CHARACTERIZATION OF CULTIVATED GROUNDNUT (*Arachis hypogaea* L.) AND GENETIC ANALYSIS OF LATE LEAF SPOT RESISTANCE IN TOGO

By

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DECLARATION

I hereby declare that except for references to works of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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ABSTRACT

Though an important crop, the yield of groundnut has been decreasing for years because of a lack of a strong breeding programme in Togo. In recent years, efforts have been going on to establish a well-functioning breeding programme to develop varieties for improving productivity and production of groundnut. The main aims of this study were to: assess farmers' perceptions of production constraints and preference criteria, characterize cultivated groundnut varieties and study genetic mechanisms for priority traits. Results will be useful to exploit genetic diversity among the cultivated groundnut accessions and to integrate farmers' knowledge and expectations in the development of high yielding and adaptable groundnut varieties that are preferred by farmers, marketers, processors and consumers in Togo. The investigation on production constraints and farmers' preferences showed that yield related traits such as pod yield (66.66%) and pod size (12.12%) were the most valued selection criteria by farmers. According to the farmers, groundnut production is mainly constrained by diseases (37.77% of the respondents). Important economic losses and various causes were associated with late leaf spot (LLS) disease in the surveyed areas. Diversity assessment of 94 accessions from Togo, Senegal and ICRISAT, using agro-morphological and molecular markers, revealed that quantitative traits such as LLS incidence, number of pods per plant and yield displayed an adequate variability (coefficient of variation > 20%). The most diverse qualitative traits based on Simpson index were primary seed colour (0.75), stem pigmentation (0.60), and growth habit (0.59). Moreover, principal component analysis underscored quantitative traits such as hundred seed weight, days to maturity, and LLS incidence, as the main traits contributing to the divergence. Correlation analysis and path analysis showed that the number of pods per plant was the main yield-related trait positively affecting yield (PC=0.84; p=0.01). Overall, SNP markers revealed high genetic variability in the genotypes and the percentage of heterozygous genotypes varied from 0 to 50% for all loci. AMOVA revealed that only 1.1% of the total

molecular variance accounted for geographical contribution to the diversity. Cluster analysis delineated three clusters harbouring useful alleles and interesting phenotypic features such as LLS resistance, a high number of pods per plant and early maturity. Structure co-analysis of phenotypic and SNP data showed that differences observed at the phenotypic level are underlined by genotypic differences. The phenotypic and genotypic diversity revealed appreciable diversity that could be exploited for the identification of parents with preferred traits for use in the breeding programme in Togo. The linkage disequilibrium (LD) in Togo groundnut collection was found to be high (0.36). Mean r^2 ranged from 0.058 to 0.99 with the genomic distance between loci ranging from 0 to 148 Mbp for intrachromosomal linkage disequilibrium. The interchromosomal LD was found to be significant at $p = 0.01$ with a certain number of loci located on different chromosomes exhibiting r^2 above 0.1. Association study revealed that out of 31 agro-morphological traits analyzed, significant associations between SNPs and agronomic traits of interest ($p \leq 10^{-7}$) were established for nine traits. Chromosomes A02, A03, A05 and B10 were significantly associated with LLS resistance with r^2 ranging from 0.50 to 0.57. Hundred seed weight, pod width, pod length, seed length, seed width, growth habit and pod reticulation were also associated with various genomic regions. Genetic mechanism analysis for LLS using sixteen F_2 progenies from full factorial design crosses indicated additive gene and maternal effects for LLS resistance with 48,3% and 3.86% of the phenotypic variance explained, respectively. Non-additive genetic variance was close to zero for LLS resistance. In contrast to LLS resistance, non-additive gene effects played a greater role than additive effects for pod weight per plot (21.79% phenotypic variance explained). For the other yield-related traits, additive genetic variance was more important than the other variance components. Accessions 43AH and ICG 7878 with the highest general combining ability could be considered as the best parents for high yielding LLS resistant lines development.

DEDICATION

To my family, brothers, sisters and friends. Your prayers and support made it easier for me to persevere to the end.

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LIST OF ABBREVIATIONS

AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
CERAAS	Centre d'etude Regionale pour l'Amelioration de l'Adaptation a la secheresse
FAO	Food and Agriculture Organization
FGDs	Focus Group Discussion
GCA	General Combining Ability
GWAS	Genome-Wide Association Study
IBPGR	International Board for Plant Genetic Resources
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
ITRA	Institut Togolais de Recherche Agronomique
LD	Linkage Disequilibrium
LLS	Late leaf spot
NC II	North Carolina II design
NGO	Non-Governmental Organism
PC	Principal Component
PCA	Principal Component Analysis
PRA	Participatory Rural Appraisal
QTL	Quantitative Trait Loci
SCA	Specific Combining Ability
WAAPP	West Africa Agriculture Productivity Program
WACCI	West African Centre for Crop Improvement

CHAPTER ONE

1. GENERAL INTRODUCTION

Groundnut (*Arachis hypogaea* L.) constitutes one of the most important legumes grown around the world (FAOSTAT, 2018). It belongs to the genus *Arachis* that includes 70 species all nearly diploid (Krapovickas & Gregory, 1994; Ferguson *et al*., 2004). But cultivated groundnut is an allotetraploid specie (2n = 4x = 40, genome AABB) divided into six botanical varieties. Based on the presence or absence of flowers on the main axis, these varieties are grouped into two subspecies, *hypogaea* and *fastigiata*. However, all cultivated groundnuts can be grouped into four market types based on morphological and agronomical traits: Runner, Virginia, Spanish and Valencia.

The seed, which is an important source of edible oil (48%) and protein (25%), occupies an important place in human and animal feeding (Jonnala *et al*., 2005; McKevith, 2005). Though the most abundant proteins in groundnut kernels, arachin and conarachin can be allergenic (Latif *et al*., 2013), groundnut as a source of dietary proteins (Arnarson, 2015), contributes significantly to the world food production.

Groundnut is an excellent cash crop in many developing countries for local and foreign markets because of the multiple uses. During the last decade, India had the largest area sown to groundnut (6 million ha) while China had the largest production (14.24 million tons) (FAOSTAT, 2018). In Africa, important groundnut producing countries are Nigeria and Senegal (FAOSTAT, 2018). Though breeding efforts combined with improved agronomic practices have led to a yield increase in most groundnut production areas (Janila *et al*., 2016a), some countries, following the example of Togo, are still dealing with low yields. Indeed, groundnut, like other crops, is extremely vulnerable to diseases such as Early Leaf Spot (ELS), Late Leaf Spot (LLS), rust, rosette, groundnut clump, groundnut mottle and bacterial wilt.

Among the biotic stresses, LLS and rust are fungal diseases widespread in most tropical countries (Sujay *et al*., 2012).

To meet the challenge of increasing food demand (Abberton *et al.*, 2016), there is a need to develop, through breeding programmes, new and improved cultivars that respond to environmental conditions for sustainable food production. The success of a breeding programme is dependent on the development of a gene pool with useful genes and a broad genetic base (Upadhyaya *et al.*, 2009; Sharma *et al.*, 2013). Despite the availability of elite lines in some countries, landraces are still considered to harbor useful genetic variation that can be exploited in breeding programme (Dwivedi *et al.*, 2017). Though molecular markers have become important for estimating genetic diversity (Milla-Lewis *et al*. 2010; Roomi *et al*., 2014; Ren *et al*., 2014; Bhad *et al*., 2016) and for the stresses management in groundnut (Mishra *et al*., 2015; Kanyika *et al*., 2015; Janila *et al*., 2016b, Pandey *et al*., 2017), morphological characters and physiological traits (IBPGR-ICRISAT, 1992) are still important for distinguishing groundnut varieties at the farm level and in breeding programmes (Janila *et al*., 2013).

Although the morphological, physiological and agronomic characteristics show significant variation in the six botanical varieties, certain studies have documented a low level of genetic diversity among cultivated lines at molecular level (Milla, 2003; Seijo *et al*., 2007; Tang *et al*., 2007; Grabiele *et al*., 2012). Thus, the slow progress in the molecular breeding of cultivated groundnut has been mostly attributed to the low level of detectable molecular genetic variation using molecular tools such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), and Simple Sequence Repeat (SSR) markers (Herselman, 2003; Tang *et al*., 2007). Nowadays, to the detriment of other markers, single nucleotide polymorphisms (SNPs) are becoming the markers of choice. They have the

advantage of being abundant in the genome (Brumfield *et al*. 2003; Pandey *et al*., 2014) and are amenable to high-throughput analysis enabling large population analysis.

Though low yielding varieties and LLS have been often reported by farmers and extension agents as causing important economic loss in Togo, no scientific study has been carried out to ascertain groundnut production constraints. LLS disease is caused by the fungus *Cercosporidium personatum* (Berk and M. A. Curtis) Deighton, and it is encountered everywhere groundnut is cultivated (Sujay *et al*., 2012). Though LLS and ELS disease are reported to be the most destructive diseases of groundnuts worldwide (Smith *et al*., 1992; Liu *et al.*, 2013), there is no data on yield loss related to these diseases in Togo. Although LLS could be managed by chemical control (Nath *et al*., 2013), resource-poor farmers cannot afford the use of chemical control measures due to lack of resources. As an alternative, many studies suggest that the use of host resistance is the most cost-effective and environmentally sustainable control measure (USDA, 2015). Recent advances in genetics and genomics provide molecular tools to facilitate groundnut breeding especially to exploit LLS resistance within cultivated species (Shoba *et al*., 2012; Sujay *et al*., 2012). Having said that, the success of the exploitation of the host resistance in a breeding programme relies on the nature of gene action and the breeding method used for traits improvement.

Indeed, knowledge of the nature of gene action involved in the expression of the trait to be improved is a prerequisite as the breeding method depends mostly on this factor. Then, the identification of the source of resistance with good breeding potential is required for the development of LLS resistant varieties. The estimation of combining ability helps in the establishment of appropriate breeding strategies (Arunachalam *et al*., 1984; Fasahat *et al*., 2016). Mating designs, in addition to establishing the gene action, provide breeders with estimates for general combining ability (GCA) and specific combining ability (SCA) (Sprague

& Tatum, 1942; Zhang *et al*., 2012; Fasahat *et al*., 2016). They are used in groundnut and other crops for the identification of the best parents for multiple traits of interest including LLS resistance (Wilson *et al*., 2013; Manivannan, *et al*., 2008; Mothilal & Ezhi, 2010; Azad *et al*., 2014; Patil *et al*., 2017). Some recent studies have identified general combiners that were used as donor parents for the improvement of certain physiological traits and for the development of high-yielding LLS resistant genotypes in breeding programmes (Vishnuvardhan *et al*., 2011; John *et al*., 2012). Most genetic studies of LLS resistance suggest that resistance is controlled by several recessive genes (Vasanthi & Reddy, 1997) and additive genetic variance seems to play the predominant role in the resistance (Jogloy *et al*., 1987; Dwivedi *et al*., 2002). Thus, LLS resistance within cultivated species can be exploited by identifying recombinant from cross combinations with good combining ability.

Groundnut is one of the most important legumes grown in Togo. However, while the world average yield is between 1.3 t ha^{-1} and 3.36 t ha^{-1} (FAOSTAT, 2016), in Togo, the average yield of groundnut is about 0.64 t ha^{-1} (DSID, 2015). Unfortunately, not much efforts have been deployed for the development of improved varieties that could boost groundnut production. There was no organized breeding programme and few improved varieties introduced into the country were made available to farmers. As a result, the yield of groundnut in Togo has been decreasing over the years (DSID, 2015).

Also, despite the economic importance, the existing variability among landraces has not been exploited through organized selection to address biotic constraints such as LLS in Togo. In fact, due to the absence of an active plant breeding programme for the genetic improvement of groundnut, there are no documented records of groundnut accessions grown by farmers in the country. Understanding the range of diversity and the genetic structure of the cultivated groundnut accessions in Togo is a useful prerequisite for the effective management and use of germplasm resources in the groundnut breeding programme. Recently, an interest has been put

on the development of improved varieties through an organized breeding programme that will boost the production of groundnut in Togo. Though no catalogue of the low yield causes is available in Togo, late leaf spot has often been reported by the extension agents as the most widespread disease. Despite the obvious presence of late leaf spot throughout the growing area, a detailed study is required for the confirmation of this constraint. Currently, efforts are going on to identifying farmers' preferences and production constraints, assembling germplasms, establishing test environments and identifying target population of environments. As part of the efforts, this study focused on understanding farmers' perceptions of production constraints and preference criteria, characterizing cultivated groundnut varieties and understanding genetic mechanisms for priority traits to exploit genetic diversity among groundnut accessions and farmer knowledge and expectations to develop high yielding groundnut varieties in Togo.

Specific objectives were to:

i. identify production constraints, farmers' perception of leaf spot disease, and their preferences of groundnut traits
ii. characterize groundnut accession using morphological, agronomic and molecular markers
iii. identify genomic regions associated with LLS and yield-related traits and
iv. determine the mode of inheritance of resistance to groundnut LLS disease.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Origin and biology

To date, nearly eighty-one species of groundnut, both annual and perennial, have been described including cultivated groundnut (Valls & Simpson, 2005; Valls *et al*., 2013). Cultivated groundnut is an allotetraploid while nearly all the other *Arachis* species are diploid (2n = 4x = 40, genome AABB) (Krapovickas & Gregory, 1994; Ferguson *et al*., 2004). The diploid progenitors contributed AA (*A. duranensis*) and BB (*A. ipaensis*) genomes to the cultivated groundnut (Kochert *et al.*, 1996). It is suggested that cultivated groundnut is a result of a single hybridization between the diploid progenitors followed by chromosome doubling (Kochert *et al.*, 1996; Moretzsohn *et al*., 2013). *A. duranensis* is encountered throughout South America while *A. ipaensis* has only been described in Southern Bolivia. For this reason, Western Brazil, Bolivia, Paraguay, and Northern Argentina are believed to be the centres of diversity (Gregory & Gregory, 1980; Jarvis *et al*., 2003). In addition, with adaption for survival in dry lands, groundnut is believed to have originated in tropical wetland areas (Stalker *et al*., 2016).

Cultivated groundnut is classified into six botanical varieties. Based on the presence or absence of flowers and lateral branches on the main axis, likely due to few major genes' variation (Wynne & Coffelt, 1982; Kochert *et al*., 1996), these varieties are grouped into two subspecies: *hypogaea* (presence) and *fastigiata* (absence). Subspecies *fastigiata* includes the varieties *fastigiata*, *vulgaris*, *aequatoriana* and *peruviana* while subspecies *hypogaea* includes two botanical varieties, *hypogaea* and *hirsuta*. These botanical types can be distinguished based on the plant, pod and seed features (IBPGR-ICRISAT, 1992; Krapovickas & Gregory, 1994). However, only three botanical varieties are now widely grown in the Americas, Asia and

Africa. These are subsp. *Hypogaea* var. *hypogaea*, subsp. *Fastigiata* var. *fastigiata* and var. *vulgaris* (Ferguson *et al*., 2004).

Groundnut is adapted to various environmental conditions, between latitudes 40º N and 40º S, including soil types, cropping patterns, abiotic and biotic stresses (Simpson *et al*., 2001; Liao & Holbrook, 2007; Mallikarjuna & Varshney, 2014). The optimal growth temperature is between 25 and 30ºC. Though groundnut is a self-pollinated crop, up to 10% of cross pollination can occur under field conditions when bees' activity is high (Knauft *et al*., 1992). Following fertilization, the peg carrying the fertilized ovule appears 4 – 10 days and grows to enter the soil to a depth of 2-4 cm and develops into pods (Pattee & Mohapatra, 1987; Xi, 1991). Sixty to eighty days after fertilization, pods should be mature. However, at the harvest, it is common to see pods at multiple maturity stages as flowering is indeterminate on groundnut.

2.2 Groundnut cytogenetics

Tetraploid (2n = 4x =40) and diploid (2n = 2x=20) species are the common species, though not the only ones, in the genus *Arachis* (Krapoviackas *et al*., 2009). Diploids are likely more ancient than tetraploids. Species of section *Arachis*, including cultivated groundnut, have metacentric chromosomes ranging from 1.4 to 3.9 nm. Three genomes have been identified in the section *Arachis*: A genome, B genome and D genome (Lavia & Fernandez, 2008). With a very large and complex genome, cultivated groundnut is an allotetraploid species (2n= 4x= 40, AABB) which cytologically behaves as a diploid. However, Leal-Bertioli *et al*. (2015) using both genetic and gene expression data, showed that groundnut can display tetrasomic genetics recombination and concluded that this genetic behaviour accounts for many of the "off types" observed in the fields.

2.3 Groundnut genetic resources

Groundnut genetic diversity is large in the centres of origin (Krapovickas & Gregory, 1994). Thus, efforts have been deployed for the identification and assembling of cultivated landraces in the areas of origin and in other places where groundnut is cultivated (Holbrook & Stalker, 2003). These accessions, which can be separated based on seed and pod morphology (Stalker & Simpson, 1995), are collected from farmers or local markets. The common method of groundnut collection has been to gather groundnut samples in small markets and from farmers. As farmers grow an array of genotypes, the diversity found in the collected germplasm is generally large (Holbrook & Stalker, 2003). When collected from farmers' fields, a greater amount of agronomic data can be obtained. Because of expeditions and collections in different places of the world, many collections are maintained or stored in different places around the world. The largest genetic resources collections, including elite germplasms, are maintained in gene banks such as International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) in India (Upadhyaya *et al*., 2011). However, groundnut germplasm is stored in many other gene banks all over the world (Holbrook, 2001).

Preservation of groundnut accession is generally simple as seeds can remain viable for more than 15 years under optimal conditions (Stalker *et al*., 2016). A large germplasm is generally difficult to phenotype because of the cost associated with evaluating all accessions for traits of interest in cultivar development. A core collection has been proposed as an alternative (Holbrook, 1999) for handling a reduced number of lines for cultivars development for traits of interest such as resistance to LLS (Holbrook & Anderson, 1995).

In Togo, as a consequence of the lack of a strong breeding programme, there is no groundnut collection. Recently, efforts have been put towards establishing a breeding programme that will respond to farmers' expectations. As a starting point, groundnut landraces are being collected

throughout the country and a core collection, useful for the breeding programme, will be established.

2.4 Genetic diversity of important traits in groundnut

Genetic variability is the base of the success of a breeding programme targeting the improvement of a particular trait. Therefore, knowledge of the genetic variation of germplasm is important for improvement, studies and conservation purposes (Falconer & Mackay, 1996). Morphological characters and physiological traits have been extensively used for grouping groundnut varieties into classes (Upadhyaya *et al*., 2011).

A large amount of variation has been observed using these descriptors in different germplasm collections (Upadhyaya *et al*., 2006). Sources of variability for disease resistance have been reported for most of the diseases from results of field, laboratory and greenhouse studies (Sujay *et al*., 2012; Tshilenge-Lukanda *et al*., 2012). Genetic variability has been reported for traits such as tolerance to drought, early maturity (Upadhyaya *et al*., 2006), and tolerance to LLS (Izge *et al*., 2007; Tshilenge-Lukanda *et al*., 2012). A certain level of variability was reported for other traits such as yield attributes (Nigam & Blummel, 2010). However, morphological traits are sensitive to environmental variation. In addition, they are not always under genetic control. In contrast, genetic markers are reliable to track genomic regions associated with a particular trait because they are unaffected by environmental fluctuations.

Although there is a significant variation in the six botanical varieties for morphological traits and physiological traits, certain studies have documented a low level of diversity among cultivated groundnut at the molecular level (Herselman, 2003; Tang *et al*., 2007). The low level of diversity in groundnut has been attributed to a combination of three causes which are: barriers to gene flow from wild diploid species to cultivated groundnut (Young *et al*., 1996), polyploidization from probably a few numbers of individuals of parental species (Halward *et al*., 1991) and use of few elite breeding lines in breeding programmes (Isleib *et al*., 2001).

However, some authors have reported that delimitation of varieties on the basis of morphological and molecular characters can exhibit a certain degree of similarity (Ferguson *et al*., 2004; Kottapalli *et al*., 2011). Thus, cultivated accessions of groundnut in the gene banks and the elite lines in the breeding programmes are still considered as sources of variability for the development of improved varieties. Molecular marker data combined with phenotypic data is a powerful tool for grouping genotypes and for selection of progenitors that will generate new breeding population (Stalker *et al*., 2016).

Despite groundnut's economic importance, the information on genetic parameters of cultivated groundnut required for breeding groundnut for biotic constraints including LLS is lacking in Togo. In fact, due to the absence of an active plant breeding programme for the genetic improvement of groundnut, there are no documented records of groundnut accessions grown by farmers in Togo.

2.5 Gene action in groundnut

Type of gene action involved in the expression of a trait is one of the considerations among others, which the breeder must look at when starting a breeding programme. Indeed, the success of a breeding programme depends on the genetic gain per selection cycle, which in turn depends mostly on how genes act or interact in the production of a phenotype (Falconer & Mackay, 1996). Also, identification and selection of parental lines can be based upon an evaluation of the performance of its offspring (Fasahat *et al*., 2016). Along with the establishment of the gene action, combining ability determined through a specific mating design offers the possibility to identify and select parent genotypes with good potential (Sprague & Tatum, 1942). This strategy has been used extensively in the development of improved cultivars of many economically important crops including corn, wheat and cotton (Khan *et al*., 2009; Gowda *et al*., 2010).

Mating designs are widely used to partition the genetic influences of a genotype into additive and non-additive components. According to Sprague and Tatum (1942), general combining ability (GCA) is related to the activity of additive genes effects as well as additive × additive interactions while specific combining ability (SCA) underlines loci with dominance non-additive effects and all the epistatic interaction components. For self-pollinated crops such as groundnut, a high GCA estimate may result in fewer gene interactions and higher achievement in selection (Fasahat *et al*., 2016).

In groundnut, likely due to the lack of effective phenotyping tools, few studies on the combining ability for traits of interest have been conducted. Also, the first studies carried out on groundnut focused mainly on the understanding of the genetic nature of yield-related traits (Dwivedi *et al*., 1989; Anderson *et al*., 1993a). Dwivedi *et al*. (1989) found that additive genetic effects largely controlled fruit and seed traits and identified parents with good breeding potential for pod and seed traits. Gene action was assessed for other traits of interest such as disease resistance, drought resistance and qualitative traits. Anderson *et al*. (1986) attributed early and late leaf spot resistance to additive genetic variance and identified best parents for incorporating both disease resistance into improved cultivars. Jogloy *et al*. (2000) ran a combining ability study and identified lines suitable for both LLS resistance and agronomic traits. Although additive gene action is more important in most of the aforementioned traits, non-additive gene effect has been reported for some of the agronomic traits (Azad *et al*., 2014). Because of the allotetraploid and self-pollinated nature of groundnut, the dominance effect cannot be exploited in the development of improved varieties as hybridization remains labour consuming.

Different breeding populations can be used for the estimation of the genetic parameters. However, F_1 hybrids are the most common plant populations used for the estimation of genetic parameters, mainly when heterosis is desired (Sprague & Tatum, 1942; Fasahat *et al*., 2016;

Patil *et al*., 2017). But the self-pollinated nature of groundnut coupled with the low number of seeds that can be obtained from each cross has forced some breeders to use F_2 and even F_3 population in diallel and North Carolina mating designs (Anderson *et al*., 1986; Jogloy *et al*., 2000). One of the major constraints of running North Carolina mating design with F_2 generation is the sample size which should be as large as possible in order to give accurate estimates of the cross means.

As far as the environment is concerned, a majority of traits are polygenic, therefore highly influenced by environment (Piepho *et al*., 2008). Therefore, the evaluation of the progenies for parents' identification should consider environmental influence. This aspect is really important in all breeding programmes as it affects the genetic gain in groundnut and other crops. Generally, stable varieties with the least GXE interaction are desirable (Piepho *et al*., 2008).

2.6 **Use of molecular markers for groundnut improvement**

It is obvious that genetic markers have increased the pace and precision of crops' genetic analysis. As a result, the use of molecular markers has tremendously increased and facilitated the development of improved cultivars with multiple traits of interest (Mammadov *et al*., 2012). However, the use of markers in groundnut improvement is in the nascent stage (Stalker *et al*., 2016). Microsatellite markers have been considered as the marker of choice in groundnut for diverse uses (Gimenes *et al*., 2007; Pandey *et al*., 2012).

Along with diversity purpose, molecular studies were conducted for the understanding of genetics underlying agronomic traits in groundnut and the association of markers with traits. The first marker-trait association report in an *A. hypogaea* x *A. hypogaea* cross was for black rot (*Cylindrocladium parasiticum*) and ELS (*C. arachidicola*) (Wynne *et al*., 1979). Since then, many studies have been carried out for marker discovery for traits of agronomic importance. QTLs for plant architecture, days to flowering, pod and seed shape, and yield-related traits

were found. Markers were also identified for traits such as pod size and seed size (Gomez *et al*., 2009; Fonceka *et al*., 2018) and for introgression of wild segments into cultivated groundnut (Fonceka *et al*., 2012). Other positive QTLs for biotic and abiotic stress management were recently identified (Mallikarjuna & Varshney, 2014; Stalker *et al*., 2016).

However, only recently, the identification of some major QTLs for LLS diseases has been reported (Sukruth *et al*., 2015). Though no marker has proved to be efficient in marker-assisted selection for groundnut foliar diseases, DNA markers linked to LLS have been identified (Gajjar *et al*., 2014; Varshney *et al*., 2014; Kanyika *et al*., 2015). For many groundnut foliar diseases such as leaf spot and viral disease, despite several years of effort, there is no validated marker that has proved to be efficient across different breeding programmes. The availability of cost-effective genomic resources, such as single nucleotide polymorphism (SNP) and genotyping by sequencing (GBS), is expected to speed up markers identification in groundnut (Pandey *et al*., 2012; Mishra *et al*., 2015; Stalker *et* al., 2016).

SNP markers have the advantage of being abundant in the genome (Brumfield *et al*. 2003) and are amenable to high-throughput analysis enabling large population analysis. Also, SNPs linked to the coding regions can be more efficiently used in breeding methodologies such as diversity study than other markers (Luikart *et al*. 2003). In addition, SNPs low mutation rate often results in more accuracy in the estimation of population structure parameters (Coates *et al*., 2009). Diversity array technology (DArT), one of the GBS tools recently developed (Jaccoud *et al*., 2001), has proved to be efficient to evaluate genetic diversity, relationships and population structure in crops such as wheat (Novoselovic´ *et al*., 2016; Nielsen *et al*., 2014) and bean (Brinez *et al*., 2012; Nemli *et al*., 2017). In groundnut, this technology has been mainly used in genome-wide association and trait mapping (Pandey *et al*., 2014; Shasidhar *et al*., 2017). In addition, GBS has the advantage of exhibiting low per sample cost. Once additional molecular markers tightly linked to the traits of interest and compatible with high-

throughput platforms are designed, the integration of the marker-assisted selection into groundnut breeding programmes will be more significant.

2.7 Constraints and genetic improvement of cultivated groundnut

Groundnut breeding history can be traced back to 20th century where the use of improved varieties has prevailed in most groundnut production areas (Mallikarjuna & Varshney, 2014). Yield has been and is the common target trait for most breeding programmes. Therefore, traits affecting yield such as disease resistance and resistance to biotic stresses have been targeted for years (Isleib *et al*., 2001).

Late Leaf Spot [*Phaeoisariopsis personata* (Berk & Curtis), Deighton], ELS [*Cercospora arachidicola* Hori], rust [*Puccinia arachidis* Sperg.], groundnut rosette virus (GRV), peanut bud necrosis (PBND), peanut stunt virus (PSV) and bacterial wilt [*Ralstonia solanacearum* E.F. Smith] are the most important foliar diseases on groundnut. However, LLS and ELS are considered as the most important biotic constraints in terms of economic losses in tropical areas (Shokes and Culbreath, 1997; Dwivedi *et al*., 2003). Regarding abiotic stresses, drought, low soil fertility and low temperature are the most important stresses on groundnut. One of the most important food safety issue on groundnut is aflatoxin contamination. Caused by *Aspergillus*, aflatoxin contamination occurs during production, processing, transportation and storage and can result in a severe economic loss (Holbrook & Stalker, 2003).

The aforementioned circumstances force groundnut breeders to develop cultivars with not only high yield but also with resistance or tolerance to important constraints. Several researchers estimate yield increase because of the use of improved cultivars, to have had an important economic impact throughout the world (Mallikarjuna & Varshney, 2014). Though there are more cultivars released in developing countries than in developed countries, this does not reflect the investment deployed in improved cultivars development. Indeed, most developing

countries, including Togo, are characterized by diversified production conditions and ineffective seed systems. Therefore, the large number of cultivars in groundnut production areas in developing countries is more due to the unsystematic agronomic practices as the adoption of the released cultivars is uncontrolled. Though many efforts have been deployed for the development of high yielding varieties, LLS remains one of the major causes of yield loss in groundnut in tropical areas (Pande *et al*., 2003).

2.8 Late Leaf Spot disease

2.8.1 Origin and occurrence

Late Leaf Spot is one of the most important fungal diseases impacting the production of groundnut in tropical areas (Mayee & Datar, 1988). The causal agent probably originated and evolved in South America along with its hosts (Subrahmanyam *et al*., 1995). The disease is widespread in tropical countries where it can cause yield loss up to 50 % (Backman & Crawford, 1984). Although LLS is commonly present wherever groundnut is cultivated, there can be fluctuation in the relative importance (Mayee & Datar, 1988) as LLS severity and the incidence are influenced by the climatic conditions. Some varieties could tolerate a low level of infection, but all levels of defoliation result in yield loss (Backman & Crawford, 1984). Depending on location and environmental conditions, LLS commonly attacks plant of 45-60 days old.

2.8.2 Symptoms

The disease damages plant production by reducing the photosynthetic area, through lesion formation and stimulation of leaflet abscission (Subrahmanyam *et al*., 1984). Ten days following the infection, small chlorotic spots are visible on the leaflets. Spots development takes about five days from the spot appearance to mature sporulating lesion (Backman & Crawford, 1984). The lesions of the LLS are similar to the ones of the ELS. However, many

characteristics can differentiate between these two diseases. Lesions of LLS disease are usually smaller, nearly circular (up to 8 mm in diameter) and darker in colour than those of the ELS disease (Mayee & Datar, 1988). Though a distinctive chlorotic halo is often present around ELS lesions, the yellow halo is not a good diagnostic tool since similar halo may be found around the spot of the LLS on the upper surface. Also, the prominence of the halo is influenced by the host-pathogen interaction (Mulder & Holliday, 1974).

The characters used for the diagnosis of the disease in the field are the colour of the lesions on the abaxial leaflet surface and the distribution of fruiting structures. Lesions of LLS are black with rough appearance and the fruiting structures are in circular rings on the abaxial surface unlike the lesions of ELS which are light brown in colour with fruiting structures randomly distributed (McDonald *et al*., 1985). The most relevant feature of the LLS lesion is the dark stroma formed at the conidial state. Lesions of LLS disease may be observed on petioles, stems, and pegs. Generally, lesions of LLS on stems vary in shape compared to lesions observed on leaves (Mayee & Datar, 1988). Stem lesions are oval to elongate and have more distinct margins on petioles and stems than the leaflet lesions. Indeed, in case of severe disease attack, chlorotic lesions become necrotic and often coalesce resulting in leaflet shed and drop (Subrahmanyam *et al*., 1984). The observed leaf drop is caused by the change in hormonal functions due to the presence of the pathogen. The defoliation occurs generally, first on lower leaves and then progresses on upper leaves (McDonald *et al*., 1985). Disease symptoms development is rapid after flowering and during pod formation.

2.8.3 Causal agents

Late Leaf Spot is a fungal disease caused by *Cercosporidium personatum* (Berk. & Curt) (Mulder & Holliday, 1974). C. *personatum* belongs to *Mycosphaerellaceae*, a family of sac fungi and to the phylum *Ascomycota*, the largest phylum. Ascomycetes are fungi that are

characterized by the production of microscopic spores inside elongated cells called as 'asci' (Crous *et al*., 2009). Asexual reproduction of the pathogen, which is the dominant form of propagation in *Mycosphaerellaceae*, favour the rapid spread of *C. personatum* into new areas (Videira *et al*., 2017). The propagation of the pathogen occurs through vegetative reproductive spores, the conidia that are dispersed by wind or water. Conidia production is restricted to the lower leaf surface where conidiophores develop concentric rings (Backman & Crawford, 1984).

C. personatum, the asexual state (Anamorph), is the most common form of the pathogen in groundnut production areas (Mayee & Datar, 1988). The existence of the perfect state (Teleomorph), called *Mycosphaerelia berkeleyii*, have been suggested in USA (Mulder & Holliday, 1974). However, in tropical areas such as sub-Saharan Africa, the pathogen exists only in the asexual state everywhere groundnut is cultivated (Mayee & Datar, 1988).

2.8.4 Epidemiology and disease cycle

Conidia produced by the conidiophores on the lower leaves are dispersed by wind, splashing water (rain), mechanical dissemination and insects (Damicone & Melouk, 2010). On a new plant tissue, conidia germinate and form germ tubes that enter host tissue through open stomata (Subrahmanyam *et al*. 1985). Then, the germ tubes penetrate the host tissue directly through the lateral faces of epidermal cells. Unlike the pathogen of the ELS, *C. personatum* does not secrete toxins that kill the host cells but produce digestive enzymes that break down organic molecules into smaller ones that are then taken up from the plant cell (Mulder & Holliday, 1974). Lesions appear as spots and become visible 10 to 14 days after the infection. Later on, the pathogen produces conidiophore and conidia for the perpetuation of the disease cycle. The new spores produced in spots serve to infect new leaves and plants (Pande *et al*., 2001).

