colour is recessive to red and is controlled by the Y locus. Linkage analyses showed that Y co-segregates with the gene coding for capsanthin capsorubin synthase (CCS) that is responsible for the synthesis of the red carotenoid pigments capsanthin and capsorubin (Lefebvre *et al.*, 1988). However, orange fruit colour of pepper can result from the accumulation of other carotenoids such as β -carotene and zeaxanthin as major pigments. However, detailed genetic analysis of the loci controlling this variation has not yet to be conducted.

2.7.5 Ripening

Fruit ripening involves many biochemical processes leading to the production of carotenoids, aroma compounds, sugars, and fruit softening. Ripening in pepper is non-climacteric (Paran and van der Knaap, 2007). In contrast, tomato fruit ripening is climacteric- it is characterised by a burst of respiration at the beginning of the process accompanied by the production of ethylene. In pepper, two ripening-related traits played a significant role during the domestication of this crop (Paran and van der Knaap, 2007). Plants are selected for reduced deciduousness and softness of fruit, which are both characteristics of wild pepper. These characters are controlled by a single locus, *S*. A candidate gene approach led to the identification of the *S* gene as the pepper homologue of the tomato fruit endopolygalacturonase (*PG*), as tomato *PG* mapped to the *S* in pepper (Rao and Paran, 2003). The tomato *PG* gene codes for a cell-wall modifying enzyme that has a role in changing the texture of the tomato fruit during ripening. In the pepper-mapping population, the soft flesh and deciduous fruit phenotypes were observed

together in all segregating individuals, indicating a pleiotropic effect of *PG* on these two traits. Expression of *PG* was detected at the ripening stage in the fruit of wild pepper but not in the non-deciduous cultivars (Paran and van der Knaap, 2007).

2.7.6 Pungency

Pungency results from the accumulation of the capsaicinoid alkaloids in the placenta of the fruit, and is unique to the *Capsicum* genus. The presence or absence of pungency is controlled by one locus, *Pun1* (formerly *C*) (Stewart Jr *et al.*, 2005). The candidate gene underlying *Pun1* was identified from genes that were differentially expressed in pungent versus nonpungent fruits. This candidate gene, *AT3*, encodes a protein with high homology to an acyltransferase and is tightly linked to *Pun1* (Stewart Jr *et al.*, 2005). Furthermore, all non-pungent accessions of *C. annuum* examined to date carry the recessive allele which contains a deletion spanning the promoter and first exon of the *AT3* gene. Moreover, virus-induced gene silencing of *AT3* resulted in reduced levels of capsaicinoids. Thus, *AT3* is very likely to underlie *Pun1*. Moreover, the wide distribution of the deletion in *AT3* across *C. annuum* indicates that it occurred early in the domestication of this species. The mechanism by which *AT3* controls pungency is unknown. It is possible that *AT3* is capsaicin synthase, the last enzyme in the capsaicinoid biosynthetic pathway, postulated to be an acyltransferase. Another gene, *CSY1*, is also suggested to be a candidate for capsaicin synthase despite its lack of similarity to acyltransferases (Prasad *et al.*, 2006). Pungent peppers differ greatly in their capsaicinoid content. A major QTL, *cap*, which controls this variation was detected on chromosome 7

(Blum *et al.*, 2003). This locus may represent a regulator of the pathway (Paran and van der Knaap, 2007) as the known capsaicinoid biosynthesis genes do not colocalise with *cap*.

2.7.7 Plant Architecture

For most crop species, plant growth habit changed dramatically as a result of domestication. This phenomenon is perhaps best described in corn where the wild progenitor teosinte exhibits branched shoots compared with modern corn which displays increased apical dominance (Clark *et al.*, 2006). Contrary to the corn shoot which is monopodial, the pepper shoot is sympodial, displaying alternate vegetative and reproductive phases. There is substantial variation in the extent of vegetative growth and axillary branching in the cultivated germplasm pool. However, very little is known about the genetic inheritance of this trait despite the fact that breeders select for increased reproductive and reduced vegetative growth. While the strategy of selecting against vegetative growth (resulting in less photosynthates for the developing fruit) often results in reduced total yield, this negative effect is easily offset by the ease of plant care, and reduction in space and nutrient requirements (Paran and van der Knaap, 2007).

2.8 Germplasm Collection, Characterisation and Conservation

Collection, selection and evaluation of germplasm is a basic step in every breeding programme (Doku *et al.*, 2013). 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). The availability of well characterised plant genetic resources is an important pre-requisite for crop improvement and genetic research (Roch *et al.*, 2010). 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 (Aktas *et al.*, 2009). In order to preserve the integrity and potency of seed samples, it is required that the whole spectrum of genetic diversity is preserved on a long term basis while, at the same time, sufficient amounts of seeds for potential use is preserved. The USDA has established a pepper gene bank from accessions collected worldwide (Wilcove and Master, 2008). The World Vegetable Center has collections in Bamako (Mali), Arusha (Tanzania), and in Shanhua (Taiwan) (AVRDC, 1993). Gene bank collections available in West Africa also include that of the Nigerian National Horticultural Research Institute in Ibadan (AVRDC, 1993).

Characterisation of collected germplasm helps breeders in selecting suitable parents for crossing experiments to develop new varieties. It eliminates duplication among closely related individuals to obtain a core collection of individuals which are different genetically. It also ensures that only genetically distinct genotypes are maintained helping to save time, space and money in germplasm conservation and maintenance (Yada *et al.*, 2010).

There have been private, national and international developments since the 20th century to collect, maintain and characterise genetic resources for most cultivated plant species. Characterised germplasm could be conserved in their natural habitats (*in-situ*) or in gene banks and tissue culture (*ex-situ*) indispensable for the continued survival of these germplasm (Se-Jong *et al.*, 2012). Vegetable species, of which pepper is part, are conserved using seed preservation. This is the most effective method of keeping large numbers of accessions and viability. It promotes the establishment of healthy seedlings as well as reduces the threat of genetic erosion by conserving the genetic diversity acquired through germplasm assembling (Ellis *et al.*, 1991).

The World Vegetable Center (AVRDC) plays a major role in the conservation and distribution of vegetable germplasm held in the public domain. The Centre holds more than 67,800 accessions of vegetable germplasm comprising 170 genera and 436 species from 156 countries of origin. With a total of 8,165 accessions, AVRDC's *Capsicum* collection is the world's largest held by a single institution, comprising about 11% of all accessions held globally (AVRDC, 1993). 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 Karatawinata, 1975).

2.9 Genetic Erosion

Central to the establishment of gene banks and other strategies to conserve plant genetic resources has been the concept of genetic erosion: the loss of variation in crops (van de Wouw *et al.*, 2009). Genetic diversity gives species the ability to adapt to changing

environments, including new pests and diseases and new climatic conditions which will allow them to respond to the challenges of the next century (Hammer *et al.*, 1999). World population is expected to increase by 2.6 billion in the next 45 years and 9.1 billion in 2050. The world needs astonishing increase in food production to feed this population. Plant genetic resources (PGR) constitute the foundation upon which agriculture and world food securities are based and the genetic diversity in germplasm collections is critical to the world's fight against hunger. Brockhaus and Oetmann (1996) defined PGR as “plant material with a current or potential value for food, agriculture and forestry”. They are the raw material for breeding new plant varieties and are a reservoir of genetic diversity (Hammer and Teklu, 2008).

Genetic erosion may be caused by factors such as urbanisation and modern agricultural practices including use of fertilisers, mechanisation, abandonment of marginal lands and crop specialisation. These could lead to loss of landraces as the habitat to which the landrace is adapted is no longer used or does no longer exist. Climate change and environmental degradation can also result in changed cropping patterns and disappearance of traditional varieties. Changes in food preferences of a growing urban population and a decreasing demand for local products may also add to the loss of diversity. Furthermore, natural disasters or human conflicts, which result in a large-scale displacement of farmers, can lead to loss of agricultural diversity that was used by the farmers involved (Richards and Ruivenkamp, 1997). Genetic uniformity leaves a species vulnerable to new environmental and biotic challenges and causes heavy damage to the society.

2.10 Morphological Characterisation

Plant species recognition has long been based on morphological characters. Plant morphology is highly polymorphic and phenotypic characters may, in principle, allow plant species taxonomy (Duminil and Di Michele, 2009). However, different individuals of the same species may present a variation in their morphology either naturally or in connection with local adaptations. Plant morphology represents a study of the development, form, and structure of plants, and, by implication, an attempt to interpret these on the basis of similarity of plan and origin (Bold *et al.*, 1987). There are four major areas of investigation in plant morphology, and each overlaps with another field of the biological sciences.

2.10.1 Comparative Morphology

Morphology is comparative, meaning that the morphologist examines structures in many different plants of the same or different species, then draws comparisons and formulates ideas about similarities (Raven *et al.*, 2005). When structures in different species are believed to exist and develop as a result of common, inherited genetic pathways, those structures are termed homologous. For example, the leaves of pine, oak, and cabbage all look very different, but share certain basic structures and arrangement of parts. The homology of leaves is an easy conclusion to make (Sattler, 1994). The plant morphologist goes further, and discovers that the spines of cactus also share the same basic structure and development as leaves in other plants, and therefore cactus spines are homologous to

leaves as well (Sattler, 1994). This aspect of plant morphology overlaps with the study of plant evolution and paleobotany (Jeune *et al.*, 2006).

2.10.2 Vegetative and Reproductive Structures

Plant morphology observes both the vegetative (somatic) structures of plants, as well as the reproductive structures. The vegetative structures of vascular plants includes the study of the shoot system, composed of stems and leaves, as well as the root system. The reproductive structures are more varied, and are usually specific to a particular group of plants, such as flowers and seeds, fern sori, and moss capsules. The detailed study of reproductive structures in plants led to the discovery of the alternation of generations found in all plants and most algae. This area of plant morphology overlaps with the study of biodiversity and plant systematics (Kirchoff *et al.*, 2008).

2.10.3 Pattern of Development

Plant morphology examines the pattern of development, the process by which structures originate and mature as a plant grows. While animals produce all the body parts they will ever have from early in their life, plants constantly produce new tissues and structures throughout their life. A living plant always has embryonic tissues (Bäurle and Laux, 2003). The way in which new structures mature as they are produced may be affected by the point in the plant's life when they begin to develop, as well as by the environment to

which the structures are exposed. A morphologist studies this process, the causes, and its result. This area of plant morphology overlaps with plant physiology and ecology.

Morphological descriptors provide essential information on genotypes by giving correct species identification (Dias *et al.*, 2013). They render simple and straight-forward approaches to distinguishing different genotypes even at the farm level. They do not require special skills in most cases and are readily available. It provides the only means of differentiation based on physical appearance. Diversity studies have led to the selection of cultivars with bigger fruits that are less pungent (Paran and van der Knaap, 2007). 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).

Morphological characterisation serves as a powerful tool in the classification of cultivars as well as study their taxonomic status (Rogers and Appan, 1973), and a very useful means of bringing to light traits of agronomic importance especially quantitative traits for crop improvement (Geleta *et al.*, 2005). Morphological and agronomical characters have been useful to evaluate local pepper collections in many countries. In the last century, chromosome morphology (Pickersgill, 1997) has been used to study genetic diversity within the genus *Capsicum*.

Earlier, diversity in *Capsicum* was studied using morphological, cytological and biological markers (Gopinath *et al.*, 2006; Pickersgill, 1988; Heiser, 1976). Conventionally morphological markers called descriptors are used for varietal identification and genetic diversity analyses in plants. They demand collection of extensive data at different

locations. Some descriptors are often considered as useful for assigning accessions to species of the genus. Hence, Baral and Bosland (2004) used morphologic descriptors to verify species assignment at the germplasm repository for the *C. chinense* and *C. frutescens* accessions they studied. Their study confirmed the usefulness of morphological descriptors in species classification, though not for all descriptors, but only two (calyx constriction and flower position). Fonseca *et al.* (2008) also assigned successfully 100% of the *C. chinense* accessions they studied to their corresponding species at the germplasm repository using mainly inflorescence characters. More recently, these results were confirmed by Ortiz *et al.* (2010) who reported the efficacy of inflorescence descriptors and seed colour to reliably distinguish among *Capsicum* species.

The level of polymorphism for morphological characteristics in elite germplasm is sometimes too limited and inadequate to allow for genotype discrimination (Geleta *et al.*, 2005). Taxonomy of *Capsicum* is confusing and it is sometimes difficult to identify accessions using only subjective morpho-agronomic data (Costa *et al.*, 2009). The use of DNA markers has opened up new opportunities and enhanced the efficiency of characterisation of accessions as well as bringing significant advancement in crop improvement programmes (Legesse *et al.*, 2007).

2.11 Molecular Characterisation

Molecular characterisation is the use of genetic traits to characterise germplasm. This method makes use of genetic markers which identify genes at given locus (Stansfield,

1986). DNA marker analysis has been suggested for the determination of genetic diversity among genotypes (Gilbert *et al.*, 1999). Molecular characterisation is very important in differentiating between closely related genotypes with accurate results due to its ability to recognise specific DNA sequences in organisms (Rocha *et al.*, 2010).

Molecular techniques related to the seedling, plant and stem, inflorescence, the fruit and seed are useful in identifying quantitative trait loci which are of agricultural importance (Rocha *et al.*, 2010). They are able to distinguish differences in nucleotide sequences irrespective of the growth stage, time, place and agronomic practices (Kwon *et al.*, 2002). Information obtained from genetic variability assessed from different DNA marker technologies should offer plant breeders ideas to address different needs of crop improvement programmes and germplasm resources conservation (Tam *et al.*, 2005).

2.11.1 Molecular Markers

Over the last decade, different molecular markers have been developed to aid plant breeding of crops. Molecular markers might play an increasingly important role in the evaluation, conservation and use of diversity in germplasm and varieties in the future. As they facilitate the purposeful utilisation of plant genetic resources (PGR), they can support efforts to broaden the genetic base of crop plants and to ensure diversity at all levels. Various markers have already been developed, with new and simple systems being designed continually. Genetic diversity of *Capsicum* has been analysed using Restriction Fragment Length Polymorphism (RFLP) (Lefebvre *et al.*, 1993); Amplified Fragment Length Polymorphism (AFLP) (Aktas *et al.*, 2009); Randomly-Amplified Polymorphic

DNA (RAPD) (Adetula, 2006); and Microsatellites or Simple Sequence Repeats (SSRs) (Pacheco-Olvera *et al.*, 2012; Stágel *et al.*, 2009; Portis *et al.*, 2007).



2.11.2 Restriction Fragment Length Polymorphism (RFLP) Markers

With the help of RFLPs, variation between different individuals or accessions can be made visible by comparing DNA sequences at the same loci in different individuals. RFLPs are detected by cutting genomic DNA with restriction enzymes (Brumlop and Finckh, 2010). Each of these enzymes has a specific recognition sequence which is typically palindromic and which leads to restriction fragments of certain length when the DNA is digested. Changes within these sequences which can be caused by point mutations, insertions or deletions, result in DNA fragments of differing length and molecular weights (Brumlop and Finckh, 2010). These fragments are size-separated with agarose gel electrophoresis and analysed by Southern blots using either locus-specific or multilocus probes. The former recognise one or a few specific regions of the genomic DNA, the latter recognise tandemly repeated DNA motifs such as microsatellites. The two main advantages of RFLP markers are co-dominance and high reproducibility. Disadvantages are the requirement of relatively large amounts of pure and intact DNA and the tedious experimental procedure (Edwards and Mccouch, 2007; Weising *et al.*, 2005). The application of RFLPs for genetic diversity studies is limited because it requires the use of radioactivity and is labour intensive (Nahm *et al.*, 1997). AFLPs and RAPDs identify only dominant alleles and are sensitive to PCR amplification.

2.11.3 Randomly-Amplified Polymorphic DNA (RAPD) Markers

RAPD markers are based on the PCR amplification of random DNA segments with single, typically short primers of arbitrary nucleotide sequence (Williams *et al.*, 1990). The primers bind to complementary sample DNA sequences and where two primers bind to the sample DNA in close enough proximity for successful PCR, a stretch of the DNA is amplified. The DNA amplification products are visualised by gel electrophoresis. No prior knowledge of the DNA sequence is needed (Brumlop and Finckh, 2010) as the primers are arbitrarily chosen. The genome is expected to be sampled randomly and the technology is especially useful if loci across an entire genome are to be assayed. A disadvantage of RAPD markers is the fact that the polymorphisms are detected only as the presence or absence of a band of a certain molecular weight, with no information on heterozygosity (Crouch, 2000). Besides being dominantly inherited, RAPDs also show some problems with reproducibility of data. Their major advantages are the technical simplicity and the independence of any prior DNA sequence information (Edwards and Mccouch, 2007; Weising *et al.*, 2005).

2.11.4 Amplified Fragment Length Polymorphism (AFLPs)

The AFLP technique combines elements of RFLP and RAPD, based on the selective PCR amplification of restriction fragments. In a first step, genomic DNA is digested and oligonucleotide adapters (defined short oligonucleotide sequences) are ligated to both ends of the resulting restriction fragments. In a second step the fragments are selectively amplified, using the adapter and restriction site sequences as primer binding sites for subsequent PCR reactions (Erwood and Truchi, 2009). As the 3' ends of the primers

extend into the restriction fragments by 1 to 4 bp, only those fragments are amplified, whose ends are perfectly complementary to the 3' ends of the selective primers (Mohan *et al.*, 1997). Thus, only a certain portion of the restriction fragments is amplified. In the last step the amplified fragments are resolved by gel electrophoresis and visualised by either autoradiography, silver staining or fluorescence, resulting in a unique reproducible fingerprint for each individual (Crouch and Ortiz, 2004).

