CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background information

Cowpea [*Vigna unguiculata* (L.) Walp.] is a tropical grain legume originating from Africa (Pasquet, 1999) and is widely grown in Africa, Latin America, Southeast Asia and Southern United States. It is estimated that Cowpea's annual production is more than 3 million tons worldwide with West and Central Africa accounting for over 64% of the 12.5 million hectares of the cultivated areas, followed by central and South America (19%), Asia (10%), East and Southern Africa (6%) (Singh el al., 1997)

This legume is composed of wild and cultivated forms with the wild forms only encountered in Africa (Pasquet, 1999). This crop plays an important role in the livelihood of millions of relatively poor people in the less developed countries in the tropics. It is used as a source of food, animal feed and as cash (Quin, 1997). However, like many other crops, cowpea is susceptible to a wide variety of pests and pathogens that attack the legume at all stages of its growth and significantly reduces its production. In order to control cowpea pests and diseases, the use of

insecticides has been the most commonly adapted method; but most farmers, due to their low income, cannot afford them (Saxena and Kidiavai, 1997), can't have the necessary equipment required, don't know how to apply them safely and effectively and they are hazardous (Saxena et al., 1989). Due to the deleterious effects of insecticides to man, the environment and livestock, alternative strategies for the insect pest management are being developed and include: (1) Habitat management by the use of mixed cropping system (Saxena and Kidiavai, 1997). Dissemond and Hindorf (1990) revealed that insect pest population was lower in sorghum/cowpea/maize intercrops than in pure cowpea strands. (2) Biological control through biotechnology, the genetic transformation aiming to develop resistant/tolerant cowpea cultivars.

Molecular markers are biochemical or DNA based molecules used to characterize population variability, evaluation of genetic diversity as well as determination of genetic relationships within and among animal and plant populations. Some of these markers have been used to address evolution in cowpea: Isozymes (Pasquet, 2000), Random Amplified Polymorphism DNA (RAPD) (Fall et al., 2003; Ba et al., 2004), Amplified Fragment Length Polymorphism (AFLP) (Coulibaly et al., 2002), Restriction Fragment Length Polymorphism (RFLP) (Menéndez et al., 1997, Feleke et al., 2006) and Simple sequence repeat (SSR) (Li et al., 2001) markers. This implies that the organization of the wild and cultivated cowpea gene

pool as well as cowpea's population genetics dynamics can be assessed using these molecular markers.

The evolution of a species is the consequence of the distribution and organisation of the genetic diversity within and between populations (Li and Rutger, 2000). Genetic diversity and relationship in many crop species and their wild relatives regarding the geographical distribution, genetic organisation and domestication region investigation have been largely assessed (Sakti and Lukasz, 1988; Peterson et al., 1994; Chan and Sun, 1997; Desplanque etal., 1999; Mace et al., 1999; Colunga-Garcia et al., 1999; Li and Rutger, 2000; Olsen and Schaal, 2001; Coulibaly et al., 2002; Ba et al., 2004; Snoussi et al., 2004; Mariac et al., 2006) and results from these studies have provided useful knowledge and strategies for efficient exploration, conservation and utilization of genetic resources in plant species. The organisation of *V. unguiculata* into eleven major subspecies have been well documented (Pasquet, 1999, Coulibaly et al., 2002; Ba et al., 2004) and the differentiation of the crop into five different cultivar groups has also been reported (Pasquet, 2000); but the geographic distribution, the organisation of the genetic variation and relationship within *V. unguiculata* have not been fully characterized

Like many other crops, wild and cultivated cowpeas are repositories of genes providing important genetic resources available. In plant species, the structure of

allozyme diversity is controlled by a variety of species-specific traits like site constraints, survival and reproduction of individuals in a population over time. The way by which genetic variation is distributed among populations is closely related to gene flow both by pollen and seed dispersal (Loveless et al., 1998). Thus, the level of isolation of populations will influence the genetic structure of a species. Highly self-pollinated species with limited pollen and seed dispersal are expected to show low level of gene flow, high inbreeding coefficient and most of the genetic variation to be observed among populations and conversely, outcrossed species with wide seed and pollen dispersal are expected to be characterized by a high level of gene flow, limited population differentiation and low inbreeding coefficients (Ronny and Jon, 2000).

Plants exhibit various breeding systems to produce their progenies and these include self-pollination, biparental inbreeding and random outcrossing (Frankham et al., 2002). The mating system is responsible of the genetic composition of future generations of a plant population. Therefore, its behaviour appears to be an important feature on the amount and distribution of the genetic variation within and among population and evolution of species (Wright, 1921, Stebbins, 1957). In addition, it is of essential importance for genetic breeding initiatives and conservation programs. Many genetic and ecological factors affect the outcrossing rate in a natural plant population (Clegg, 1980). These include degree of self-compatibility (Murawski and Hamrick, 1991), population density (Ellstrand et al.,

1978; Routley et al., 1999; Field et al., 2005), rainfall distribution (Rice, 1990; Adel et al., 2004), air temperature (del Rio and Búrquez, 1986; Adel et al., 2004,), availability of competing flower species (Johanna, 1983) and pollinator activity (Hirao et al., 2006). Because of these factors, the mating system in plant populations is subject to changes and can range from a high level of inbreeding due to self-fertilization to extensive outcrossing. Cowpea (*V. unguiculata* (L.) Walp.) is a hermaphrodite diploid plant species that is primarily self-pollinated. It has perfect coloured flowers adapted to self-fertilization as well as to outcrossing. They attract insects, especially large bees, *Xylocopa caffra*, *Xylocopa senior* and *Xylocopa flavorufa,* although smaller ones like megachiles are also effective pollinators (Pasquet et al. 2008). These insects are mainly responsible for the cross-pollination.

1.2 Justifications of the study

Wild and cultivated cowpeas are not resistant to insect pests. For this reason, Scientists have been working to produce genetically modified cowpea plants with insect resistant gene, producing insect specific toxin, *Bacillus thuringiensis* (Bt) toxin (Murdock et al., 2002). This protein is specific in their activities against Coleoptera and Lepidoptera (Schnepf et al., 1998), especially for Maruca Pod Borer (*Maruca vitrata),* which heavily reduce cowpea productivity in Africa (Machuka et al., 1999).

Therefore, with the upcoming introduction of Bt cowpea in Africa, there are possibilities that the Bt gene will be transferred through hybridization and backcross processes from transgenic plants to other cultivars and wild relatives because of the existence of weak genetic barriers within cowpeas (Fatokun, 1991). The first plants with the engineered insect resistant gene will tend to be more developed and aggressive than others. This implies that there will be a possible genetic swamping of the inserted gene affecting cowpea gene pool. It is therefore important to estimate gene flow in order to predict how fast the distribution of the Bt gene might be, to study the genetic relationship within cowpeas as well as the breeding systems. This is especially important, since the likely diffusion of Genetically Modified (GM) cowpea can interfere with the equilibrium that exists between wild and domesticated cowpea.

Moreover, knowledge on the organization of cowpea gene pool until recently has been largely assessed using allozymes (Panella and Gepts, 1992; Pasquet, 1993b, 1999) because of its simplicity in screening large samples. Using both isozymes and DNA based marker will give more light on the genetic relationships and phylogenetic studies in cowpea as opposed to the use of a single marker. Previous studies on cowpea have been mostly carried out using accessions. Using cowpea populations will allow the access of others parameters in population genetics

studies: the level of genetic exchange or gene flow and population outcrossing rates.

Wild cowpea plants are found only in tropical Africa and Madagascar (Ba et al., 2004) and therefore, there is no doubt on the African origin of the crop. However, the domestication area of *V. unguiculata* is still under speculation. Molecular investigations highlight a unique domestication event in *V. unguiculata* (Panella and Gepts, 1992; Pasquet, 1999). Several domestication centres have been proposed and are summarised in Ba et al. (2004). Despite the fact that several genetic analysis studies were undertaken in *V. unguiculata* (Panella and Gepts, 1992; Vaillancourt et al., 1993; Pasquet, 1993b; 1998; 1999; 2000; Coulibaly et al., 2002; Ba et al., 2004; Feleke et al., 2006), the genetic diversity and relationship between wild and cultivated cowpea prior to domestication area investigation is poorly documented.

1.3 Hypothesis

1) There is a genetic relationship between wild and cultivated cowpeas.
2) There are cultivated specific markers unevenly distributed within wilds.
3) Populations are in Hardy-Weinberg equilibrium.
4) There is isolation by distance in populations of wild *V. unguiculata.*
5) There is a genetic exchange within cowpeas.

6) Outcrossing rate in cowpea is dependent on temperature, rainfall distribution and flower density.

1.4 Objectives

1.4.1 General objective:

To determinate the population genetics structure, the dynamics of the mating system, the wild crop complex dynamics and genetic relationship within *Vigna unguiculata ssp. unguiculata* using isozymes and PCR-RFLP markers an access the organization of cowpea gene pool, its genetic diversity, its breeding system as well as gene flow.

1.4.2 Specific objectives:

1) Assess the genetic relationship between wild and cultivated cowpea
2) Identify cultivated specific markers, assess their distribution within wild and locate the origin of the cultivated species of cowpea.
3) Assess and test the genetic structure of cowpea populations relative to the Hardy – Weinberg expectations

4) Determine and test the relationship between genetic and geographic distance in cowpea populations

5) Estimate the populations outcrossing rates and level of gene flow between populations

6) Assess the dynamics of the mating system of a cowpea population through time and its relation to plant density, temperature and rainfall distribution

CHAPTER TWO

LITERATURE REVIEW

2.1 COWPEA (*Vigna unguiculata* (L.) walp.)

2.1.1. Cytotaxonomy, nomenclature, origin and domestication

2.1.1.1. Cytotaxonomy and nomenclature

The Cytotaxonomy of Cowpea like other vigna species is relatively simple; the plant is a diploid species (2n = 2x = 22) with little genetic and no chromosomal divergence between the cultivars and their putative wild ancestors (Steele, 1976). Cowpea (*Vigna unguiculata* (L.) Walp) is one of the 80 species of the genus *Vigna* (Pasquet, 2001) that was heavily researched during the 20th century. It is a Dicotyledonea belonging to the order Fabales, family Fabaceae, subfamily Faboideae, tribe Phaseoleae, subtribe Phaseolinae, genus Vigna (Padulosi and Ng, 1997)). Cowpea has several common names. In the United States, it is referred to as black-eyed pea, crowder pea, southern pea (Duke, 1981) or black-eyed bean (Miller, 1988). This legume is also called bean in Anglophone West African counties and niébé in French speaking.

2.1.1.2 Origin and domestication

Since the wild cowpea forms are endemic to Africa, the African origin of cowpea has never been a point of contention (Pasquet, 1999). The precise

origin of cowpea has been a matter of speculation and discussion for many years. Several sites in West, Central and East Africa have been proposed as centres of origin of cowpea (Harlan, 1971; Steele, 1972; Coulibaly et al., 2002; Popelka et al., 2004). Wild plants in nature are highly variable. Some can be cultivated without naturalization while others require genetic acclimatization for domestication (Kupzow, 1980). The nature of the initial material seems therefore to be of great importance on the success of domestication. Since many years, the domestication of wild plants have progressively become an important feature; this in connection with the intensity of agriculture through the increase of animal rearing and human population requiring more hay, pastures and foods for consumption, development of industries and the rising needs of medicinal therapeutics. Ng (1995) postulated that there was a change in growth habit during the evolution of *Vigna unguiculata* with evolvement from the wild, cultivated cowpea through the domestication process. The species was brought under cultivation through natural selection and there was a loss in seed hardness and pod dehiscence corresponding to an increase in pod and seed size (Lush and Evans, 1981).

The cultivated cowpeas form a genetically coherent group and are closely related to the annual wild cowpea, ssp. *unguiculata* var. *spontanea* (Schweinf) *Pasquet*. (Pasquet, 1999). The morphology and growth habits of this wild legume are very similar to those of cultivated cowpea except that its mature pod contains small seeds (wild-like attribute) and are dehiscent, much smaller than those of cultivated cowpea (Padulosi and Ng, 1997). The precise location

where the crop was domesticated is still under speculation, but the wide geographical distribution of wild cowpea in sub-Saharan Africa indicates that *V. unguiculata* could have been brought under cultivation in any part of the region. According to Ng and Marechal (1985), the selection of cowpea as a pulse as well as for fodder may have brought under cultivation the cultigroup *unguiculata* and selection for cowpea with long peduncles for fibers and fodders has resulted to the cultigroup *textilis*. From West Africa, the cultigroup *unguiculata* was introduced to East Africa, then to Europe and India where further diversification produced the cultigroup *sesquipedalis* and *biflora* (Steele and Mehra, 1980)

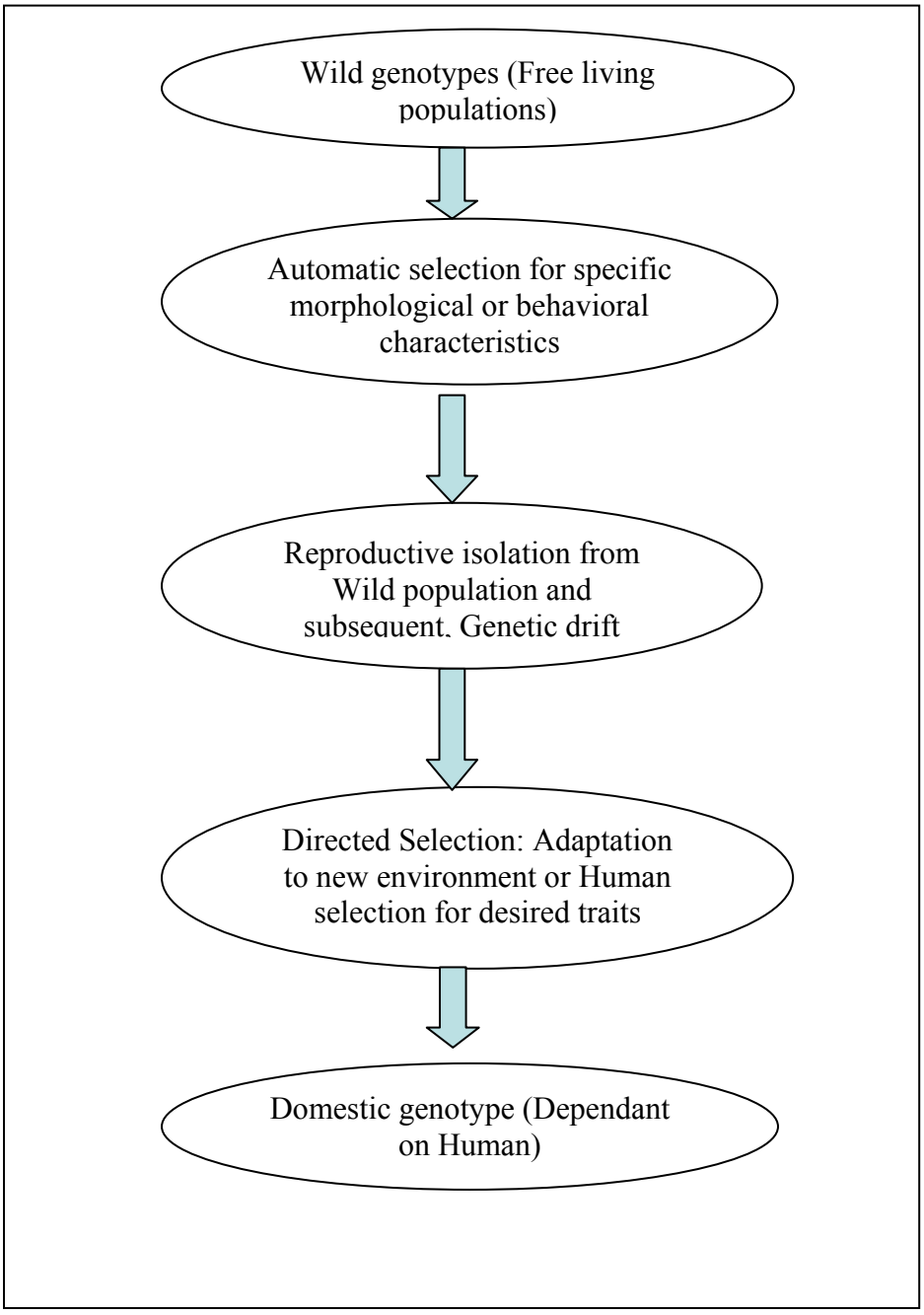


Figure.2.1. Domestication process in plants (Zeder, 2006)

2.1.2 Ecology, biology and reproduction

Cowpea is an herbaceous legume grown in tropical areas. As a warm season crop, cowpea is well adapted in many areas of the humid tropics and temperate zones. It tolerates heat and dry conditions but is intolerant to frost (Duke, 1981). Cowpea also perform well on a wide variety of soils and soil conditions, but perform best on well-drained sandy loams or sandy soils from highly acid to neutral; and is less tolerant in alkaline conditions (Duke, 1981). Cowpea germination is rapid at a temperature above 18°C with an optimum at 28°C (Craufurd et al., 1997). Seeds germination is epigeal with the cotyledons emerging from the ground. The first two leaves above cotyledons are simple and opposite, the others are alternate, petiolate and trifoliate (Pasquet and Baudoin, 2001). Peduncle that arises from the leaf axial contains commonly two or three pods and sometimes can carry four or more pods. Cowpea plant generally sets flowers 30 to 40 days after germinating with a life cycle of 60-240 days (Duke, 1981; Miller et al., 1989).

Cowpea is strongly tap rooted in general, with a strong principal root and many spreading laterals root on the soil surface. This disposition enables the plant to explore the soil for moistures (Pasquet and baudoin, 2001). Cowpea's roots have nodules containing nitrogen-fixing bacteria. It can be grown in polyculture, associated with cassava, corn, sorghum or millet (Duke, 1981). Annual cowpea primarily is a self-pollinating plant (Fery, 1985), the open display of flowers above the foliage and the presence of floral nectarines attract insects that some have been identified as cowpea pollinators. The cultivated

cowpea flowers open at the end of the night and close late morning. Some wild cowpea forms are considered to be allogamous, due to a particular arrangement of the anthers and stigma that prevent self-pollen to reach the stigma (Pasquet and Baudoin, 2001). In inbred plants (autogamous), the stigmatic surface and the anthers are in contact (Lush, 1979). The low fertility in allogamous flower, which range from 0 to 40% versus 40 to 70% for autogamous flowers, can be compensated by manual fertilization or insect tripping (Pasquet and Baudoin, 2001).

2.1.3 Organisation of *Vigna unguiculata*

Vigna unguiculata (L) Walp. is composed of cultivated cowpea (*V. unguiculata ssp unguiculata*), subdivided in five cultigroups or varieties namely *unguiculata ,biflora*, *melanophthalmus*, *sesquipedalis and textilis* (Pasquet, 1998). Wild gene pool includes annual wild cowpea (*Vigna unguiculata ssp. unguiculata var. spontanea)* and ten wild perennial subspecies divided in two groups according to their breeding system. The perennial allo-autogamous subspecies (*dekindtiana, stenophylla, tenius, alba* and *pubescens*) are the most closely related to the annual forms than do the perennial allogamous subspecies (*pawekiae, burundiensis, letouzeyi, baoulensis* and *aduensis*) (Pasquet and Baudoin, 2001). The organization of the different cowpea subspecies is shown in the figure 2.2.

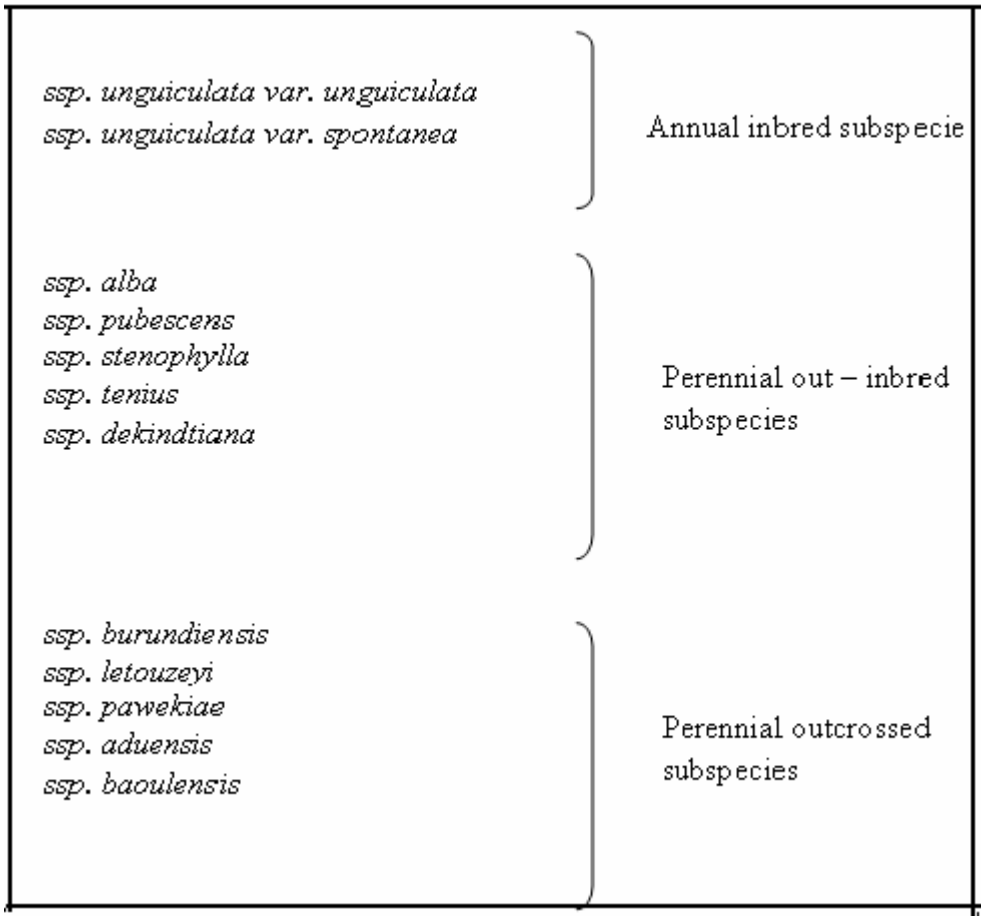


Figure 2.2 Structuration of *V. unguiculata* subspecies (Pasquet, 1999)

2.1.4. Diversity of cowpea

2.1.4.1. Morphological diversity

Cowpea is morphologically very diverse (Pasquet, 1999). From the wild, cowpea has been selected for various traits, giving rise to several varieties with a lot of differences in their morphology. The cultivated forms of cowpea show a great diversity in their seed and pods while the wild forms present a lot of

variability in their floral morphology and vegetative characters (Pasquet, 1993a). Cultivated cowpeas differ from wild forms by having larger seeds and pods, non-dormant seeds and non-dehiscent pods (Lush and Evans, 1981). Cowpea plants can be found erect, semi-erect, prostrale or climbing in their growing areas. Fruits of the cowpea plant are pods that vary in size, colour and texture; they can be erect, crescent-shaped or coiled and are usually yellow when ripe but can also be brown or purple in colour. The length of the pod, which usually can reach 30 cm, contains 8-20 seeds that vary in size, shape and color. Seed color is determined by the C gene, a dominant gene associated with genes controlling pigments synthesis (Pasquet and Baudoin, 2001). They are very diverse, ranging from white, black, brown, purple, green, and red to various types of mottled seeds. Cowpea stems are smooth or slightly hairy and sometimes tinged with purple. Stigma orientation can be vertical or horizontal (Pasquet, 1993a). Self-pollinating flowers are arranged in raceme or intermediate inflorescence in alternate pair and can be white, dirty yellow, pink, pale blue or purple in color. Flower length ranges from 21 to 43 mm (Pasquet, 1993a). Are found in wild cowpea characteristics such as perenniality, hairiness, small size of pods and seeds, pod shattering, outbreeding and bearded stigma (Padulosi and Ng, 1997)

2.1.4.2. Genetic diversity

Many studies carried out on cowpea reveal that the crop exhibits important genetic diversities and variabilities (Pasquet, 1999; Li et al., 2001; Fall et al., 2003). Using allozymes variations to study 199 germplasm accessions of wild

and cultivated cowpea, Pasquet (1999) showed that cowpea taxa (ranked as subspecies) could be considered as different species considering the high genetic distances observed between accessions from different taxa.

2.1.5. Uses of cowpea

Cowpea has a wide variety of uses for human as well as for livestock feed. It can be used at all stages of its growth (fresh leaves, peas and pods) as a vegetable crop. Several snacks and meal dishes are also prepared from its grains (Quin, 1997). Its tender green leaves are an important food source in Africa and are prepared as a potherb, like spinach. In industrialized countries, cowpea green seeds are cooked, canned or frozen to make them ready to serve. Dried mature seeds are also suitable for boiling and canning. With its high protein content (20-25%), cowpea has been referred as a poor man's meat (Fall et al., 2003) and is considered as a source of cheap protein in both rural and urban tropical African diet with its protein digestibility higher than that of other legumes (Marconi et al., 1990). Proteins in cowpea seeds are rich in amino acid lysine and tryptophan compared to cereal grains; however it is deficient in methionine and cystine when compared to animal proteins. Therefore, cowpea seeds are valued as a nutritional supplement to cereals and an extender to animal proteins. In many regions around the word, cowpea is used as high quality legume hay for livestock feed.

Cowpea is also used to improve soil fertility (Ogbuinya, 1997). As nitrogen fixing crop through the symbiotic association with the bacteria *bradyrhizobium ssp,* cowpea contributes to the available N level in the soil and in that case, increasing the yield of cereal crops when grown in rotation. Therefore, cowpea is a versatile crop feeding people, their livestock and the next crop. With its deep roots, cowpea helps to stabilize the soil preventing land deterioration and minimizing soil erosion (Davis et al., 1991). The cultivar group *textilis* with its long floral peduncles is used for fiber production in West Africa. Cowpea plant is also used for medicinal purposes (Padulosi and Ng, 1990).

2.1.6. Cowpea production constraints

2.1.6.1. Biotic stresses.

2.1.6.1.1 Diseases

A wide range of parasites and pests limits cowpea production and this include: Bacteria (*Xanthomonas axonopodis pv. vignicola, Clavibacter michiganensis subsp. michiganensis* and *Acidovorax avenae subsp. citrulli)* (Gitaitis et al., 2004); Fungi with *Pythium ssp, Rhyzoctonia solani* and *Phytophtora ssp* mainly attacking seed while *Corynespora cassiicola, Cercospora canescens* attacking leaves and *Cladosporium vignae*, *Choanephora cucurbitarum* attacking the pods (Allen, 1983). Cowpea is also attacked by virus like CCMV (Cowpea Chlorotic Mottle Virus), Yellow Mosaic Virus, transmitted by white fly (*Bemisia tabaci*) vector and affect both vegetative and reproductive part of the plant (Yousaf et al., 2002). Yellow Mosaic Virus may cause 14-54%

decrease in plant height, 30 to 95% decrease in dry stem weight of cowpea (Yousaf et al., 2002). Cowpea is further attacked by pests throughout its growth cycle like nematodes (*Meloidogyne*, *Rotylenchus and Pratylenchus*) on the roots (Pasquet and Baudoin, 2001).

2.1.6.1.2 Insect pests

Cowpea also suffers from insect pests both in the field as well as when the seeds are dried and stored after harvesting (Oghiakhe, 1995). In Africa, no other crop suffers such high yield losses due to a plethora insect pests as cowpea does (Hans, 1996) and these pests constitute the greatest constraint on cowpea production in Africa (Oghiakhe, 1995). Among them is cowpea aphid (*Aphid craccivora*) (Nuessly et al., 2004); they feed by piercing plant tissues and sucking plant juices. Their feeding, especially on the fruiting stem, reduces considerably the quantity of the plant nutrients available for pod and pea development. Other insect pests include Maruca Pod Borer *(Maruca vitrata),* very destructive*,* pod sucking bug (*Chlavigralla tementosicollis*) (Oghiakhe, 1995; Hans, 1996), bean fly (*Ophiomyia phaseoli*), leafhoppers (*Empoasca spp.*) and cowpea storage weevil (*Collosobruchus maculates*) (Oghiakhe, 1995). We also have cowpea curculio which causes blister like spots on the surface of the pod; Leaf Feeding Beetles that, with their foliage feeding habits cause irregular shaped holes in the leave. Another most important cowpea insect pest is legume or bean-flower thrips (*Megalurothrips sjostedti*). It is the most destructive, attacking the reproductive structures of the cowpea during plant development (Saxena and Kidiavai, 1997).

2.1.6.2 Abiotic stresses

Environment stresses that include extreme temperatures, soil salinity and drought represent major limitation factors for agricultural productivity. Some plant species have developed various mechanisms to adapt in such stressful conditions (Hirt and Shinozaki, 2003). The result of the environmental effects on plant growth may be the difference of the damage effect or stress caused by the environment and the adaptative response controlled by the plant (Fitter and Hay, 1987). When the environmental stress is dominant, damages may occur and are manifested by the death of all or part of the plant reducing growth rate and productivity. Drought is a sustained period of time without significant rainfall (Linsley et al., 1959). Cowpea is largely cultivated in tropical and semi-arid zones where drought frequently occurs and may be the most serious environmental agent capable to limit cowpea production.

2.1.7 Genetic improvement of cowpea: Bt technology

Grain yield losses in cowpea are particularly due to biotic stresses and especially insect pests that include Maruca Pod Borer (*Maruca vitrata*), pod sucking bug, aphids, thrips and bruchids. Conventional insecticides are not the answer to the insect problems because many cowpea growers can't afford them. To introduce insect resistance trait into cowpea genome, advanced research in molecular biology has made it possible (Higgins et al., 2007). Studies carried out on some main cowpea insect pests indicated that these insects could be controlled by *Bacillus thuringiensis* crystal protein or (Bt) toxin produced by *Bacillus thuringiensis* (Bt), a bacterium occurring naturally

in the soil (Lambert and Peferoen, 1992). The Bt toxin act by binding to the membrane of the insect midgut epithelial cell leading to the lyses of the cell and eventually kills the insect (Schnepf et al., 1998).

The Bt technology starts with the identification of the Bt gene producing the desired lethal protein and then follows four steps. (1). The Bt gene is combined with a marker gene with antibiotic resistance characteristic. (2). The Combined Bt gene + marker is then inserted into plant cells. (3). Plant cells are grown in the presence of antibiotics. (4). Plant cell carrying the Bt and the antibiotic resistance gene grows in the plant and the plant survives with insect attack.

2.1.8. Environmental risks linked to the introduction of transgenic or Bt cowpea.

Scientists have made possible the development through biotechnology of the genetically modified cowpea called Bt (*Bacillus thuringiensis*) cowpea with a highly effective insect resistant gene (Murdock et al., 2002; Higgins et al., 2007). However, the introduction of this Bt cowpea like other Bt crops is viewed with many perceived risks: (1) That they can have a harmful effect on non-target and beneficial insects; (2) Widespread use, they will transform insects to species resistant to Bt toxin, the pest that they are intended to control; (3) Accumulation though gene flow of the transgene into the native materials with the possibility of affecting the genetic diversity of landraces and wild plants; (4) The possible transfer of that insect resistant gene (Bt gene) through pollen flow from the transgenic plant to other cultivars and the wild relatives

with the evolution of more aggressive weeds, difficult to control (Ellstrand and Hoffman, 1990); and specially within *Vigna unguiculata* subspecies where genetic barriers are weak (Fatokun, 1991). This happens because genetically engineering plants very often have the potential to spontaneously hybridize with the wild relatives growing in proximity (Ellstrand and Hoffman, 1990).

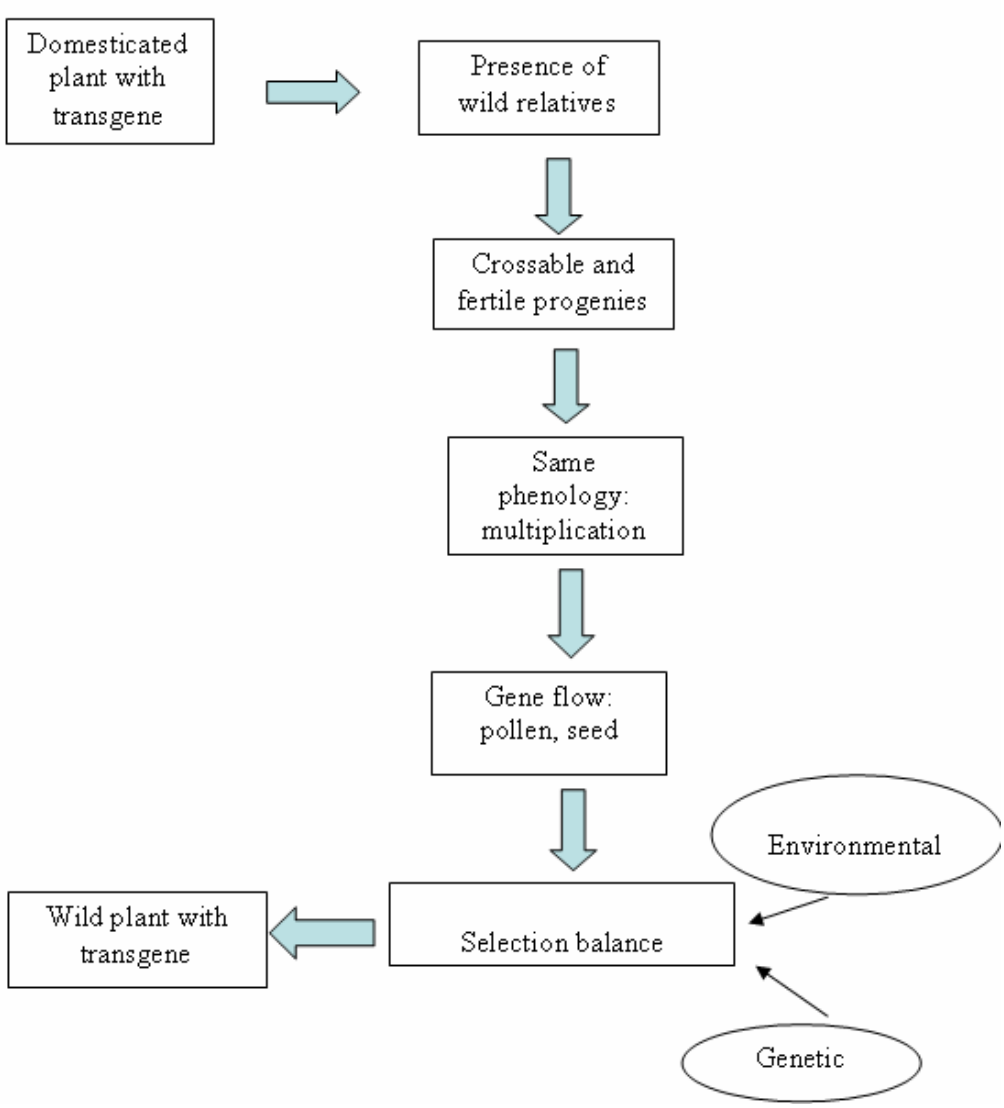


Figure 2.3: Escape process of transgene from domesticated plants into wild relatives (Gepts and Papa, 2003)

2.1.9 Genetic exchange within cowpeas

Gene flow is the movement of gene among populations or within a population. It has a significant influence on the distribution of the genetic traits (Hamrick, 1989). Gene flow occurs through reproductive means such as cross-pollination or directly through "horizontal gene transfer" occurring between species like during genetic engineering. Between *Vigna unguiculata* subspecies, reproductive barriers are weak (Fatokun, 1991) and thus, should facilitate gene movements or gene flow.

Allozyme markers suggest that within *Vigna unguiculata*, gene flow is quite widespread between the wild and the cultivated cowpea, giving a large crop-weed complex well distributed in the entire Sub Saharan Africa (Pasquet, 1999). In a recent study with Malawian cowpeas using randomly amplified polymorphism DNA (RAPD) markers, Nkongolo (2003) showed that there is an important variation among cowpea accessions, variation accounted for 96% and sustaining an uncontrolled gene flow. Coulibaly et al. (2002) also highlighted an extensive gene flow between wild and cultivated cowpeas when they evaluated genetic relationships in 117 accessions of cowpeas (*Vigna unguiculata* (L.) Walp) using amplified fragment length polymorphism (AFLP), with the wild cowpea more diverse than the cultivated.

2.2 Experimental approaches used in plant domestication investigation.

Plant domestication for sustaining Humans survival started 12,000 years ago (Salamini et al., 2002) and today has led to the stratification of our society and development of key technologies.

2.2.1. Molecular markers

In recent years, various DNA based techniques have been used to trace the domestication area of many animal and plant species. This has been possible by the existence of polymorphism at a nucleus or organelle DNA loci, making differences between individuals. Among these DNA based methods are restriction fragment length polymorphism with the use of restriction enzymes (Sonnante et al., 1994; Peterson et al., 1994; Desplanque et al., 1999) random amplified polymorphic DNA (Chan and Sun 1997; Ba et al., 2004), microsatellites (Olsen and Schaal, 2001; Viard et al., 2002; Yoshihiro et al., 2002; Grassi et al., 2003; Mariac et al., 2006), amplified fragment length polymorphism (Mace et al., 1999; Coulibaly et al., 2002) detected through PCR based procedures and Iso-enzymes (Sakti and Lukasz, 1988; Colunga-Garcia et al., 1999; Li and Rutger 2000). These approaches are useful in determining the origin of the cultivated species of plants with wild relatives.

2.2.2. Genetic distances

Genetic distance is the measure of the relatedness of the genetic material between populations or species (Shriver et al., 1995). Using these molecular marker techniques, various algorithms exist for genetic distance calculations.

Some infer distance on the basis of the presence or absence of character while others infer an estimate for the number of nucleotide substitution that may have occurred between populations or species.

2.2.3. Phylogenies

A phylogeny is a graph that illustrates the relatedness of individuals, populations or species (Graur and Li, 2000). Distance methods can be used to construct a phylogenetic tree from a matrix of pair wise genetic distances. Another different method involves the construction of trees of species or populations on the basis of the overall similarity of their allele frequencies.

2.2.4. Cytological methods

The genetic variation among related species can be assessed by comparing the organization of their chromosomes. It would be therefore possible to appreciate chromosome inversion, duplication, translocation and ploidy changes that can distinguish crop plants from their wild progenitors

2.3 Isozymes

2.3.1 Definition

Isozymes or isoenzymes are different forms of proteins encoded by structural gene and sharing a common substrate. They can also be considered as close related variant of enzymes that differ in amino acid sequence and catalyze the

same chemical reaction. Isozymes generally arise through gene duplication, polyploidisation or hybridization.

2.3.2 Isozyme as molecular Marker

Whilst catalyzing the same reaction, change in the sequence of the amino acid affect the electric charge of the enzyme. They then differ in electric mobility detectable after gel electrophoresis and enzyme staining. Electrophoresis is done using various support media, which are cellulose acetate membranes, agarose, polyacrylamide and starch gels. The two first support media are not generally employed in the study of enzyme polymorphism because of the lack of sufficient resolving power (Wendel and Weeden, 1989).

Isozymes were discovered by Hunter and Market in 1957 and so far have been mainly investigated to assess genetic diversity in the *Vigna unguiculata* complex (Sonnante et al., 1997; Pasquet, 1999) as well as in many other animal and crops species. They are one of the most likely materials for genome analysis by geneticists (Pasteur et al., 1987). Miroslav, (2000) identified isozymes as a powerful tool for gene variability within and between animal and plant populations. Isozymes can be encoded by alternate alleles of the same gene and in these case, allelic products called allozymes are very useful for genetic structure and closely related species studies (Kephart, 1990; May, 1992). Isozymes and allozymes have been extensively used to describe genome of many plant species including cowpea (Pamela and Gepts, 1992; Pasquet, 1993b, 1999).

2.3.3. Advantages and drawback of isozymes

Although they are now being largely supplanted by DNA-based approaches more informative like single nucleotide polymorphism and microsatellites, protein gel electrophoresis remains a great and powerful research tool for population genetic and diversity studies because: (1.) A proportion of coding gene is highly polymorphic with two or more allele per gene. (2.) The gene alleles coding for protein synthesis are generally codominant (Brown et al., 1989) and thus, a heterozygous can be easily distinguished. (3) They are amongst the cheapest and quickest marker to develop. However, the main drawback of isozymes and allozymes is their low abundance, the limited number of allele and loci available which are only from the DNA coding regions

2.4 Chloroplast DNA (cpDNA)

2.4.1 Definition

Chloroplast DNA is a circular molecule of DNA found in all photosynthetic plant cell which codes for the function of the photosynthesis. Up to 900 chloroplasts can be found in a plant cell (Frey, 1999) and the size of the DNA present in each chloroplast range from 30 to 200 Kbp (Martin and Hermann, 1998). Chloroplast DNA has a high conservative rate and a relatively stable gene content and therefore suitable for evolutionary studies (Palmer, 1985).

2.4.2 Chloroplast DNA and molecular evolution investigation

Chloroplast DNA is the most widely studied plant genome with regards to both molecular evolution and phylogenetic relationships (Clegg et al., 1994). It is generally inherited uniparently, mainly maternally in Angiosperm plants and paternally in gymnosperm (Palmer et al., 1988). This mode of inheritance as well as several other features of the cpDNA has facilitated the evolutionary investigation of many species. This include: (1) the genome is small in size and can be screened easily. (2) cpDNA is highly characterized at the molecular level, providing basic information to support comparative evolutionary research (Clegg et al., 1994). (3) The nucleotide substitution rate in cpDNA is low compared to nuclear and mitochondrial DNA (Wolf et al., 1987) and therefore is an appropriate window of resolution to study plant phylogeny and evolution (Clegg, 1983).