After the production and release of the conidia, the germination occurs on newly infected tissue within 10 to 14 days when the environmental conditions are adequate (Subrahmanyam *et al*., 1995). The optimal conditions required for LLS disease occurrence and development are warm temperatures and long periods of high humidity (Damicone & Melouk, 2010). However, the pathogen can survive through inoculum reservoir on infected plant debris favouring the perpetuation of the disease from season to season (Subrahmanyam *et al*., 1984, 1995). This survival occurs by the means of conidia and mycelium (Mondal & Wahhab, 2001). It is suggested that the survival can last from 30 to 60 days on groundnut residue that are submerged under the soil but can increase up to 12 months if the residues are stored indoors (Mondal & Wahhab, 2001).

2.8.5 Disease control

Many chemical substance tests demonstrated that LLS can be effectively controlled by fungicides. Indeed, several fungicides that provide excellent control when applied adequately are available (Garren & Jackson, 1973; Damicone & Melouk, 2010). However, in addition to being harmful to the environment, these fungicides are expensive and developing countries' farmers cannot afford their use.

Thus, cultural practices and the use of genetic resistance have been adopted in most groundnut production areas for sustainable disease control. Cultural practices include the choice of less susceptible varieties, adequate irrigation, rotation of crops and management of residues by tillage. Rotation of groundnut with other crops and management of groundnut residue help delay the onset of the disease and slow its development (Damicone & Melouk, 2010). The most important component of the best cultural practices is the use of host plant resistance to managing the disease (McDonald *et al*., 1985).

2.8.6 Resistance to LLS disease

Resistance to LLS is complex (Anderson *et al*., 1993b) as it has several components contributing to the resistance. These components include initial infection, lesion size, sporulation, and defoliation (Anderson *et al*., 1993b). According to Subrahmanyam *et al*. (1985), resistance appeared to be associated with small stomatal apertures. After penetration, resistance is associated with cell wall thickening around the infection site and with the deposition of pectic substances on cell walls and in the intercellular space resulting in the difficulties for the pathogen in cell penetration. In response to *C. personatum*, Pattee & Young (1982) found that necrotic defense was operative in resistant cultivars.

Also, resistance to LLS has been often associated with late maturity and alternative branching with dark green leaves. However, a breeding effort through recombination has resulted in improved lines carrying different combinations of these features (Stalker *et al*., 2016).

2.8.7 Genetic resources for LLS resistance

Many *A. hypogaea* have been found to have a desirable level of resistance to LLS disease (Branch & Fletcher, 2001). Most of the LLS resistant accessions originated from Bolivia (Subrahmanyam *et al*., 1995; Isleib *et al*., 2001). It is suggested that the pathogen and its host have long been associated in their centres of origin and coevolved, developing complementary genetic systems (Branch & Fletcher, 2001). Though complete resistance to LLS has not been found in cultivated groundnut (Vishnuvardhan *et al*. 2011), exploration of resistance gene centres provided germplasm for varietal improvement for resistance to LLS diseases. Efforts are being made for the incorporation of the high level of LLS resistance found in wild relatives into some cultivars (Fonceka *et al*., 2012; Khera *et al*., 2018).

Recently, many efforts have been deployed at ICRISAT to broaden the genetic base of *A. hypogaea* (Malikarjuna & Varshney, 2014) through the development of synthesized tetraploids

(Shilpa *et al*., 2013). When some of these lines were crossed with cultivated groundnut, some of the progenies had LLS resistance genes. From these results, synthesized tetraploids have been considered as a good way of introgressing LLS resistance into cultivated groundnut (Shilpa *et al*., 2013). Thus, breeding progress has been made in the suppression of LLS disease but combining a high level of resistance and high yield with acceptable quality traits continues to be difficult.

In addition to the identification of the lines with good breeding potential, most genetic studies of LLS resistance suggest that resistance is controlled by several recessive genes (Dwivedi *et al*., 2002) and additive genetic variance seems to contribute predominantly to the resistance (Dwivedi *et al*., 2002; Pasupuleti *et al*., 2013). It was earlier suggested that the resistance to LLS is controlled by more than five genes (Luo *et al*., 2005). More recently, Leal-Bertioli *et al*. (2009), through a QTL analysis, concluded that LLS resistance is controlled by additive or partial dominance gene action. Other QTL studies have enabled the identification of genomic regions conferring resistance to LLS and rust (Khedikar *et al*., 2010; Sujay *et al*., 2012).

2.8.8 Identification and use of molecular markers for LLS

The use of molecular markers in groundnut breeding programmes is slowly increasing. Groundnut has been one of the most important orphan crops when talking about a genetic advance in crop improvement. Fortunately, recent decades have witnessed the deployment of molecular tools to overcome the short comings encountered in conventional breeding (Janila *et al*., 2013).

Because of the tight link between LLS and rust disease, most of the few studies conducted to map the resistance to both diseases have been carried out together (Wang *et al*., 2011; Sujay *et al*., 2012; Gajjar *et al*., 2014). For instance, Khedikar *et al*. (2010) have conducted a QTL analysis for rust and LLS using recombinant inbred line (RILs) population and reported only a

minor QTL for LLS resistance. Similarly, with the objective of identifying major QTL for LLS, Sujay *et al*. (2012) using two RILs population reported two candidate genomic regions containing the major QTLs with 10 to 62% phenotypic variance explained. More recently, many genomic regions linked to LLS resistance have been reported to be located on linkage group A03, A05 and B10 (Han *et al.*, 2018; Agarwal *et al*., 2018) through QTL analysis or Genome Wide Association Study (GWAS).

In spite of the aforementioned advances, unlike the markers for rust resistance, the validation of linked markers for LLS resistance is still awaiting. Thus, studies are still going on for the identification of new genomic regions affecting LLS resistance. Their validation will probably accelerate the process of introgression of LLS disease resistance into preferred groundnut varieties (Gajjar *et al*., 2014; Stalker *et al*., 2016).

2.9 Demand-led breeding in groundnut

The search for alternative approaches to generating information for the development of new technologies in response to the failure of most of the agricultural projects has become crucial (Frediani & Boano, 2012). As an alternative, PRA was pioneered by the Plant Science programmes of the Overseas Development Institute (PSODI) at the University of Wales in Bangor and was based on Witcombe and Joshi's work on participatory crop improvement in Asia (Witcombe *et al*., 2006). The term participatory rural appraisal has however been slightly modified over time and called Client-oriented breeding (COB) or participatory varietal selection (PVS) (Witcombe *et al*., 2006; Soleri & Cleveland, 2004). Participatory Rural Appraisal (PRA) enables local farmers, in addition to influencing the research, to carry out their own analysis (Abedi & Vahidi, 2011; Sattar *et* al., 2017). The participation of the most important stakeholders ensures the direct involvement of the project beneficiary (Almekinders

& Elings 2001; Kolech *et al*., 2017) and in turn, guarantees the adoption and rapid diffusion of new varieties (Kraaijvanger *et al*., 2016; Kolech *et al*., 2017).

There are many examples of PRAs that have enabled agricultural project leaders to identify and incorporate farmers' perception in the project development and implementation. For instance, Abedi and Vahidi (2011), through a PRA, identified labour and lack of finance as the most important constraint to the production of Bambara groundnut in Southern Guinea Savana of Nigeria. Seed spoilage of smallholder farmers and trade regulations have been identified as very important to ensure the sustainability of groundnut production through PRA study conducted by Prasad *et al*. (2006). In Malawi, a survey on farmers between 2010 and 2013 enabled Fitzgerald (2015) to make a recommendation for lasting changes in the groundnut stakeholder's life and for the development of sustainable groundnut value chains. With the goal to assess the main production constraints of groundnut in Ghana (Oppong-Sekyere *et al*., 2015), the constraints to the production such as drought and diseases were identified by farmers through a PRA study. Thus, PRA is not only conducted upstream but also downstream the projects, as it offers the opportunity to measure the efficiency of the previous projects in addition to the identification of the prevailing constraints that need to be incorporated in the new projects.

The objective of the participatory breeding approaches, since its adoption in many development programmes, was to exploit farmers' perceptions and preferences in setting the breeding goals in order to increase the chance of adoption (Morris & Bellon, 2004; Ortiz-Ferrara *et al*., 2007; Manzanilla *et al*., 2011). Also, PRA results helps the breeder in the identification of genotypes (parents) to be crossed to generate new breeding populations (Bellon & Reeves 2002; Danial *et al*., 2007).

Gender differences in farmers preferences were reported in many African countries (Aguilar *et al*., 2014; Ali *et al*., 2015; Backiny-Yitna & McGee, 2015; Oseni *et al*., 2015). Thus, as gender affects adoption of new technologies (Danial *et al*., 2007), implementing gender in breeding projects will yield greater impact on farmers (Johnson *et al*., 2016). As a consequence, studies for the identification of production constraints and farmers' preferences, should not ignore the role played by men and women (Kristjanson *et al*., 2017).

In Togo, no such study has ever been conducted on groundnut for the identification of production constraints. The new breeding programme could exploit this tool for more impactful outcomes from the breeding products to be developed.

2.10 Groundnut production in Togo

Groundnut is mainly cultivated in mixed cropping with cereals in the northern part of Togo. However, recent statistics revealed that farmers in the southern part are increasingly interested in growing groundnut (DSID, 2015). Both male and female farmers of various ages are involved in groundnut production which occurs from June to September.

Groundnut yield has drastically increased in many parts of the world as a result of improved varieties development (Isleib *et al*., 2001; Wan *et al*., 2003; Mallikarjuna & Varshney, 2014). In Togo, groundnut has been profitable for decades, despite the lack of a strong breeding programme probably due to the introduction of improved varieties developed elsewhere. The high profitability could also be partly attributed to the crop's low production inputs and its ability to withstand climatic conditions. Indeed, the output/input ratio of groundnut has been higher than those of many food crops in Togo (DSID, 2015). Unfortunately, for nearly two decades now, low soil fertility, biotic stresses and erratic rainfall in the context of changing climate are negatively impacting groundnut production in Togo. As a matter of fact, groundnut yield has not been increasing, rather steadily over the years. There is a big yield gap between

the world average of about 1.7 t ha^{-1}(FAOSTAT, 2018) and the average yield in Togo (0.67 t ha^{-1}). Thus, in spite of breeding efforts combined with improved agronomic practices that have led to yield increase in most groundnut production areas (Janila *et al*., 2016b), some countries including Togo are still experiencing low yields.

CHAPTER THREE

3. GROUNDNUT PRODUCTION CONSTRAINTS AND FARMERS' TRAIT PREFERENCES

3.1 Introduction

Participatory Rural Appraisal (PRA) has been an alternative approach to generating information for the development of new agricultural technologies in response to the failure of most of the agricultural projects to meet the need of farmers (Duraiappah *et al*., 2005; Frediani & Boano, 2012). It allows the beneficiary farmers to influence the direction of the research by enabling them to carry out their own analysis (Abedi & Vahidi, 2011; Sattar *et al*., 2017). In turn, the diffusion of the new agricultural technologies is insured through the involvement of the most important stakeholder (Kraaijvanger *et al*., 2016; Kolech *et al*., 2017).

The objective of the participatory breeding approaches, since their adoption, was to exploit farmers' perceptions and preferences in setting the breeding goals in order to increase the likelihood of adoption (Morris & Bellon, 2004; Ortiz-Ferrara *et al*., 2007; Manzanilla *et al*., 2011). Thus, selection of parent genotypes to be crossed for the development of breeding populations has been based on PRA results. Some authors reported difference in farmers' preferences and perception in developing countries (Aguilar *et al*., 2014; Ali *et al*., 2015; Backiny-Yitna & McGee, 2015; Oseni *et al*., 2015). These authors suggested that implementing gender in agricultural project results in greater impact on the project beneficiary (Johnson *et al*., 2016) as gender affects adoption of new agricultural technologies (Doss & Morris, 2001; Danial *et al*., 2007). Thus, studies for the identification of production constraints and farmers' preferences should not ignore differences in the roles played by men and women (Kristjanson *et al*., 2017).

In Togo, few improved groundnut varieties have been introduced and made available to farmers because of the absence of an organized breeding programme. Consequently, yields have been decreasing. Though late leaf spot (LLS) and low yielding cultivars have been blamed for the decreasing groundnut yield in Togo by extension agents, no study has been carried to ascertain production constraints. Therefore, a PRA was carried out in three Northern regions of Togo with the aim of identifying groundnut production constraints, assessing farmers' knowledge of LLS, and assessing farmers' preferred traits.

3.2 Methodology

3.2.1 Description of the study areas

The survey was carried out in Centrale, Kara and Savanes (Table 3.1; Figure 3.1). The study area is in Sudano-Sahelian zone (Adewi *et al*., 2010; Batebana *et al*., 2015) and experiences one rainy season. All the three regions share farming and trading as major socio-economic activities. However, Savanes is characterized to a lesser extent by livestock keeping. The survey was carried out in nine villages made up of three villages per region. Villages were selected on the basis of the importance of groundnut in the farming system and the representativeness of farmers using the national statistics data.

3.2.2 Data collection

Preliminary data obtained from the Directorate of National Agricultural Statistics (DSID, 2015) on groundnut production guided the survey team in the identification of the extension services and farmers. Preliminary data included groundnut farming practices and the production constraints as reported by the extension agents. The mean number of groundnut farmers was 73 per village (DSID, 2015), and 20 farmers including males and females were randomly selected and interviewed in each village from the list of farmers provided by the extension service. In total, 180 groundnut growers were interviewed from the nine villages (Table 3.1).

Farmers were individually contacted and visited according to their availability. A semi-structured questionnaire was used for individual interviews (Appendix 4A).

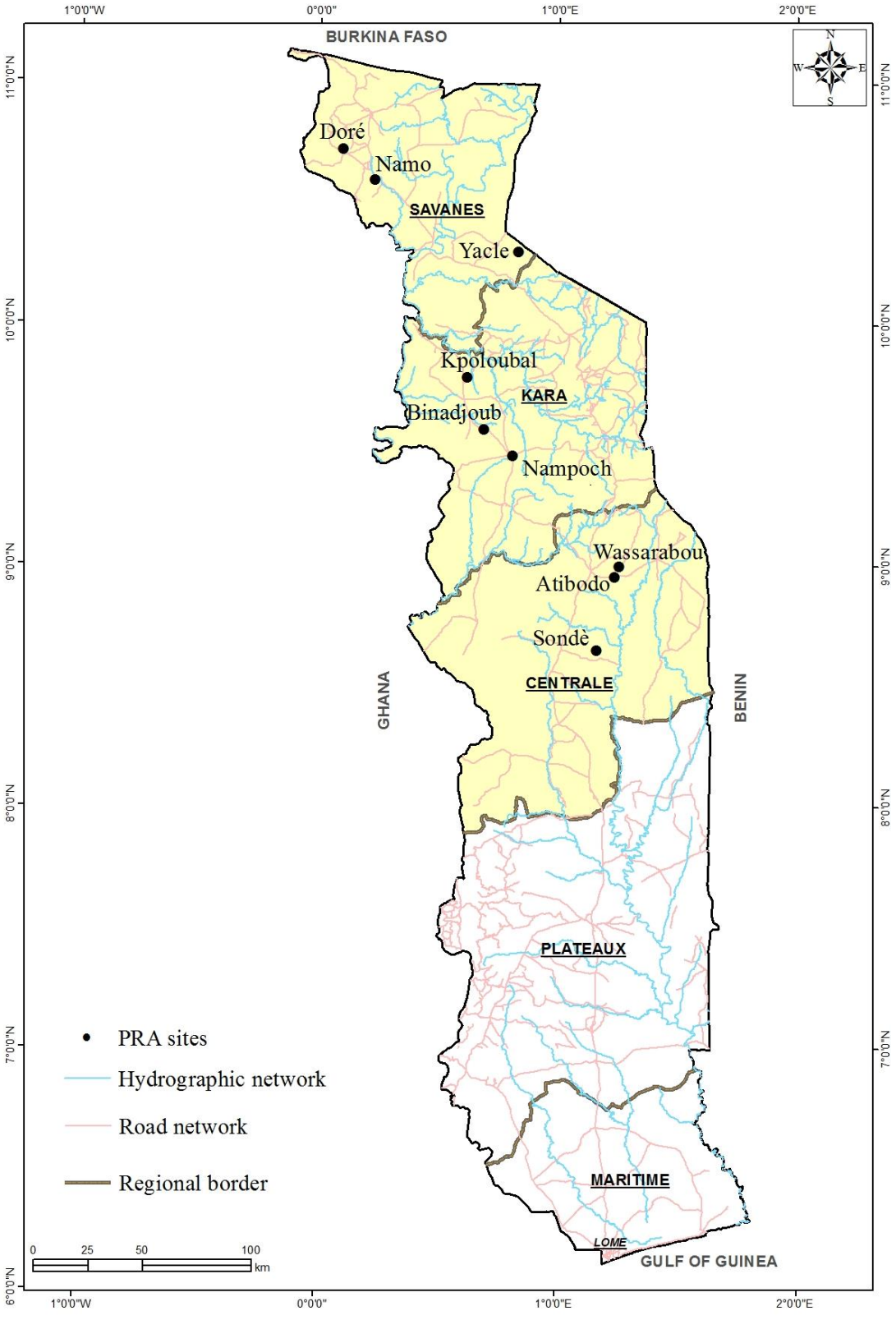

Figure 3.1 Map of Togo showing the surveyed area (ArcGIS 10.2.2)

One focus group discussion (FG) was also carried out in each village. In addition, two focal group discussions were carried in Maritime. These discussions were intended to check the assumption that farmers were interested in growing groundnut in the southern Togo. Each focus group was made up of 10 males and 10 females, thus 20 farmers per group discussion. To ensure that all topics were covered, a checklist (Appendix 4B) was used by the moderator. The FGs occurred in the morning and in the afternoon but mostly in the afternoon.

Because of their familiarity with farmers, village chiefs were of a great help in the organization of the FGs and the administration of the questionnaire. Data on the agronomic practices, cultivated varieties, the use of groundnut, the preferences and the constraints in groundnut production were collected. Farmers' preferences and their perceptions of constraints were assessed using scoring and ranking. Also, data related to farmers' knowledge and management options of diseases, with a focus on LLS, were collected. Along with the farming data, demographic information and gender differences were assessed for the perception and preferences. Groundnut seed samples were collected from farmers, where possible.

3.2.3 Data analysis

The statistical analysis was carried in R version 3.3.1 using the package "surveydata" (Lumley, 2011). Analysis of variance was carried for quantitative data collected from individual farmer interviews. For the presentation of the results, percentage and means were used. The Chi-square test was used as statistical test for the comparison of the perceptions between gender and between regions. Pearson correlation analysis (Benesty *et al*., 2009) was carried for the determination of the association between social and farming system parameters.

Table 3.1 Sites of PRA and the number of respondents

Region	Village	Community	Location	No. of FG	No. of informants
Kara	Kpoloubal	Bassar	N 09.77990	1	20
			E 00.62039		
	Binadjoub	Kabye	N 09.56500	1	20
			E 00.69254		
	Nampoch	Lamba	N 09.22001	1	20
			E00.71510		
Savanes	Yacle	Moba	N 10.29908	1	20
			E 00.79510		
	Dore	Bissa	N 10.72191	1	20
			E 00.09526		
	Namo	Moba	N 10.33601	1	20
			E 00.74864		
Centrale	Wassarabou	Kotocoli	N 08.95293	1	20
			E 01.21332		
	Atibodo	Kabye	N 08.95458	1	20
			E 01.24128		
	Sonde	Kabye	N 08.562680	1	20
			E 00.973260		
Maritime	Gboto	Ewe	N 06.67739	1	0
			E 01.53206		
	Tabligbo	Ewe	N 06.588117	1	0
			E 01.499870		
Total	**12**	**7**		**11**	**180**

No. of FG = number of focus groups, No. of informants = number of farmers interviewed

For an in-depth understanding of farmers' preference and perceptions, content analysis was carried out using qualitative data collected at both individual and group level (Noble & Smith, 2014). The map of Togo showing the PRA sites was drawn in ArcGIS version 10.2.2 (Wang, 2014) using collected geographic data.

3.3 Results

3.3.1 Demographic characteristics of farmers

- ***Age of farmers***

Across the surveyed area, the most predominant age group was between 41-50 years (33.89%) followed by age group less than 40 years (28.89%) and between 51-60 years (26.11%) while age group over 61 years (11.11%) were minorities (Table 3.2). A comparison of the regions showed that the most important age group was of farmers aged between 41-50 years both in Savanes (31.67%) and Centrale (36.67%) and less than 40 years in Kara (38.33%). The statistical analysis of variance revealed a significant difference between regions ($P< 0.01$). Savanes exhibited the highest mean age, while Kara presented the lowest (results not shown). No statistical difference was observed at the village level within regions.

- ***Educational qualification of groundnut farmers and typical household size***

A large proportion of farmers (42.22%) were illiterate. Only 20.55% and 19.45% of the farmers had primary school certificate and secondary school certificate, respectively. The rest of the respondents had some form of literacy tuition. Statistical comparison between regions showed no significant differences ($\chi^2(6) = 11.79$, $p > 0.05$) though Centrale seemed more educated with 36.66% illiteracy. With respect to gender, men were more literate than women ($\chi^2(3) = 62.52$, $p = 0.001$) with the highest proportion of illiteracy in women in Kara and Savanes (Figure 3.2).

Table 3.2 Ages of groundnut farmers in the surveyed area

Region	Village	Age							
		<40		41-50		51-60		>61	
		Num.*	Perc.	Numb.	Perc.	Numb.	Perc.	Numb.	Perc.
Kara	Kpoloubal	7	35	10	50	2	10	1	5
	Binadjoub	8	40	6	30	4	20	2	10
	Nampoch	8	40	4	20	8	40	0	0
	Total/Mean	**23**	**38.33**	**19**	**33.33**	**14**	**23.34**	**3**	**5**
Savanes	Yacle	4	20	6	30	6	30	4	20
	Dore	3	15	7	35	5	25	5	25
	Namo	4	20	6	30	6	30	4	20
	Total/Mean	**11**	**18.33**	**19**	**31.67**	**17**	**28.33**	**13**	**21.67**
Centrale	Sonde	4	20	10	50	6	30	0	0
	Kitambouli	6	30	6	30	6	30	2	10
	Wassarabou	8	40	6	30	4	20	2	10
	Total/Mean	**18**	**30**	**22**	**36.67**	**16**	**26.67**	**4**	**6.66**
Grand Total/Mean		**52**	**28.89**	**61**	**33.89**	**47**	**26.11**	**20**	**11.11**

*Num = Number, Perc = Percentage

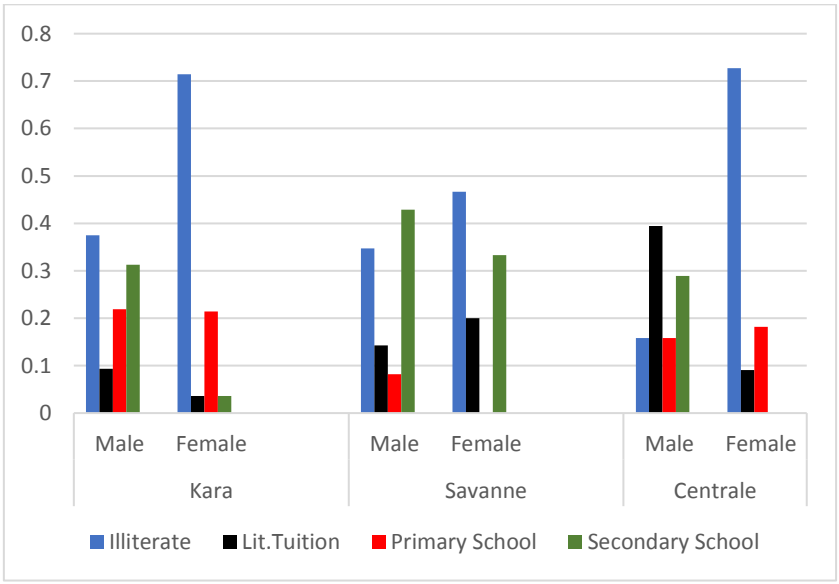

Figure 3.2 Educational qualification. *Axes are in proportion*

From the survey results, it was established that the mean family size was: nine (9) in Savanes, eight (8) in Kara and seven (7) in Centrale (Table 3.3). Analysis of variance revealed significant difference between Savanes and Kara districts (P = 0.05). Chi-square test showed no significant difference between gender for the household size.

- ***Gender and marital status of farmers***

Sex and marital status of the groundnut growers are summarized in Table 3.4. Overall, 62.22% and 37.78% of the respondents representing male and female, respectively, are engaged in groundnut production. There was no significant difference between regions on proportion of males and females ($\chi^2(2) = 4.30$, $p > 0.05$). However, in Kara, Nampoch exhibited the highest proportion of females engaged in groundnut production (80%). Marital status showed that majority of farmers were married (91.11%) with only 5.56% unmarried and 3.33% widowers. A significant difference between regions and community on the marital status was observed ($\chi^2(4) = 10.19$, $p = 0.05$). Detailed information on the marital status revealed that more than half of the married farmers were polygamous.

Table 3.3 Educational level and household size of groundnut farmers in the surveyed area in Togo.

Region	Village	Qualification										Typical Household
		Illiterate		**Lit. Tuition**		**PS**		**SS**		**Degree**		
		Num.	Perc.	Num.	Perc.	Num.	Perc.	Num.	Perc.	Num.	Perc.	
Kara	Kpoloubal	9	45	6	30	4	20	1	5	0	0	9
	Binadjoub	8	40	2	10	5	25	5	25	0	0	7
	Nampoch	10	50	0	0	8	40	2	10	0	0	6
	Total/Mean	**27**	**45**	**8**	**13.33**	**17**	**28.33**	**8**	**13.34**	**0**	**0**	**7.33ab****
Savanes	Yacle	12	60	0	0	2	10	6	30	0	0	6
	Dore	3	15	7	35	6	30	4	20	0	0	11
	Namo	12	60	0	0	2	10	6	30	0	0	6
	Total/Mean	**27**	**45**	**7**	**11.66**	**10**	**16.66**	**16**	**26.68**	**0**	**0**	**8.33a**
Centrale	Sonde	9	45	4	20	3	15	4	20	0	0	7
	Attibodo	7	35	6	30	3	15	4	20	0	0	6
	Wassarabou	6	30	7	35	4	20	3	15	0	0	6
	Total/Mean	**22**	**36.66**	**17**	**28.33**	**10**	**16.67**	**11**	**18.34**	**0**	**0**	**6.33b**
Grand total/mean		**76**	**42.22**	**32**	**17.78**	**37**	**20.55**	**35**	**19.45**	**0**	**0**	**7.33**

*Num = Number, Perc = Percentage; **Means within a column followed by the same letter (s) are not significantly different

Table 3.4 Gender and marital status of groundnut farmers in the surveyed area in Togo

Region	Village	Gender				Marital status					
		Male		Female		Married		Single		Widower	
		Num.	Perc.	Num.	Perc.	Num.	Perc.	Num.	Perc.	Num.	Perc.
Kara	Kpoloubal	15	75	5	25	20	100	0	0	0	0
	Binadjoub	13	65	7	35	19	95	1	5	0	0
	Nampoch	4	20	16	80	20	100	0	0	0	0
	Total/Mean	**32**	**53.33**	**28**	**46.67**	**59**	**98.33**	**1**	**1.67**	**0**	**0b**
Savanes	Yacle	18	90	2	10	18	90	0	0	2	10
	Dore	12	60	8	40	18	90	2	10	0	0
	Namo	13	65	7	35	15	75	2	10	3	15
	Total/Mean	**43**	**71.66**	**17**	**28.33**	**51**	**85**	**4**	**6.67**	**5**	**8.33a**
Centrale	Sonde	16	80	4	20	18	90	2	10	0	0
	Attibodo	11	55	9	45	19	95	0	0	1	5
	Wassarabou	10	50	10	50	17	85	3	15	0	0
	Total/Mean	**37**	**61.66**	**23**	**38.33**	**54**	**90**	**5**	**8.33**	**1**	**1.67b**
Grand Total/Mean		**112**	**62.22**	**68**	**37.78**	**164**	**91.11**	**10**	**5.56**	**6**	**3.33**

*Num = Number, Perc = Percentage; **Means within a column followed by the same letter (s) are not significantly different.

3.3.2 Practices in groundnut production

Land preparation for groundnut cultivation usually starts around April (in Central), May in Kara or June in Savanes with the onset of the rainy season. The main equipment used were axes, hoes and tractors. In addition, the use of ox-plows was common in Savanes. Usually, sowing is done after plowing and the first rains, and weeding was carried out twice or thrice if necessary. All the described activities were carried by both men and women. There was no use of inorganic fertilizers on groundnut. However, in mixed cropping with maize or sorghum, groundnut can benefit from the fertilizers applied to those cereals. Unlike Kara and Centrale, where manure incorporation is not common, Savanes is characterized by the systematic incorporation of manure in some areas.

3.3.3 Farm characteristics

Groundnut farm size ranged from 0.44 hectare to 1.66 hectares in the surveyed area (Table 3.5). Statistical difference based on the analysis of variance was observed between regions (P = 0.001) and between community (P = 0.01). The largest groundnut plots were observed in Savanes (1.35 hectares) and the smallest in Centrale (0.6 hectare) and Kara (0.58 hectare). Gender analysis revealed a clear difference in land access as men had larger land than women (Figure 3.3).

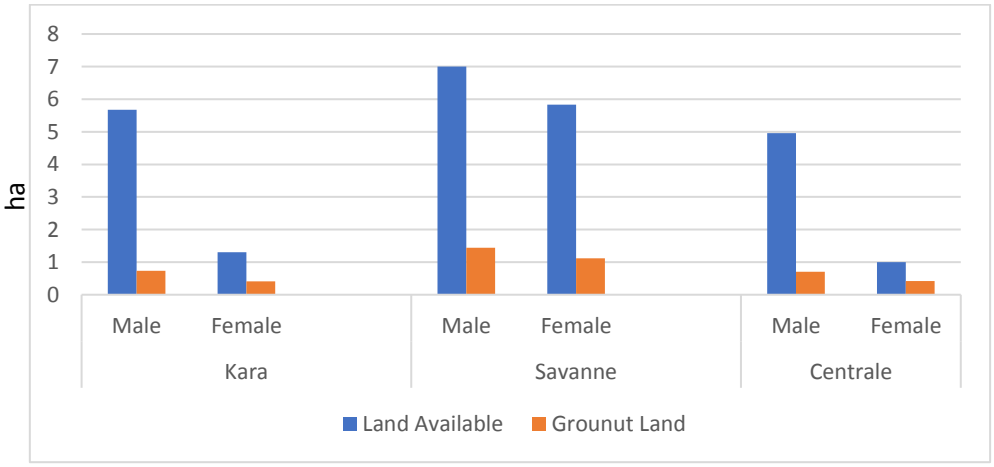

Figure 3.3 Land available for agriculture and land area (ha) used for groundnut production as estimated by interviewees

Table 3.5 Farm size, seed sources, cropping system and proportion of groundnut sold

Region	Village	Farm size (ha)			Seed Sources			% M. C.**	Sold proportion
		Smallest	Mean	Largest	Market	Saved	Others		
Kara	Kpoloubal	0.25	0.51	0.75	30.00	60.00	10.00	60.00	60.00
	Binadjoub	0.25	0.71	2.00	45.00	50.00	5.00	95.00	75.5
	Nampoch	0.25	0.52	0.75	05.00	75.00	20.00	80.00	59.2
	Mean	**0.25**	**0.58b**	**1.16**	**26.67**	**61.67**	**11.66**	**78.33**	**64.90b**
Savanes	Yacle	1	1.47	2.00	20.00	65.00	15.00	60.00	93.50
	Dore	0.5	1.12	2.00	25.00	75.00	0.00	7.00	83.75
	Namo	1	1.47	2.00	30.00	70.00	0.00	53.00	93.50
	Mean	**0.83**	**1.35a**	**2.00**	**25.00**	**70.00**	**5.00**	**40.00**	**90.25a**
Centrale	Sonde	0.25	0.77	2.00	85.00	5.00	10.00	35	82.50
	Attibodo	0.25	0.53	1.00	65.00	25.00	10.00	40	93.6
	Wassarabou	0.25	0.49	1.00	45.00	40.00	15.00	30	88.75
	Mean	**0.25**	**0.6b**	**1.33**	**65.00**	**23.33**	**11.66**	**35**	**88.28a**
Grand Mean		**0.44**	**0.84**	**1.66**	**38.89**	**51.67**	**9.44**	**51.11**	**81.14**

**% M. C.: % Mixed cropping

*Means within a column followed by the same letter (s) are not significantly different.

Most of the farmers relied on own saved seeds (51.67% of farmers) for next season planting. However, 38.89% of the farmers reported purchasing seeds from the surrounding markets (Table 3.5). The remaining farmers (9%) obtained their seeds for planting by the means of borrowing or donation from Non-governmental organizations (NGOs). Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ), an NGO in Togo, has been often reported by the interviewed farmers as the source of the groundnut seeds. Nonetheless, there were differences between regions. The proportion of farmers purchasing seeds from market was higher in Centrale than in the other regions ($\chi^2(4) = 32.52$, $p = 0.001$). Also, a slight gender difference was observed as women tended to obtain planting materials by means of donation and borrowing than men (Figure 3.4).