Possible reasons for AFLP-Polymorphisms are (i) sequence variations in a restriction site (the same as in RFLPs), (ii) insertions or deletions within an amplified fragment (also the same as in RFLPs) and (iii) differences in the nucleotide sequence immediately adjoining the restriction site (not detected with RFLPs) (Ashraf *et al.*, 2008). Thus, the usage of AFLP technologies results in the detection of higher levels of polymorphisms compared with RFLPs. AFLPs also have a much higher multiplex ratio (more markers per study) and better reproducibility than RAPDs. However, AFLPs require greater technical skill and as they require the use of polyacrylamide gels for detection with equally larger investments in equipment. On the whole, AFLP markers allow the rapid generation of highly replicable markers, thus permitting high-resolution genotyping of fingerprinting quality (Bernardo, 2008). A drawback can be that, most AFLP markers are dominant rather than co-dominant, due to the complex banding patterns. In some cases, the scoring of AFLP polymorphisms as co-dominant marker loci is possible, because, for a single character, diploid homozygous individuals cause a more intense peak than heterozygous individuals. Specialised algorithms and software packages that are capable of finding such markers and scoring them co-dominantly have been developed (Meudt and Clarke, 2007).

2.11.5. Microsatellite Markers

Microsatellites are also known as simple sequence repeats (SSRs) and the resulting markers are variously called simple sequence length polymorphisms (SSLPs), sequence-tagged microsatellite sites (STMS), SSR markers or microsatellite markers. SSRs are DNA stretches, consisting of tandemly repeated short nucleotide units (1-5 bases per unit). Such repeats are distributed throughout the genomes of all eukaryotic species (Brumlop and Finckh, 2010). In microsatellite analysis, sequence information of the regions flanking the repeats is used for creating locus specific PCR primer pairs. The resulting amplification products are separated on polyacrylamide gels and visualised (Edwards and Mccouch, 2007). The differences in the numbers of repeated units cause differences in band size, which are locus-specific, co-dominantly inherited and highly polymorphic. The technique reveals allele size differences even of a single base pair. A further advantage is the fact, that microsatellite markers can easily be distributed between labs by sharing primer sequences (Weising *et al.*, 2005; Nybom, 2004).

Expressed sequence tag (EST) sequencing projects have provided sequence data available in online databases and can be scanned for identification of SSRs, so called EST-SSRs or genic microsatellites (Brumlop and Finckh, 2010). Genic SSRs are quickly obtained by electronic sorting and have an expected high transferability because the primers are derived from conserved coding regions of the genome. This makes genic microsatellites a useful tool in characterisation of genetic variation within natural populations or between breeding lines. Especially, because the variation in transcribed genes with known function can be assessed, genic microsatellites are expected to enhance the role of genetic markers in evaluating germplasm (Varshney *et al.*, 2005).

SSR markers were used in this study due to their numerous advantages over the other PCR-based markers in genetic diversity studies. They are locus-specific and co-dominant in nature and provide 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 (Weising *et al.*, 2005; Powell *et al.*, 1996).

The only disadvantage 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). The biochemical and molecular techniques of UPOV has identified SSR as the most widely used marker system for plant variety studies characterisation (UPOV-BMT, 2002). SSR markers have been developed for pepper (Se-Jong *et al.*, 2012; Jang *et al.*, 2004; Moon *et al.*, 2003; Lefebvre *et al.*, 2001; Paran *et al.*, 1998; Kang *et al.*, 1997; Prince *et al.*, 1992). These markers have been proven to be useful in assessing genetic diversity and phylogeny, characterisation of germplasm and to detect duplication, parental verification in crosses, gene tagging in marker assisted breeding and gene cloning in genetic transformation (Costa *et al.*, 2006).

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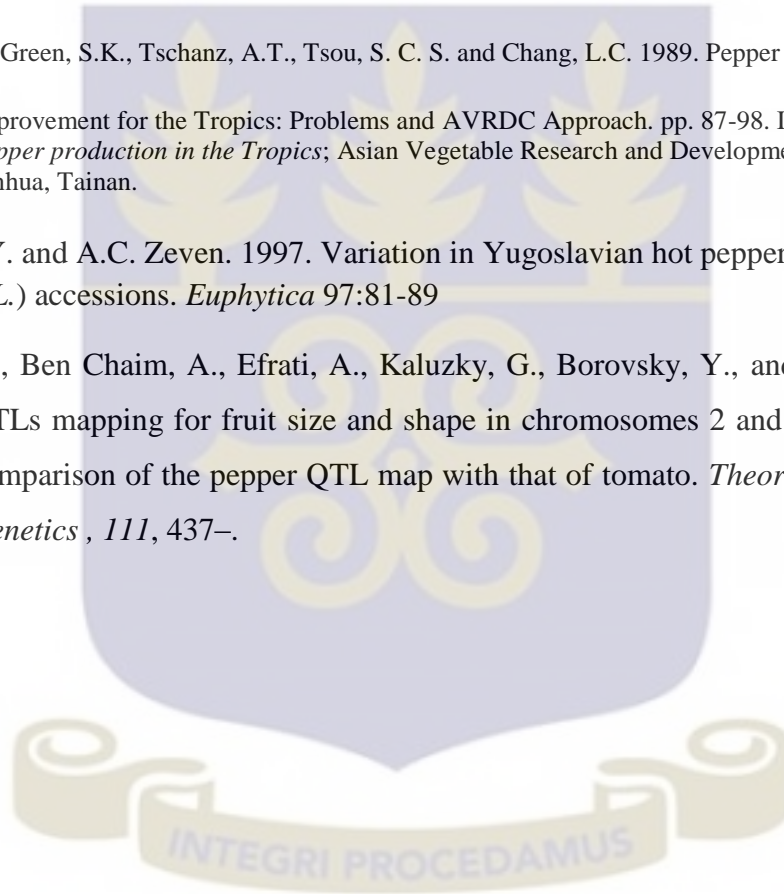
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CHAPTER THREE

AGROMORPHOLOGICAL CHARACTERISATION OF TWENTY ACCESSIONS OF HOT PEPPER (*Capsicum spp L.*).

3.1. Introduction

The general term used for the study of the developmental forms and structure of plants is plant morphology, useful in plant identification. Plant breeders have used morphological traits in characterising pepper for decades by the help of descriptors. Several accessions of *Capsicum* have been morphologically characterised, using descriptors according to the guidelines from the International Plant Genetic Resources Institute (IPGRI, 1995).

The descriptor list shows a set of individual traits that are attributed to a particular species and ways to measure them. It contains information on germplasm from registration, characterisation and evaluation, management and uses of the plant. The IPGRI descriptors include 79 phenotypic traits divided into 25 vegetative, 16 inflorescence, 22 fruit, 6 seed and 10 yield and quality characters (Theresa *et al.*, 2013). The descriptor list serves as a standard for data collection on morphological traits and it is useful as basis for botanical classification of plant species. Morphological characterisation is the only means of differentiation based on physical appearance. It is very useful in bringing to light traits of agronomic importance especially quantitative traits for crop improvement (Geleta *et al.*, 2005). Even though morphological characterisation is important in genotype identification, its application is highly influenced by prevailing environmental factors (Geleta *et al.*, 2005; Gepts, 1993) making the results location specific. Its major advantages are that the traits are observed by the farmers at the farm level making it possible for evaluation of the performance of the crop in different environments.

In Ghana, Quartey *et al.* (2014) used phenotypic characters to assess the agronomic performance of eight pepper genotypes (six exotic and two local genotypes) under rain fed conditions. The result indicates that exotic hybrid varieties matured earlier than the local genotypes. They also performed better in terms of fruit weight, fruit length and yield. Nkansah *et al.* (2011) evaluated the performance of bird's eye pepper for their morphology, growth and yield in two ecological zones in Ghana. The results indicate significant differences in growth parameters as well as yield and yield components of the four cultivars in both seasons. Agyare (2013) classified 50 genotypes of pepper based on their phenotypic and molecular attributes. The results indicate substantial variation among the 50 accessions at both morphological and molecular level especially in fruit traits.

These researchers used fewer local cultivars in their work. They did not analyse the nutritional content of the pepper genotypes used very few of them used their molecular attributes in the classification. In this study, morphological traits will be used to characterise the nineteen local and one exotic pepper varieties.

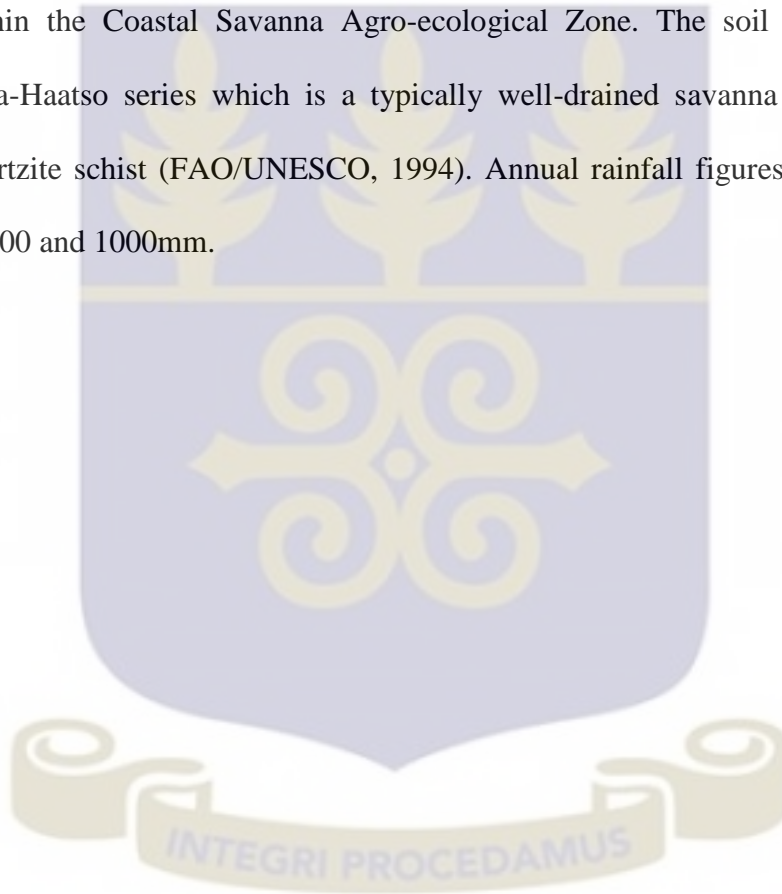
3.2. Objective of the Study

The objective of the study was to characterise 20 accessions of indigenous cultivars of hot pepper and their wild relatives collected from eight geographical regions of Ghana using morphological traits to identify genotypes with desirable characteristics for future breeding work.

3.3. Materials and Methods

3.3.1. Experimental Site

The study was conducted at the research farms of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC). The field is located at 05 40'N, 013'W with an elevation of 76 meters above sea level within the Coastal Savanna Agro-ecological Zone. The soil at the site is the Nyigbenya-Haatso series which is a typically well-drained savanna Ochrosol derived from Quartzite schist (FAO/UNESCO, 1994). Annual rainfall figures in the area range between 700 and 1000mm.



3.3.2 Collection of Germplasm

Twenty accessions of hot pepper were collected from eight regions of Ghana for the study as indicated in Table 1 below.

Table 3.1: Accessions of hot pepper (*Capsicum* spp L.) used in the study.

| Accession (Code) | Region | Locality | Type |
|------------------|---------------|---------------|-------------------|
| Acc 01 | Greater Accra | Osu | Bird's eye |
| Acc 02 | Greater Accra | Dome | Bird's eye |
| Acc 03 | Greater Accra | Madina | Bird's eye |
| Acc 04 | Greater Accra | Makola | Bird's eye |
| Acc 05 | Greater Accra | Lapaz | Exotic |
| Ash 01 | Ashanti | Adanse | Bird's eye (wild) |
| Ash 02 | Ashanti | Assin Fosu | Bird's eye (wild) |
| Ash 03 | Ashanti | Obuase | Bird's eye (wild) |
| Bra 01 | Brong Ahafo | Techiman | Bird's eye (wild) |
| Eat 01 | Eastern | Akpamu | Bird's eye (wild) |
| Eat 02 | Eastern | Klo Agogo | Bird's eye (wild) |
| Nor 01 | Northern | Tamale | Bird's eye |
| Nor 02 | Northern | Tamale | Cherry |
| Nor 03 | Northern | Tamale | Bird's eye |
| Upp 01 | Upper West | Bole | Scotch bonnet |
| Vot 01 | Volta | Kpando | Bird's eye (wild) |
| Vot 02 | Volta | Kpando | Bird's eye (wild) |
| Vot 03 | Volta | Peki | Bird's eye (wild) |
| Vot 04 | Volta | Peki | Bird's eye (wild) |
| Wes 01 | Western | Bonsu Nkwanta | Bird's eye (wild) |

3.3.3. Experimental Plot and Design

A field was ploughed and demarcated into four main blocks (replicates) with 80 subplots of 3.0 m x 2.1 m in a Randomised Complete Block Design (RCBD). The planting distance used was 0.7 m x 0.5 m giving 24 plants per plot including border plants.

3.3.4. Agronomic Practices

Seeds of the 20 accessions were nursed on the 4th of August 2014 and transplanted one seed per hill on 15th September 2014. Weeding was done manually every two weeks till the fruits were harvested. Fertiliser starter solution of 20:20:20 of NPK at 7g/L was applied to seedlings at transplanting. Also NPK 15:15:15 at 220kg/ha was applied at 7 days and 220kg/ha of Sulphate of Ammonia was applied 14 days after the NPK application. Watering was done twice a week until the fruits were harvested.

3.3.5 Data Collection

Data were collected using the International Plant Genetic Resource Institute (IPGRI, 1995) descriptor list for *Capsicum* with little modification. Data were taken on four main growth stages: vegetative stage, inflorescence, fruit and seed, yield and quality characteristics were also determined.

- (a) **Vegetative Characteristics:** Data collected at this stage include; hypocotyl colour (HYC) and pubescence (HYP), cotyledonous leaf colour (CLC) and shape (CLS), cotyledonous leaf length (CLL) and width (CLW), life cycle (LIC), stem colour (STC), nodal anthocyanin (NOA), stem shape (STS), stem pubescence (STP), plant height (PLH), growth habit (GRH), canopy width (CAW), stem length (STL), stem branching habit (STB), leaf density (LED), leaf colour (LEC), leaf shape (LES), lamina margin (LAM), leaf pubescence (LEP), mature leaf length (MAL).

- (b) **Inflorescence Characteristics:** Data taken include; days to 50% flowering (DAF), number of flowers per axis (FLA), flower position (FLP), corolla colour (COC), corolla spot colour (CSS), corolla shape (COS), corolla length (COL), anther colour (ANC), anther length (ANL), filament length (FIL), filament colour (FIC), Calyx pigmentation (CAP), calyx annular constriction (CAC),
- (c) **Fruit Characteristics:** Data taken include; days to 50% fruiting (DFR), fruit colour at intermediate stage (FRS), fruit colour at maturity stage (FCM), fruit shape (FRS), fruit length (FRL), fruit width (FWD), fruit weight (FRW), fruit shape at pedicel attachment (SPA), neck at base of fruit (NBF), fruit shape at blossom end (SBE), number of locules (NUL), fruit surface (FRS), ripe fruit persistence (RFP), varietal mixture condition (VMC),
- (d) **Seed Characteristics:** Data taken include; seed colour (SEC), seed surface (SES), seed diameter (SED), hundred seed weight (HSW), number of seed per fruit (SPF).

3.3.6 Data Analyses

Data collected were analysed using One-way Analysis of Variance (ANOVA) and Tukey's Honestly Significant Difference Test (HSD) for mean separation. Data were also subjected to principal components analysis and correlation analysis to determine percentage contribution of measured traits to total genetic variance and the degree of association among the accessions. Cluster analysis based on city block similarity matrix was employed to obtain a dendrogram of the relatedness of the accessions. The General

Statistical Software Programme (Statistix, version 8, USA) and Microsoft Excel were used for all the data analyses.

3.4 Results

3.4.1 Variation in morphological characteristics of some accessions on the field.



Acc04



Upp 01

Plate 3.1: Variation in vegetative and reproductive growth of some accessions on the field



Eat 02



Wes 01



Nor 02



Ash 02



Acc 01



Vol01



Vol 02



Ash 03

Plate 3.1: Cont'd

3.4.2 Variability in 13 Quantitative Agromorphological Traits of *Capsicum* spp L.

Table 3.2 shows phenotypic variability in 13 quantitative traits among 20 accessions of hot pepper (*Capsicum* spp L.). The accessions exhibited statistically significant variation with respect to all thirteen quantitative traits. Wes 01 recorded the highest number of days to 50% flowering (44 days) and fruiting (57 days), stem width (2.85cm), and matured leaf length (3.42cm); while Nor 03 recorded the highest fruit length (4.60cm), number of seeds per fruit (38.5), least number of days to 50% flowering (25 days) and fruiting (37 days). Acc 03 recorded the highest plant height (68.17cm) with Ash 01 and Eat 01 recording the lowest plant height (21.50cm). Similarly, Acc 05 recorded the highest 100 seed and fruit weight , and the lowest number of seeds per fruit (15) and

also least fruit pedicel length. However, Bra 01 recorded the highest number of seeds per fruit (39) and the lowest 100-seed weight (0.325g). Upp 01 recorded highest fruit weight and Acc 04 the least fruit weight (0.32g).