2.5 Polymerase Chain Reaction and Restriction Fragment Length Polymorphism

2.5.1 The technique

The technique of PCR-RFLP begins by the amplification of the target DNA region with specific or universal primers using Polymerase Chain Reaction then, restriction digestion with restriction enzymes and electrophoretic separation of the fragments to detect possible mutations. Mutations may generate or remove restriction sites resulting in changes in fragment numbers and length.

2.5.2 PCR-RFLP as molecular marker

The restriction fragment length polymorphism analyses use restriction enzymes to cut DNA at specific 4-6 bp recognition sites (Dowling et al., 1990). The variations that may exist at a recognition site have made the PCR-RFLP technique one important tool in genome analysis. Vekemans et al. (1998) and Yu et al. (1999) demonstrated the use of PCR-RFLP as marker in investigating phylogentic relationships in Phaseolus species and Acanthamoeba spp respectively. It has also been used in genetic diversity studies (Lakshmi et al., 2000; Fattouch et al., 2005)

CHAPTER THREE

GENERAL MATERIAL AND METHODS

3.1 Plant materials, description and preparations

3.1.1 Plant materials

Cowpea seeds of accessions and populations were used as plant materials for isozyme analysis and cowpea young leaves from accessions were used for the PCR-RFLP analysis of the cpDNA.

3.1.2 Description and preparations

The accessions of *V. unguiculata ssp. Unguiculata* comprised wilds (*var. spontanea*) ones and accessions from five different cultivar groups of the cultivated species (*var. unguiculata*). Differences in number of accessions per cultivar group, country or region of origin were dictated by the availability of the seed materials that were obtained from the world *phaseolinae* collections maintained in the Jardin Botanique Nationale de Belgique (BR), Meise, Belgium, the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, the International Plant Genetic Resources Institute (IPGRI), Harare, Zimbabwe, the ORSTOM collection Maintained in Montpellier, France and USDA-ARS collections, Charleston, USA. Codes, countries of origin and coordinates of these accessions are provided in Appendix 1 and 2.

The populations of wild cowpea were from four countries in West Africa: Benin, Burkina Faso, Ghana and Niger (Table 3.1, Figure 3.1). Niger and Benin populations were sampled in September-October 1995, populations from Ghana in September- October 2002 and those from Burkina Faso in October 2003. Five to thirty seven cowpea individual plants were sampled per population mainly based on the availability. The seeds present in each pod were kept in separated envelopes and stored in the freezer until analyses were carried out. Seeds from a natural population of wild *V. unguiculata* were also regularly sampled on an area of 0.9 ha at Milanani, coastal Kenya, located at 04^{o} 27' S, 39^{o} 26' E and 30 m altitude above the sea level. Cowpea seeds were collected on a monthly basis from November 2005 to October 2007 in this population. Flowers were counted in the whole sampling site. Rainfall and temperature data were also recorded on a daily basis.

While some seeds were soaked in distilled-deionised water for isozyme electrophoresis, others were planted at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya under green house conditions. Only one seed was planted per pot and the growth was monitored throughout the plant life cycle. Very young leaves when formed were harvested in the morning before strong sunshine and kept in liquid nitrogen for DNA extraction.

Table 3.1 Collection localities, longitude and latitude of natural population of wild *V. unguiculata* in West Africa

Population	Code	Country	Localities	Number of plants	Latitude	Longitude
BEN02	302	Benin	Quenou	05	09º 47' N	02º 38' E
BEN03	303	Benin	Angaradebou	08	11º 19' N	03º 02' E
BEN04	304	Benin	Gamya	13	10º 23' N	02º 43' E
BEN05	305	Benin	Nattitingou	19	10º 18' N	01º 22' E
BEN06	306	Benin	Parakou	20	09º 20' N	02º 38' E
BUR03	403	Burkina Faso	Koupela	07	12º 11' N	00º 20' W
BUR05	405	Burkina Faso	Dionkoudougou	06	12º 25' N	00º 09' W
BUR06	406	Burkina Faso	Bicanga-yango kolokoni	05	12º 30' N	00º 04' W
BUR07	407	Burkina Faso	Kouri	05	12º 37' N	00º 04' W
BUR16	416	Burkina Faso	Djibo	05	14º 06' N	01º 36' W
BUR23	423	Burkina Faso	Tenkodogo	05	11º 46' N	00º 21' W
BUR27	427	Burkina Faso	Tampionkin	12	12º 27' N	00º 05' W
BUR29	429	Burkina Faso	Ouagadougou kongousi	11	12º 24' N	01º 32' W
BUR32	432	Burkina Faso	Ouagadougou sapouy leo	08	12º 17' N	01º 31' W
GHA08	508	Ghana	Bolgatanga - Tamale	07	10º 44' N	00º 47' W
GHA18	518	Ghana	Near Bawku	07	11º 04' N	00º 08' W
GHA20	520	Ghana	Near Bawku	05	10º 58' N	00º 05' W
GHA21	521	Ghana	Near Bawku	05	11º 03' N	00º 03' W
GHA24	524	Ghana	Zebila - Bawku	05	11º 00' N	00º 21' W
GHA25	525	Ghana	Near Zebila	05	10º 56' N	00º 29' W
GHA26	526	Ghana	Bolgatanga - Zebila	13	10º 52' N	00º 45' W
GHA29	529	Ghana	Navrongo - Bolgatanga	05	10º 48' N	00º 55' W
NIG01	601	Niger	Dosso-lago	06	13º 03' N	03º 12' E
NIG02	602	Niger	Hamdallay	11	13º 33' N	02º 24' E
NIG03	603	Niger	Niamey-tillabery	10	13º 34' N	02º 01' E
NIG04	604	Niger	Magou-Torodi	37	13° 06' N	01° 44' E
NIG05	605	Niger	Farie	15	13º 45' N	01º 41' E
NIG06	606	Niger	Gotheye	11	13º 46' N	01º 37' E
NIG08	608	Niger	Gotheye	05	13º 39' N	01º 46' E
NIG09	609	Niger	Falmey	24	12° 36' N	02° 51' E
NIG10	610	Niger	Koudié - Falmey	20	12° 33' N	02° 52' E
NIG11	611	Niger	Boumba	18	12° 24' N	02° 50' E
NIG12	612	Niger	Bara	28	12° 23' N	03° 26' E
NIG13	613	Niger	Bengou	17	11° 59' N	03° 34' E
NIG14	614	Niger	Sabon Birni	13	11° 53' N	03° 36' E

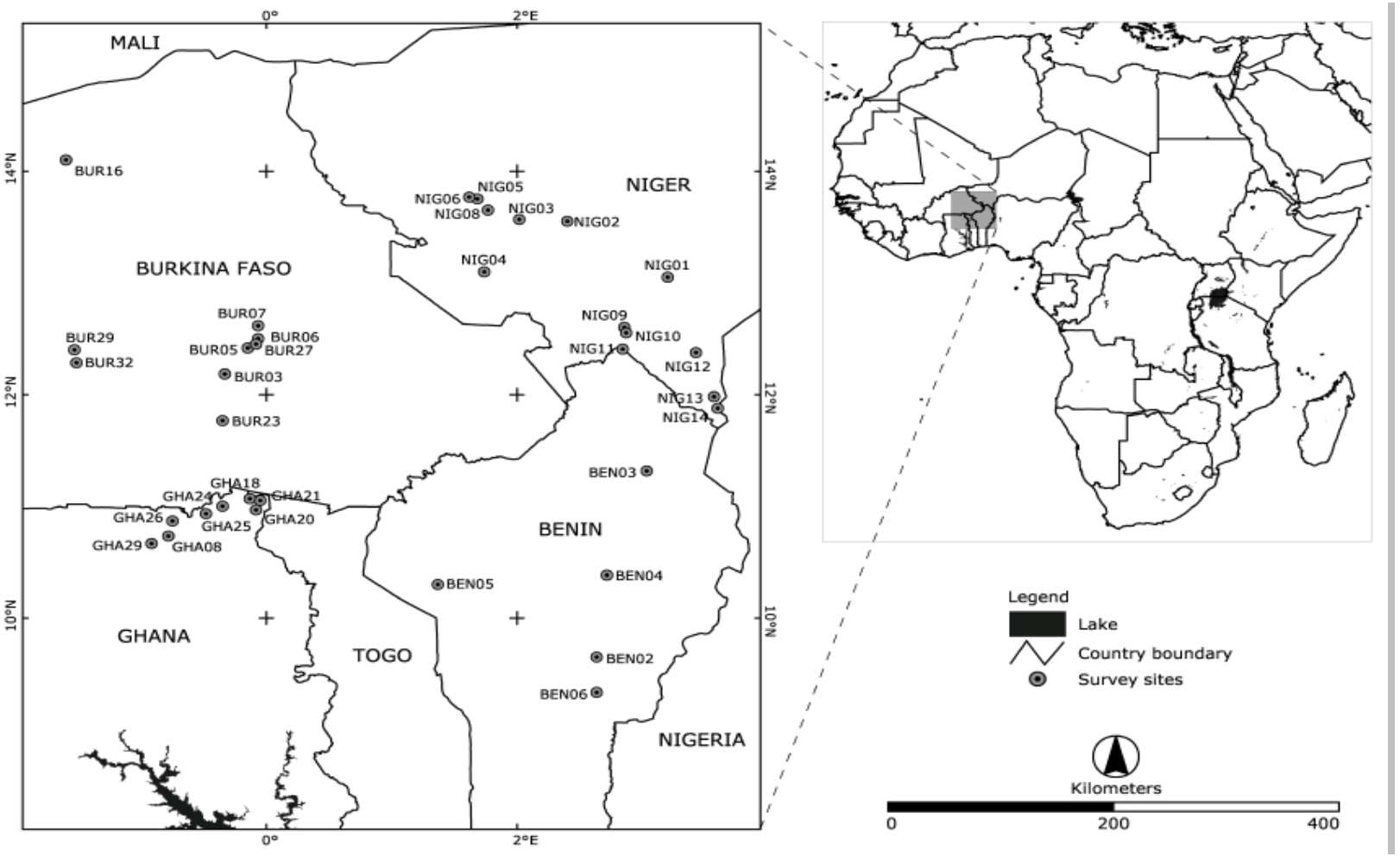


Figure 3.1 Map showing the sampling site locations of populations in West Africa

3.2 Isozyme analysis.

3.2.1 Starch gel preparation

Starch gel was prepared using 14% (w/v) of Starch in the gel buffer: 5mM L-histidine mono HCL, 2.5mM NaCL titrated to pH 6.0 with 5M NaOH (Second and Trouslot, 1980). Appropriate quantity of starch and gel buffer was mixed in an Erlenmeyer flask and heated using a microwave oven with vigorous stirring until it boiled. The solution was then degassed using an aspirator and poured into an acrylic gel mould already leveled and allowed to cool at room temperature. After cooling, the gel was then covered to prevent dehydration and left overnight in the cold room.

3.2.2 Enzyme extraction

Cowpea seeds were nicked and imbibed in water overnight to initiate germination and enzyme expression. Each seed was homogenized with the extraction buffer composed of 9.866mM Tris-HCl, 0.990mM EDTA and 0.004% NADP at pH 6.80 (Pasteur et al., 1987) using a porcelain mortar and pestle.

3.2.3 Gel loading and electrophoresis

Samples including standards (samples with known genotypes) and Bromophenol blue tracker dye were loaded to the 14% starch gel as extracts absorbed by wicks (2mmX4mm) from Whattman 3MM filter paper. The enzyme electrophoresis was resolved using as electrode buffer 0.41M citric

acid, trisodium salt titrated to pH 6.0 with citric acid solution. Contact between the gel and the electrode buffer was established using folded Whattman 3MM filter paper. The electrophoresis was carried out at 200V in the cold room (4°C) to avoid enzyme denaturation and for about 3 hours depending on the anodic position of the tracker dye.

3.2.4 Enzyme visualization

The gel after electrophoresis was notched at the right corner to mark gel orientation. Gel slices were obtained using pulled nylon thread and each slice was then placed in a staining tray for a specific stain leading to the enzyme visualization under visible or UV light depending on the enzyme system. Each enzyme stain was resolved according to Wendel and Weeden (1989). The recipe composition and quantities of each enzyme are described in Table 3.2

Table 3.2: List of enzyme system screened and stain recipes required.

Enzyme system	Abbreviation	E.C. Number*	Organ Studied	Chemical and stains involved (1)	Quantity
Alcohol dehydrogenase	Adh		seed	β-NAD	10 mg
				Ethanol	0.5 ml
				Tris-HCl 0.5M pH 8.50	10 ml
				NBT (10 mg/ml)	1 ml
				PMS (1 mg/ml)	1 ml
				H2O	62.5 ml
Alanine aminopeptidase	Aap	3.4.11.2	Seed	DL-alanine β-naphtylamide hydrochloride	60 mg
				N,N dimethyl formamide	1 ml
				$MgCl_2$ (50 mg/ml)	1 ml
				0.2M tris-maleate pH 6.0 with NaOH 5M	30 ml
				H_2O	45 ml
				Fast black K salt	30 mg
Leucine aminopeptidase	Lap	3.4.11.1	Seed	L-leucine β-naphtylamide hyrochloride	30 mg
				N,N dimethyl formamide	1 ml
				$MgCl_2$ (50 mg/ml)	1 ml
				0.2M tris-maleate pH 6.0 with NaOH 5M	30 ml
				H_2O	45 ml
				Fast black K salt	30 mg
Endopepidase	Enp	3.4.--.--**	Seed	αN-benzoyl DL-arginine β-naphtylamide hydrochloride	35 mg
				N,N dimethyl formamide	1 ml
				$MgCl_2$ (50 mg/ml)	1 ml
				0.2M tris-maleate pH 5.50 with NaOH 5M	37.5 ml
				H_2O	37.5 ml
				Fast black K salt	30 mg
Formate dehydrogenase	Fdh	1.2.1.2	Seed	Formic acid	150 mg
				β-NAD	10 mg
				Tris-HCl 0.5M pH 8.50	20 ml
				H2O	60 ml
				NBT (10 mg/ml)	1 ml
				PMS (1 mg/ml)	1 ml
Fluorescent esterase	Fle	--.--.--.--**	Seed	4-methylumbelliferyl acetate	10 mg
				Acetone	3 ml
				Sodium acetate 0.05M pH 5.0	15 ml
				Agar (2%)	15 ml
Isocitrate dehydrogenase	Idh	1.1.1.42	Seed	DL-isocitric acid	100 mg
				β-NADP	5 mg
				Tris-HCl 0.5M pH 8.50	12 ml
				$MgCL_2$ (50 mg/ml)	1 ml

				NBT (10 mg/ml)	1 ml
				PMS (1 mg/ml)	1 ml
				Agar (2%)	15 ml
Malate dehydrogenase	Mdh	1.1.1.37	Seed	β-NAD	10 mg
				Malate 1M pH 8.0	3 ml
				Tris-HCl 0.5M pH 8.50	10 ml
				NBT (10 mg/ml)	1 ml
				PMS (1 mg/ml)	1 ml
				H_2O	69 ml
Phosphogluconate dehydrogenase	Pgd	1.1.1.43	Seed	6-Phosphogluconic acid	10 mg
				β-NADP	5 mg
				Tris-HCl 0.5M pH 8.50	12 ml
				$MgCL_2$ (50 mg/ml)	1 ml
				NBT (10 mg/ml)	1 ml
				PMS (1 mg/ml)	1 ml
				Agar (2%)	15 ml
Phosphoglucoisomerase	Pgi	5.3.1.9	Seed	Fructose 6-phosphate disodium salt	20 mg
				β-NADP	5 mg
				Tris-HCl 0.5M pH 8.50	12 ml
				$MgCL_2$ (50 mg/ml)	1 ml
				Glucose 6-phosphate dehydrogenase	50 ul
				NBT (10 mg/ml)	1 ml
				PMS (1 mg/ml)	1 ml
				Agar (2%)	15 ml
Phosphoglucomutase	Pgm	5.4.2.2	Seed	α-D-glucose 1-phosphate	90 mg
				NADP	5 mg
				Tris-HCl 0.5M pH 8.50	12 ml
				$MgCL_2$ (50 mg/ml)	1 ml
				Glucose 6-phosphate dehydrogenase	50 ul
				NBT (10 mg/ml)	1 ml
				PMS (1 mg/ml)	1 ml
				Agar (2%)	15 ml
Shikimate dehydrogenase	Sdh	1.1.1.25	Seed	Shikimic acid	30 mg
				β-NADP	5 mg
				Tris-HCl 0.5M pH 8.50	12 ml
				$MgCL_2$ (50 mg/ml)	1 ml
				NBT (10 mg/ml)	1 ml
				PMS (1 mg/ml)	1 ml
				Agar (2%)	15 ml

*: Nomenclature of the International Union of Biochemistry and Molecular Biology

**: Non specific

(1): All the enzymes were stained according to Wendel and weeden (1989) with some modifications

3.3 DNA analysis

3.3.1 Total DNA isolation

Total DNA was extracted from cowpea's young leaves tissues according to the Cetyltrimehyl Ammonium Bromide (CTAB) procedure described by Doyle and Doyle (1987). About 1g of cowpea's leaves tissue was frozen in liquid nitrogen and then crushed in 1.5 ml microfuge tubes using a mini-pestle. To the ground tissue was added 400 µl of CTAB extraction buffer containing: 2% CTAB, 1.4M NaCl, 20mM EDTA, 100mM Tris-HCl (pH 8.0) and 1% 2-mercaptoethanol. The mixture was incubated at 65°C for 20 minutes followed by the addition of 400µl of chloroform: isoamyl alcohol (24:1). The solution was then mixed on a shaker for 15 minutes, centrifuged at 13,000 revolutions per minute (RPM) for 5 minutes and transferred to a new tube with 400 µl of phenol: chloroform (1:1), and mixed gently for 15 minutes followed by a centrifugation at 13,000 RPM for 5 minutes. The supernatant was then transferred to a new tube with 400 µl of isopropanol, mixed gently and left at room temperature for 5 minutes. The DNA was pelleted by centrifugation for 3 minutes at 13,000 RPM and then washed 3 times with 70% ethanol and air dried for 10 minutes by inverting the tube. A hundred microliters of 0.1 TE buffer (10mM Tris-HCl pH 8.0, 0.1mM EDTA) was used to re-suspend the pellet followed by the addition of 4ul of RNAse A (10mg/ml) and then incubated for 15 minutes. The pellet was precipitated with 1 ml of absolute ethanol for 15 minutes and then centrifuged at 13,000 RPM for 5 minutes to pellet the DNA. The DNA pellet was dried for 2 hours, and then suspended in

50ul sterile distilled water. The quality of the DNA was tested using 0.7% (w/v) agarose gel electrophoresis and the concentration was determined by the help of BECKMAN DU® 640B spectrophotometer (USA). The DNA samples were then stored at -20°C until PCR amplification was carried out.

3.3.2 PCR – Amplification

3.3.2.1 PCR Components

The amplification of the region that contain the Bam H1 restriction site of the chloroplast DNA was carried out using forward primer (Primer CAPF) and as reverse primer (Primer DWR) (Feleke et al., 2006). Their sequences are shown in Table 3.3. Each fifty micro litres reaction was containing 0.1 µg of genomic DNA, 0.3 µM of each primer, 1.5 units of Taq DNA polymerase (Genscript, USA), 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1 % Triton® X-100, 2.5 mM MgCl2 and 200 µM of each dNTPs

Table 3.3: Polymerase Chain Reaction primer sequences

Code	Type	Sequence
CAPF	Forward	5' - ATGCTACCTCCCATAAACTTA – 3'
DWR	Reverse	5' - GAACTTAGCTCGAATCAAC - 3'

3.3.2.2 PCR conditions

The PCR cocktail was vortexed and centrifuged at 13,000 rotations per minute in order to settle the mixture. Using 9800 Fast Thermal Cycler of Applied Biosystems (Singapore), the reaction mixture was initially incubated for 2 minutes at 94^{o}C followed by 35 cycles of 30 seconds denaturation at 94^{o}C, 30 seconds annealing at 55^{o}C and 90 seconds extension at 72^{o}C. An additional extension of 10 minutes at 72^{o}C was performed at the end of the 35 cycles. The amplification was confirmed by the presence of bands on a 1 % (w/v) agarose gel like the one shown in Figure 4.13.

3.3.3 Fragment digestion and agarose gel electrophoresis

The DNA fragment amplified using the above two primer sets were digested with 10 units of Bam H1 enzyme (SIGMA) at 37^{o}C overnight. Amplified DNAs from wild (SP219) and cultivated (524B) prototypes with known cpDNA haplotype were also exposed to enzyme digestion and used as controls. At the end of the digestion, the digested fragments were electrophoresed on a 1 % (w/v) agarose gel using 1 X TBE (0.1M Tris-HCl, 0.1M Boric Acid, 2mM EDTA, pH. 8.0) buffer systems with ethidium bromide staining to reveal the DNA bands (Figure 4.14).

3.4 Statistical analysis

3.4.1 Enzyme scoring and inference of maternal genotype

For each enzyme system, we allocated "1" to the presumed locus encoding the most isozyme migration toward the anode and additional loci were numbered sequentially with the decrease of the electrophoretic mobility. According to Pasquet (1999), the most common allele was designated by 100 for each locus and others were estimated in millimeters increase or decrease in relation to this standard.

The genotype of each mother plant was estimated from the progeny array following Brown and Allard (1970) method using MLTR computer program for windows version 2.2 (Ritland, 2002). Hierarchical arrangements of data were established at the group level so that the data could be analyzed within and among the groups.

3.4.2 Gene diversity estimation

Genetic diversity indices were calculated with the help of Popgene version 1.3 (Yeh et al., 1999), Genetix computer program version 4.02 (Belkir et al., 2001) and Fstat software Version 2.9.3 (Goudet, 2002)**.** The level of gene variability were assessed by calculating allele frequencies, the proportion of polymorphic loci (P), the mean number of allele among all loci (A), the mean number of alleles among polymorphic loci (A_p), observed heterozygosity (calculated as the ratio between number of heterozygous genotypes and the total number of

genotypes analysed for each locus) and the total diversity (H_t) (Nei, 1973), partitioned into the diversity within group (H_s) and between group gene diversity (D_{st}). $(H_t) = (H_s) + (D_{st})$. The proportion of total allelic diversity found among group (G_{st}) was calculated: $(G_{st}) = D_{st}/H_t$ (Nei, 1973). For each locus, χ^2 test (Workman and Niswander 1970) was used to detect differences in allele frequencies among populations

3.4.3 Fixation indices and gene flow estimations

Fixation indices (F_{IT}, F_{IS} and F_{ST}) were estimated to characterize the genetic structure within and among populations using F-statistics (Wright, 1978) of the Fstat software Version 2.9.3 (Goudet, 2002)**.** F_{IS} and F_{IT} measure heterozygote excess or deficiency relative to the panmictic expectations within populations and in the total population respectively. F_{ST} measure the populations' differentiation. These indices were calculated using Weir and Cockerham (1984) estimators. Standard errors were estimated by jack-knifing and confidence intervals by bootstrapping, all given by the Fstat program (Goudet, 2002). Gene flow between populations measured in term of number of migrant per generation (*Nm*) was estimated using both Wright's (1951) formula ($Nm = (1 - F_{ST})/4F_{ST}$) and Slatkin's (1985) method ($\ln[p(1)] = a\ln(Nm) + b$ with $a = -0.505$ and $b = -2.440$) based on the frequency of private allele, found in only one population.

3.4.4 Estimation of genetic distance

Genetic distance for each pair wise combination of populations or accessions was estimated according according to Nei (1972) and using Popgene software package version 1.3 and Genetix computer program version 4.02 (Yeh et al., 1999; Belkir et al., 2001) and using this formula: $D = - \ln \left[G_{xy} \Big/ \sqrt{G_x . G_y} \right]$ With: $G_x = \sum p_i^2$, $G_y = \sum q_i^2$, $G_{xy} = \sum p_i . q_i$, p_i and q_i are frequencies of the i^{th} allele in the population X and Y respectively.

3.4.5 Phylogenetic relationships

The phylogenetic tree indicating how close or distant populations or accessions are was constructed by clustering the genetic distances using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) phylogenetic tree of MEGA version 2.1 software (Kumar et al., 2001).

3.4.6 Isolation by distance

Isolation-by-distance was tested by examining the relationship between geographic distance and genetic differentiation among populations, using Rousset's (1997) method. Assuming a two-dimensional habitat and isolation-by-distance, Rousset showed that the ratio Fst / (1-Fst), estimated between pairs of populations, is linearly related to the logarithm of the Euclidean geographical distance separating these populations. The significance of the regression of Fst / (1-Fst) against distance was assessed using a permutation method and the Spearman rank correlation coefficient. Isolation by distance

through a mantel test (Manly, 1985) was also performed in order to assess the correlation between the genetic and the geographic distance.

3.4.7 Mating system parameters estimations

The mating system parameters were estimated using MLTR computer program of Ritland (2002), an extension of the original program of Ritland and Jain (1981) based on the mixed mating model (mixture of outcrossing and self-fertilization events). With the maximum likelihood procedure of this program, we were able to estimate (1) multi loci outcrossing rate (tm), (2) Average single locus outcrossing rate (ts), (3) average single locus inbreeding coefficient of meternal parents (F), (4) Outcrossing rate between relatives (tm-ts). Standard errors of these estimates were calculated based on 500 bootstraps with the unit of resampling being the maternal family array. We tested the significance level of ts and tm by a one tailed Student's t-test based on the null hypothesis that ts or tm=1 (Sokal and Rohlf, 1995). Pearson correlations between the rate of outcrossing and environmental agents and their significance were calculated using SAS 9.1.2 software package (SAS Institute Inc, 2004)

CHAPTER FOUR

RESULTS

4.1 Isozyme diversity, wild-crop complex dynamics and domestication hypothesis in cowpea (*v. unguiculata l. walp)*

4.1.1 Isozyme banding patterns

4.1.1.1 Alcohol dehydrogenase (*Adh*)

Alcohol dehydrogenase zymogramme showed two distinct zones of activities indicating two different loci, Adh_1 and Adh_2 that are observed in Figure 4.1. A homozygous individual displayed three distinct bands with the middle band position depending on the position of the two extremes. A heterozygous individual was not identified in the samples screened. The two loci were polymorphic with two alleles each: Adh_1^{100}, Adh_1^{104} for Adh_1 locus and Adh_2^{091}, Adh_2^{100} for the locus Adh_2

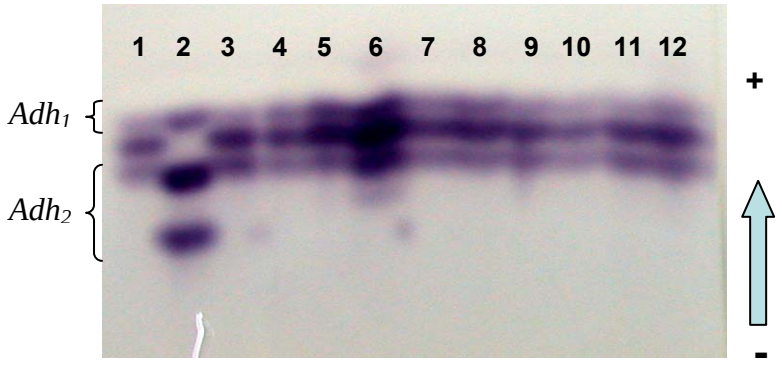


Figure 4.1: Zymogramme of Alcohol dehydrogenase (Adh)
Lane 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12: *Adh1 100/100, Adh2 100100*
Lane 2: *Adh1 100/100, Adh2 091/091*

4.1.1.2 Alanine Aminopeptidase (*Aap*)

Two zones of activity for alanine aminopeptidase enzyme representing two distinct loci Amp_2 and Amp_4 were observed (Figure 4.2). Both loci appear to be polymorphic and monomeric with a heterozygous presenting two bands of equal intensity. Three electrophoretic variants were observed at the locus Amp_2: Amp_2^{098}, Amp_2^{100} and Amp_2^{102} and four at the locus Amp_4: Amp_4^{093}, Amp_4^{096}, Amp_4^{100} and Amp_4^{104}

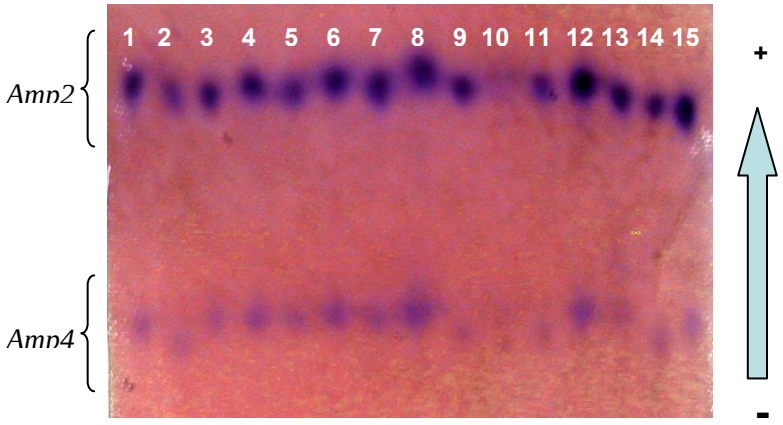


Figure 4.2: Zymogramme de l'alanine amino peptidase (*aap*)
Lane 3, 5, 7, 13, 15: *Amp2 100/100* and *Amp4 100/100*
Lane 1, 4, 6, 8, 12: *Amp2 102/102* and *Amp4 100/100*
Lane 2, 9, 11, 14: *Amp2 100/100* and *Amp4 096/096.* Lane 10*: Amp2 100/102*

4.1.1.3 Endopeptidase (*Enp*)

The zymogramme of the endopeptidase in cowpea is characterized by one zone of activity representing a single locus. The figure 4.3 is a typical zymogramme and does not shows heterozygous individuals. Others endopeptidase gel stains reveal that in cowpea, *Enp* is a monomeric enzyme with heterozygous showing

two bands of the same intensity. The entire sample screened reveal five allelic variants: *Enp*^{095}, *Enp*^{098}, *Enp*^{100}, *Enp*^{103} and *Enp*^{105}

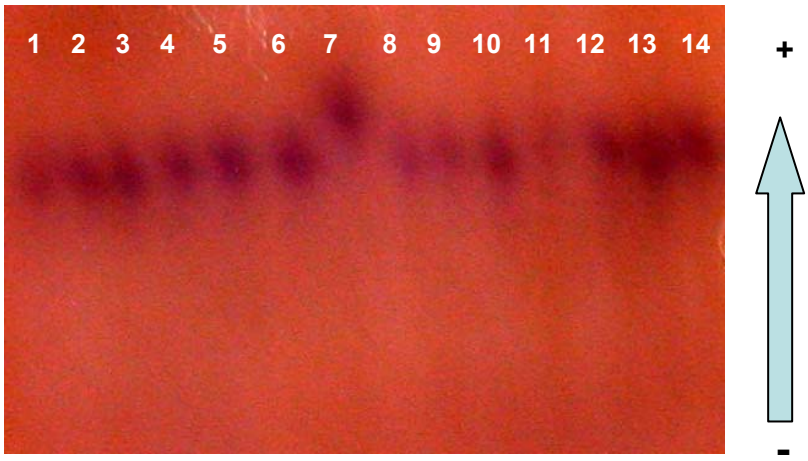


Figure 4.3: Zymmogramme of the endopeptidase (*Enp)*
Line 1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, 14: *Enp 100/100*
Line 8: *Enp 098/098.* Line 7: *Enp 103/103*

4.1.1.4 Formate dehydrogenase (*Fdh*)

Gels stained for *Fdh* enzyme showed one zone of activity controlled by a single locus (Figure 4.4). *Fdh* locus was polymorphic demonstrating five electrophoretic variants in the sample screened: *Fdh*^{096}, *Fdh*^{098}, *Fdh*^{100}, *Fdh*^{102} and *Fdh*^{104}

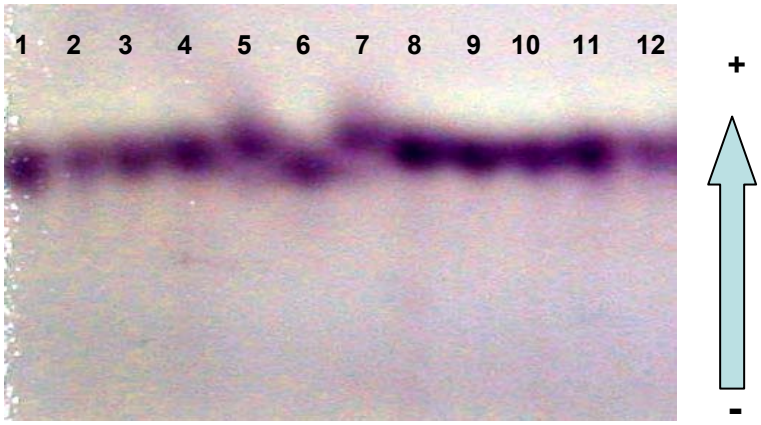


Figure 4.4: Formate dehydrogenase (*Fdh*) zymogammes from cowpea
Lane 1, 2, 3, 4, 8, 9, 10, 11, 12: *Fdh100/100*
Lane 5 and 7: *Fdh102/102*
Lane 6: *Fdh096/096*

4.1.1.5 Fluorescent esterase (*Fle*)

Zymogrammes for *Fle* enzyme showed two main zones of activity controlled by two distinct loci Fle_1 and Fle_3 (Figure 4.5). Fle_1 was the most anodal with three electrophoretic variants: Fle_1^{094}, Fle_1^{097} and Fle_1^{100} while Fle_3 were polymorphic and dimeric with three bands phenotype for heterozygous individuals. Fle_3 showed five different alleles: Fle_3^{092}, Fle_3^{096}, Fle_3^{098}, Fle_3^{100} and Fle_3^{104}

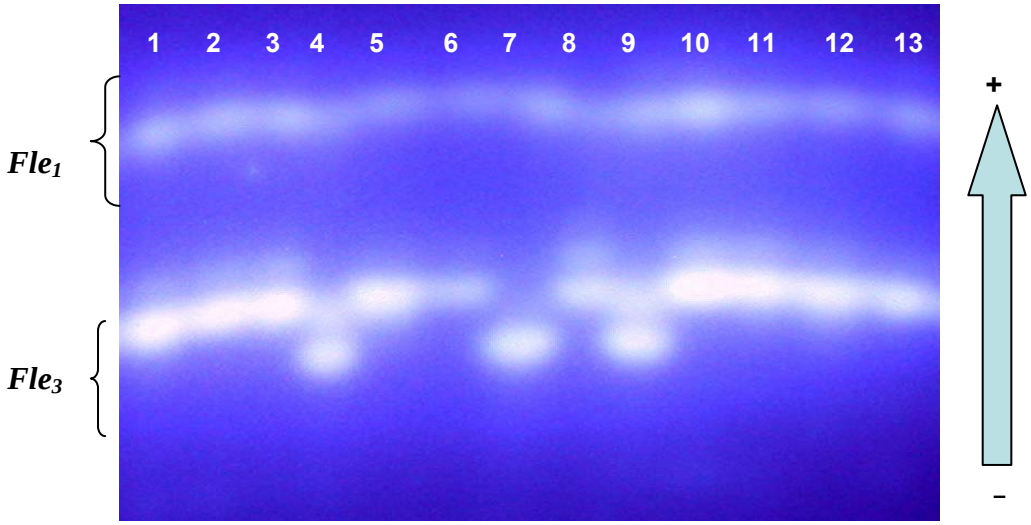


Figure 4.5: Fluorecent esterase (Fle) zymogammes
Lane 1, 2, 3, 5, 6, 8, 10, 11, 12 and 13: *Fle1 100/100 ; Fle3 100/100*
Lane 4, 7, and 9: *Fle1 100/100 ; Fle3 096/096*

4.1.1.6 Isocitrate dehydrogenase (*Idh*)

Two zones of activity representing two distinct loci were observed and were polymorphic. The most anodal (Idh_1) with three electrophoretic variants: Idh_1^{090}, Idh_1^{100} and Idh_1^{105}compared to the most cathodal (Idh_2), with two variant alleles Idh_2^{095} and Idh_2^{100}. Zymogramme of *Idh* enzymes are shown in Figure 4.6

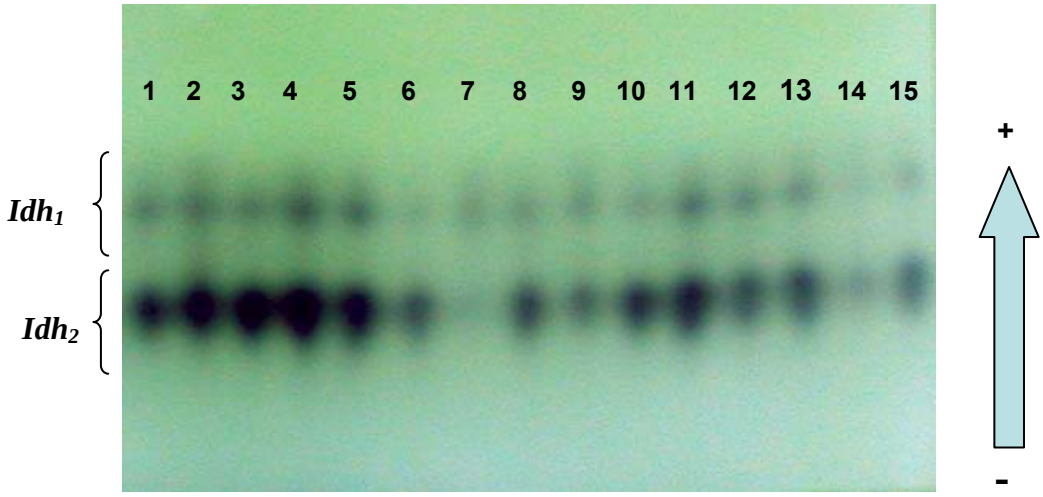


Figure 4.6: Zymogramme of Isocitra dehydrogenase enzyme (*Idh*) Lane 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15: *Idh1 100/100, Idh2 100/100*

4.1.1.7 Leucine aminopeptidase (*Lap*)

Three zones of activity were revealed. They represent three distinct loci Amp_1, Amp_2 and Amp_3 (Figure 4.7). The locus Amp_1 which appear in this gel popymorphic with two variants Amp_1^{096} and Amp_1^{100} was not visible on all the gels stained for *Lap* enzyme and therefore was not included in our analysis. Amp_2 and Amp_{3a} were monomeric and polymorphic with three allelic variants for the locus Amp_2: Amp_2^{098}, Amp_2^{100} and Amp_2^{102}; four: Amp_{3a}^{093}, Amp_{3a}^{096}, Amp_{3a}^{100} and Amp_{3a}^{103} for the locus Amp_{3a}.

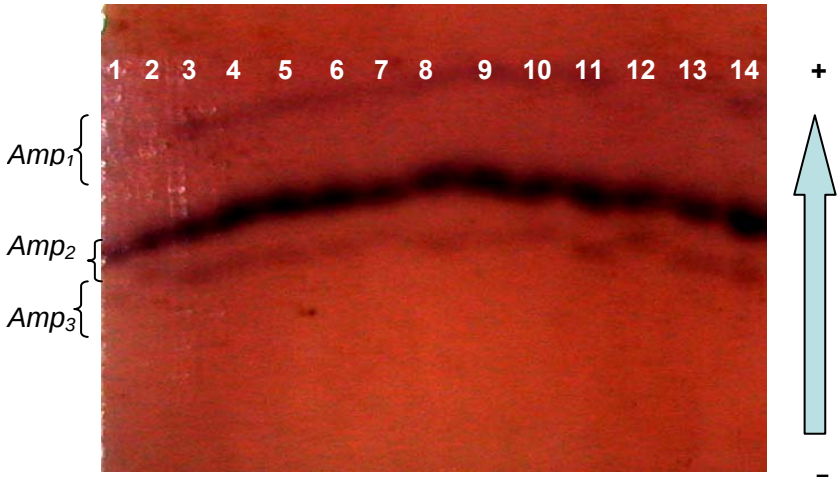


Figure 4.7: Zymogramme of Leucine amino peptidase (*Lap*)
Lane 1, 3, 4, 5, 6, 7, 9, 10, 13, 14: *Amp2 100/100, Amp3 100/100*
Lane 8: *Amp2 102/102, Amp3 100/100.* Lane 11: *Amp2 100100, Amp3 097/097.* Lane 2 12: *Amp2 100/100; Amp3 103/103*

4.1.1.8 Malate dehydrogenase (*Mdh*)

The figure 4.8 shows the electrophoretic banding patterns of *Mdh* enzyme. Five zone of activity from four distinct loci were observed. All loci were monomorphic with only one electrophoretic variant at each. The most anodal locus (Mdh_1) migrates faster compared to Mdh_4 bands which were closed to the starting point. Band intermediate of those controlled by Mdh_2 and Mdh_3 loci is suggested to be an intergenic reaction bands.