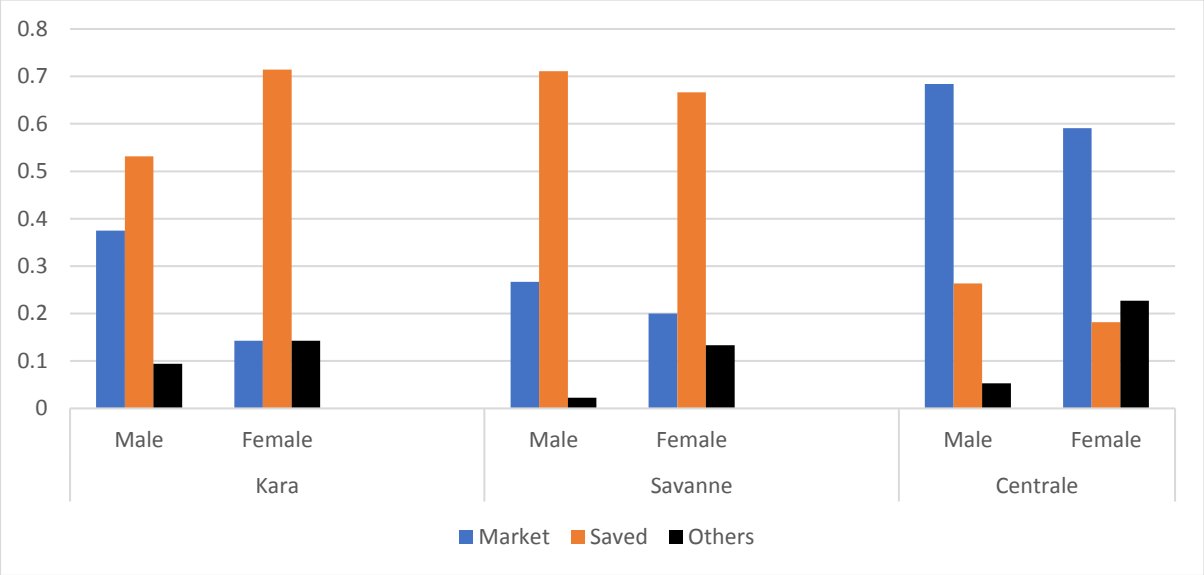

Figure 3.4 Source of groundnut seeds (in percentage)

In the surveyed area, groundnut was grown mostly in mixed cropping with cereals such as maize, millet and sorghum (Table 3.5). A large proportion of the harvest (81%) is sold at the market soon after harvest (Table 3.5). The sold proportion of the harvest was higher in Savanes (90%) and Centrale (88%) than in Kara ($p = 0.001$) where a substantial part is kept for home consumption (Figure 3.5).

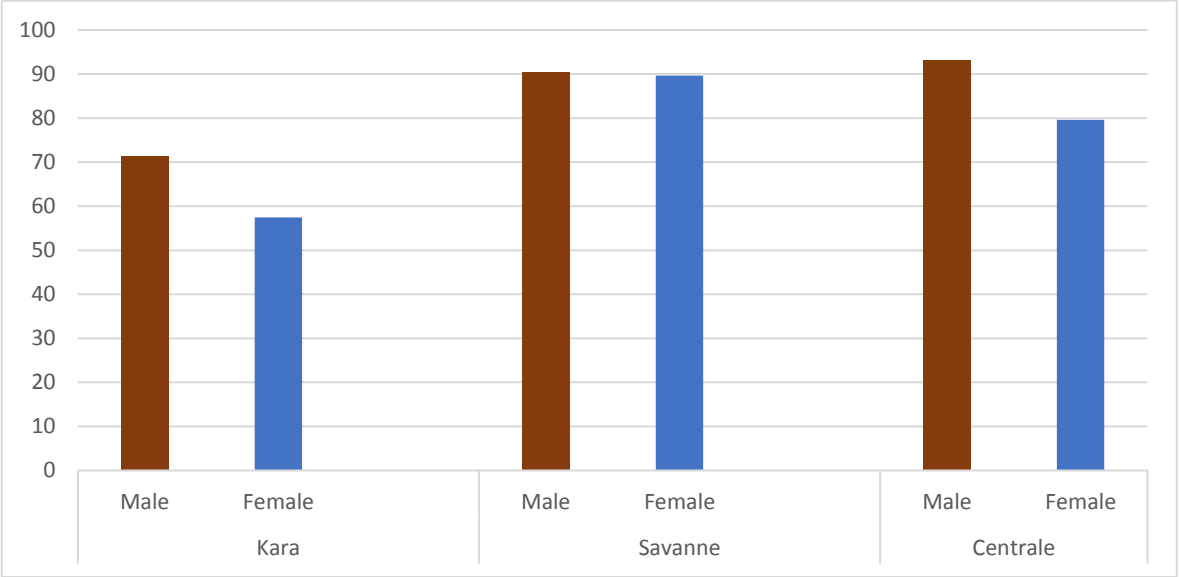

Figure 3.5 Proportion of groundnut sold

3.3.4 Cultivated groundnut varieties

The rank of groundnut based on the importance in the community varied from one region to the other. Groundnut ranked second in Kara and Savanes behind sorghum or maize and third in Centrale behind millet and maize. Assessment of the groundnut varieties grown by farmers revealed that few local landraces were cultivated in the surveyed area (Table 3.6). For instance, no more than one variety has been reported in Centrale in each of the community visited. Improved varieties, introduced from foreign countries were reported by farmers. The most common improved varieties were T3, SORAD, SOTOCO, RMP12 and ICIAR19B. The most widespread improved variety across the regions was SORAD, also called SOTOCO. These names given to the same variety were the names of the national companies that carried out the distribution of the variety when it was introduced into the country. SORAD was appreciated in the past by farmers because of the large pod and seed size. According to the farmers, the variety has become low yielding over the years. It is worthy to mention that no local improved varieties were reported by the respondents.

Table 3.6 Characteristics of cultivated varieties according to farmers in the surveyed area

Region	Villages	Varieties	Characteristics
Savanes	Yacle	Ntifofo	
		Koka	
		Soulare	
		SORAD	Big pods and grains
	Dore	RMP12	Small pods and grains
		T3	
	Namo	SORAD	Big pods and grains
		Koumongou	
Kara	Binadjoub	SOTOCO	Big pod and grains/ Low yield
		Tchamba	
	Kpoloubal	Ngbengbeng	Small pods / Low yield
		Djafo	
		Tchana	
	Nampoch	Smagbengbe	Small grains/Low yield
		Oukandjassina	
		Tchana	
Centrale	Sonde	Nale-Nale	2 kernels per pod / Low yield
	Kitambouli	Lossoketo	Small pods / Low Yield
	Wassarabou	SORAD	Big pod and grains

3.3.5 Preferred characteristics of groundnut varieties

Overall, farmers had similar preferences as yield-related traits were the most often mentioned by the respondents (Table 3.7). However, a significant difference was observed between regions based on chi-square test ($\chi^2(16) = 60.52$, $p = 0.001$). In Kara, pod yield was mentioned by 65% of the respondents as the most important. The other preferred traits in Kara were drought (13.33%), pod size (6.66%) and taste (6.66%). In Savanes, pod yield (64.44%) followed by traits such as pod size (12.22%) and high oil content (6.11%) were emphasized by

farmers. In addition to pod yield and large pod size which were the most preferred traits, disease resistance and a large number of seeds per pod were also indicated as traits of interest by farmers in Centrale. Red seed colour (by those who mentioned colour) and early maturity were also identified by farmers as important characteristics to have in mind when developing improved varieties. Comparison of the preferences between gender showed no significant differences.

Table 3.7 Percentage of time preferred characteristic of groundnut varieties were mentioned

Region	Village	Large Pod Size	Yield	Oil	Drought	Colour	Disease Resistance	Taste	Early Maturity	Seed/pod
Kara	Kpoloubal	5	55	10	20	10	-	-	-	-
	Binadjoub	5	90	-	-	-	5	-	-	-
	Nampoch	10	50	-	20	-	-	20	-	-
	Mean	**6.66**	**65**	**3.33**	**13.33**	**3.33**	**1.66**	**6.66**	-	-
Savanes	Yacle	30	60	10	-	-	-	-	-	-
	Dore	-	75	25	-	-	-	-	-	-
	Namo	25	65	10	-	-	-	-	-	-
	Mean	**18.33**	**66.66**	**15**	**-**	**-**	**-**	**-**	**-**	
Centrale	Sonde	35	50	-	-	-	-	-	10	5
	Attibodo	-	70	-	-	-	5	-	10	15
	Wassarabou	-	65	-	-	-	25	-	-	10
	Mean	**11.66**	**61.67**	**-**	**-**	**-**	**10**	**-**	**6.67**	**10**
Grand mean		**12.22**	**64.44**	**6.11**	**4.44**	**1.11**	**3.33**	**2.22**	**2.22**	**3.33**

3.3.6 Perception of farmers on constraints to groundnut production

Generally, diseases and insects were the most widespread constraints limiting groundnut production in the surveyed area (Table 3.8). Disease was identified by 37.77% of the respondents while insects' attacks were mentioned by 27.77% of the farmers. Other minor constraints mentioned by farmers were the lack of high yielding varieties by 8.33% of farmers and Striga by 4.5% of the respondents.

Though no differences were observed between gender on the perception of the constraints, chi-square test revealed that the importance of the constraints varied between regions ($\chi^2(18) = 84.70$, $p = 0.001$). Diseases were more important in Kara and Centrale while insects were considered as most important in Savanes. The mention of Striga as one of the most important constraints on groundnut occurred only in Kara (13.33% of the respondents). The other minor constraints raised by the farmers were the lack of groundnut seeds in Savanes (by 6.66% of the respondents) and the lack of high yielding varieties in Centrale (15% of the respondents).

Table 3.8 Percentage of times that constraints were mentioned

Region	Village	Disease	Insects	Striga	Drought	Low Yield	Labour	Lack of Seed	Flooding	Soil Fertility	No constraint
Kara	Kpoloubal	60	10	20	-	-	-	-	-	-	10
	Binadjoub	25	35	20	5	10	5	-	-	-	-
	Nampoch	30	20	-	20	20	-	-	-	-	10
	Mean	**38.33**	**21.66**	**13.33**	**8.33**	**10**	**1.66**	**-**	**-**	**-**	**6.66**
Savanne	Yacle	20	60	-	10	-	-	10	-	-	-
	Dore	25	25	-	50	-	-	-	-	-	-
	Namo	20	60	-	10	-	-	10	-	-	-
	Mean	**21.66**	**48.33**	**-**	**23.33**	**-**	**-**	**6.66**	**-**		**-**
Centrale	Sonde	65	15	-	-	-	-	10	10	-	-
	Attibodo	60	15	-	-	15	-	-	-	10	-
	Wassarabou	35	10	-	-	30	-	-	20	5	-
	Mean	**53.33**	**13.33**	**-**	**-**	**15**	**-**	**3.33**	**10**	**5**	**-**
Grand Mean		**37.77**	**27.77**	**4.44**	**10.55**	**8.33**	**0.55**	**3.33**	**3.33**	**1.66**	**2.22**

3.3.7 Awareness of Late Leaf Spot disease

A large majority of farmers were aware of LLS disease (95.55% of the farmers) and the three regions exhibited similar awareness of the disease (Table 3.9). Nonetheless, a large proportion of farmers ignore the cause of the disease (60.55%) and nearly all the farmers do not have measures to control the disease.

Table 3.9 Farmers' perception of LLS

Regions	Community	% of respondents knowing LLS symptoms	% of respondents knowing the cause of LLS	Available control measure
Kara	Kpoloubal	100	0	10
	Binadjoub	100	10	0
	Nampoch	90	10	0
	Total	**96.67**	**10**	**0**
Savanes	Yacle	100	60	0
	Dore	100	50	0
	Namo	100	60	0
	Total	**100**	**56.67**	**0**
Centrale	Sonde	95	70	0
	Attibodo	85	30	0
	Wassarabou	90	55	0
	Total	**90**	**51.67**	**0**
Total		**95.55**	**39.44**	**1.11**

Overall, the disease was associated to pods' maturity by 27.22% of the respondents while 7.22% mentioned drought to be the probable cause of LLS (Figure 3.6d). Further investigations on the probable cause of the disease reported by farmers, revealed that the perception of the

cause of LLS disease was not the same between region ($\chi^2(12) = 64.04$, $p = 0.001$). In Centrale and Savanes, respectively 48.33% and 30% of the farmers considered LLS symptoms as maturity symptoms (Figure 3.6a). Other causes such as drought and sun burn (Sunbeams) were mentioned respectively by 20% and 6.65% of the interviewed in Savanes (Figure 3.6b). In Centrale, divine punishment was (Div.Punishement) was reported by farmers as one the causes among others (Figure 3.6c).

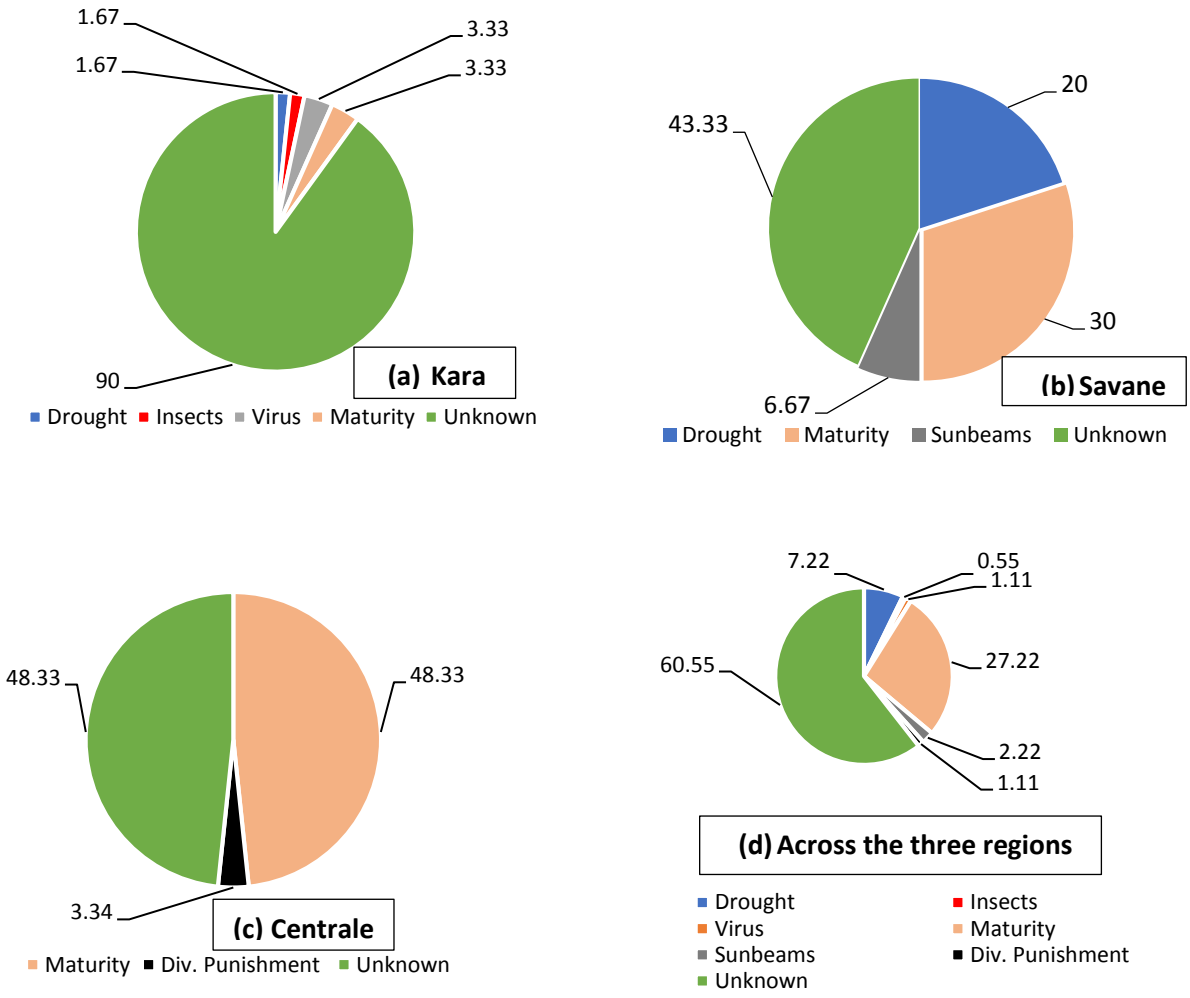

Figure 3.6 Perception on the cause of LLS disease on groundnut (figures are in percentage)

3.3.8 Correlation among social and farming system parameters

A significant correlation was observed between farmers' age and farmers' experience (P = 0.01). Similarly, a significant correlation was observed between the household size and farmers' age, the land available for agriculture and groundnut farm size, land available for agriculture and gender (Table 3.10).

Table 3.10 Correlation among social and farming system parameters

	Age	Household size	Sex	Land Available	Education	Marital Status	Groundnut Land	Farmer Exp
Age		***	*	**	NS	NS	***	***
Household size	**0.53**		NS	***	NS	***	NS	***
Sex	-0.15	-0.08		***	***	NS	***	NS
Land Available	0.30	0.30	**0.51**		***	NS	***	**
Education	0.008	0.13	0.32	0.29		NS	NS	NS
Marital Status	0.19	-0.21	0.12	-0.07	-0.11		*	**
Groundnut Land	0.19	0.04	0.36	**0.63**	0.12	0.15		***
Farmers' Experience	**0.81**	0.43	0.13	0.36	0.09	0.25	0.36	

Significance codes: '***' 0.001; '**' 0.01; '*' 0.05, 'NS' Non-significant

3.4 Discussion

A diversity of farming practices was observed in the surveyed area. These practices, though characterized by rationality, seemed untidy and unsystematic. The observed practices, in the context of the climate change, may partly be the cause of the low yield observed in groundnut production in Togo. Moreover, the changing environment calls for developing new technologies that adapt to farmers growing conditions. It is well known that the adoption of new agricultural technologies is affected by gender and educational level. This study has revealed that most groundnut farmers in Togo are illiterate. Hence, any diffusion effort of new varieties should take into account the issue in its approach. For instance, farmers in Centrale because of their high education level tend to purchase seeds than the other farmers. The tendency to purchase seeds from market could be translated as the propensity to adopt new improved varieties if available. Preliminary socio-economic data analysis revealed that most of the farmers in Centrale originated from Kara and nearly all of them or their parents have migrated in search of agricultural land. This assumption was confirmed by this survey as Kara exhibited the smallest land surface available for agriculture.

Also, there was a clear gender bias in land ownership as men had larger lands available for agriculture than women. Doss *et al*. (2015) and Kieran *et al*. (2015) previously reported similar findings. The correlation between gender and land ownership highlight that gender should not be ignored when implementing agricultural projects. However, in some areas such as Savanes, the gender bias was very low. Financially sound women in Savanes, who are mostly traders, own plots as large as men's plots. In Nampoch, it was reported that, because of the decreasing yield in groundnut production, men are shifting to more profitable crops such as soybean and yam. Mainly because of the home consumption, and also likely marginalized by the development of cash crops

(Wooten, 2003), women in that locality are forced to keep growing groundnut. The observed bias between gender for access to groundnut seeds and educational level underlines the necessity to develop and diffuse breeding products with gender in mind (Kristjanson *et al*., 2017).

Except slight differences, farmers' preferences were very similar across the surveyed areas. Generally, yield-related traits such as pod yield and large pod were the most preferred. Also, a large number of pods per plant is viewed by farmers as yield selection criterion. Region wise, respondents in Kara preferred taste and drought resistant in addition to the yield-related traits. In the contrast to the other regions, a substantial proportion of groundnut production is locally consumed in Kara. This could explain why taste is a valued trait for farmers in that area. In Savanes and Centrale, secondary traits such as disease resistance, number of seeds per pod, and oil content were raised as traits to be incorporated into improved varieties. The diversity of farmers preferences in Savanes and Centrale could be related to the groundnut uses and the market demand because nearly all the production in these areas is sold. In Savanes, groundnut buyers come mainly from the surrounding foreign countries such as Ghana and Benin. In contrast, farmers in Centrale sell their groundnut to local buyers and processors. In case of home consumption, groundnut is mainly used for sauce in the surveyed area. However, boiled and roasted groundnut as well as processed into cake are also the common use of groundnut throughout the country. Thus, the diversity of the use of groundnut could partly explain the slight differences in farmers' preferences.

Overall, farmers face a wide range of constraints in groundnut production. Nonetheless, diseases, insects and drought could be considered as the most important factors limiting groundnut production according to the farmers. Recently, a survey carried out in Ghana in groundnut production revealed similar results (Yussif *et al*. 2014; Oppong-Sekyere *et al*., 2015) with drought, low yield and diseases raised by farmers as the limiting factors in groundnut production. The

perceptions of farmers on the importance of the constraints were not the same from one region to the other. In fact, insects were considered as the major constraint in groundnut production in Savanes. The explanation of this perception could be found in the untidy agronomic practices in that area. Harvested groundnut is left in the field for up to fifteen days for drying and facilitating of easy pod stripping. Farmers in that area reported that the attacks of the insects occur during the period between harvest and pod stripping. This issue could be addressed by adopting best agronomic practices.

Nonetheless, insects' attacks have been reported during storage in the other regions. This forces farmers to sell their groundnut soon after harvest in order to relieve themselves from quality maintenance and storage insects' issues. In addition to the storage issues, farmers often sell their groundnut soon after harvest, when the price is low, in order to meet school fees and healthcare costs. There is a need to finance the development of groundnut storage facilities. These facilities were made available to farmers, through agricultural credits, for other crops such as maize (ROPPA, 2013). Extending these credits on groundnut will likely help farmers increase their income through the reduction of the loss in storage. Also, such facilities will allow farmers to keep their harvest for a higher price in the future. The profitability of such initiative depends also on the collaboration between a strong breeding programme, an efficient extension service and the farmers for coordinated agricultural activities. Though clearly defined groundnut farmers organization does not exist in the surveyed area, the existence of other organizations of which most farmers are members could be exploited by the research and the extension service.

In Kara, Striga has been strikingly mentioned by some farmers as a constraint on groundnut production. The perception of striga as a constraint on groundnut may be partly related to the cropping system in that region. Indeed, farmers in Kara mostly grow groundnut in mixed cropping

with other cereal such as maize and sorghum. Thus, *Striga hermonthica* could have been mistaken to affect groundnut. Therefore, cereal breeding programmes in Togo should be aware that improved varieties targeting this region should incorporate Striga resistance.

Farmers are aware of the widespread status of LLS disease in their area. When asked to rank the foliar diseases on the basis of the importance, majority of farmers identified LLS as the most widespread foliar disease confirming previous reports that spotted LLS as one of the most important fungal disease in groundnut production areas (Sujay *et al*., 2012). The disease severity has increased over the years according to the respondents. Farmers in Centrale reported that there were local resistant varieties about two decades ago. Because of the changing climate, which results often times in the shortening of the rainy season (Adewi *et al.*, 2010; Batebana *et al*., 2015), new early maturing varieties were likely adopted to the detriment of the landraces with long maturity period. It is well known that LLS resistance is associated with late maturing landraces (Janila *et al*., 2013).

The cause of LLS disease seemed unknown to most of the interviewed. To farmers opinion, LLS is a natural disease occurring when the pod reached the full maturity. Farmers have been using the symptoms as an indication of the pods' maturity for long, and therefore neglected the development of disease control methods. However, farmers reported that the symptoms are no longer used nowadays as an indication of pod maturity because of the unmatured pods that were often found using LLS as indicator on the currently cultivated varieties. It is worthy mentioning that the same belief on LLS has been reported in Congo (Izge *et al*., 2007). These results underline the ignorance of groundnut farmers on the cause of the diseases affecting groundnut production. For instance, groundnut rosette has been associated with witchcraft in the surveyed area. Altogether, these

results suggest that groundnut farmers should be educated on the important diseases through an awareness creation.

Overall, few improved varieties are available for farmers and most farmers rely on the old landraces for groundnut production. SORAD, also named SOTOCO, was introduced twenty years ago to boost groundnut production. The variety was quickly adopted by farmers because of its large pod size and the high yield. This is confirmed by this survey, as SORAD was the most widespread variety in the surveyed area. However, though the variety is still cultivated, the groundnut farmers complained about its performance. SORAD is a late maturing variety that often exhibits low yield in the context of erratic rainfall. Early maturing varieties such RMP12 and T3 were recently introduced to Togo as alternatives to the existing low yielding varieties. However, this survey revealed that few farmers adopted these varieties. The main reproaches to these varieties are their small pod and seed size. It could be concluded that farmers in the surveyed area would reject improved varieties with small pod and seed size. In fact, difficulty in manual shelling and low yield were associated with small pod size. Introduced six years ago from Nigeria, Samnut 24 is another variety that is currently promoted by the research institute because of the high number of pods per plant. Unfortunately, multiple tests and field observations showed that Samnut 24 is highly susceptible to early and late leaf spot diseases. Altogether, there was a clearly expressed need for higher-yielding varieties that can boost groundnut production in Togo.

Groundnut breeders in Togo could make use of the opportunity created by the identified challenges for the development of improved varieties that will likely meet farmers' preferences. A participatory breeding involving all the stakeholders in groundnut production would probably increase the chance of adoption of the new improved varieties.

3.5 Conclusion

This study provided a basis for a participatory groundnut breeding programme through the identification of the constraints and preferences as well as the characterization of agronomic practices in groundnut production in Togo. This study has revealed that, with few exceptions, yield-related traits such as pod yield and pod size were the most important selection criteria of farmers. Diseases were considered to be the most important factor limiting groundnut production. Among diseases, farmers indicated that LLS was of economic importance. Thus, in Togo, a breeding programme on groundnut should consider that groundnut farmers perceive diseases as the major limiting factor to production. High yielding groundnut varieties that incorporate traits such as large pod size and resistance to LLS are likely to be adopted by groundnut farmers in Togo. Thus, the next studies will focus on mining the existing germplasm and breeding tools to use in the breeding programme.

CHAPTER FOUR

4. MOLECULAR AND PHENOTYPIC DIVERSITY OF GROUNDNUT CULTIVARS FROM TOGO

4.1 Introduction

The success of a breeding programme is dependent on the development of a gene pool with useful genes and a broad genetic base (Upadhyaya *et al.*, 2009; Sharma *et al.*, 2013). Despite the availability of elite lines in some countries, landraces are still considered to harbor useful alleles that need to be exploited in plant breeding (Dwivedi *et al.*, 2017), justifying the need to collect and create a core collection when starting a breeding programme. Genes discovered in diverse germplasm resources can be introgressed into farmers' preferred varieties for the improvement of yield and other traits of interest (Cobb *et al.*, 2013).

Morphological and molecular diversity analyses have been very useful in characterizing germplasm collections and identifying potential parents for a breeding programme. Morphological characters and physiological traits are visible descriptors that are important for diversity studies. Also, morphological descriptors remain important for identifying accessions at the farm level, as farmers rely on observable differences to describe landraces. In addition, the knowledge of the importance of an agronomic trait, in terms of contribution toward diversity, is becoming very crucial in plant breeding (Dwivedi *et al.*, 2017).

Nowadays, molecular markers have become more important for estimating genetic diversity (Milla-Lewis *et al.* 2010; Roomi *et al*., 2014; Ren *et al*., 2014; Bhad *et al*., 2016). They complement morphological markers in a diversity study. Among the available markers, single nucleotide polymorphisms (SNPs) are becoming the marker of choice to the detriment of other

genetic markers. SNPs linked to the coding regions can be more efficiently used in breeding methodologies such as diversity study than other markers (Luikart *et al*. 2003). In addition, SNPs low mutation rate often results in more accuracy in the estimation of population structure parameters (Coates *et al*., 2009). DArTseq-based SNPs is one of the low-cost microarrays-based methods used for genetic studies including genetic diversity and structure analysis in many crops (Jaccoud *et al*., 2001) that has proven to be efficient.

As a starting point for the new breeding programme, diversity assessment of the cultivated groundnut accessions from Togo, using morphological and molecular marker traits, was the aim in the present study. The activities focused on collection of groundnut accessions grown by farmers; description of accessions and classification into groups; assessment of the interrelationships among accessions, among traits and the estimation of morphological and molecular diversity in the collected germplasm.

4.2 Materials and Methods

4.2.1 Collection of accessions

The identification of the collections area was based on the importance of groundnut in local farming system and 'visiting a maximum number of sites' has been adopted as collection strategy. In each region, the collection sites were determined with the aid of the agricultural extension agents. A meeting was organized with the extension service to define the best itinerary that would facilitate the capture of the diversity of groundnut genotypes grown in the area. Thus, forty-one villages from four regions were visited: 16 villages in Savanes, 14 in Kara, 8 in Centrale, and 3 in Maritime (Figure 4.1). In each village, groundnut germplasm (seeds and pods) were obtained from farmers, mostly in storages or from local markets that are nearly 10 km apart. Shelled groundnut samples were mostly found at the market while samples from farmers' storage were, in a large

part, unshelled. Geographical coordinates were collected using a GPS instrument. In addition, a data form was used to collect data such as name of the locality, agroecological zone, geographical zone, local name of the accession, ethnic group and farmer's name. Overall, 126 groundnut samples were collected and put in envelops before their transportation to the Centre de Recherche Agronomique de la Savane Seche (CRASS), the arid savanna agronomic research centre in Kara, Togo.

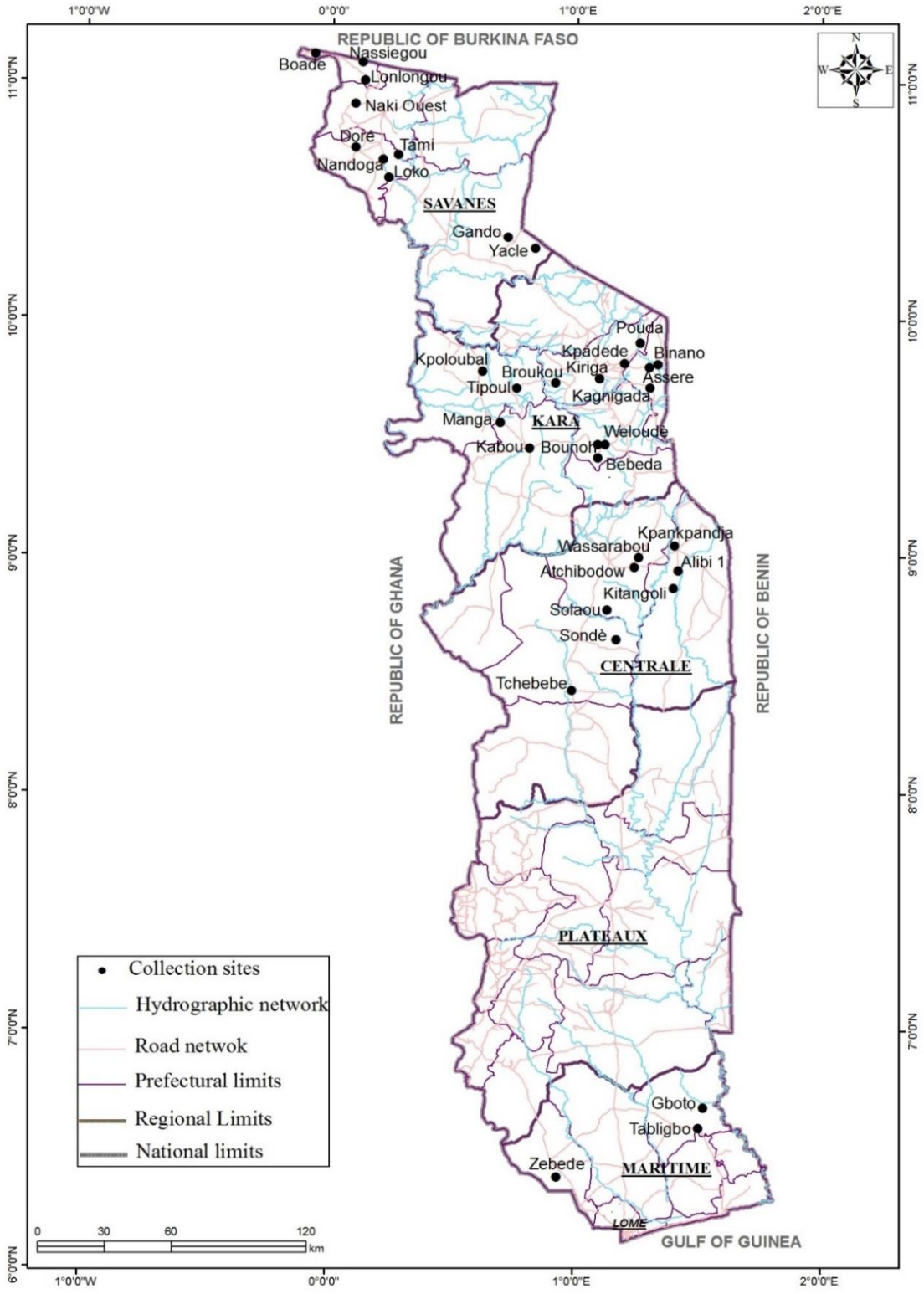

Figure 4.1 Map of Togo showing the collection sites (Source: Banla, ArcGIS 10.1)

4.2.2 Plant material

Prior to the morphological characterization, a preliminary field evaluation was carried out for the 126 collected groundnut samples and 9 lines from Senegal, obtained from the Togolese Agronomic Research Institute (ITRA-CRASS). Collected samples were reduced to 94 accessions, for facilitating genotyping on standard 96 well plate. Results of preliminary data analysis provided information for eliminating the duplicates and facilitated the identification of the 94 genotypes. The germplasm collection (94 genotypes) consisting of collected accessions from Togo (n=83), lines from Senegal (n=9) and lines from ICRISAT (n=2 only genotyped), representing three geographical regions were used in this study.