Table 3.2: Variability in 13 Quantitative Agromorphological Traits of *Capsicum* spp L.

| Traits | Plant Height | Plant canopy width | Stem width | Matured Leaf Length | Matured Leaf width | Days to 50% Flowering | Days to 50% Fruiting | Fruit Length | Fruit Width | Fruit Weight | Fruit Pedicel Length | 100 Seed Weight | Number of seeds/Fruit |
|---------------|---------------------------|---------------------------|--------------------------|--------------------------|--------------------------|---------------------------|---------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|---------------------------|
| Acc 01 | 37.975 ^{bcde} | 53.500 ^{abc} | 2.175 ^{ab} | 6.850 ^{bc} | 3.075 ^{abc} | 26.250 ^{gh} | 38.500 ^{fgh} | 4.225 ^{ab} | 0.800 ^{cde} | 0.800 ^b | 3.075 ^{ab} | 0.500 ^{cde} | 24.500 ^{cdef} |
| Acc 02 | 33.250 ^{defgh} | 44.000 ^{bcd} | 2.100 ^{ab} | 3.225 ^{ij} | 2.300 ^{defg} | 28.500 ^{efgh} | 40.500 ^{defgh} | 2.250 ^e | 0.525 ^{efg} | 0.475 ^{cd} | 2.175 ^{defgh} | 0.750 ^{ab} | 24.250 ^{cdef} |
| Acc 03 | 68.175^a | 60.000 ^b | 2.025 ^{ab} | 5.625 ^{cde} | 2.875 ^{abcd} | 30.750 ^{def} | 43.000 ^{bcdef} | 4.000 ^{bc} | 0.750 ^{cdef} | 0.675 ^{bc} | 3.150^a | 0.700 ^{abc} | 18.500 ^{gh} |
| Acc 04 | 47.875 ^b | 54.500 ^{ab} | 2.075 ^{ab} | <u>2.325^j</u> | 1.675 ^{gh} | 27.250 ^{fgh} | 40.000 ^{efgh} | 1.925 ^{ef} | 0.575 ^{defg} | <u>0.325^d</u> | 1.775 ^{ghi} | 0.600 ^{abcd} | 20.250 ^{fg} |
| Acc 05 | 27.000 ^{fghi} | 26.250 ^{fgh} | 1.925 ^{ab} | 3.800 ^{hi} | 2.700 ^{abcde} | 27.750 ^{efgh} | 37.750 ^{gh} | 4.175 ^{bc} | 2.150 ^b | 1.325^a | <u>1.625ⁱ</u> | 0.800^a | <u>15.000^h</u> |
| Ash 01 | <u>21.500ⁱ</u> | 31.750 ^{fg} | 1.975 ^{ab} | 4.200 ^{fghi} | 2.075 ^{efgh} | 31.250 ^{def} | 43.000 ^{bcdef} | 1.700 ^{fg} | 0.500 ^{fg} | 0.300 ^d | 3.075 ^{ab} | 0.450 ^{de} | 21.250 ^{efg} |
| Ash 02 | 22.500 ⁱ | <u>20.500^h</u> | 2.075 ^{ab} | 3.950 ^{ghi} | 1.825 ^{fgh} | 28.750 ^{efgh} | 42.250 ^{cdefg} | 1.875 ^{ef} | 0.450 ^g | 0.350 ^d | 3.050 ^{ab} | 0.425 ^{de} | 22.000 ^{defg} |
| Ash 03 | 22.00 ⁱ | 60.500^a | 2.000 ^{ab} | 4.375 ^{efghi} | 1.775 ^{fgh} | 34.000 ^{cd} | 45.500 ^{bcd} | 1.700 ^{fg} | 0.475 ^{fg} | 0.350 ^d | 3.075 ^{ab} | 0.400 ^{de} | 37.000 ^b |
| Bra 01 | 23.875 ^{hi} | 36.000 ^{def} | 1.650 ^b | 4.800 ^{efgh} | 2.375 ^{cdefg} | 32.000 ^{cde} | 46.750 ^{bc} | 3.200 ^d | <u>0.450^g</u> | 0.400 ^d | 2.575 ^{bcde} | <u>0.325^e</u> | 34.500 ^c |
| Eat 01 | 21.500 ⁱ | 27.750 ^{fgh} | 2.000 ^{ab} | 6.775 ^{bcd} | 2.800 ^{abcde} | 40.250 ^{ab} | 53.500 ^b | 1.375 ^{gh} | 0.500 ^{fg} | 0.375 ^d | 2.275 ^{defg} | 0.500 ^{cde} | 21.000 ^{efg} |
| Eat 02 | 28.250 ^{efghi} | 61.750^a | <u>1.750^b</u> | 5.350 ^{efg} | 2.200 ^{defgh} | 41.750 ^b | 53.000 ^c | 1.150 ^h | 0.525 ^{efg} | 0.375 ^d | 1.925 ^{fghi} | 0.575 ^{bcd} | 35.000 ^{ab} |
| Nor 01 | 46.000 ^{bc} | 36.500 ^{def} | 2.000 ^{ab} | 8.550^a | 3.200 ^{ab} | 30.250 ^{defg} | 41.500 ^{defgh} | 1.175 ^h | 1.100 ^c | 0.800 ^b | 2.675 ^{abcd} | 0.475 ^{de} | 33.000 ^b |
| Nor 02 | 26.375 ^{ghi} | 23.750 ^{gh} | 2.100 ^{ab} | 4.575 ^{efghi} | 2.375 ^{cdefg} | 28.000 ^{efgh} | 39.250 ^{fgh} | 3.800 ^c | 0.850 ^{cd} | 0.725 ^b | 3.075 ^{ab} | 0.575 ^{bcd} | 24.500 ^{cdef} |
| Nor 03 | 39.000 ^{bcd} | 58.000 ^c | 2.100 ^{ab} | 5.400 ^{def} | 2.600 ^{bcde} | <u>24.500^h</u> | <u>37.000^h</u> | 4.600^a | 1.025 ^{bc} | 0.650 ^{bc} | 2.925 ^{abc} | 0.500 ^{cde} | 38.500^a |
| Upp 01 | 45.500 ^{bc} | 51.500 ^{bc} | 2.125 ^{ab} | 5.625 ^{cde} | <u>1.525^h</u> | 25.750 ^h | 37.250 ^{gh} | 3.900 ^{bc} | 2.225^a | 1.125 ^b | 2.425 ^{cdef} | 0.525 ^{cde} | 18.250 ^{gh} |
| Vol 01 | 26.675 ^{ghi} | 36.550 ^{def} | 2.075 ^{ab} | 7.025 ^{bc} | 2.700 ^{abcde} | 34.250 ^{cd} | 45.000 ^{bcde} | 1.150 ^h | 0.500 ^{fg} | 0.350 ^d | 1.700 ^{hi} | 0.4750 ^{de} | 24.000 ^{cdef} |
| Vol 02 | 34.500 ^{defg} | 55.000 ^{ab} | 2.050 ^{ab} | 3.425 ^{hij} | 2.150 ^{defgh} | 41.750 ^b | 53.750 ^b | <u>1.125^h</u> | 0.550 ^{efg} | 0.300 ^d | 2.125 ^{efghi} | 0.500 ^{cde} | 26.500 ^{cd} |
| Vol 03 | 27.750 ^{fghi} | 32.875 ^{efg} | 2.175 ^{ab} | 5.750 ^{cde} | 2.450 ^{bcdef} | 36.250 ^{bc} | 47.250 ^{bc} | 2.200 ^e | 0.550 ^{efg} | 0.400 ^d | 2.300 ^{defg} | 0.500 ^{cde} | 33.250 ^b |
| Vol 04 | 31.000 ^{defghi} | 43.125 ^{cde} | 2.000 ^{ab} | 4.550 ^{efghi} | 1.825 ^{fgh} | 36.250 ^{bc} | 47.500 ^b | 3.325 ^d | 0.525 ^{efg} | 0.325 ^d | 2.575 ^{bcde} | 0.500 ^{cde} | 25.500 ^{cde} |
| Wes 01 | 36.750 ^{cdef} | 29.500 ^{fgh} | 2.850^a | 8.125 ^{ab} | 3.425^a | 43.750^a | 56.750^a | 1.400 ^{gh} | 0.575 ^{defg} | 0.425 ^d | 2.200 ^{defgh} | 0.425 ^{de} | 28.000 ^d |
| LoS | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| Mean | 33.372 | 42.165 | 2.061 | 5.215 | 2.396 | 32.462 | 44.45 | 2.513 | 0.543 | 0.78 | 2.49 | 0.525 | 26.2 |
| CV (%) | 2.621 | 32.426 | 10.937 | 31.27 | 22.075 | 18.02 | 13.503 | 49.324 | 53.242 | 66.191 | 21.5 | 25.3 | 26.1 |

LoS = Level of significance, CV = Co-efficient of variation; **= significant at P<0.05; Mean represents average of the individual characters

measured for all accessions under study. **Bolded** and underlined values represent maximum and minimum values respectively for each trait

measured.

3.4.3: Correlations between 13 Quantitative Traits of *Capsicum* spp L.

Table 3.3 below shows Pearson product moment correlations between each pair of agromorphological traits. These correlation coefficients range between -1 and +1 and measure the strength of the linear relationship between these characters. There was positive significant relationship between plant canopy width and plant height ($r = 0.51$). Mature leaf width recorded very significant correlation with mature leaf length ($r = 0.738$) but had a negative non-significant correlation with plant canopy width ($r = -0.22$).

Days to 50% fruiting had a very strong positive correlation with days to 50% flowering ($r = 0.983$) and a negative non-significant correlation with plant height and plant canopy width. Fruit traits such as fruit length, fruit width and fruit weight exhibited negative significant correlations with days to 50% fruiting ($r = -0.663$; -0.47 ; -0.549) and days to 50% flowering ($r = 0.65$; 0.54 ; 0.60), respectively. However, number of seeds per fruit reveals a negative significant correlation with 100 seed weight ($r = -0.524$).



Table 3.3: Pearson Correlation Analysis of 13 Quantitative Traits of *Capsicum* spp L.

| Traits | PH | PCW | SW | MLL | MLW | D50Fl | D50Fr | FL | FWd | FWt | FPL | 100SWt | NS/Fr |
|---------------|---------------|--------|--------|----------------|--------|-----------------|-----------------|----------------|-----------------|--------|--------|----------------|-------|
| PH | | | | | | | | | | | | | |
| PCW | 0.510* | | | | | | | | | | | | |
| SW | 0.202 | -0.182 | | | | | | | | | | | |
| MLL | 0.154 | -0.145 | 0.379 | | | | | | | | | | |
| MLW | 0.204 | -0.225 | 0.384 | 0.738** | | | | | | | | | |
| D50Fl | -0.267 | -0.042 | 0.192 | 0.260 | 0.187 | | | | | | | | |
| D50Fr | -0.260 | -0.051 | 0.191 | 0.249 | 0.187 | 0.983*** | | | | | | | |
| FL | 0.314 | 0.131 | -0.084 | -0.128 | 0.014 | -0.663** | -0.654** | | | | | | |
| FWd | 0.268 | -0.016 | -0.006 | 0.051 | -0.011 | -0.474 | -0.540* | 0.539* | | | | | |
| FWt | 0.320 | -0.052 | 0.000 | 0.153 | 0.217 | -0.549* | -0.607** | 0.647** | 0.934*** | | | | |
| FPL | 0.092 | 0.049 | -0.011 | 0.090 | -0.015 | -0.336 | -0.280 | 0.347 | -0.163 | -0.021 | | | |
| 100SWt | 0.373 | 0.113 | -0.065 | -0.333 | 0.062 | -0.282 | -0.361 | 0.329 | 0.424 | 0.495 | -0.341 | | |
| NS/Fr | -0.209 | 0.295 | -0.101 | 0.235 | 0.093 | 0.241 | 0.259 | -0.189 | -0.377 | -0.354 | 0.185 | -0.524* | |

* = significant ($P < 0.05$); **=very significant ($P < 0.001$); ***= highly significant ($P < 0.0001$) computed using standard linear Pearson correlation.

PH = Plant height; PCW = Plant canopy width; SW = Stem width; MLL = Matured leaf length; MLW = Matured length width; D50Fl = Days to 50% flowering; D50Fr = Days to 50% fruiting; FL = Fruit length; FWd = Fruit width; FWt = Fruit weight; FPL = Fruit pedicel length; 100SWt = 100 Seed weight; NS/Fr = Number of seeds per fruit.

3.4.4: Cluster Analysis of 13 Quantitative Traits of *Capsicum* spp L.

Genetic relationships among the 20 accessions of hot pepper, based on 13 quantitative traits are displayed in the form of dendrogram (Figure 3.2), generated using the Coefficient of Cityblock, Complete Linked Similarity Matrix. Two clusters A and B were formed at 36.90% similarity, each re-grouping into three sub-clusters, making a total of six subclusters at 57.60 % genetic similarity. Cluster A is made up of sub-clusters 1, 2 and 3 which include accessions Upp 01, Acc 05, Acc 04, Acc 02, Acc 03, Nor 02, Nor 03, Acc 01; all of which are cultivated lines. Cluster B is made up of sub-clusters 4, 5 and 6 which comprises accessions Ash 01, Ash 02, Bra 01, Vol 03, Vol 04, Eat 01, Vol 01, Eat 02, Vol 02, Nor 01, Wes 01; all of which are wild pepper lines.

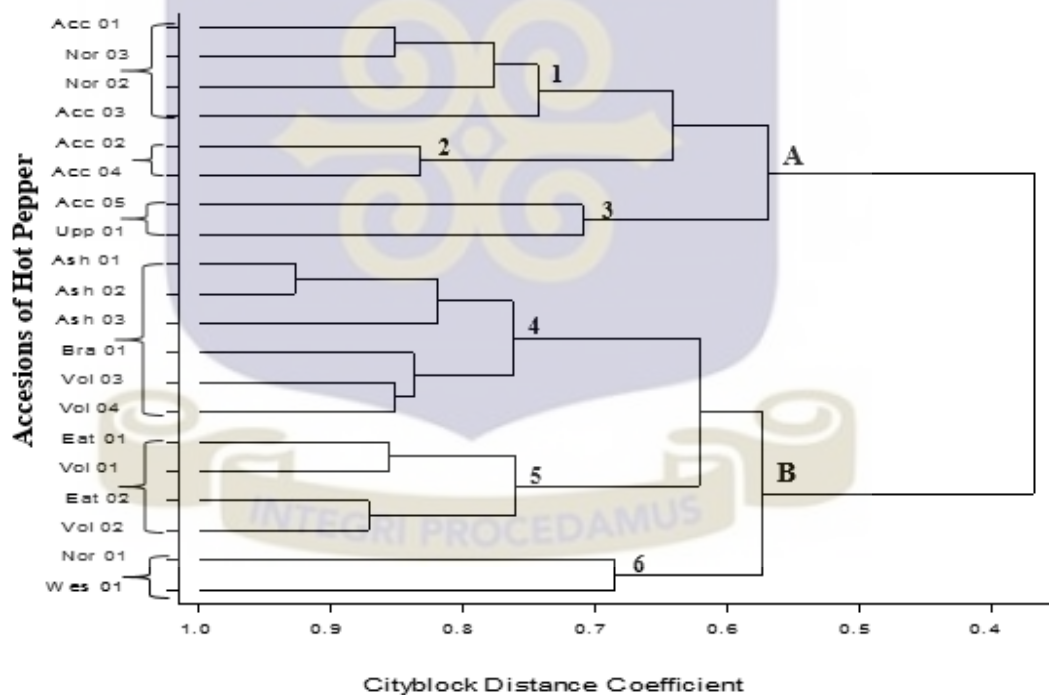


Figure 3.2: Dendrogram showing genetic relationships among 20 accessions of hot pepper based on 13 quantitative traits using the Coefficients of Cityblock, Complete Linked Similarity Matrix.

3.4.5: Principal Components Analysis of 13 Quantitative Traits

Table 3.4 displays the results of Principal Components Analysis (PCA) of the 13 quantitative traits showing the factor scores of each character among the 20 accessions of hot pepper, and percentage total variance accounted for by five principal components (PCs). The five PCs accounted for about 97.57% of total variance with the first principal component (PC₁) recording the highest (56.12%). The purpose of the analysis is to obtain a small number of linear combinations of the 13 characters (variables) which account for most of the variability in the data. The second, third, fourth and fifth principal components (PC₂, PC₃, PC₄ and PC₅) accounted for 24.32%, 12.48%, 6.25% and 0.40% of the total genetic variation, respectively. PC₁, which accounted for the highest proportion (56.12%) of total variation is associated with plant canopy width, plant height, fruit length and number of seeds per fruit.

Table 3.4: Principal Components Analysis of 13 Quantitative Traits

| Traits | PC ₁ | PC ₂ | PC ₃ | PC ₄ | PC ₅ |
|---------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 100 Seed weight (g) | 0.00183 | 0.00516 | 0.00042 | -0.00764 | -0.00955 |
| Days to 50% flowering | -0.07300 | -0.34845 | -0.58481 | -0.11695 | 0.10290 |
| Days to 50% fruiting | -0.07600 | -0.35513 | -0.60539 | -0.08146 | -0.15871 |
| Fruit length (cm) | 0.02055 | 0.05351 | 0.07331 | 0.01351 | 0.08438 |
| Fruit pedicel length (cm) | 0.00310 | 0.00477 | 0.01851 | 0.03002 | 0.01087 |
| Fruit weight (g) | 0.00416 | 0.02497 | 0.01665 | -0.00912 | 0.12402 |
| Fruit width (cm) | 0.00239 | 0.01611 | 0.00998 | 0.00124 | 0.08513 |
| Matured leaf length (cm) | -0.00432 | 0.00173 | -0.08808 | 0.15081 | 0.93250** |
| Matured leaf width (cm) | -0.00247 | 0.00822 | -0.02963 | 0.04373 | 0.19346 |
| Number of seeds per fruit | 0.05102 | -0.47012 | 0.13673 | 0.86084** | -0.11224 |
| Plant canopy width (cm) | 0.79612** | -0.45762 | 0.21873 | -0.32036 | 0.07300 |
| Plant height (cm) | 0.59329* | 0.56405* | -0.45835 | 0.33178 | -0.09251 |
| Stem width (cm) | -0.00070 | 0.00414 | -0.01272 | 0.00699 | 0.02825 |
| % Variance | 56.12 | 24.32 | 12.48 | 6.25 | 0.40 |

3.4.6 Variability in Qualitative Agromorphological Traits of *Capsicum* spp L.

Table 3.5 shows variation in qualitative traits among the 20 accessions. Fruit colour at intermediate state, leaf and stem pubescence and life cycle of the plants did not show any variation among the accessions. All the fruits were green, leaf pubescence was sparse for all, stem pubescence was also sparse with biennial life cycle. Fruit shape exhibited the widest variation as elongate, triangular, almost round and campanulate. Thirty five per cent of the accessions were elongate, 45% triangular, 15% were almost round and 5% campanulate. Slight variation was seen in leaf colour as green and light green, with 55% showing green and the rest were light green coloured.

Leaf density and branching habit varied as dense, intermediate and sparse among the accessions. Majority of the mature fruits were red, 15% were light red and 5% were orange yellow. With respect to plant growth habit; three types emerged, intermediate, prostrate and erect. Three different types of leaf shape emerged; namely deltoid, ovate and lanceolate with majority showing deltoid shape. Fruit shape at blossom end ranged from pointed sunken to blunt with most of them as pointed. Fruit set was categorised as high, low and intermediate. 45% of the accessions exhibited high fruit set, 30% low and 25% intermediate fruit set.