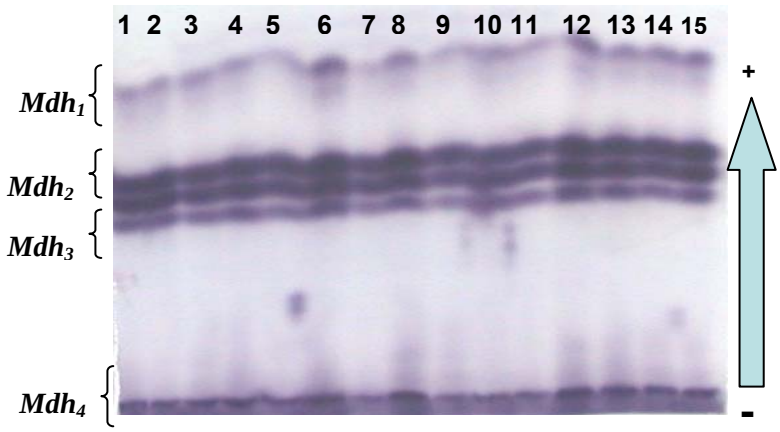


Figure 4.8: Malate dehydrogenase (*Mdh*) zymogramme.
Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15: $Mdh_1^{100/100}$; $Mdh_2^{100/100}$; Mdh_3^{100100}; $Mdh_4^{100/100}$

4.1.1.9 Phosphogluconate dehydrogenase (*Pgd*)

Two frequent zones of activity controlled by two loci characterize the zymogramme of Pgd enzyme in cowpea (Figure 4.9). The two loci were polymorphic with two alleles for the locus Pgd_1: Pgd_1^{096} and Pgd_1^{100} and three for the locus Pgd_2: Pgd_2^{096} Pgd_2^{100} and Pgd_2^{102}

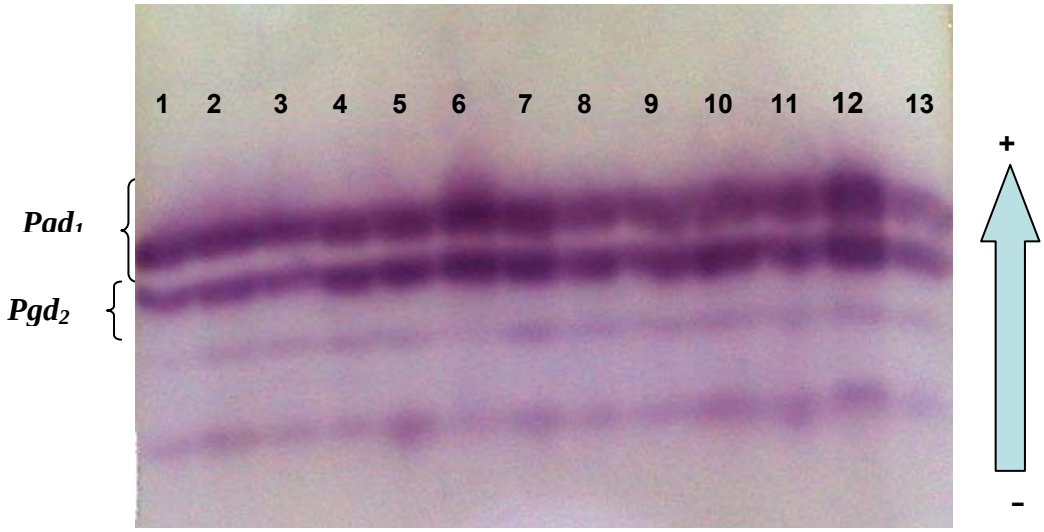


Figure 4.9: Zymogramme of Phosphogluconate dehydrogenase (*Pgd*). Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13: $Pgd_1^{100/100}$; $Pgd_2^{100/100}$

4.1.1.10 Phosphoglucoisomerase (*Pgi*)

Four zones of activity representing the product of three distinct genes were observed for *Pgi* zymogramme (Figure 4.10). The most anodal loci (Pgi_1) was monomorphic. Pgi_2 and Pgi_3 were polymorphic and dimeric, generating a triple-banded phenotype for heterozygous individuals. Pgi_3 enzymes migrate through the cathode. Bands from the zone of activity intermediate of the zone controlled by Pgi_2 and the one controlled by Pgi_3 appear as an intergenic reaction bands with their position and number depending on those controlled by Pgi_2 and Pgi_3. Only one allele were observed for Pgi_1 (Pgi_1^{100}), four electrophoretic variants for Pgi_2 (Pgi_2^{090}, Pgi_2^{100}, Pgi_2^{107} and Pgi_2^{115}) and three for Pgi_3 (Pgi_3^{092}, Pgi_3^{100} and Pgi_3^{108}).

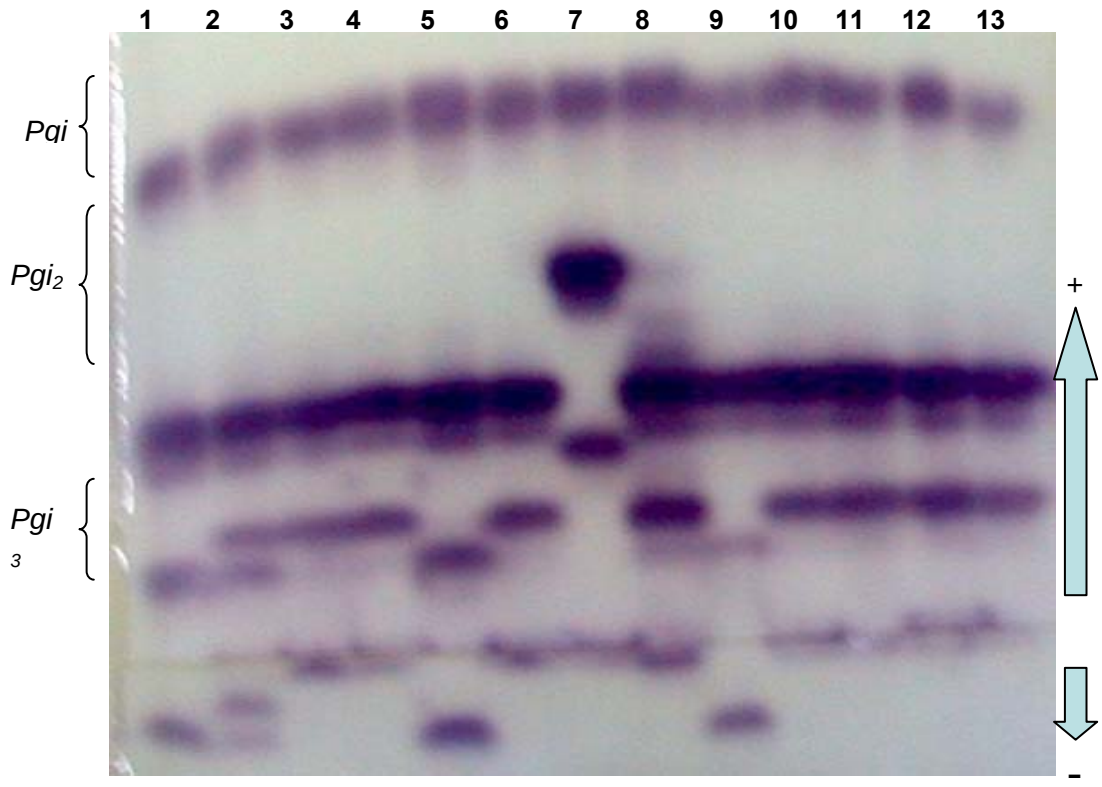


Figure 4.10: Zymogramme of phosphoglucoisomerase (*Pgi*) of cowpea. Lanes 1, 5, 9: $Pgi_1^{100/100}$; $Pgi_2^{100/100}$; $Pgi_3^{096/096}$ Lanes 3, 4, 6, 8, 10, 11, 12, 13: $Pgi_1^{100/100}$; $Pgi_2^{100/100}$; $Pgi_3^{100/100}$ Lane 2: $Pgi_1^{100/100}$; $Pgi_2^{100/100}$; $Pgi_3^{096/100}$. Lane 7: $Pgi_1^{100/100}$; $Pgi_2^{130/130}$; $Pgi_3^{100/100}$

4.1.1.11 Phosphoglucomutase (*Pgm*)

Pgm electrophoretic banding patterns are shown in Figure 4.11. Two zones of activity representing two distinct loci Pgm_1 and Pgm_2 were observed. Two alleles were observed at each locus: Pgm_1^{096} and Pgm_1^{100} at the Pgm_1 locus; Pgm_2^{095} and Pgm_2^{100} at Pgm_2 locus. Heterozygous individual appear as two bands of equal intensity at each of the *Pgm* locus, conferring a monomeric structure of the phosphoglucomutase in cowpea.

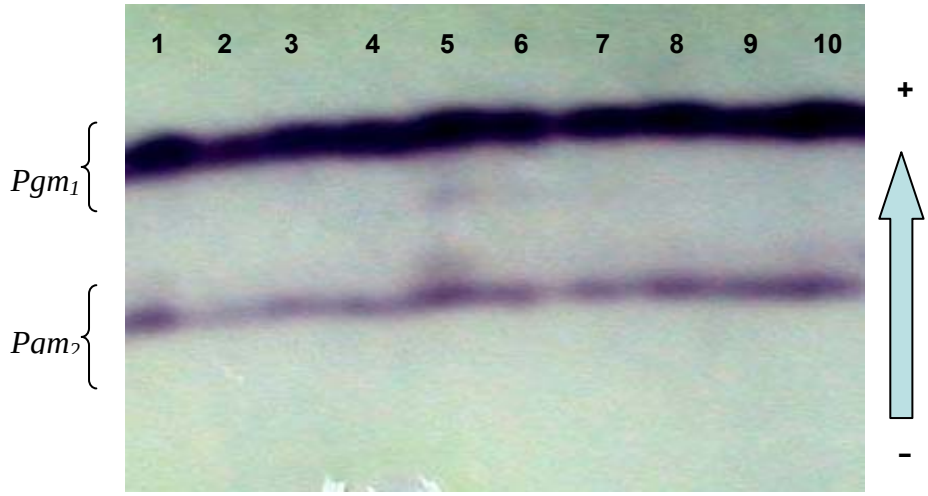


Figure 4.11: Phosphoglucumutase (*Pgm)* zymogramme of cowpea.
Lane 1, 2, 3, 4, 5, 6, 7, 8, 9, 10: $Pgm_1^{100/100}$; $Pgm_2^{100/100}$

4.1.1.12 Shikimate dehydrogenase (*Sdh*)

Gels stained for *Sdh* enzyme showed two close zones of activity. An illustration is shown in Figure 4.12. It is suggested that both zones are controlled by a single locus with one of the two bands being a shadow band rather than the two zone of activities coming from two tightly link loci. A heterozygous was represented by three bands with the middle band being the shadow band. Three electrophoretic variants were observed: Sdh^{095}, Sdh^{100} and Sdh^{105}.

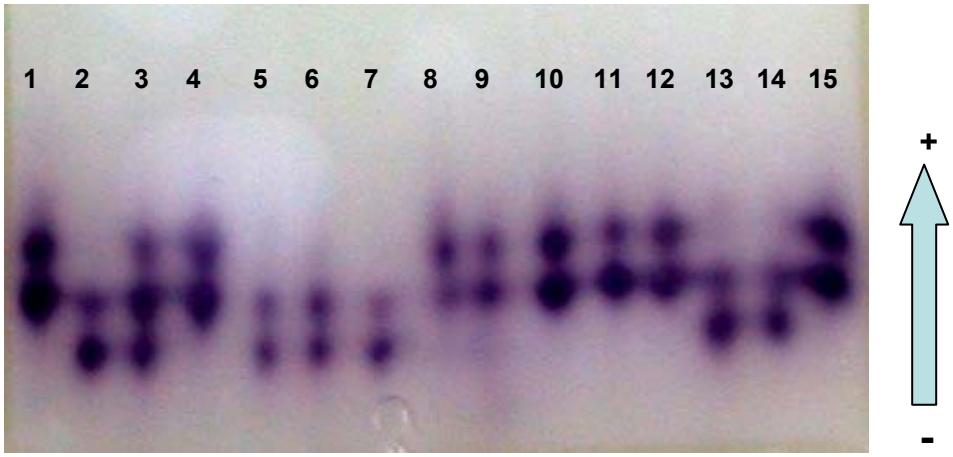


Figure 4.12: Shikimate dehydrogenase (*Sdh*) zymogammes from cowpea seeds
Lane 1, 4, 8, 9, 10, 11, 12, 15: *Sdh100/100*
Lane 3: *Sdh 095/100*
Lane 2, 5, 6, 7, 13, 14: *Sdh 095/095*

4.1.2 Chloroplast DNA banding

Chloroplast DNA amplification using CAPF and DWR primer set (Feleke et al., 2006) generated for each sample a unique band of approximately 1560 bp (Figure 4.13). The polymorphism were observed when amplified fragments were exposed to *Bam*H1 restrictions enzyme (Figure 4.14)

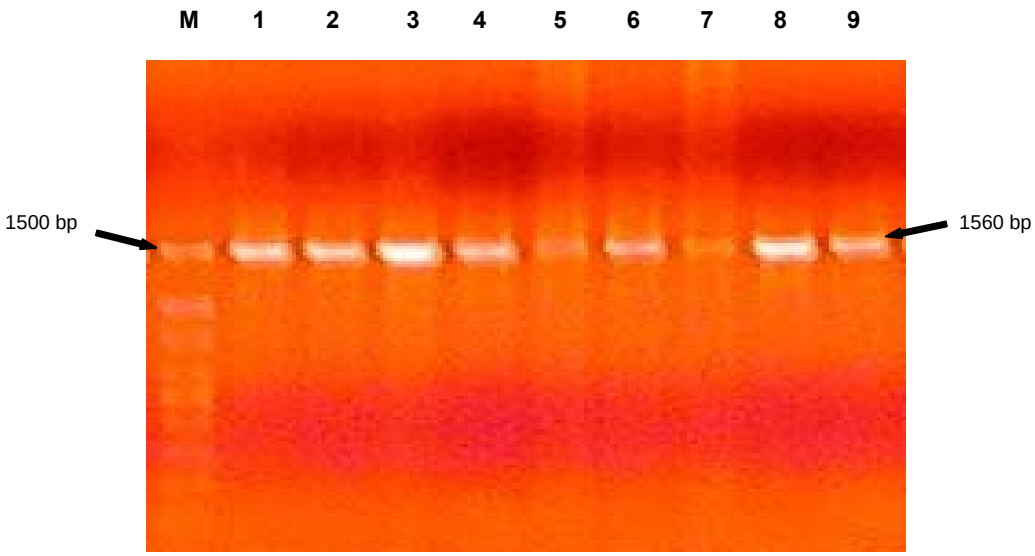


Figure 4.13: Ethidium bromide stained 1% (w/v) agarose gel showing amplification results using CAPF and DWR primers set.
Lane M is Molecular ladder. Lane 4 and 5 are respectively cultivated (524B) and wild (219-01) controls. Lanes 1, 2, 3, 6, 7, 8, 9 are wild samples (SP423 (ETH), SP265 (KEN), TVNU308 (TCD), SP112 (CMR), SP873 (BEN), SP817 (GHA), NI1381 (BWA)

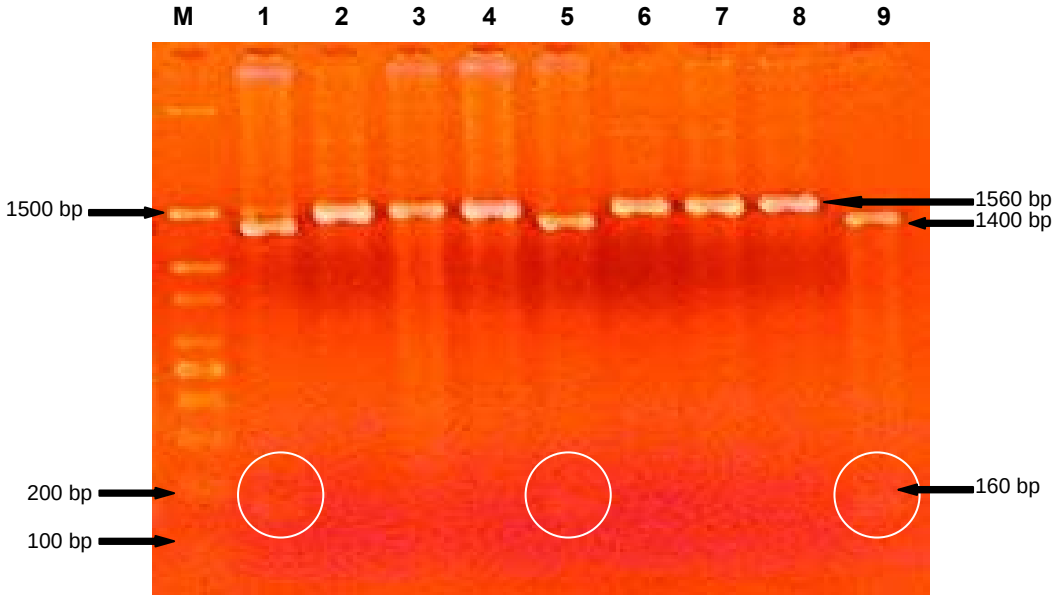


Figure 4.14 Ethidium bromide stained 1% (w/v) agarose gel showing digestion results with *Bam*H1 restriction enzyme following PCR amplification using CAPF and DWR primers set.
Lane M is Molecular ladder. Lane 4 and 5 are respectively cultivated (524B) and wild (219-01) controls. Lanes 1, 2, 3, 6, 7, 8, 9 are wild samples (SP423 (ETH), SP265 (KEN), TVNU308 (TCD), SP112 (CMR), SP873 (BEN), SP817 (GHA), NI1381 (BWA)

4.1.3 Composition and distribution of locus and alleles

With the use of 23 isozyme loci, a total 314 accessions of *V. unguiculata* were screened. Sixty one alleles were identified across the wild cowpea and thirty alleles in cultivated accessions. Twenty nine alleles were common in both cultivated and wild cowpea.

4.1.3.1 Composition and distribution within wild cowpeas

Within wild, 5 of the 23 loci were monomorphic (*Mdh_1, Mdh_2, Mdh_3, Mdh_4,* and *Pgi_1*) with all the 250 individuals possessing bands with identical mobility at any of these loci. The other 18 loci were polymorphic (*Adh_1, Adh_2, Amp_2, Amp_3, Amp_4, Enp, Fdh, Fle1, Fle_3, Idh_1, Idh2, Pgd_1, Pgd_2, Pgi_2, Pgi_3, Pgm_1, Pgm_2* and *Sdh*). Allele frequencies for the polymorphic loci are presented in Tables 4.1, 4.2, and 4.3. The loci *Fdh and Fle_3* were the most polymorphic with five given alleles each (*Fle_3^{092}, Fle_3^{096} Fle_3^{098}, Fle_3^{100}* and *Fle_3^{104}* for *Fle_3* locus, *Fdh^{096}, Fdh^{098}, Fdh^{100}, Fdh^{102} and Fdh^{104}* for the locus *Fdh*) followed with loci having four alleles each, *Pgi_2* (*Pgi_2^{092}, Pgi_2^{100}, Pgi_2^{105},Pgi_2^{115}*); *Enp* (*Enp^{095}, Enp^{100}, Enp^{103}, Enp^{105}*); *Amp_3* (*Amp_3^{094}, Amp_3^{097}, Amp_3^{100}, Amp_3^{103}*); *Amp_4* (*Amp_4^{093} Amp_4^{096}, Amp_4^{100}, Amp_4^{104}*), then, those with three alleles each: *Amp_2* (*$Amp_2^{098,}$ Amp_2^{100}, Amp_2^{102}*), *Fle_1* (*Fle_1^{094}, Fle_1^{097}, Fle_1^{100}*), *Idh_1* (*Idh_1^{090}, Idh_1^{100}, Idh_1^{105}*), *Pgd_2* (*Pgd_2^{096}, Pgd_2^{100}, Pgd_2^{102}*), *Pgi_3* (*Pgi_3^{092}, Pgi_3^{100}, Pgi_3^{108}*) and *Sdh* (*Sdh^{095}, Sdh^{100}, Sdh^{105}*); those of two alleles each: *Adh_1*

(Adh_1^{100}, Adh_1^{104}), Adh_2 (Adh_2^{091}, Adh_2^{100}), Idh_2 (Idh_2^{095}, Idh_2^{100}), Pgd_1 (Pgd_1^{096}, Pgd_1^{100}), Pgm_1 (Pgm_1^{096}, Pgm_1^{100}) and Pgm_2 (Pgm_2^{095}, Pgm_2^{100}). Among the 18 polymorphic loci, half were highly polymorphic Amp_2, Amp_3, Amp_4, *Enp*, *Fdh*, Fle_3, Pgi_2, Pgi_3 and *Sdh* with the most common allele frequency being less than 90%. The others were weakly polymorphic (Adh_1, Adh_2, *Fle1*, Idh_1, *Idh2*, Pgd_1, Pgd_2, Pgm_1 and Pgm_2) with the most common allele highly represented.

According to regions, nine alleles were only present in southern Africa (Adh_1^{104}(2.4%), Fle_1^{094}(17.1%), Fle_1^{097} (7.3%), Fle_3^{092} (4.9%), Fle_3^{104} (17.1%), Idh_1^{105} (2.4%), Pgd_1^{096} (36.6%), Pgd_2^{096} (4.9%) and Pgi_2^{107} (4.9%)), height in eastern Africa (Amp_3^{094} (1.3%), Amp_4^{104} (1.3%), Enp^{095} (1.3%), Enp^{105} (0.7%), Fdh^{098} (10.4%), Fle_3^{098} (1.3%), Idh_1^{090} (1.3%) and Pgd_2^{102}(1.3%). None was specific in either west or central Africa. Adh_1, Fle_1 and Pgd_1 were found to be polymorphic only in southern Africa.

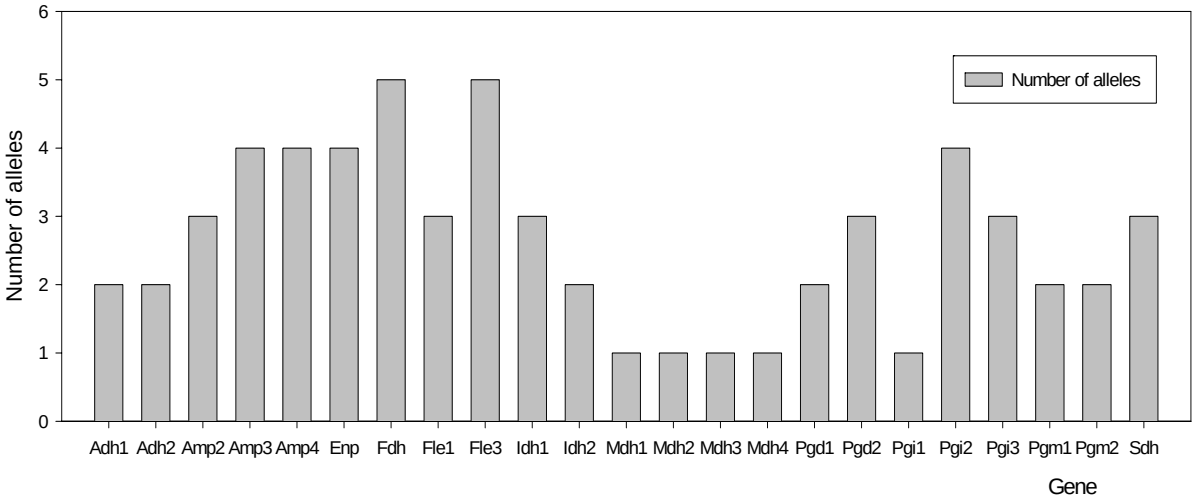


Figure 4.15: Distribution of number of alleles according to genes in wild cowpea (*var. spontanea*)

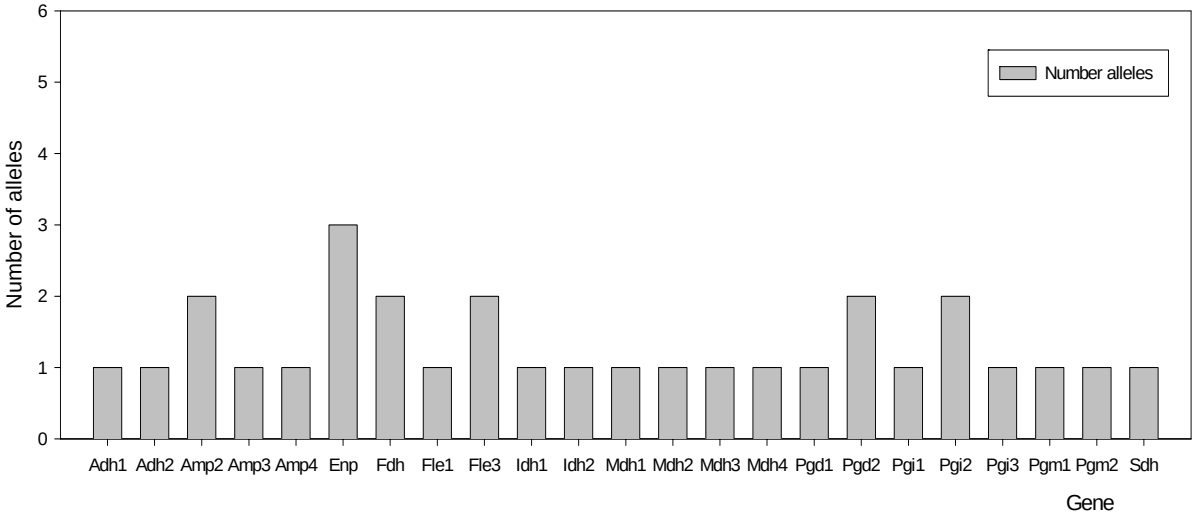


Figure 4.16: Distribution of number of alleles according to genes in cultivated cowpea (*var. unguiculata*)

4.1.3.2 Composition and distribution within cultivated cowpeas

Twelve isozyme loci polymorphic within wild *V. unguiculata* were found monomorphic across cultivated accessions and all the polymorphic loci in cultivated cowpea were polymorphic within wilds. Cultivated cowpeas were polymorphic in six out of the twenty three loci scored. These polymorphic loci in cultivated accessions are Amp_2, *Enp*, *Fdh*, Pgd_2 and Pgi_2. *Enp* appears with three given alleles (Enp^{098}, Enp^{100}, Enp^{103}) followed with two alleles: *Fdh* (Fdh^{100} and Fdh^{102}), Amp_2 ($Amp_2{}^{100}$ and $Amp_2{}^{102}$), Fle_3 ($Fle_3{}^{096}$ and $Fle_3{}^{100}$), Pgd_2 ($Pgd_2{}^{096}$ and $Pgd_2{}^{100}$), Pgi_2 ($Pgi_2{}^{100}$ and $Pgi_2{}^{115}$). Adh_1, Adh_2, Amp_{3a}, Amp_4, *Fle1*, Idh_1, *Idh2*, Mdh_1, Mdh_2, Mdh_3, Mdh_4, Pgd_1, *Pgi1*, Pgi_3, Pgm_1, Pgm_2 and *Sdh* were monomorphic within cultivated accessions. With the exception of Enp^{098}, all the alleles found in cultivated groups were found within wilds. $Pgi_2{}^{115}$ were only found in accessions from Cameroon (5.9%), in cultivar group *textilis* (25%); $Pgd_2{}^{096}$ were found in Ethiopia (16.7%), in eastern Africa (5.0%) and in cultivar group *biflora* (6.7%). Enp^{103} were only in eastern Africa (25%).

Table 4.1 Allele frequencies in wild *V. unguiculata* (West and Central Africa)

		All	GHA	BUR	NIG	BEN	NGA	WA	CMR	COG	RCA	TCD	CA
Gene	Allele												
Adh1	*100*	**0.996**	1.000	1.000	1.000	1.000	1.000	**1.000**	1.000	1.000	1.000	1.000	**1.000**
	104	**0.004**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
Adh2	*91*	**0.016**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
	100	**0.984**	1.000	1.000	1.000	1.000	1.000	**1.000**	1.000	1.000	1.000	1.000	**1.000**
Amp2	*98*	**0.010**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.011	0.000	0.000	0.000	**0.007**
	100	**0.810**	0.294	0.700	0.875	0.800	0.833	**0.656**	0.756	1.000	1.000	0.800	**0.809**
	102	**0.180**	0.706	0.300	0.125	0.200	0.167	**0.344**	0.233	0.000	0.000	0.200	**0.184**
Amp3	*94*	**0.004**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
	97	**0.065**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.167	0.000	0.000	**0.015**
	100	**0.895**	1.000	1.000	1.000	1.000	1.000	**1.000**	0.956	0.833	0.857	1.000	**0.941**
	103	**0.036**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.044	0.000	0.143	0.000	**0.044**
Amp4	*93*	**0.028**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
	96	**0.207**	0.059	0.500	0.313	0.000	0.167	**0.266**	0.078	0.200	0.143	0.200	**0.112**
	100	**0.761**	0.941	0.500	0.688	1.000	0.833	**0.734**	0.922	0.800	0.857	0.800	**0.888**
	104	**0.004**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
Enp	*95*	**0.004**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
	100	**0.874**	1.000	1.000	1.000	1.000	1.000	**1.000**	1.000	1.000	1.000	1.000	**1.000**
	103	**0.121**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
	105	**0.002**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
Fdh	*96*	**0.059**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.167	0.000	0.000	**0.015**
	98	**0.032**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
	100	**0.677**	0.500	0.825	0.667	0.800	0.833	**0.702**	0.611	0.833	0.714	0.500	**0.625**
	102	**0.216**	0.500	0.175	0.333	0.200	0.167	**0.298**	0.367	0.000	0.286	0.400	**0.331**
	104	**0.016**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.100	**0.029**
Fle1	*94*	**0.028**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
	97	**0.012**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
	100	**0.960**	1.000	1.000	1.000	1.000	1.000	**1.000**	1.000	1.000	1.000	1.000	**1.000**
Fle3	*92*	**0.008**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
	96	**0.289**	0.824	0.250	0.375	0.400	0.417	**0.461**	0.568	0.000	0.143	0.150	**0.410**
	98	**0.004**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
	100	**0.671**	0.177	0.750	0.625	0.600	0.583	**0.539**	0.432	1.000	0.857	0.850	**0.590**
	104	**0.028**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
Idh1	*90*	**0.004**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
	100	**0.992**	1.000	1.000	1.000	1.000	1.000	**1.000**	1.000	1.000	1.000	1.000	**1.000**
	105	**0.004**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**

Idh2	*95*	**0.006**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.022	0.000	0.000	0.000	**0.015**
	100	**0.994**	1.000	1.000	1.000	1.000	1.000	**1.000**	0.978	1.000	1.000	1.000	**0.985**
Pgd1	*96*	**0.060**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
	100	**0.940**	1.000	1.000	1.000	1.000	1.000	**1.000**	1.000	1.000	1.000	1.000	**1.000**
Pgd2	*96*	**0.008**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
	100	**0.988**	1.000	1.000	1.000	1.000	1.000	**1.000**	1.000	1.000	1.000	1.000	**1.000**
	102	**0.004**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
Pgi2	*92*	**0.014**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.143	0.000	**0.015**
	100	**0.778**	1.000	1.000	0.875	1.000	0.333	**0.906**	0.333	1.000	0.786	0.300	**0.434**
	107	**0.008**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**
	115	**0.200**	0.000	0.000	0.125	0.000	0.667	**0.094**	0.667	0.000	0.071	0.700	**0.552**
Pgi3	*92*	**0.143**	0.794	0.200	0.313	0.200	0.167	**0.383**	0.133	0.000	0.000	0.300	**0.134**
	100	**0.807**	0.206	0.800	0.688	0.800	0.583	**0.938**	0.867	1.000	1.000	0.700	**0.866**
	108	**0.050**	0.000	0.000	0.000	0.000	0.250	**0.023**	0.000	0.000	0.000	0.000	**0.000**
Pgm1	*96*	**0.010**	0.088	0.000	0.000	0.000	0.000	**0.023**	0.000	0.000	0.000	0.000	**0.000**
	100	**0.990**	0.912	1.000	1.000	1.000	1.000	**0.977**	1.000	1.000	1.000	1.000	**1.000**
Pgm2	*95*	**0.032**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.022	0.167	0.000	0.000	**0.029**
	100	**0.968**	1.000	1.000	1.000	1.000	1.000	**1.000**	0.978	0.833	1.000	1.000	**0.971**
Sdh	*95*	**0.173**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.022	0.250	0.000	0.000	**0.037**
	100	**0.807**	1.000	1.000	1.000	1.000	1.000	**1.000**	0.978	0.750	1.000	1.000	**0.963**
	105	**0.020**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	**0.000**

Table 4.2 Alleles frequencies in Wild *V unguiculata* (Eastern and Southern Africa)

		All	**SDN**	**UGA**	**KEN**	**ETH**	**TZA**	**EA**	**RSA**	**MWI**	**ZMB**	**ZWE**	**BWA**	**SA**
Gene	Allele													
Adh1	*100*	**0.996**	1.000	1.000	1.000	1.000	1.000	**1.000**	0.875	1.000	1.000	1.000	1.000	**0.976**
	104	**0.004**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.125	0.000	0.000	0.000	0.000	**0.024**
Adh2	*91*	**0.016**	0.050	0.083	0.000	0.000	0.000	**0.026**	0.125	0.000	0.000	0.125	0.000	**0.049**
	100	**0.984**	0.950	0.917	1.000	1.000	1.000	**0.974**	0.875	1.000	1.000	0.875	1.000	**0.951**
Amp2	*98*	**0.010**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.125	0.000	0.111	**0.049**
	100	**0.810**	0.950	0.917	0.842	0.846	0.885	**0.890**	0.875	0.875	0.875	1.000	0.889	**0.902**
	102	**0.180**	0.050	0.083	0.158	0.154	0.115	**0.110**	0.125	0.125	0.000	0.000	0.000	**0.049**
Amp3	*94*	**0.004**	0.000	0.000	0.000	0.000	0.077	**0.013**	0.000	0.000	0.000	0.000	0.000	**0.000**
	97	**0.065**	0.000	0.333	0.053	0.000	0.000	**0.065**	0.143	0.125	0.250	0.143	0.556	**0.256**
	100	**0.895**	0.700	0.667	0.947	1.000	0.923	**0.844**	0.857	0.875	0.750	0.857	0.444	**0.744**
	103	**0.036**	0.300	0.000	0.000	0.000	0.000	**0.078**	0.000	0.000	0.000	0.000	0.000	**0.000**
Amp4	*93*	**0.028**	0.000	0.000	0.000	0.000	0.077	**0.013**	0.125	0.250	0.000	0.250	0.111	**0.146**
	96	**0.207**	0.075	0.250	0.026	0.039	0.077	**0.084**	0.250	0.000	0.813	0.750	0.667	**0.500**
	100	**0.761**	0.925	0.750	0.921	0.962	0.846	**0.890**	0.625	0.750	0.188	0.000	0.222	**0.354**
	104	**0.004**	0.000	0.000	0.053	0.000	0.000	**0.013**	0.000	0.000	0.000	0.000	0.000	**0.000**
Enp	*95*	**0.004**	0.000	0.083	0.000	0.000	0.000	**0.013**	0.000	0.000	0.000	0.000	0.000	**0.000**
	100	**0.874**	1.000	0.917	1.000	1.000	0.692	**0.935**	0.250	0.875	0.500	0.000	0.111	**0.338**
	103	**0.121**	0.000	0.000	0.000	0.000	0.269	**0.046**	0.750	0.125	0.500	1.000	0.889	**0.663**
	105	**0.002**	0.000	0.000	0.000	0.000	0.039	**0.007**	0.000	0.000	0.000	0.000	0.000	**0.000**
Fdh	*96*	**0.059**	0.000	0.000	0.000	0.000	0.115	**0.020**	0.250	0.000	0.250	0.000	0.888	**0.293**
	98	**0.032**	0.200	0.167	0.000	0.154	0.000	**0.104**	0.000	0.000	0.000	0.000	0.000	**0.000**
	100	**0.677**	0.750	0.667	0.921	0.769	0.731	**0.779**	0.500	0.875	0.625	0.625	0.111	**0.537**
	102	**0.216**	0.050	0.167	0.079	0.077	0.154	**0.097**	0.250	0.125	0.000	0.250	0.000	**0.122**
	104	**0.016**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.125	0.125	0.000	**0.049**
Fle1	*94*	**0.028**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.250	0.000	0.556	**0.171**
	97	**0.012**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.250	0.111	**0.073**
	100	**0.960**	1.000	1.000	1.000	1.000	1.000	**1.000**	1.000	1.000	0.750	0.750	0.333	**0.756**
Fle3	*92*	**0.008**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.125	0.125	0.000	**0.049**
	96	**0.289**	0.050	0.000	0.105	0.000	0.423	**0.110**	0.250	0.250	0.125	0.000	0.167	**0.159**
	98	**0.004**	0.050	0.000	0.000	0.000	0.000	**0.013**	0.000	0.000	0.000	0.000	0.000	**0.000**
	100	**0.671**	0.900	1.000	0.895	1.000	0.577	**0.877**	0.750	0.750	0.500	0.875	0.278	**0.622**
	104	**0.028**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.125	0.000	0.556	**0.171**
Idh1	*90*	**0.004**	0.050	0.000	0.000	0.000	0.000	**0.013**	0.000	0.000	0.000	0.000	0.000	**0.000**
	100	**0.992**	0.950	1.000	1.000	1.000	1.000	**0.987**	1.000	1.000	1.000	0.875	1.000	**0.976**
	105	**0.004**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.125	0.000	**0.024**

Idh2	*95*	**0.006**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.063	0.000	0.000	0.000	**0.012**
	100	**0.994**	1.000	1.000	1.000	1.000	1.000	**1.000**	1.000	0.938	1.000	1.000	1.000	**0.988**
Pgd1	*96*	**0.060**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.250	0.000	0.250	0.375	0.889	**0.366**
	100	**0.940**	1.000	1.000	1.000	1.000	1.000	**1.000**	0.750	1.000	0.750	0.625	0.111	**0.634**
Pgd2	*96*	**0.008**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.125	0.000	0.000	0.125	0.000	**0.049**
	100	**0.988**	1.000	1.000	0.947	1.000	1.000	**0.987**	0.875	1.000	1.000	0.875	1.000	**0.951**
	102	**0.004**	0.000	0.000	0.053	0.000	0.000	**0.013**	0.000	0.000	0.000	0.000	0.000	**0.000**
Pgi2	*92*	**0.014**	0.000	0.000	0.000	0.000	0.115	**0.020**	0.000	0.000	0.000	0.000	0.111	**0.024**
	100	**0.778**	1.000	0.833	0.895	0.885	0.808	**0.896**	1.000	0.750	1.000	1.000	0.889	**0.927**
	107	**0.008**	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.250	0.000	0.000	0.000	**0.049**
	115	**0.200**	0.000	0.167	0.105	0.115	0.077	**0.084**	0.000	0.000	0.000	0.000	0.000	**0.000**
Pgi3	*92*	**0.143**	0.000	0.000	0.000	0.039	0.115	**0.026**	0.000	0.000	0.000	0.000	0.000	**0.000**
	100	**0.807**	1.000	1.000	0.947	0.962	0.808	**0.948**	1.000	0.563	0.625	0.688	1.000	**0.781**
	108	**0.050**	0.000	0.000	0.053	0.000	0.077	**0.026**	0.000	0.438	0.375	0.313	0.000	**0.220**
Pgm1	*96*	**0.010**	0.000	0.000	0.053	0.000	0.000	**0.013**	0.000	0.000	0.000	0.000	0.000	**0.000**
	100	**0.990**	1.000	1.000	0.947	1.000	1.000	**0.987**	1.000	1.000	1.000	1.000	1.000	**1.000**
Pgm2	*95*	**0.032**	0.050	0.167	0.053	0.077	0.077	**0.078**	0.000	0.000	0.000	0.000	0.000	**0.000**
	100	**0.968**	0.950	0.833	0.947	0.923	0.923	**0.922**	1.000	1.000	1.000	1.000	1.000	**1.000**
Sdh	*95*	**0.173**	0.000	0.182	0.316	0.000	0.385	**0.171**	0.750	0.063	0.750	0.750	1.000	**0.671**
	100	**0.807**	1.000	0.727	0.684	1.000	0.539	**0.803**	0.250	0.688	0.125	0.250	0.000	**0.256**
	105	**0.020**	0.000	0.091	0.000	0.000	0.077	**0.026**	0.000	0.250	0.125	0.000	0.000	**0.073**

Table 4.3 Allele frequencies in Cultivated *V. unguiculata*

Gene	Allele	BEN	BUR	GHA	NIG	CMR	ETH	KEN	SDN	UGA	ZAF	All	CG-B	CG-M	CG-S	CG-T	CG-U
Amp2	*100*	0.000	0.000	0.000	0.000	0.118	0.167	0.000	0.000	0.000	0.000	**0.047**	0.000	0.000	0.000	0.250	0.078
	102	1.000	1.000	1.000	1.000	0.882	0.833	1.000	1.000	1.000	1.000	**0.953**	1.000	1.000	1.000	0.750	0.922
Enp	*98*	0.500	0.167	0.400	0.000	0.177	0.000	0.250	0.500	0.500	0.429	**0.277**	0.267	0.273	0.000	0.250	0.250
	100	0.500	0.833	0.600	1.000	0.824	0.667	0.500	0.500	0.333	0.571	**0.646**	0.600	0.727	1.000	0.750	0.656
	103	0.000	0.000	0.000	0.000	0.000	0.333	0.250	0.000	0.167	0.000	**0.077**	0.133	0.000	0.000	0.000	0.094
Fdh	*100*	0.500	0.667	1.000	1.000	0.588	1.000	0.857	0.750	1.000	0.714	**0.773**	0.833	0.818	1.000	0.500	0.774
	102	0.500	0.333	0.000	0.000	0.412	0.000	0.143	0.250	0.000	0.286	**0.227**	0.167	0.182	0.000	0.500	0.226
Fle3	*96*	0.500	1.000	1.000	0.667	0.941	0.333	0.688	0.500	0.833	0.857	**0.792**	0.633	0.818	1.000	1.000	0.813
	100	0.500	0.000	0.000	0.333	0.059	0.667	0.312	0.500	0.167	0.143	**0.208**	0.367	0.182	0.000	0.000	0.188
Pgd2	*96*	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000	**0.016**	0.067	0.000	0.000	0.000	0.000
	100	1.000	1.000	1.000	1.000	1.000	0.833	1.000	1.000	1.000	1.000	**0.984**	0.933	1.000	1.000	1.000	1.000
Pgi2	*100*	1.000	1.000	1.000	1.000	0.941	1.000	1.000	1.000	1.000	1.000	**0.984**	1.000	1.000	1.000	0.750	1.000
	115	0.000	0.000	0.000	0.000	0.059	0.000	0.000	0.000	0.000	0.000	**0.016**	0.000	0.000	0.000	0.250	0.000

4.1.4 Genetic diversity within wild cowpeas

4.1.4.1 Isozyme diversity

Wild cowpea showed high level of isozyme diversity between accessions and very little within accessions. The average diversity index of wild cowpea was 0.149 ± 0.04 (SE) with an average of 2.652 ± 0.251 (SE) alleles per locus. Gene diversity at individual isozyme loci varied considerably, ranging from 0 for *Mdh_1, Mdh_2, Mdh_3, Mdh_4 and Pgi_1* to 0.489 for *Fdh.*

Regarding the geographic distribution pattern, each group had a different degree of genetic variation. The table 4.4 shows the geographic distribution of the genetic variation indices: Total gene diversity (Ht), Number of alleles per locus (A) and proportion of polymorphic loci (P) at the 18 polymorphic loci. It reveals that the diversity of wild *V. unguiculata* decreases from southern to western Africa, through eastern and central. Accessions from southern Africa had the highest diversity indices (Ht = 0.214, A = 2.087, P (0.99) = 0.696) followed by eastern Africa (Ht = 0.110, A = 2.130, P (0.99) = 0.610). Central and western Africa were less diverse with (H = 0.104, A = 1.652 P (0.99) = 0.435) and (Ht = 0.107, A = 0.348, P (0.99) = 0.304) respectively. Using Pearson correlation coefficient, allelic richness correlated significantly with observed heterozygosity (r = 0.64, P = 0.0011), gene diversity (r = 0.62, P = 0.0016. There was also a positive correlation between observed heterozygosity and gene diversity (r = 1.00, P < 0.001).