4.2.3 Morphological evaluation

The genotypes were phenotyped during the 2017 and 2018 rainy seasons. The phenotyping trials were established in LLS hot spot environments to increase the chance of disease occurrence during the growing seasons from March to June at Ativeme (6°25'22" N; 1°6'38" E). Ativeme, with 1270 mm rainfall annually and 23°C mean temperature, is characterized by a grey sandy loam soil. An alpha lattice design with three replications six blocks per replication was adopted as experimental design with two checks added to the 94 genotypes. A row of 3 meters containing 10 plants with 30 cm between plants represented an experimental unit. A spacing of 50 cm between rows and 1 m between blocks was adopted. The field was kept weed free at an interval of one weeding every three weeks. IBPGR-ICRISAT (1992) descriptors were used to assess agro-morphological characteristics. Morphological descriptors included:

- Growth habit: recorded at podding stage as 1 = spreading, 2 = bunch and 3 = erect
- Main stem: scored as 1 = presence and 2 = absence

- Number of branches: scored as 1 = primary (n+1), 2 = secondary (n+2) and 3 = Tertiary (n+3)
- Branching pattern: determined on the (n+1) cotyledonary lateral branches as 1 = Alternate and 2 = Sequential
- Stem surface: recorded as 1 = glabrous, 2 = Sub-glabrous and 3 = Moderately Hairy
- Stem pigmentation: recorded on mature plant as 1 = green, 2 = purple and 3 = mixed
- Leaflet shape: on fully expanded apical leaflet on the main stem and recorded as 1 = Narrow Elliptic, 2 = Oblong Elliptic, 3 = Wide Elliptic and 4 = Obovate
- Leaflet tip: on fully expanded apical leaflet on the main stem recorded as 1 = acute and 2 = obtuse
- Flowers on main axis: recorded on the main stem as 1 = presence or 2 = absence
- Colour of standard petal marking: colour of markings on the front face of the standard petal
- Pod beak: recorded on dried pods as 1 = slight, 2 = moderate and 3 = prominent
- Pod constriction: recorded on dried pods as 0 = none, 1 = slight, 2 = moderate and 3 = prominent
- Pod reticulation: recorded as 0 = none, 1 = slight, 2 = moderate and 3 = prominent
- Seed colour: recorded two weeks after harvest and complete drying on mature seeds

The agronomic descriptors included:

- Plant height (cm): measured from cotyledonary axil up to terminal bud, mean of five plants recorded 75 days after planting
- Plant width (cm): measured at the widest point from branch tip to tip on 10 plants recorded 60 days after planting

- Number of primary branches: counted at 60 days after emergence on five plants
- Leaflet length: (mm): measured on the leaf of the main stem, fully expanded apical leaflet. Recorded on five leaflets from five different plants
- Leaflet width (mm), measured on the leaf of the main stem, fully expanded apical leaflet, at widest point. Recorded on five leaflets from five different plants
- Days to emergence: counted from sowing to 50% emergence
- Days to maturity: counted from emergence to maturity determined by checking 80% pods maturity
- Days to 50% flowering: counted from emergence to 50 % of plants flowering
- Pod length (mm): recorded on 10 random mature pods
- Pod width (mm): measured at the widest point on 10 random mature pods
- Pod volume (cm^3): volume of 10 fresh mature pods measured by water dispersal using a graduated test tube
- Seeds per pod: number of seeds per pod counted on 10 random mature pods
- Seed length (mm): recorded on 10 random mature seeds
- Seed width (mm): measured at the midpoint on 10 random mature seeds
- 100 pod weight (g): weight of 100 random mature pods
- 100 seed weight (g): weight of 100 random mature wrinkle-free seeds
- Shelling percentage $=$ Weight of seeds obtained from 100 pods/100 pods weight
- Pod yield (g) per plant: Mean weight of pods harvested on five random plants.

Disease scoring for LLS was carried out at 60 days and 80 days after sowing using a 9 points scale (Subrahmanyam *et al*., 1995). Data were collected at the plant level on five plants per plot for all variables except for days to emergence, days to maturity and days to 50% flowering.

4.2.4 DNA Extraction and DArT Analysis

Leaf samples were collected from three weeks old plants using the LGC plant sample collection kit and dried overnight at 35℃ in an oven. Dried samples were then sent to Integrated Genotyping Service and Support (IGSS) platform at Biosciences for east and central Africa laboratory at the International Livestock Research Institute (BecA/ILRI) for DNA extraction and genotyping using Diversity Arrays Technology (DArT) markers. Whole genome genotyping was carried out using Genotyping-By-Sequencing (GBS) technology (Elshire *et al*., 2011).

For quality control, derived DArTseq SNPs with the following criteria were removed using package "dartR" in R statistical software version 3.3.1: SNPs with call rate less than 70%, SNPs with PIC less than 0.1, and loci with trimmed sequence tags that were too similar. After filtering and quality control, 990 DArTseq SNPs were retained for the diversity study.

4.2.5 Data analysis

4.2.5.1 Morphological data analysis

Analyses for the morphological data were performed in R version 3.1.1 using packages "agricolae", "cluster", "plyr", "magrittr", "Rtsne" and "ggbiplot". Analysis of variance was run for quantitative data after imputation of the missing data (Harrell, 2017) with a linear model considering genotypes and blocks as fixed. To handle the data with different units, Gower's metric (Gower, 1971; Pavoine *et al*., 2009; Henry *et al*., 2015) and UPGMA clustering (Langfelder *et al*.,2007) were used for determining the structure of germplasm collections (Podani & Shmera, 2006; Kaufman & Rousseeuw, 2009; Johnson & Wichern, 2014). The average Silhouette method in Partitioning Around Medoids (PAM) was used for estimating the optimal number of clusters (K) and to estimate the probability of membership (Pedregosa *et al*., 2011). Cophenetic correlation

coefficient (CPCC) was used to measure how faithfully the original dissimilarity structure is preserved by the dendrogram and as an indicator for the strength of subgroup differentiation (Sokal & Rohlf, 1973; Odong *et al*., 2011). The contribution of individual traits to the diversity was assessed through principal component analysis (PCA) (Coghlan, 2013), and Simpson's diversity index (D) (Simpson, 1949) was used to estimate diversity in qualitative traits. The estimation of the correlation among variables was carried out using Pearson correlation analysis (Benesty *et al*., 2009). Path analysis was carried out for yield-related traits using structural causal models as described by Kline (2015) and Byrne (2016) using the R package 'lavaan'.

4.2.5.2 Molecular data analysis

Genetic diversity and analysis of molecular variance (AMOVA) were done in R software version 3.3.1 using package "dartR", "StAMPP", "ggplot2" and "PopGenReport". The identification of clusters of genetically similar genotypes was done using the Bayesian method in R software. The optimal number of clusters, K-value, was identified using PAM Silhouette (Pedregosa *et al*., 2011). Principal component analysis (PCA) and Neighbour-joining tree were used to reveal structure in the germplasm. The genetic distances among the clusters were calculated as Nei's minimum distance and pairwise F_{st} (Hedrick, 2005).

4.3 Results

4.3.1 Morphological diversity

4.3.1.1 Analysis of variance in quantitative traits

The analysis of variance revealed a significant variability in the collected germplasm ($p = 0.01$) for all quantitative traits. Traits of agronomic importance such as LLS, pods per plant, and pod yield showed a highly significant variation (Table 4.1). However, the coefficient of variation was very low for most traits. On the 20 quantitative traits, only four (number of lateral branches, LLS,

number of pods per plant and yield) exhibited a coefficient of variation above 20%. The coefficient of variation for days to emergence, leaflet width, pod width, and seed length and width were even close to zero.

Table 4.1 Mean square, range and coefficient of variation of quantitative traits

Traits	Mean Square		Range			CV (%)
	Genotypes (d.f=95)	Error (d.f= 175)	Min	Mean	Max	
Days Emergence	128.326*	0.023	05.33	6.50	8.66	2.331
Days Flowering	2233.39**	1.98	23.00	27.19	32.66	5.17
Days Maturity	26998.5**	2.3	80.00	94.18	120.00	1.592
Plant Height	159.837***	33.667	15.84	32.51	48.75	17.849
Plant Width	154.87**	82.53	39.55	58.69	76.53	15.477
Num.Lat. Bran.	30.15***	7.230	05.92	9.67	22.07	27.797
Leaflet Length	1.7444***	0.14	03.60	05.51	07.38	6.852
Leaflet Width	28.4667**	0.037	01.90	3.06	3.87	6.299
LLS 60 Days	3.3131***	0.7899	01.66	03.96	05.00	22.423
LLS 90 Days	59.176***	0.234	01.8	04.29	06.5	11.264
Pods / plant	298.35***	136.73	31.31	54.44	80.05	21.476
Pod Volume	2.1612***	0.082	1.98	3.23	6.05	8.889
Pod Length	0.1452***	0.014	01.95	02.42	3.28	4.94
Pod Width	0.190***	0.001	01.00	01.18	01.40	3.28
Shelling %	30.687**	4.479	64.85	73.48	88.69	2.880
Seed Length	5.047**	0.004	01.07	01.29	01.59	5.106
Seed Width	2.3124**	0.001	00.76	00.87	01.02	3.337
100 Pod weight	25.3244**	3.619	66.52	73.66	88.69	2.582
100 Seed Weight	98.740***	20.290	34.33	45.868	64.666	9.902
Pod Yield	70.021***	58.821	01.43	02.62	03.92	22.144

Significance codes: '***' 0.001; '**' 0.01; '*' 0.05, 'NS' Non-significant, Num.Lat. Bran. = number of lateral branc hes, CV = coefficient of variation, d.f = degree of freedom

4.3.1.2 Principal component analysis

Principal component analysis (PCA) underscored traits contributing mostly to the divergence in the germplasm. For the quantitative traits, the fist principal component (PC1) accounted for 32.7% and the second principal component (PC2) for 15.3% of the variance (Figure 4.2). The PC1 was strongly correlated with plant height, LLS at 90 days (LLS.90D), number of lateral branches (Lat.Brach) and days to maturity (Days_Mat). Furthermore, based on the loadings, PC1 is primarily a measure of contrast between plant height and days to maturity. The PC2 can be viewed as a measure of variance in hundred seed weight (Hun.SeedWeight), pod width (Pod.Width), pod length (Pod.length), seed length (Seed_Length) and days to flowering (Days_Flow). Both PCs accounted for 48% of the total variance. The highest loadings in the third PC were obtained with number of pods per plant and yield while PC5 strongly correlated with Days to emergence and pod volume (Table 4.2). Also, the first five PCs accounted for 76% of the total variation.

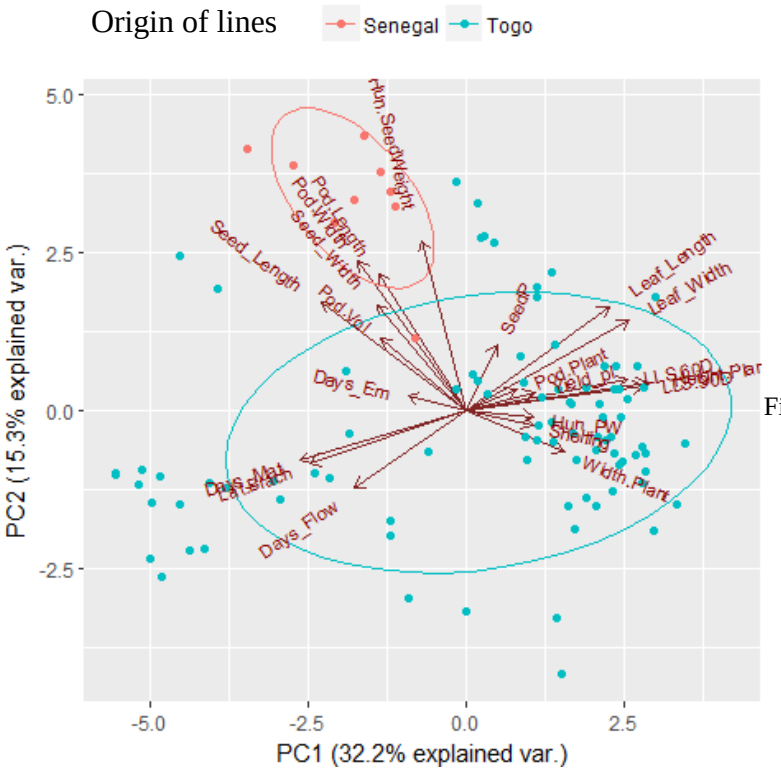

Figure 4.2 A principal component analysis showing similarity relationships among 92 accessions based on quantitative traits. Days_Em = days to emergence, Days_Mat = days to maturity, Days_Flow = days to flowering, Width.Plant = plant width, Plant_H = Plant height, Pod.Vol = pod volume, Hun_PW = hundred pods weight, Hun.SeedWeight = hundred seed weight, Pod.plant = number of pods per plant, SeedP = seed per pod, LLS.60D = LLS at 60 days, LLS.80D = LLS at 80 days.

Table 4.2 Correlation between variables and factors (Eigenvectors)

Traits	Principals components and variance explained				
	PC1 (33.68) *	**PC2 (15.7)**	**PC3 (10.6)**	**PC4 (08.9)**	**PC5 (07.10)**
Hundred pods weight	0.124	-0.029	-0.20	**0.634**	-0.032
Hundred seeds weight	-0.086	**0.465**	-0.021	0.006	-0.189
Letteral branches	-0.290	-0.145	0.218	0.100	0.059
Late leaf spot	**0.329****	0.067	-0.103	-0.078	-0.056
Pod volume	-0.162	0.174	-0.257	0.121	**0.413**
Plant width	0.182	-0.119	0.020	-0.020	**0.432**
Number of pods per plant	0.094	0.083	**0.619**	0.145	0.141
Pod width	-0.205	**0.395**	-0.065	0.024	-0.005
Pod length	-0.166	**0.360**	-0.141	0.025	**0.307**
Yield per plants	0.127	0.073	**0.572**	0.259	0.107
Seed length	-0.272	0.294	0.026	0.051	-0.092
Seed width	-0.166	**0.308**	0.230	0.055	-0.212
Leaf length	0.263	0.284	-0.030	-0.092	-0.136
Leaf width	0.299	0.257	0.065	-0.090	0.029
Days to emergence	-0.109	0.035	-0.030	0.052	0.533
Days to flowering	-0.209	-0.220	0.035	-0.107	0.082
Days to maturity	**-0.309**	-0.138	0.061	0.003	0.021
Shelling %	0.123	-0.046	-0.175	**0.649**	-0.101

**Account of PCs for the variability are in bracket. **Characters that contributed most to the variation (>0.3) of the particular component are in bold.*

For the qualitative traits, the first two PCs accounted for nearly 40.4% of the variation (Figure 4.3). The first PCA accounted for 28.1% of total variance whereby, presence of flower on main axis (FOMA), branching pattern (Branching_P), and growth habit (GrowthH) were the variables that contributed most to the divergence. The second component exhibited pod reticulation (Pod_R) and leaf shape (Leaf_S) as most important variables contributing to the divergence.

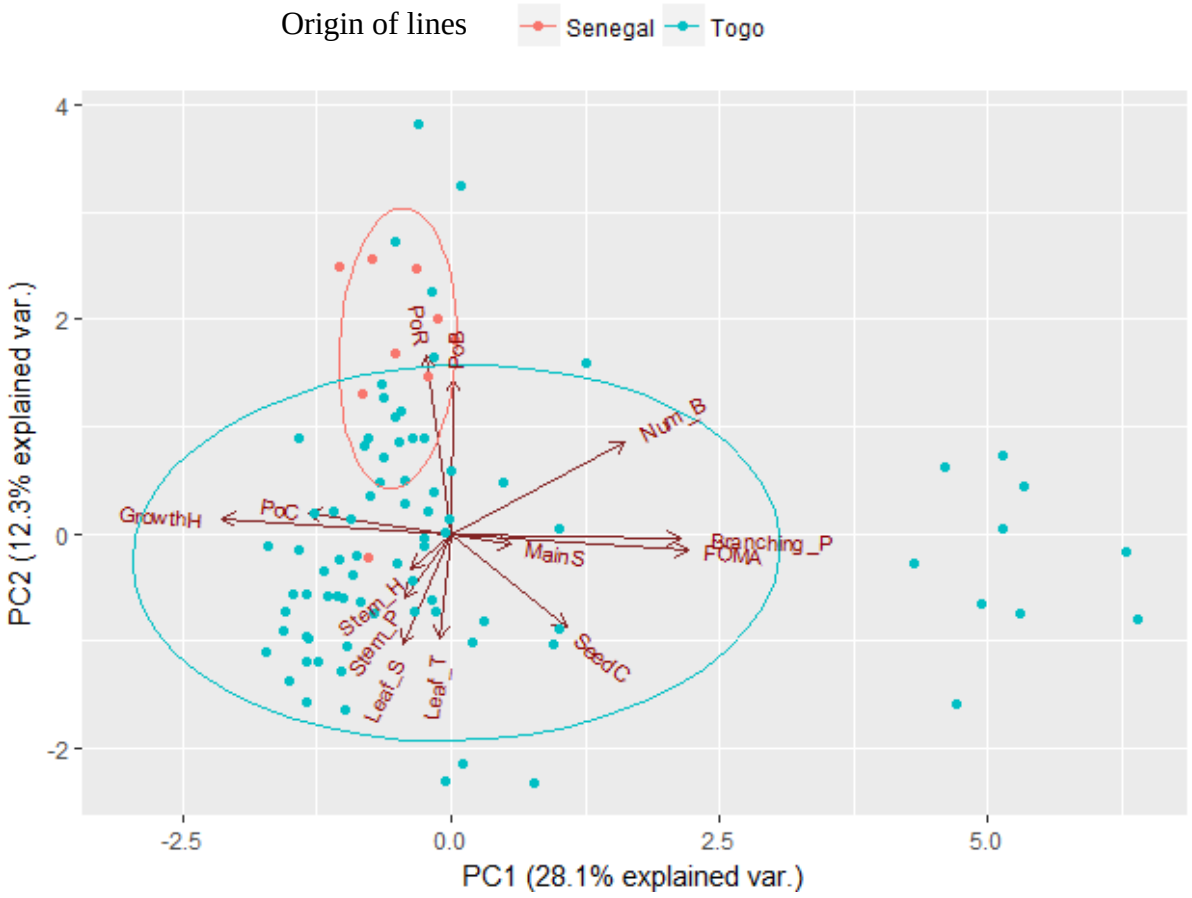

Figure 4.3 A principal component analysis showing similarity relationships among 92 accessions based on qualitative traits. GrowthH = growth habit, Num_B = number of branching, Branching_P = branching pattern, MainS = main stem presence, FOMA = flower on the main axis, Stem_H = stem hairs presence, Stem_P = stem pigmentation, Leaf_S = leaf shape, Leaf_T = leaf tip, PoC = pod constriction, PoR = pod reticulation, PoB = pod beak, SeedC = seed color.

Overall, the most common qualitative characteristics (in terms of richness) were bunch growth habit with main stem somewhat apparent, sub-glabrous stem surface, sequential branching pattern, wide elliptic leaflet shape, acute leaflet tip, flower on the main axis, slight pod reticulation, moderate pod constriction, slight pod beak and red seed colour type.

4.3.1.3 Correlations among quantitative traits

The test for strength and significance of the correlation among quantitative traits revealed that there was a significant positive correlation between pod yield and number of pods per plant (r =

0.95, p = 0.01) and between hundred pod weight and shelling percentage (r = 0.86, p = 0.01). However, the correlation of yield with hundred pod weight and hundred seed weight were not significant (Table 4.3). Late leaf spot correlated negatively with days to maturity (r = -0.64, p = 0.01), and positively with plant height (r = 0.55, p = 0.01). Also, a significant negative correlation was observed between days to maturity and plant height (r = -0.59, p = 0.01).

Table 4.3 Correlation analysis for quantitative traits

	HPW	HSW	Heit	LLS	PV	NPP	PW	PL	Yi	SL	SW	DF	DM
HPW													
HSW	-0.05												
Heit	0.10	-0.11											
LLS	0.15**	-0.03	0.55**										
PV	-0.01	0.14*	-0.19**	-0.29*									
NPP	-0.05	0.01	0.26**	-0.02	-0.06**								
PW	-0.14*	0.53**	-0.30**	-0.31**	0.48**	-0.22*							
PL	-0.05	0.41**	-0.15*	-0.26**	0.57**	-0.06	0.54**						
Yi	0.11	0.03	0.30**	0.03	-0.19*	0.95**	-0.13*	-0.08					
SL	-0.14*	0.45**	-0.43**	-0.48**	0.31**	-0.06	0.60**	0.51**	-0.10				
SW	-0.14*	0.42**	-0.23**	-0.29**	0.14*	0.16*	0.45**	0.25**	0.14*	0.60**			
DF	-0.17**	-0.06	-0.42**	-0.38**	0.10	-0.08	-0.03	0.04	-0.14*	-0.14*	0.01		
DM	-0.27**	0.02	-0.59**	-0.64**	0.61**	-0.08	0.21**	0.13*	-0.12*	-0.12*	0.20**	0.48**	
Sh%	0.86**	-0.01	0.10	0.18**	-0.02	-0.01	-0.13*	-0.13*	0.18**	-0.17**	-0.15*	-0.23**	-0.17**

Significance codes: '**' 0.01; '*' 0.05; DM = days to maturity, DF = days to flowering, DE = days to emergence, SL = seed length, PL = pod length, PW = pod width, PV = pod volume, Heit = plant height, NPP = Number of pods per plant, HPW = hundred pod weight, HSW = hundred seed weight, Sh% = shelling percentage, LLS = late leaf spot at 80 days.

4.3.1.4 Path analysis for yield-related traits

Number of pods per plant (NPod.Pl) seemed to have the strongest direct effect on yield (p = 0.05) with a path coefficient (Pc) of 0.85. Though hundred pod weight and hundred seed weight contributed to yield, the direct effect was very low and not significant (Figure 4.4). Also, comparison of multiple models revealed no indirect effect between pod size and seed size and between hundred seed weight and hundred pod weight. Pod length contributed significantly to the hundred pod weight. Negative indirect effect was observed between pod size (pod length and width) and yield as increasing pod size caused reduction in the number of pods per plant which in turn reduced yield. A path coefficient of 0.26 was found for extraneous variables showing that the traits considered in this analysis are the ones contributing mostly to the yield. The path coefficient for extraneous variable was high for hundred pod weight (Pc = 0.70) and hundred seed weight (Pc = 0.64).

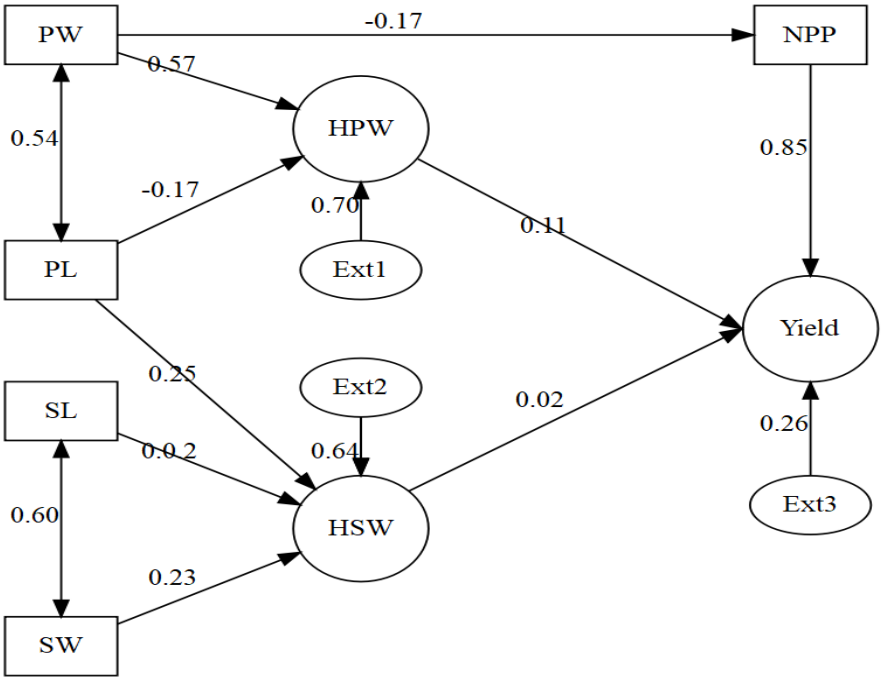

Figure 4.4 Plot of the direct and indirect effect of pod and seed traits on yield. Straight arrow represents direct effect. Double arrow represents correlation. NPP = number of pods per plants, HPW = hundred pod weight, HSW = hundred seed weight, PL = Pod length, PW = pod width, SL = seed length, SW = seed width and Ext = Extraneous variables

4.3.1.5 Population structure and relationship among genotypes

In the quantitative traits scatter plots, the second PC separated Senegal cultivars from Togolese cultivars while PC2 mostly separated local accessions into groups (Figure 4.2). In the qualitative data plot, no clear separation was achieved based on the origin (Figure 4.3). However, the first PC seemed to identify a group of 10 local accessions as outliers.

The average Silhouette width of the PAM clustering showed a weak structure (silhouette width < 0.50) in the studied germplasm. Despite the weak silhouette mean, two clusters (K = 2) appeared to be the optimal number of clusters (Appendix 2; Figure 4.5). However, in the search for more dissimilarity between genotypes, following the average Silhouette width's rank of clusters, three clusters (K = 3) were also retained.

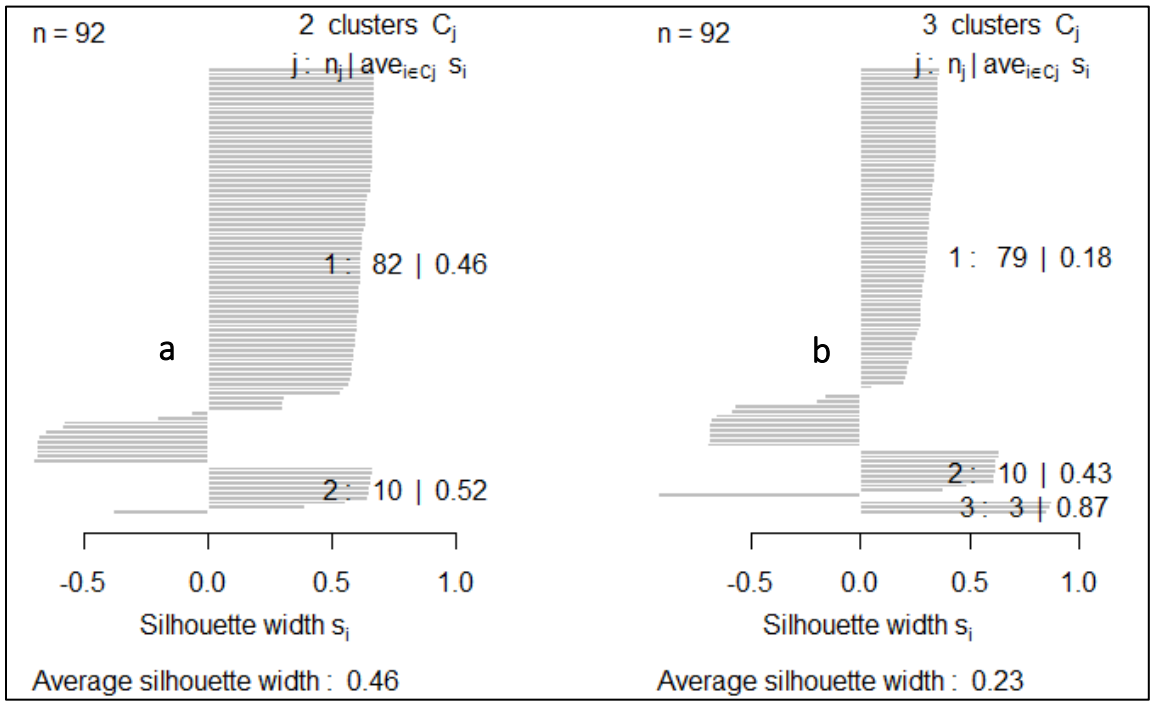

Figure 4.5 Optimal number of clusters, a-K = 2 and b-K = 3 with a probability of membership of genotypes

In regards to the optimal two clusters (K=2), a large number of the genotypes (82 lines), including lines from Senegal, were grouped in cluster I (Figure 4.6) and the other local accessions were grouped in cluster II. The probability of membership of genotypes was 0.46 and 0.52 for cluster I and II, respectively when k = 2 (Figure 4.6a). Owing to the low Silhouette width, these probabilities can be considered reasonable. Trait means of the optimal two clusters are summarized in Table 4.4. A comparison of clusters revealed that cluster II (C2) was mainly grouping late maturing genotypes with a low LLS incidence, low hundred pod weight and yield. Conversely, cluster I (C1) seemed to be made of early maturing, susceptible to LLS genotypes and exhibited a slightly higher yield.

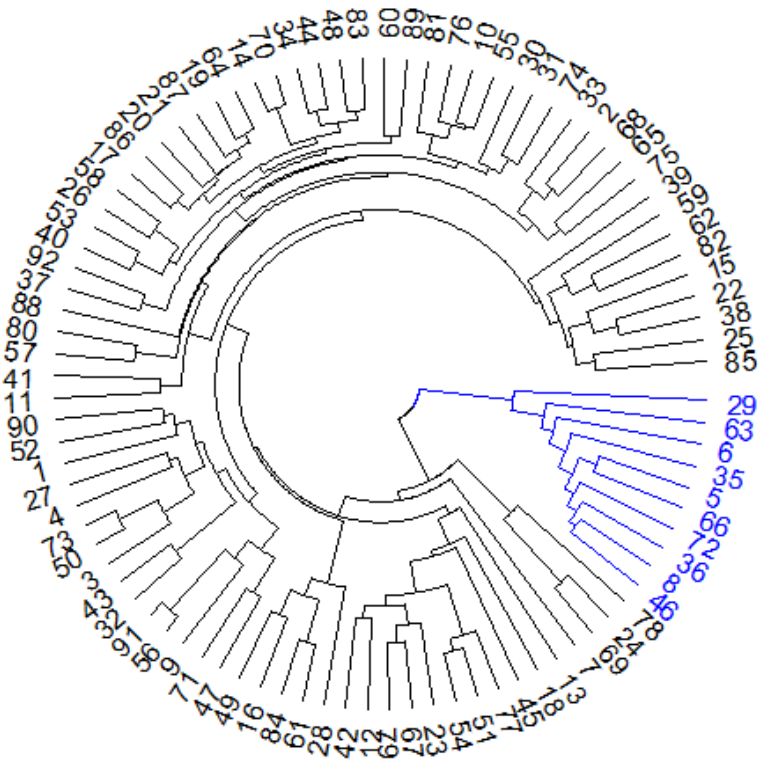

Figure 4.6 Dendrogram showing optimal two clusters. Branches are outlined with respect to the clusters: Cluster I= black, cluster II =blue

Table 4.4 Mean of yield-related traits for optimal two clusters

Cluster	Genotypes origin		Traits				
	Togo	Senegal	LLS	Days to maturity	Hundred Pod weight	Hundred seed weight	Yield
C1	72	9	4.49 (1.8-6.1)*	91.76 (80-120)	73.79 (66.52-81.04)	46.07 (34.33-66.66)	2.65 (1.51-3.90)
C2	10	0	2.60 (2-3.5)	109.16 (90-110)	72.79 (70.9-75.07)	43.97 (38.56-49.53)	2.33 (1.43-3.23)

*Ranges

When K=3, the majority of the studied genotypes, including lines from Senegal, were grouped in cluster I (C1) as in K = 2 (Figure 4.7). The particularity in K= 3 was cluster 3 (C3), which was comprised of 3 local accessions that exhibited quite high probability of membership (0.84). In relation to the probability of membership, genotypes in CI, with a score of 0.18 carried the highest within cluster diversity (Figure 4.5).