Table 3.5: Variability in Qualitative Agromorphological Traits of *Capsicum* spp L.

| Accession | Life cycle | Stem colour | Stem pubescence | Plant growth habit | Branching habit | Leaf density | Leaf colour | Leaf shape | Lamina margin | Flower position | Number of flower per axile | Leaf pubescence | Anther colour | Calyx Margin |
|-----------|------------|-------------|-----------------|--------------------|-----------------|--------------|-------------|------------|---------------|-----------------|----------------------------|-----------------|---------------|--------------|
| Acc 01 | biennial | Green | Sparse | intermediate | intermediate | intermediate | light green | deltoid | entire | erect | one | sparse | purple | intermediate |
| Acc 02 | biennial | Green | Sparse | prostrate | dense | dense | green | lanceolate | | erect | one | sparse | yellow | intermediate |
| Acc 03 | biennial | Green | Sparse | erect | dense | intermediate | green | deltoid | undulate | erect | one | sparse | yellow | intermediate |
| Acc 04 | biennial | Green | Sparse | erect | sparse | sparse | green | deltoid | entire | erect | two | sparse | yellow | intermediate |
| Acc 05 | biennial | Green | Sparse | erect | sparse | sparse | green | deltoid | entire | intermediate | one | sparse | yellow | dentate |
| Ash 01 | biennial | Green | Sparse | erect | dense | dense | light green | deltoid | undulate | erect | two | sparse | yellow | entire |
| Ash 02 | biennial | Green | Sparse | erect | dense | dense | green | deltoid | entire | erect | two | sparse | yellow | entire |
| Ash 03 | biennial | Green | Sparse | erect | dense | intermediate | green | deltoid | entire | erect | two | sparse | yellow | entire |
| Bra 01 | biennial | Green | Sparse | erect | intermediate | intermediate | green | deltoid | entire | erect | two | sparse | pale blue | entire |
| EAT 01 | biennial | Green | Sparse | erect | dense | dense | green | ovate | undulate | erect | two | sparse | purple | dentate |
| Eat 02 | biennial | Green | Sparse | intermediate | intermediate | intermediate | light green | deltoid | entire | erect | one | sparse | yellow | entire |
| Nor 01 | biennial | Green | Sparse | intermediate | sparse | intermediate | green | ovate | entire | intermediate | two | sparse | yellow | intermediate |
| Nor 02 | biennial | Green | Sparse | erect | sparse | sparse | green | deltoid | entire | intermediate | two | sparse | yellow | intermediate |
| Nor 03 | biennial | Green | Sparse | intermediate | intermediate | intermediate | light green | deltoid | undulate | erect | one | sparse | light purple | intermediate |
| Upp 01 | biennial | Green | Sparse | intermediate | intermediate | intermediate | green | ovate | entire | intermediate | two | sparse | light purple | intermediate |
| Vot 01 | biennial | Green | Sparse | erect | dense | dense | light green | deltoid | undulate | erect | two | sparse | purple | intermediate |
| Vot 02 | biennial | Brown | Sparse | erect | intermediate | intermediate | light green | deltoid | entire | erect | two | sparse | yellow | entire |
| Vot 03 | biennial | Green | Sparse | erect | sparse | sparse | light green | ovate | entire | erect | one | sparse | yellow | entire |
| Vot 04 | biennial | Green | Sparse | intermediate | intermediate | intermediate | green | lanceolate | entire | erect | two | sparse | yellow | intermediate |
| Wes 01 | biennial | Green | Sparse | erect | intermediate | dense | light green | ovate | undulate | erect | two | sparse | purple | dentate |

Table 3.5 (cont'd).

| Accession | Fruit colour at intermediate | Fruit colour at maturity | Fruit shape | Neck at base of fruit | Fruit shape at blossom end | Fruit surface | Ripe fruit persistence | Varietal mixture condition | Corolla colour | Calyx annular constriction | Fruit set |
|-----------|------------------------------|--------------------------|--------------|-----------------------|----------------------------|---------------|------------------------|----------------------------|----------------|----------------------------|--------------|
| Acc 01 | Green | Red | elongated | absent | pointed | semi wrinkled | persistent | slight | white | absent | high |
| Acc 02 | Green | light red | triangular | absent | pointed | smooth | persistent | slight | green yellow | absent | low |
| Acc 03 | Green | light red | elongated | present | pointed | semi wrinkled | persistent | slight | green yellow | absent | high |
| Acc 04 | Green | light red | elongated | absent | sunken | semi wrinkled | persistent | slight | green yellow | absent | intermediate |
| Acc 05 | Green | Orange yellow | almost round | absent | sunken | semi wrinkled | intermediate | slight | yellow | present | low |
| Ash 01 | Green | Red | triangular | absent | pointed | semi wrinkled | persistent | slight | light yellow | absent | high |
| Ash 02 | Green | Red | triangular | absent | pointed | smooth | intermediate | slight | light yellow | absent | intermediate |
| Ash 03 | Green | Red | triangular | absent | blunt | semi wrinkled | intermediate | slight | light yellow | absent | low |
| Bra 01 | Green | Red | triangular | absent | blunt | wrinkled | intermediate | slight | green yellow | absent | high |
| EAT 01 | Green | Red | triangular | absent | blunt | smooth | persistent | slight | light yellow | absent | intermediate |
| Eat 02 | Green | Red | triangular | absent | sunken | smooth | persistent | slight | white | present | high |
| Nor 01 | Green | Red | almost round | absent | pointed | Semi wrinkled | persistent | slight | green yellow | absent | high |
| Nor 02 | Green | Red | elongated | present | pointed | Semi wrinkled | intermediate | slight | green yellow | absent | low |
| Nor 03 | Green | Red | elongated | absent | sunken | Semi wrinkled | persistent | slight | green yellow | absent | high |
| Upp 01 | Green | Red | campanulate | absent | blunt | Semi wrinkled | persistent | slight | yellow | absent | intermediate |
| Vot 01 | Green | Red | triangular | absent | pointed | smooth | persistent | slight | light yellow | present | low |
| Vot 02 | Green | Red | elongated | absent | blunt | Semi wrinkled | persistent | slight | light yellow | absent | high |
| Vot 03 | Green | Red | triangular | absent | pointed | Semi wrinkled | persistent | slight | light yellow | absent | intermediate |
| Vot 04 | Green | Red | elongated | absent | pointed | smooth | persistent | slight | green yellow | absent | low |
| Wes 01 | Green | Red | almost round | absent | pointed | smooth | persistent | slight | light yellow | absent | high |

3.5 DISCUSSION

3.5.1 Variability in Qualitative and Quantitative Agromorphological Traits of 20 accessions of hot pepper.

In this study, 38 agro-morphological traits (25 qualitative and 13 quantitative) were studied for genetic diversity in 20 accessions of hot pepper. The accessions varied extensively in vegetative and reproductive characteristics as shown in Tables 3.2 and 3.3 and also in Tables 3.4 and 3.5.

Minimal variation was seen in fruit colour at intermediate stage, leaf colour and pubescence, stem colour and pubescence. All the fruits were green at intermediate stage which agrees with work done by Ballina- Gomez *et al.*, (2013) when *Capsicum annum* L. accessions from Mexico were characterised. Leaf colour was green with sparse pubescence. Manju and Sreelathakumary (2002) reported that 90% of leaf colour was green and 87.55% had sparse leaf pubescence in *Capsicum chinense* germplasm. In the present study, 55% of the accessions had green leaves and 45% light green with all accessions showing sparse leaf pubescence. Datta and Das (2013) reported that most of the genotypes had sparse leaf pubescence (67.92 %) with green (62.26 %) and dark green (33.96 %) leaves in *Capsicum annum* germplasm. This implies that these traits may not be useful in studying genetic diversity in *Capsicum*.

Plant growth habit varied from prostrate to erect and erect growth habit was dominant (65 %) followed by intermediate (30 %). Majority of the genotypes showed intermediate branching habit (40%), with 35% dense and 25% sparse. These observations disagree with findings by Datta and Das (2013) who observed intermediate as the dominant

(58.49%) plant growth habit followed by prostrate (35.85%) and dense as the dominant branching habit (50.94%) followed by intermediate. These differences could be attributed to the differences in the germplasm and the number of accessions used. Most of the fruits were triangular, had red colour, smooth surface, pointed at blossom end and neck at the base of fruit was absent.

With respect to flower position and number of flowers per axil, most of the accessions, especially the wild varieties had erect positions with one or two flowers per axil. These results are consistent with those by Castañón-Nájera *et al.* (2008) and Chavez and Castillo (1999) working with accessions of *C. pubescens*, and *C. annuum* genotypes. These researchers found that commercial peppers have an oblique flower position, in contrast to erect flower position of wild peppers.

The highest CV in quantitative characters was seen in fruit weight, fruit width and fruit length with values 66.19%, 53.24% and 49.32%, respectively. These characteristics could therefore be established as important descriptors in genetic diversity study of pepper genotypes. Panthee *et al.* (2004) stated that the variation found in quantitative traits is useful for developing varietal descriptors and in variety identification since quantitative traits are of agronomic interest for crop improvement programmes. Ukkund *et al.* (2007); Sreelathakumary and Rajamony (2002); Ibrahim *et al.* (2001) also documented that fruit yield per plant, number of fruits per plant, and plant height contribute to high variation in *C. annuum* genotypes with corresponding significant CV (s). On the other hand, minor variations were seen in plant height, stem width and days to 50% fruiting with CV values

ranging from 2.62%, 10.93% and 13.50%, respectively. In other studies, low variation in days to 50% fruiting have also been observed. Sharma *et al.* (2010) found 8.18% CV in days to flowering in a collection of accessions of sweet pepper (*C. annuum*).

The significant phenotypic variation observed within the qualitative and quantitative agromorphological characters of the accessions corresponds to the diverse collection sites (eight geographical regions). Agromorphological diversity studies in pepper by Dagnoko *et al.* (2013); Aktas *et al.* (2009); Geleta *et al.* (2005) and Ravindran *et al.* (1997) are consistent with findings of this study.

3.5.2 Correlations among 13 Quantitative Agromorphological Traits of *Capsicum* spp L.

Correlation measures the intensity of association between traits (Steel and Torrie, 1984). Selection for a single character may increase likelihoods for all traits that are positively correlated but declines for characters that are negatively correlated. Significant correlations revealed among some of the agromorphological traits suggest that these traits could be used to predict the other. Traits that show significant positive correlations could be improved simultaneously. However, traits that exhibited significant inverse relationships could be improved independently (Nyadanu *et al.*, 2014).

Weak correlation between fruit length and number of days to 50% fruiting, fruit length and number of days to 50% flowering, number of days to 50% fruiting and fruit width, number of days to 50% fruiting and fruit weight, number of days to 50% fruiting and fruit length, number of days to 50% fruiting and fruit width, number of days to 50% fruiting

and fruit weight, number of days to 50% flowering and fruit weight, number of seeds per fruit and 100 seed weight, 100 seed weight and number of seeds per fruit as found in this study is inconsistent with results obtained by Dias *et al.* (2013); Pacheco-Olvera *et al.* (2012); Bosland and Votava (2000).

The positive interrelationship between the characters, plant height and plant canopy width, matured leaf length and matured leaf width, number of days to 50% flowering and number of days to 50% fruiting, fruit length and fruit weight, fruit weight and fruit width indicate that these attributes are most important components for direct selection for yield as confirmed by Olawuyi1 *et al.* (2014) and Nwangburuka *et al.* (2012). Traits that show significant positive correlations could be improved simultaneously. However, traits that exhibited significant inverse relationships can only be improved independently (Nyadanu *et al.*, 2014).

3.5.3 Genetic Relationship among 20 Accessions of *Capsicum* spp L. based on Cluster Analysis

In the hierarchical cluster analysis, two major clusters of pepper genotypes A and B were formed, each re-grouping into three sub-clusters making a total of six subclusters. Cluster A is made up of sub-clusters 1, 2 and 3 which include accessions Upp 01, Acc 05, Acc 04, Acc 02, Acc 03, Nor 02, Nor 03, and Acc 01 of which are cultivated lines. Sub cluster 1 is made up of accessions Acc 01, Nor 03, Nor 02, Acc 03. These four accessions had in common the following characteristics; green stem colour, sparse stem pubescence, erect or intermediate plant growth habit, intermediate leaf density, light colour or green

leaf colour, deltoid leaf shape, entire or undulate lamina margin, erectile flower position, sparse leaf pubescence, intermediate carlyx margin, greenish leaf colour, semi-wrinkled fruit shape and high fruit set.

Subcluster 2 has accessions Acc 02, and Acc 04. They are cultivated types of bird's eye pepper lines collected from the Greater Accra Region. These have light red fruits at maturity, fruit shape at blossom end is pointed, anther colour is yellow among others. Sub cluster 3 is made up of accessions Acc 05 and Upp 01. These have larger fruit sizes, yellow corolla colour and the fruit colour at intermediate stage is green among others.

Cluster B is is also made up of three subclusters, 4, 5 and 6. Sub- cluster 4 has accessions Ash 01, Ash 02, Ash 03, Bra 01, Vol 03 and Vol 04; all of which are wild bird's eye pepper lines. These are characterised by small fruit sizes, erect fruit positions, detoid leaf shape, and red fruit colour at maturity. Sub-cluster 5 contain accessions Eat 01, Vol 01, Eat 02, and Vol 02. They were collected from Eastern and Volta regions and characterised by small fruit types, rect fuit positions, sparse leaf pubescence and also wild bird's eye pepper lines. Sub-cluster 6 has accessions Nor 01 and Wes 01. They are characterised by erect plant growth habit, ovate leaf shape, two flowers per axile and fruit shape is almost round.

The clustering pattern showed a clear distinction between the pepper genotypes. Cultivated genotypes were noticeable in cluster A. These results are similar to those found by Chavez and Castillo (1999) working with accessions of *C. pubescens*, and Castañón-Nájera *et al.* (2008) working with *C. annuum* genotypes. The accessions Acc 02 and Acc 04, Acc 05 and Upp 01, Nor 01 and Wes 01 were clustered in sub-clusters 2,

3 and 6, respectively as the most diverse and therefore could be potentially useful as sources of variable traits in pepper improvement programme. Irwin *et al.* (1998) suggested that closely related accessions are normally located within 80–90% genetic similarity. Although, the dendrogram shows low genetic relatedness, crosses between accessions with similarity indices of 80–100% may not be desirable; but the potential for successful crossing of unrelated varieties may produce an array of genotypes from which useful agronomic types may be selected (Gulsen *et al.*, 2007).

None of the accessions was identified as duplicate from the cluster analysis. For accessions to be considered as duplicate, genetic similarity index (GSI) must exceed 0.95 (95%) (Andersson *et al.*, 2007). This contrasts with results obtained by other researchers such as Ahiakpa *et al.* (2013), Kirchoff *et al.* (2008) and Andersson *et al.*, (2007).

3.5.4 Variability in Agro-morphological Traits as Revealed by Principal Component Analysis

The most significant variables with substantial phenotypic variation are defined by principal component (PC) analysis in genetic diversity studies using agro-morphological traits. The PC analysis in this study showed that 56.12% of the total genetic variance among the pepper accessions was accounted for by the first principal component taking into account all the 13 quantitative traits studied. This disagrees with finding of Ballina-Gomez *et al.* (2013) where the first principal component explained only 22% of the total variation in *Capsicum annum* L. accessions from Southern Mexico.

The PC scores of plant canopy width, plant height, number of seeds per fruit and matured leaf length were mostly correlated with the first, second, fourth and fifth principal components (PC₁, PC₂, PC₄ and PC₅), respectively of the Principal Components Axes. Large plant canopy width provides large leaf surfaces with enhanced interception of solar radiation with subsequent increase in the amount of photosynthetic activities which may correspondingly increase the plant's assimilatory ability (Payakhapaab *et al.*, 2012). Thus, factor scores of these four characters indicate their substantial contribution to total genetic variation among the 20 accessions of hot pepper (*Capsicum* spp L.) studied. This finding is consistent with that of Doku *et al.* (2013) and Ortiz *et al.* (2010), in which factor scores of nine and twelve characters for rice respectively accounted for variance among accessions and were mostly correlated with PC₁, PC₂, PC₃ and PC₄ of the Principal Components Axes.

The first two and last two principal axes accounted for over 87.09% of total variation among the 13 characters describing the accessions. These characters can be employed in differentiating genotypes of hot pepper (Nsabiyera *et al.*, 2013). The total contribution of the four principal components of this study was higher (87.09%) than observations made by other workers (Ahiakpa *et al.*, 2013; Akotkar *et al.*, 2010, Costa *et al.*, 2010) where the principal component axes contributed 66.37%, 76.62% and 64.5% variation, respectively. The factor scores of the four characters imply that, high priorities should be given to these genetic traits, if selection is to be made for future breeding programmes.

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CHAPTER FOUR

MOLECULAR CHARACTERISATION OF TWENTY ACCESSIONS OF HOT PEPPER(*Capsicum* spp l.)

4.1 Introduction

Farmers in Ghana have selected genotypes that best meet their needs culminating in a large number of traditional varieties. This has led to numerous vernacular names given to the same varieties depending on ethnic origin. This phenomena has led to confusion in the exact number of the varieties of pepper under cultivation in the country hence the need to characterise the genetic resources of the crop. In the past, researchers have used chromosome morphology (Pickersgill, 1971) and protein/enzyme profiling (Kumar *et al.*, 2010; Posch *et al.*, 1994) to study genetic diversity within the genus *Capsicum*. However, these methods are constrained by influence of environment on trait phenotype, epistatic interactions, pleiotropic effects etc. Furthermore, the taxonomy of *Capsicum* is so confusing and sometimes it is difficult to identify accessions using only subjective morpho-agronomical data (DaCosta *et al.*, 2006).