Table 4.4: Estimates of the genetic variation indices in wild *V. unguiculata*

Country/Region	N	A	Ap	P (0.95)	P (0.99)	Ho	Hs	Ht
GHA	17	1.261	2.000	0.261	0.261	0.005		0.079
BUR	20	1.217	2.000	0.217	0.217	0.002		0.083
NIG	16	1.261	2.000	0.261	0.261	0.000		0.096
BEN	5	1.174	2.000	0.174	0.174	0.000		0.063
NGA	6	1.304	2.167	0.261	0.261	0.029		0.101
West Africa	64	**1.348**	**2.143**	**0.261**	**0.304**	**0.005**		**0.107**
CMR	45	1.522	2.100	0.261	0.435	0.010		0.104
COG	6	1.217	2.000	0.217	0.217	0.007		0.066
RCA	7	1.261	2.200	0.217	0.217	0.006		0.065
TCD	10	1.304	2.167	0.261	0.261	0.004		0.101
Central Africa	**68**	**1.652**	**2.500**	**0.304**	**0.435**	**0.008**		**0.104**
SDN	20	1.435	2.250	0.348	0.348	0.002		0.066
UGA	12	1.478	2.111	0.391	0.391	0.000		0.120
KEN	19	1.522	2.091	0.478	0.478	0.009		0.081
ETH	13	1.304	2.167	0.174	0.261	0.010		0.049
TZA	13	1.696	2.600	0.435	0.435	0.030		0.145
East Africa	**77**	**2.130**	**2.857**	**0.435**	**0.610**	**0.010**		**0.110**
RSA	8	1.565	2.182	0.478	0.478	0.000		0.164
MWI	8	1.478	2.100	0.435	0.435	0.016		0.134
ZMB	8	1.609	2.300	0.435	0.435	0.012		0.183
ZWE	8	1.522	2.091	0.478	0.478	0.005		0.160
BWA	9	1.522	2.333	0.391	0.391	0.015		0.136
Southern Africa	**41**	**2.087**	**2.500**	**0.478**	**0.696**	**0.010**		**0.214**
All	**250**	**2.652**	**3.111**	**0.435**	**0.609**	**0.008**		**0.149**

4.1.4.2 Chloroplast DNA (cpDNA) diversity

The cpDNA analysis within wild and cultivated cowpea accessions using primers CAPF and DWR generated a unique expected amplified fragment of approximately 1560 bp. The digestion of the wild samples with *Bam*H1 restriction enzyme was negative for some and generated two bands of 1400 and

160 bp in others. Therefore, the wild cowpea was characterized by two distinct haplotype at that amplicon of the cpDNA: the amplicon with loss of BamH1 restriction site (Haplotype 1) and where restriction site is present (Haplotype 0).

Out of the 253 wild accessions investigated for chloroplast DNA variation, 99 showed haplotype 1 and 154 accessions with haplotype 0. The geographic distribution of these haplotypes was uneven. While haplotype 1 distribution goes decreasing from the western part of the continent to the southern via central and eastern, the haplotype 0 distributions goes increasing. Central Africa appears to be the region with approximately balanced distribution of the two haplotypes. At the country level, Ghana had the most haplotype 1 distribution with 16 cases out of the 17 accessions analysed compared to Uganda (0/12), Ethiopia(0/13), South Africa (0/8), Zambia (0/8), Zimbabwe (0/8) and Botswana (0 haplotype1 out of 9 accessions).

4.1.5 Genetic variation within cultivated cowpeas

4.1.5.1 Isozyme

Cultivated accessions compared to wild exhibited low genetic variation. Within accessions gene diversity was quite low (Hs = 0.001) and therefore, the variation between accessions was responsible for most of the genetic diversity. This was dependent on only six loci (*Amp_2, Enp, Fdh, Fle_3, Pgd_2* and *Pgi_2*) out of twenty three scored and was characterised by the high coefficient of genetic

differentiation found between accessions (Gst = 0.977). The genetic diversity indices considering all cultivated accessions were (Ht = 0.058 ± 0.029 (SE), A = 0.130 ± 0.117 (SE), P (0.99) = 0.261). Cultivated accessions from eastern (Ht = 0.061, A = 1.261, P (0.99) = 0.217) and southern Africa (Ht = 0.056, A = 1.174, P (0.99) = 0.174) were slightly higher than those of western (Ht = 0.045, A = 1.130, P (0.99) = 0.125) and central Africa (Ht = 0.052, A = 1.217, P (0.99) = 0.217). The unequal distribution of sampling size too variable among different cowpea cultivar groups could not allow a proper comparison of the genetic variation between these groups. Pearson correlation coefficients indicated positives correlations between allelic richness and gene diversity (r = 0.39, P = 0.06), observed heterozygosity (r = 0.39, P = 0.06) and between observed heterozygosity and gene diversity (r = 1.00, P < 0.001).

Table 4.5: Estimates of the genetic variation indices in cultivated *V. unguiculata*

Country/Region/Group	N	A	Ap	P (0.95)	P (0.99)	Ho	Hs	Ht
Benin	4	1.130	2.000	0.130	0.130	0.000	0.000	0.065
Burkina Faso	6	1.087	2.000	0.087	0.087	0.000	0.000	0.031
Ghana	5	1.044	2.000	0.044	0.044	0.000	0.000	0.021
Niger	3	1.044	2.000	0.044	0.044	0.000	0.000	0.019
Western Africa	**18**	**1.130**	**2.000**	**0.125**	**0.125**	**0.000**	**0.000**	**0.045**
Cameroon	17	1.217	2.000	0.217	0.217	0.000	0.000	0.052
Ethiopia	6	1.174	2.000	0.174	0.174	0.000	0.000	0.063
Kenya	8	1.174	2.333	0.130	0.130	0.005	0.003	0.057
Sudan	2	1.130	2.000	0.130	0.130	0.022	0.011	0.060
Uganda	6	1.130	2.500	0.087	0.087	0.000	0.000	0.039
Easten Africa	**22**	**1.261**	**2.200**	**0.130**	**0.217**	**0.004**	**0.002**	**0.061**
South Africa	7	1.174	2.000	0.174	0.174	0.000	0.006	0.056
CG-Biflora	15	1.217	2.250	0.174	0.174			0.064
CG-Melanophthalmus	11	1.130	2.000	0.130	0.130			0.045
CG-Sesquipedalis	2	1.000						0.000
CG-Textilis	5	1.174	2.000	0.174	0.174			0.081
CG-Unguiculata	31	1.217	2.250	0.174	0.174			0.060
All	**64**	**1.304**	**2.167**	**0.174**	**0.261**	**0.001**	**0.001**	**0.058**

4.1.5.2 Chloroplast DNA

In cultivated cowpea, 108 accessions in sub-Saharan Africa were assessed for the cpDNA variation. All these accessions generated a unique haplotype characterised by the absence of the *Bam*H1 restriction site on the 1560 bp fragment amplified by CAPF and DWR primers (Haplotype 1).

4.1.6 Diversity and differentiation between wild and cultivated cowpea

To assess the differences in diversity between wilds and cultivated samples, various diversity indices were compared using student's two tailed t test. The average total gene diversity in wild accessions was 0.149 ± 0.040 (SE) and was significantly higher (Student t test, t = -2.91, P = 0.008) compared to cultivated accessions with 0.058 ± 0.029 (SE) as its mean gene diversity. In order to compare the number of alleles in cultivated accessions to those found in the wilds, the effect of sampling size being of great consideration, this effect was corrected by calculating using Fstat version 2.9.3 (Goudet, 2001) the allelic richness. A significant lower allelic richness (Table 4.7) in the cultivated accessions compared to wilds was found (t = -5.77, P < 0.001). The average allelic richness in cultivated was 1.304 ± 0.117 (SE) compared to 2.487 ± 0.243 in wilds. The cultivated accessions had 61% fewer gene diversity and 48% lower allelic richness than in wild accessions. Other genetic diversities indices were also significantly high in wild compared to cultivars. These are observed heterozygosity (Ho) (t = 2.82 P = 0.010), diversity expected within accessions (Hs) (t = 2.02, P = 0.056), diversity expected among accessions (Dst) (t = 2.89, P = 0.009) and the proportion of allelic diversity found among accessions (Gst) (t = 4.89, P < 0.001).

The genetic differentiation between the wild and cultivated accessions was statistically different from zero (p < 0.001) with an average of 0.255, 95% confidence interval (0.071 – 0.432); this differentiation varied from – 0.003 to

0.684 depending on the locus studied and was significant ($P < 0.050$) for 12 of the 18 polymorphic loci. The average Fit was 0.959 ($P < 0.001$) in the wilds accessions and 0.977 ($P < 0.001$) in the cultivars.

Table 4.6: Number of alleles or haplotype at different loci in wild and cultivated cowpea and the most frequent genotype or haplotype.

	Number of alleles / haplotype				**Most frequent genotype / haplotype**	
Gene	**Total**	**wild**	**cultivated**	**common**	**Wild**	**cultivated**
Adh1	2	2	1	1	100100	100100
Adh2	2	2	1	1	100100	100100
Amp2	3	3	2	2	100100	**102102**
Amp3	4	4	1	1	100100	100100
Amp4	4	4	1	1	100100	100100
Enp	5	4	3	2	100100	100100
Fdh	5	5	2	2	100100	100100
Fle1	3	3	1	1	100100	100100
Fle3	5	5	2	2	100100	**096096**
Idh1	3	3	1	1	100100	100100
Idh2	2	2	1	1	100100	100100
Mdh1	1	1	1	1	100100	100100
Mdh2	1	1	1	1	100100	100100
Mdh3	1	1	1	1	100100	100100
Mdh4	1	1	1	1	100100	100100
Pgd1	2	2	1	1	100100	100100
Pgd2	3	3	2	2	100100	100100
Pgi1	1	1	1	1	100100	100100
Pgi2	4	4	2	2	100100	100100
Pgi3	3	3	1	1	100100	100100
Pgm1	2	2	1	1	100100	100100
Pgm2	2	2	1	1	100100	100100
Sdh	3	3	1	1	100100	100100
Total (Isozyme)	**62**	**61**	**30**	**29**		
cpDNA	**2**	**2**	**1**	**1**	Haplotype 0	**Haplotype 1**

Table 4.7: Diversity indices and differentiation in Wild and Cultivated *V. unguiculata*

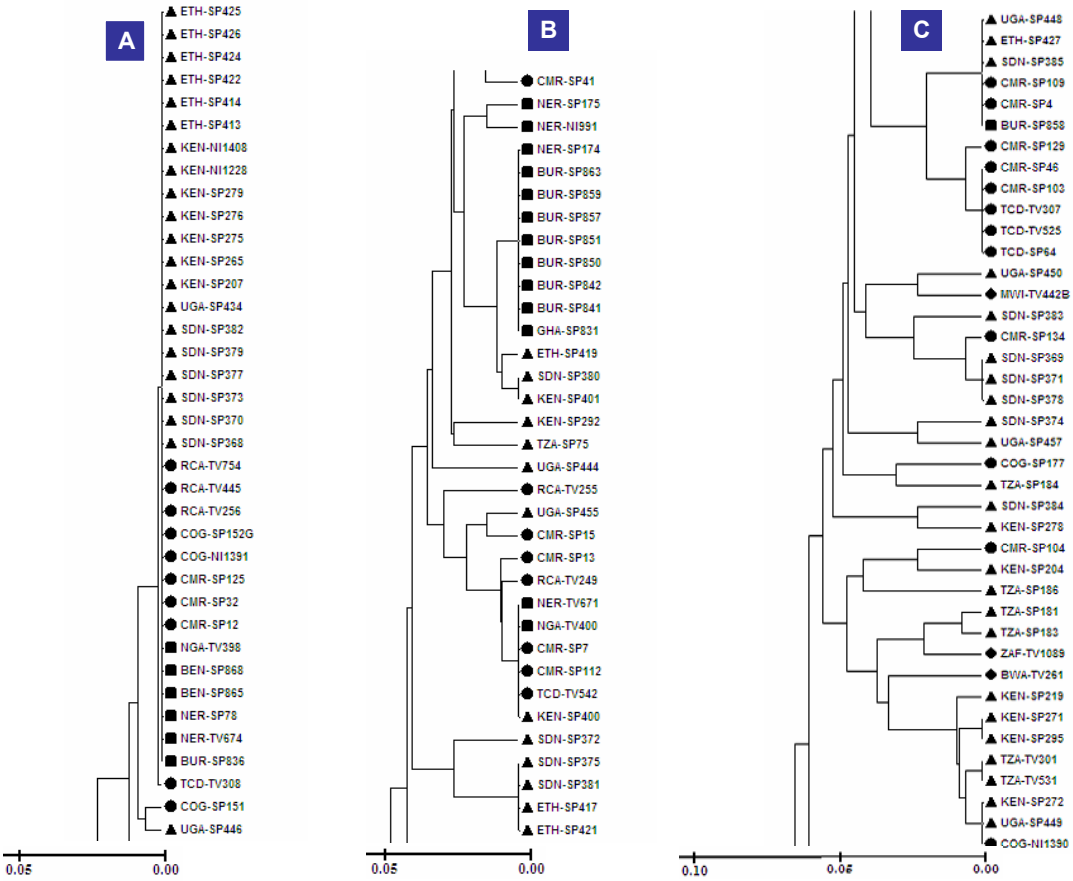
	Ar		**Ho**		**Hs**		**Dst**		**Gst**		**Ht**		**Fst**
	Wild	Cult	Wild	Cult	Wild	Cult	Wild	Cult	Wild	Cult	Wild	Cult	
Loci													
Adh1	1.681	1.000	0.000	0.000	0.000	0.000	0.008	0.000	1.000	0.000	0.008	0.000	0.003
Adh2	1.990	1.000	0.000	0.000	0.000	0.000	0.032	0.000	1.000	0.000	0.032	0.000	0.005
Amp2	2.924	2.000	0.008	0.000	0.005	0.016	0.304	0.088	0.984	0.849	0.309	0.104	0.684*
Amp3	3.686	1.000	0.000	0.000	0.000	0.000	0.194	0.000	1.000	0.000	0.194	0.000	0.047*
Amp4	3.682	1.000	0.028	0.000	0.014	0.000	0.363	0.000	0.963	0.000	0.377	0.000	0.140*
Enp	3.119	3.000	0.020	0.000	0.010	0.000	0.213	0.493	0.955	1.000	0.223	0.493	0.173*
Fdh	4.990	2.000	0.014	0.016	0.008	0.008	0.481	0.346	0.985	0.978	0.489	0.354	0.009*
Fle1	2.968	1.000	0.000	0.000	0.000	0.000	0.078	0.000	1.000	0.000	0.078	0.000	0.015*
Fle3	4.582	2.000	0.020	0.016	0.011	0.008	0.455	0.325	0.976	0.977	0.466	0.333	0.342*
Idh1	2.362	1.000	0.000	0.000	0.000	0.000	0.016	0.000	1.000	0.000	0.016	0.000	0.001
Idh2	1.820	1.000	0.004	0.000	0.002	0.000	0.010	0.000	0.832	0.000	0.012	0.000	0.000
Mdh1	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	m
Mdh2	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	m
Mdh3	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	m
Mdh4	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	m
Pgd1	2.000	1.000	0.000	0.000	0.000	0.000	0.113	0.000	1.000	0.000	0.113	0.000	0.034*
Pgd2	2.580	2.000	0.000	0.000	0.000	0.000	0.024	0.031	1.000	1.000	0.024	0.031	0.003
Pgi1	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	m
Pgi2	3.881	2.000	0.028	0.000	0.014	0.000	0.341	0.031	0.961	1.000	0.355	0.031	0.114*
Pgi3	3.000	1.000	0.032	0.000	0.016	0.000	0.313	0.000	0.951	0.000	0.329	0.000	0.100*
Pgm1	1.943	1.000	0.012	0.000	0.006	0.000	0.014	0.000	0.697	0.000	0.020	0.000	0.003
Pgm2	2.000	1.000	0.000	0.000	0.000	0.000	0.062	0.000	1.000	0.000	0.062	0.000	0.016*
Sdh	2.997	1.000	0.008	0.000	0.004	0.000	0.314	0.000	0.987	0.000	0.318	0.000	0.113*
All			0.008	0.001	0.004	0.001	0.145	0.057	0.961	0.977	0.149	0.058	0.255*

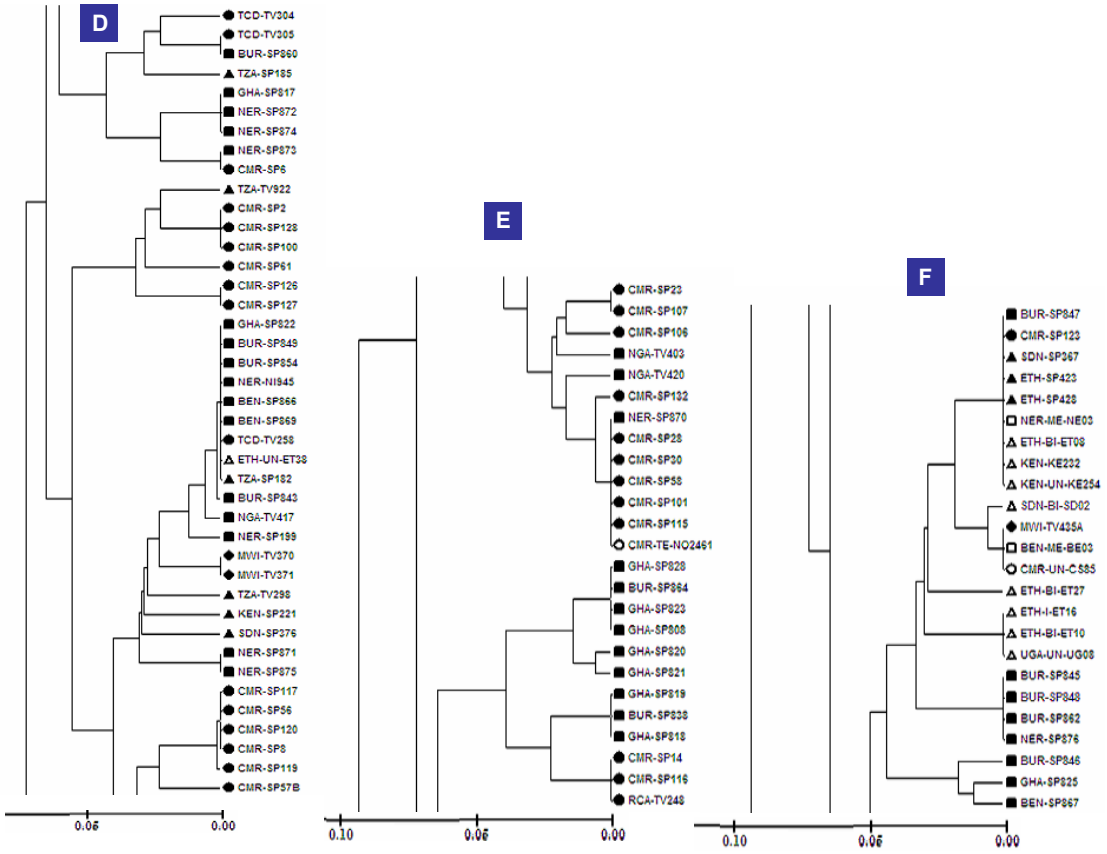
Ar = Allele richness; Ho = Observed heterozygosity; Hs = Heterzygosity expected within accessions; Dst = Heterozygosity expected among accesions; Gst = Proportion of alleleic diversity foud between accessions, Ht = Total gene diversity; Fst = Genetic differentiation found beween wild and cultivated V. unguiculata. *: Significant at 0.050 probability level

4.1.7 Phylogenetic relationships in *V. unguiculata*

For cluster analysis, Nei (1972) genetic distances were calculated for all possible pairwise comparison among the 314 wilds and cultivated accessions of *V. unguiculata.* Genetic distances between groups according to regions were also calculated. Therefore the genetic relationship between cowpea accessions and groups was assessed with the construction of the UPGMA (Unweighted Pair Group Method with Arithmetic mean) phylogenetic tree from the genetic distance matrices. The cluster analysis at the accession level generated a unique dendogram with several clusters (Figure 4.17). Basically, the domesticated accessions were homogenous and grouped in the same cluster at the middle of the dendogram while those wilds from southern Africa were in the bottom part of the tree. Twenty four wild accessions were amidst the cultivated cluster. The majority, fifteen of them were from western Africa (SP832, SP835, SP829, SP826, SP827, SP824, SP300, SP847, SP845, SP848, SP862, SP876, SP846, SP825 and SP867); four were from Cameroon (central Africa) (SP1, Sp19, SP22and SP123); four from eastern Africa (SP367, SP423, SP426 and NI1048) and one from Malawi (southern Africa) (TV435A). The majority of these wild accessions where characterised by cpDNA haplotype 1 (18 out of 24), Isosyme Amp_2^{102} (23 out of 24) and Fle_3^{096} (12 out of 24) profiles, highly represented within cultivated species. Two domesticated accessions appear within the wild clusters: TE-NO2461 and UN-ET38. Although they were having the cpDNA haplotype 1 like all the cultivated accessions, their isozyme *Amp* profile was like most wild (Amp_2^{100}). It is therefore indicated that not all the 250 wild or 64

cultivated accessions could be clearly separated by the use of isozyme markers (Figure 4.17). The dendogram partly reflected the spatial distribution of accessions. However if all cowpea accessions could not be clearly differentiated at the accession level, an obvious relationship associated with the spatial distribution of wild cowpea was observed progressively at the country and at the region level. Figures 4.18, 4.19, 4.20 and 4.21 show the dendogram relating wild and also cultivated cowpea according to countries and regions. The tables 4.8 and 4.9 indicate that wilds from Western Africa and especially Ghana and Benin were the most closest to the domesticated taxon than do other wilds.

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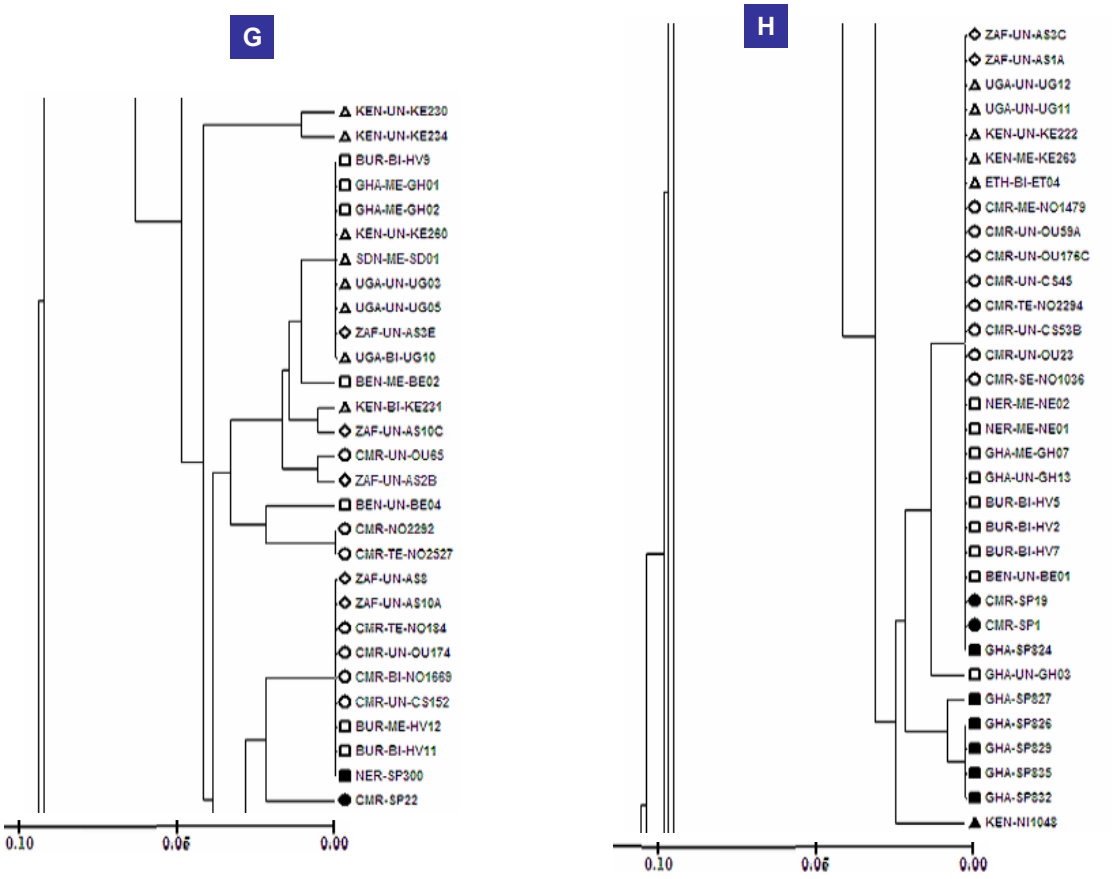
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GHA-UN-GH13
BUR-BI-HV5
BUR-BI-HV2
BUR-BI-HV7
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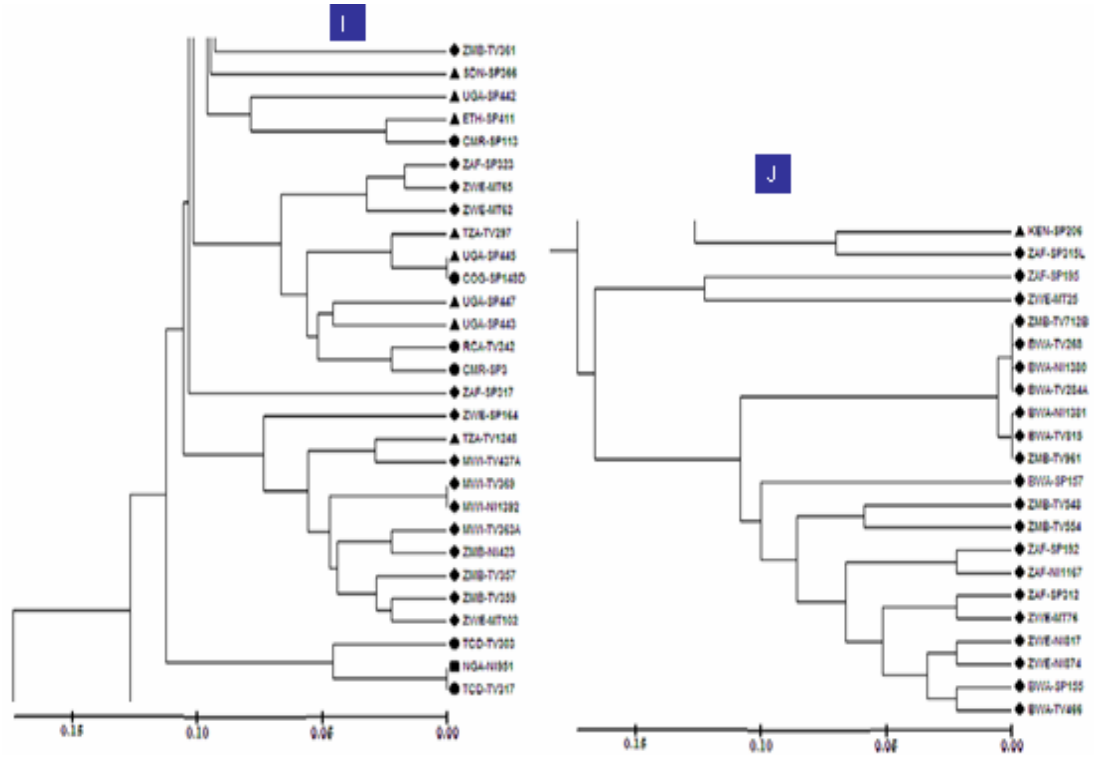

Figure 4.17: UPGMA phylogenetic tree showing the genetic relationship of 314 accessions of *V. unguiculata* using Nei's (1972) genetic distance values. A, B, C, D, E, F, G, H, I and J represent the order of the tree from the top to the bottom

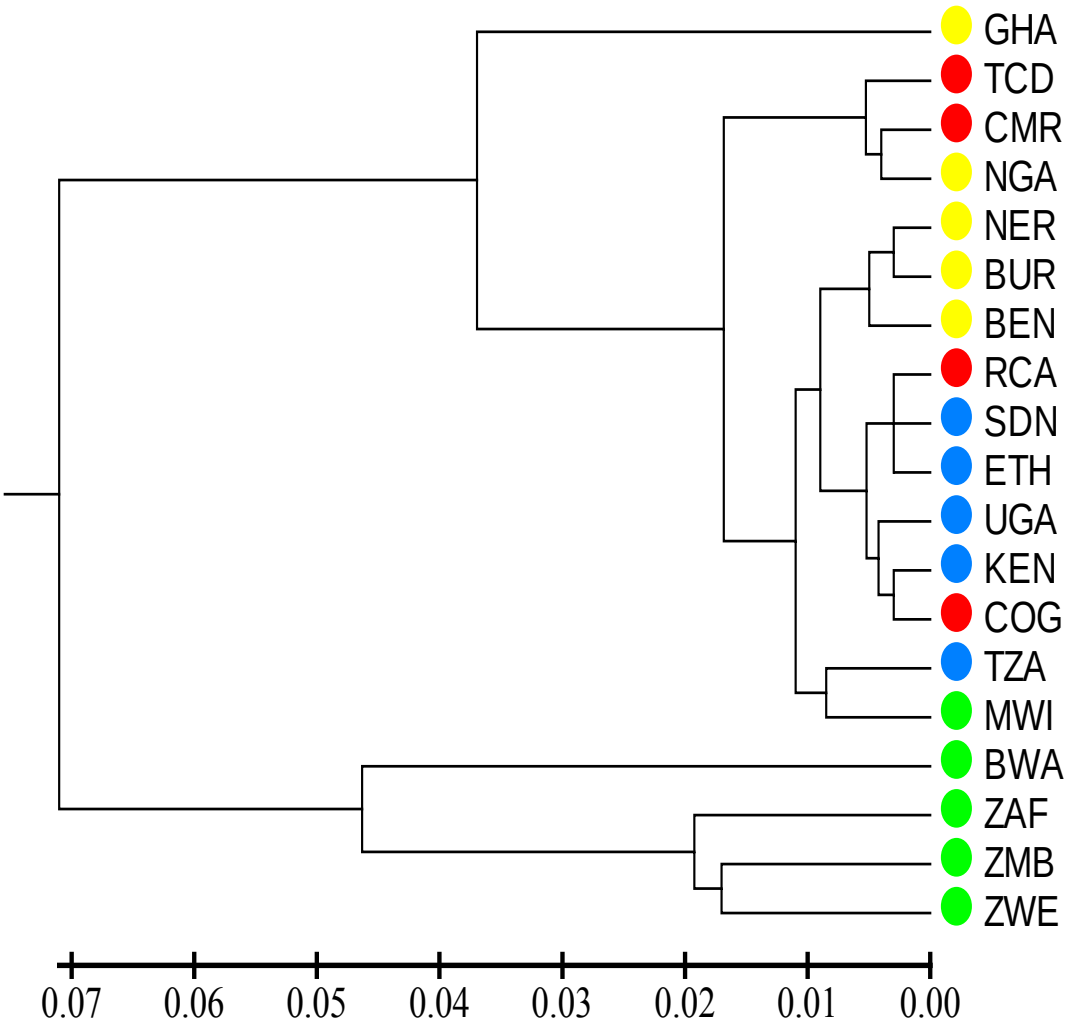

Figure 4.18: Phylogenetic relationships of wild cowpea using Nei's (1972) genetic distance at the country level.

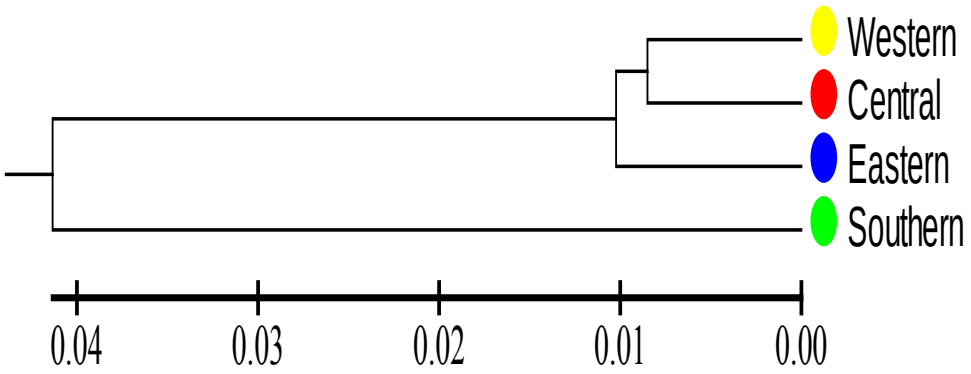

Figure 4.19: Phylogenetic relationships of wild cowpea using Nei's (1972) genetic distance at the region level

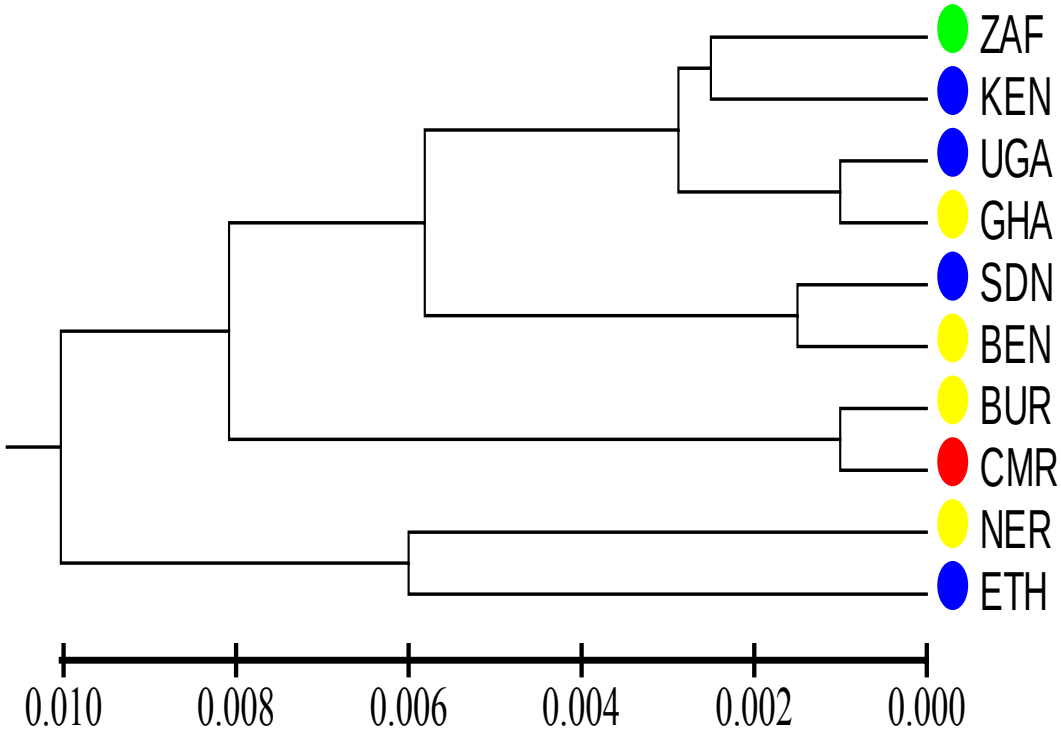

Figure 4.20: Phylogenetic relationships of cultivated cowpea using Nei's (1972) genetic distance at the country level

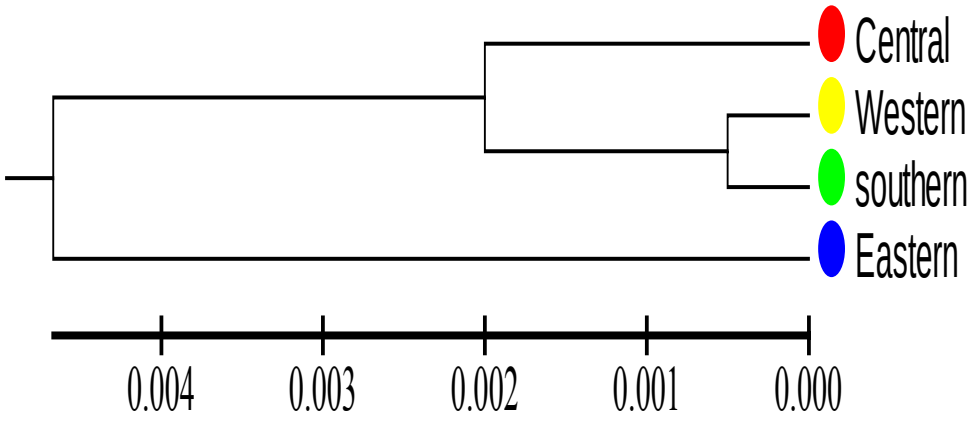

Figure 4.21: Phylogenetic relationships of cultivated cowpea using Nei's (1972) genetic distance at the region level

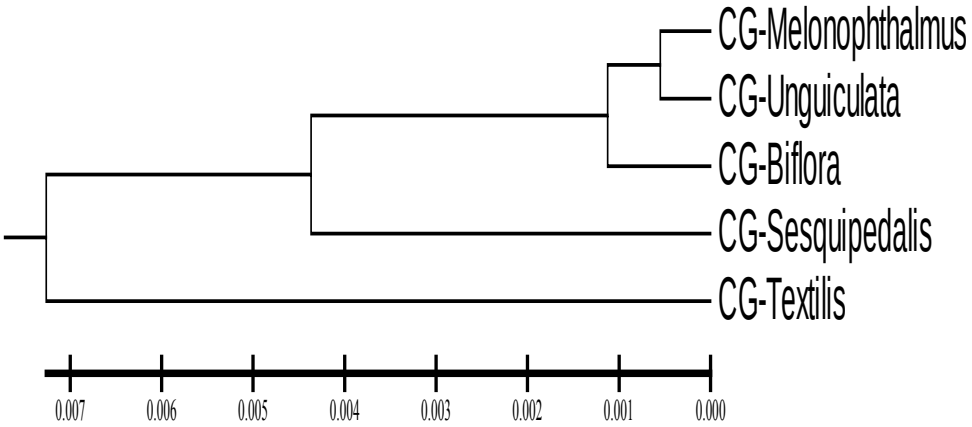

Figure 4.22: Phylogenetic relationships of cultivar groups of cowpea using Nei's (1972) genetic distance

4.1.8 Distribution of alleles and haplotype characteristic of cultivated cowpea within wilds

The distribution of chloroplast DNA haplotype 1, Amp_2^{102} and Fle_3^{096}, highly represented in cultivated cowpea at 100%, 95.3% and around 80% respectively was particularly investigated within wilds and are presented in Figures 4.23, 4.24, 4.25, 4.26, 4.27 and 4.28. All of these alleles and haplotype were found to be highly represented in the western part of the continent. At the country level, positive and significant correlations were found between Haplotype 1 and Amp_2^{102} distribution ($r = 0.60$ $P < 0.010$, Figure 4.29); Haplotyp1 and Fle_3^{096} distribution ($r = 0.72$, $P < 0.001$, Figure 4.31); Amp_2^{102} and Fle_3^{096} distribution ($r = 0.77$, $P < 0.001$, Figure 4.33). At the regional level, Positive and strong correlations were also observed between Haplotype 1 and Amp_2^{102} ($R^2 = 0.82$, Figure 4.30), Haplotype 1 and Fle_3^{096} ($R^2 = 0.97$, Figure 4.32) and between Amp_2^{102} an Fle_3^{096} ($R^2 = 0.74$, Figure 4.34)