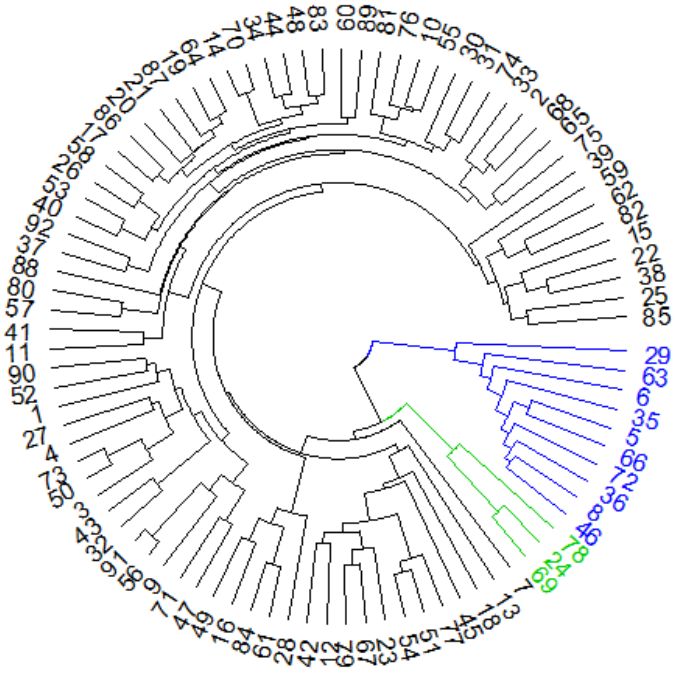

Figure 4.7 Dendrogram showing three clusters. Branches are outlined with respect to the clusters: Cluster I=black, cluster II=blue, cluster III=green

Quantitative and qualitative features of the three clusters are shown, respectively, in Table 4.5 and Table 4.5. Cluster III (C3) contained local early maturing genotypes, high LLS incidence, large pod volume and a higher hundred pod weight, while in C2 genotypes were essentially late maturing with a low incidence of LLS (Table 4.5). Indeed, C2, in marked contrast to cluster I, displayed the longest maturity period, the lowest hundred pod weight and the lowest incidence of LLS. The majority of the studied genotypes, including lines from Senegal, were grouped in cluster I. However, when the number of clusters is brought to K = 9, lines from Senegal were separated into a cluster that showed moderate resistance to LLS (result not shown). Overall, the clusters that displayed a high number of pods per plant seemed to yield more than the others. Also, clusters that tended to be less susceptible to LLS were late maturing.

Table 4.5 Ranges of traits in three cluster grouping of the 92 groundnut accessions

	Cluster		
Trait	**C1**	**C2**	**C3**
Days Emergence	6.38	6.2	6.66
Days Flowering	26.87	29.93	24.33
Days Maturity	92.02	109.16	85
Plant Height (cm)	33.76	21.46	39.22
Plant Width (cm)	58.51	55.55	65.54
Number of Lateral Branches	8.89	15.04	7.07
Leaflet Length (cm)	5.61	4.42	6.83
Leaflet Width (cm)	3.13	2.49	3.22
LLS 90 Days	4.45	2.60	5.16
Pods / plant	55.69	49.63	43.73
Pod Volume (cm^3)	3.04	3.98	5.05
Pod Length (cm)	2.39	2.43	2.86
Pod Width (cm)	1.17	1.20	1.31
Shelling %	73.80	73.14	76.94
Number of Seeds per pod	1.7	1.4	3
Seed Length (cm)	1.27	1.41	1.29
Seed Width (cm)	0.87	0.92	0.81
100 Pod weight	73.70	72.79	76.16
100 Seed Weight	46.01	43.97	45.42
Yield	2.69	2.32	2.14

Table 4.6 Qualitative features of k = 3 clusters

Trait	Descriptors	C1	C2	C3	Simpson Index (H)
Growth Habit	Bunch	34.37%	10%	66.66%	0.59
	Erect	65.63%	-	33.34%	
	Spreading	-	90%	-	
Main stem	Non-apparent	08.26%	-	25.00%	0.43
	Somewhat apparent	79.74%	33.33%	62.5%	
	Apparent	12%	66.67%	12.5%	
Stem surface	Sub-glabrous	98.30%	100%	100%	0.04
	Moderately Hairy	1.70%	-	-	
Branching Pattern	Sequential	100%	-	100%	0.22
	Alternate	-	100%	-	
Number of Branches	Primary (n+1)	38.00%	-	33.33%	0.57
	Secondary (n+2)	62.00%	10%	66.67%	
	Tertiary	-	90%	-	
Stem Pigmentation	Green	42.16%	60%	-	0.60
	Purple	-	-	100%	
	Mixed	57.84%	40%	-	
Leaflet Shape	Narrow Elliptic	18.66%	-	100%	0.33
	Oblong Elliptic	16.94%	70%	-	
	Wide Elliptic	64.40%	10%	-	
	Obovate	-	20%	-	
Leaflet Tip	Acute	93.22%	90%	100%	0.14
	Obtuse	6.78%	10%	-	
Flower on Main Axis	Presence	100%	-	100%	0.19
	Absence	-	100%	-	
Pod reticulation	None	1.69%	-	66.66%	0.51
	Slight	66.10%	90%	33.34%	
	Moderate	30.50%	10%	-	
	Prominent	1.69%	-	-	
Pod Constriction	None	-	30%	-	0.55
	Slight	40.68%	60%	100%	
	Moderate	59.32%	10%	-	
	Deep	-	-	-	
Pod Beak	Slight	72.30%	70%	33.33%	0.42
	Moderate	26.50%	30%	66.67%	
	Prominent	1.20%	-	-	
Primary Seed Colour	Pink	1.69%	-	-	0.75
	Light Red	50.84%	-	-	
	Red	16.94%	-	-	
	Dark Red	25.42%	60%	-	
	Dark purple	3.38%	-	100%	
	Variegated	1.69%	40%	-	

Most genotypes within the clusters were very similar on qualitative traits bases (Table 4.6). Genotypes in C1 were erect types (65.68%), sub-glabrous stem surface (98.30%), sequential branching pattern (100%) with flower on the main axis (*Fastigiata* subspecies). In contrast, C2 genotypes were spreading types with alternate branching and absence of flowers on the main axis (*hypogaea* subspecies). The highest uniformity of cluster members was observed in C3. Indeed, a total uniformity was observed on growth habit, stem surface, branching pattern, number of branches, flowers on the main axis and pod reticulation. In relation to the calculated Simpson index, the most diverse traits were primary seed colour (0.75), stem pigmentation (0.60), Growth habit (0.59), and number of branches (0.57) (Table 4.5).

4.3.1.5 Quantitative versus qualitative traits-based Clustering

To check the collinearity of quantitative traits and qualitative traits in grouping genotypes, two trees were generated from both separate data sets. In general, while lines from Senegal clustered together based on the quantitative data (Figure 4.8a), the reverse was observed on the qualitative trait dendrogram (Figure 4.8b). Also, some genotypes, following the example of accessions 05AH, 03AH, 07AH, 13AH, were quite similar, when clustered using qualitative traits but distinct on quantitative trait bases. Altogether, the low correlation coefficient ($r = 0.33$) obtained between trees based on the quantitative and qualitative traits confirm the spatial divergence observed on the dendrograms.

The evaluation of the performance of hierarchical clustering methods with respect to germplasm collections revealed that qualitative traits tree had a higher cophenetic coefficient (0.88) than the quantitative traits tree (0.79).

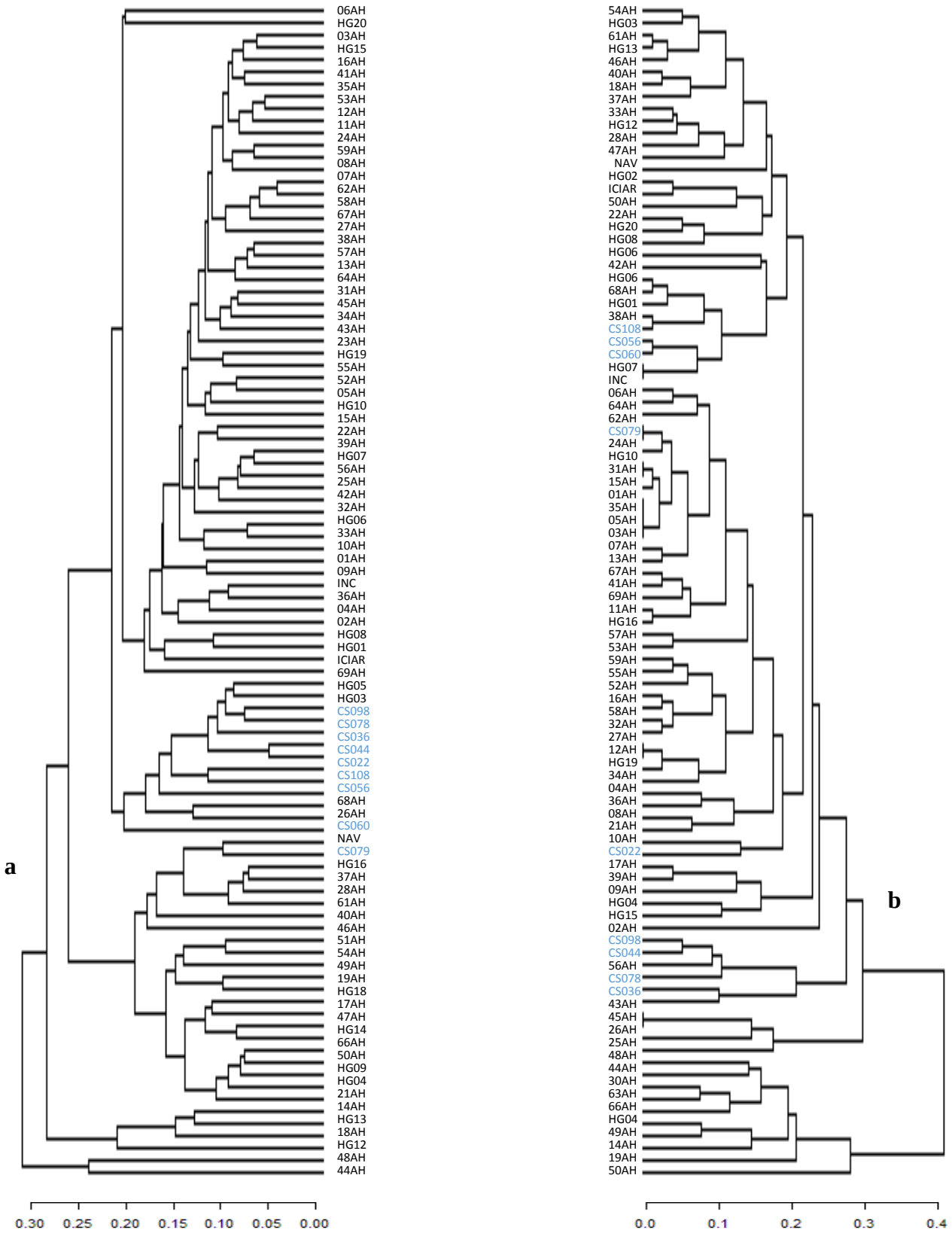

Figure 4.8 Dendrogram based on quantitative traits (a) and qualitative traits (b) in diversity assessment of 92 groundnut accessions. Black=lines from Togo, Blue= lines from Senegal

4.3.2 Molecular diversity

4.3.2.1 Allelic diversity

Genotyping of the germplasm collections from Togo, breeding lines from Senegal and ICRISAT has allowed the identification of nearly 7072 diverse SNPs. However, only 990 SNPs well distributed over the genome with polymorphism information content (PIC) higher than 0.1 were retained for the diversity analysis (Annex 3). The PIC values ranged from 0.1 to 0.5 with a mean of 0.24. The distribution of the PIC and of minor allele frequencies (MAF) is shown in Figure 4.9.

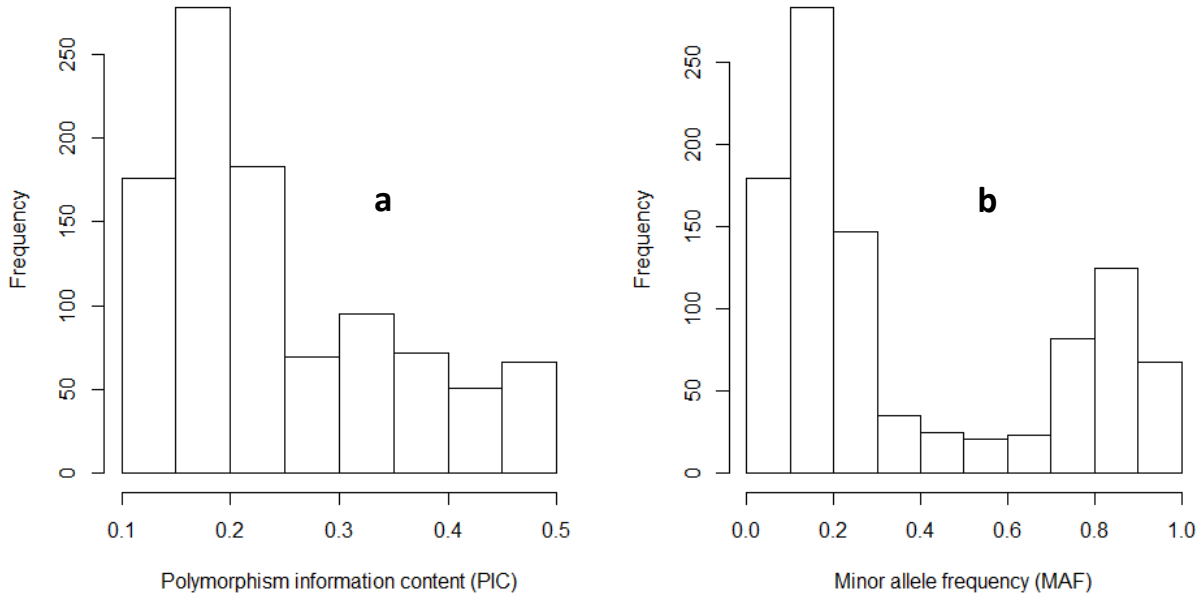

Figure 4.9 Distribution of SNPs based on PIC (a) and MAF (b)

Overall, the percentage of heterozygous genotypes varied from 0 to 50% for all loci. However, less than 10% of the genotypes were heterozygous for more than 30% of the investigated loci (Figure 4.10a). The most heterozygous genotype, HG09 carried only 30% homozygous loci. The most homozygous genotypes, 39AH, 38AH, 34AH, and 59AH carried less than 5% heterozygous loci. Varying proportions of homozygosity were observed in the genotypes from Togo unlike in genotypes from Senegal where less than 7% of heterozygous loci was detected in all genotypes. The genetic diversity was well distributed across the majority of the loci

(Figure 4.10C). The test for Hardy Weinberg Equilibrium (HWE) shows that there was a clear significant departure from HWE in the population.

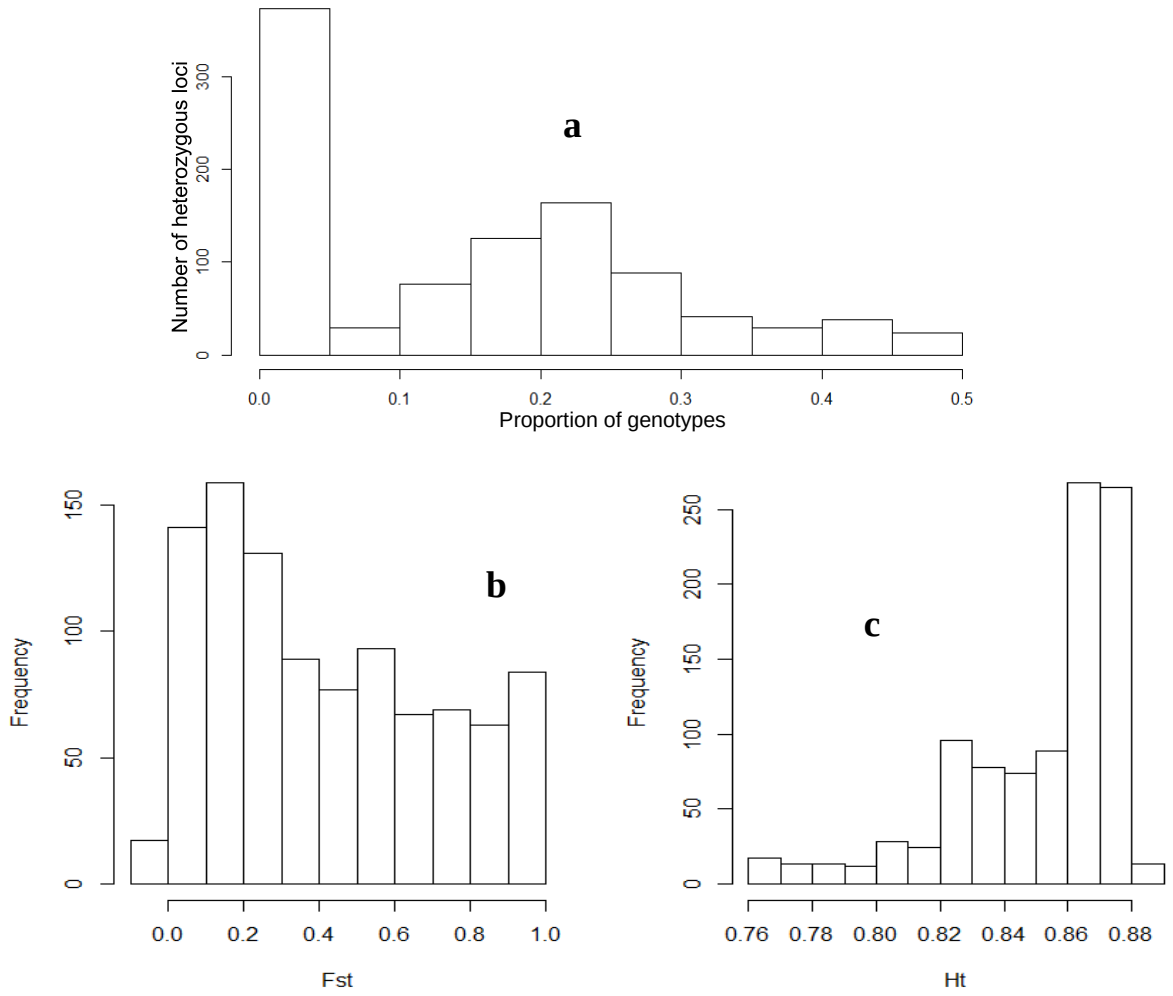

Figure 4.10 a-Structure of heterogeneity detected in 92 groundnut accessions; b-Distribution of fixation index (Fst) and c-Frequency distribution of total genetic diversity (Ht)

4.3.2.3 Geographical analysis of diversity

The analysis of molecular variance to partition the total genetic variation within and between geographical origin revealed that only 1.1% of the total variation accounted for between geographical origin with nearly all the observed diversity being among genotypes. Moreover, the isolation by distance analysis of the relationship between genetic dissimilarity (Kosman & Leonard, 2005) and geographical diversity showed that the difference between populations was more based on their genetic differences than based on their geographical origin (Figure 4.11). Also, there was no correlation between genetic diversity and geographical diversity.

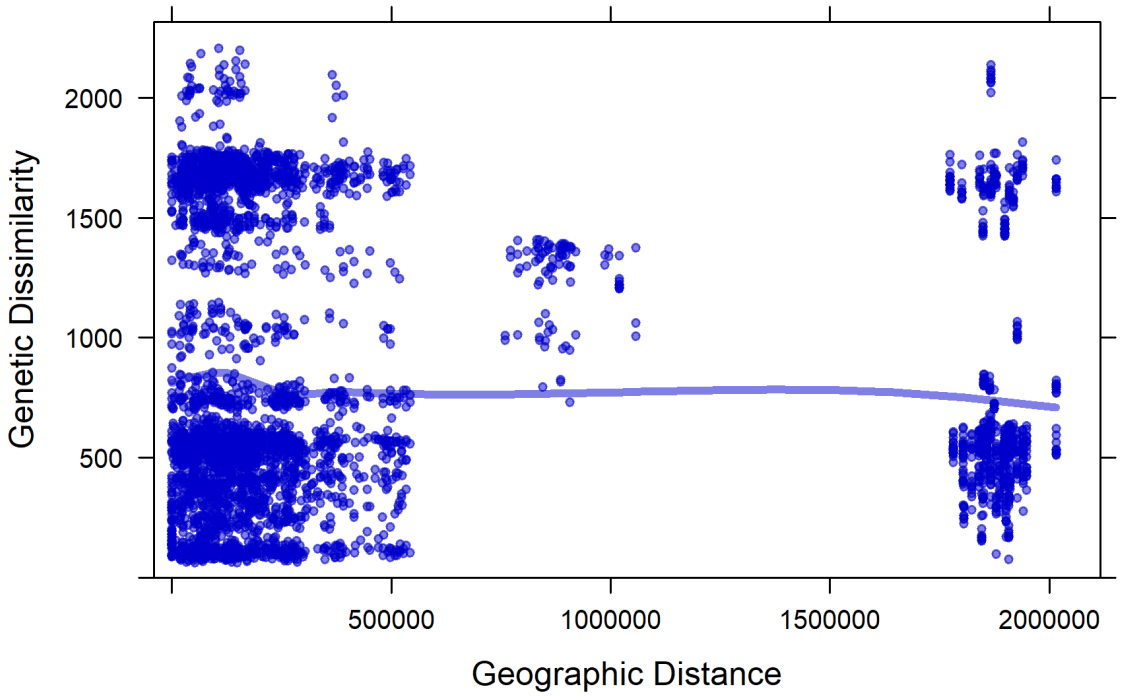

Figure 4.11 Multilocus genetic dissimilarity versus geographic distance. The line represents the running average.

4.3.2.4 Principal component analysis of populations

Discriminant analysis of principal components (DAPC) was used for the visualization of the emergent population structure and group assignment in the groundnut accessions. DAPC revealed that the first two axes, explaining more than 10% of total variation each, were adequate for discriminating genotypes. The first two principal components (PC) explained 67% of the total variance. The emergent structure is depicted in a two-dimensional scatter plot involving investigated groundnut accessions (Figure 4.12). The main result from the scatter plot is the extremely low genetic variability of the introduced lines from Senegal compared to the cultivated accessions in Togo. Indeed, lines from Senegal formed a compact group but were to a large extent overlaid by a group of genotypes from Togo. Genotypes from Togo were the most dispersed as they covered the whole range of genetic diversity observed in this study with some tendency to form specific subclusters. Though ICG 7878 from ICRISAT was somewhat different from the other genotypes, it had some similarities with some lines from Togo.

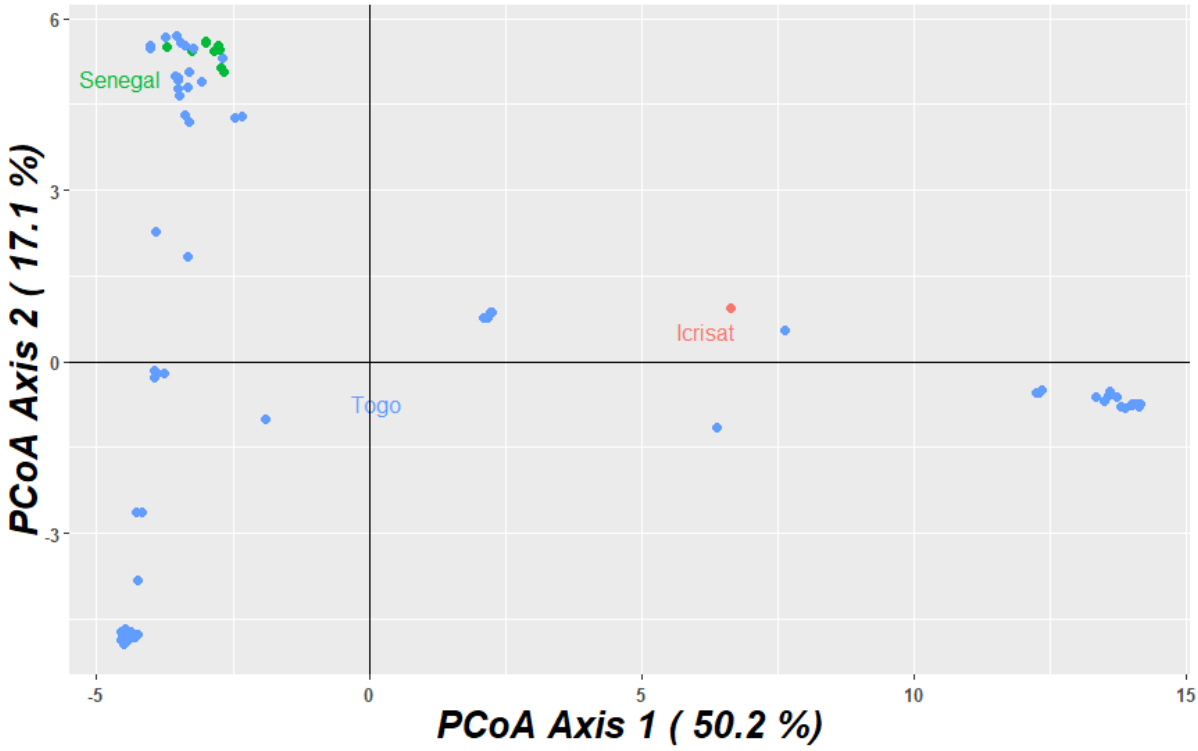

Figure 4.12 A principal component analysis biplot diagram showing similarity relationships among populations based on SNP markers

The absence of evidence for population structure was confirmed by the low Fst value for the relationship between lines from Senegal and lines from Togo. However, the relatively high value of Fst between a line from ICRISAT and lines from Senegal confirmed the spatial distance observed between them (Table 4.7).

Table 4.7 Pairwise Fst and Nei's genetic distance value for populations

	Togo	Senegal	ICRISAT
Togo	-	0.034	0.108
Senegal	0.181	-	0.148
ICRISAT	0.304	0.471	-

*The top diagonal is Nei's Gst; **The bottom diagonal is pairwise Fst

4.3.2.5 Cluster analysis

PAM Silhouette revealed that two clusters (K = 2) were optimal in both the analysis of marker and the co-analysis of marker-phenotypic data with respectively an average silhouette of 0.7 and 0.69 (Figure 4.13 and 4.15). However, the three-cluster solution (K = 3) was described, to understand the characteristic of the three groups, in the co-analysis of phenotypic and molecular data because it was the next in rank of the silhouette width. In Figure 4.13, Cluster 1 grouped majority of lines from Togo and lines from Senegal while cluster 2 grouped 19 lines from Togo that clustered with the line from ICRISAT. A probability of membership of 0.71 and 0.67 was observed respectively for cluster 1 and cluster 2 with quite a high average silhouette width (0.7) denoting a strong structure in the collected germplasm.

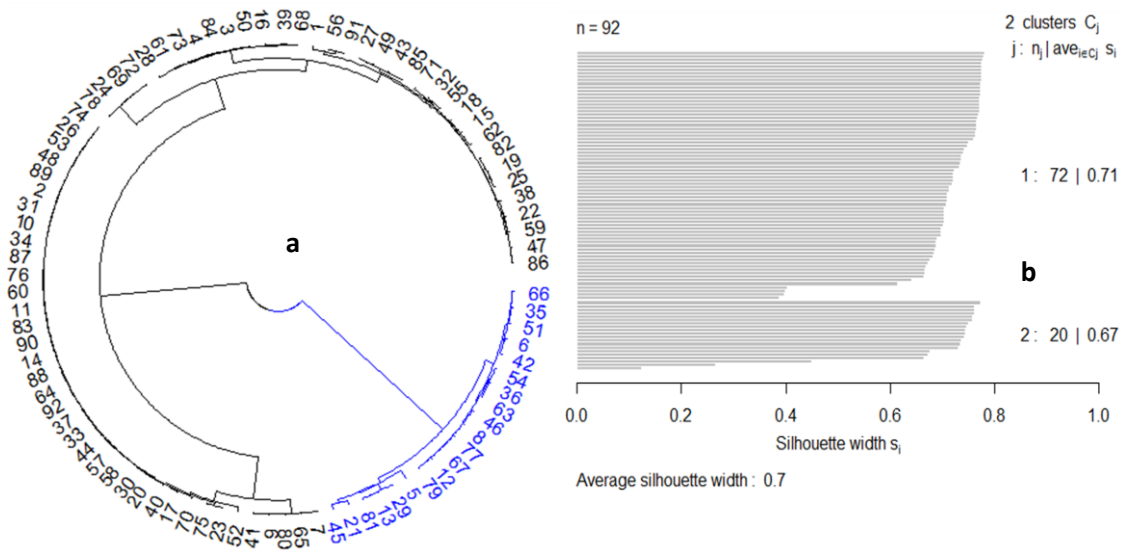

Figure 4. 13 (a) Molecular data dendrogram showing the optimal two clusters: Cluster 1 = Black; Cluster 2 = Blue; (b) Silhouette width of the optimal clusters.

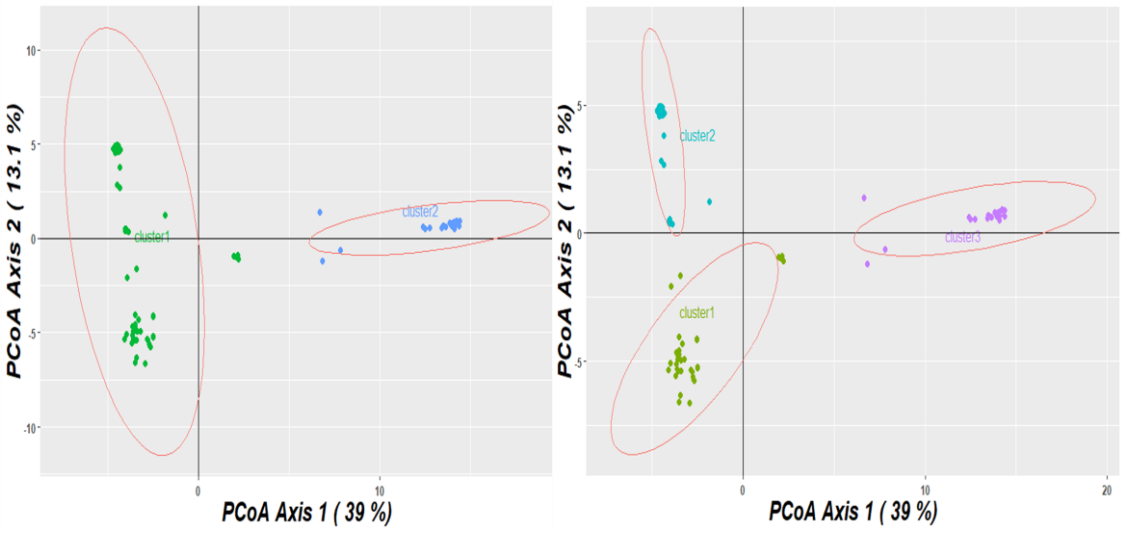

Figure 4.14 Cluster analysis: two clusters solution (left); three cluster solution (right).

Co-analysis of the marker and phenotypic data revealed two optimal clusters that were not different from the clusters obtained in molecular data analysis. However, the probability of membership of the genotypes to the clusters, 0.70 and 0.66 respectively for cluster 1 (CI) and cluster 2 (CII), was slightly lower than the probability in the molecular analysis. Further structure analysis of the germplasm revealed that cluster 1 in the two-cluster solution (K=2) could be split into two clusters (Figure 4.14). However, the new cluster 1 (CI), made up of lines from Togo and lines from Senegal, exhibited a low probability of membership (0.23). The two other clusters CII and CIII, made up of a group of local genotypes and the ICRISAT line, exhibited a probability of membership of 0.65 (Figure 15Bb).

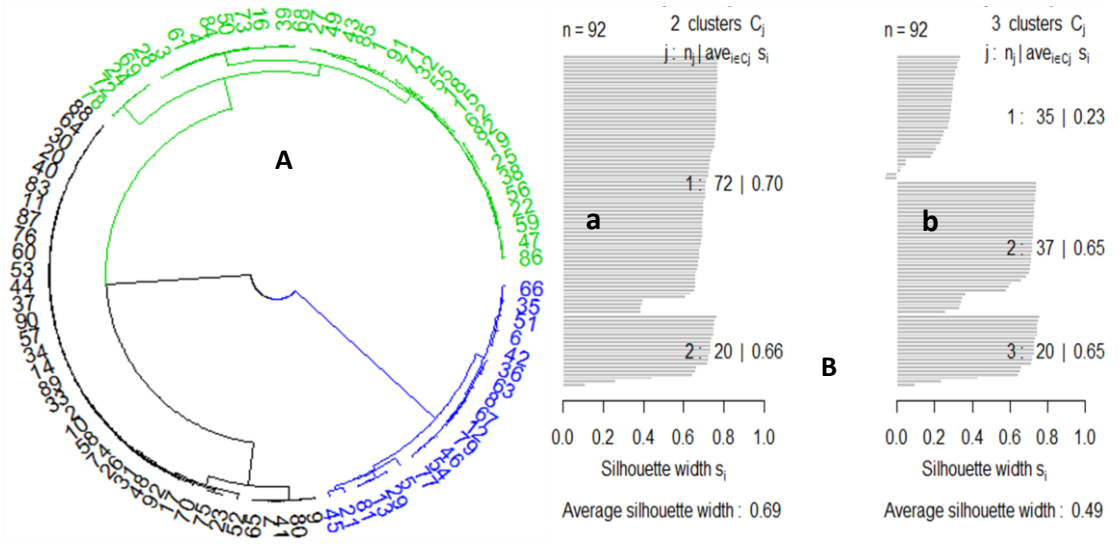

Figure 4.15 (A)Dendrogram showing the three clusters based on the marker and phenotypic data: CI=Green; CII=Black; CIII= Blue; B = Optimal number of clusters: a = Silhouette width of k = 2, b = Silhouette width of k = 3 clusters

A correlation analysis of the marker and phenotypic dendrograms revealed a positive correlation (0.46). Analysis of molecular features of the clusters revealed that the distribution of private alleles (PA), minor allele frequency (MAF) and richness was not the same across clusters (Figure 4.16). Indeed, CII carried the lowest number of private alleles and richness while CIII, with the smallest sample size, exhibited the highest number of private alleles (PA), minor allele frequencies (MAF) and richness. Altogether, the three clusters were not fixed for alternate alleles. There was a significant correlation between allelic richness, number of private alleles and total alleles ($p = 0.05$).