DNA markers based on polymerase chain reaction (PCR) technology are efficient in genetic diversity studies and varietal authentication in crop plants, and are simple and easy to use (Lal *et al.*, 2010; Powell *et al.*, 1996). These molecular markers differ in their purpose, time requirements, ease of application, cost and ability to detect variability. Molecular markers such as Restriction fragment length polymorphism (RFLP), Random

amplified polymorphic DNA (RAPDs), amplified fragment length polymorphism (AFLPs), and simple sequence repeats (SSRs) have been developed for pepper (Jang *et al.*, 2004; Lee *et al.*, 2004; Moon *et al.*, 2003; Kang *et al.*, 2001; Lefebvre *et al.*, 2001; Paran *et al.*, 1998; Prince *et al.*, 1992). These markers have been proven to be useful in assessing genetic diversity and phylogeny, characterisation of germplasm and to detect duplication, parental verification in crosses, gene tagging in marker assisted breeding and gene cloning in genetic transformation (Da Costa *et al.*, 2006).

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). The working group of biochemical and molecular techniques of UPOV has identified SSR markers as most widely used system for plant varietal characterisation (UPOV-BMT, 2012) due to the fact that SSR markers are locus-specific and co-dominant in nature. They offer better resolution than the other PCR-based markers (Soni *et al.*, 2010). There is a huge extent of allelic diversity (polymorphic information content) making it possible to reveal variation among closely related individuals. They are abundant and well distributed throughout the genome, reproducibility is high and amplification is easy (Weising *et al.*, 2005; Powell *et al.*, 1996).

4.2 Objectives of the Study

The objective of the study was to molecularly characterise 20 accessions of indigenous cultivars of pepper and their wild relatives collected from eight geographical regions of Ghana using SSR markers to assess their genetic diversity.

4.3 Materials and Methods

4.3.1 Experimental Site

The study was conducted at the Molecular Laboratory of the Biotechnology and Nuclear Agricultural Research Institute (BNARI) with the assistance of the Kirk House Trust Mobile Laboratory facility of the Cocoa Research Institute of Ghana (CRIG).

4.3.2 DNA Extraction

Genomic DNA was extracted from the 20 pepper accessions using CTAB method described by Doyle and Doyle (1990) with slight modifications. Young and tender leaves were taken from the pepper genotypes into an ice chest and sent to the laboratory for DNA extraction.

Twenty (20) milligrams of leaf samples were ground in 2.0 ml Eppendorf tubes into fine powder with liquid nitrogen. Eight hundred μ l of 2% CTAB and 0.5 μ l 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 μ l) 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 μ l) and shaken 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 μ l of washing buffer. The washing buffer was decanted and the pellet was washed in 400 μ l 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 μ l of TE buffer and centrifuged at high speed for 30 seconds and stored in the fridge at 4°C . Quality of the DNA of each accession was confirmed by electrophoresis on 2% agarose gel and polymorphic bands were obtained indicating good results.

4.3.3 SSR (microsatellite) Markers and PCR Amplification

Simple sequence repeats primers (SSR) used to detect polymorphism among the pepper genotypes are presented in the Appendix . 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 μ l reaction mixture in 96-well plates.

PCR kits procured from Metabion International were used for the amplification. The kits composed 2X PCR master mix containing DNA Polymerase (0.2 U per 10 µl reaction), PCR buffer, dNTPs (0.2 mM each at 1X), MgCl₂ (1.5 mM at 1X), stabilisers 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 programmed as initial denaturation at 95°C for 3 mins, followed by cycles of 95°C for 10 sec, 52°C for 10 sec and 72°C for 10 sec. The reactions were 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.

4.3.4 Gel Electrophoresis

PCR products were separated on a 3% agarose gel matrix in a horizontal electrophoresis set up pre-stained with ethidium bromide (0.5µg/ml). Approximately 8µl of amplified products was loaded into agarose wells then electrophoresed at 100V for 1 hour. All products were run alongside a 1kb plus ladder (Invitrogen).

4.4 Results

Fig4.1 shows the banding pattern for primer CAMS493 of the 20 pepper accessions from DNA amplicons. The figure shows the polymorphic nature of the bands obtained. Fifteen SSR primers were used for the study but only eight yielded polymorphic bands.

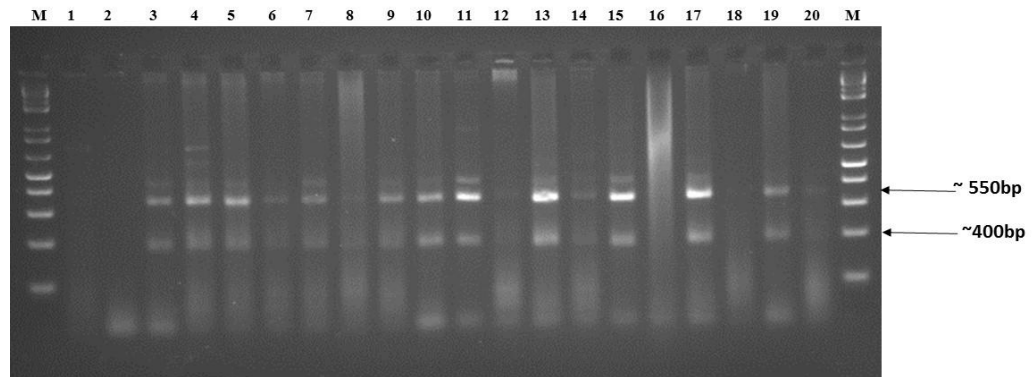


Figure 4.1: Banding pattern of primer CAMS493 on 20 hot pepper accessions (1- 20).

Lane M 1 = Acc 01; 2 = Nor 01; 3 = Ash 03; 4 = Bra 01; 5 = Nor 03; 6 = Eat02; 7 = Wes 01; 8 = Ash 02; 9 = Vol 03; 10 = Vol 01; 11 = Nor 02; 12 = Eat 01; 13 = Vol 02; 14 = Ash 01; 15 = Acc03; 16 = Acc 04; 17 = Vol 04; 18 = Upp 01; 19 = Acc02; 20 = Acc 05.

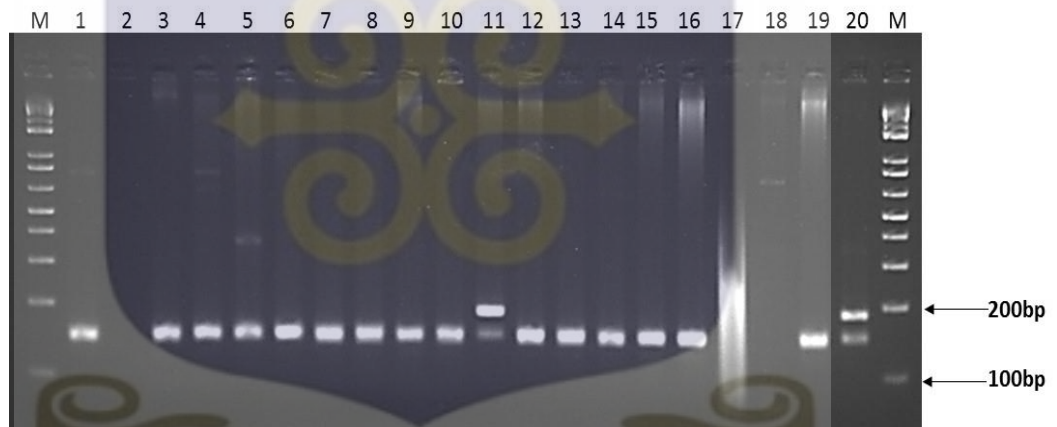


Figure 4.2: Banding pattern of primer CAMS 871 on 20 hot pepper accessions (1- 20).

Lane M 1 = Acc 01; 2 = Nor 01; 3 = Ash 03; 4 = Bra 01; 5 = Nor 03; 6 = Eat02; 7 = Wes 01; 8 = Ash 02; 9 = Vol 03; 10 = Vol 01; 11 = Nor 02; 12 = Eat 01; 13 = Vol 02; 14 = Ash 01; 15 = Acc03; 16 = Acc 04; 17 = Vol 04; 18 = Upp 01; 19 = Acc02; 20 = Acc 05.

4.4.1 Microsatellite Variation Statistics for all loci

A total of 14 alleles were generated by eight primers with a mean of 1.75 alleles per locus. Six out of the eight loci produced two alleles each and loci CAMS 396 and CAMS 493 produced the least number of alleles. Locus CAMS 823 recorded the highest variability with Shannon information index of 0.693 and expected heterozygosity of 1.00.

Table 4.1: Genic variation for all Loci

| Locus | *Na | **Ne | ***I |
|----------------|-------------|--------------|-------------|
| CAMS 396 | 1.0 | 1.00 | 0.00 |
| CAMS 406 | 2.0 | 1.98 | 0.68 |
| CAMS 476 | 2.0 | 1.14 | 0.24 |
| CAMS493 | 1.0 | 1.00 | 0.00 |
| CAMS 823 | 2.0 | 2.00 | 0.69 |
| CAMS 871 | 2.0 | 1.11 | 0.21 |
| CAMS 117 | 2.0 | 1.05 | 0.12 |
| CAMS 885 | 2.0 | 1.12 | 0.22 |
| Total | 14 | 10.39 | 2.16 |
| Mean | 1.75 | 2.00 | 0.27 |
| St Dev. | 0.46 | 0.95 | 0.27 |

*Na= Observed number of alleles; **Ne = Effective number of alleles; ***I= Shannon's information index.

4.4.2 Differentiation of Pepper Population

From Table 4.2, two populations made up of cultivated bird's eye pepper showed the lowest genetic diversity with 0.09 of Shannon's diversity index, 0.06 of Nei's genetic diversity and expected heterozygosity of 0.07. Three populations made up of non-bird's

eye pepper exhibited the highest genetic diversity with 0.44 of Shannon's diversity index, Nei's genetic diversity index of 0.30 and expected heterozygosity of 0.43. The overall percentage polymorphism was 75% (Table 4.2).

Table 4.2: Estimated Genetic Diversity among 20 accessions of hot pepper based on polymorphism of 8 SSR loci.

| Pop | Na | Ne | PL | P% | H _o | H _e | Nei's | I | F _{is} | F _{it} | F _{st} | t |
|----------------|-------------|------------|----------|-----------|----------------|----------------|-------------|-------------|-----------------|-----------------|-----------------|-------------|
| Pop1 | 1.25 | 1.25 | 2 | 25 | 0.25 | 0.19 | 0.12 | 0.17 | | | | |
| Pop2 | 1.14 | 1.11 | 1 | 12.5 | 0.09 | 0.07 | 0.06 | 0.09 | | | | |
| Pop3 | 1.71 | 1.5 | 5 | 62.5 | 0.38 | 0.43 | 0.30 | 0.44 | | | | |
| Overall | 1.75 | 1.3 | 6 | 75 | 0.27 | 0.24 | 0.17 | 0.27 | -0.61 | 0.00 | 0.38 | 0.72 |

Na = Average number of alleles; P = percentage of polymorphic loci; PL = polymorphic loci; H_o = Observed heterozygosity; H_e = Expected heterozygosity; I = Shannon diversity index; F_{is}, F_{it} and F_{st} = Estimates of F-statistics of sub- and regional populations (Hartl and Clark, 1989); t = outcrossing rate $(1 - F_{it}) / (1 + F_{it})$.

4.4.3. Genetic Diversity of Population of 20 accessions of hot pepper

Table 4.3 shows the genetic diversity at the population level. Genetic diversity ranges from 0.04 to 0.959 indicating a diverse population. Populations 1 and 2 are closer in identity with population 3 and 1 being the widest.

Table 4.3: Nei's Unbiased Measures of Genetic Identity and Genetic Distance

| Pop ID | 1 | 2 | 3 |
|--------|--------|-------|-------|
| 1 | **** | 0.959 | 0.888 |
| 2 | 0.0413 | **** | 0.917 |
| 3 | 0.118 | 0.085 | **** |

Nei's genetic identity (above diagonal) and genetic distance (below diagonal); Population 1= Bird's eye pepper wild, Population 2 = Cultivated bird's eye pepper; Population 3 = Non bird's eye pepper.

4.4.4 Genetic Similarity and Cluster Analysis

Fig 4.3 shows a dendrogram based on Nei's (1972) genetic distance using Unweighted Pair Group Method (UPGM) modified from NEIGHBOUR procedure of PHYLIP version 3.5. Populations 1 and 2 show the closest similarity in cluster 1.



Figure 4.3: Dendrogram of Genetic distance using 8 SSR markers.

Table 4.4: Length of Genetic Distance between Populations

| Between | And | Length |
|---------|------|---------|
| 2 | 1 | 1.60509 |
| 1 | pop1 | 1.24063 |
| 1 | pop2 | 1.24063 |
| 2 | pop3 | 2.84572 |

4.4.5: Cluster Analysis based on 8 SSR Markers.

Fig 4.4 shows the genetic relationship among the accessions, revealed by eight primers based on Jaccard coefficient using complete link similarity matrix method. Two clusters were formed at genetic similarity (0.092). Cluster 1 is made up of accessions Acc 01, Acc

04, Upp 01 and Nor 01. Cluster two is subdivided into two groups at genetic similarity 0.18.

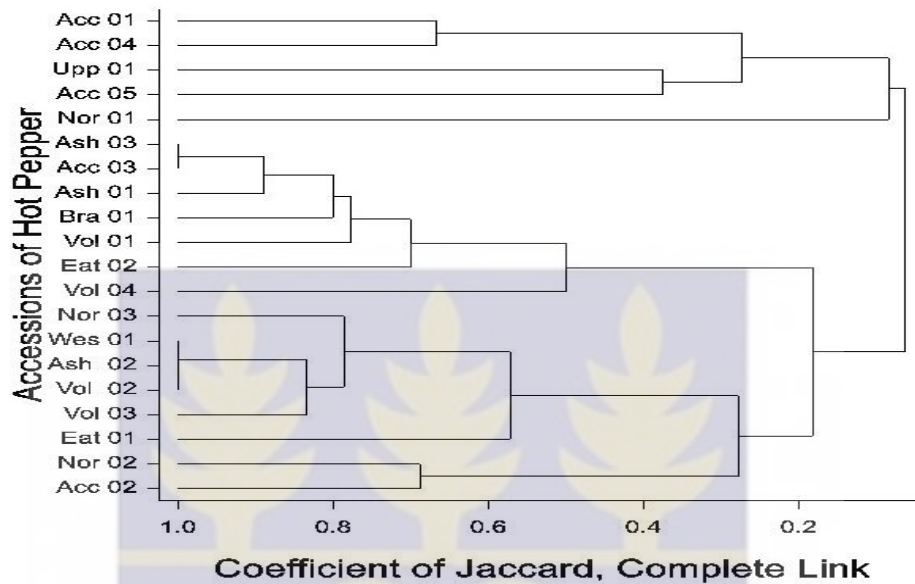


Figure 4.4: Dendrogram showing Genetic Relationships revealed by 8 primers among the among 20 accessions of hot pepper.

4.5 Discussion

4.5.1 Genetic variation among population of hot pepper

Genetic variability was evaluated among 20 accessions of hot pepper grouped into three populations using SSR markers. The results indicate that the primers were informative. The average number of alleles (N_a) was 1.7; Nei's expected heterozygosity was 0.17; and Shannon's diversity index was 0.27. The low value of polymorphic information content implies a high level of genetic similarity within the pepper accessions under study.

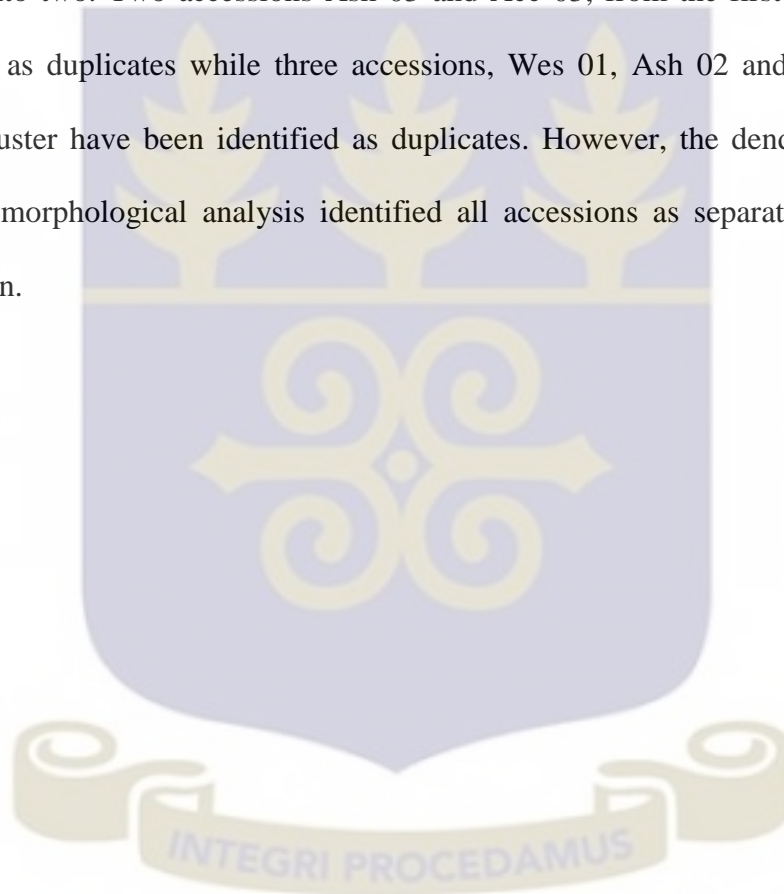
However, the SSR analysis revealed that the overall percentage polymorphism was fairly high (75%) compared to some earlier reports. Paran *et al.* (1998) using 10 SSR primer pairs detected 13% polymorphism in 34 Israeli gene bank pepper accessions. Also, Akatas *et al.* (2009) using 4 primer pairs found 26% polymorphism in Turkish pepper germplasm. The obtained number of alleles per locus (1.75) is lower than that of Minamiyama *et al.* (2006) and Kwon *et al.* (2007) who reported 2.9 and 3.0, respectively. The high out crossing rate (0.72) and low F_{is} (-0.61), F_{it} (0.00), F_{st} (0.38) indicates that the accessions under study are generally open pollinated.

4.5.2 Genetic Divergence

Estimates of Nei's unbiased measures of genetic identity and genetic distance also confirm the genetic resemblance among the accessions. Populations 1 and 2 had the highest similarity and population 3 was widely diverse from population 1. The short genetic distance and the high genetic identity among the populations indicate closeness among accessions in those populations. Population 1 is made up of wild species of bird's eye pepper and population 2 contains cultivated lines of bird's eye pepper. This high level of resemblance further confirms that they are of a common ancestry (Ahiakpa *et al.*, 2014). Population 3 is made up of three accessions which are not bird's eye pepper, one exotic and two local lines hence registered the widest genetic distance from the other populations.