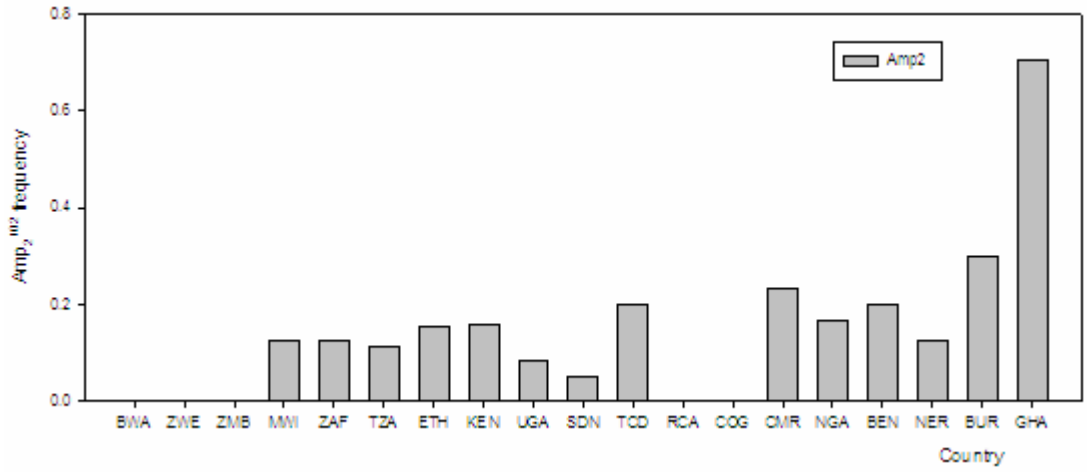

Figure 4.23: Distribution of Amp_2^{102} within countries

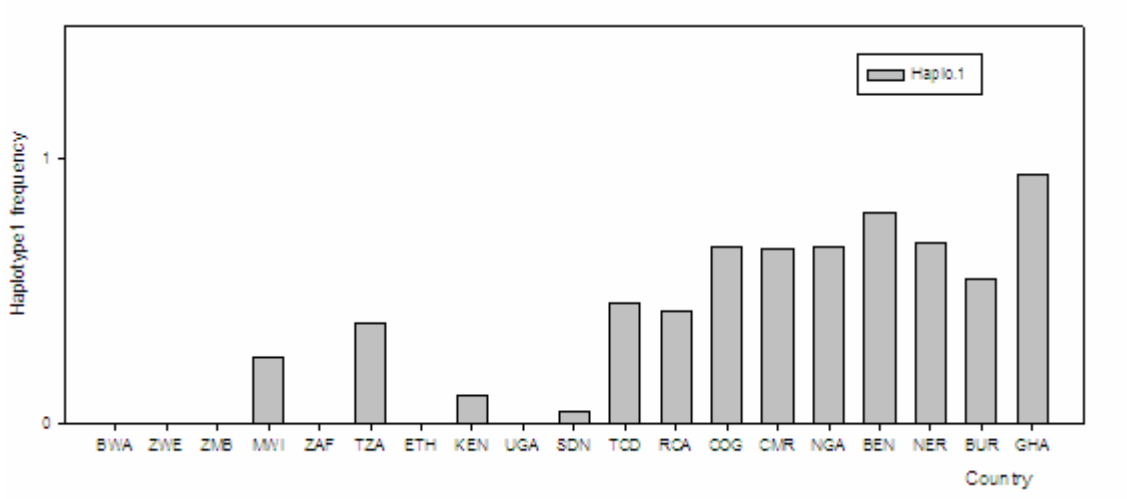

Figure 4.24: Distribution of cpDNA haplotype 1 within countries

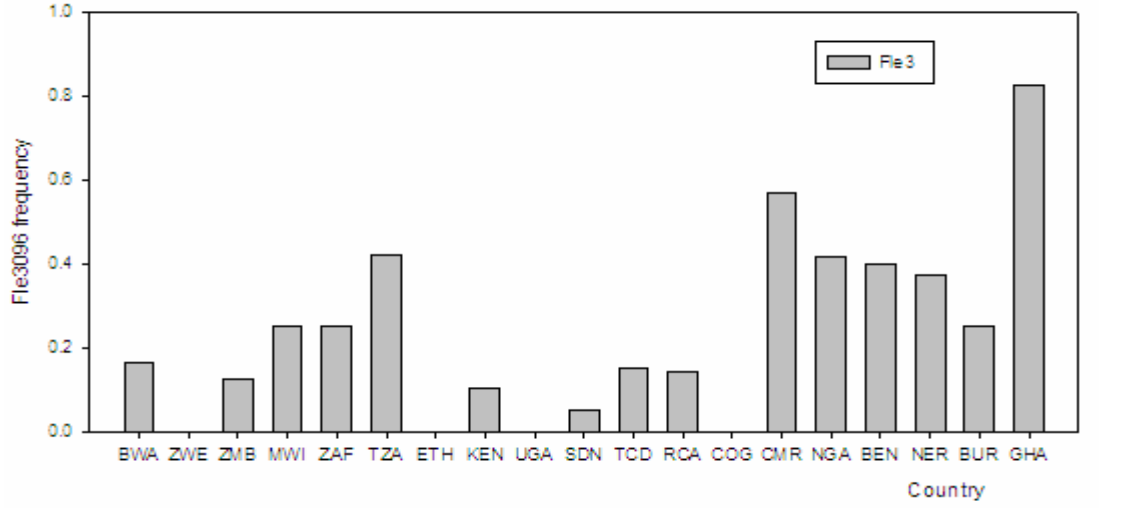

Figure 4.25: Distribution of Fle_3^{096} within countries

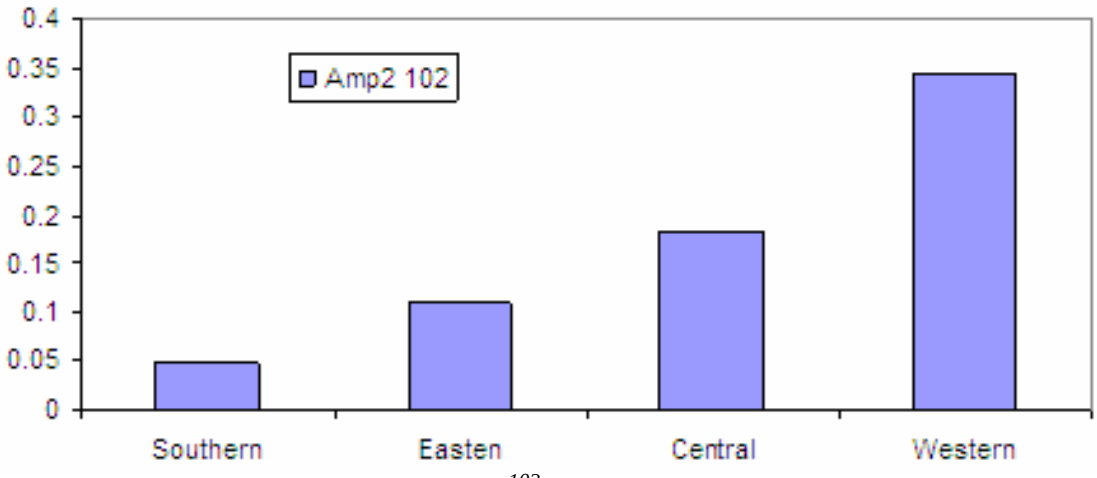

Figure 4.26: Distribution of Amp_2^{102} according to regions

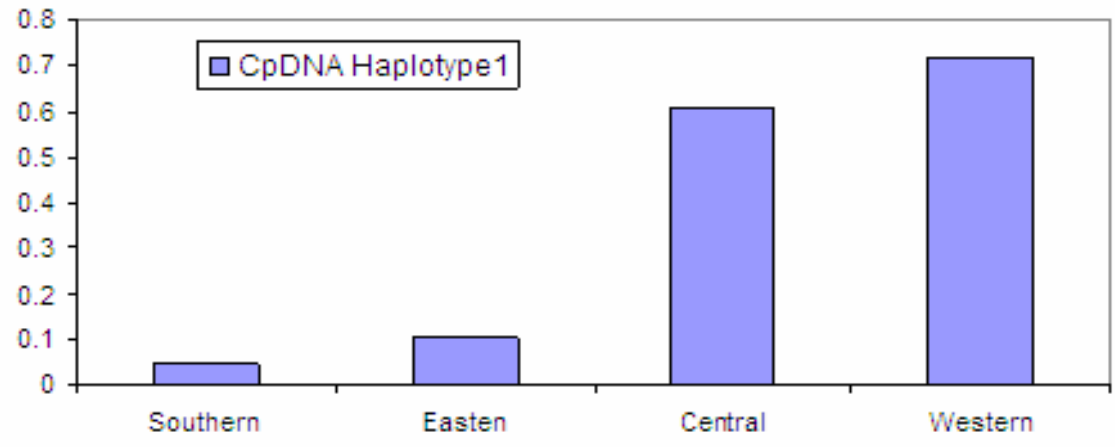

Figure 4.27: Distribution of cpDNA Haplotype 1 according to regions

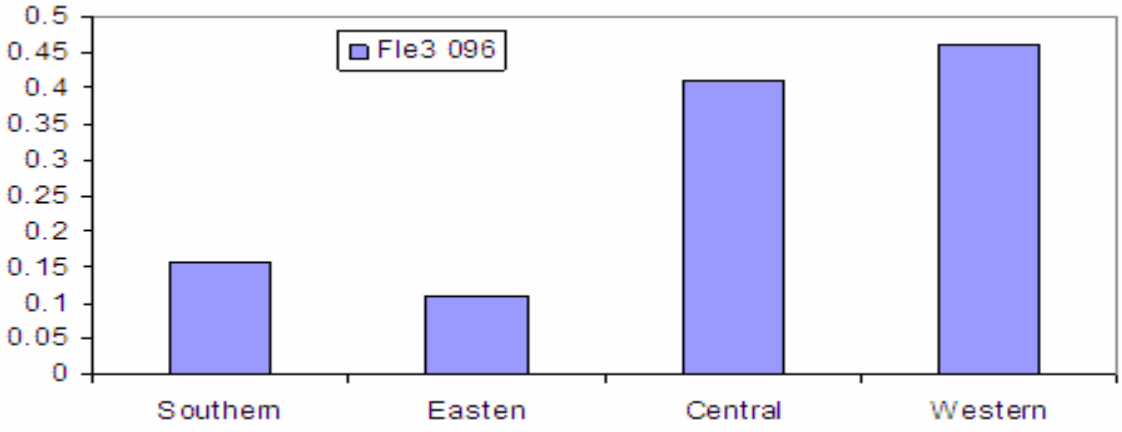

Figure 4.28: Distribution of Fle_3^{096} according to regions

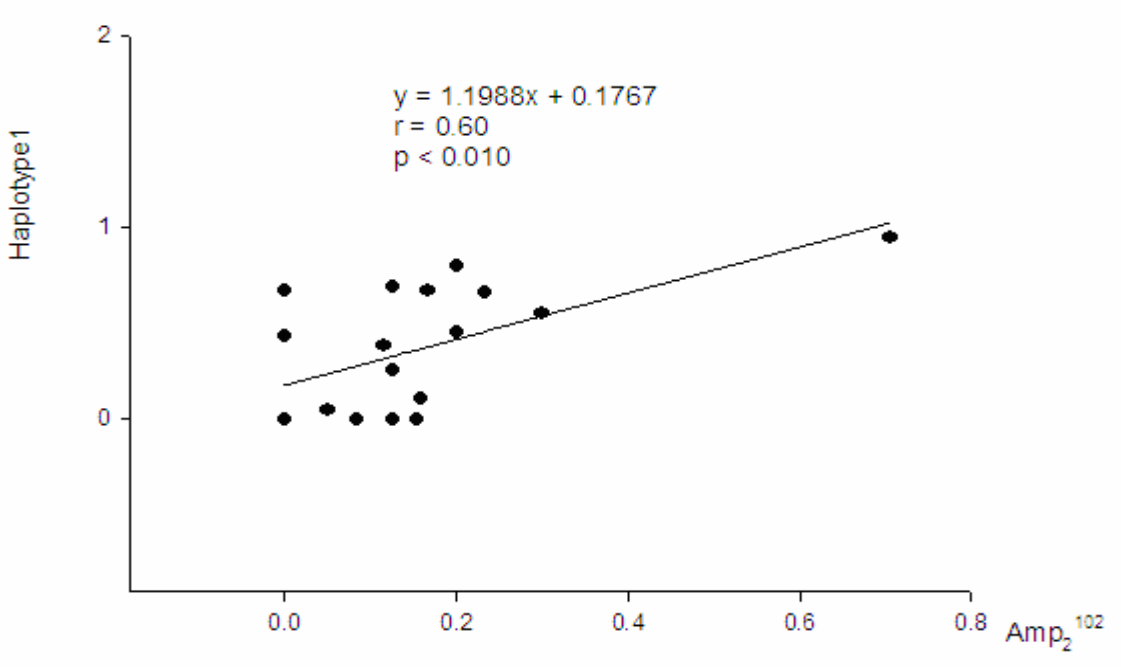

Figure 4.29: Regression plot between Amp_2^{102} and cpDNA Haplotype 1

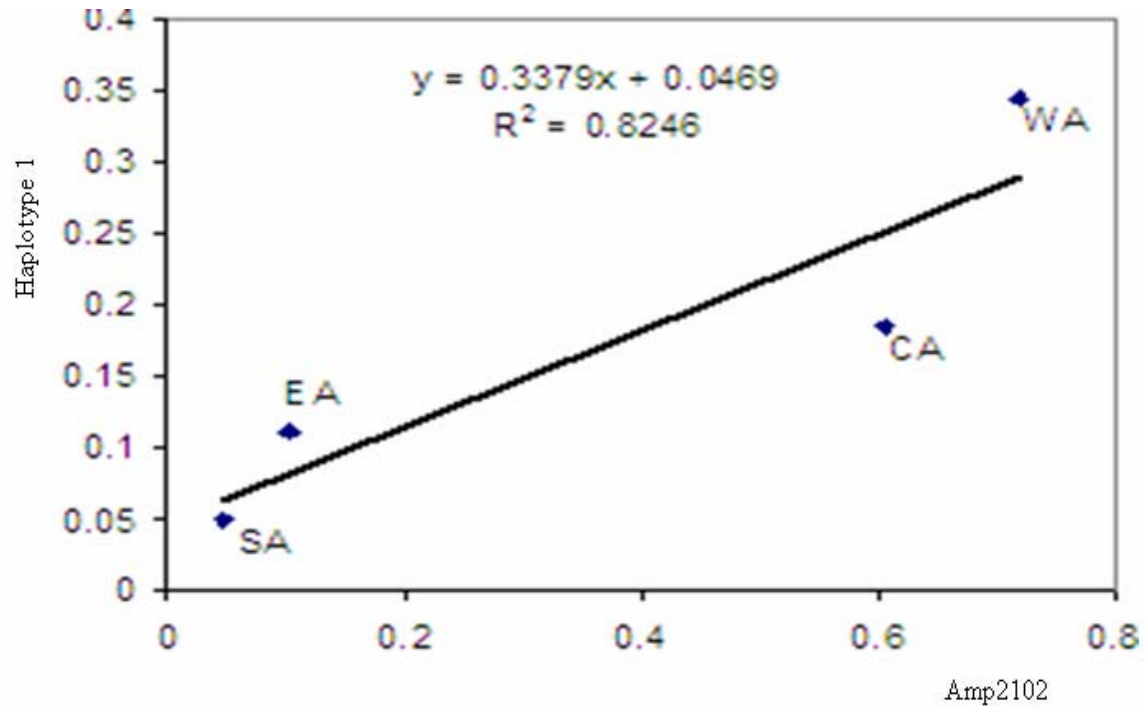

Figure 4.30: Regression plot between cpDNA haplotype 1 and Amp_2^{102} at the region level

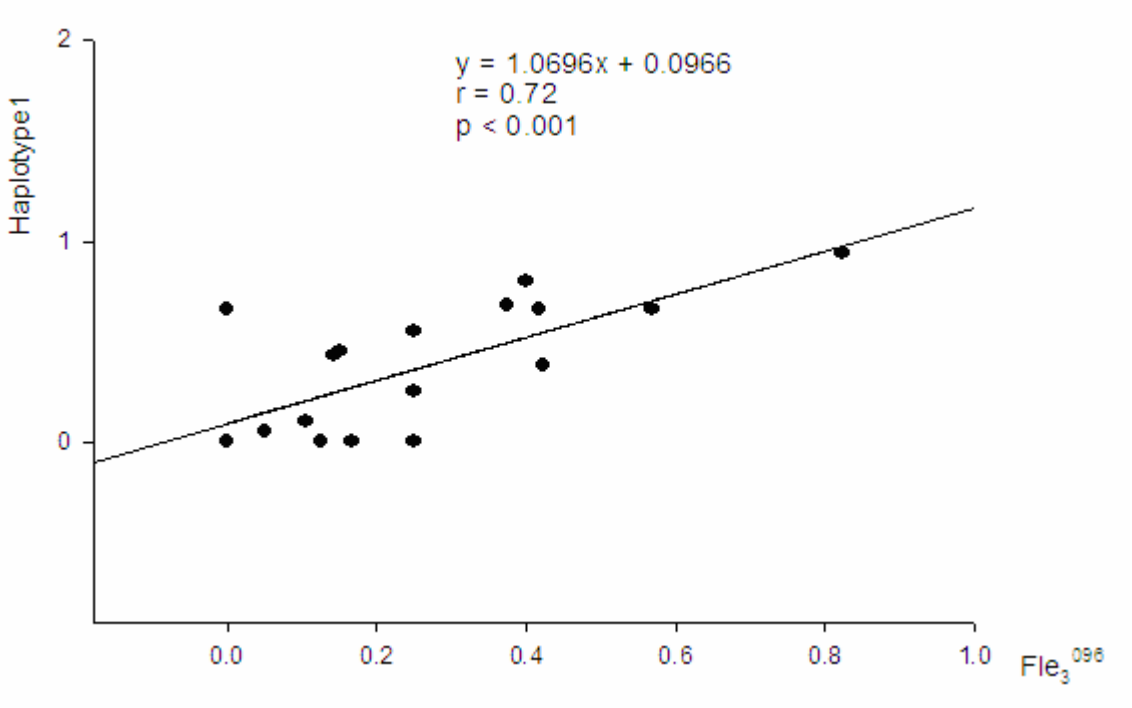

Figure 4.31: Regression plot between Fle3096 and cp DNA Haplotype 1

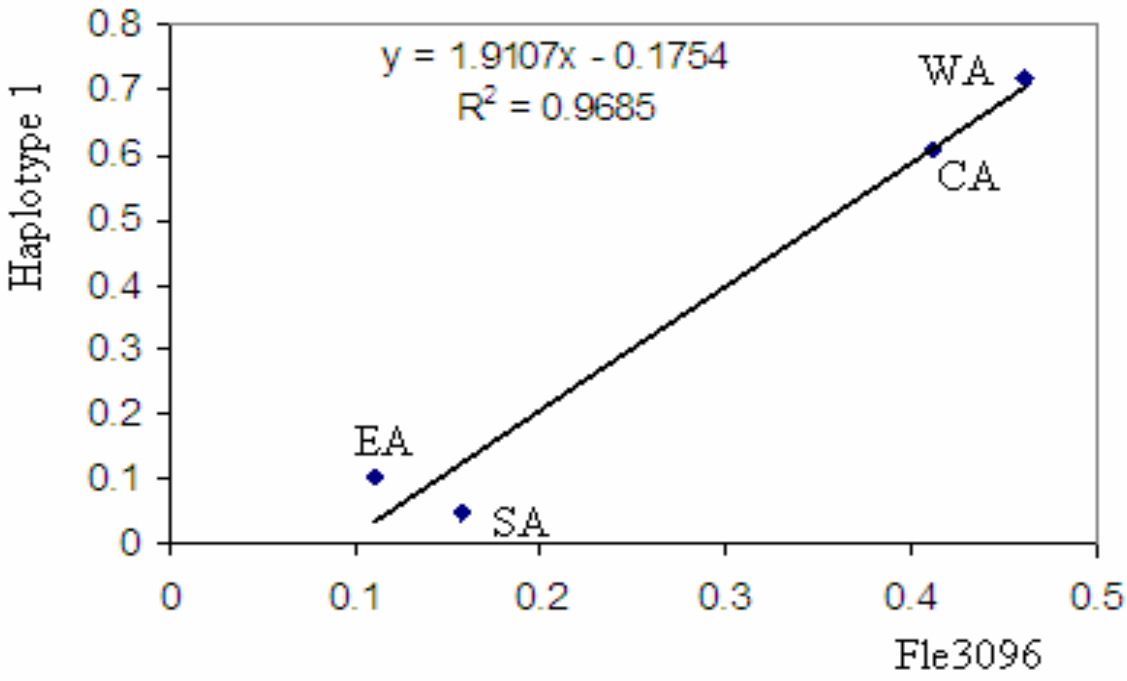

Figure 4.32: Regression plot between cpDNA haplotype 1 and Fle3096 at the region level

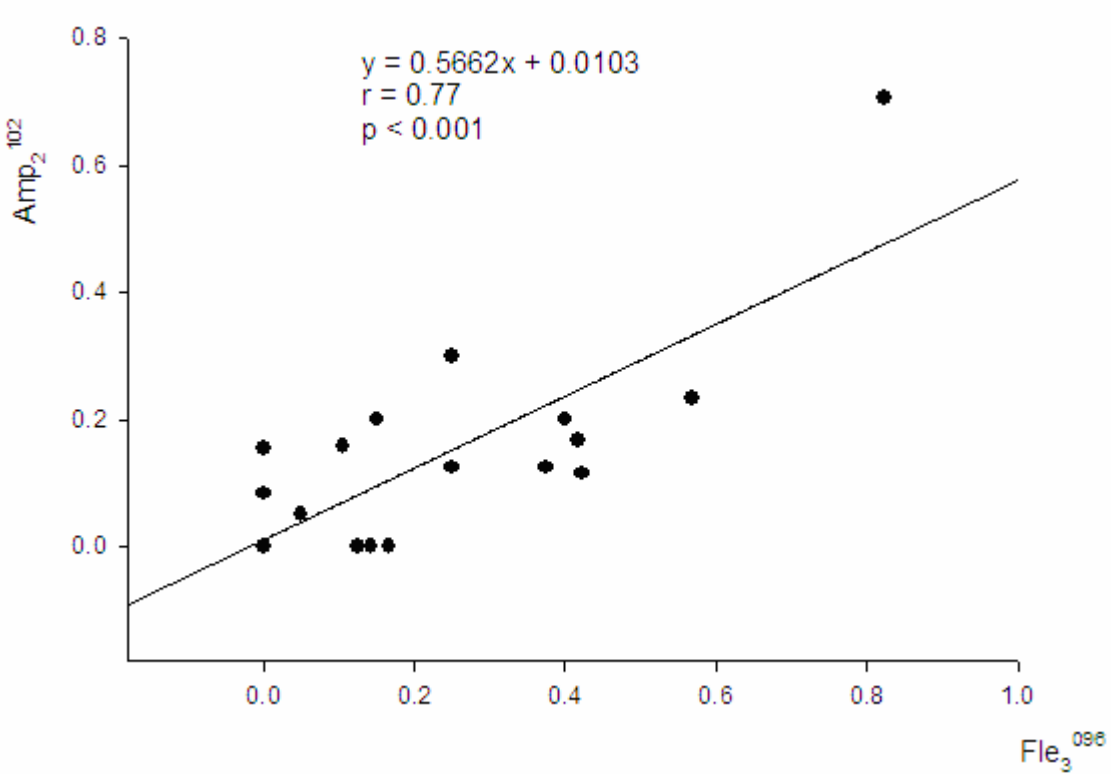

Figure 4.33: Regression plot between Amp_2^{102} and Fle_3^{096}

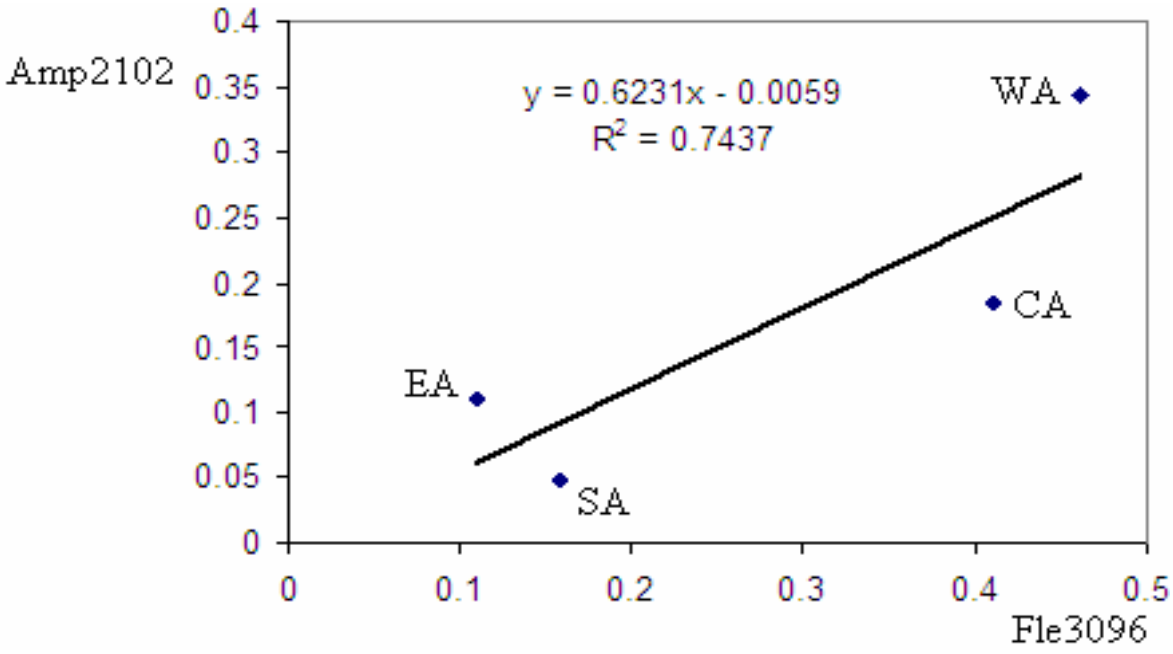

Figure 4.34: Regression plot between Amp_2^{102} and Fle_3^{096} at the region level

Table 4.8: Nei's (1972) genetic distance matrix of cowpea accessions at the regional level

	C.West	C.Central	C.East	C.South	W.West	W.Central	W.East	W.South
	18	17	22	7	64	68	77	41
C.West	0.000							
C.Central	0.003	0.000						
C.East	0.006	0.015	0.000					
C.South	0.001	0.005	0.007	0.000				
W.West	0.042	0.038	0.042	0.048	0.000			
W.Central	0.062	0.052	0.061	0.068	0.017	0.000		
W.East	0.070	0.070	0.059	0.078	0.021	0.020	0.000	
W.South	0.144	0.143	0.122	0.147	0.087	0.096	0.065	0.000

Table 4.9: Nei's (1972) genetic distance between cultivated cowpea and wild accessions from West African countries

Cultivated	C.West	C.Central	C.East	C.South	All Cultivated
	18	17	22	7	64
Wilds					
Ghana (17)	0.042	0.034	0.054	0.045	0.044
Burkina Faso (20)	0.057	0.057	0.050	0.064	0.057
Niger (16)	0.061	0.054	0.059	0.067	0.060
Benin (5)	0.044	0.041	0.041	0.051	0.044
Nigeria (6)	0.075	0.068	0.071	0.082	0.074

Table 4.10: Nei's (1972) genetic distance matrix of cowpea cultivar groups and wild accessions from West African countries

	W.GHA	**W.BUR**	**W.NIG**	**W.BEN**	**W.NGA**	**CG.Bif**	**CG.Mel**	**CG.Ses**	**CG.Tex**	**CG.Ung**
W.GHA	0.0000									
W.BUR	0.0562	0.0000								
W.NIG	0.0431	0.0064	0.0000							
W.BEN	0.0425	0.0133	0.0069	0.0000						
W.NGA	0.0656	0.0319	0.0191	0.0252	0.0000					
CG.Bif	0.0472	0.0507	0.0580	0.0407	0.0717	0.0000				
CG.Mel	0.0426	0.0559	0.0608	0.0346	0.0741	0.0020	0.0000			
CG.Ses	0.0459	0.0634	0.0686	0.0492	0.0780	0.0111	0.0060	0.0000		
CG.Tex	0.0383	0.0624	0.0526	0.0437	0.0580	0.0178	0.0119	0.0192	0.0000	
CG.Ung	0.0416	0.0540	0.0561	0.0401	0.0707	0.0025	0.0011	0.0091	0.0092	0.0000

4.2 Genetic diversity and population structure of wild cowpea (*Vigna unguiculata*) in a region of West Africa

4.2.1 Distribution and compositions of locus and alleles

A total of 32 alleles at the 21 isozyme loci were identified across the 35 populations studied. 13 loci were monomorphic (*Fle_1 Idh_1 Idh_2, Mdh_1, Mdh_2, Mdh_3, Mdh_4, Pgd_1, Pgd_2, Pgi_1 Pgm_1, Pgm_2* and *Sdh*) with all the 396 individuals possessing bands with identical mobility at each locus. The other 9 loci were polymorphic (*Amp_2, Amp_{3a}, Amp_4, Enp, Fdh, Fle_3, Pgi_2, Pgi_3,* and *Pgm_2*) each at least in one population. Allele frequencies for the polymorphic loci are presented in tables 4.11, 4.12, 4.13 and 4.14. The locus Amp_2 and Fle_3 were the most polymorphic with two given alleles each (Amp_2^{100}, Amp_2^{102}) and (Fle_3^{096}, Fle_3^{100}) respectively with the two electrophoretic variants present in 17 populations out of the 35 studied followed *Fdh* with also two given alleles (Fdh^{100} and Fdh^{102}) and polymorphic in 14 populations. Although Pgm_1 locus exhibited three given alleles, two of them were only present in 3 populations in Niger. Considering all the 35 populations, were highly polymorphic the loci, *Fdh, Fle_3, Fdh, Amp_2, Pgi_3 and Amp_4* whose allele frequency of the most common allele were less than 90%. The remaining polymorphic loci were weakly polymorphic with the frequency of the most common allele superior to 95%. Allele frequencies of 8 of the 9 polymorphic loci measured by χ^2 test differed significantly ($P<0.001$) among the 35 populations (Table 4.16). Some isozyme alleles were found in only one population. For example Enp^{102} was

detected in Burkina Faso (BUR05) at the frequency of 8.33%, also Pgm_1^{094} in Niger (NIG12) at 7.14% frequency.

Table 4.11: Allele frequencies estimated at 9 polymorphic loci in Burkina Faso

Population Code	**BUR03** 403	**BUR05** 405	**BUR06** 406	**BUR07** 407	**BUR16** 416	**BUR23** 423	**BUR27** 427	**BUR29** 429	**BUR32** 432	**Burkina Faso**
Allele										
Amp_2^{100}	1.000	0.833	0.900	0.200	1.000	1.000	1.000	0.546	0.813	**0.810**
Amp_2^{102}	0.000	0.167	0.100	0.800	0.000	0.000	0.000	0.455	0.188	**0.190**
Amp_{3a}^{100}	1.000	0.917	1.000	1.000	1.000	1.000	1.000	0.909	1.000	**0.977**
Amp_{3a}^{102}	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.091	0.000	**0.023**
Amp_4^{096}	0.143	0.833	1.000	0.200	0.700	0.000	0.417	0.000	0.125	**0.336**
Amp_4^{100}	0.857	0.167	0.000	0.800	0.300	1.000	0.583	1.000	0.875	**0.664**
Enp^{096}	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.046	0.000	**0.023**
Enp^{100}	1.000	0.917	1.000	1.000	1.000	1.000	0.917	0.955	1.000	**0.969**
Enp^{102}	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.008**
Fdh^{100}	0.000	0.917	1.000	0.200	1.000	0.200	0.583	0.818	0.625	**0.602**
Fdh^{102}	1.000	0.083	0.000	0.800	0.000	0.800	0.417	0.182	0.375	**0.398**
Fle_3^{096}	0.714	0.167	0.000	0.800	0.500	0.300	0.000	0.364	0.625	**0.359**
Fle_3^{100}	0.286	0.833	1.000	0.200	0.500	0.700	1.000	0.636	0.375	**0.641**
Pgi_2^{100}	0.857	1.000	1.000	1.000	1.000	1.000	1.000	0.909	1.000	**0.969**
Pgi_2^{130}	0.143	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.000	**0.031**
Pgi_3^{096}	0.857	0.000	0.000	0.000	0.100	0.800	0.542	0.409	0.375	**0.383**
Pgi_3^{100}	0.143	1.000	1.000	1.000	0.900	0.200	0.458	0.591	0.625	**0.617**
Pgm_1^{094}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**
Pgm_1^{100}	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**1.000**
Pgm_1^{104}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**

Table 4.12: Allele frequencies estimated at 9 polymorphic loci in Benin

Population Code	**BEN02 302**	**BEN03 303**	**BEN04 304**	**BEN05 305**	**BEN06 306**	**Benin**
Amp_2^{100}	0.800	1.000	0.115	0.816	1.000	**0.754**
Amp_2^{102}	0.200	0.000	0.885	0.184	0.000	**0.246**
Amp_{3a}^{100}	1.000	1.000	1.000	1.000	1.000	**1.000**
Amp_{3a}^{102}	0.000	0.000	0.000	0.000	0.000	**0.000**
Amp_4^{096}	0.000	0.000	0.000	0.000	0.000	**0.000**
Amp_4^{100}	1.000	1.000	1.000	1.000	1.000	**1.000**
Enp^{096}	0.000	0.000	0.000	0.000	0.000	**0.000**
Enp^{100}	1.000	1.000	1.000	1.000	1.000	**1.000**
Enp^{102}	0.000	0.000	0.000	0.000	0.000	**0.000**
Fdh^{100}	1.000	1.000	0.000	1.000	1.000	**0.800**
Fdh^{102}	0.000	0.000	1.000	0.000	0.000	**0.200**
Fle_3^{096}	0.000	1.000	0.000	0.000	1.000	**0.431**
Fle_3^{098}	0.000	0.000	0.000	0.000	0.000	**0.000**
Fle_3^{100}	1.000	0.000	1.000	1.000	0.000	**0.569**
Pgi_2^{100}	1.000	1.000	1.000	0.895	1.000	**0.969**
Pgi_2^{130}	0.000	0.000	0.000	0.105	0.000	**0.031**
Pgi_3^{096}	0.000	0.000	1.000	0.000	0.000	**0.200**
Pgi_3^{100}	1.000	1.000	0.000	1.000	1.000	**0.800**
Pgm_1^{094}	0.000	0.000	0.000	0.000	0.000	**0.000**
Pgm_1^{100}	1.000	1.000	1.000	1.000	1.000	**1.000**
Pgm_1^{104}	0.000	0.000	0.000	0.000	0.000	**0.000**

Table 4.13: Allele frequencies estimated at 9 polymorphic loci in Ghana

Population	**GHA08**	**GHA18**	**GHA20**	**GHA21**	**GHA24**	**GHA25**	**GHA26**	**GHA29**	**Ghana**
Code	508	518	520	521	524	525	526	529	
Alleles									
Amp_2^{100}	0.071	1.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.144**
Amp_2^{102}	0.929	0.000	1.000	1.000	1.000	1.000	1.000	1.000	**0.856**
Amp_{3a}^{100}	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**1.000**
Amp_{3a}^{102}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**
Amp_4^{096}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**
Amp_4^{100}	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**1.000**
Enp^{096}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**
Enp^{100}	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**1.000**
Enp^{102}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**
Fdh^{100}	0.000	0.000	0.000	0.500	0.800	0.000	0.000	0.000	**0.125**
Fdh^{102}	1.000	1.000	1.000	0.500	0.200	1.000	1.000	1.000	**0.875**
Fle_3^{096}	1.000	0.643	1.000	1.000	1.000	0.300	1.000	0.900	**0.875**
Fle_3^{098}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**
Fle_3^{100}	0.000	0.357	0.000	0.000	0.000	0.700	0.000	0.100	**0.125**
Pgi_2^{100}	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**1.000**
Pgi_2^{130}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**
Pgi_3^{096}	1.000	1.000	1.000	0.400	0.300	1.000	1.000	1.000	**0.875**
Pgi_3^{100}	0.000	0.000	0.000	0.600	0.700	0.000	0.000	0.000	**0.125**
Pgm_1^{094}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**
Pgm_1^{100}	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**1.000**
Pgm_1^{104}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**

Table 4.14: Allele frequencies estimated at 9 polymorphic loci in Niger

Population	NIG01	NIG02	NIG03	NIG04	NIG05	NIG06	NIG08	NIG09	NIG10	NIG11	NIG12	NIG13	NIG14	Niger	West Africa
Alleles															
Amp_2^{100}	1.000	1.000	1.000	0.027	0.933	1.000	0.200	0.792	1.000	0.471	0.732	0.765	0.462	**0.657**	**0.630**
Amp_2^{102}	0.000	0.000	0.000	0.973	0.067	0.000	0.800	0.208	0.000	0.529	0.268	0.235	0.539	**0.344**	**0.370**
Amp_{3a}^{100}	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**1.000**	**0.996**
Amp_{3a}^{102}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**	**0.004**
Amp_4^{096}	0.000	0.000	0.000	0.000	0.233	0.000	1.000	0.833	0.600	0.059	0.250	0.059	0.231	**0.245**	**0.187**
Amp_4^{100}	1.000	1.000	1.000	1.000	0.767	1.000	0.000	0.167	0.400	0.941	0.750	0.941	0.769	**0.755**	**0.813**
Enp^{096}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**	**0.004**
Enp^{100}	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**1.000**	**0.995**
Enp^{102}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**	**0.001**
Fdh^{100}	1.000	0.000	0.000	0.081	0.133	0.000	1.000	0.913	0.650	1.000	0.964	0.941	1.000	**0.574**	**0.556**
Fdh^{102}	0.000	1.000	1.000	0.919	0.867	1.000	0.000	0.087	0.350	0.000	0.036	0.059	0.000	**0.427**	**0.444**
Fle_3^{096}	1.000	1.000	0.000	1.000	0.433	0.000	0.000	0.125	0.368	0.438	0.518	0.882	0.885	**0.559**	**0.547**
Fle_3^{100}	0.000	0.000	1.000	0.000	0.567	1.000	1.000	0.875	0.632	0.563	0.482	0.118	0.115	**0.441**	**0.453**
Pgi_2^{100}	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.821	1.000	1.000	**0.949**	**0.962**
Pgi_2^{130}	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.179	0.000	0.000	**0.051**	**0.038**
Pgi_3^{096}	0.000	1.000	1.000	0.000	0.900	1.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	**0.228**	**0.333**
Pgi_3^{100}	1.000	0.000	0.000	1.000	0.100	0.000	1.000	1.000	1.000	1.000	0.875	1.000	1.000	**0.772**	**0.667**
Pgm_1^{094}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.000	**0.009**	**0.005**
Pgm_1^{100}	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.929	0.765	0.923	**0.967**	**0.982**
Pgm_1^{104}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.235	0.077	**0.023**	**0.013**

4.2.2 Genetic diversity analysis

4.2.2.1 Genetic diversity at the population level

For each population, standard parameters used to describe the genetic variation were estimated. These are percentage of polymorphic loci *(P)*; mean number of allele per locus *(A)*; mean number of allele per polymorphic locus *(AP)*; the observed *(Ho)* and the expected heterozygosity *(He)*. These parameters were all estimated from the 21 putative loci scored and are presented in table 4.6. All five parameters indicated that the genetic diversity at the population level in West Africa (P = 11.29%, A = 1.1129, AP = 2.0000, Ho = 0.0094 and He = 0.0345). Populations from Burkina Faso (P = 19.59 %, A = 1.1959, AP = 2.0000, Ho = 0.0144, He = 0.0622) and Niger (P = 12.45%, A = 1.1245, AP = 2.0000, Ho = 0.0084, He = 0.0356) were having more polymorphism and gene diversity compared to populations from Ghana (P = 4.78%, A = 1.0476, AP = 2.0000, Ho = 0.0082 and He = 0.0173) and Benin (P = 3.81%, A = 1.0381, AP = 2.0000, Ho = 0.0050, He = 0.0096).

4.2.2.2 Genetic diversity at the species level

The five genetic diversity parameters at the species level were (P = 0.4286; A = 1.5238, AP = 2.2222, Ho = 0.0086 and He = 0.1109) and in general much higher than those observed at the population level except in observed heterozygosity (Ho), which was almost similar at the species and

population level. The proportion of polymorphic loci for example at the species level (P = 42.86%) was approximately four times higher than that of mean population level (P = 11.18%).