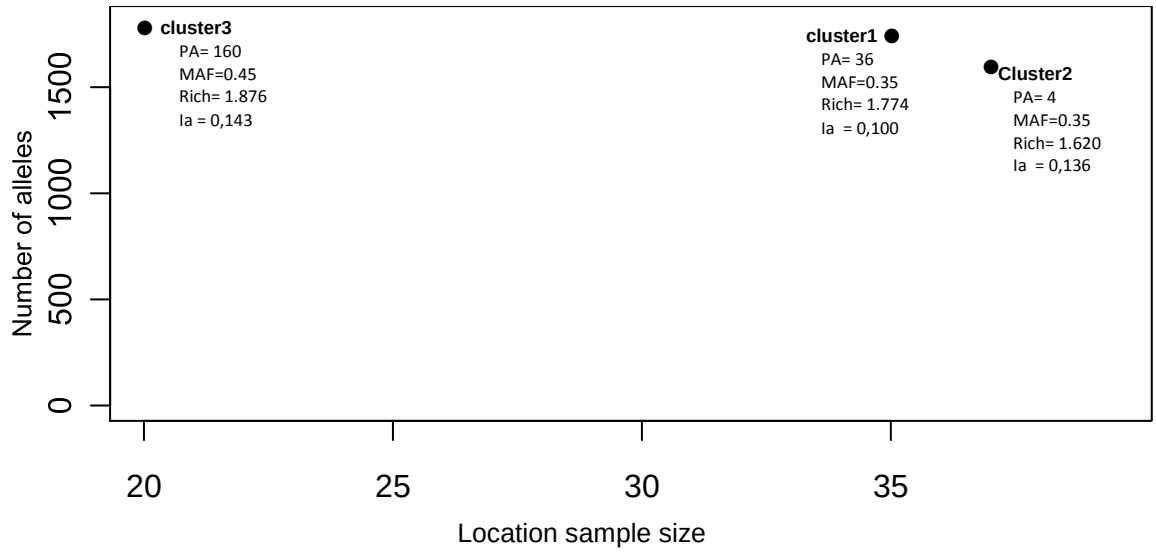

Figure 4.16 Number of alleles vs location sample size. PA=Number of private alleles; Rich= Mean Allelic richness; Ia= Index of association

Pairwise comparison of genetic distance between clusters using Nei's Gst and Fst was done and a high global Fst (0.54) confirmed the difference observed between clusters. The Fst estimates for clusters ranged from 0.399 to 0.67 (Table 4.8) and cluster 3 showed a significant level of genetic differentiation ($p<0.05$).

Table 4.8 Pairwise Fst value and Nei's genetic distance for clusters

	CI	CII	CIII
CI	-	0.0029*	0.0309
CII	*0.399***	-	0.0375
CIII	*0.563*	*0.67*	-

*The top diagonal is Nei's Gst; **The bottom diagonal is pairwise Fst

Analysis of phenotypic characteristics of the three clusters revealed that there were differences in agronomic features. Comparison of agronomic characteristics of clusters revealed that genotypes in CI and CII were early maturing and susceptible to LLS (Figure 4.17). In contrast, CIII was made up of genotypes that are medium and late maturing with partial resistance to LLS disease. Also, CII showed a higher yield than the other clusters.

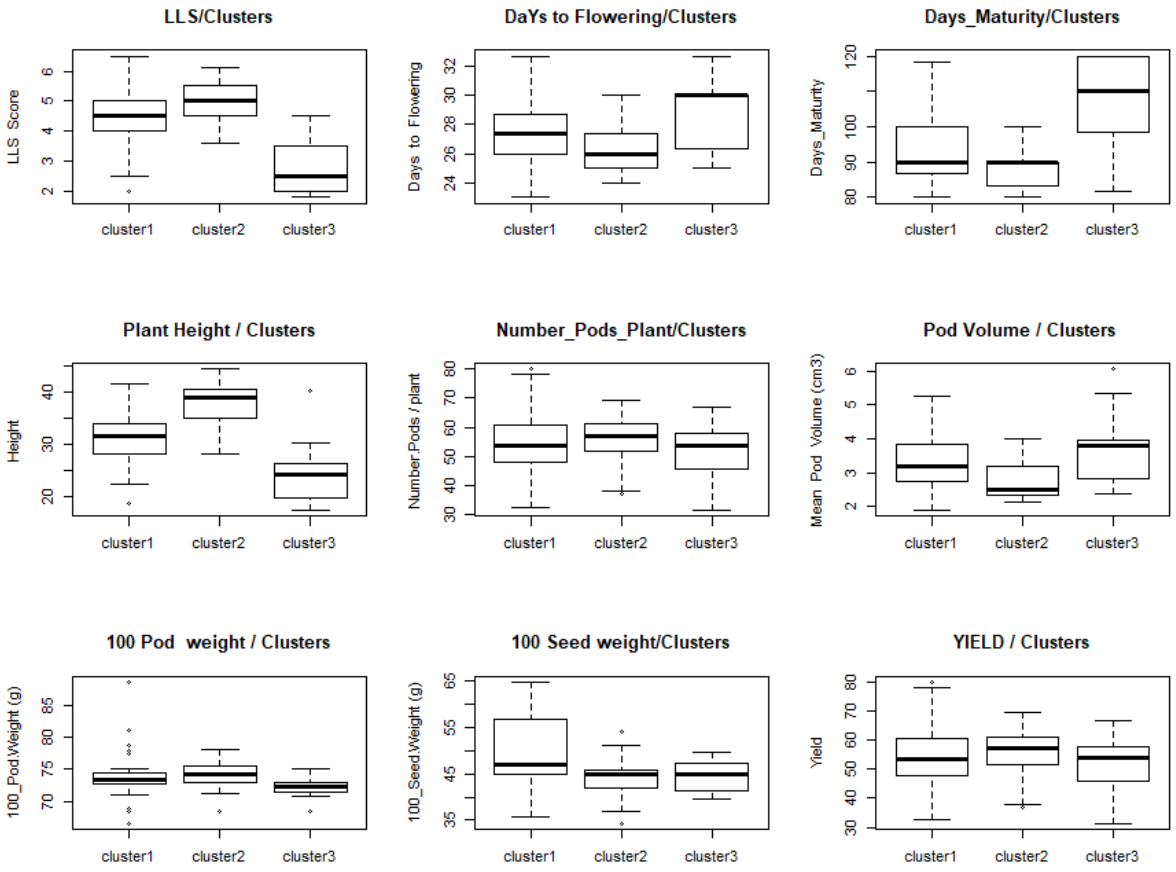

Figure 4.17 Agronomic characteristics of the three clusters in the co-analysis of phenotypic and molecular data.

CI and CII differed mainly on the LLS score, days to flowering and pod volume. Differences were also observed between the two clusters based on other phenotypic characteristics such as plant height, plant width, pod volume, pod length, seed length, growth habit, number of lateral branches, pod reticulation and constriction (result not shown). A quite high cophenetic correlation coefficient (0.95) was obtained for the clustering of the marker and phenotypic data.

4.4 Discussion

4.4.1 **Phenotypic diversity among collected accessions**

The analysis of variance suggests an adequate relative variability for some quantitative traits. Indeed, traits such as number of lateral branches, number of pods per plant, pod yield and LLS displayed a high coefficient of variation (CV) showing that improvement could be achieved

through selection for higher productivity. However, the low CV for other traits may suggest a directional selection which reduced cover-wide phenotypic variance. Previous diversity studies using phenotypic traits reported a low variation in days to emergence, leaflet width, pod width, and seed length and width (Upadhyaya *et al.*, 2003; Kushwah, 2011). This uniformity underscores the necessity to increase diversity through the incorporation of new alleles into the germplasm for these traits' improvement.

Correlation-based principal component analysis highlighted that traits of importance such as hundred seed weight and resistance to LLS contributed significantly to the diversity. Indeed, the higher the trait diversity in a germplasm, the higher the probability of the presence of genotypes with particularly important traits. Qualitative trait wise, most traits that showed a high Simpson index, correlated with the first two PCs. However, traits of value for farmers such as pod constriction, seed length, seed width and primary seed colour (Janila *et al*., 2016b) which were not retained by the first two PCs, exhibited a high Simpson index. Previous diversity studies indicated the relatively low importance of these traits as groundnut descriptors (Upadhyaya *et al*., 2003). The high Simpson index for qualitative traits of interest indicates that several different trait features with similar abundance are available in the germplasm collection and could be used in breeding to respond to farmers' preferences.

Nonetheless, the seemingly contrasting result between PCA and Simpson index could be explained by the fact that the two approaches do not measure diversity at the same level. While Simpson index measures diversity in an individual trait, PC analysis measures contribution of an individual trait to the overall diversity. Also, the high loadings observed in the PCs for high Simpson index traits suggest a correlation between Simpson index value and trait contribution to overall diversity in principal component analysis.

Population structure analysis of phenotypic traits delineated germplasm accessions into distinct groups. However, the silhouette width of PAM cluster showed a low population structure in the germplasm collection implying that there were more similarities than differences between the collected genotypes. This result was unexpected, because, as there is no modern breeding programme in Togo and lines were collected from different areas of the country, one might expect more diversity in the germplasm. Artificial selection by farmers over decades to adapt to similar environmental constraints across regions may have narrowed the phenotypic diversity in cultivated groundnut in Togo. Also, the low number of introduced varieties coupled with the free exchange of seeds among farmers could explain the similarity between genotypes.

Nonetheless, useful differences were conspicuous across clusters within the germplasm collections. Overall, the clusters that displayed a high number of pods per plant seemed to yield more than those that were characterized by a low number of pods per plant. Also, pod yield was strongly correlated with the number of pods per plant suggesting that groundnut pod yield is more influenced by the number of pods per plant than the pod weight and seed weight in the germplasm. Path analysis confirmed this assumption. This result agreed with the findings of Kushwah (2011) and Haghpanah *et al*. (2018) who suggested the use of the number of pods per plant as a selection index for yield improvement. This is interesting for indirect selection, as the number of pods per plant is a visible descriptor that could easily be used for selecting plants in the field, for yield improvement, without the need for costly equipment. Large pod size is another preferred characteristic at farmers' level. In this study, one cluster exhibited a large pod size. However, because of their low number of pods per plant, they were low yielding. In addition, pod size did not correlate with seed size and hundred seed weight. Only genotype HG11, a long duration variety, combined a high number of pods per plant and a large pod size. With the erratic rainfall, a consequence of the changing climate, such genotypes should be improved through incorporation of earliness and other beneficial traits.

Variability was found among clusters in the germplasm for LLS incidence. With few exceptions, resistance to LLS was positively associated with late maturity in this study, as clusters that showed a low incidence of LLS tended to be late maturing. This tendency was confirmed by correlation analysis of quantitative traits. Such a finding could be a genetically meaningful trend, but could also be attributed to the fact that disease was scored at the same time on all plants, and the incidence of the disease could increase with growth on the late maturing plants. However, this confirms the difficulty to find landraces combining LLS resistance and early maturity (Janila *et al*., 2016b). These results imply that the development of early maturing LLS resistant lines will necessitate the use of breeding tools such as genetic and chromosome shuffling. For this purpose, most resistant and late maturing genotypes could be used as parents for incorporation of LLS resistance into early and susceptible varieties.

Though the quantitative and qualitative traits showed a weak convergence in grouping accessions in this study, phenotypic cluster VII and VIII, which exhibited the highest probability of membership, grouped genotypes that were homogenous based on both trait types. All *hypogaea* subspecies genotypes (alternate branching and absence of flower on the main axis) were grouped in CII while the *fastigiata* subspecies with sequential branching and presence of flower on the main axis (nearly 90 % of the accessions), were scattered in other clusters.

4.4.2 Molecular diversity

Overall, SNP markers provided estimates of population genetic parameters useful for population structure analysis. Principal component analysis showed that the diversity observed in the studied germplasm was not associated with the geographical origin. The relatively low value of Fst in this study means that there was not much difference between the populations based on their geographical origin. Also, the isolation by distance relationship did not

hold for these populations, as there was no correlation among matrices of genetic (Fst) and geographical distances of the populations used in this study. The common alleles between lines from Togo and other lines could represent, at least in part, a kind of core allele for groundnut.

Besides, the common alleles could indicate that lines from Senegal and most lines from Togo showed a high level of genetic exchange between them. This result is surprising as one might expect a much more genetic difference between breeding lines (Senegal and ICRISAT) and cultivated landraces from Togo. However, this could be partly explained by the fact that the genetic background of the parents of the breeding lines was very similar to the cultivated groundnut landraces in Togo. Also, a small proportion of the collected accessions from Togo were introductions from various sources. These lines, mostly those that clustered with lines from Senegal, may have a certain parentage with Senegal breeding lines. The ICG 7878 from ICRISAT was genetically different from the other genotypes in the scatter plot and the difference was even higher with lines from Senegal as evidenced by the Fst value. This result suggests that ICG 7878 shares few alleles in common with lines from Senegal. However, cluster analysis showed that ICG 7878 could be grouped with some cultivated local Togolese accessions. Thus, some of these lines from Togo may originally have come from ICRISAT confirming the idea that population structure is associated with origin of germplasm (Würschum *et al*., 2013; Dwivedi *et al*., 2017).

Overall, genetic parameters indicated appreciably broad genetic base and diverse nature of the germplasm collection evaluated in this study. AMOVA proved that the majority of the genetic variation observed was due to differences between individuals. The distribution of the minor allele frequency (MAF) was skewed toward the low and high value of MAF with an intermediate group of genotypes between the two. Thus, cluster analysis distinguished clusters where genotypes, from different geographical origins, shared a similar pattern of variation. The

cluster of co-analysis of the phenotypic and molecular data was very similar to the clusters of the marker data alone.

A comparison of the resulting clusters distinguished between three groups of genotypes with specific diversity accumulation index. The first group (CI) grouped lines from Senegal and lines from Togo which carried a medium number of alleles and of private alleles. The second group (CII) made up of cultivated landraces from Togo, showed the lowest number of alleles and private alleles and carried the lowest proportion of heterozygosity for the highest number of accessions. The third group (CIII) with the highest allelic richness and private alleles represented lines from Togo and the ICRISAT line. This variation in the number of alleles across clusters reflects natural variation and diversity.

Regarding all diversity criteria, CIII showed the lowest genetic diversity compared to other clusters. The low-frequency of private alleles in CII suggests that this group of genotypes may have undergone no or very low selection pressure (Zhu *et al*., 2004). Identification of private alleles in a cluster provides specificity of that group of genotypes. Private alleles associated with groups of genotypes have been previously reported in groundnut (Kottapalli *et al*., 2011; Varshney *et al*., 2009) and found to be useful for diversity creation. Thus, genotypes carrying these alleles will likely be useful for introducing diversity in the current breeding programme in Togo.

4.4.3 Diversity in co-analysis of phenotypic and marker traits

The difference observed between the clusters at marker level was associated with phenotypic differences in agronomic traits. ICG 7878 was bred for resistance to LLS disease and it is interesting to observe that CIII to which it belongs exhibited the lowest disease infection. However, genotypes of this cluster tended to be late maturing. Lines from Senegal were phenotypically similar mainly in quantitative traits analysis with moderate resistance to LLS. As these lines were selected among a high number of genotypes introduced into the country,

the directional selection based on quantitative traits have probably contributed to the uniformity among them. Though qualitative traits showed a certain dissimilarity between them, molecular analysis confirmed the similarity of genotypes from Senegal.

Other agronomic traits of interest such as flowering date, number of pods per plant, pod volume, hundred pod and seeds weight were variously associated with the clusters distinguished in this study. In addition, general separation of genotypes into subspecies was observed in this study as CIII grouped subsp. *hypogaea* while CI and CII groups were mostly from subsp. *fastigiata* confirming that population structure in groundnut is associated with botanical variety types (Wang *et al.*, 2011). However, some genotypes, as evidenced by the scatter plot, may contain mixtures of genetic components from different subspecies as a result of breeding effort in other countries.

Low level of detectable genetic polymorphism has been considered as a constraint in undertaking molecular breeding in groundnut (Varshney *et al*, 2009). However, the present study reports a high level of genetic dissimilarity in the studied germplasm. For instance, the highest dissimilarity was observed between HG06 and HG09, two accessions from Togo. HG06 is an early maturing genotype highly susceptible to ELS and LLS, which is widely cultivated in the northern regions of Togo. HG09 is a late maturing genotype that is resistant to ELS and LLS diseases. These genotypes could be used in the development of breeding populations for resistance to foliar diseases. Moreover, such populations can be used for genetic mapping and QTL analysis.

It is worth mentioning that the high diversity observed in the collected germplasm may be explained by the lack of breeding programme in Togo which with trait-oriented breeding tends to decrease diversity over time (Dwivedi *et al*., 2017). Overall, SNPs generated by DArTseq technology have been efficient in the identification of SNPs that are well distributed throughout

the groundnut genome and the population structure provided means for exploiting heterogeneity potential among genotypes in the germplasm collection. Therefore, clusters delineated in this study could guide the development of a core collection useful for the new groundnut breeding programme in Togo.

Overall, the cophenetic coefficient revealed that the clustering method was efficient in this study. Also, the clustering techniques proved to be more effective for the marker data and qualitative data than the quantitative data as evidenced by the high cophenetic coefficient observed in this study for qualitative traits and marker data. Apparently, this is because qualitative traits and marker data divide individuals into distinct types with little or no intermediate value in contrast to quantitative traits where individual do not fall into sharply demarcated groups but form continuous series. In this sense, categorical traits seem more effective at capturing variability in trait values than continuous traits. However, qualitative data are less used in cluster analysis. The findings of this study indicate that qualitative data could be very useful in complementing quantitative data, for grouping genotypes in cluster analysis, if the appropriate clustering method is applied. The high cophenetic coefficient, in this study, confirms that the choice of UPGMA with Gower metric, as clustering method, was appropriate.

4.5 Conclusion

Phenotypic diversity was low compared to genotypic diversity in this study. Phenotypic traits such LLS resistance, days to maturity and hundred seed weight exhibited a high variability. A high proportion of SNPs harbouring high genetic variability were detected in this study. The allele frequencies distribution found in this study suggests that there was a quite high genetic diversity. Altogether, an adequate and useful diversity for breeding purposes was observed in the 92 groundnut accessions studied. Diversity observed at the molecular level was associated with phenotypic variation, mainly in quantitative agronomic traits suggesting that the generated

genotypic data may allow capturing the effect of most of the QTLs associated with productive traits. The delineation, in this study, of accessions harbouring desired traits/alleles into groups is valuable information in the starting of groundnut breeding programme and for improving the management and utilization of the collected germplasm in the development of new varieties.

CHAPTER FIVE

5. ANALYSIS OF LINKAGE DISEQUILIBRIUM AND MARKER-TRAIT ASSOCIATION STUDY FOR LLS AND YIELD-RELATED TRAITS

5.1 Introduction

Linkage disequilibrium (LD) is one of the statistical methods used to identify loci that are associated when conducting population molecular parameters. Thus, knowledge of LD pattern along groundnut genome is crucial in diversity study for multiple reasons. Indeed, it is useful for the determination of the distribution of crossing-over and regions of genome subjected to different selection pressures (Porto-Neto *et al.*, 2014). Also, the success of implementation of a large number of breeding methodologies used in genetics nowadays such as genome-wide association studies (GWAS), marker-assisted selection (MAS), quantitative trait loci (QTL) mapping is dependent on the LD level between markers in the population (Bejarano *et al.*, 2018). Thus, LD analysis is a step that cannot be ignored as far as GWAS and QTL mapping strategies are concerned.

GWAS is a genome-trait association study in which the whole genome of different individuals is assessed to see if there is any significant association between any variant and agronomic traits. In contrast to other methods, GWAS as a non-candidate driven approach investigates the entire genome for a potential association between SNPs/genomic regions and traits (Pearson & Manolio, 2008). It has the advantage of using a broader genetic variation and exploiting recombination events from a large numbers of meiosis accumulated throughout the germplasm development history (Abdurakhmonov & Abdukarimov, 2008).

Single nucleotide polymorphisms (SNPs), compared to other markers, have the advantage of being abundant in the genome (Brumfield *et al*. 2003) and are amenable to high-throughput analysis. Thus, identification of SNPs linked to the coding regions as result of GWAS could be exploited in breeding methodologies such markers assisted selection or genomic selection

(Luikart *et al*. 2003, Bush & Moore, 2012). The objectives of this study were to determine the level of linkage disequilibrium in the germplasm collection and identify candidate genomic regions associated with LLS and yield-related traits.

5.2 Materials and methods

Genotypes consisting of collected accessions from Togo (n=83), lines from Senegal (n=9) and lines from ICRISAT (n=2), were used in this study. These genotypes are a subset of germplasm collection used in the diversity study.

5.2.1 DNA Extraction and DArT Analysis

Dried leaf samples were sent to Integrated Genotyping Service and Support (IGSS) platform at Biosciences for east and central Africa laboratory at the International Livestock Research Institute (BecA/ILRI) for DNA extraction and genotyping using Diversity Arrays Technology (DArT) markers as described in the diversity study. Genotyping was carried out using Genotyping-By-Sequencing (GBS) technology (Elshire *et al*., 2011).

5.2.2 Data analysis

5.2.2.1 Linkage disequilibrium analysis

For LD and GWAS analysis, SNPs with unknown genomic positions were removed (Huang *et al*., 2012). Genotypic data were subjected to LD analysis and LD was calculated both at the whole germplasm level and within the germplasm for the clusters using a square correlation of allele frequencies (r^2). LD analysis was done using both web application KDCompute, and R statistical software version 3.3.1 (Branca *et al*., 2011; Esteras *et al*., 2013; Delourme *et al*., 2013; Li *et al*., 2014; Vos *et al*., 2017). The r^2 value ≥ 0.2 was considered as a threshold to declare significant association between loci pairs.

5.2.2.1 GWAS of Agronomic traits

SNPs with polymorphism information content (PIC) > 0.2 representing 12% of the total generated SNPs were selected for the association analysis using general linear model with GGT2 software (van Berloo, 2008). The following formula was used for the calculation of the association:

$$Y = Xa + Qb + Ku + e$$

where: Y represents the phenotype, X represents the genotype, a is the vector of fixed systematic effects, Q is the population structure, b is the vector of allele substitution effects of the major QTL, K is the relationship between samples, u is the vector of additive genetic effects explained by the polygenes, and e the error. Bonferroni multiple test threshold was used for significance of the association. The plot of association was done in R software version 3.3.1 using "qqman" package (Turner, 2014).

5.3 Results

5.3.1 Linkage disequilibrium

Nearly 42% of the analysed SNPs pairs were in linkage disequilibrium ($p = 0.001$) and r^2 ranged from 0.058 to 0.99 (Table 5.2). The mean LD for the whole germplasm set was 0.33. However, there were large differences in r^2 values among SNP pairs and only 13.3% of SNPs pairs evaluated exhibited r^2 higher than 0.5.

Intrachromosomal r^2 ranged from 0.25 to 0.425 for the entire genome (Table 5.2). Chromosome A08, A10 and B10 exhibited the lowest mean r^2 (< 0.30) while the highest intrachromosomal mean r^2 value was observed on chromosome A04 and B05 (> 0.40). No difference was observed between genome A and genome B for the r^2 values.

Table 5.1 Intrachromosomal linkage disequilibrium pattern

A GENOME				B GENOME			
Chrom.	% LP	Mean r^2	r^2 range	Chrom.	% PL	Mean r^2	r^2 range
A01	0.389	0.376	0.061 - 0.998	B01	0.588	0.328	0.059 - 0.999
A02	0.638	0.329	0.059 - 0.999	B02	0.708	0.383	0.058 - 0.999
A03	0.573	0.329	0.058 - 0.990	B03	0.601	0.377	0.059 - 0.999
A04	0.706	0.425	0.060 - 0.990	B04	0.617	0.389	0.058 - 0.999
A05	0.513	0.338	0.058 - 0.998	B05	0.661	0.414	0.058 - 0.999
A06	0.692	0.384	0.059 - 0.999	B06	0.550	0.304	0.058 - 0.999
A07	0.365	0.321	0.059 - 0.999	B07	0.587	0.37	0.058 - 0.99
A08	0.496	0.25	0.059 - 0.972	B08	0.665	0.371	0.058 - 0.998
A09	0.758	0.351	0.059 - 0.999	B09	0.501	0.311	0.058 - 0.999
A10	0.722	0.269	0.052 - 0.999	B10	0.469	0.282	0.059 - 0.990
Mean A	0.572	0.33	0.058 - 0.99	Mean B	0.582	0.345	0.058 - 0.99
T-Intra	0.34	0.34	0.058 - 0.99				
T-Inter	0.30	0.18	0.006 - 0.23				

Chrom. = Chromosome, %PL = % loci pair in LD with $P < 0.001$, T-Intra = Total intrachromosomal, T-Inter = Total interchromosomal.

Structure analysis revealed three distinguishable clusters in the entire germplasm (k=3). Three clusters were confirmed by principal component analysis with a low level of admixture between clusters (Figure 5.1). The squared allele frequencies (r^2) were assessed for all pairwise SNP combinations and Index of association (Ia) was used as a measure of multi-locus linkage disequilibrium comparison between clusters. Monomorphic loci in clusters were removed prior to the LD computation. The highest LD was observed in CIII (0.63) while the lowest was exhibited by CI (0.39). For CI, CII, and CIII respectively 3.85%, 6.26% and 18.33% of the SNP pairs were in LD with r^2 value above 0.5 (Table 5.2).

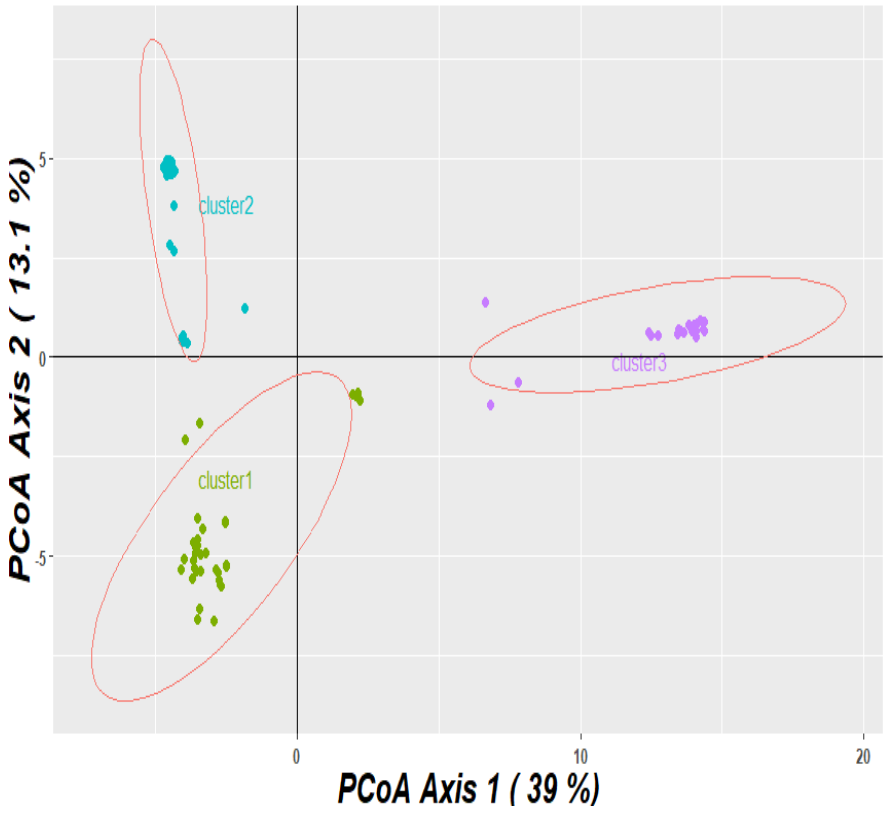

Figure 5.1 A principal component biplot diagram showing the three clusters identified by structure analysis.

These observations were confirmed by the significant index of association (Ia) in the entire germplasm collection (P = 0.001). CIII showed the highest Ia (0.143) while CI exhibited the lowest (0.10). The distribution of the LD in clusters followed the same trend observed in Ia showing a certain correlation. The plot of r^2 as a function of genetic distance revealed no significant difference in LD decay between clusters.

Table 5.2 LD characteristics of clusters

	CI	CII	CIII	Overall
Proportion of polymorphic loci (%)	0.68	0.45	0.85	1
Proportion of Significant LD (p = 0.001)	16.04 %	18.04%	30.82%	58.21%
Mean LD	0.39	0.50	0.63	0.33
Range	0.15-0.99	0.14-0.99	0.27-0.99	0.058-0.99
LD>0.5	3.85%	6.26%	18.73%	13.30
PIC	0.25	0.27	0.24	0.24

The plot of LD over the genetic distance in base pairs (Mbp) showed a decay that appeared to be low (Fig 11). Furthermore, the distance at which half of the maximum LD has decayed was quite high (113.14 Mbp). In this study, the genomic distance at which LD value (r^2) decreases below the threshold (0.2) was around 140–145 Mbp (Figure 5.2). No significant difference in LD decay was observed between genome A and B as well as between chromosomes.

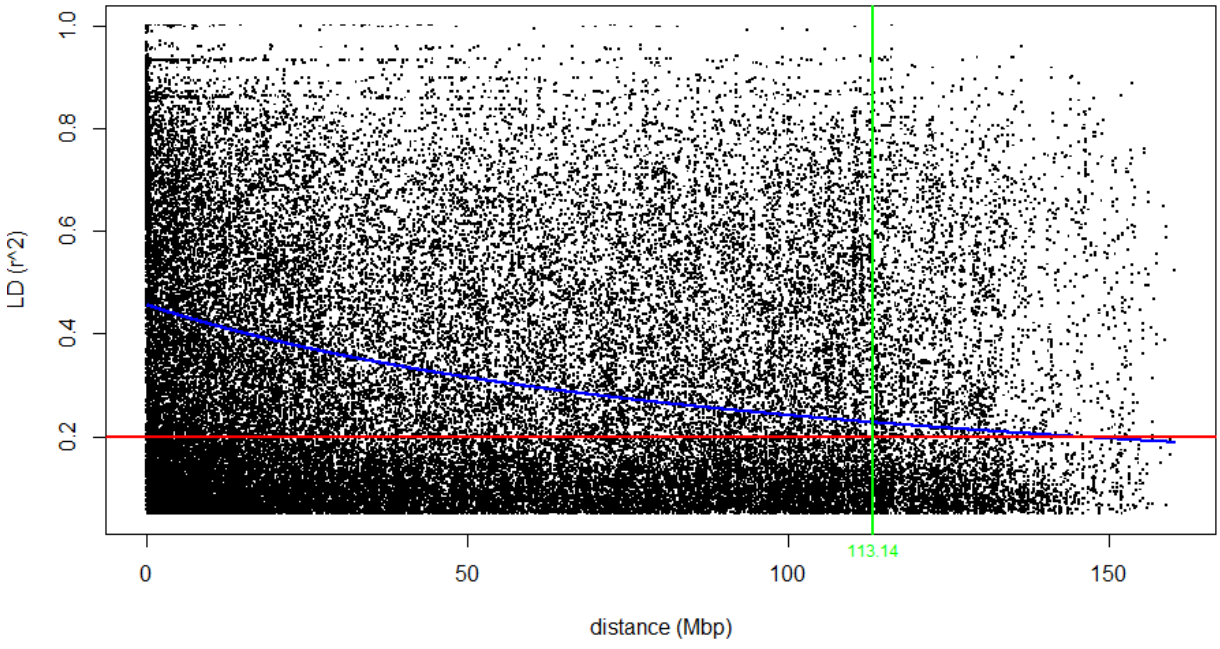

Figure 5.2 Linkage Disequilibrium decay in 92 groundnut accessions; Blue line= trend line for the mean LD; Green line= half decay; red line= r^2 threshold for LD

5.3.2 Genome-wide association

Calculated r^2 revealed that among 31 phenotypic traits scanned, a significant association was found with only 13 traits (Table 5.3). Nearly 56 SNP peaks in total reached the optimal P value (-log(P) = 7.21). Most of the significant markers ($p< 10^{-5}$) showed r^2 below 0.5. However, SNPs with high correlation ($r^2 > 0.50$) were found to be associated with LLS resistance. These SNPs were mainly located on chromosome A02, A03, A05 and B10. Thus, in this study, LLS resistance was mainly associated with the A than the B genomes in groundnut.

Table 5.3 Linkage groups for agronomic traits and r^2 range

Traits	No. of Sig. SNPs	Chromosome	r^2 range
LLS	12	A02; **A03; A05; B10***	0.50-0.57
100Seed Weight	36	**A07**; A08; B10	0.22-0.39
Pod width	16	**A08**; B04; **B05**; B6	0.30-0.42
Pod length	14	**A07**; B05	0.25-0.31
Seed width	18	**B05**	0.21-0.31
Seed length	36	A03; A04; A08; A10; B03; B04; **B05; B06**; B10	0.30-0.56
Growth Habit	122	A01; A04; A06; **A07**; A08; A09; A10; B07; B08	0.30-0.46
Pod Reticulation	35	A01; A02; A07; A08; B02; B07; B08; **B10**	0.30-0.42
Seed Color	33	A04; A08; B01; B02; **B07**	0.30-0.41

*Previously reported genomic region are in bold, *No. of Sig. SNPs* = Number of significant associations

SNPs with high association to specific chromosomes were discovered for other traits such as growth habit, pod width, pod length and seed length. SNPs on chromosome A07, A08 and B10 were found to be significantly associated with hundred seed weight with r^2 ranging from 0.22 to 0.39. Among the chromosomes associated to hundred seed weight, chromosome A07 harboured the highest number of SNPs (6 SNPs). Pod length and pod width were significantly associated, respectively with A07 and B05 ($0.25 \leq r^2 \leq 0.31$) and with A08, B04, B05, B6 ($0.30 \leq r^2 \leq 0.42$). Also, pod width was found to be more associated with B genome while pod length was significantly associated with both A and B genomes. Seed coat colour was found to be specifically associated with chromosome A04, A08, B01, B02 and B07 with r^2 ranging from 0.30 to 0.41.