4.5.3 Genetic Diversity among hot pepper Accessions as Revealed by SSR Primers.

The dendrogram further confirmed the relationship among the accessions. The results showed that all 20 accessions were grouped in two major clusters at genetic similarity of 0.18. The first cluster consist of five accessions and the second cluster had fifteen accessions. The first cluster was further divided in two groups and the second also further divided into two. Two accessions Ash 03 and Acc 03, from the first cluster have been identified as duplicates while three accessions, Wes 01, Ash 02 and Vol 02 from the second cluster have been identified as duplicates. However, the dendrogram generated from the morphological analysis identified all accessions as separate entities with no duplication.



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CHAPTER FIVE

ESSENTIAL MINERAL ELEMENT COMPOSITION IN FRUITS OF HOT PEPPER (*Capsicum* spp L.)

5.1 Introduction

Pepper (*Capsicum* spp L.) is an important agricultural crop, due to its economic importance as well as nutritional value of its fruits. Peppers are used worldwide as spices, condiments and vegetables (Lahbib *et al.*, 2012). The fruit contains phosphorus, manganese, copper, zinc and selenium in abundance. They are also prominent sources of Vitamins A and C (Nadeem *et al.*, 2011).

Essential mineral elements are those that can not be produced by the body yet indispensable to the body (Ahiakpa *et al.*, 2014). They are the major components of bones and teeth and help to control composition of body fluids and cells and serve as essential adjuncts to many enzymes and other functional proteins (Dashti *et al.*, 2004). Although, they constitute a small fraction of whole diets, macro- and micro-nutrients play an important role in various metabolic processes essential for maintaining good health and prevention of diseases and their deficiency is usually detrimental to normal biochemical function of the human body (Akhter *et al.*, 2004).

Elements considered essential are the major (or macro) elements (sodium, magnesium, phosphorus, chloride, potassium, and calcium) and the minor (or micro) elements (chromium, manganese, iron, cobalt, copper, zinc, selenium, molybdenum, bromine and iodine). In addition, there are the newer trace elements, which are possibly essential

including lithium, boron, fluorine, silicon, vanadium, nickel, arsenic, tin and lead (Crews, 1998).

The World Health Organisation (WHO) recommends daily intake of 400 g of fresh fruits and vegetables, due to their high mineral and vitamin contents which play very critical role in reducing risk of diseases such as diabetes and cancer (Matthews (2006); WHO/FAO, (2005). About 100 g of pepper is estimated to contain 166 kJ (Cal) energy, 8.8 g carbohydrates, 5.3 g sugars, 1.5 g dietary fibre, 0.4 g fat, 1.9 g protein, 48 µg vitamin A equivalent, 534 µg beta-carotene, 0.51 mg vitamin B6 and 144 mg vitamin C (Crews, 1998).

The concentrations of these essential elements may vary from one region to the other as they are affected by various agricultural practices such as type of soil, type of fertiliser and other agrochemicals including pesticides and herbicides. These essential elements are present in vegetables in trace and ultra-trace quantities. Hence, an analytical technique with sufficient sensitivity is required for the accurate determination of these elements in their edible parts. The major techniques employed are Flame Atomic Absorption Spectrometry (FAAS); Graphite Furnace Atomic Absorption Spectrometry (GFAAS); Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Szefer and Nriagu, 2007). Techniques such as Differential Pulse Cathode Stripping Voltamperometry (DPCSV) and Instrumental Neutron Activation Analysis (INAA) have also been shown as excellent tools for trace and ultra-trace elements analysis (Inam and Somer, 2000).

Neutron Activation Analysis (NAA), is an important technique for quantitative multi-element analysis of major, minor, trace, and rare elements and is based on the

measurement of characteristic radiation from radionuclides formed directly or indirectly by neutron irradiation of the material of interest (Parthasarathy, 1998). It is a less frequently used technique in the determination of essential elements in food samples because of the necessity of accessing a nuclear reactor. In spite of this, INAA possesses a number of advantages and possibilities which includes the lack of necessity for chemical destruction, no need for chemical pretreatment or digestion of sample and sources of systematic errors are few. A good accuracy is achieved because the isotopes are identified twice: by the γ -radiation and the half-life of the activated isotopes (Żukowska and Biziuk, 2008).

The Ghana Research Reactor-1 (GHARR-1), established in 1995, has been used extensively to determine elemental compositions in a number of biodegradable substances including fruits and vegetables (Ahiakpa *et al.*, 2014; Quartey *et al.*, 2012; Adotey *et al.*, 2009) as well as in spices and medicinal plants (Serfor-Armah *et al.*, 2002; 2001). Fruits of hot pepper have been identified to contain a number of mineral elements such as potassium, calcium, magnesium, phosphorus and manganese. in different concentrations. Concentrations may vary due to varietal differences and geological locations. In the previous study, twenty (20) accessions of hot pepper were grown and characterised according to their morphological descriptions. Thirteen (13) accessions out of the 20 were chosen for elemental analysis due to availability of fruits at the time of elemental analysis. This work will be the first of its kind with regards to the method used for analysis and as well as offer insight into the nutritional potential of hot pepper.

5.2 Objective of the study

The objective of the study was to determine the nutritional (elemental) potential of fruits of hot pepper with emphasis on:

- i. Major elements (magnesium, potassium, calcium, sodium and chlorine);
- ii. Minor elements (manganese, bromine and aluminium).

5.3 Materials and Methods

5.3.1 Lyophilisation and Milling

Samples of freshly harvested pepper weighing approximately 100 g were freeze-dried using a Christ Gamma 1-16 lyophilisator (Adotey *et al.*, 2011). After freeze drying, the samples were pulverised in a vibratory disc mill (Retsch RS 100) to obtain fine powdery lyophilised homogenate of pepper samples. The powdery samples were stored in 100 mL polyethylene containers. The containers were placed in hermetically-closed polyethylene bags. The samples were kept in refrigerators at 4° C. A small portion of the lyophilised homogenate of pepper samples were taken and used for the analysis.

5.3.2 Analysis of lyophilised pepper samples

The levels of Na, K, Br, Mg, Al, Cl, Ca and Mn in the lyophilised pepper samples were determined using the Instrumental Neutron Activation Analysis (INAA). The method involves irradiation of samples and standards in a nuclear reactor to convert the stable isotopes of the elements of interest or one of the stable isotopes of interest into their radioactive form. ‘Cooling’ of the radioactive sample so that the activity reaches safe

levels for human handling, and measurement of the γ -radiation intensity of the induced radionuclides (in samples and standards) by γ -ray spectrometry using high purity germanium (HPGe) γ -ray detector. Accumulation and quantification of the γ -ray spectrum followed, and calculation of the concentration of the elements of interest [based on the relative standardisation method of neutron activation analysis (NAA)].

5.3.3 Instrumentation

A calibrated weighing balance, Mettler Toledo AE 163 (Zurich, Switzerland) was used for weighing of samples and standards. Sample irradiations for Neutron Activation Analysis (NAA) were carried out in Ghana's 15 kW Miniature Neutron Source Research Reactor (MNSR) situated at the Ghana Atomic Energy Commission, Kwabenya, Accra, at a neutron flux of 5×10^{11} neutrons $\text{cm}^{-2} \text{s}^{-1}$. Measurement of γ -radiation intensity of the radionuclides induced in samples and standards were done on a γ -ray spectrometric system, made up of an N-type HPGe detector (model GR 2518), connected to an ACCUSPEC multi-channel analyser (MCA) emulation software (Canberra, Australia). The detector has an efficiency of 25% and a resolution of 1.8 keV at the 1332.5 keV γ -line of ^{60}Co . The ORTEC MAESTRO-32 γ -spectroscopy software was used for γ -ray spectrum acquisition and the HPGe semi-conductor detector γ -spectrum evaluation software, WinSPAN-2010, Version 2.10 (China).

5.3.4 Preparation of samples for Irradiation

Three replicates of small samples (200 mg) of each lyophilised homogenised pepper sample were wrapped in thin polyethylene foil, heat-sealed and packed in irradiation capsules. The three replicate samples for each were packed into the same polyethylene container. The polyethylene container was heat-sealed, followed by irradiation of the samples in the nuclear reactor.

5.3.5 Irradiation of samples

The lyophilised and homogenised pepper samples were irradiated for short-lived and medium-lived radionuclides. The irradiation, decay and counting times for the three categories of radionuclides are presented in Table 5.1.

The samples were sent into the reactor by means of a pneumatic transfer system. At the end of the irradiation, the capsules were retrieved from the reactor and allowed to cool down until the level of activity was within the acceptable limit for handling. This was followed by γ -radiation intensity measurement. During irradiation, the neutrons interact with the stable isotopes of the elements present in the pepper samples converting them into the radioactive isotopes through the (n, γ) reaction (Table 5.2).

Table 5.1: Irradiation, decay and γ -radiation measurement schemes used for INAA

| Type of Radionuclide | Irradiation, decay and γ -radiation measurement | | |
|----------------------|--|----------|----------|
| | t_i | t_d | t_m |
| Short-lived | 60s | Variable | 600s |
| Medium-lived | 1 hr | 24 hrs | Variable |

t_i is irradiation time t_d is decay time; t_m is γ -radiation measurement time

Table 5.2: Radioisotopes produced during the interaction of stable isotopes with neutrons

| Element | Isotope | Stable (n, γ) reaction | Characteristics of radioisotope | | |
|-----------|---------|---|---------------------------------|-------------------------|----------------------------|
| | | | Radioisotope | Half-life ($t_{1/2}$) | γ -ray energy (KeV) |
| Sodium | Na | $^{23}\text{Na}(n, \gamma)^{24}\text{Na}$ | ^{24}Na | 15.02 h | 1368.6; 2754.1 |
| Potassium | K | $^{41}\text{K}(n, \gamma)^{42}\text{K}$ | ^{42}K | 12.36 h | 1524.7 |
| Bromine | Br | $^{81}\text{Br}(n, \gamma)^{82}\text{Br}$ | ^{82}Br | 35.3 h | 554.3; 776.5 |
| Magnesium | Mg | $^{26}\text{Mg}(n, \gamma)^{27}\text{Mg}$ | ^{27}Mg | 9.45 min | 1014.4 |
| Aluminium | Al | $^{27}\text{Al}(n, \gamma)^{28}\text{Al}$ | ^{28}Al | 2.24 min | 1778.0 |
| Chlorine | Cl | $^{37}\text{Cl}(n, \gamma)^{38}\text{Cl}$ | ^{38}Cl | 37.3 min | 1642.4; 2167.5 |
| Calcium | Ca | $^{48}\text{Ca}(n, \gamma)^{49}\text{Ca}$ | ^{49}Ca | 8.7 min | 3084.4 |
| Manganese | Mn | $^{55}\text{Mn}(n, \gamma)^{56}\text{Mn}$ | ^{56}Mn | 2.58 hr | 846.7; 1810.7 |

5.3.6 Measurement of γ -radiation intensity

The radioactive samples were each placed on an HPGe γ -ray detector for measurement of the γ -radiation intensity. Measurement time for short and medium lived radionuclides is shown in Table 5.2. The MAESTRO-32 γ -ray spectrum acquisition software was used for γ -spectrum acquisition. A plexi-glass source support was mounted on the detector during measurement of γ -radiation intensity in order to ensure easy and reproducible source positioning (De Corte, 1987).

5.3.7 Evaluation of γ -spectrum

The net peak areas of the acquired γ -ray spectra for samples and standards were evaluated at their respective γ -ray energies (Table 5.2), using the HPGe semi-conductor (Fig. 5.1) γ -ray spectrum evaluation software, WinSPAN-2010, Version 2.10 (China).



Figure 5.1: A high purity germanium (HPGe) γ -ray detector

5.3.8 Calculation of Concentration

After accumulation of the spectra (sample and standard), their respective net peak areas were evaluated. This was followed by evaluation of decay corrections (that is the decay factor and the measurement factor). By comparing the net peak area of sample with that of the standard, the concentration of the analyte was evaluated based on the relative standardisation method of NAA, through the following equation (Kucera *et al.*, 2004):

$$C_{\text{analyte_sam}} = \frac{A_{\text{sam}} \cdot M_{\text{std}} \cdot C_{\text{analyte_std}}}{A_{\text{std}} \cdot M_{\text{sam}}}$$

$$A_{\text{sam}} = \left(\frac{N_p}{t_m \cdot D \cdot C_m} \right)_{\text{sam}} ;$$

$$A_{\text{std}} = \left(\frac{N_p}{t_m \cdot D \cdot C_m} \right)_{\text{std}} ;$$

- A_{sam} and A_{std} are the activities of the sample and standard, respectively,
- N_p is the net γ -photo peak area of radioisotope produced at a specific γ -energy line,
- $D = e^{-\lambda t_d}$ is decay factor (i.e. Correction factor for decay between start of measurement of sample and start of measurement of standard).
- $C_m = \frac{1 - e^{-\lambda t_m}}{\lambda t_m}$ is measurement factor (that is correction factor for decay during the measurement).
- t_m is measuring time (sec.)
- λ is the decay constant;
- $\lambda = \frac{\ln 2}{T_{1/2}}$;
- $T_{1/2}$ is the half-life

- t_d is the decay time between measurements of sample and standard (sec.);
- M_{std} is the mass of the standard used for analysis (g);
- M_{sam} is the mass of the sample used for the analysis (g);
- $C_{analyte_std}$ is the concentration of the analyte in the standard ($\mu\text{g g}^{-1}$)
- $C_{analyte_sam}$ is the concentration of the analyte being determined ($\mu\text{g g}^{-1}$)

5.3.9 Reference Material

The validity of the INAA method used was checked by analysing compositionally appropriate Standard Reference Material, NIST 1547 (Peach Leaves). The reference material was analysed together with the samples under the same experimental conditions. The results obtained shows excellent agreement with certified values (Table 5.3).

Table 5.3: Present Study Compared with Certified Values for NIST 1547

| Analyte | Concentration (mg/kg) | |
|---------|-----------------------|--------------------|
| | Present Study | Certified Value |
| Na | 22.30 ± 3.60 | 24.00 ± 2.00 |
| *K% | 2.40 ± 0.40 | 2.43 ± 0.30 |
| Br | | |
| Mg | 0.36 ± 0.05 | 0.432 ± 0.01 |
| Al | 255.10 ± 3.70 | 249.00 ± 8.00 |
| Cl | 348.40 ± 5.10 | 360.00 ± 19.00 |
| *Ca% | 2.10 ± 0.30 | 1.56 ± 0.02 |
| Mn | 100.30 ± 14.72 | 98.00 ± 3.00 |

* The unit for **K** and **Ca** is % and not mg/kg

5.3.10 Data Collection and Analysis

The concentrations were calculated on dry weight basis and each concentration is an average of three individual determinations. Data were analysed using One-way ANOVA with Statgraphics Centurion XVI.I. Microsoft excel was used in the drawing of graphs.

5.4 Results

Concentrations of five essential macro elements (Ca, Cl, K, Mg and Na), two micro elements (Al and Mn) and one trace element (Br) detected by INAA in fruit samples of 13 accessions of hot pepper are shown in Table 5.4.

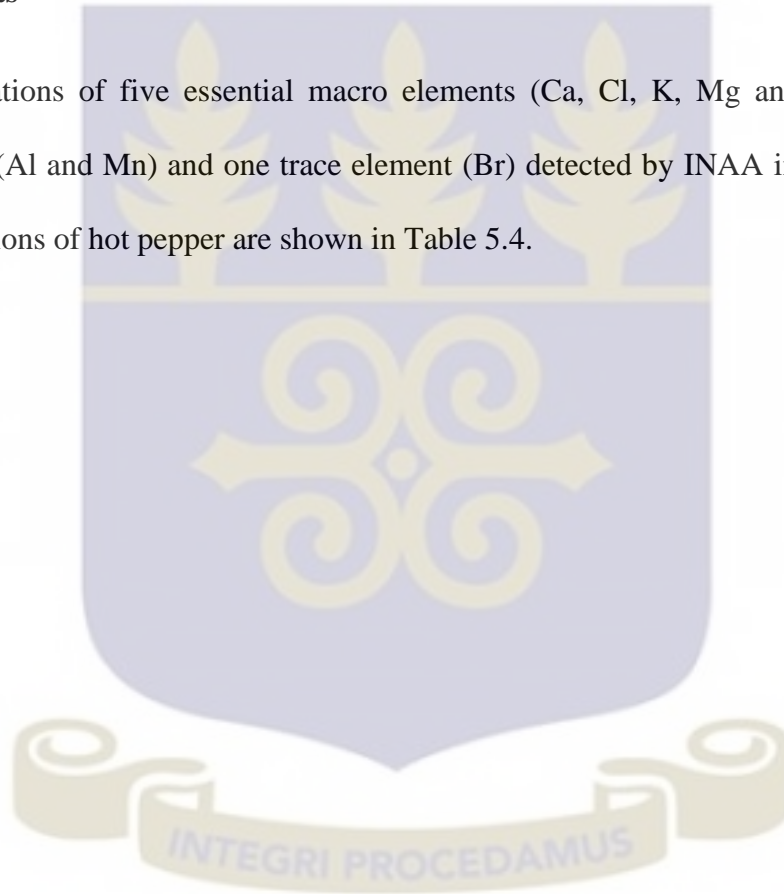


Table 5.4: Concentrations of 8 Mineral Elements in fresh Fruits of Hot Pepper Accessions