Table 4.15: Estimates of genetic variation for 35 populations of *V. unguiculata* in West Africa

Population	Code	P	AP	A (SE)	Ho (SE)	He (SE)
Benin						
BEN02	302	0.0476	2.0000	1.0476 (0.0690)	0.0190 (0.0276)	0.0152 (0.0221)
BEN03	303	0.0000	-	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
BEN04	304	0.0476	2.0000	1.0476 (0.0428)	0.0037 (0.0033)	0.0097 (0.0087)
BEN05	305	0.0952	2.0000	1.0952 (0.0488)	0.0025 (0.0019)	0.0233 (0.0123)
BEN06	306	0.0000	-	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Average	Pop. level	**0.0381**	**2.0000**	**1.0381 (0.0321)**	**0.0050 (0.0066)**	**0.0096 (0.0086)**
	Specie level	**0.2381**	**2.0000**	**1.2381 (0.0383)**	**0.0029 (0.0012)**	**0.0743 (0.0135)**
Burkina Faso						
BUR03	403	0.1905	2.0000	1.1905 (0.1075)	0.0000 (0.0000)	0.0544 (0.0319)
BUR05	405	0.2857	2.0000	1.2857 (0.1336)	0.0397 (0.0259)	0.0615 (0.0304)
BUR06	406	0.0476	2.0000	1.0476 (0.0690)	0.0095 (0.0138)	0.0086 (0.0124)
BUR07	407	0.1905	2.0000	1.1905 (0.1273)	0.0000 (0.0000)	0.0610 (0.0407
BUR16	416	0.1429	2.0000	1.1429 (0.1134)	0.0286 (0.0227)	0.0524 (0.0448)
BUR23	423	0.1429	2.0000	1.1429 (0.1134)	0.0095 (0.0138)	0.0505 (0.0405)
BUR27	427	0.1905	2.0000	1.1905 (0.0821)	0.0278 (0.0146)	0.0772 (0.0359)
BUR29	429	0.3333	2.0000	1.3333 (0.1030)	0.0087 (0.0058)	0.1027 (0.0376)
BUR32	432	0.2381	2.0000	1.2381 (0.1091)	0.0060 (0.0068)	0.0919 (0.0441)
Average	Pop. level	**0.1959**	**2.0000**	**1.1959 (0.1065)**	**0.0144 (0.0115)**	**0.0622 (0.0354)**
	Specie level	**0.3810**	**2.1250**	**1.4286 (0.0528)**	**0.0149 (0.0022)**	**0.1110 (0.0116)**
Ghana						
GHA08	508	0.0476	2.0000	1.0476 (0.0583)	0.0068 (0.0083)	0.0063 (0.0077)
GHA18	518	0.0476	2.0000	1.0476 (0.0583)	0.0204 (0.0250)	0.0219 (0.0268)
GHA20	520	0.0000	-	1.0000 (0,0000)	0.0000 (0.0000)	0.0000 (0.0000)
GHA21	521	0.0952	2.0000	1.0952 (0.0951)	0.0095 (0.0138)	0.0467 (0.0466)
GHA24	524	0.0952	2.0000	1.0952 (0.0951)	0.0095 (0.0138)	0.0352 (0.0355)
GHA25	525	0.0476	2.0000	1.0476 (0.0690)	0.0095 (0.0138)	0.0200 (0.0290)
GHA26	526	0.0000	-	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
GHA29	529	0.0476	2.0000	1.0476 (0.0690)	0.0095 (0.0138)	0.0086 (0.0124)
Average	Pop. level	**0.0476**	**2.0000**	**1.0476 (0.0556)**	**0.0082 (0.0111)**	**0.0173 (0.0197)**
	Specie level	**0.1905**	**2.0000**	**1.1905 (0.0394)**	**0.0073 (0.0021)**	**0.0430 (0.0089)**
Niger						

NIG01	601	0.0000	-	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
NIG02	602	0.0000	-	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
NIG03	603	0.0000	-	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
NIG04	604	0.0952	2.0000	1.0952 (0.0350)	0.0000 (0.0000)	0.0096 (0.0039)
NIG05	605	0.2381	2.0000	1.2381 (0.0797)	0.0349 (0.0137)	0.0659 (0.0251)
NIG06	606	0.0000	-	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
NIG08	608	0.0476	2.0000	1.0476 (0.0690)	0.0190 (0.0276)	0.0152 (0.0221)
NIG09	609	0.1905	2.0000	1.1905 (0.0581)	0.0000 (0.0000)	0.0469 (0.0149)
NIG10	610	0.1429	2.0000	1.1429 (0.0567)	0.0048 (0.0034)	0.0667 (0.0265)
NIG11	611	0.1429	2.0000	1.1429 (0.0606)	0.0000 (0.0000)	0.0524 (0.0252)
NIG12	612	0.3333	2.0000	1.3333 (0.0645)	0.0289 (0.0084)	0.0943 (0.0214)
NIG13	613	0.2381	2.0000	1.2381 (0.0748)	0.0112 (0.0061)	0.0547 (0.0197)
NIG14	614	0.1905	2.0000	1.1905 (0.0789	0.0110 (0.0072)	0.0571 (0.0266)
Average	Pop. level	**0.1245**	**2.0000**	**1.1245 (0.0444)**	**0.0084 (0.0051)**	**0.0356 (0.0143)**
	Specie level	**0.3333**	**2.1428**	**1.3810 (0.0284)**	**0.0087 (0.0007)**	**0.1103 (0.0091)**
West Africa	**Pop. level**	**0.1129**	**2.0000**	**1.1129 (0.0612)**	**0.0094 (0.0083)**	**0.0345 (0.0201)**
	Specie level	**0.4286**	**2.2222**	**1.5238 (0.0242)**	**0.0086 (0.0005)**	**0.1109 (0.0068)**

4.2.3 Genetic structure of populations

The parameter F_{IS} is a measure of the deviation from Hardy – Weinberg expectations or random mating within populations. The average F_{IS} within the 35 cowpea's populations was 0.788 ranging from –0.034 (Locus Enp) to 0.880 (Loci Pgi_2) (Table 4.20). The observed positive and significant ($P<0.05$) F_{IS} value (0.788) indicates that there was a significant deficit of heterozygous and subsequent inbreeding in the populations studied. This high F_{IS} indicates a low level of outcrossing. The analysis of fixation indices (F_{IS}), calculated for all polymorphic loci in each population (table 4.) showed up to 87% of values were positives (66 out of 76), of which 58 (76.3%) deviated significantly from zero ($P<0.05$). Seventeen values of

fixation indices (22.4%) were negatives and none of them departed significantly from zero.

Using Nei's genetic diversity statistics, the estimates of population genetic structure are shown in Table 4.16. The average total heterozygosity (H_T) and intrapopulation genetic diversity (H_S) were 0.114 and 0.039 respectively. Total and within population gene diversity showed the highest values for the loci Fdh (H_T = 0.499, H_S = 0.130); Fle_3 (H_T = 0.498, H_S = 0.213); Pgi_3 (H_T = 0.489, H_S = 0.112); Amp_2 (H_T = 0.475, H_S = 0.163) and Amp_4 (H_T = 0.309, H_S = 0.130). The interpopulaion genetic diversity (D_{ST}) and coefficient of genetic heterogeneity among population (G_{ST}) varied from 0.000 (Locus Amp3a and Enp) to 0.377 (locus Pgi3) and from – 0.010 (Locus Amp3a) to 0.771 (locus Pgi3) respectively with a mean for all 21 loci of 0.075 and 0.658 respectively. This result indicates that in wild *V. unguiculata*, 65.8% of the total allozyme diversity detected is between populations while 34.2% represents intrapopulation genetic diversity. Population genetic structure of five population groups is recorded in Table 4.21. Burkina Faso and Niger population groups showed a relatively high genetic diversity, H_T = 0.112 and 0.115 respectively compared to Benin (H_T = 0.073) and Ghana (H_T =0.050). Of these four population groups, Burkina Faso showed the greater part of their genetic diversity distributed within populations, G_{ST} = 0.351 compared to Benin (G_{ST} = 0.858); Niger (G_{ST} = 0.666) and Ghana (G_{ST} = 0.579) of which the most part of their allozyme variation was among populations.

Table 4.16: Nei's statistics of genetic diversity and χ^2 values for 9 polymorphic loci in *Vigna Unguiculata*

Loci	χ2	Df	P	Ht	Hs	Dst	Gst
Amp2	322.548	1	< 0.001	0.475	0.163	0.312	0.658
Amp3a	262.999	1	< 0.001	0.010	0.010	0.000	-0.010
Amp4	322.065	1	< 0.001	0.309	0.130	0.179	0.579
Enp	0.008	3	0.999	0.012	0.012	0.000	0.012
Fdh	361.246	1	< 0.001	0.499	0.130	0.369	0.739
Fle3	335.761	1	< 0.001	0.498	0.213	0.285	0.572
Pgi2	354.486	1	< 0.001	0.083	0.029	0.054	0.652
Pgi3	353.447	1	< 0.001	0.489	0.112	0.377	0.771
Pgm1	806.960	3	< 0.001	0.022	0.020	0.001	0.064
All 21 loci				**0.114**	**0.039**	**0.075**	**0.658**

Df, Degree of freedom; P, Probability; H_T, overall gene diversity; H_S, within population gene diversity, D_{ST}, Amount of gene diversity between population; G_{ST}, Proportion of allelic diversity among populations

Table 4.17: Wright's fixation indices and Chi-square test for populations of *V. unguiculata* in Burkina Faso and Benin

Population	BUR03	BUR05	BUR06	BUR07	BUR16	BUR23	BUR27	BUR29	BUR32	BEN02	BEN03	BEN04	BEN05	BEN06
Loci														
Amp_2	-	-0.2	-0.111	1.000^{**}	-	-	-	1.000^{***}	0.590^{*}	-0.25	-	0.623^{**}	0.825^{***}	-
Amp_{3a}	-	-0.091	-	-	-	-	-	1.000^{***}	-	-	-	-	-	-
Amp_4	1.000^{***}	1.000^{***}	-	1.000^{**}	0.524	-	1.000^{***}	-	1.000^{***}	-	-	-	-	-
Enp	-	-0.091	-	-	-	-	-0.091	-0.048	-	-	-	-	-	-
Fdh	-	-0.091	-	1.000^{**}	-	1.000^{**}	0.657^{*}	1.000^{***}	1.000^{**}	-	-	-	-	-
Fle_3	1.000^{***}	1.000^{***}	-	1.000^{**}	0.6	0.524	-	1.000^{***}	1.000^{**}	-	-	-	-	-
Idh_1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pgi_2	1.000^{***}	-	-	-	-	-	-	1.000^{***}	-	-	-	-	1.000^{***}	-
Pgi_3	1.000^{***}	-	-	-	-0.111	1.000^{**}	0.497	0.812^{**}	1.000^{**}	-	-	-	-	-
Pgm_1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pgm_2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	1	0.255	-0.111	1	0.338	0.841	0.516	0.823	0.918	-0.25	-	0.623	0.913	-

*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.
- indicates monomorphic locus

Table 4.18: Wright's fixation indices and Chi-square test for populations of *V. unguiculata* in Niger

Population	NIG01	NIG02	NIG03	NIG04	NIG05	NIG06	NIG08	NIG09	NIG10	NIG11	NIG12	NIG13	NIG14
Loci													
Amp_2	-	-	-	1.000^{***}	-0.071	-	-0.25	1.000^{***}	-	1.000^{***}	0.545^{**}	1.000^{***}	1.000^{***}
Amp_{3a}	-	-	-	-	-	-	-	-	-	-	-	-	-
Amp_4	-	-	-	-	0.814^{***}	-	-	1.000^{***}	0.792^{***}	1.000^{***}	0.429^{*}	-0.063	1.000^{***}
Enp	-	-	-	-	-	-	-	-	-	-	-	-	-
Fdh	-	-	-	1.000^{***}	-0.154	-	-	1.000^{***}	1.000^{***}	-	1.000^{***}	1.000^{***}	-
Fle_3	-	-	-	-	0.864^{***}	-	-	1.000^{***}	1.000^{***}	1.000^{***}	0.785^{***}	0.433^{*}	0.623^{**}
Idh_1	-	-	-	-	-	-	-	-	-	-	-	-	-
Pgi_2	-	-	-	-	-	-	-	-	-	-	0.757^{***}	-	-
Pgi_3	-	-	-	-	-0.111	-	-	-	-	-	0.837^{***}	-	-
Pgm_1	-	-	-	-	-	-	-	-	-	-	1.000^{***}	1.000^{***}	-0.083
Pgm_2	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	-	-	-	1	0.268	-	-0.25	1	0.931	1	0.765	0.674	0.635

*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.
- indicates monomorphic locus

Table 4.19: Wright's fixation indices and Chi-square test for populations of *V. unguiculata* in Ghana

Population	**GHA08**	GHA18	GHA20	GHA21	GHA24	GHA25	GHA26	GHA29
Loci								
Amp_2	-0.077		-	-	-	-	-	-
Amp_{3a}	-		-	-	-	-	-	-
Amp_4	-		-	-	-	-	-	-
Enp	-		-	-	-	-	-	-
Fdh	-		-	0.6	1.000**	-	-	-
Fle_3	-	0.067	-	-	-	0.524	-	-0.111
Idh_1	-		-	-	-	-	-	-
Pgi_2	-		-	-	-	-	-	-
Pgi_3	-		-	1.000*	0.524	-	-	-
Pgm_1	-		-	-	-	-	-	-
Pgm_2	-		-	-	-	-	-	-
Mean	-0.077	0.067	-	0.8	0.762	0.524	-	-0.111

*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.
- indicates monomorphic locus

4.2.4 Population subdivision and Gene flow

Population subdivision is very High as shown by 7 of the 9 F_{ST} Values being significantly different from zero, reaching even 0.807 at the locus Pgi_3 (table 4.20). The mean F_{ST} value was 0.641 indicating a low level of gene flow. The magnitude of the genetic divergence within population groups of Benin, Burkina Faso, Ghana and Niger, measured by F_{ST} was also very high: 0.325 (Burkina Faso), 0.619 (Niger), 0.643 (Ghana) and 0.888 (Benin) and were all statistically different from Zero ($P<0.001$) (table 4.21). Estimates of the number of migrant pre generation or gene flow both based on Wright's (1951) equation or Slatkin (1985) formula were very low as predicted by the high F_{ST} values despite the proximity of many pair of population. The mean estimate of gene flow based on wright's equation and Slatkin method were $Nm^W = 0.211$ and $Nm^S = 0.868$ respectively (Table 4.21)

Table 4.20: Summary of F-statistic estimated for all 35 *vigna unguiculata* populations sampled in West Africa.

Loci	FIT ± SE	FIS ± SE	FST ± SE
Amp2	**0.905 ± 0.038**	**0.757 ± 0.084**	**0.608 ± 0.108**
Amp3a	**0.666 ± 0.731**	**0.661 ± 0.669**	0.015 ± 0.033
Amp4	**0.902 ± 0.056**	**0.800 ± 0.092**	**0.513 ± 0.112**
Enp	- 0.002 ± 0.003	- 0.034 ± 0.017	0.031 ± 0.016
Fdh	**0.960 ± 0.023**	**0.843 ± 0.092**	**0.746 ± 0.063**
Fle3	**0.925 ± 0.025**	**0.819 ± 0.055**	**0.585 ± 0.099**
Pgi2	**0.932 ± 0.081**	**0.880 ± 0.125**	**0.432 ± 0.418**
Pgi3	**0.945 ± 0.024**	**0.716 ± 0.102**	**0.807 ± 0.065**
Pgm1	**0.856 ± 0.242**	**0.843 ± 0.243**	**0.084 ± 0.099**
Over loci	**0.924 ± 0.013**	**0.788 ± 0.021**	**0.641 ± 0.055**
95% CI	0.892 - 0.943	0.735 - 0.823	0.535 - 0.823

F_{IT}, inbreeding coefficient in the total population; F_{IS}, inbreeding coefficient within populations; F_{ST}, genetic differentiation among populations. Standard errors were estimated by jack-knifing and confidence intervals by bootstrapping. Estimates significantly different from zero ($P < 0.05$) are indicated in bold.

Table 4.21: Separate genetic diversity and F-statistic estimates for population groups of *V. unguiculata* in West Africa

Population group	N	Genetic diversity estimates				F-statistic estimates			Gene flow	
		H_T	H_S	D_{ST}	G_{ST}	F_{IT}	F_{IS}	F_{ST}	Nm^W	Nm^S
Benin	65	0.073	0.010	0.063	0.858	0.969	0.724	0.888	0.032	0.026
Burkina Faso	64	0.112	0.072	0.039	0.351	0.873	0.811	0.325	0.519	1.094
Ghana	52	0.050	0.021	0.029	0.579	0.830	0.523	0.643	0.139	-
Niger	215	0.115	0.038	0.077	0.666	0.926	0.808	0.619	0.154	1.484
Mean	**99**	**0.088**	**0.035**	**0.052**	**0.614**	**0.900**	**0.717**	**0.619**	**0.211**	**0.868**

Table 4.22: Estimated values with range in parentheses of Genetic identity (GI) and Genetic distance (GD) within and between population groups

	GI	GD
Within population groups		
Benin	0.9140 (0.8003 - 1.0000)	0.0860 (0.0000 - 0.1997)
Burkina Faso	0.9400 (0.8401 - 0.9964)	0.0600 (0.0036 - 0.1599)
Ghana	0.9624 (0.8827 - 1.0000)	0.0376 (0.0000 - 0.1173)
Niger	0.9062 (0.7482 - 1.0000)	0.0938 (0.0000 - 0.2518)
Between population groups		
Ghana and Benin	0.8765 (0.8051 - 0.9950	0.1235 (0.0050 - 0.1949)
Ghana and Niger	0.8874 (0.7567 - 0.9939)	0.1126 (0.0061 - 0.2433)
Ghana and Burkina Faso	0.8900 (0.7386 - 0.9969)	0.1100 (0.0031 - 0.2614)
Benin and Niger	0.9169 (0.7401 - 0.9939)	0.0831 (0.0061 - 0.2599)
Benin and Burkina Faso	0.9290 (0.8091 - 0.9798)	0.0710 (0.0202 - 0.1909)
Niger and Burkina Faso	0.9278 (0.7881 - 0.9934)	0.0722 (0.0066 - 0.2119)
All	**0.9109 (0.7386 - 1.0000)**	**0.0891 (0.0000 - 0.2614)**

Table 4.23 Pairwise population differentiation quantified by F_{ST} values of 35 populations of V. unguiculata in West Africa

	BUR03	BUR05	BUR06	BUR27	BUR07	BUR16	BUR23	BUR29	BUR32	BEN02	BEN03	BEN04	BEN05	BEN06	NIG01	NIG02	NIG03	NIR05	NIG06	NIG08	GHA08	GHA18	GHA20	GHA21	GHA24	GHA25	GHA26	GHA29	NIG04	NIG09	NIG10	NIG11	NIG12	NIG13	NIG14
BUR03	0.000																																		
BUR05	0.615	0.000																																	
BUR06	0.765	-0.040	0.000																																
BUR27	0.359	0.225	0.336	0.000																															
BUR07	0.454	0.495	0.713	0.480	0.000																														
BUR16	0.568	-0.001	0.250	0.242	0.486	0.000																													
BUR23	0.027	0.551	0.750	0.151	0.471	0.526	0.000																												
BUR29	0.338	0.270	0.415	0.179	0.223	0.221	0.188	0.000																											
BUR32	0.200	0.271	0.453	0.161	0.174	0.145	0.108	-0.012	0.000																										
BEN02	0.698	0.392	0.782	0.280	0.607	0.450	0.607	0.088	0.231	0.000																									
BEN03	0.743	0.690	0.964	0.594	0.685	0.591	0.782	0.331	0.236	0.887	0.000																								
BEN04	0.687	0.837	0.944	0.605	0.746	0.869	0.646	0.514	0.626	0.905	0.965	0.000																							
BEN05	0.756	0.485	0.678	0.392	0.695	0.521	0.670	0.222	0.384	-0.095	0.733	0.862	0.000																						
BEN06	0.851	0.818	0.982	0.715	0.827	0.757	0.884	0.487	0.416	0.940	NA	0.978	0.805	0.000																					
NIG01	0.772	0.762	0.971	0.679	0.758	0.742	0.822	0.480	0.502	0.928	1.000	0.969	0.817	1.000	0.000																				
NIG02	0.144	0.867	0.985	0.646	0.787	0.869	0.582	0.567	0.492	0.966	1.000	0.937	0.901	1.000	1.000	0.000																			
NIG03	0.487	0.828	0.979	0.407	0.823	0.860	0.222	0.504	0.538	0.947	1.000	0.862	0.855	1.000	1.000	1.000	0.000																		
NIG05	0.007	0.545	0.655	0.229	0.473	0.509	-0.042	0.307	0.207	0.585	0.660	0.502	0.658	0.755	0.728	0.297	0.199	0.000																	
NIG06	0.506	0.837	0.981	0.422	0.834	0.869	0.243	0.519	0.555	0.951	1.000	0.867	0.860	1.000	1.000	1.000	NA	0.209	0.000																
NIG08	0.787	0.270	0.628	0.487	0.642	0.515	0.777	0.407	0.509	0.792	0.952	0.921	0.732	0.976	0.958	0.978	0.970	0.696	0.972	0.000															
GHA08	0.516	0.815	0.948	0.657	0.550	0.825	0.642	0.471	0.492	0.915	0.956	0.777	0.881	0.976	0.961	0.888	0.937	0.472	0.940	0.925	0.000														
GHA18	-0.057	0.742	0.900	0.441	0.632	0.730	0.095	0.407	0.319	0.846	0.901	0.788	0.824	0.945	0.920	0.377	0.664	0.034	0.678	0.901	0.699	0.000													
GHA20	0.557	0.825	0.980	0.670	0.556	0.842	0.691	0.471	0.497	0.954	1.000	0.860	0.896	1.000	1.000	1.000	1.000	0.508	1.000	0.958	-0.027	0.787	0.000												
GHA21	0.516	0.624	0.816	0.559	0.067	0.599	0.547	0.210	0.230	0.713	0.754	0.771	0.758	0.868	0.813	0.819	0.873	0.516	0.881	0.750	0.449	0.677	0.455	0.000											
GHA24	0.618	0.626	0.838	0.582	0.209	0.599	0.633	0.193	0.253	0.728	0.767	0.843	0.748	0.875	0.837	0.890	0.917	0.591	0.923	0.769	0.669	0.768	0.700	-0.094	0.000										
GHA25	0.516	0.751	0.917	0.541	0.527	0.781	0.506	0.372	0.455	0.863	0.950	0.196	0.840	0.975	0.952	0.910	0.873	0.406	0.881	0.879	0.491	0.681	0.643	0.528	0.682	0.000									
GHA26	0.711	0.899	0.989	0.762	0.734	0.913	0.823	0.606	0.652	0.975	1.000	0.902	0.922	1.000	1.000	1.000	1.000	0.611	1.000	0.978	0.049	0.868	NA	0.654	0.829	0.788	0.000								
GHA29	0.519	0.803	0.958	0.643	0.516	0.817	0.636	0.442	0.470	0.926	0.976	0.788	0.882	0.988	0.978	0.944	0.966	0.474	0.968	0.933	-0.067	0.732	0.000	0.417	0.656	0.470	0.110	0.000							
NIG04	0.829	0.881	0.945	0.823	0.197	0.886	0.866	0.668	0.688	0.915	0.911	0.905	0.888	0.929	0.938	0.923	0.947	0.780	0.948	0.927	0.819	0.891	0.841	0.495	0.675	0.861	0.865	0.827	0.000						
NIG09	0.684	-0.088	-0.052	0.310	0.587	0.082	0.625	0.389	0.400	0.395	0.647	0.798	0.464	0.726	0.742	0.821	0.775	0.606	0.780	0.239	0.805	0.750	0.813	0.684	0.671	0.764	0.852	0.801	0.838	0.000					
NIG10	0.488	0.040	0.183	0.195	0.403	-0.007	0.417	0.270	0.163	0.285	0.425	0.729	0.380	0.537	0.603	0.688	0.650	0.438	0.659	0.416	0.706	0.584	0.715	0.554	0.555	0.668	0.777	0.700	0.758	0.122	0.000				
NIG11	0.623	0.357	0.539	0.411	0.356	0.316	0.542	0.065	0.168	0.150	0.394	0.750	0.257	0.520	0.628	0.781	0.765	0.569	0.773	0.504	0.714	0.692	0.722	0.404	0.321	0.670	0.791	0.703	0.728	0.409	0.317	0.000			
NIG12	0.431	0.162	0.289	0.244	0.288	0.058	0.354	0.056	0.047	0.103	0.161	0.616	0.178	0.239	0.348	0.576	0.573	0.413	0.580	0.351	0.569	0.499	0.574	0.333	0.280	0.544	0.639	0.561	0.616	0.241	0.142	0.052	0.000		
NIG13	0.573	0.454	0.617	0.487	0.382	0.305	0.549	0.218	0.137	0.410	0.068	0.798	0.490	0.158	0.530	0.725	0.778	0.563	0.785	0.649	0.709	0.659	0.715	0.418	0.355	0.720	0.783	0.705	0.721	0.516	0.336	0.189	0.099	0.000	
NIG14	0.600	0.402	0.588	0.496	0.286	0.285	0.583	0.190	0.171	0.440	0.262	0.794	0.530	0.400	0.582	0.767	0.807	0.581	0.814	0.556	0.690	0.692	0.692	0.297	0.183	0.694	0.777	0.679	0.693	0.472	0.345	0.114	0.088	0.056	0.000

4.2.5 Cluster analysis and isolation by distance

The cluster analysis based on Nei's (1992) genetic distance D between any pair from the 35 populations generated a UPGMA dendogram that illustrates the genetic relationship among populations (Figure 4.35). This dendogram reveals a complex pattern that only partly reflects the spatial distribution of populations. Populations from Ghana tend to be grouped in the bottom part of the dendogram, but they are included in two other adjacent clusters. Four of the five populations of Benin belong to the same cluster while the fifth part is classified into another cluster. Most of the Niger populations are clustered in the upper and central part of the dendogram and Burkina Faso populations are scattered across the dendogram.

In the whole study, the relationship between geographical and genetic differentiation using Rousset's (1997) method generated a significant and positive correlation ($r = 0.142$, $p < 0.001$, Figure 4.36). Using mantel test (Manly 1985), it was found approximately the same positive and significant correlation between the genetic distance and the logarithm of the geographic distance ($r = 0.140$, $P < 0.001$, Figure 4.37)

Table 4.24 Genetic distance (above diagonal) and geographic distance (below) martice of 35 populations of V
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unguiculata in West Africa

	BEN02	BEN03	BEN04	BEN05	BEN06	BUR03	BUR05	BUR06	BUR07	BUR16	BUR23	BUR27	BUR29	BUR32	GHA08	GHA18	GHA20	GHA21	GHA24	GHA25	GHA26	GHA29	NIG01	NIG02	NIG03	NIR05	NIG06	NIG08	NIG04	NIG09	NIG10	NIG11	NIG12	NIG13	NIG14
BEN02	**0.000**	0.051	0.127	0.001	0.051	0.122	0.037	0.050	0.087	0.040	0.072	0.034	0.020	0.034	0.179	0.127	0.192	0.106	0.090	0.142	0.192	0.182	0.103	0.158	0.103	0.094	0.103	0.068	0.125	0.036	0.033	0.015	0.019	0.042	0.048
BEN03	190.6	**0.000**	0.200	0.052	0.000	0.095	0.073	0.101	0.069	0.037	0.090	0.084	0.041	0.023	0.148	0.108	0.154	0.071	0.056	0.184	0.154	0.155	0.049	0.100	0.154	0.100	0.154	0.135	0.090	0.076	0.044	0.029	0.020	0.006	0.018
BEN04	82.1	109.5	**0.000**	0.129	0.200	0.069	0.165	0.191	0.089	0.179	0.048	0.078	0.070	0.087	0.043	0.060	0.050	0.083	0.110	0.005	0.050	0.040	0.260	0.089	0.038	0.047	0.038	0.157	0.102	0.159	0.143	0.121	0.130	0.168	0.157
BEN05	156.6	214.5	148.1	**0.000**	0.052	0.121	0.038	0.051	0.089	0.040	0.072	0.035	0.021	0.035	0.182	0.128	0.195	0.109	0.093	0.145	0.195	0.185	0.093	0.109	0.104	0.095	0.104	0.070	0.128	0.034	0.033	0.016	0.018	0.043	0.050
BEN06	35.3	225.1	117.2	175.7	**0.000**	0.095	0.073	0.101	0.069	0.037	0.090	0.084	0.041	0.023	0.148	0.108	0.154	0.071	0.056	0.184	0.154	0.155	0.049	0.100	0.154	0.100	0.154	0.135	0.090	0.076	0.044	0.029	0.020	0.006	0.018
BUR03	429.7	379.4	388.6	280.0	453.8	**0.000**	0.130	0.160	0.076	0.103	0.013	0.055	0.066	0.036	0.049	0.003	0.057	0.083	0.109	0.063	0.057	0.055	0.134	0.007	0.028	0.007	0.028	0.198	0.093	0.132	0.079	0.113	0.085	0.094	0.111
BUR05	432.8	367.8	386.1	288.0	458.7	32.8	**0.000**	0.004	0.092	0.010	0.101	0.034	0.053	0.050	0.202	0.150	0.215	0.131	0.117	0.176	0.215	0.206	0.127	0.175	0.136	0.100	0.136	0.024	0.146	0.001	0.011	0.043	0.028	0.062	0.054
BUR06	433.2	362.4	384.4	290.6	459.7	45.7	13.0	**0.000**	0.126	0.018	0.124	0.041	0.076	0.074	0.247	0.181	0.261	0.171	0.154	0.209	0.261	0.251	0.155	0.212	0.155	0.122	0.155	0.024	0.190	0.003	0.021	0.064	0.046	0.089	0.080
BUR07	442.7	367.3	392.4	301.6	469.7	56.3	24.0	13.0	**0.000**	0.086	0.083	0.095	0.049	0.038	0.056	0.090	0.058	0.019	0.029	0.070	0.058	0.057	0.123	0.090	0.123	0.079	0.123	0.100	0.006	0.090	0.062	0.045	0.053	0.049	0.037
BUR16	676.7	590.8	625.8	532.1	703.1	253.7	244.5	243.6	234.2	**0.000**	0.087	0.037	0.045	0.031	0.180	0.122	0.190	0.110	0.095	0.180	0.190	0.186	0.089	0.133	0.133	0.085	0.133	0.050	0.133	0.011	0.008	0.036	0.016	0.035	0.035
BUR23	402.4	372.4	368.7	248.7	424.2	46.4	75.6	87.3	99.5	293.0	**0.000**	0.043	0.040	0.027	0.068	0.010	0.080	0.091	0.110	0.055	0.080	0.073	0.145	0.028	0.008	0.005	0.008	0.160	0.108	0.101	0.063	0.083	0.067	0.086	0.104
BUR27	430.4	362.2	382.4	286.9	456.7	40.3	8.1	5.9	18.6	246.5	81.4	**0.000**	0.032	0.029	0.130	0.059	0.145	0.119	0.122	0.095	0.145	0.135	0.140	0.089	0.036	0.029	0.036	0.075	0.142	0.033	0.025	0.056	0.039	0.077	0.084
BUR29	448.6	511.9	515.2	393.5	569.2	132.7	150.4	159.8	161.2	189.4	146.8	157.7	**0.000**	0.011	0.082	0.069	0.090	0.043	0.038	0.069	0.090	0.084	0.085	0.085	0.070	0.050	0.070	0.076	0.072	0.053	0.041	0.012	0.013	0.030	0.029
BUR32	540.0	507.3	508.2	384.5	560.1	129.2	149.4	159.5	161.9	202.4	139.4	157.0	13.1	**0.000**	0.074	0.042	0.082	0.043	0.044	0.081	0.082	0.079	0.075	0.049	0.062	0.030	0.062	0.095	0.062	0.051	0.024	0.023	0.013	0.019	0.025
GHA08	393.3	422.1	385.0	240.2	405.7	168.7	199.7	211.6	223.7	385.2	124.4	205.8	202.8	190.2	**0.000**	0.047	0.001	0.031	0.057	0.020	0.001	0.000	0.205	0.042	0.086	0.054	0.086	0.207	0.050	0.200	0.158	0.126	0.128	0.127	0.115
GHA18	341.5	346.9	320.9	185.0	359.3	126.2	150.3	159.7	172.7	373.4	81.4	154.1	212.9	202.7	80.2	**0.000**	0.056	0.090	0.117	0.056	0.056	0.053	0.163	0.006	0.020	0.006	0.020	0.219	0.106	0.151	0.097	0.122	0.099	0.107	0.127
GHA20	331.7	342.6	313.1	175.1	348.8	138.2	161.6	170.7	183.7	385.8	93.7	165.1	224.6	214.3	80.8	12.4	**0.000**	0.030	0.056	0.024	0.000	0.001	0.211	0.049	0.100	0.064	0.100	0.216	0.049	0.212	0.170	0.132	0.136	0.133	0.118
GHA21	332.6	338.0	311.6	176.0	350.6	129.9	152.5	161.4	174.4	379.0	86.2	155.9	220.7	210.8	87.6	9.3	10.0	**0.000**	0.005	0.056	0.030	0.031	0.125	0.082	0.146	0.086	0.136	0.128	0.016	0.129	0.103	0.049	0.061	0.052	0.036
GHA24	359.6	371.2	342.4	203.3	375.9	131.7	159.2	169.8	182.6	370.9	85.3	164.0	202.3	191.3	55.9	24.8	29.4	33.3	**0.000**	0.083	0.056	0.057	0.109	0.109	0.164	0.108	0.164	0.112	0.030	0.115	0.099	0.034	0.048	0.039	0.021
GHA25	370.0	386.5	355.4	214.3	385.2	140.1	169.1	180.2	192.8	372.8	93.9	174.4	199.4	187.8	39.6	41.0	43.9	49.1	16.4	**0.000**	0.024	0.018	0.243	0.074	0.054	0.049	0.054	0.165	0.075	0.171	0.149	0.117	0.127	0.153	0.139
GHA26	394.6	416.3	383.1	240.1	408.2	153.5	184.5	196.5	208.6	371.6	109.3	190.7	190.9	178.5	15.3	71.0	73.7	79.2	46.2	30.1	**0.000**	0.001	0.211	0.049	0.100	0.064	0.100	0.216	0.049	0.212	0.170	0.132	0.136	0.133	0.118
GHA29	405.1	437.7	398.9	253.3	416.5	180.4	212.0	224.2	236.0	389.4	137.2	218.3	204.3	191.5	16.4	96.5	97.1	103.9	72.2	55.9	28.8	**0.000**	0.213	0.050	0.091	0.059	0.091	0.205	0.050	0.203	0.164	0.127	0.132	0.133	0.118
NIG01	383.5	193.8	301.5	365.6	418.3	395.8	370.5	359.9	357.8	532.4	411.5	362.7	519.0	519.3	504.7	424.8	426.1	418.0	448.8	465.2	494.1	521.1	**0.000**	0.154	0.211	0.156	0.211	0.192	0.143	0.130	0.097	0.081	0.053	0.057	0.069
NIG02	434.9	258.0	354.2	378.9	470.1	333.4	304.0	291.9	286.9	436.7	358.6	295.9	445.5	447.7	467.2	390.3	394.5	385.3	412.4	428.0	454.6	483.0	103.0	**0.000**	0.049	0.020	0.049	0.252	0.098	0.177	0.113	0.136	0.110	0.104	0.123
NIG03	441.2	273.8	362.5	370.5	476.0	297.9	267.6	255.2	249.4	395.4	325.9	259.5	406.4	409.1	438.5	363.5	368.7	359.2	384.6	399.9	425.4	454.0	140.5	41.5	**0.000**	0.013	0.000	0.192	0.152	0.135	0.099	0.129	0.112	0.146	0.166
NIG05	468.0	308.1	391.3	385.6	502.5	279.8	248.1	235.3	227.8	356.9	312.2	240.1	379.8	383.5	429.8	358.1	364.6	354.8	377.6	392.2	416.0	444.8	181.8	80.7	41.4	**0.000**	0.013	0.154	0.104	0.100	0.064	0.096	0.074	0.094	0.107
NIG06	471.5	313.2	395.2	386.9	505.8	275.3	243.5	230.6	222.9	349.5	308.5	235.5	373.9	377.8	426.8	355.7	362.4	352.5	374.9	389.4	412.9	441.6	189.1	88.1	48.7	7.4	**0.000**	0.192	0.152	0.135	0.099	0.129	0.112	0.146	0.166
NIG08	455.2	294.0	378.1	375.5	489.7	280.3	249.1	236.4	229.6	367.3	311.1	241.0	384.0	387.3	427.1	354.1	360.1	350.4	374.2	389.0	413.4	442.2	169.0	69.4	28.6	14.3	20.8	**0.000**	0.149	0.020	0.054	0.058	0.060	0.106	0.074
NIG04	396.0	243.3	320.4	314.0	430.3	246.8	218.4	206.7	202.9	377.9	270.9	210.3	363.4	364.7	380.2	304.1	309.0	299.6	325.7	341.2	367.3	395.9	158.8	87.8	60.5	72.9	75.7	61.7	**0.000**	0.144	0.105	0.069	0.084	0.069	0.056
NIG09	329.4	144.2	247.3	303.2	364.5	349.8	327.2	317.7	317.4	510.6	360.9	319.8	477.4	476.5	447.8	367.6	368.2	360.5	292.1	408.4	437.8	464.2	62.5	116.6	140.8	180.3	186.7	166.0	133.4	**0.000**	0.011	0.041	0.028	0.064	0.055
NIG10	324.1	138.6	242.0	299.6	359.2	350.8	328.6	319.2	319.2	514.0	361.2	321.2	478.9	477.9	446.8	366.7	367.1	359.4	391.1	407.5	436.9	463.2	66.0	122.3	146.1	185.3	191.7	171.1	137.2	5.7	**0.000**	0.036	0.019	0.039	0.043
NIG11	307.7	123.1	225.7	284.4	342.9	346.2	325.3	316.3	316.9	516.9	354.6	318.0	475.7	474.1	437.0	356.9	356.9	349.5	381.5	397.9	427.5	453.4	81.5	136.0	157.1	195.3	201.4	181.0	142.5	21.6	16.4	**0.000**	0.008	0.017	0.012
NIG12	315.5	125.4	234.9	322.3	349.5	409.5	388.8	379.9	380.5	577.4	416.6	381.6	539.2	537.6	494.1	414.4	413.4	406.6	439.2	455.4	485.3	510.4	79.0	171.7	202.4	243.2	250.0	229.2	200.3	66.9	63.5	63.6	**0.000**	0.013	0.013
NIG13	278.9	94.2	200.7	304.8	311.9	425.5	407.7	399.8	401.8	608.3	427.7	400.9	557.3	554.6	495.0	416.5	414.2	408.2	441.3	457.3	487.4	511.1	125.7	216.1	244.2	284.2	290.7	270.0	234.9	104.0	99.2	92.4	46.7	**0.000**	0.008
NIG14	269.4	87.7	192.2	300.5	302.2	429.5	412.5	404.9	407.2	615.9	430.5	405.8	561.6	558.7	495.0	417.1	414.4	408.6	441.8	457.7	487.8	511.1	137.7	227.3	254.8	294.5	301.0	280.3	243.9	114.3	109.3	101.4	58.7	12.0	**0.000**

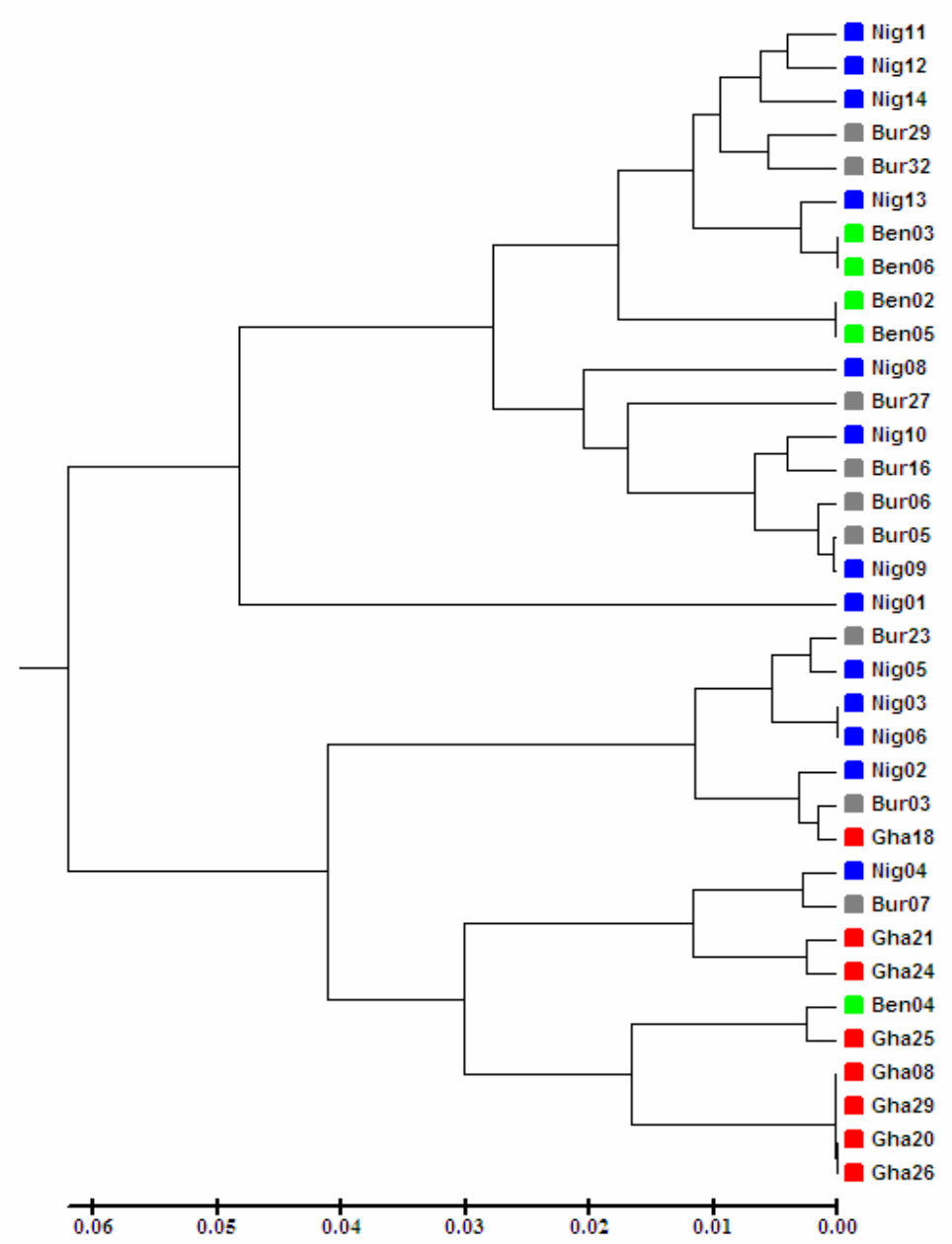

Figure 4.35 UPGMA phylogenetic trees based on Nei's (1972) genetic distance showing the genetic relationship between the 35 populations of *V. unfuiculata* studied