Various genomic regions were significantly associated with growth habit, pod reticulation, and seed width (Figure 5.3). Also, seed length and seed width seemed to be more associated with the B genome than the A genome in this study. Growth habit exhibited the highest number of

SNPs significantly associated with up to nine chromosomes that mostly belong to the A genome.

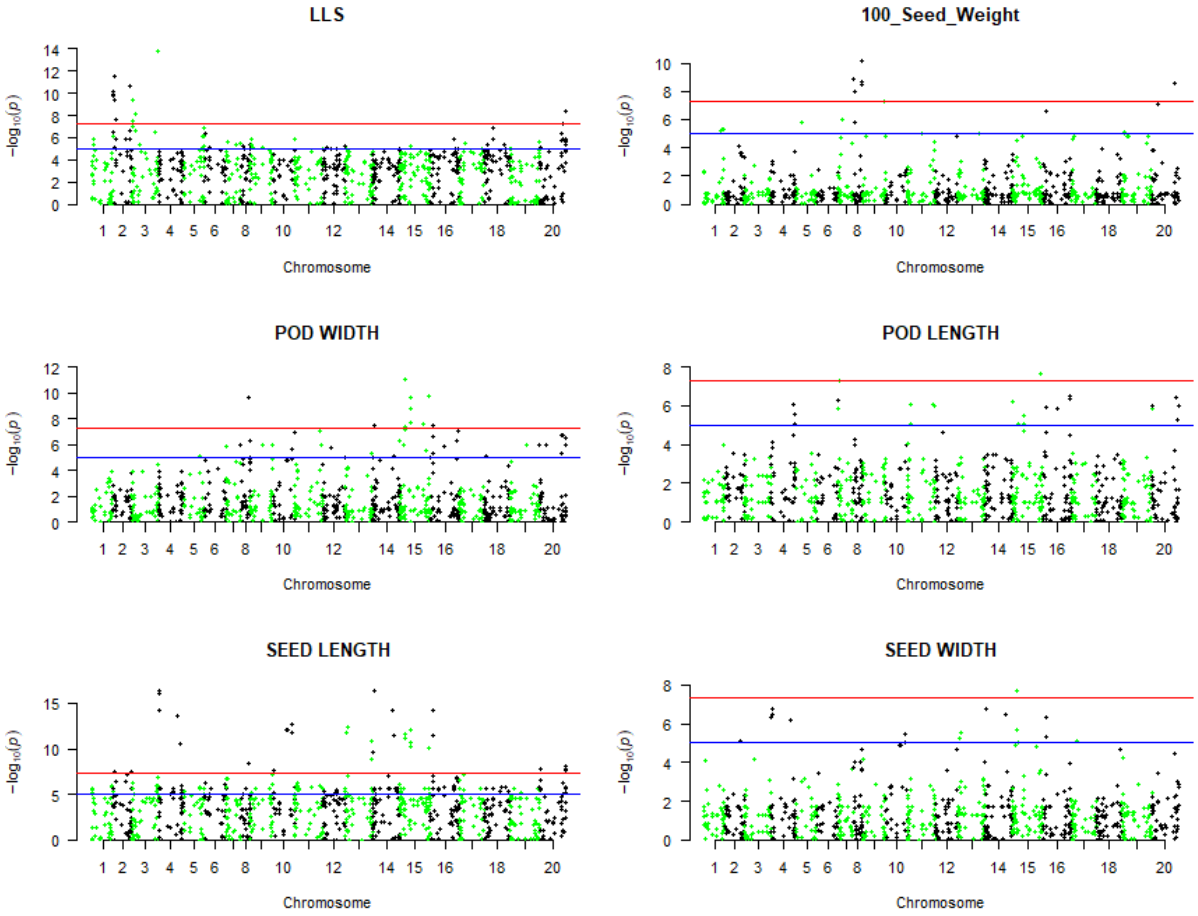

Figure 5.3 Illustration of Manhattan plot depicting strongly associated markers to different traits. The Bonferroni multiple test threshold is shown by a red line. Associated significant SNPs are at the top of the red line. Chromosomes were numbered as 1 – 20 where the A genome is represented by 1-10 and the B genome is represented as 11-20.

5.4 Discussion

Knowledge of the structure and linkage disequilibrium in a germplasm collection is a prerequisite for the trait association study. In this study, structure analysis revealed three distinguishable clusters sharing similar molecular features. The presence of three clusters in the germplasm collection was further confirmed by principal component analysis with a low level of admixture between clusters. A large number of SNP pairs were found to be in linkage disequilibrium in the studied germplasm with an r^2 value above 0.3. The high proportion of

SNPs with high values of minor allele frequency (MAF) (>0.4) found in the diversity study may be related with the high LD (r^2>0.3) observed in this study. This confirms the idea that LD distance increase with the increase of MAF (Yan *et al*., 2009).

The plot of r^2 as a function of the distance showed a decay. However, the half decay position (Branca *et al*., 2011; Vos *et al*., 2017) in this study was high confirming the high level of linkage disequilibrium observed in this study. Similar high LD values have been reported on groundnut germplasm collection from 48 countries using SSR and SNP markers by Pandey *et al*. (2014). Also, previous reports of LD study in groundnut mini core using SSR markers, showed that a large number of marker pairs were in linkage equilibrium (Belamkar *et al*., 2011). Though the difference may be partly due to the population studied, in comparison to the LD reported by these authors, in this study a higher LD in germplasm collection from Togo with the SNP markers was detected. Besides, the low decay observed in this study could partly be explained by the self-pollinated nature of groundnut (Vos *et al*., 2017) and the limited occurrence of recent genetic introgression events.

Linkage disequilibrium useful for association study was found to be very high in this population (143 Mb). However, several studies reported similar findings for highly self-pollinating crops (Abdurakhmonov & Abdukarimov, 2008; Agrama & Eizenga, 2008). In addition, the single domestication event (single wild progenitor has generated all the cultivated groundnut), probably resulting in fewer alleles being passed on to subsequent generations, may have induced the high LD observed in groundnut (Krapovickas & Gregory, 1994).

Overall, population structure influenced LD in this study, as the mean r^2 varied between clusters identified. Cluster III, consisting of groundnut accessions from Togo and a line from ICRISAT, exhibited the highest LD while cluster II, made up of lines from Togo and from Senegal, showed the lowest LD. Belamkar *et al*. (2011) reported similar findings with SSR

markers in the USA and suggested that any association study using the studied population should consider population structure. Significant low LD between unlinked loci was found in this study. This confirmed the existence of other LD generating factors suggested by previous studies (Stich *et al*., 2007). The existence of distinct clusters is one factor that could explain the LD pattern in this study as relatedness could generate LD between unlinked loci (Malysheva-Otto *et al*., 2006). However, the low level of LD between unlinked loci suggests that an association study would be effective with the present germplasm. In addition, the high LD observed for the intrachromosomal loci pairs suggests that few markers are required for the association mapping (Abdurakhmonov, & Abdukarimov, 2008).

For a more accurate estimate of the association, a highly significant threshold ($r^2 \geq 0.2$) was set in this study. Indeed, using $r^2 = 0.2$ as a threshold (Delourme *et al*., 2013; Li *et al*., 2014), linkage disequilibrium decay showed that 143 Mbp is the approximate extent of LD distance measure useful for association study in the collected germplasm.

GWAS yielded useful information that could be exploited for the improvement of multiple agronomic traits of interest. Many SNPs (56) were found to be associated with useful agronomic traits such as LLS resistance, Pod size, seed size, growth habit and seed coat colour suggesting there is a group of QTLs controlling these traits. The association analyses revealed up to 12 SNPs significantly associated with LLS resistance. Some of the genomic regions described in this study for LLS resistance genes on chromosome A03, A05 and B10 are well known. Recently, Zhang *et al.* (2017) in a GWAS study reported genes encoding a protein that is involved in LLS resistance to be distributed mainly on chromosome A03. Many QTLs are reported to be located on linkage group A05 and B10 (Agarwal *et al*., 2018). Also, the A genome seems to harbour more SNPs for LLS resistance than the B genome in this study as suggested by Leal-Bertioli *et al.* (2015). However other genomic regions such as the SNPs located on chromosome A02 for LLS are new. LLS is one of the major foliar diseases

worldwide causing significant yield loss. Thus, the identification of new genomic regions controlling resistance is worthy of further investigation.

Along with LLS, many SNPs were found to be associated with yield component traits. Among the significant linkage group associated to pod size, only A08 and B05 (for pod width) and B10 (pod length) have been previously reported to be associated with pod width and pod length (Fonceka *et al*., 2012). Also, new SNPs were found to be associated with seed width and seed length and were located on linkage groups different from those reported by previous studies (Fonceka *et al*., 2012; Pandey *et al*., 2014). In addition, significant associations were also identified for hundred seed weight (36 SNPs), growth habit (122 SNPs), pod reticulation (32 SNPs) and seed colour (33 SNPs). Overall, and at the best of our knowledge, most of the identified marker-trait association in this study are new. Thus, further analysis of agronomic traits using SNPs identified in this study could allow the identification of candidate genes for the future breeding programmes. The association study confirmed that GWAS is a useful method for the analysis of complex agronomic traits (Fahrenkrog *et al*., 2017).

5.5 Conclusion

A high LD has been observed in this study with the germplasm collection and was found to be consistent with other LD reported on highly self-pollinating crops. Several marker-trait associations were found for LLS resistance, hundred pod weight, pod length, pod width, seed length, seed width, pod reticulation, seed colour and growth habit. For LLS resistance, six new SNPs (100027806, 100015404, 100024878, 100052500, 100005206 and 100002029) were detected on chromosome A02. The identification of SNPs and linkage groups harbouring useful genes in this study could play a key role in the identification of candidate genes and the development of markers for the future breeding programme in Togo. These markers could be

deployed in marker-assisted selection for the development of high yield LLS resistant genotypes adapted to farmers growing conditions in Togo and worldwide.

CHAPTER SIX

6. GENETIC ANALYSIS OF LATE LEAF SPOT RESISTANCE, YIELD AND YIELD COMPONENTS

6.1 Introduction

Nature of gene action involved in the expression of a trait, is one of the considerations among others that the breeder must look at when starting a breeding programme. Indeed, the success of a breeding programme depends on the genetic gain per selection cycle which in turn depends mostly on how genes act or interact in the production of a phenotype (Falconer & Mackay, 1996). Another important step in plant breeding programmes is the identification of best performing lines to use in future crosses. Selection of parental lines can be based upon an evaluation of the performance of their offspring (Fasahat *et al*., 2016).

In parallel with establishing the gene action, combining ability determined through a specific mating design offers the possibility to select parents that would generate improved progenies (Sprague & Tatum, 1942). To predict the response to the selection, breeders rely on additive genetic variance. Thus, partitioning the phenotypic variation into genotypic and environmental variance and further into additive and non-additive is crucial (Ortiz & Golmirzaie, 2002). Also, the estimation of maternal effect is important as it influences the evolution of progenies based on the correlation between maternal and offspring traits (Räsänen & Kruuk, 2007).

Full factorial design (also called North Carolina II design) is one of the best methods for simultaneous estimation of additive, non-additive and maternal variances that explain phenotypic traits (Neff & Pitcher 2005; Neff *et al*. 2011). The objectives of the present study were to i) determine the type of gene action conditioning late leaf spot (LLS) resistance and yield-related traits and ii) identify parents with a good combining ability to initiate groundnut breeding programme in Togo.

6.2 Materials and Methods

6.2.1 Plant materials

Eight parent genotypes were crossed in a full factorial mating design (North Carolina II design). Four farmers' varieties from Togo, 38AH, 09AH, 68AH and 43AH were used as males and four genotypes 12CS_22 and 12CS_36 from Senegal and ICG 7878 and ICGV 02271 from ICRISAT were used as females. The farmers' local varieties were highly susceptible to LLS and exhibited small pods and seed size. The lines from Senegal were moderately resistant to LLS but with large pod and seed size. In contrast to local genotypes from Togo, ICRISAT genotypes were resistant to LLS with medium pod and seed size (Table 6.1). F1 hybrids were developed at ICRISAT-WCA in Bamako. Male parents were planted in one row of 40 plants per male parent two weeks before female parents. Four rows of 4 m long and 30 cm between hills and 1 m between rows were adopted for female parents. Hand emasculation in the afternoon and pollination early in the morning were carried out by skilled workers at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, Bamako) under the supervision of the researcher and precaution were taken to avoid contamination. F1 seeds were then planted with both parents in unreplicated trial during the off season to check the hybridity based on morphological traits such us plant height, leaf color and shape and pod traits. Selected hybrids were then sent to CERAAS for genotyping using a set of polymorphic markers. Confirmed hybrids were then advanced to F_2 population for multilocation trials.

Table 6.1 Parent genotypes characteristics

Genotype	Status	Pedigree	Growth habit	LLS resistance	Pod and seed size
38AH	Male	Accession (Togo)	Erect	Low	Small
09AH	Male	Accession (Togo)	Erect	Low	Small
43AH	Male	Accession (Togo)	Erect	Low	Small
69AH	Male	Accession (Togo)	Bunch	Moderate	Large
12CS_22	Female	Fleur11 x AiAB	Bunch	Moderate	Medium
12CS_36	Female	Fleur11 x AiAB	Bunch	Moderate	Medium
ICG 7878	Female	Accession (North Carolina)	Bunch	High	Medium
ICGV 02271	Female	TAG 24 x ICGV 86031	Bunch	High	Medium

6.2.2 Test environments and experimental design

Sixteen cross combinations F_2 populations and their parents were evaluated using an alpha-lattice design in four agroecological environments representing the major ecological zones of groundnut production: Talo_Kara (9.546° N, 1.193° E), Tantigou_Dapaong (10.8733° N, 0.2010° E), Sotouboua_Central (8.565° N, 0.977° E) and Mission Tove_Maritime (6.322°N, 1.126°E). A plot of four rows of 5 m length containing 33 plants per row with 15 cm between plants represented an experimental unit. A spacing of 50 cm between rows and 1 m between blocks was adopted. The field was kept weed free at an interval of one weeding every three weeks by hoeing.

6.2.3 Data collection

Disease scoring for LLS was done at 60 days and 90 days after sowing using a 9 points scale (Subrahmanyam *et al*., 1995) on individual F_2 plants as described in Table 6.2. In parallel with establishing the gene action for the improvement of LLS, observation on yield parameters such as pod weight (weight/plant, 100 pods), number of pods per plant, were recorded on 120 F_2

plants per cross combination. Hundred seed weight was collected on a plot basis. Pod length, pod width, seed length, and seed width were collected on plot basis on 30 randomly selected pods and seeds.

Table 6.2 Description of late leaf spot rating scale

Leaf spot score	**Description**	**Disease severity (%)**
1	No disease	0
2	Lesions largely on lower leaves; no defoliation	1-5
3	Lesions largely on lower leaves; very few lesions on middle leaves; defoliation of some leaflets evident on lower leaves	6-10
4	Lesions on lower and middle leaves, but severe on lower leaves; defoliation of some leaflets evident on lower leaves	11-20
5	Lesions on all lower and middle leaves; over 50% defoliation of lower leaves	21-30
6	Lesions severe on lower and middle leaves; lesions on top leaves but less severe; extensive defoliation of lower leaves; defoliation of some leaflets evident on middle leaves	31-40
7	Lesions on all leaves but less severe on top leaves; defoliation of all lower and some middle leaves	41-60
8	Defoliation of all lower and middle leaves; lesions severe on top leaves and some defoliation of top leaves evident	61-80
9	Defoliation of almost all leaves leaving bare stems; some leaflets may be present, but with severe leaf spot	81-100

6.2.4 Data analysis

6.2.4.1 Variance component estimates

Phenotypic variances were partitioned into additive, non-additive and maternal effect using the Restricted Maximum Likelihood (REML) model (Neff & Pitcher 2005; Bates *et al*., 2015) in "*fullfact"* R package as follow:

$$Y_{ijklmn} = \mu + B_i + L_j + M_k + F_l + I_m + e_{ijklmn}$$

where Y_{ijklmn} is the phenotypic response, μ is the mean value, *Bi* is the effect of block, L_j is the effect of location, M_k is the kth effect of male, F_l is the lth effect of female, I_m is the mth effect of interaction between male and female and *eijklmn* is the random residual. The random and fixed effect of all the factors were estimated.

The significance for block, location, male, Female and the interaction was estimated using the likelihood ratio test with '*observLmer3'* function in the "*fullfact"* R package (Houde & Pitcher, 2016) for the normally distributed traits (Appendice 3). The phenotypic variance was then apportioned into male (V_m), female (V_f) and the interaction between male and female (V_I). The calculation of additive (V_A), non-additive (V_{NA}) and maternal variance (V_M) was as follows: $V_A = 4V_m$; $V_{NA} = 4V_I$; $V_M = V_f - V_m$ (Lynch & Walsh, 1998).

6.2.4.2 Combining ability estimates

The estimation of GCA and SCA was done using excel and package "lme4" in R software version 3.3.1. The following model was used in R:

$$X_{ijklmn} = \mu + g_i + g_j + s_{ij} + (\frac{1}{b})\Sigma_k e_{ijklmn}$$

where μ = the population mean; g_i = the general combining ability effect of the ith parent; g_i = the general combining ability effect of the jth parent; s_{ij} = the specific combining ability

effect of the cross between ith and jth parents; e_{ijklmn}= the environmental effect associated with ijkth observation; b = number of blocks/replication. Using parent's genotyping data, Euclidian genetic distance was computed between the 8 parents for the evaluation of the association between genetic distance and SCA (Hedrick, 2005).

6.3 Results

6.3.1 Variance components in LLS and yield-related traits

A significant random effect for location (8.09%) and significant interactions between the environment and variance components for LLS (Table 6.3) and most yield-related traits was observed. Additive gene effect played the major role in all the three locations, but the estimation of non-additive and maternal effect varied between environments for LLS resistance. The maternal effect was higher in Kara whereas non-additive effect was not detected. Analysis of variance component across environment for LLS resistance showed that additive genetic explained the largest part of the phenotypic variance observed (87.84%) while non-additive variance was very low (4.3). Maternal variance (8.6) contributed to the rest of the phenotypic variance. With the addition of the residual, the variance components sum up to more than 100% showing that there may be epistasis.

Table 6.3 Variance component for LLS by sites

	Variance components (%)					
Sites	V_I	V_m	V_f	V_A	V_{NA}	V_M
Savanes	1.2	21.9	13.3	87.84	4.83	-8.6
Kara	0.00	18.13	0.00	72.52	0.00	-18.26
Sotouboua	1.1	22.3	14.5	89.2	4.4	-7.3
Mission_Tove	0.8	15.3	15.2	61.2	3.1	-0.1.1

V_I, V_m and V_f represent the phenotypic variance components (%) male x female interaction, male and female effects, respectively. V_A, V_{NA} and V_M represent additive genetic, non-additive genetic and maternal effect variance components, respectively.

Additive genetic variance seemed to prevail over non-additive variance for all the other traits except for pod weight per plant (Table 6.4). Non-additive genetic variance played a major role (21.79% of the phenotypic variance explained) in the expression of the pod weight per plant. For the number of pods per plants, additive genetic variance explained 7.3% of the total phenotypic variance while non-additive (0.56%) and maternal effect (-1.82%) were very low.

Table 6.4 Variance components for yield and yield-related traits across sites

	Variance components (%)						
Traits	**V_L**	**V_I**	**V_m**	**V_f**	**V_A**	**V_{NA}**	**V_M**
LLS	9.25	2.35e-3	10.87	19.73	43.48	9.4E-3	3.86
Pods / plant	53.7	0.142	1.82	0.00	7.30	0.56	-1.82
Pod length	1.49	0.00	0.48	46.04	1.93	0.00	45.56
Pod width	14.83	0.00	0.13	63.66	0.53	0.00	63.72
Seed length	24.8	0.00	0.51	16.4	2.07	0.00	15.8
Seed width	10.54	0.00	10.02	1.01	4.05	0.00	9.01
100 Pod weight	14.30	$2.22e^{-13}$	2.34	2.21	9.38	0.00	-0.12
100 Seed weight	10.6	0.00	3.21	11.66	12.84	7.15E-15	8.44
Pod weight/plant	41.01	5.44	1.42	0.64	5.69	21.79	-0.78
Shelling %	69.92	1.67	0.79	2.60	3.18	6.69	1.81

V_L, V_I, V_m and V_f represent the phenotypic variance components (%) explained by location, male x female interaction, male and female effects, respectively. V_A, V_{NA} and V_M represent additive genetic, non-additive genetic and maternal effect variance components, respectively.

Overall, LLS resistance, pod length, pod width, seed length, seed width and hundred seed weight showed a high additive effect. For Pod length, pod width, seed length, seed width pod weight per plant and shelling percentage, the additive effect was significant but low. Posotive maternal effects were observed for the LLS, pods/plant, pod width, seed size (length and

width), hundred seed weight and shelling percentage. In contrast, hundred pod weight and pod weight per plant showed a negative maternal effect.

6.3.2 GCA for LLS and yield-related traits

Significant interactions were observed between the environments and GCA in the number of pods per plant, pod length, pod width, seed length, seed width, hundred pod weight, and shelling percentage and at a lesser extent in LLS incidence (result not shown). GCA in Kara and Sotouboua showed a high correlation for most traits while Sotouboua and Mission_Tove showed a weak but significant correlation ($p = 0.05$). Estimates of GCA effect across environment for the tested genotypes are presented in Table 6.4 and 6.5. As suggested by Bookmyer *et al*. (2009) and Mukankusi *et al*. (2011), on the basis of the LLS scale where less disease incidence is associated with the lowest value, negative combining ability effects are preferable for disease resistance.

Table 6.5 General combining ability for LLS and yield-related traits of the genotypes used as male parents

Traits	GCA of male parents				Significance
	43AH	68AH	09AH	38AH	
LLS	**-0.137** (±0.046)	-0.007 (±0.047)	0.039 (±0.046)	0.1042 (±0.047)	*
Pod/Plant	**0.164** (±9.4E-3)	**0.311** (±9.3E-3)	0.005 (±9.3E-3)	-0.481 (±9.3E-3)	*
Pod Length	0.026 (±0.009)	-0.037 (±0.009)	-0.005 (±0.009)	0.016 (±0.009)	NS
Pod Width	**0.018** (±0.004)	0.006 (±0.004)	-0.011 (±0.003)	-0.002 (±0.003)	*
Seed Length	-0.007 (±0.007)	-0.016 (±0.007)	0.021 (±0.007)	0.001 (±0.007)	NS
Seed Width	**0.018** (±0.004)	-0.007 (±0.004)	-0.005 (±0.004)	-0.006 (±0.004)	*
Hund PW	**1.082** (±0.126)	**2.613** (±0.127)	-2.01 (±0.166)	-1.685 (±0.124)	*
Hund SW	-0.427 (±0.080)	-1.263 (±0.080)	**0.385** (±0.082)	**1.304** (±0.081)	*
PW/Plant	0.280 (±0.264)	**0.577** (±0.264)	-0.12 (±0.264)	-0.737 (±0.264)	*
Shelling %	**1.657** (±0.619)	-0.757 (±0.620)	0.045 (±0.620)	-0.944 (±0.620)	*

The best High GCA were highlighted (in bold) and ranked (rank in parenthesis). Significance codes: '***' 0.001; '**' 0.01; '*' 0.05, 'NS' Non-significant; Hund PW = hundred pod weight, Hund SW = hundred seed weight, PW/Plant = pod weight per plant; SD = Standard deviation

Among male parents, 43AH showed a slightly higher GCA effect in the desired direction for LLS resistance (Table 6.5). Among the female parents, ICG 7878 exhibited the highest GCA effect in the desired direction (Table 6.6). Thus, these two parents were good general combiners for LLS resistant genotypes' development. Line 43AH exhibited, along with LLS resistance, good GCA effect for yield-related traits such as number of pods per plant, pod width, hundred pod weight, and shelling percentage (Table 6.4 and 6.6). The best general combiner for LLS

resistance, ICG 7878, exhibited negative GCA for most yield-related traits such as hundred pod weight and hundred seed weight.

Table 6.6 Female parents GCA for LLS and yield-related traits

	GCA of female parents				
Traits	12CS_22	12CS_36	ICGV 02271	ICG 7878	Significance
LLS	0.771 (±0.146)	0.641 (±0.145)	0.215 (±0.145)	**-1.627** (±0.145)	**
Pod/Plant	0.54 (±0.187)	0.151 (±0.186)	-0.849 (±0.186)	0.158 (±0.186)	NS
Pod Length	-0.01 (±0.036)	0.115 (±0.036)	-0.065 (±0.036)	-0.038 (±0.036)	NS
Pod Width	-0.02 (±0.027)	-0.116 (±0.026)	**0.054**(2) (±0.026)	**0.08**(1) (±0.026)	*
Seed L	0.011 (±0.019)	0.044 (±0.018)	-0.06 (±0.018)	0.005 (±0.019)	*
Seed W	0.006 (±0.008)	-0.027 (±0.008)	0.024 (±0.008)	-0.002 (±0.008)	*
Hund PW	1.646 (±3.333)	-1.794 (±3.313)	**8.556** (±3.323)	-8.407 (±3.325)	**
Hund SW	**2.051**(2) (±1.571)	0.571 (±1.571)	**2.461**(1) (±1.571)	-5.083 (±1.571)	**
PW/Plant	**0.657(1)** (±0.199)	**0.289(2)** (±0.198)	-0.365 (±0.199)	-0.581 (±0.198)	*
Shelling %	**0.764(2)** (±0.760)	**1.436(1)** (±0.761)	0.0301 (±0.762)	-2.229 (±0.762)	*

The best High GCA were highlighted (in bold) and ranked (rank in parenthesis). Significance codes: '***' 0.001; '**' 0.01; '*' 0.05, 'NS' Non-significant; Hund PW = hundred pod weight, Hund SW = hundred seed weight, PW/Plant = pod weight per plant

Genotypes such as 12CS_22 and 12CS_36 which did not show significant negative GCA effect for LLS resistance, are good combiners for yield related traits such as pod weight per plant and shelling percentage. The other two male parents, 09AH and 38AH displayed positive GCA effect for only seed length and hundred seed weight. No significant GCA effect was observed for pod length, seed length on the male side, and the number of pods per plant and pod length on the female side.

6.3.3 SCA for LLS and yield-related traits

Multi-environment SCA analysis revealed a significant interaction between environment and SCA for yield-related traits (result not shown). The interaction was even high with the ranking of SCA changing across different environments. Estimation of SCA across environments revealed positive and negative cross combinations. For LLS resistance, eight hybrids exhibited negative SCA effects and the top three were 12CS_22x38AH, ICG 7878x68AH, ICGV 02271x43AH (Table 6.7). These cross combinations had as parent combinations resistant x susceptible, resistant x moderately resistant, and moderate resistant x susceptible confirming the non-additive gene action observed in the variance component analysis. Most of the combinations with LLS resistance generally displayed an undesirable SCA effects for pod/plant and pod weight per plant, except 12 CS_36x38AH (Table 6.7).

Table 6.7 Specific combining ability of cross combination for LLS and Pod traits

	Traits				
Cross combination	LLS	Pod/Plant	PW/Plant	Pod Length	Pod Width
12CS_22 x 43AH	0.2106 (±0.048)	**1.231**(2) (±0.261)	**0.809**(2) (±0.266)	-0.006 (±0.039)	-5E-04 (±0.002)
12CS_36 x 43AH	0.0069 (±0.047)	-0.547 (±0.262)	-0.228 (±0.266)	-0.014 (±0.039)	-0.007 (±0.005)
ICGV 02271 x 43AH	**-0.16(3)** (±0.047)	-0.519 (±0.263)	0.2025 (±0.267)	-0.009 (±0.038)	-0.012 (±0.006)
ICG 7878 x 43AH	-0.058 (±0.047)	-0.165 (±0.262)	-0.784 (±0.266)	0.0285 (±0.040)	0.019 (±0.006)
12CS_22 x 68AH	-0.104 (±0.047)	-0.054 (±0.262)	-0.872 (±0.266)	0.034 (±0.041)	-0.014 (±0.006)
12CS_36 x 68AH	0.1736 (±0.047)	**1.279**(1) (±0.262)	0.640(4) (±0.266)	0.0137 (±0.038)	0.022 (±0.021)
ICGV 02271 x 68AH	0.1921 (±0.048)	-0.498 (±0.262)	-0.176 (±0.266)	0.0073 (±0.041)	0.0049 (±0.002)
ICG 7878 x 68AH	**-0.262**(2) (±0.048)	-0.727 (±0.262)	0.408(5) (±0.266)	-0.055 (±0.041)	-0.012 (±0.266)
12CS_22 x 09AH	0.1829 (±0.047)	-0.332 (±0.262)	**1.114**(1) (±0.266)	0.031 (±0.039)	0.020 (±0.021)
12CS_36 x 09AH	-0.095 (±0.048)	-0.832 (±0.261)	-1.078 (±0.266)	0.032 (±0.038)	0.0004 (±0.003)
ICGV 02271 x 09AH	-0.113 (±0.047)	**0.613**(3) (±0.262)	-0.09 (±0.266)	-0.031 (±0.037)	-0.011 (±0.002)
ICG 7878 x 09AH	0.0255 (±0.048)	0.550 (±0.262)	0.0543 (±0.266)	-0.032 (±0.039)	-0.009 (±0.004)
12CS_22 x 38AH	**-0.289**(1) (±0.048)	-0.845 (±0.262)	-1.051 (±0.267)	-0.058 (±0.040)	-0.005 (±0.003)
12CS_36 x 38AH	-0.086 (±0.046)	0.099 (±0.263)	0.666(3) (±0.267)	-0.032 (±0.039)	-0.016 (±0.021)
ICGV 02271 x 38AH	0.081 (±0.048)	0.405 (±0.261)	0.0638 (±0.266)	0.032 (±0.037)	0.018 (±0.021)
ICG 7878 x 38AH	0.294 (±0.047)	0.342 (±0.262)	0.3208 (±0.266)	0.058 (±0.390)	0.0028 (±0.0018)
Significance	*	*	**	**NS**	NS

The top High SCA were highlighted (in bold) and ranked (rank in parenthesis). Significance codes: '***' 0.001; '**' 0.01; '*' 0.05, 'NS' Non-significant; PW/Plant = pod weight per plant

Other interesting cross combination were: 12CS_22 x 43AH, ICGV 02271 x 09AH, ICG 7878 x 09AH for the number of pods per plants; 12CS_22 x 09AH for pod weight per plant; ICG 7878 x 43AH for shelling percentage; ICGV 02271 x 43AH for hundred seed weight and

hundred pods weight (Table 6.8). Also, these crosses were mostly between high GCA x high GCA or high GCA and low GCA for the different traits. However, another cross between low GCA x low GCA, following the example of ICGV 02271 x 09AH, exhibited a high SCA effect for the number of pods per plant. Altogether, these results underlined the implication of non-additive gene action in the yield-related trait expression, even though additive genes play the major role. Specific combining ability for pod length, pod width, seed length and seed width displayed no statistical difference between cross combinations (Table 6.7 and 6.8).