| Accessions | Macro Elements | | | | | Micro Elements | | Trace Element |
|------------|---|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|--|--------------------------------------|--------------------------------------|
| | Na (mg/kg)±SD | K (%)±SD | Mg (g/kg)±SD | Cl (g/kg)±SD | Ca (g/kg)±SD | Al (mg/kg)±SD | Mn (mg/kg)±SD | Br (mg/kg)±SD |
| ACC 01 | 368.53±9.95 ^f | 3.50±0.10 ^e | 1.44±0.22^g | 5.62±0.84 ^{bc} | 5.36±0.80 ^a | <u>595.55±89.33^a</u> | 18.14±2.72 ^{cde} | 22.04±1.98 ^{bc} |
| ACC 02 | 593.13±7.12 ^{de} | 3.32±0.06 ^f | 2.05±0.17 ^{cde} | 3.46±0.05 ^{fgh} | <u>5.55±0.34^a</u> | 329.10±18.43 ^{cdef} | 19.48±1.37 ^{bcd} | 33.07±3.01 ^b |
| ACC 03 | 519.60±6.76 ^e | 3.33±0.04 ^f | 1.66±0.09 ^{efg} | 3.72±0.05 ^{efg} | 3.33±0.22 ^{def} | 255.3±3.58^f | 16.49±1.14 ^{de} | 20.15±2.10 ^{cd} |
| ACC 04 | 315.4±4.74^f | 3.48±0.05 ^e | 2.25±0.34 ^{bcd} | 2.66±0.40 ^{hi} | 3.87±0.00 ^{cde} | 312.12±46.82 ^{def} | <u>36.17±5.43^a</u> | 24.91±2.17 ^b |
| ACC 05 | 540.1±7.03 ^{de} | 3.66±0.05 ^d | 1.59±0.15 ^{efg} | <u>7.19±0.09^a</u> | 4.17±0.23 ^{bcd} | 587.9±7.65 ^a | 10.33±1.55 ^{fg} | 10.37±1.54^f |
| ASH 01 | 714.4±6.85 ^b | 3.14±0.04 ^g | 1.74±0.26 ^{defg} | 6.32±0.95 ^{ab} | 3.06±0.46 ^{efg} | 295.44±44.28 ^{ef} | 21.45±3.22 ^{bc} | 20.67±2.77 ^{bcd} |
| ASH 02 | 618.3±7.42 ^{cd} | 3.30±0.05 ^f | 1.63±0.10 ^{efg} | 2.25±0.03 ⁱ | 3.70±0.15 ^{cdef} | 291.1±4.37 ^{ef} | 9.79±0.96^g | 29.76±2.27 ^b |
| BRA 01 | 689.5±7.59 ^{bc} | 2.88±0.04^h | 1.96±0.29 ^{cdef} | 4.34±0.65 ^{def} | 2.99±0.45 ^{fg} | 352.65±52.90 ^{bcd} | 23.18±3.48 ^b | 14.75±2.11 ^e |
| NOR 01 | 1070.0±10.00 ^a | <u>4.15±0.05^a</u> | 2.09±0.31 ^{cde} | 2.13±0.32ⁱ | 4.27±0.84 ^{bc} | 405.13±60.77 ^{bc} | 17.45±2.62 ^{cde} | <u>65.62±3.75^a</u> |
| NOR 02 | 1047.0±10.00 ^a | 3.83±0.05 ^c | 2.43±0.36 ^{bc} | 4.73±0.71 ^{cd} | 3.96±0.59 ^{cd} | 386.34±57.95 ^{bcd} | 21.54±3.23 ^{bc} | 24.25±2.84 ^{bc} |
| NOR 03 | 730.7±8.04 ^b | 3.79±0.05 ^d | <u>4.17±0.63^a</u> | 3.27±0.79 ^{gh} | 2.34±0.35^g | 374.23±56.13 ^{bcd} | 14.89±2.23 ^{def} | 21.40±2.60 ^{bc} |
| VOL 01 | <u>1114.0±167.00^a</u> | 4.04±0.05 ^b | 1.47±0.22 ^{fg} | 4.52±0.68 ^{de} | 4.98±0.75 ^{ab} | 433.03±64.95 ^b | 14.17±2.13 ^{efg} | 16.73±2.51 ^{de} |
| VOL 02 | 542.7±6.52 ^d | 3.16±0.04 ^g | 2.65±0.40 ^b | 2.94±0.44 ^{ghi} | 3.01±0.45 ^{fg} | 406.22±60.93 ^{bc} | 15.87±2.38 ^{de} | 24.46±2.82 ^b |
| Mean | 682.03±251.89 | 3.51±0.37 | 2.09±0.75 | 4.09±1.59 | 3.89±1.03 | 386.47±110.34 | 18.38±6.91 | 25.24±13.32 |
| CV (%) | 36.93 | 10.42 | 36.12 | 38.92 | 26.54 | 28.55 | 37.60 | 52.74 |
| RDA | 300-3500 | 3.50 | 0.13-0.42 | 0.75-0.90 | 0.20-1.3 | 3-10 | 2-11 | 2-5 |

Note: Values **bolded and underlined** refer to the highest concentration and lowest concentration respectively of a particular element; ± = standard deviation of the mean mineral element concentration in the accession; CV = Coefficient of Variation; RDA = recommended daily allowance

5.4.1 Sodium content in hot pepper fruits

The highest sodium content of 1114.00 ± 167.00 mg/kg was recorded in Vol 01 while the least was recorded in Acc 04 (315.40 ± 4.74 mg/kg). Average sodium content recorded was 682.03 ± 251.89 mg/kg. There were no significant differences in sodium content in fruit samples among accessions Vol 01, Nor 01 and Nor 02. Similarly, the sodium contents of accessions Bra 01 and Nor 03 were not statistically different. Also, there were no significant differences among accessions Acc 02, Acc 05 and Vol 02.

5.4.2 Magnesium content in hot pepper fruits

Highly significant differences ($p < 0.01$) were recorded among the accessions with reference to the magnesium content in the fruits. Nor 03 recorded the highest magnesium content of 4.17 ± 0.63 , while Acc 01 recorded the least with magnesium content of 1.44 ± 0.22 g/kg. There were, however, no significant differences in magnesium content between accessions Acc 02 and Nor 01. The same can also be inferred for accessions Acc 03, Acc 05 and Ash 02. Magnesium content averaged 2.09 ± 0.75 g/kg for all accessions.

5.4.3 Chlorine content in hot pepper fruits

Chlorine content in fruits of the various accessions studied ranged between 2.13 ± 0.32 - 7.19 ± 0.09 g/kg. The highest chlorine content was recorded in accession Acc 05, while the least was recorded in Nor 01. Mean chlorine content was 4.09 ± 1.59 g/kg. However, there were no significant differences between accessions Acc 05 and Ash 01. Neither was there any difference in chlorine content between accessions Ash 01 and Acc 01.

5.4.4 Calcium content in hot pepper fruits

Accession Acc 02 recorded the highest calcium (Ca) content in fruit samples among all the accessions, while accession Nor 03 recorded the least. Calcium content ranged between 5.55 ± 0.34 – 2.34 ± 0.35 g/kg. Recorded mean calcium content was 3.89 ± 1.03 g/kg. There were no significant differences in Ca content between accessions Acc 05 and Nor 03.

5.4.5 Aluminium content in hot pepper fruits

With respect to aluminium (Al), Acc 01 recorded the maximum concentration of 595.55 ± 89.33 mg/kg. However, this was not statistically different from accession Acc 05 which recorded Al content of 587.9 ± 7.65 mg/kg. Acc 03 recorded the minimum concentration of 255.3 ± 3.58 mg/kg. There were no significant differences in Al content among accessions Bra 01, Nor 01, Nor 02, Nor 03 and Vor 02. Similarly, there were no differences among accessions Acc 02, Acc 04, Ash 01 and Ash02. Mean Al content recorded was 386.47 ± 110.34 mg/kg.

5.4.6 Manganese content in hot pepper fruits

Manganese (Mn) content in fruit samples of the accessions studied was highest in Acc 04 with a value of 36.17 ± 5.43 mg/kg. Ash 02 recorded the least Mn content of 9.79 ± 0.96 mg/kg. Highly significant differences ($p < 0.01$) were observed in the Mn content of all accessions under study (Table 5.6). There were no significant differences among

accessions Acc 01, Acc 03, Nor 01, Nor3 and Vol 02. Average manganese content was 18.38 ± 6.91 mg/kg.

5.4.7 Bromine content in hot pepper fruits

Concentration of bromine (Br) in hot pepper fruit samples ranged from 10.37 ± 1.54 mg/kg in accession Acc 05 to 65.62 ± 3.75 mg/kg in accession Nor 01 . There were no significant differences in Br content among accessions Acc 01, Nor 02 and Nor 03. Neither were there any significant differences among accessions Acc 02, Acc 04, Ash 02 and Vol 02. Similarly, significant differences were not observed between accessions Bra 01 and Vol 01. Mean bromine content recorded a value of 25.24 ± 13.32 mg/kg.

5.4.8 Potassium content in hot fruit samples

Potassium concentrations ranged between $2.88\% \pm 0.04$ - $4.15\% \pm 0.05$, with Nor 01 recording the highest potassium content while Bra 01 recorded the least. There were no significant differences in potassium content between accessions Acc 05 and Nor03, and as well as among accessions Acc 02, Acc 03 and Ash 02. Likewise, there were no differences in potassium content between accessions Acc 01 and Acc 04. Mean potassium content was $3.51\% \pm 0.37$.

5.4.9 Relationship among Mineral Elements in Fruits of Hot Pepper Accessions

Table 5.5 shows the association between pairs of elements in fruits of the various accessions of hot pepper studied. There was a strong and positive correlation between Na and K. Its association with Br was positive but not strong. However, the associations between Na and Mg and Ca as well as Al, Cl and Mn were positive and negative, respectively, but statistically significant. Similarly significant and positive associations were recorded between K and Br as well as between Al and Ca. The correlation between contents of Al and Cl in the fruit was positive and significant. Also a highly significant and positive association was recorded between contents of Al and Cl in the fruit. In contrast the associations between Mg and Cl as well as Mg and Ca were negative and significant.

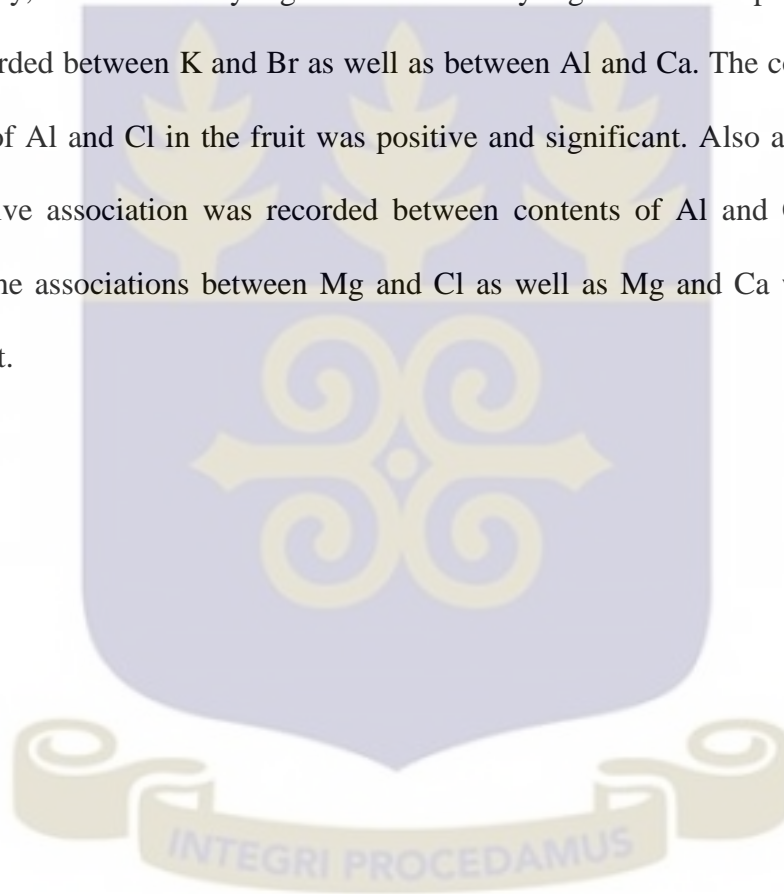


Table 5.5: Correlations among 8 Mineral Elements in fruits of 13 accessions of hot pepper

| Mineral Element | Na | K | Br | Mg | Al | Cl | Ca | Mn |
|-----------------|---|---|---|---|---|---|--|----|
| Na | | | | | | | | |
| K | 0.5903*** (0.0001) | | | | | | | |
| Br | 0.3329* (0.0384) | 0.4134** (0.0089) | | | | | | |
| Mg | 0.0681^{ns} (0.6803) | 0.1523^{ns} (0.3547) | 0.0822^{ns} (0.6188) | | | | | |
| Al | -0.0568^{ns} (0.7314) | 0.3515* (0.0282) | -0.1232^{ns} (0.4551) | -0.0898^{ns} (0.5867) | | | | |
| Cl | -0.0676^{ns} (0.6826) | -0.0769^{ns} (0.6418) | -0.5831** (0.0001) | -0.3347* (0.0373) | 0.4966*** (0.0013) | | | |
| Ca | 0.0218^{ns} (0.8953) | 0.3255* (0.0432) | 0.2063^{ns} (0.2077) | -0.4289** (0.0064) | 0.4309** (0.0062) | 0.1207^{ns} (0.4644) | | |
| Mn | -0.2266^{ns} (0.1654) | -0.1657^{ns} (0.3134) | 0.0383^{ns} (0.8169) | 0.1362^{ns} (0.4082) | -0.2418^{ns} (0.1381) | -0.1269^{ns} (0.4413) | 0.0086^{ns} (0.9587) | |

Below each correlation coefficient (**bolded**) is P-value (underlined). *, **, *** = significant at $P \leq 0.05$, 0.01, 0.001 respectively; Ns = not significant at $P \leq 0.05$.



5.5 DISCUSSION

5.5.1 Macro Elements

The analysis revealed significant variation in macro, micro as well as the trace elements. K and Na are required for bone and teeth development. They are important for normal muscle function, transmission of nerve impulses, metabolism and utilisation of B-group vitamins (Paul and Southgate, 1988).

Sodium content varied depending on the accession. Fruits from all three accessions collected from the Northern Region had high sodium content. Since all the accessions were grown on an experimental plot and replicated three times, the variations in the content of sodium may be attributed to factors such as the preferential uptake of sodium by these lines as well as their need for them in fruit formation and overall plant development (Ahiakpa *et al.*, 2014; Quarthey, *et al.*, 2012; Adotey *et al.*, 2009). The accessions from the Northern Region, as well as, VOL-01 which recorded the highest Na content, may be natural accumulators of Na and are adapted to such environments.

Values recorded in this study are far higher than those reported by Ahiakpa *et al.* (2014); Quarthey *et al.* (2010) and Adotey *et al.* (2009) in fruits of okro, wild tomato and pepper, respectively. Some medicinal plants have also been reported to contain sodium concentrations of 2.16 mg/kg (Debrah *et al.*, 2011) and 5.02 mg/g (Zafar *et al.*, 2010).

Potassium is the primary electrolyte located inside the body's cells (intracellular) and stored in muscle fibres along with glycogen. It plays a critical role by helping transporting glucose into the muscle cell (WHO, 2012b). Potassium also interacts

with both sodium and chlorine to control fluid and electrolyte balance and assists in the conduction of nerve impulses (Kawasaki *et al.*, 1998). Potassium also facilitates the transmission of nerve impulses. Potassium deficiency symptoms are nausea, slower reflexes, vomiting, muscle weakness, muscle spasms, cramping, and rapid heart rate (Geleijnse *et al.*, 2003). All three accessions from the Northern Region, as well as accession VOL-1, recorded high K content in the fruit samples. Attributed reason could be genetic. The plants are possibly adapted to soils with high contents of these elements and hence had developed a natural mechanism to uptake these nutrients from the soil irrespective of the nutritional composition of the soil. Potassium content recorded in this study is also higher than those reported by Ahiakpa *et al.* (2014); Debrah *et al.* (2011) and Adotey *et al.* (2009), in okro (92.14 mg); some medicinal plants (0.08-1.83%) and pepper (0.74-3.07 mg/kg), respectively. Quartey *et al.* (2012) however reported higher K concentrations in wild tomato (maximum of 7.31 g/kg). The RD1 for potassium is 3500 mg which is equivalent to 3.5 g (WHO, 2012b). Regular diets including fruits of hot pepper will contribute significantly to the overall potassium requirements that the body needs.

Magnesium content reported in this study is higher than those reported by Ahiakpa *et al.* (2014) in okra fruits (49.99 mg/kg) and Adotey *et al.* (2009) in fruits of pepper (0.04-0.30 g/kg). Some medicinal plants have been reported to contain between 0.14–0.23 g/kg of magnesium (Dim *et al.*, 2004; Razic *et al.*, 2003 ; Serfor-Armah *et al.*, 2002). Magnesium is a co-factor in more than 300 enzyme systems that regulate diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control and blood pressure regulation (Rude, 2010). Magnesium also plays a role in the active transport of calcium and potassium ions

across cell membranes, a process that is important to nerve impulse conduction, muscle contraction and normal heart rhythm (Larsson *et al.*, 2012).

Chlorine is an essential nutrient in human nutrition, required for healthy functions of nervous and digestive systems (IOM, 2004). Chloride is formed as a by-product of the electrolytes: K, Mg and Na. Chloride is also found in the stomach as HCl, where it maintains pH. To function properly, organisms must actively pump sodium and chloride out of cells and actively take in potassium (Selinus *et al.*, 2005). The RDI for Cl is about 750-900 mg (Brazin, 2006). Concentrations reported in this study varied between 2.13-7.19 g/kg which is lower than the RDI but more than those reported by Adotey *et al.* (2009) in pepper. Suslow (2006) reported Cl content of 250-400 mg/kg in chilli pepper. Likewise, concentrations of Cl recorded for fresh broccoli, cucumber, melon, green onion, tomato and apples ranged from 100-150 mg/kg; 100-150 mg/kg; 100-150 mg/kg; 100-150 mg/kg; 200-350 mg/kg and 100-1500 mg/kg respectively (Suslow, 2006). Zaichick (2002) also reported concentrations of 1.97 mg/kg, 0.58 mg/kg, 0.34 mg/kg, 16.8 mg/kg, 0.50 mg/kg and 1.62 mg/kg in potato, tomato, onion, parsley, carrot and turnip. Variations in this study could be attributed to influence of soil geochemical processes, anthropogenic influences and previous agronomic practices on experimental site (Ahiakpa *et al.*, 2014; Suslow, 2006).