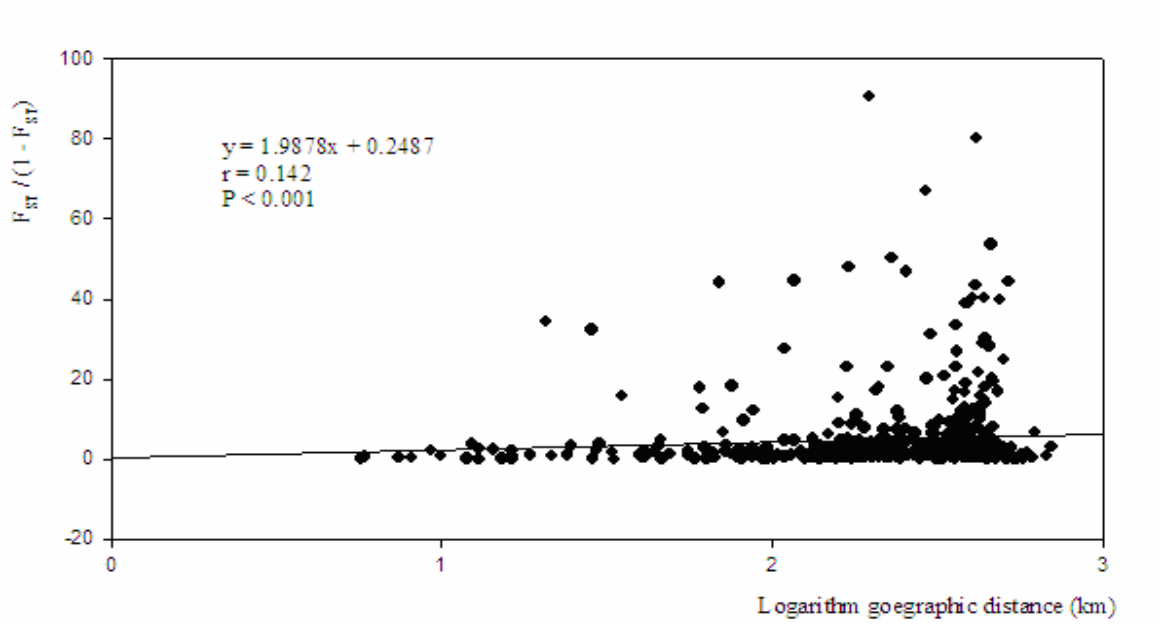

Figure 4.36: Relationship between F_{ST} / (1 – F_{ST}) and the logarithm of geographic distance (km) of 35 populations of *V. unguiculata* in West Africa

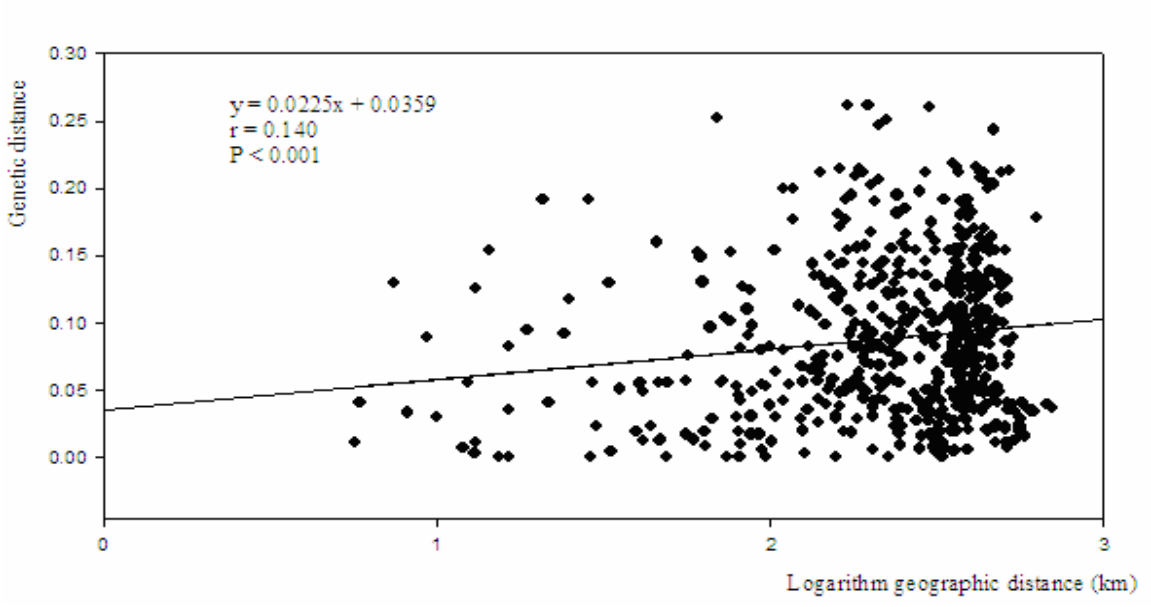

Figure 4.37: Relationship between genetic distance and the logarithm of geographic distance (km) of 35 populations of *V. unguiculata* in West Africa

4.2.6 Outcrossing rates

Fifteen populations showing sufficient variability were used to estimate the mating system of wild cowpea. The analysis of the mating system indicated that wild *V. unguiculata* is significantly ($P < 0.001$) highly inbred with up to 97% self-pollination (Table 4.25). Outcrossing rates estimates of tm ranged from 0.01 (BUR29, BUR32, NIG09 and NIG11) to 0.095 (BUR05). The multilocus and mean single-locus outcrossing estimates were tm = 0.034±0.007 and ts = 0.024±0.006, respectively (Table 4.25). Significant self-fertilization meaning tm and ts statistically lower than one was detected in the fifteen populations in which tm and ts were calculated simultaneously. Accordingly, the means of tm and ts were significantly lower than one ($P < 0.001$). All the fifteen selected populations did not show any deviation from zero for (tm-ts). The mean biparental inbreeding were 0.010±0.003 and was not statistically different fron zero ($P > 0.050$) indicating the absence of crossing between relatives. The inbreeding coefficients were significantly high for all the fifteen populations with a mean at 0.815±0.028.

Table 4.25: Outcrossing rates estimates for 15 populations of *V. unguiculata*

Population	tm±SE	ts±SE	(tm-ts)±SE	F
BUR03	0.014±0.007*	0.014±0.007*	0.000±0.000	0.649*
BUR05	0.095±0.035*	0.064±0.024*	0.031±0.017	0.458*
BUR07	0.061±0.007*	0.044±0.006*	0.017±0.004	0.990*
BUR16	0.013±0.002*	0.014±0.002*	0.000±0.000	0.736*
BUR23	0.028±0.014*	0.020±0.014*	0.008±0.003	0.753*
BUR27	0.050±0.008*	0.038±0.007*	0.012±0.003	0.689*
BUR29	0.010±0.000*	0.010±0.000*	0.000±0.000	0.906*
BUR32	0.010±0.000*	0.010±0.000*	0.000±0.000	0.879*
NIG05	0.063±0.019*	0.020±0.010*	0.044±0.014	0.471*
NIG09	0.010±0.000*	0.010±0.000*	0.000±0.000	0.892*
NIG10	0.016±0.002*	0.012±0.001*	0.004±0.001	0.948*
NIG11	0.010±0.000*	0.010±0.000*	0.000±0.000	0.889*
NIG12	0.037±0.003*	0.025±0.002*	0.012±0.001	0.990*
NIG13	0.034±0.004*	0.022±0.003*	0.012±0.002	0.990*
NIG14	0.058±0.004*	0.040±0.003*	0.017±0.002	0.990*
	0.034±0.007*	**0.024±0.006***	**0.010±0.003**	**0.815±0.028***

*P<0.050

4.3 Outcrossing rates dynamics in cowpea (V*igna unguiculata* (l.) walp.) and its relation to rainfall distribution, temperature and flower density

4.3.1 Outcrossing rates and inbreeding estimates

Multi and single locus outcrossing rate estimates (tm and ts respectively) differed from one month to another and between the two years (Figure 4.38, 4.39 and Table 4.26). Single locus estimates during the first year ranged from 0.024 to 0.783 with a mean of 0.235 (Table 4.26). The multi loci estimate was 0.268. For the second year, ts estimates ranged from 0.132 to 0.820 with a mean of 0.355. The tm estimates was 0.385 (Table 4.26). The month of March surprisingly indicated a very high rate of outcrossing in both years. Averaged across months over the two years analysis, the multi and single locus outcrossing rates were 0.327 ± 0.044 and 0.295 ± 0.042 respectively. These rates were statistically different between the two years. The proportion of outcross seeds was also estimated at each of the four loci. Enp, Fdh and Pgd2 displayed relatively the same level of outcrossing: 0.364 ± 0.068, 0.333 ± 0.055 and 0.316 ± 0.056 respectively while the rate of outcrossing at Fle3 locus was quite high (ts = 0.704 ± 0.130) (Table 4.26).

A negative inbreeding coefficient (F = - 0.059) was found. The negative value is an indication of excess of heterozygosity. To examine the possibility of biparental inbreeding, arising from the cross between related plants, the difference between

the multilocus (tm) and the average single locus (ts) estimates were calculated. The mean (tm – ts) is 0.032 ± 0.006 (Table 4.16) and was significantly different from zero suggesting the existence of crossing between relatives.

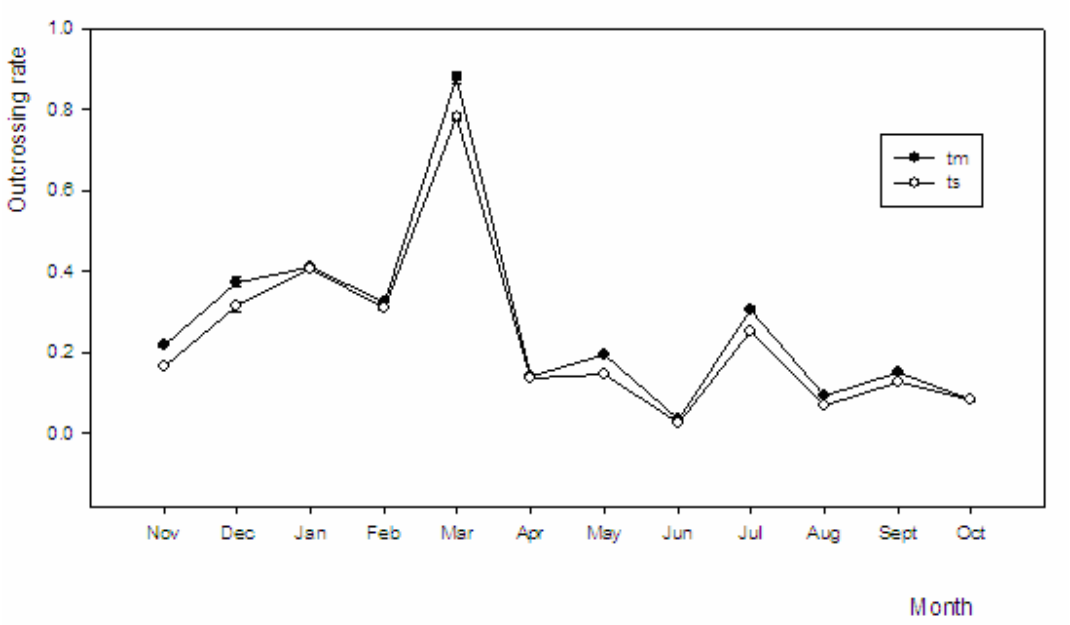

Figure 4.38: Evolution Outcrossing rate November 2005 – October 2006

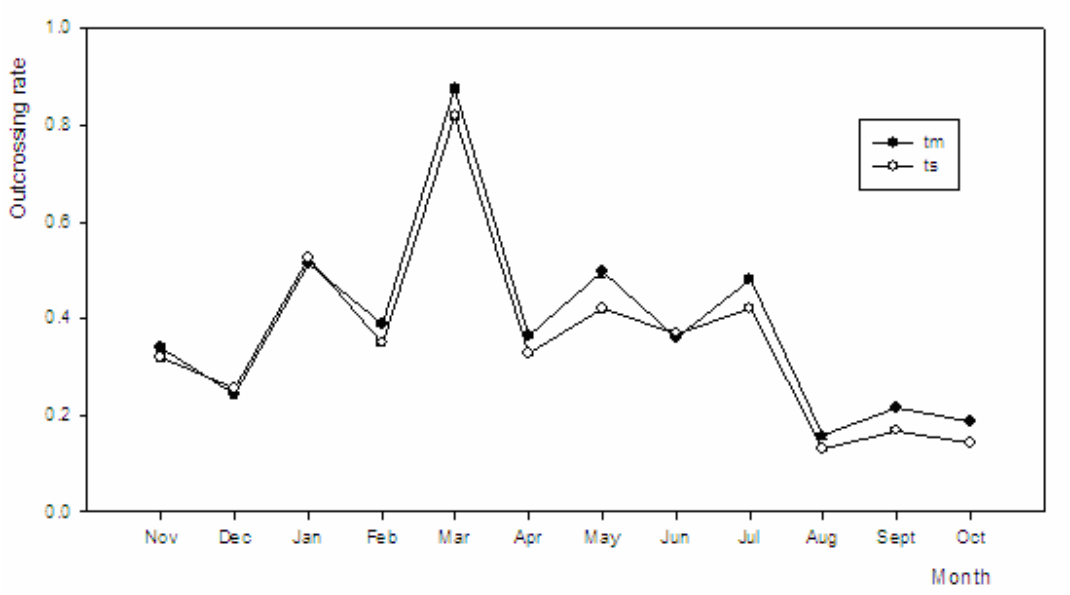

Figure 4.39: Evolution Outcrossing rate November 2006 – October 2007

Table 4.26: Indices of mating system parameters for *V. unguiculata*

	All	Milanani 06	Milanani 07	Level of confidence (P)
Outcrossing rates parameters				
Enp	0.364 (0.068)	0.454 (0.119)	0.274 (0.058)	0.1629
Fdh	0.333 (0.055)	0.336 (0.094)	0.329 (0.062)	0.9217
Fle3	0.704 (0.130)	0.489 (0.110)	0.918 (0.225)	0.0890
Pgd2	0.316 (0.056)	0.184 (0.049)	0.448 (0.087)	0.0174*
Average single locus (ts)	0.295 (0.042)	0.235 (0.059)	0.355 (0.055)	0.0036**
Multi loci (tm)	0.327 (0.044)	0.268 (0.065)	0.385 (0.056)	0.0081**
(tm-ts)	0.032 (0.006)***	0.033 (0.008)**	0.030 (0.009)**	0.8231
F	- 0.059 (0.068)	- 0.035 (0.103)	- 0.082 (0.093)	0.7501
Rainfall (mm)	145.565 (27.666)	139.417 (34.422)	152.274 (45.721)	0.4723
Temperature (^{o}C)	25.792 (0.308)	25.833 (0.438)	25.750 (0.444)	0.6742
Flower density / ha	1822 (435.125)	1291 (739.970)	2131 (540.784)	0.4786

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

4.3.2 Correlations between outcrossing rate and environmental agents

The mating system parameters and environmental factors indicated the same trend as shown in Table 4.27. Using Pearson correlation coefficients, the relationship between the monthly specific outcrossing rate and environmental factors were quantified and are illustrated in Figures 4.40, 4.41 and 4.42. It reveals the dependence of cross-pollination on temperature and rainfall distribution. Air temperature significantly correlates positively ($r = 0.51$, $P = 0.0116$) with the level of outcrossing, while rainfall inversely correlates with the same ($r = - 0.66$, $P < 0.001$). No significant correlation occurred between flower density and outcrossing

rate (r = 0.43, P = 0.0712). Obviously, a negative correlation between rainfall and air temperature was also observed (r = - 0.44, P = 0.0364).

Table 4.27: Correlations between 2005-2006 and 2006-2007 parameters

Parameters	Pearson correlation coefficient
tm (05-06) – tm (06-07)	0.81**
ts (05-06) – ts (06-07)	0.82**
(tm-ts) (05-06) - (tm-ts) (06-07)	0.31
Temperature (05-06) – Temperature (06-07)	0.91***
Rainfall (05-06) – Rainfall (06-07)	0.85***
Flower density (05-06) - Flower density (06-07)	0.82*

*: P < 0.05; **: P < 0.01; ***: P < 0.001

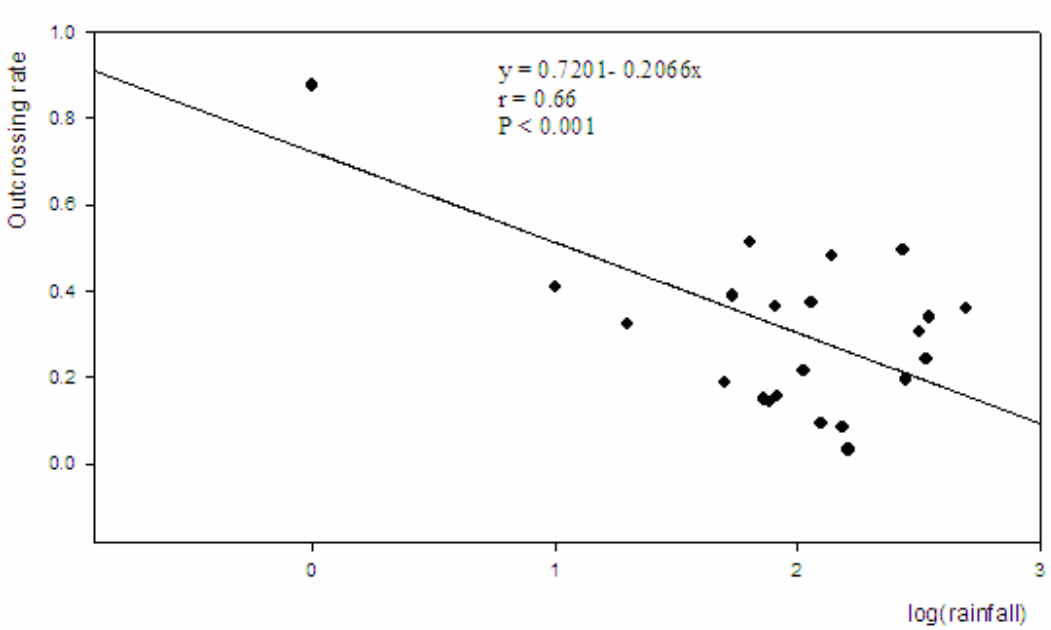

Figure 4.40: Relation between outcrossing rate and Rainfall

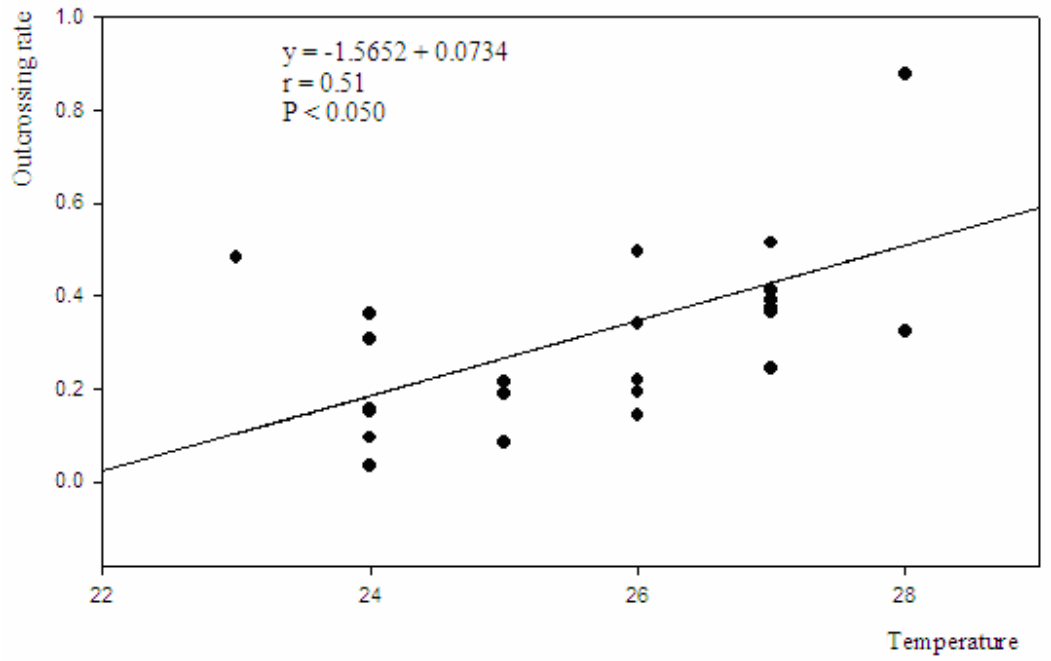

Figure 4.41: Relation between outcrossing rate and air temperature

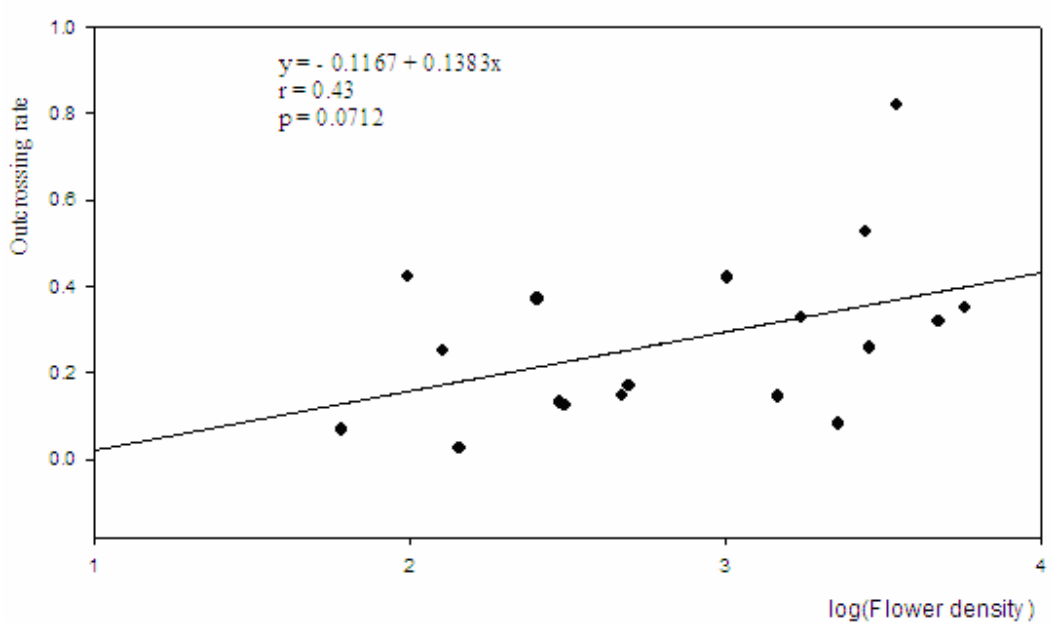

Figure 4.42: Relation between outcrossing rate and flower dendity

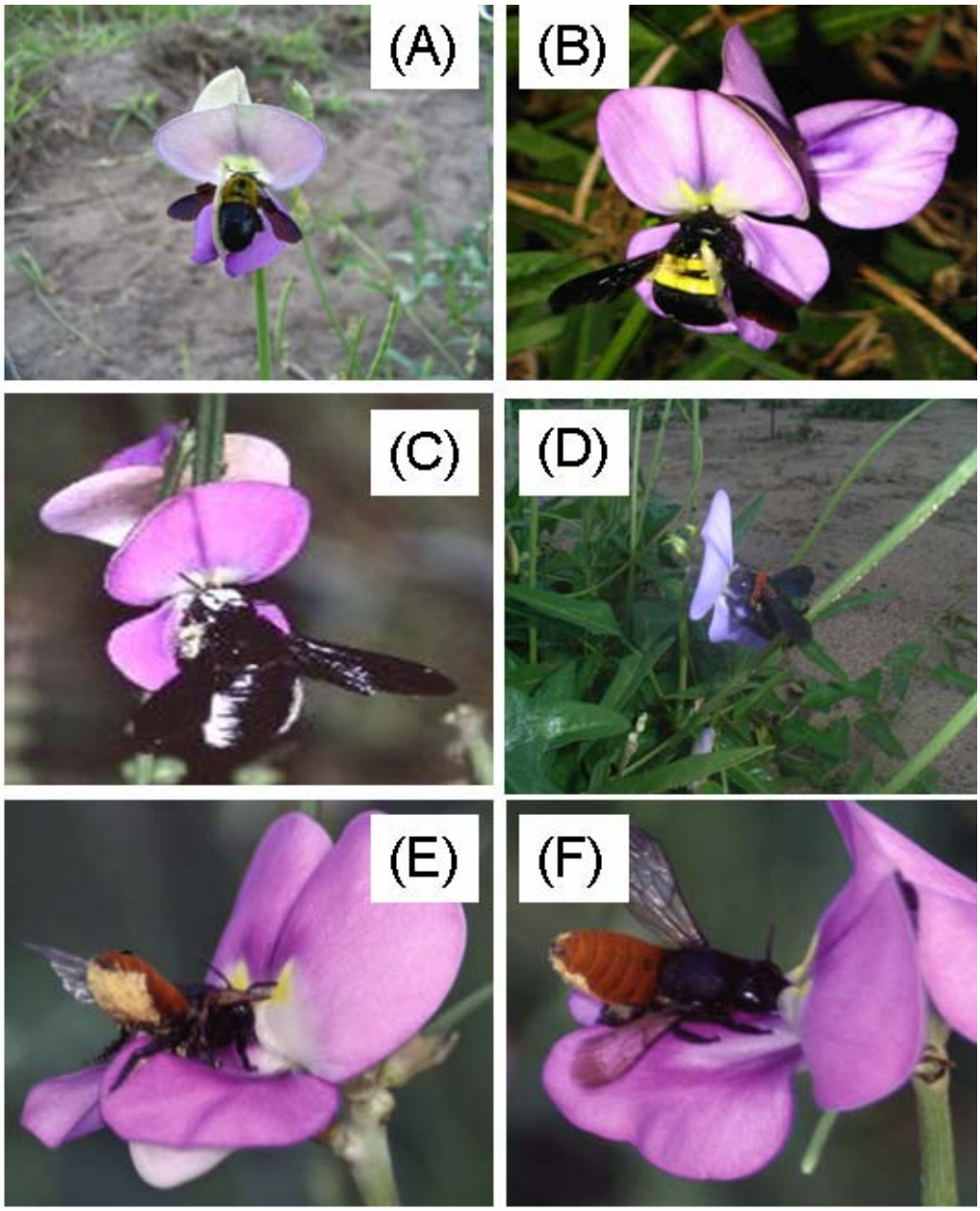

Figure 4.43 Bees species of the genus xylocopa and megachile visiting wild V. unguiculata flowers. (A): *Xylocopa senior*. (B): *Xylocopa caffra.* (C): *Xylocopa nigrita*. (D): *Xylocopa flavorufa.* (E) and (F): *Megachile bombiformis*

CHAPTER FIVE

DISCUSSION

5.1 Isozyme diversity, wild-crop complex dynamics and domestication hypothesis in cowpea (*V. unguiculata L. walp)*

5.1.1 Wild cowpea (*var. spontanea*) gene pool

According to Nei (1978), the best estimates of the genetic diversity parameters require a large number of accessions widely distributed and studied in a great number of loci. In attempting to fulfil these above considerations, Two hundred and fifty wild accessions well distributed in western, central, eastern and southern part of Africa and twenty three iso-enzyme loci were used. Iso-enzymes are commonly used for genetic diversity and gene flow analysis in animal and plant species (Pasteur et al., 1987; Sonnante et al., 1997; Pasquet, 1999; Miroslav, 2000). The total gene diversity obtained in this study was H_T = 0.149 and the proportion of polymorphic loci considering the most frequent allele to have a percentage less than 95% was 43.5% for wild *V. unguiculata*, which is consistent with what is expected in most self-pollinated plant species (Hamrick and Godt, 1990). This gene diversity indice is within those inferred from isozymes data: (H_T = 0.090, 24 loci, 56 accessions) for Panella and Gepts (1992); (H_T = 0.153, 26 loci) for Vaillancourt et al. (1993) and (H_T = 0.199, 35 loci, 91 accessions) for Pasquet

(1999). However, the gene diversity found in this study was low compared to other studies on cowpea using DNA based markers. Ba et al. (2004) obtained with 202 RAPD markers and 30 accessions a gene diversity of 0.223. Coulibaly et al. (2002) also using 188 ALFP loci and 52 accessions obtained H_T = 0.175; thus, demonstrating the superiority of DNA based markers.

Different degrees of polymorphism were observed within each group of wild *V. unguiculata (var. spontanea)* suggesting some wild representing a significant source of genetic variation in terms of genetic resource management. Within *var. spontanea*, isozyme analysis showed southern Africa group genetically very distinct from other regions with high total gene diversity (H_T = 0.214), high frequencies of polymorphic loci and number of allele per locus (Table 4.4). The second centre of diversity was in east Africa (H_T = 0.110) with also a high proportion of polymorphic loci and number of alleles per locus (Table 4.4). West Africa was the less diverse (H_T = 0.107) with low proportion of polymorphic loci and number of alleles per locus compared to southern and eastern. Although theses genetic diversity indices were low, this ranking with iso-enzymes of *var. spontanea* diversity centres follow the same ranking as those of Ba et al. (2004) with Random Amplified Polymorphic DNA markers and Coulibaly et al. (2002) with Amplified Fragment Length Polymorphism. The central Africa group added to this study contrary to previous ones were found to be the third centre of diversity after southern and eastern Africa with a total diversity of 0.104,

proportion of polymorphic loci of 30.4% and a mean number of alleles per locus (A = 1.65). The within countries variations follows the same ranking as the total gene diversity according to regions with the highest recorded in southern Africa (H_S = 0.165) followed by eastern (H_S = 0.095), central and western with H_S = 0.088 and 0.089 respectively. This ranking does not corroborate with Ba et al. (2004) and Coulibaly et al. (2002) who obtained high H_S values in eastern, 0.196 and 0.168 respectively suggesting eastern Africa as the origin area of the taxon. Besides heterozygote deficits observed in wild cowpea (*var. spontanea*), positives and significant F_{IS} and F_{IT} were observed suggesting some degree of inbreeding within wilds. This observation is not surprising, taking into account the biology of this taxa.

There is no generally known genetic self-incompatibility system within *var. spontanea.* The mechanism of seed dispersal where dried dehiscent pods explode spontaneously does not promote long distance gene flow. It favours mating among relatives. There was no significant difference in inbreeding coefficients found in different regions: southern (F_{IT} = 0.958±0.016, F_{IS} = 0.942±0.022); eastern (F_{IT} = 0.914±0.033, F_{IS} = 0.904±0.038); central (F_{IT} = 0.938±0.011, F_{IS} = 0.926±0.017) and western Africa (F_{IT} = 0.963±0.017, F_{IS} = 0.953±0.023). However, the genetic differentiation of accessions varied among regions with the lowest being found in eastern Africa (F_{ST} = 0.103±0.031) with a high potential of genetic exchange and the highest being from southern Africa (F_{ST} = 0.280±0.034).

5.1.2 Cultivated cowpea (*Var. unguiculata*) gene pool

When a species is brought into domestication, population bottleneck, founder effects and artificial selection are likely to be expected with the reduction of the genetic diversity of the crop in relation to its wild progenitor (Doebley, 1989). Compared with the variation in wild accessions, cultivated cowpea is more homogenous. This study showed some polymorphism with isozymes as previously revealed by Pasquet (2000); Vaillancourt et al. (1993) and Panella and Gepts (1992). The total gene diversity obtained from 23 loci and 64 accessions was H_T = 0.058 and like in the wilds is within the range of those reported using izozymes: from 0.018 to 0.061 (Pasquet, 2000; Vaillancourt et al., 1993 and Panella and Gepts, 1992). Ba et al. (2004) with RAPD and Coulibaly et al. (2002) using ALFP obtained high values: H_T = 0.123 and 0.108 respectively. This gene diversity was quite homogenous in the different regions sampled (Table 4.5) and does not agree with Pasquet (2000) which showed eastern Africa to be more diverse compared to western Africa. The difference with the current results may be that Pasquet (2000) compared only the cultivar group *biflora* while in this study, the cultivar groups from these regions was not compared but the cultivated samples of the regions since there was a large disproportion in sampling size for the different groups. However comparing the proportion of polymorphic loci and number of alleles per locus, eastern Africa were found to have higher values as found by Pasquet (2000)

5.1.3 Loss of the genetic diversity from wilds cowpea (*var. spontanea*) to cultivars (*var. unguiculata*)

A careful examination of the results at isozyme loci show a significant difference in diversity indices that are allele richness, proportion of polymorphic loci, observed heterozygosity and gene diversity between wild and cultivated accessions. Chloroplast DNA hapotype frequencies also show a clear genetic divergence, cultivars are less diverse than wilds. Wild cowpea was more diverse (Ht = 0.149) compared to cultivated cowpea (Ht = 0.058) as previously demonstrated in a direct comparison of diversity between landrace and wilds accessions by Ba et al. (2004) using RAPD markers; Coulibaly et al. (2002) using ALFP; Pasquet, 1999; Vaillancout et al., 1993; Panella and Gepts, 1992 using iso-enzyme markers. The reduction in diversity of cultivated cowpea (*var. unguiculata*) compared to wild (*var. spontanea*) was 61%, which is omparable to previous studies: 45% (Ba et al., 2004); 38% (Coulibaly et al., 2002); 69% (Pasquet, 1999); 80% (Panella and Gepts, 1992) and 81% (Vaillancourt et al., 1993). Besides cowpea, other wild plants species commonly display high level of variation compared to cultivated species (Chan and Sun, 1997; Saghai Maroof et al., 1994; Sharma et al., 1995). The survival of wild plants under natural conditions is advantaged by the maintenance of a high level of genetic variability (Chan and Sun, 1997). Cultivated plants come from selection pressure from a limited portion of the wild gene pool and therefore are expected to show low level

polymorphism than their wild counterparts. Although slightly higher in wild accessions compared to cultivated, both wild and cultivated cowpea displayed very little diversity within accessions, which is expected because of their breeding systems. Cultivated cowpea and their putative wild progenitors are monoecious exhibiting a mixed mating system highly self pollinated with the close disposition of the reproductive structures (Lush, 1979). The most significant loss of the genetic variation within cultivated cowpea accessions could have occurred as a result of artificial selection for breeding lines.

The average genetic differentiation among subspecies of *Vigna unguiculata* estimated by Pasquet (1999) is 0.341. This value was calculated from several wild subspecies and cultivated specie. It was found in this study that the *spontanea-unguiculata* differentiation is associated with a large allelic frequencies differentiation at many isozyme loci. The value of genetic differentiation found by Pasquet (1999) is high and comparable to the value found in this study (0.255) between wild (*var. spontanea*) and cultivated (*var. unguiculata*) of only one subspecie (*unguiculata)*. If genetic differentiation between wild and cultivated cowpea was high, the average Nei's genetic distance (GD) was however low (GD = 0.0506) and genetic identity (GI) very high (GI = 0.9507). Low genetic distance and high gene identity are to be expected between any crop plant and their presumed wild progenitor. Such results were obtained previously in *Vigna*

unguiculata (Coulibaly et al., 2002), *Vigna subterranea* (Pasquet et al., 1999) and *Phaseolus vulgaris* (Sonnante et al., 1994)

5.1.4 Evolutionary origin of cultivated cowpea (*var. unguiculata*)

As Doebley (1992) noted concerning the plants domestications; it seems a reasonable assumption that the first farmers used only a small fraction of the variation present within the progenitor species of today's crops and probably as domestication process proceeded, these farmers selected only the best phenotypes and this should have left the domesticated species with less genetic variation. Both isozymes and cpDNA through PCR-RLFP demonstrated some intra specific variation in *V. unguiculata.* The generally lower value of allozyme and cpDNA polymorphism in the cultivated specie in comparison to the wild may suggest that the crop specie could have passed through genetic bottleneck during the process of speciation and / or experienced strong selection under domestication. *Vigna unguiculata* seems to be one of the most important legume species out of the 80 species of the genus *Vigna* (Pasquet, 2001) and most likely experienced high selection pressure under domestication with subsequent low genetic variation as shown by both markers. Domestication is an introduction of a few accessions to a new geographic region with subsequent inbreeding (Chan and Sun, 1997). The prediction by Doebley (1989) in the case of wild-cultivated derivatives analysed by the help of iso-enzymes is that (1) the cultigens variation fall within the range of those of the putative wild progenitor. Many of our cultivated accessions

electrophenotypes are the same as some of those wild accessions in the same regions and agree with the prediction. (2) the cultigens have a subset of the allelic diversity found in the wild progenitor. In this study, Enp^{098}, one electrophoretic variant of the endopeptidase enzyme was not identified within wilds but the 29 other variants present in the cultivated gene pool belonged to the *var. spontanea* gene pool.

Several points can be made regarding the evolutionary and origin of the cultivated species of *V. unguiculata.* Isosyme alleles and cpDNA haplotype characteristic within the domesticated gene pool were found to be unequally distributed in the wilds in the different countries and regions screened. The question is: can a more specific area of origin of the cultivated cowpea be located? Based on these isozyme alleles and cpDNA characteristic of the cultivated cowpea, the results support a common view of the domestication of *V. unguicultata* in West Africa as did Ba et al. (2004) with RAPD markers, Coulibaly et al. (2002) with ALFP markers, Feleke et al. (2006) with RFLP markers. The grouping of almost all the cultivated taxa in the same cluster of the dendogram although the intrusion of some wilds suggest a single domestication event of cowpea in Africa; this agrees with the cluster analysis of Panella and Gepts (1992), Pasquet (1999), Coulibaly et al. (2002) and Ba et al. (2004). Table 4.8 indicates that wild accessions from West Africa are the most close to the domesticated taxa than do wild groups from central, eastern and southern Africa. Coulibaly et al. (2002) and Ba et al. (2004)

made the same observation. More precisely at the country level, table 4.9 shows that it is those wild accessions, especially from Ghana and Benin that are most close to the domesticated cowpea. Isosyme alleles Amp_2^{102}, Fle_3^{096} and cpDNA haplotype1 are marked highly cultivated plants (*var. unguiculata*) and just few wilds (*var. spontanea*). Although the presence of these domesticated markers within wilds could be viewed as a genetic swamping of the wild gene pool by the domesticated pool as hypothesised by Feleke et al. (2006) referring to cpDNA haplotype 1, they are much likely to be linked to the domestication events of *V. unguiculata*.

In this study, accessions of *var. spontanea* from north east Africa that were not present in previous studies preventing an undeniable location of the centre of domestication of cowpea were included. The included accessions especially from Sudan (20) and Ethiopia (13) were found to be very poor (1/33) in each cultivated specific markers making the whole of east Africa unlikely to be the origin of the cultivated specie of cowpea. For the 250 wild accessions studied, cpDNA, Amp_2 and Fle_3 profiles were recorded for each of them. The domesticated markers were found to be disproportional within wilds. cpDNA haplotype 1, Amp_2^{102} and Fle_3^{096} were highly distributed in west Africa at the region level as demonstrated by Feleke et al. (2006) with cpDNA haplotype 1. These are illustrated in Figure 4.26. 4.27 and 4.28. Each of those markers was also highly represented in Ghana at the country level (Figure 4.23, 4.24 and 4.25). cpDNA haplotype 1 is therefore

frequent in West Africa and especially in Ghana. This distribution correlates positively and significantly with Amp_2^{102} and Fle_3^{096} distributions. With these significant and positive correlations between cpDNA haplotype 1 and Amp_2^{102}, cpDNA haplotype 1 and Fle_3^{096} and between Amp_2^{102} and Fle_3^{096} found within wild cowpea (Figures 4.29, 4.30, 4.31, 4.32, 4.33 and 4.34), it is agreeable that cowpea was domesticated in a region where these cultivated specific markers are highly represented within wilds (*var. spontanea*) and this means in west Africa, especially in Ghana. The presence even though at low frequencies of these cultivated specific markers in other regions were noted, raising the possibility though little of a domestication outside West Africa. The cowpea crop-weed complex is therefore widely distributed in the sub-Saharan Africa although unevenly. Coulibaly et al. (2002) reviewed arguments speculating on regions where cowpea was domesticated. West Africa was proposed according to some authors and others in north east Africa mainly based on morphological diversity of wild cowpea in the region. At the gene level, isosymes and cpDNA data of the thirty three accessions from north east Africa screened in this study did not show significant diversity and were very poor in cultivated specific markers. This makes this latter region unlikely regarding the domestication area. However, the existence of weedy, intermediate between wild and cultivated cowpea in West Africa demonstrated by Coulibaly et al. (2002) was confirmed by isosymes and cpDNA in this study using more accessions. It highlights the dynamics of the cowpea crop-weed complex in the region as also did Feleke et al. (2006) and supports also

earlier work of Vaillancourt and Weeden (1992) who concluded West Africa was the origin of the cultivated cowpea based on cpDNA profiles.