Table 6.8 Specific combining ability of cross combination for yield and seed related traits

	Traits				
Crosses	100 PodW	100 SeedW	Shelling %	Seed Length	Seed Width
12CS_22 x 43AH	1.6035 (±1.783)	-1.446 (±4.4E-8)	-1.187 (±0.710)	-5E-05 (±0.012)	0.007 (±0.075)
12CS_36 x 43AH	-8.2078 (±1.720)	-0.259 (±4.4E-8)	0.587 (±0.720)	0.0043 (±0.013)	-0.0143 (±0.076)
ICGV 02271 x 43AH	**5.321**(1) (±1.746)	**2.993**(1) (±4.4E-8)	-1.21 (±0.720)	-0.013 (±0.012)	0.023 (±0.076)
ICG 7878 x 43AH	1.2838 (±1.720)	-1.289 (±4.3E-8)	**1.81**(1) (±0.720)	0.0083 (±0.013)	-0.015 (±0.077)
12CS_22 x 68AH	-1.6436 (±1.735)	-0.795 (±4.3E-8)	**1.739**(2) (±0.740)	-0.008 (±0.012)	-0.0171 (±0.076)
12CS_36 x 68AH	1.3563 (±1.720)	0.3344 (±4.4E-8)	-1.552 (±0.720)	-0.004 (±0.011)	0.012 (±0.073)
ICGV 02271 x 68AH	1.279 (±1.720)	-0.256 (±4.1E-8)	-0.449 (±0.700)	0.002 (±0.011)	0.0047 (±0.076)
ICG 7878 x 68AH	-0.9918 (±1.820)	0.716(4) (±4.2E-8)	0.262 (±0.710)	0.011 (±0.012)	5E-05 (±0.076)
12CS_22 x 09AH	**3.3693**(3) (±1.952)	**1.452**(2) (±4.2E-8)	1.337(4) (±0.730)	-0.004 (±0.013)	0.009 (±0.076)
12CS_36 x 09AH	2.445 (±1.920)	0.2903 (±4.4E-8)	-0.351 (±0.720)	-0.009 (±0.013)	-0.0005 (±0.071)
ICGV 02271 x 09AH	-3.2738 (±1.820)	-1.834 (±4.2E-8)	0.254 (±0.720)	0.044 (±0.012)	-0.015 (±0.076)
ICG 7878 x 09AH	-2.54 (±1.820)	0.0921 (±4.4E-8)	-1.239 (±0.770)	-0.031 (±0.010)	0.0062 (±0.066)
12CS_22 x 38AH	-3.3292 (±1.820)	**0.789**(3) (±4.4E-8)	-1.889 (±0.750)	0.012 (±0.012)	0.0013 (±0.007)
12CS_36 x 38AH	**4.407**(2) (±1.710)	-0.366 (±4.3E-8)	1.317(5) (±0.740)	0.010 (±0.011)	0.0023 (±0.078)
ICGV 02271 x 38AH	-3.3258 (±1.700)	-0.904 (±4.4E-8)	**1.406**(3) (±0.730)	-0.033 (±0.014)	-0.0123 (±0.076)
ICG 7878 x 38AH	2.248(5) (±1.820)	0.481(5) (±4.3E-8)	-0.834 (±0.720)	0.012 (±0.012)	0.009 (±0.076)
Significance	*	*	**	NS	NS

Top three top SCA were highlighted (in bold) and ranked (rank in parenthesis). Significance codes: '***' 0.001; '**' 0.01; '*' 0.05, 'NS' Non-significant; 100 SeedW = hundred seed weight; 100 PodW = hundred pod weight

6.3.4 Traits correlation for SCA and GCA

GCA effect correlation analysis of LLS and other agronomic traits revealed a positive correlation between LLS and hundred seed weight (r = 0.9; p = 0.01), LLS and shelling

percentage (r =0.73; p = 0.05) (Table 6.9). Conversely, LLS exhibited negative GCA effect correlation with pod width (r =-0.71; p = 0.05). Positive correlation was also observed between other pairs traits such as number of pods per plant and pod weight per plant (r = 0.72; p = 0.05), pod length and seed length (r = 0.76; p = 0.05), hundred pods weight and hundred seed weight (r = 0.73; p = 0.04). Surprisingly, negative correlation for GCA effects between pod length and pod width (r= -0.88; p = 0.01), seed length and seed width (r = -0.80; p = 0.05) were observed.

Table 6.9 Correlation between traits' GCA for all cross combination

	LLS	**PPP**	**PL**	**PW**	**SL**	**SW**	**HPW**	**HSW**	**PWP**	**SH**
LLS										
PPP	0.01									
PL	0.39	0.26								
PW	-0.71*	-0.31	-0.88**							
SL	0.11	0.61	0.76*	-0.69						
SW	-0.09	-0.36	-0.65	0.67	-0.80*					
HPW	0.61	-0.41	-0.29	0.01	-0.69	0.57				
HSW	0.90**	-0.37	0.14	-0.41	-0.19	0.22	0.73*			
PWP	0.53	0.72*	0.22	-0.48	0.22	-0.09	0.31	0.19		
SH	0.73*	0.18	0.59	-0.66	0.21	0.08	0.43	0.59	0.61	

LLS=late leaf spot, PPP=Number of pods per plant, PL=pod length, PW=pod width, SL=Seed length, SW=Seed width, HPW= 100 pod weight, 100Seed weight, PWP=pod weight per plant, and SH = Shelling %

6.3.5 Correlation between SCA and genetic distance

Genetic distances between parent genotypes were calculated based on SNP data (Table 6.10) and a correlation analysis of SCA and genetic distance revealed no significant correlation between SCA and genetic distance. Thus, for most of the traits' SCA varied independently from the genetic distance. However, some traits such as pod weight per plant (PWP) and shelling percentage (SH) seemed to exhibit high SCA when the genetic distance between parents increases (Table 6.11).

Table 6.10 Euclidian distance between parent genotypes based on SNP data

	09HA	38AH	43AH	68AH	12CS_22	12CS_36	ICG 7878	ICGV 02271
09AH	0.00							
38AH	9.63	0.00						
43AH	9.64	2.59	0.00					
68AH	11.59	12.01	12	0.00				
12CS_22	11.65	12.12	12.07	5.89	0.00			
12CS_36	11.53	11.82	11.89	5.33	4.6	0.00		
ICG 7878	15.54	15.54	15.47	14.57	14.67	14.57	0.00	
ICGV 02271	13.54	14.63	14.23	13.82	14.53	14.22	10.21	0.00

Table 6.11 Correlation pattern between SCA and the genetic distance between parents.

	LLS	PP	PWP	PL	PW	HPW	HSW	SH	SL	SW	GD
LLS											
PP	0.56*										
PWP	0.51*	0.49*									
PL	0.63**	0.16	-0.03								
PW	0.57*	0.25	0.18	0.59*							
HPW	0.47	0.42	0.23	0.18	0.62**						
HSW	0.24	0.31	0.09	0.15	0.66**	0.41					
SH	-0.39	0.10	0.10	-0.36	-0.49*	-0.08	-0.37				
SL	-0.33	-0.34	-0.36	-0.01	-0.18	-0.06	-0.27	0.02			
SW	0.65**	0.28	0.10	0.51*	0.91**	0.66**	0.74**	-0.66**	-0.22		
GD	-0.06	-0.22	0.05	-0.20	-0.11	-0.13	-0.23	0.22	-0.39	-0.17	

LLS=late leaf spot, PP=Number of pods per plant, PL=pod length, PW=pod width, SL=Seed length, SW=Seed width, HPW= 100 pod weight, 100Seed weight, PWP=pod weight per plant, SH= Shelling % and GD= Genetic distance

6.4 Discussion

Determination of the mode of inheritance of agronomic traits helps adopt better planning and execution of the breeding programme. Nature of gene action controlling agronomic traits is crucial as the breeding method and response to a selection from breeding population depends on it. In this study, the additive genetic effects were higher than the non-additive and maternal effects for most traits. Indeed, LLS resistance, pod length, pod width, seed length, seed width and hundred seed weight showed a high additive gene action. Other traits such us number of

pods per plant, hundred pods weight, and shelling percentage showed significant but low additive gene action. This result agrees with previous studies that reported that LLS resistance and yield-related traits are controlled by additive gene action (Dwivedi *et al*.,1989; Pasupuleti *et al*., 2013). However, some authors reported a predominance of non-additive gene effect for pod yield and related traits using line by tester (Manivannan *et al*., 2008; Mothilal & Ezhil, 2010; Patil *et al*., 2017) or diallel population (Azad *et al*., 2014).

In this study, non-additive gene effect prevailed over additive gene effect only for pod weight per plant. The discrepancy between these results and those of other researchers highlights the importance of GxE in the estimation of the genetic variance components for agronomic traits. In addition, the estimates of heritability for LLS resistance in this study was much higher than those previously reported. Also, the estimates of the additive gene effects for yield-related traits were either lower or higher than those reported in previous studies. The differences in additive genetic effects between this study and previous studies may be due to the differences in the populations used or difference in the experimental designs and analytical methods. It is known that strong selection reduces additive genetic variance, thus populations subjected to different selection pressure can exhibit a different distribution of genetic variance components.

Although gene action has been investigated in various agronomic traits of interest, few studies focused on the estimation of maternal effects. In this study, the maternal effect was significantly high for LLS resistance and pod size (length and width) but small for most of the yield-related traits. It is worthy to mention that maternal effect has been previously reported for LLS resistance (Singh *et al*.; 1997; Pasupuleti *et al*., 2013). The highly significant maternal effect in LLS resistance suggests that the choice of the resistance donor as female parent for the development of the breeding population is the best approach. Also, the hundred pods weight and pod weight per plant showed significant female effect than male effect. Overall, the maternal effect was lower than additive gene effect for all traits suggesting that maternal effect,

though significant, contributes less than additive genes to the phenotypic expression of the agronomic traits studied. The observed differences between traits in the magnitude of the additive, non-additive and maternal effect is not surprising as different traits can exhibit a different level of heritability (He *et al*., 2017). In this study, LLS resistance, pod size and seed size exhibited higher additive gene action than the other agronomic traits whereas pod weight per plant and shelling outturn had a higher non-additive genetic effect than the other traits. These differences in gene action indicate that the agronomic traits studied will have different responses to selection.

The success of a breeding programme relies upon the accuracy in the choice of the parent genotypes, and combining ability has been considered as an efficient tool for the identification of the best parents (Fasahat *et al*., 2016). In this study, combining ability analysis has allowed the identification of parents for LLS resistance and yield-related traits for utilization in genetic improvement of groundnut. The GCA estimate revealed genotypes such us 43AH and ICG 7878 as the best general combiners for LLS resistance. Interestingly, 43AH also exhibited good GCA effect for yield-related traits such as pod width, hundred pod weight, pod weight per plant, and shelling percentage. These two genotypes could be used as sources of LLS resistance for the development of resistant inbred lines. However, the best general combiner for LLS resistance, ICG 7878 seems to carry negative genes for most of the yield component traits. Thus, the use of such genotypes for the introgression of resistant genes in a breeding programme should be done using a breeding methodology that allows getting rid of these negative alleles.

In addition, males' GCA effect did not show a significant difference for the number of pods per plant, pod length and seed width. These genotypes are preferred by farmers because of their earliness and relatively high number of pods per plant. This could explain the similarity of the GCA effect observed. In contrast, 12CS_22 and 12CS_36 which share common genetic origin

(common parents) displayed different combining ability effects for all the traits except LLS and seed length. This result implies that similar genetic background does not always infer similar combining ability.

High SCA effects were obtained for LLS, number of pods per plant, pod weight per plant, hundred seed weight, hundred pod weight and shelling percentage. These SCA were obtained by high x high, high x low, and low x low GCA effect parents. The pedigree breeding method should be efficient for the development of high yielding varieties using crosses such ICGV 02271 x 43AH and ICG7878 x 68AH as the crosses involved parents with high GCA and displayed high SCA effect for LLS resistance. In that case, the hope is to get good transgressive segregants as SCA cannot be exploited in the development of pure lines.

It is evident that a majority of quantitative characters, in particular yield-related traits, show varying degree of association among themselves. Even if the combining ability is carried out for a number of traits simultaneously, there was a difficulty in identifying best parents or cross combinations for a number of traits. For that purpose, correlation analysis of GCA and SCA effects among evaluated traits was used in this study. Various degree of association both desirable and undesirable were observed between LLS and yield-related traits for combining ability. GCA effect for LLS resistance, hundred seed weight and shelling percentage were correlated in this study. Conversely, LLS exhibited negative GCA effect correlation with pod width. Other yield-related traits exhibited a positive correlation between themselves. GCA and SCA positive correlations for different traits suggest that these traits could be improved together through selection using parents with good GCA for at least one of the correlated traits. Significant interactions between environment and GCA in yield related traits indicate that developing breeding products that target specific environments would be more effective. It has been often suggested that the use of extremely divergent parents results in high SCA. In this study, only three traits out of eleven seem to confirm that assumption. The overall result of the

analysis of the correlation between molecular divergence and SCA suggests that the use of extremely divergent parents to obtain heterotic combinations for agronomic traits of interest in groundnut does not hold for this study.

6.5 Conclusion

The results suggest that additive gene action predominates over the other types of gene action for LLS resistance and most yield-related traits. Maternal effect seems to play an important role in the phenotypic variance of LLS resistance, pod and seed size. Furthermore, selection could be more effective when based on performance in specific environment as there was a significant environment by GCA effect. For that purpose, genotypes such us 43AH and ICG7878 are the best parents for LLS resistance and yield-related traits improvement.

CHAPTER SEVEN

7. GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 General conclusions

The results of the present study would help in guiding the new breeding programme from setting the breeding goals and strategies to the product delivery. The PRA survey revealed that, with few exceptions, farmers in Togo face similar production constraints. Overall, information on the farming practices, constraints in groundnut production, and farmers preferred characteristics provide the basis for a participatory breeding programme. The breeding programme on groundnut should consider foliar diseases, mainly LLS, as a major constraint. In the present state, high yielding groundnut varieties with large pod size and resistance to LLS are likely to be adopted by groundnut farmers.

The diversity analysis of the cultivated groundnut cultivars in Togo revealed an appreciable diversity for phenotypic traits as well as molecular markers. Traits of interest such LLS resistance, days to maturity and hundred seed weight showed significant variation within the germplasm collection. At the molecular level, many SNPs harbouring large genetic variability were found in the groundnut collection. Besides, the diversity observed at the molecular level was associated with phenotypic variation, particularly for quantitative agronomic traits. The identification of three groups of accessions harbouring desired traits/alleles is valuable information for starting the groundnut breeding programme.

The GWAS analysis revealed several new marker-trait associations for LLS resistance, hundred pod weight, pod length, pod width, seed length, seed width, pod reticulation, seed colour and growth habit. The identification of SNPs and linkage groups harbouring useful genes is worthy of further investigation. These SNPs could play a key role in the development of markers for the future breeding programme in Togo.

Most of the traits mentioned by farmers are quantitative traits with a complex mode of inheritance. In this study, eight parents selected based on farmers preferences were evaluated together with their progenies for combining ability for LLS resistance and yield-related traits. The results indicated that additive gene effect is predominant over other variance components except for pod weight per plant. Thus, applying selection for these traits in later generations would be efficient in biparental breeding populations. In addition, GCA effect estimation has identified ICG 7878 and 43AH as the best combiner for LLS resistance. The significance of genotypes x environment in this study for most yield-related traits, suggests that selection for specific environment would be efficient.

7.2 Recommendations

Based on the results of this study, the following points are important on the way forward for developing improved groundnut varieties and increasing their adoption:

- An awareness creation to educate farmers on the important diseases and how they can be managed for higher productivity at harvest.
- The establishment of a groundnut seed system to respond to the clearly expressed demand.
- The new groundnut breeding programme should exploit the genetic diversity observed in groundnut collection from Togo. For the other traits that showed low diversity, it is recommended to introduce genotypes harboring these traits into the germplasm collection.
- Some SNPs identified in this study are new and further investigation are recommended for the confirmation of these genomic regions associated with useful traits.
- The breeding populations generated from ICG 7878 x 43AH and ICG 7878 x 68AH need to be further evaluated for the development of high yielding LLS resistant lines.

However, because of the negative yield-related alleles carried by ICG 7878 the newly developed varieties should contain a low proportion of ICG 7878 genetic background.

- For LLS resistant lines development, it is preferable to use the resistant donor as the female parent in order to take advantage of the maternal effect.
- Selection for yield should be applied in specific environments for more efficiency as significant GxE was observed in this study.
- The use of single seed descent or pedigree as breeding strategies could be employed when targeting LLS resistance and most of the yield components due to additive gene effect.
- The use of the number of pods per plant as visual selection criteria would be important for rapid and low-cost selection of high yielding varieties.

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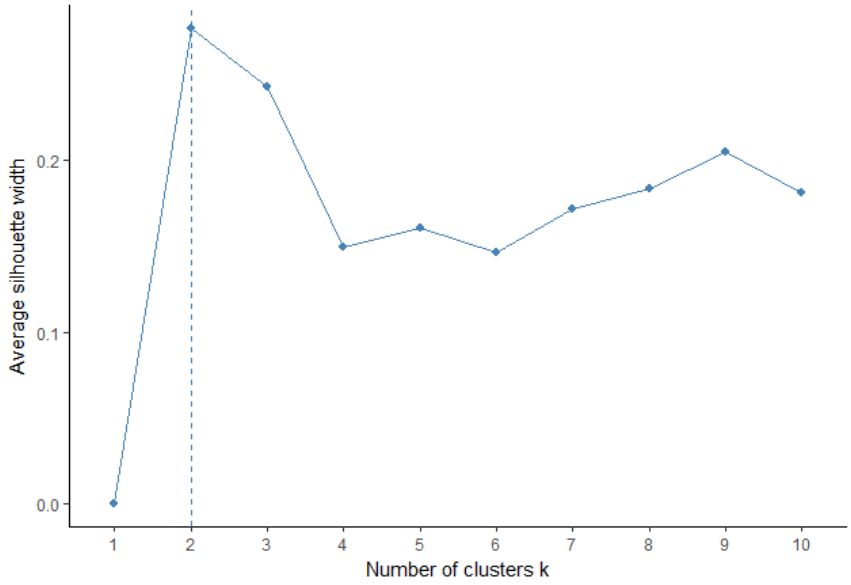
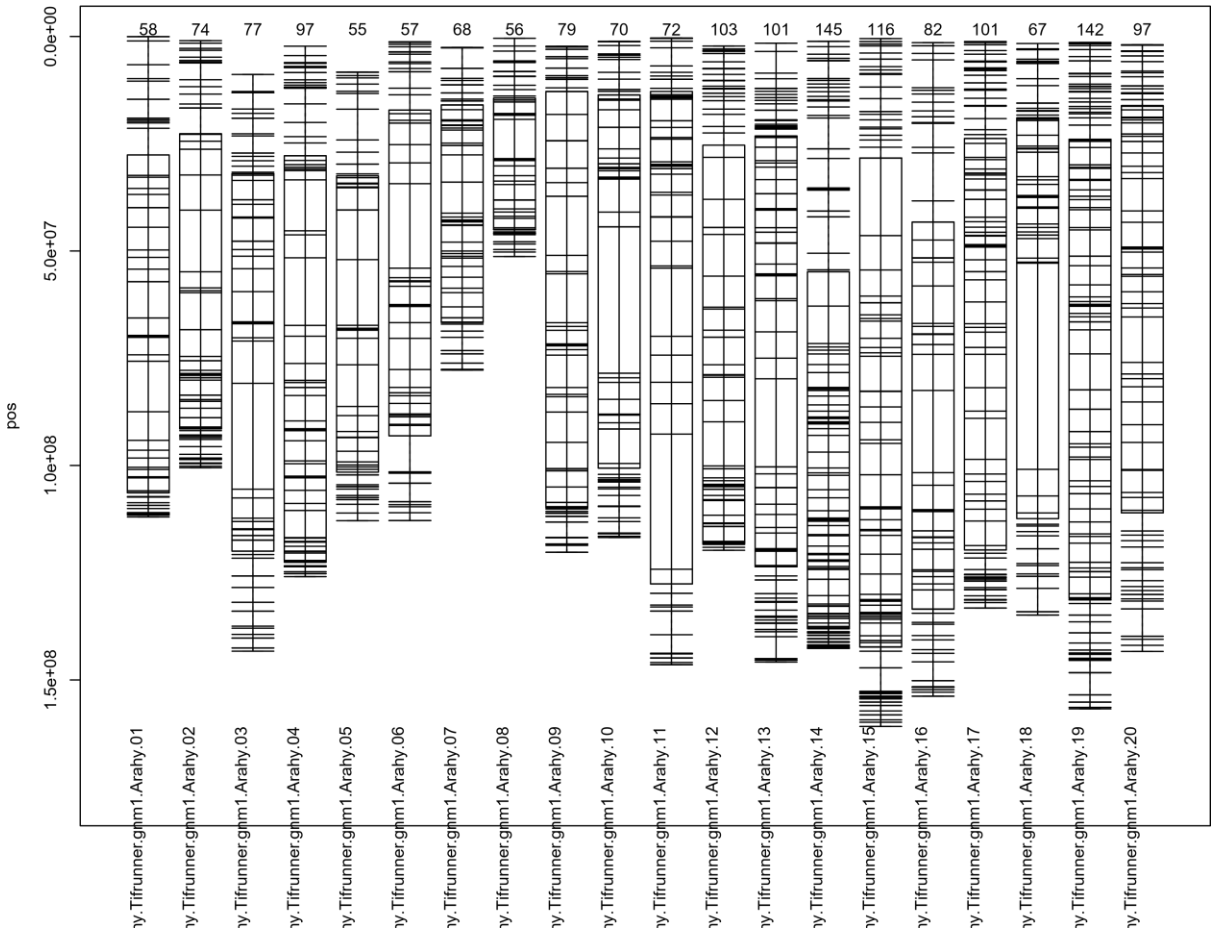
APPENDICES

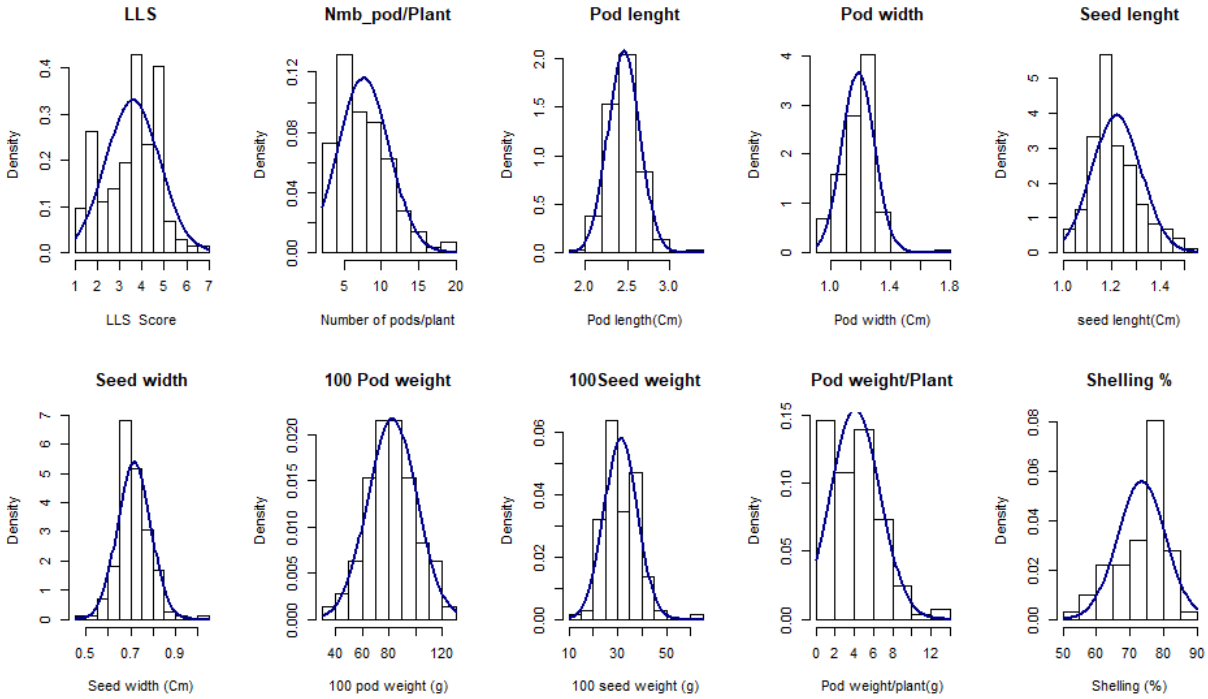
Appendix 1 Optimal number of clusters in phenotypic data



Appendix 2 SNPs distribution over chromosomes



Appendix 3 Distribution of the evaluated traits in combining ability study



Appendix 4. PRA Questionnaire

A-Individual questionnaire

PRA QUESTIONNAIRE: Groundnut's Farmer

Nom de l'enquêteur……Date…….

I. IDENTIFICATION

1.1.Caractéristiques sociodémographiques

Région :	**Préfecture:**	**Village:**
Nom du répondant :		
Age :	**Sexe :** ☐ **Masculin** ☐ **Féminin**	
Niveau d'éducation ?	1= Aucun ; 2 = Alphabétisé ; 3 = Primaire 4 = Secondaire ; 5 = Supérieur	
Situation matrimoniale ?	1= Marié ; 2= Veuf (ve) ; 3= Divorcé ; 4= Célibataire	
Nombre de personne en charge ?	1= Enfants……..; 2 = Adultes…… ;	

1.2. Activités agricole

Taille de l'unité de production	**Droit de propriété terrien**
1 Surface total disponible :…….. ha 2 Surface exploitée :…….....ha 3 Surface en jachère :……....ha 4 Surface cultivée d'arachide……ha	☐ Privée ……..……ha ☐ Familiale……....ha ☐ Communale……ha ☐ Gouvernemental……..ha
5 Depuis quand êtes-vous agriculteur ?	☐
6 Etes-vous agriculteur en plein temps ou en temps partiel ?	☐ Plein temps ☐ Temps partiel
7 Si temps partiel, combien de quel proportion de votre temps consacrez-vous à l'agriculture ?	☐ Moins de la moitié ☐ Moitié ☐ Plus de la moitié

8. Citez 5 cultures cultivées les deux dernières années par ordre d'importance, et les personnes impliquées dans la production

	Culture	**Superficies**	**Genre**	**Rendement**	**Proportion vendue**
1					

2					
3					
4					
5					

1.3. Main d'œuvre

a- De quelle main d'œuvre disposez-vous pour les activités agricoles ?

Age	Travailleurs en temps plein				Travailleurs en temps plein			
	Masculin		Féminin		Masculin		Féminin	
	Familiale	Loué (ouvrier)	Familiale	Loué (ouvrier)	Familiale	Loué (ouvrier)	Familiale	Loué (ouvrier)

b- De quels types main d'œuvre louée employé vous chaque saison ?

1= Occasionnelle 2= Permanente 3= Entraide villageoise

c- Pour quelles activités employez-vous de la main d'œuvre loué ?

	Activité	Occasionnelle	Permanente	Entraide villageoise
1	Labour			
2	Semi			
3	Sarclage			
4	Récolte			
5	Post récolte			
6	Autres (Spécifier)			

II. Production d'arachide

2.1. Système de production d'arachide

1. Quelle place occupe la culture l'arachide dans vos activités ?	1 = Important ; 2 = Non négligeable ; 3 = négligeable
2. Quels équipements utilisez-vous pour la culture d'arachide ?	
3. Quel sont les périodes de semi ?	
4. Quel sont les périodes de récoltes ?	
5. Utilisez l'engrais pour la culture d'arachide ? Quel type d'engrais ?	

6. Combien de sarclage effectuez-vous du semi à la récolte de l'arachide ?	
7. A quel moment effectuez-vous le sarclage ?	
8. Cultivez-vous l'arachide en culture ou associée ?	1= Culture pure ; 2= Culture associé
9. Etes –vous membre d'un groupement agricole ?	1 = Oui 2 = Non
10. Quel type de groupement ?	
11. Depuis quand êtes-vous membre ?	
12. Quels problèmes discutez-vous dans le groupement ?	

2.2. Production d'arachide au cours des deux dernières campagnes

Cultures	**Superficie emblavée**		**Rendements**	
	2013/2014	2014/2015	2013/2014	2014/2015
Arachide				
Culture associé				

III. Existence de contraintes liées à la production et barrières à la commercialisation

3.1.Rencontrez-vous des difficultés dans la production d'arachide?.....

Citez-les par ordre d'importance :

Contraintes	**Causes**	**Perte de rendements estimée**	**Moyens de lutte**

3.2.Quelles sont les variétés cultivées ? Par ordre de préférence.

Variétés	**Qualités**	**Défauts**

3.3. Quels sont les variétés les plus exigeantes (main d'œuvre et intrants)

Ordre	Variétés	Expliquer les raisons
1		
2		
3		
4		

3.4. Quel couleur préférez-vous pour les graines d'arachides ?

1= Rouge 2= Blanchâtre 3= Pas de préférence 4= Autres (Spécifier)

3.5. Mode et système de production

Activités	Périodes	Main d'œuvre impliqué		
		Hommes	Femmes	Enfants
Labour				
Semi				
Sarclage				
Récolte				
Transport				
Séchage				
Décorticage				
Tri				
Vente				
Autres (Spécifier)				

3.6. Caractéristiques désirées par ordre de préférence pour une variété d'arachide :

Ordre	Caractéristiques par ordre de préférence
1	
2	
3	

3.7.Comment sélectionnez-vous les semences d'arachides pour la saison suivante ?

……
……
……

3.8. Rencontrez-vous des problèmes dans l'obtention des semences d'arachides ?.....

Si oui,
lesquels ?.....
....

……
……

3.9. Quelles insectes / maladies rencontrez-vous sur vos cultures d'arachides ? Par ordre d'importance.

Maladies / Insectes	**Causes**	**Perte de rendements estimée**

3.10. Comment lutez-vous contre ces insectes /maladies ?

Maladies / Insectes	**Moyens de luttes**

3.11. Connaissez-vous la « Cercosporiose » (montrer une photo des symptômes) ?.....

- Quel est le nom local ?.....
- Quels est la cause, selon vous ?.....
- Comment est transmit la maladie selon vous ?.....
- Estimer les pertes liées à la cercosporiose d'arachide ?

1= Faible (moins de 20%) ; 2= Modéré (21-41%) ; 3 = Elevé (plu de 50%) ; Total (100%)

- Quels moyens de luttes employées vous?..... ;

3.12. Connaissez-vous des variétés non sensibles à la « Cercosporiose » ? Citez-les.

……
……
……
……
……

3.13. Selon vous, quel est l'évolution de la maladie dans votre zone ces dernières années?

1= Progresse (augmente) 2= Reste constante 3= Diminue

3.14. Quels problèmes rencontrez-vous pendant le stockage et la conservation de vos récoltes d'arachides ? Par ordre d'importance.

Problèmes	**Causes**	**Perte de rendements estimée**

3.15. Existe-t-il des obstacles à la vente de l'arachide dans votre zone ?.....

Enumérez-les :……...

……..

.

IV. Revenus liée à la production d'arachide au cours des deux dernières années

4.1. Quelles affectations aviez fait de vos récoltes d'arachide précédentes?

		AFFECTATION		
Année	Production totale	Proportion consommée (fraction)	Proportion vendue (Fraction)	Autres (préciser)…. ……. …….
2013/2014				
2014/2015				

- Qui décide de l'affectation des récoltes d'arachides ?.....
- Quand vendez-vous vos récoltes d'arachides ?.....
- Vendez-vous l'arachide en coque ou en graines ?.....

4.2. Quelles sont vos revenus ?

Année	Production totale	Coût de production	Prix moyen de vente	Revenu
2013/2014				
2014/2015				

V. Appui conseil technique

5.1. Avez-vous accès aux services d'appui-conseil technique ? 1= Oui 2 = Non

5.2. Si oui, auprès de quel(s) structure(s) et pour quelles activités ?

……
……
……

5.3.A quel moment de la saison, recevez-vous la visite des techniciens ?

Opération	Nombre de visite
Labour	
Semi	
Sarclage	
Récolte	

Avez-vous eu à visité une parcelle de démonstration ?.....

VI. Accès au crédit agricole

Etes-vous membre d'une microfinance ?	1= Oui 2 = Non
Si oui, quelle microfinance ?	
Depuis quand êtes-vous membre ?	
Obtenez-vous des crédits pour la production agricoles ?	
Obtenez-vous des crédits pour la production d'arachide ?	
Type de crédit ?	1 = crédits espèces ; 2 = crédits intrants
Types d'intrants ?	1= Semences ; 2 = Engrais ; 3 = Semences et engrais ; 4 = Prdts phytosanitaires
Le crédit est-il adapté ? Justifier.	

VII. Avez-vous un commentaire ou des suggestions liés à la production agricole et surtout à la culture d'arachide ?

B-Focus group discussion

PRA QUESTIONNAIRE : Focus group discussion

Nom de

l'enquêteur……Date…….

Région :	**Préfecture :**	**Village :**

CHECK LIST OF THE FOCUS GROUP DISCUSSION

1. Production végétale	
	- Citez 5 cultures majeures cultivées dans votre zone par ordre de d'importance
2. Identification et caractérisations de variétés cultivées d'arachides	
	- Combien de variété d'arachides cultivez-vous ? - Pouvez-vous décrire chacune d'elle ? - Pouvez-vous classer les variétés par ordre d'importance ?
3. Disponibilité et gestion des semences	
	- Comment sélectionnez-vous les semences pour la saison suivante ? - Comment stocker vous l'arachide? - Avez-vous des problèmes de stockage/ conservation des semences ? - Comment gerez-vous les problèmes de stockage/ conservation des semences ? - D'où proviennent vos semences ? - Eprouvez-vous des difficultés à obtenir des semences d'arachide ?
4. Système de culture	
	- Quelle est la taille moyenne d'une parcelle d'arachide dans votre zone ? - Quels types d'équipement utilisez-vous pour la culture d'arachide ? - Pouvez-vous décrire comment vous cultivez l'arachide ? - Quelle sont les périodes de semi d'arachide ? - A quelles périodes récoltez-vous l'arachide ? - Appliquez-vous des engrais ? De quel type d'engrais s'agit-il ? - Combien de fois sarclez-vous vos champs d'arachide ?
5. Contraintes à la production d'arachide	

	- Pouvez-vous citer les insectes et maladies rencontrés sur vos cultures d'arachide ? - Comment luttez-vous contres ces contraintes ? - Quelles contraintes abiotiques rencontrez-vous dans la culture d'arachide ? - Pouvez-vous classer les contraintes par ordre d'importance ? - Connaissez-vous la « Cercosporiose d'arachide » ? Cause ? Moyen de lutte ? - Existe-t-il des variétés résistantes à la « Cercosporiose »
6. Consommation d'arachide	
	- Comment consommez-vous l'arachide ? - Nourrissez-vous vos troupeaux avec les fanes d'arachide ?
7. Commercialisation de l'arachide	
	- Comment vendez-vous vos récoltes d'arachide ? - Avez-vous des difficultés à vendre vos récoltes ? Citez-les.
8. Préférence des producteurs d'arachide	
	- Que souhaitez-vous avoir comme caractéristiques dans une variété d'arachide ?
	- Avez-vous des commentaires ou des suggestions concernant la production d'arachide ?