Calcium is required for vascular contraction and vasodilation, muscle function, nerve transmission, intracellular signaling and hormonal secretion, though less than 1% of total body calcium is needed to support these critical metabolic functions (Bailey *et al.*, 2010). The functions of calcium in the human system demand sufficient intake of this mineral (Martin Jr., *et al.*, 1985).

All the accessions used in this study recorded high concentrations of Ca in the fruits. Two accessions from Greater Accra Region, ACC-01 and ACC-02 had very high concentrations of the element. These accessions could have over the years inherently developed the mechanism to metabolise calcium for their use. These two accessions can be explored for future breeding work. Adotey *et al.* (2009) reported in their work that calcium content ranged between 0.26–0.53 g/kg in market-purchased pepper. In okra and tomato, Ahiakpa *et al.* (2014) and Quartey *et al.* (2012) have reported values of 10.34-99.18 mg/kg and 0.04-0.30 g/kg respectively. Zaichick (2002) reported that the calcium content in some commercial tomatoes is 0.31 g/kg. Concentrations reported in this study are far higher than those reported by these authors.

5.5.2 Micro elements

Aluminum is very abundant in the earth crust and in the sea. It is present in only small amounts in animal and plant tissues (Hass, 2006). The amount of aluminum in the human body ranges between 50 and 150 mg, with an average of about 65 mg. Most of this mineral is found in the lungs, brain, kidneys, liver and thyroid. Daily intake of aluminum may range from 10-110 mg (Hass, 2006), but the body will eliminate most of this in the faeces and urine and some in sweat. This may vary depending on location and from person to person. In Europe, aluminium intake from food is estimated at 3-10 mg. To prepare, preserve and store food and drinks, aluminium is used in foils, menu trays, cans, etc. Aluminium intake from cans, foil or saucepans is very small (about 0.1 mg/day) (Hass, 2006).

Values reported in this study are far higher than the RDI and again higher than the concentration reported by Ahiakpa *et al.* (2014) in okro (42.50 mg/kg). In medicinal plants, Debrah *et al.* (2011) reported Al content of between 18.28-504.20 mg/kg.

Appropriate balance and intake of manganese plays a vital role in preserving bone density and thus preventing osteoporosis. Manganese is also noted to play an important role in preventing diabetes, reducing symptoms related to premenstrual syndromes in women and preventing epilepsy (Zhao *et al.* 2002). Adotey *et al.* (2009) have reported concentrations of 16.10-22.50 mg/kg in fruits of pepper. Values reported in this study fall within the limits of those reported by Ahiakpa *et al.* (2014) for okro (36.35 mg/kg), and Quartey *et al.* (2012) for tomato fruits (11.50 mg/kg).

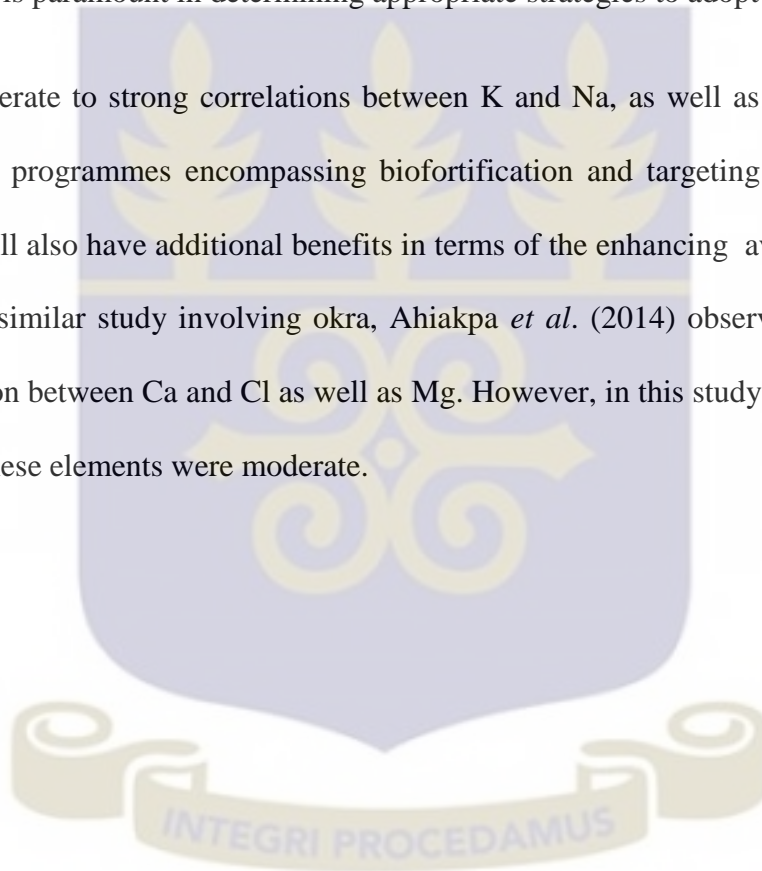
5.5.3 Trace elements

Concentrations of bromine reported in this study are not too different from that of Adotey *et al.* (2009) who reported Br content in accessions of pepper to be between 7.56-29.10 mg/kg. The same workers recorded concentrations of 15.26-37.93 mg/kg, 22.40-47.50 mg/kg, 70.20-142.60 mg/kg and 12.97-29.70 mg/kg in fruits of tomato, garden egg, onion and carrot, respectively. Ahiakpa *et al.* (2014) reported a concentration of between 18.45-104.60 mg/kg. The RDI is 2-5 mg/kg which is lower than values recorded in the current study as well as studies carried out by the earlier workers.

5.5.4 Correlations among 8 Mineral Elements in Fruits of 13 accessions of Hot Pepper

In recent years, there has been a growing interest on the part of breeders to develop new varieties of crops which combine desirable agronomic attributes such as high yield and disease resistance with high elemental composition (Nestel *et al.*, 2006; Gregorio, 2002). Adequate knowledge of association between major essential elements is paramount in determining appropriate strategies to adopt in breeding.

The moderate to strong correlations between K and Na, as well as Br are desirable. Breeding programmes encompassing biofortification and targeting certain elements like K will also have additional benefits in terms of the enhancing availability Na and Br. In a similar study involving okra, Ahiakpa *et al.* (2014) observed a very strong correlation between Ca and Cl as well as Mg. However, in this study, the relationships among these elements were moderate.



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CHAPTER SIX

GENERAL CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Three separate studies, morphological characterisation using qualitative and quantitative traits, molecular characterisation using SSR markers and essential elements using INAA technique, were employed to assess genetic diversity in accessions of hot pepper. Based on the information obtained from the results, the following conclusions can be made:

- 1(a) Substantial variation existed among the 20 accessions of hot pepper with respects to agro-morphological characteristics especially in plant height, plant canopy width and length, fruit shape, colour, weight, length and width.
- (b) The output of the Principal Components Analysis (PCA) revealed diverse contributions of distinct characters to total genetic variance.
- (c) The first two principal components accounted for 80.44% of the total genetic variance among the accessions. Significant variance was accounted for by plant canopy width, number of seeds per fruit, plant height and matured leaf length.
- d) Cluster analysis based on agromorphological traits revealed genetic divergence of 36.90-94.53%. Acc 05 and Upp 01, Nor 01 and Wes 01 exhibited the greatest diversity while the highest degree of similarity existed between Ash 01 and Ash 02.
- e) No duplicates were detected from the agro-morphological traits under study.

- f) Positive interrelationship was established between plant height and plant canopy width, matured leaf length and matured leaf width, number of days to 50% flowering and number of days to 50% fruiting, fruit length and fruit weight, fruit width and fruit length; thus indicating that these attributes are the most essential components for direct selection for improvement in yield in hot pepper.
- g) Accessions Wes 01 and Eat 02 may be particularly suitable for improvement for export due to their high fruit set ability, smooth fruit shape and red fruit colour.
- 2a)** The study also revealed a high level of genetic similarity within the accessions.
- b) Populations 1 and 2 had the greatest similarity and population 3 was widely diverse from population 1.
- c) SSR markers identified accessions Ash 03 and Acc 03 as duplicates and also Wes 01, Ash 02 and Vol 02 as duplicates.
- 3a)** All three accessions from the Northern Region, Nor-01, Nor-02 and Nor- 03, as well as VOL-01 from the Volta Region recorded high amounts of sodium in their fruit samples.
- b) Moderate to strong correlation was observed between K and Ca, as well as Br with varied correlations existing between the rest of mineral elements detected.

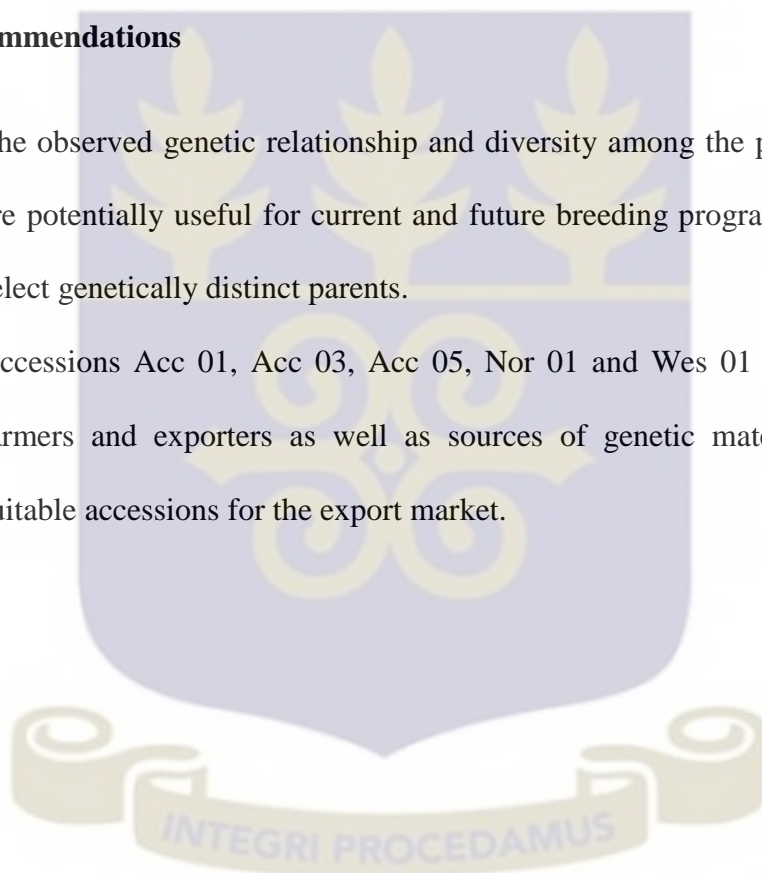
c) Concentrations of potassium and bromine were highest in NOR-01 but varied among the rest of the accessions.

d) Concentrations of aluminium and manganese in the fruits exceeded their recommended daily intake but most of the mineral elements were either moderate or within range of their respective RDAs.

6.2 Recommendations

a) The observed genetic relationship and diversity among the pepper accessions are potentially useful for current and future breeding programmes in order to select genetically distinct parents.

b) Accessions Acc 01, Acc 03, Acc 05, Nor 01 and Wes 01 may be ideal for farmers and exporters as well as sources of genetic materials to develop suitable accessions for the export market.



APPENDICES**Appendix1 ANOVA Table for Na by Accessions**

| <i>Source</i> | <i>Sum of Squares</i> | <i>Df</i> | <i>Mean Square</i> | <i>F-Ratio</i> | <i>P-Value</i> |
|----------------|-----------------------|-----------|--------------------|----------------|----------------|
| Between groups | 2.3538E6 | 12 | 196150. | 89.09 | 0.0000 |
| Within groups | 57246.1 | 26 | 2201.77 | | |
| Total (Corr.) | 2.41105E6 | 38 | | | |

Appendix11 ANOVA Table for K by Accessions

| <i>Source</i> | <i>Sum of Squares</i> | <i>Df</i> | <i>Mean Square</i> | <i>F-Ratio</i> | <i>P-Value</i> |
|----------------|-----------------------|-----------|--------------------|----------------|----------------|
| Between groups | 4.99419 | 12 | 0.416182 | 143.26 | 0.0000 |
| Within groups | 0.0755333 | 26 | 0.00290513 | | |
| Total (Corr.) | 5.06972 | 38 | | | |

Appendix111 ANOVA Table for Mg by Accessions

| <i>Source</i> | <i>Sum of Squares</i> | <i>Df</i> | <i>Mean Square</i> | <i>F-Ratio</i> | <i>P-Value</i> |
|----------------|-----------------------|-----------|--------------------|----------------|----------------|
| Between groups | 19.1378 | 12 | 1.59482 | 17.14 | 0.0000 |
| Within groups | 2.41923 | 26 | 0.0930475 | | |
| Total (Corr.) | 21.5571 | 38 | | | |

Appendix.IV. ANOVA Table for Cl by Accessions

| <i>Source</i> | <i>Sum of Squares</i> | <i>Df</i> | <i>Mean Square</i> | <i>F-Ratio</i> | <i>P-Value</i> |
|----------------|-----------------------|-----------|--------------------|----------------|----------------|
| Between groups | 88.1028 | 12 | 7.3419 | 23.41 | 0.0000 |
| Within groups | 8.15403 | 26 | 0.313616 | | |
| Total (Corr.) | 96.2569 | 38 | | | |

Appendix V ANOVA Table for Ca by Accessions

| <i>Source</i> | <i>Sum of Squares</i> | <i>Df</i> | <i>Mean Square</i> | <i>F-Ratio</i> | <i>P-Value</i> |
|----------------|-----------------------|-----------|--------------------|----------------|----------------|
| Between groups | 34.0408 | 12 | 2.83673 | 11.40 | 0.0000 |
| Within groups | 6.46913 | 26 | 0.248813 | | |
| Total (Corr.) | 40.5099 | 38 | | | |

Appendix VI ANOVA Table for Al by Accessions

| <i>Source</i> | <i>Sum of Squares</i> | <i>Df</i> | <i>Mean Square</i> | <i>F-Ratio</i> | <i>P-Value</i> |
|----------------|-----------------------|-----------|--------------------|----------------|----------------|
| Between groups | 395683. | 12 | 32973.6 | 12.80 | 0.0000 |
| Within groups | 66987.9 | 26 | 2576.46 | | |
| Total (Corr.) | 462671. | 38 | | | |

Appendix VII. ANOVA Table for Mn by Accessions

| <i>Source</i> | <i>Sum of Squares</i> | <i>Df</i> | <i>Mean Square</i> | <i>F-Ratio</i> | <i>P-Value</i> |
|----------------|-----------------------|-----------|--------------------|----------------|----------------|
| Between groups | 1618.15 | 12 | 134.846 | 17.83 | 0.0000 |
| Within groups | 196.668 | 26 | 7.56417 | | |
| Total (Corr.) | 1814.82 | 38 | | | |

Appendix VIII ANOVA Table for Br by Accessions

| <i>Source</i> | <i>Sum of Squares</i> | <i>Df</i> | <i>Mean Square</i> | <i>F-Ratio</i> | <i>P-Value</i> |
|----------------|-----------------------|-----------|--------------------|----------------|----------------|
| Between groups | 6568.01 | 12 | 547.334 | 83.82 | 0.0000 |
| Within groups | 169.779 | 26 | 6.52996 | | |
| Total (Corr.) | 6737.79 | 38 | | | |

APPENDECE 1X: LIST OF PRIMERS

| | |
|-----------|---------------------------------------|
| CAMS032 | Forward:5'-TGCCACATAGGTTGGCTTTC-3' |
| CAMS032 | Reverse:5'-CAAAGCCAATGCACATAATCA-3' |
| CAMS066 | Forward:5'-AAAAACATGCACCAGTCCTT-3' |
| CAMS066 | Reverse:5'-CAACCGCCTGAATTTTCTCT-3' |
| CAMS212 | Forward:5'-TTCCCTTTCCCAACATGGTA-3' |
| CAMS212 | Reverse:5'-ACACCCGAAGATGGGTTAGA-3' |
| CAMS228 | Forward:5'-GAGGGCTAAGCAAAGCAGAA-3' |
| CAMS228 | Reverse:5'-TGCATGTTCCCTTAGTTTCC-3' |
| CAMS396 | Forward:5'-GTCGGCCGTCATTCACTATT-3' |
| CAMS396 | Reverse:5'-AGCTTGATGCACCTGGTCTT-3' |
| CAMS406 | Forward:5'-TAAAAATCGCGGAAAGTTGC-3' |
| CAMS406 | Reverse:5'-GTCGTTCTATGCGGCATTTT-3' |
| CAMS476 | Forward:5'-TTTTCCCTTTCCAGTTGTTCA-3' |
| CAMS476 | Reverse:5'-ATGGGTGAAGTGTGAAAAGAA-3' |
| CAMS493 | Forward:5'-TCGATGACGAAAAAGTGTGAA-3' |
| CAMS493 | Reverse:5'-AGGGCAAAAGACCCATTCTT-3' |
| CAMS823 | Forward:5'-TCCTCCTCTTCTCGTGTTTC-3' |
| CAMS823 | Reverse:5'-AAAGAAGCAGCAGGTGAAGA-3' |
| CAMS871 | Forward:5'-ACAAAGCATCGGCTGAAAAT-3' |
| CAMS871 | Reverse:5'-GCGACCAAGTACCAACAGGT-3' |
| AVRDCPP32 | Forward:5'-ATGGAGGATTACCTCGCAAC-3' |
| AVRDCPP32 | Reverse:5'-CATGATGACCATCCATCCAT-3' |
| CAMS 117 | Forward:5'-TTGTGGAGGAAACAAGCAAA-3' |
| CAMS 117 | Reverse:5'-CCTCAGCCCAGGAGACATAA-3' |
| CAMS 072 | Forward:5'-CCCGCGAAATCAAGGTAAT-3' |
| CAMS 072 | Reverse:5'-AAAGCTATTGCTACTGGGTTCCG-3' |
| CAMS 885 | Forward:5'-ACCGAAAAGCAACCCAATCA-3' |
| CAMS 885 | Reverse:5'-TTGAAATTGCTGAAACTCTGAA-3' |
| CAMS 492 | Forward:5'-GTTCAAACACTTCCCCCTCA-3' |
| CAMS 492 | Reverse:5'-TGTCATCGTTGGTCGTTACC-3' |