Nei's (1972) genetic distance between cultivated and wild groups at the region level ranged from 0.038 to 0.147 (Table 3.10). It shows that wild accessions from west Africa are closer to the domesticated types than do the wild types from central, east or southern Africa. Therefore like previous results with isosymes (Pasquet, 1999), ALFP (Coulibaly et al., 2002), RAPD (Ba et al., 2004) or cpDNA (Feleke et al., 2006), the current results confirm that domestication in east or southern Africa is unlikely. Coulibaly et al. (2002) with ALFP and Ba et al. (2004) also found the shortest genetic distance between wild and cultivated cowpea in West Africa and proposed a possible origin of the cultivated cowpea in the area. Ng (1995) suggested a domestication of cowpea by ethnic groups in west Africa using cowpea primarily for fodder. cpDNA haplotype 1 characterized all cultivated cowpea and this include primitive landrace from cultivar group *textilis* (Pasquet, 2000). Because of its long inflorescent peduncles, the seeds of this cultivar group in earlier times were not generally consumed and were cultivated as fibres (Pasquet and Baudoin, 2001). The low genetic distance found between the cultivar group *textilis* and the wild *var. spontanea* in Ghana (Table 4.10) once again supports the theory that the domestication of cowpea took place in Ghana a west African country. Table 4.10 as well as the dendogram in the figure 4.22 also show that the cultivar group *textilis* is far distant from the four other cultivar

groups, hypothesising that the cultivar group *textilis* from Ghana could have been introduced to other regions of Africa where further inbreeding and selection produced other cultivars, more homogenous and therefore distant to the primitive *textilis* that appears heterogeneous as shown by Pasquet (2000). In addition, the finding of the oldest archaeological evidence of cowpea dated 1500 BC dated in Ghana (D'Andrea et al., 2007) strongly agrees with both our isosymes and cpDNA results in this study where domesticated specific allozyme alleles at cpDNA haplotype 1 were all highly present in Ghana at the country level and west Africa at the regional level.

5.2 Genetic diversity and population structure of wild cowpea (V*igna unguiculata*) in a region of West Africa

5.2.1 Genetic diversity at the population and species level

The level of genetic diversity in this study for wild *V. unguiculata* at the species level (P=42.86; A=1.52; H=0.114) and population level (P=11.29%; A=1.11; H=0.0345) are closer to those reported for self fertilized plant species using isozymes as marker (P = 41.8%, A = 1.69, H = 0.124) at the species level and (P = 20%, A = 1.31, H = 0.074) at the population level (Hamrick and Godt, 1990). Wild *V. unguiculata* is endemic to sub-saharan Africa from West to East and in the

southern part (Pasquet, 1999). The data presented here are low compared to the previous studies on cowpea using allozymes: (H=0.290; A=4.08) reported by Pasquet (1999); (H=0.168; A=2.42) by Vaillancourt et al. (1993) and (H=0.110; A=2.00) by Panella and Gepts (1992). Their studies were conducted not only using *V. unguiculata ssp. unguiculata Var. spontanea* that is used in the present survey, but also included several perennial subspecies. Pasquet (1999) results were from eight different subspecies well distributed in the entire tropical Africa. This explains the high level of diversity obtained compared to Vaillancourt et al. (1993), Panella and Gepts (1992) both with surveys on 6 subspecies and to the present result with only one subspecies. In addition this study was conducted using 21 loci, which are low compared to 35 loci used by Pasquet (1999), 26 by Vaillancourt et al. (1993) and 24 loci by Panella and Gepts (1992) and also explains the results observed. It is expected that plant species that are predominantly inbred to have a low amount of genetic diversity (Loveless and Hamrick, 1984). In cowpea, stigma and anthers are in contact and the pollen is normally shed before the full opening of the flower (Lush, 1979), disposition that reduces cross-pollination. The low level of gene diversity and allele richness in the population studied could then be attributed to high selfing rate coupled with a disposition preventing heterozygous individuals. Some natural and environmental phenomenon such as restricted habitat range, homogeneity of the environment, genetic bottleneck following a habitat fragmentation and founder effects as well

are likely to produce low genetic variation as it was observed in this study (Hedrick et al., 1976; Frankham et al., 2002)

5.2.2 Hardy – Weinberg expectations

The level of inbreeding in natural population helps to appreciate the deviation from hardy – Weinberg expectations or departure from random mating. The inbreeding coefficient or fixation index (F_{IS}) can range from –1 to +1. Negative values indicate heterozygosity excess and positive values indicate heterozygosity deficiency relative to random mating. Of the 35 populations screened, 8 (BE03, BE06, GHA20, GHA26, NIG01, NIG02, NIG03, and NIG06) were uniform. Five present excess of heterozygosity (BE02, BUR06, GHA08, GHA29 and NIG08) while the remaining 22 have a deficiency in heterozygotes. The tendency for all the population is that there is a significant deficiency in heterozygotes indicating a deviation from the Hardy-Weinberg expectation. This is confirmed by the positive mean and significant inbreeding coefficient obtained within population (F_{IS} = 0.788, P <0.050). According to the specie's biology, given that cowpea is predominantly an inbreed plant, some most extreme form of positive assortative mating favoring homozygous individuals or non random mating is the most likely reason of the deficiency since in cowpea, most pollinator movements are within few flower patches and mostly between flowers of the same plant (Pasquet et al. 2008). However, mutations are unlikely to produce this deficiency but changes

through natural selection, restricted gene flow and genetic drift would need to be taken in consideration.

5.2.3 Distribution of the genetic diversity

The breeding system is a major factor influencing G_{ST} and F_{ST} values (Hamrick and Godt, 1996). Although Burkina Faso populations demonstrate most of their genetic variation within Population (G_{ST} = 0.351), the majority of the genetic diversity in the present study occurs among the thirty-five cowpea populations (G_{ST} = 0.658). Hence, 65.8% of the genetic variation occurs between populations. This proportion is far higher than those reported for outcrossed animal pollinated plant species (18.7%), predominantly outbred plant species (24.3%), but follows the expected pattern for most autogamous plant species where the great part of the diversity occurs between populations (Hamrick, 1989; Ronny and Jon, 2000) because of the high self-fertilization. Recent studies by Pasquet et al. (1999) and Bi et al. (2003) showed the same trend when analyzing the genetic diversity in bambara groundnut (G_{ST} = 0.712) and wild lima bean (G_{ST} = 0.575) respectively. The within population genetic diversity was H_S = 0.039. This value of H_S obtained from this study is four times lower compared to those from ALFP of Coulibaly et al. (2002). This is explained by the fact that isozymes compared to AFLP, occupy very few loci in the DNA sequence and low number of allele within each locus.

5.2.4 Population structure and differentiation

As summarized by Loveless and Hamrick (1984), inbred species tend to show lower genetic variation within population and higher genetic divergence among populations. Genetic differentiation was found mainly among population at the level of Nei's (1972) unbiased genetic distance D of 0.0891) (Table 4.22). This value corresponds to mean distance found between any two populations and ranged from 0.0000 to 0.2614. Divergence within Ghana populations was D = 0.0376, low value compared to Benin where all populations were well distanced (D = 0.0860). This observation corroborates with equilibrium models of isolation by distance that predict an increase in genetic distance with geographic distance (Sohini et al., 2005).

The level of genetic divergence, measured by D among populations both between and within population groups was low. However, F_{ST} values were relatively high (F_{ST} = 0.641) suggesting the presence of a significant genetic differentiation even among geographically closed populations. This differentiation found in west Africa populations was high compared to those obtained from 25 populations in coastal Kenya in East Africa (0.349) (Yusuf, unpublished) indicating high gene flow based on Wright formula in these populations compared to west Africa. There are no published estimates of fixation indices in cowpea populations. This study reveals a high genetic differentiation among populations and extremely high levels of inbreeding coefficients (F_{IS} = 0.788; F_{IT} = 0.924). High self-pollinated

plant species are expected to show High inbreeding coefficients (Ronny and Jon, 2000). All the five population groups investigated show significant genetic differentiation among patches within populations. The high inbreeding coefficients suggest the occurrence of non-random mating system for the population studied. The authors also suggested that this high inbreeding in *V. unguiculata* were caused by limited seed dispersal. Wild cowpea seeds are dispersed explosively when the mature dry pod walls suddenly split open and curl as a spring, sending seeds a few centimetres from the mother plant. This clustering of relatives is likely to generate high inbreeding coefficients (Frankham et al., 2002). Bi et al. (2003) documented similar high inbreeding coefficients (F_{IS} = 0.866; F_{IT} = 0.932) in wild populations of *Phaseolus lunatus (Fabaceae)*, a mixed mating plant species that is predominantly self-pollinated (Baudoin et al., 1998).

Although both the genetic distance (D) and the population differentiation (F_{ST}) were positively correlated with the geographic distance: (r = 0.140, P < 0.001, Figue 4.37) and (r = 0.142, P < 0001, Figure 4.36) respectively, high genetic identity (I) was also observed among populations. This pattern of genetic relationship among populations showing some effect of geography only explains a fraction of the genetic variation in wild *V. unguiculata*. The high level of population differentiation observed is consistent with other related inbred plant species: *Corrigiola Litoralis L*. (Durka, 1999); *Calystegia collina* (Wolf et al.,

2000) and *Phaseolus lunatus* (Bi et al., 2003) and may reflect inefficient gene dispersal among cowpea populations.

5.2.5 Gene flow

Highly self-pollinated species with limited pollen and seed dispersal are expected to show low level of gene flow (Ronny and Jon, 2000). The indirect estimates of Nm either based on Wright's (1951) equation or Slatkin (1985) method suggest restricted gene flow among populations as predicted by the high genetic differentiation estimates. The mean estimates of gene flow within population groups were $Nm^W = 0.211$ and $Nm^S = 0.868$ respectively, both indicating a long-term historical isolation considers that Nm values greater than 1.0 is considered necessary to prevent divergence resulting from genetic drift (Wright, 1951). It was observed that Nm^S is approximately four times higher than Nm^S; this difference could be attributed to the low frequencies of the private alleles. Private alleles frequencies found were: 0.0714 for Pgm_1^{094} in NIG12; 0.0833 for Enp^{102} in BUR05 and 0.1053 for Pgi_2^{130} in BEN05. Similar low level of gene flow was reported by Bi et al. (2003) when they studied the population genetic structure of wild lima bean, an autogamous plant species. Hence, gene flow via seed or pollen is quite limited in wild cowpea because of limited seed dispersal and self-pollination.

5.2.6 Outcrossing rates

In natural populations of cowpea, mixed genotypes may grow in the same area and cross-pollination by insects such as large bees may produce heterozygous progeny. The mean single and multi locus outcrossing rate in these populations are 3.4 and 2.4% respectively. The general close disposition of the reproductive organs of the wild cowpea (*var. spontanea*) plant as well as the phenomenon of clustering of relatives as mentioned earlier are likely to generate this low rate of outcrossing in wild cowpea, *var. spontanea* being known as predominantly self pollinated plant species (Pasquet, 1999). This rate of outcrossing in wild cowpea is within the range of those reported for other highly self-pollinated plant species such as wild G*lycine soja,* 13% (Fujita et al., 1997), C*orrigiola litoralis*, 14% (Durka, 1999), *Phaseolus lunatus*, 9.6% (Bi et al., 2005), *Avena fatua*, 3-6% (Jain and Rai, 1974), *Avena barbata*, 3.7% (Hamrick and Holden 1979) and *Hordeum jubatum*, 1-3% (Babbel and Wain, 1977)

5.3 Outcrossing rate dynamics of cowpea (*vigna unguiculata* (l.) walp.) and its relation to rainfall distribution, temperature and flower density

5.3.1 Outcrossing rate estimates

V. unguiculata is an open pollinated herbaceous tropical legume requiring insects, especially large bees for cross pollination events. In this study, estimates of outcrossing based on single and multilocus analyses showed big differences between months and the two years over which the study was undertaken. The outcrossing rate ranged from 2.4 to 87.6% (Figures 4.38 and 4.39). This remarkable scale of variation is associated with striking differences in climatic conditions throughout the year. It is consistent to those reported in *A. canadensis* and *C. tembloriensis* fields by Routley et al. (1999) and Holtsford and Ellstrand (1989) respectively.

The mean population multi loci outcrossing rate was 0.327 ± 0.044 (Table 4.26) indicating that *V. unguiculata* progenies are derived from a mixture of outcrossing and self-fertilization events, predominantly by the latter. The general disposition of reproductive structures in cowpea flower with anther and stigma being in contact (Lush, 1979) explains the high selfing rate found in this study. The average outcrossing rate obtained in this study is comparable to those previously reported for self-pollinated plant species. Becker et al. (1992) reported similar outcrossing rate (one-third) and Routley et al. (1999) obtained tm = 0.29 when studying

Brassica napus and *Aquilegia Canadensis* respectively. The outcrossing rate obtained in March indicated wild cowpea largely outcrossing. The diminution of the population density and a sudden increase of pollinator population beyond the natural level is a plausible explanation for this observed peak. The outcrossing rate in this study is supported by the frequent observation of a large number of insect pollinators on the cowpea flowers of this population, especially large bees from the genus *Xylocopa*. However, some monthly outcrossing rates indicated values significantly above or below the average (0.327); this rise and fall reveal the dynamics of the mating system through time. This extensive variation likely reflects the perpetual changes of environmental conditions. Outcrossing rate evolution indicates the same tendency during the two consecutive years (Figure 4.38, 4.39 and Table 4.27), although significant difference was found between the two years (Table 4.26). Multi locus outcrossing rate estimate was found to be statistically different from the single locus estimate, indicating significant crossing between relatives (Ritland, 1990). This biparental inbreeding (3.2%) was consistent during the two years. Wild cowpea seeds are generally found a few centimetres from the mother plant and this clustering of relatives is likely to generate significant biparental inbreeding. Spatial variation in the rate of outcrossing has been highlighted in a number of studies (Ge et al., 2003; Suso and Moreno, 1999). Our results indicate quite a wide variation significantly linked to air temperature and rainfall distribution as reported by Adel et al. (2004) when estimating outcrossing rate in *H. spontaneum*.

5.3.2 Dependence on environmental agents

The dependence of insect activity on temperature has been demonstrated (del Rio and Búrquez, 1986). The present data indicates a significant positive correlation between the monthly level of outcrossing and the average temperature. The main *V. unguiculata*'s pollinators are large bees and the efficiency of their activities depends on the ambient temperature. They seem to perform well in relation to pollination at high temperatures. Diana and Avitabile, (1998) indicated sunny days and air temperature between 15.6 and 32.2^{o}C as optimum flying conditions for bees. Lyon, (1992) also demonstrated that bees' pollination in *S. Canadensis* cannot occur until the temperature reaches 17 – 20^{o}C. The finding of this study is in agreement with these results, as well as with those of del Rio and Búrquez, (1986) who showed that pollination efficiency in *Mirabilis jalapa L.* increase linearly with temperature. Rain and wet condition do not allow bees to fly. It is then obvious that the rate of self-fertilization in *V. unguiculata* will increase in the absence of pollinators as was shown in *S Canadensis* by Lyon, (1992). Generally, vegetables are badly affected by rain when they are in the flowering stage. The rain is likely to destroy flowers and pollination wouldn't take place. Lyon, (1992) indicated that rainy weather hinder flower opening in *S. canadensis*. The current result indicates a significant negative correlation between the level of outcrossing and rainfall distribution values. This is supported by the report of Picard (1960) who demonstrated the influence of local climate on the extent of natural cross-pollination, high in drier areas and low in wet zones. The results are contrary to

the report of Adel et al. (2004) who found in *H. spontaneum* (wild barley) a positive correlation between the season specific outcrossing rate and annual precipitation, and a negative correlation between the level outcrossing and monthly temperature. This difference can be due to the degree of genetic adaptation and physical properties of the two species. Cowpea is known to be well adapted in dry areas (Duke, 1981) compared to barley with drought stress being the major constraint to its development (Adel et al., 2004).

Some research findings have found positive correlation between outcrossing and plant density (Routley et al. 1999; Field et al., 2005) while others have found inverse correlation (Ellstrand et al., 1978). The result here does not show significant relationship between flower density and the level of outcrossing likewise the pollinator efficiency in cowpea population isn't density dependant.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Starch gel electrophoresis of isozymes from cowpea seed tissue homogenates was highly valued in this study. Isozyme analyses continue to be the more suitable method for phylogenetic investigations and studies of the evolutionary origin of crop species because appearing to be the most practical ones requiring scoring of several loci in a large number of individuals (Chan and Sun, 1997; Pasquet, 1999; Bi et al., 2003). However several other techniques using nuclear markers such as microsatellites (Li et al., 2001), ALFP (Coulibaly et al., 2002), RAPD (Ba et al., 2004), RFLP (Feleke et al., 2006) have revealed to be helpful in the genetic studies of cowpea genome and should be considered at a high scale with huge sample size widely distributed in further additional lines of investigations for future research in cowpea genetics

Using isozymes and PCR-RFLP markers, the genetic variability of wild and cultivated cowpea in various regions in sub-saharan Africa and evolutionary origin of the domesticated specie were assessed. Approximately 78% of the 23 isozyme loci in cowpea primary gene pool were polymorphic. A total of 62 alleles were scored at these 23 isozyme loci giving an average of 2.70 alleles per locus. A

significantly lower diversity indices was found in cultivated cowpea than in wild seeds. There is a genetic relationship between wild and cultivated cowpea although significant differentiation was found. The wild-cultivated Nei's (1972) genetic distance estimation and the distribution of cultivated specific allele and haplotype within wilds suggest a single domestication in West Africa at the regional level and in Ghana at the country level. The current results with isozymes and cpDNA indicated an unlikely domestication of cowpea in eastern and southern Africa as previously demonstrated by Coulibally et al. (2002) with AFLP markers and Ba et al. (2004) with RAPD markers.

Both wild and cultivated cowpea displayed almost all of their genetic variation among accessions: 96.1 and 97.7% respectively indicating the need of expanding the number of accession for the optimum estimation of the cowpea genetic resource. This implies sampling all places where cowpea plant may be found. The aim in the conservation of plant genetic resource is to maintain as much genetic variability as possible. The planning therefore require identification of the desired population, continuous management and monitoring (Bi et al., 2003)

Data from allozyme electrophoresis in cowpea whether at the accession or at the population level revealed low allele richness, observed and expected heterozygosity, interpopulation gene flow and a genetic diversity mainly among localities or populations like other self pollinated plant species. The low genetic

diversity indices were associated to an effect of inbreeding since high inbreeding coefficients were found. The essential of the genetic variation were found within the wild gene pool highlighting the need for the conservation of the wild ancestor regarding the conservation of cowpea genetic resource. Therefore, it is mportant to preserve entirely their ecological habitat because populations are severely threatened of crash in many areas following land uses and road constructions.

Evidence of outcrossing in wild *V. unguiculata* was shown at the population level, providing useful information on the genetic structure, gene flow among populations and in the improvement of the cultivated cowpea species. The genetically modified (GM) cowpea in Africa is intended to help African farmers to overcome the low production they have been facing due to various pest, diseases and abiotic stresses. With this GM cowpea, there will be a possible dissemination of the inserted gene toward other cultivars and wild relatives. The estimation of the level of genetic exchange or gene flow for grouped populations in a region of West Africa either based on Wright method (Nm^W) and slatkin formula (Nm^S) were low: 0.211 and 0.868 respectively minimising the gene spreading that may be associated with the introduction of GM cowpea in Africa. The GM cowpea problem can furthermore be minimised if GM crops will be well separated from other crops and wild cowpea populations or the design as GM cowpea, a plant that does not produce pollen or produce in small quantities, a plant that can produce seed without fertilisation or plan with closed flowers (Gepts and Papa, 2003).

This study shows that gene dissemination within cowpea can't be totally eliminated because of the presence of bees, pollinator agents. Gene flow within a wild cowpea population will depend on the breeding system of that population and the environment prevailing in its locality. GM cowpea risks will be therefore associated to the type and proximity of the cowpea plant or population around those genetically modified. Gene flow in term of outcrossing rate varied big between cowpea populations and was more pronounced in Milalani population in coastal Kenya when compared to populations fromWest Africa. In this region of West Africa, gene flow within populations measured by tm and ts was high when compared to gene flow between populations measured by the number of migrant per generation (Nm); Nm barely exceeding one. This fits with the just published results on cowpea pollinator movements by Pasquet et al. (2008) revealing that bees are doing a lot of flower to flower flights within the same flower patch than flower to flower flight between two different flower patches

The mating system dynamics studies using the milalani population as an example showed that wild *V. unguiculata* possesses a mixed mating system predominantly inbred. This mating system decreases significantly with rainfall distribution and increases with flower density and temperature. Environmental factors therefore affect the outcrossing rate in wild *V. unguiculata* and merit further studies. A better understanding of the influence of these factors on outcrossing will be

important in cowpea breeding as testified in rapeseed (*Brassica napus*) (Becker et al., 1992). The success of the breeding of cowpea pure line would therefore require a maximum reduction of the level of outcrossing, meaning low temperature and plant density and more rain while breeding of synthetic varieties of cowpea will be successful when increasing the outcrossing rate (high temperature, plant density and less rain). Also for the breeding of hybrids, a reliable high level of outcrossing should be a prerequisite for cowpea seed production.

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APPENDICES

Appendix 1: Country of origin, codes and coordinates of wild accessions of *V. unguiculata*

	Accession Code	Country	Latitude	Longitude
Ghana				
	SP 808	GHA	10 44 N	00 47 W
	SP 817	GHA	11 03 N	00 10 W
	SP 818	GHA	11 04 N	00 08 W
	SP 819	GHA	11 05 N	00 07 W
	SP 820	GHA	10 58 N	00 05 W
	SP 821	GHA	11 03 N	00 03 W
	SP 822	GHA	11 04 N	00 11 W
	SP 823	GHA	11 00 N	00 18 W
	SP 824	GHA	11 00 N	00 21 W
	SP 825	GHA	10 56 N	00 29 W
	SP 826	GHA	10 52 N	00 45 W
	SP 827	GHA	10 52 N	00 45 W
	SP 828	GHA	10 52 N	00 44 W
	SP 829	GHA	10 48 N	00 55 W
	SP 831	GHA	10 53 N	01 05 W
	SP 832	GHA	10 52 N	01 06 W
	SP 835	GHA	10 47 N	00 50 W
Burkina Faso				
	SP 836	BUR	12 09 N	00 30 W
	SP 838	BUR	12 12 N	00 20 W
	SP 841	BUR	12 25 N	00 09 W
	SP 842	BUR	12 30 N	00 04 W
	SP 843	BUR	12 37 N	00 04 W
	SP 845	BUR	13 15 N	00 12 W
	SP 846	BUR	13 35 N	00 15 W
	SP 847	BUR	14 02 N	00 03 W
	SP 848	BUR	14 26 N	00 14 W
	SP 849	BUR	14 06 N	01 36 W
	SP 850	BUR	13 41 N	02 09 W
	SP 851	BUR	13 38 N	02 19 W
	SP 854	BUR	11 46 N	00 22 W
	SP 857	BUR	11 55 N	00 22 W

	SP 858	BUR	12 27 N	00 05 W
	SP 859	BUR	12 39 N	00 33 W
	SP 860	BUR	12 24 N	01 33 W
	SP 862	BUR	12 44 N	01 31 W
	SP 863	BUR	13 04 N	01 04 W
	SP 864	BUR	12 17 N	01 31 W
Niger				
	TV 671	NER	14 05 N	05 55 E
	TV 674	NER	14 05 N	05 55 E
	SP 78	NER	11 59 N	03 19 E
	SP 174	NER	No coordinates	
	SP 175	NER	No coordinates	
	NI 945	NER	12 23 N	03 26 E
	NI 991	NER	13 29 N	01 57 E
	SP 199	NER	12 22 N	03 25 E
	SP 300	NER	13 05 N	01 44 E
	SP 870	NER	13 03 N	03 12 E
	SP 871	NER	13 33 N	02 24 E
	SP 872	NER	13 34 N	02 01 E
	SP 873	NER	13 45 N	01 41 E
	SP 874	NER	13 46 N	01 37 E
	SP 875	NER	13 45 N	01 39 E
	SP 876	NER	13 39 N	01 46 E
Benin				
	SP 865	BEN	09 47 N	02 38 E
	SP 866	BEN	11 19 N	03 02 E
	SP 867	BEN	10 23 N	02 43 E
	SP 868	BEN	10 18 N	01 22 E
	SP 869	BEN	09 20 N	02 38 E
Nigeria				
	NI 951	NGA	No coordinates	
	TV 398	NGA	No coordinates	
	TV 400	NGA	No coordinates	
	TV 403	NGA	No coordinates	
	TV 417	NGA	No coordinates	
	TV 420	NGA	No coordinates	
Cameroon				
	SP 1	CMR	10 36 N	13 59 E
	SP 2	CMR	10 11 N	14 31 E
	SP 3	CMR	11 08 N	14 18 E
	SP 4	CMR	11 08 N	14 18 E
	SP 6	CMR	No coordinates	
	SP 7	CMR	10 59 N	14 31 E

	SP 8	CMR	10 07 N	14 08 E
	SP 12	CMR	10 53 N	13 47 E
	SP 14	CMR	10 34 N	13 56 E
	SP 15	CMR	10 41 N	13 36 E
	SP 19	CMR	10 12 N	14 11 E
	SP 22	CMR	10 40 N	14 20 E
	SP 23	CMR	09 34 N	13 31 E
	SP 28	CMR	09 19 N	13 24 E
	SP 30	CMR	10 27 N	14 46 E
	SP 32	CMR	10 07 N	14 08 E
	SP 41	CMR	10 59 N	14 12 E
	SP 46	CMR	11 24 N	14 34 E
	SP 58	CMR	10 28 N	13 41 E
	SP 60	CMR	No coordinates	
	SP 100	CMR	09 04 N	12 59 E
	SP 101	CMR	08 49 N	14 11 E
	SP 103	CMR	08 42 N	12 48 E
	SP 104	CMR	08 55 N	13 31 E
	SP 106	CMR	10 04 N	14 08 E
	SP 107	CMR	09 16 N	14 16 E
	SP 109	CMR	11 02 N	14 27 E
	SP 112	CMR	10 57 N	14 39 E
	SP 115	CMR	10 56 N	14 49 E
	SP 119	CMR	10 45 N	14 34 E
	SP 120	CMR	10 45 N	14 34 E
	SP 123	CMR	08 34 N	12 43 E
	SP 125	CMR	10 57 N	14 39 E
	SP 126	CMR	09 45 N	13 34 E
	SP 127	CMR	09 43 N	13 33 E
	SP 128	CMR	10 04 N	14 29 E
	SP 132	CMR	09 36 N	13 30 E
	SP 134	CMR	09 36 N	13 30 E
	SP 113	CMR	10 56 N	14 48 E
	SP 116	CMR	10 46 N	14 39 E
	SP 117	CMR	10 46 N	14 39 E
	SP 129	CMR	10 04 N	14 29 E
	SP 13	CMR	10 53 N	13 47 E
	SP 56	CMR	10 28 N	13 41 E
	SP 57 B	CMR	10 28 N	13 41 E
	SP 61	CMR		
Congo				
	NI 1390	COG	02 52 S	15 29 E
	NI 1391	COG	02 23 S	15 44 E
	SP 148 D	COG	02 20 S	15 48 E

	SP 151	COG	03 59 S	15 24 E
	SP 152G	COG	03 58 S	15 35 E
	SP 177	COG	00 44 S	16 52 E
Centrafica Republic				
	TV 242	RCA	05 02 N	18 26 E
	TV 248	RCA	06 24 N	18 00 E
	TV 249	RCA	06 40 N	19 10 E
	TV 255	RCA	05 15 N	21 07 E
	TV 256	RCA	05 20 N	21 03 E
	TV 445	RCA	05 35 N	20 04 E
	TV 754	RCA	05 44 N	20 25 E
Tchad				
	TV 258	TCD	10 29 N	15 31 E
	TV 303	TCD	11 28 N	15 20 E
	TV 304	TCD	11 26 N	15 21 E
	TV 305	TCD	11 01 N	15 29 E
	TV 307	TCD	10 44 N	15 33 E
	TV 308	TCD	10 14 N	15 19 E
	TV 315	TCD	10 32 N	16 41 E
	TV 317	TCD	08 34 N	17 05 E
	TV 525	TCD	10 48 N	15 25 E
	TV 542	TCD	No coordinates	
	SP 64	TCD	No coordinates	
Sudan				
	SP 366	SDN	12 48 N	30 06 E
	SP 367	SDN	11 57 N	34 18 E
	SP 368	SDN	11 33 N	34 11 E
	SP 369	SDN	11 24 N	29 38 E
	SP 370	SDN	11 43 N	29 46 E
	SP 371	SDN	11 02 N	29 41 E
	SP 372	SDN	12 29 N	29 47 E
	SP 373	SDN	11 47 N	34 28 E
	SP 374	SDN	11 59 N	29 40 E
	SP 375	SDN	11 41 N	34 16 E
	SP 376	SDN	11 55 N	29 41 E
	SP 377	SDN	12 32 N	29 53 E
	SP 378	SDN	11 46 N	29 46 E
	SP 379	SDN	11 44 N	34 19 E
	SP 380	SDN	11 46 N	34 22 E
	SP 381	SDN	13 32 N	35 41 E
	SP 382	SDN	13 22 N	35 48 E
	SP 383	SDN	12 18 N	34 11 E
	SP 384	SDN	11 16 N	29 40 E

	SP 385	SDN	11 13 N	29 25 E
Uganda				
	SP 434	UGA	01 30 N	33 34 E
	SP 442	UGA	02 48 N	32 17 E
	SP 443	UGA	03 17 N	32 07 E
	SP 444	UGA	03 27 N	32 04 E
	SP 445	UGA	03 33 N	32 04 E
	SP 446	UGA	02 37 N	32 08 E
	SP 447	UGA	02 37 N	32 03 E
	SP 448	UGA	02 35 N	31 56 E
	SP 449	UGA	02 21 N	31 37 E
	SP 450	UGA	02 25 N	31 31 E
	SP 455	UGA	02 00 N	31 29 E
	SP 457	UGA	00 59 N	32 29 E
Kenya				
	SP 204	KEN	01 29 N	35 02 E
	SP 206	KEN	00 29 N	34 18 E
	SP 207	KEN	02 44 S	38 00 E
	SP 219	KEN	04 16 S	39 15 E
	SP 221	KEN	04 27 S	39 26 E
	SP 265	KEN	04 00 S	39 37 E
	SP 271	KEN	02 47 S	36 56 E
	SP 272	KEN	03 56 S	39 32 E
	SP 275	KEN	03 41 S	39 46 E
	SP 276	KEN	03 52 S	39 46 E
	SP 278	KEN	02 20 S	40 20 E
	SP 279	KEN	02 23 S	40 24 E
	SP 292	KEN	00 38 S	34 20 E
	SP 295	KEN	02 32 S	37 51 E
	SP 400	KEN	00 32 N	35 32 E
	SP 401	KEN	00 28 N	35 40 E
	NI 1048	KEN	03 57 S	39 32 E
	NI 1228	KEN	03 57 S	39 32 E
	NI 1408	KEN	03 37 S	39 51 E
Ethiopia				
	SP 411	ETH	12 49 N	37 22 E
	SP 413	ETH	13 02 N	37 18 E
	SP 414	ETH	13 12 N	37 14 E
	SP 417	ETH	13 53 N	36 53 E
	SP 419	ETH	14 08 N	36 54 E
	SP 421	ETH	12 36 N	36 54 E
	SP 422	ETH	12 35 N	36 49 E
	SP 423	ETH	12 36 N	36 44 E

	SP 424	ETH	12 38 N	36 37 E
	SP 425	ETH	12 40 N	36 32 E
	SP 426	ETH	12 53 N	36 13 E
	SP 427	ETH	12 55 N	36 12 E
	SP 428	ETH	11 07 N	36 23 E
Tanzania				
	SP 75	TZA		
	SP 181	TZA	06 50 S	38 00 E
	SP 182	TZA	06 50 S	38 10 E
	SP 183	TZA	06 40 S	38 10 E
	SP 184	TZA	06 20 S	37 50 E
	SP 185	TZA	06 00 S	38 00 E
	SP 186	TZA	06 10 S	37 50 E
	TV 297	TZA	05 10 S	29 50 E
	TV 298	TZA	06 11 S	39 11 E
	TV 301	TZA	06 10 S	39 26 E
	TV 531	TZA	06 16 S	39 24 E
	TV 922	TZA	05 18 S	39 02 E
	TV 1248	TZA	06 08 S	39 17 E
South Africa				
	SP 192	ZAF	22 35 S	31 15 E
	SP 195	ZAF	26 53 S	32 53 E
	SP 312	ZAF	23 41 S	30 42 E
	SP 315 L	ZAF	28 09 S	32 19 E
	SP 317	ZAF	27 29 S	32 01 E
	SP 323	ZAF	24 42 S	30 47 E
	NI 1167	ZAF	No coordinates	
	TV 1089	ZAF	No coordinates	
Malawi				
	TV 363 A	MWI	14 05 S	33 53 E
	TV 369	MWI	13 04 S	33 31 E
	TV 370	MWI	10 35 S	34 07 E
	TV 371	MWI	11 24 S	33 50 E
	TV 435 A	MWI	15 35 S	35 10 E
	TV 437A	MWI	11 52 S	33 44 E
	TV 442 B	MWI	09 51 S	33 47 E
	NI 1392	MWI	11 22 S	33 56 E
Zambia				
	TV 961	ZMB	16 39 S	27 09 E
	TV 548	ZMB	15 14 S	23 06 E
	TV 554	ZMB	13 29 S	32 47 E
	TV 712 B	ZMB	16 03 S	27 31 E
	NI 423	ZMB	13 08 S	28 25 E

	TV 357	ZMB	17 28 S	24 17 E
	TV 359	ZMB	17 46 S	25 46 E
	TV 361	ZMB	12 30 S	28 04 E
Zimbabwe				
	SP 164	ZWE	20 21 S	32 20 E
	MT 25	ZWE	19 50 S	28 25 E
	MT 62	ZWE	20 15 S	32 50 E
	MT 65	ZWE	20 15 S	32 55 E
	MT 76	ZWE	19 57 S	32 05 E
	MT 102	ZWE	18 24 S	32 55 E
	NI 817	ZWE	18 12 S	31 34 E
	NI 874	ZWE	18 12 S	31 34 E
Botwana				
	SP 155	BWA	20 01 S	21 00 E
	SP 157	BWA	23 17 S	26 49 E
	TV 261	BWA	20 10 S	25 50 E
	TV 268	BWA	20 10 S	23 10 E
	TV 284 A	BWA	19 10 S	22 40 E
	TV 466	BWA	19 00 S	22 40 E
	TV 815	BWA	20 25 S	22 50 E
	NI 1380	BWA	20 00 S	23 25 E
	NI 1381	BWA	20 10 S	23 10 E

Appendix 2: Country of origin, codes and coordinates of cultivated accessions of *V. unguiculata*

	Accession Code	Country	Latitude	Logitude	Cultivar Group
Benin					
	BE01	BEN	07 53 N	02 13 E	Biflora
	BE02	BEN	08 40 N	02 35 E	Biflora
	BE03	BEN	10 40 N	02 38 E	Melanophtalmus
	BE04	BEN	07 01 N	01 46 E	UnguiculaTextilisa
Burkina Faso					
	HV 7	BUR	10 10 N	2 55 W	Biflora
	HV9	BUR	11 20 N	4 55 W	Biflora
	HV 2	BUR	11 15 N	3 30 W	Biflora
	HV 5	BUR	10 42 N	5 14 W	Biflora
	HV11	BUR	No coordinates		Biflora
	HV12	BUR	No coordinates		UnguiculaTextilisa
Ghana					
	G03	GHA	10 32 N	02 42 E	Unguiculata
	G13	GHA	09 14 N	02 21 W	Unguiculata
	G01	GHA	07 40 N	01 01 W	Melanophtalmus
	G02	GHA	08 23 N	00 33 W	Melanophtalmus
	G07	GHA	09 32 N	01 09 W	Melanophtalmus
Niger					
	NE01	NER	13 03 N	03 12 E	Melanophtalmus
	NE02	NER	13 59 N	03 12 E	Melanophtalmus
	NE03	NER	13 39 N	01 46 E	Melanophtalmus
Cameroon					
	CS 152	CMR	04 51 N	14 16 E	Unguiculata
	NO 1036	CMR	11 47 N	15 06 E	Sesquipedalis
	OU 23	CMR	04 22 N	09 06 E	Unguiculata
	NO 1669	CMR	10 54 N	14 12 E	Biflora
	OU 174	CMR	05 45 N	11 03 E	Unguiculata
	NO 2292	CMR	08 29 N	13 19 E	
	CS 53 B	CMR	04 39 N	09 53 E	Unguiculata
	NO 2294	CMR	10 47 N	13 46 E	Textilis
	CS 45	CMR	04 52 N	11 15 E	Unguiculata
	OU 176C	CMR	05 32 N	10 05 E	Unguiculata
	OU 59A	CMR	04 59 N	09 26 E	Unguiculata
	NO 184	CMR	10 39 N	13 52 E	Textilis
	NO 1479	CMR	No coordinates		Melanophtalmus
	NO 2527	CMR	08 32 N	13 10 E	Textilis

	NO 2461	CMR	08 38 N	12 38 E	Textilis
	OU65	CMR	No coordinates		Unguiculata
	CSB5	CMR	No coordinates		Unguiculata
Ethiopia					
	ET 16	ETH	No coordinates		Biflora
	ET 27	ETH	No coordinates		Biflora
	ET 08	ETH	No coordinates		Biflora
	ET 38	ETH	No coordinates		Unguiculata
	ET 04	ETH	No coordinates		Biflora
	ET 10	ETH	No coordinates		Biflora
Kenya					
	KE254	KEN	04 22 S	39 30 E	Unguiculata
	KE203	KEN	03 12 S	39 55 E	Unguiculata
	KE263	KEN	04 30 S	39 14 E	Melanophtalmus
	KE222	KEN	04 27 S	39 26 E	Unguiculata
	KE260	KEN	04 00 S	39 29 E	Unguiculata
	KE234	KEN	03 59 S	39 35 E	Unguiculata
	KE231	KEN	01 27 N	35 28 E	Biflora
	KE232	KEN	01 29 N	35 02 E	
Sudan					
	SD01	SDN	12 14 N	29 44 E	Biflora
	SD02	SDN	11 03 N	29 41 E	Melanophtalmus
Uganda					
	UG 03	UGA	No coordinates		Unguiculata
	UG 05	UGA	No coordinates		Unguiculata
	UG 08	UGA	No coordinates		Unguiculata
	UG 10	UGA	No coordinates		Biflora
	UG 11	UGA	No coordinates		Unguiculata
	UG 12	UGA	No coordinates		Unguiculata
South Africa					Unguiculata
	AS 10c	ZAF	No coordinates		Unguiculata
	AS 2b	ZAF	No coordinates		Unguiculata
	AS 1a	ZAF	No coordinates		Unguiculata
	AS 3c	ZAF	No coordinates		Unguiculata
	AS 8	ZAF	No coordinates		Unguiculata
	AS 10a	ZAF	No coordinates		Unguiculata
	AS 3e	ZAF	No coordinates		Unguiculata

Appendice 3: Publications

3.1 Abstracts:

Kouam, E.B, Elteraifi, I. Muluvi G.M and Pasquet, R.S. Genetic structure of wild cowpea populations in Sudan (2008). Presented at the International Symposium on Biotechnology, 4th – 8th May, Sfax, Tunisia, International workshop on Making Markers Meaningful in Agroforestry and Tree Genetics, ICRAF, 29th September – 3rd October 2008, Nairobi, Kenya

Kouam, E. B. (2008). Bt gene escape assessment from cowpea to wild relatives. Presented at the international workshop on Risk, Benefits and Opportunities of the release of GMOs in Africa regions, 15 – 19 September, University of Cape Town, South Africa

3.2 Proceeding:

Kouam, E.B, Elteraifi, I. Muluvi G.M and Pasquet, R.S (2007). Population genetic structure of natural populations of wild cowpea. In proceedings of the Fourteenth annual conference of Bioscience on "Biosciences and genetically modified products. Held in Bambui - Bamenda, Cameroon on 6, 7 and 8 December 2007

3.3 Thesis:

Kouam, E.B (2004). Antioxidant status of bilirubin and uric acid in malarial patients. M.Sc research thesis submitted and defended at the Department of Biochemistry, Faculty of Science, University of Douala, Cameroon, in partial fulfilment of the award of Master's Degree in Biochemistry

3.4 Article published:

Kouam, E.B., Ndomou, M., Gouado, I. and Fotso, K.H. (2008). Antioxidant status of bilirubin and uric acid in malaria patients in Douala. Pak. J. Biol. Sci. 11 (12) 1646 – 1649

3.5 Articles under review

Kouam, E.B., Pasquet, R.S., Thoen, K., Tignegre, J.B., Gaudin, R., Ouedraogo, J.T., Salifu, A.B., Muluvi, G.M. and Gepts, P. Genetic structure and mating system in wild cowpea (*Vigna unguiculata ssp unguiculata var. spontanea*) populations in a region of West Africa.

Heredity

Kouam, E.B., Pasquet, R.S. Muluvi G.M. and Gepts, P. Outcrossing rate dynamics of cowpea (*Vigna unguiculata* (L.) Walps), a diploid plant species (Fabaceae) using allozyme polymorphisms.

Molecular Ecology

3.6 Article in preparation:

Kouam, E.B., Pasquet, R.S. Muluvi G.M. and Gepts, P. Isozyme diversity, wild-crop complex dynamics and domestication hypothesis in cowpea (*Vigna unguiculata*)