GENETIC STUDIES AND MAPPING OF BENEFICIAL ALLELES FROM WILD SPECIES TO IMPROVE AGRONOMIC TRAITS IN CULTIVATED GROUNDNUT (*Arachis hypogaea L.*)

BY

AISSATOU SAMBOU

(10496579)

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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.

.....

Aissatou Sambou

(Student)

.....

Prof. Samuel Kwame Offei (Supervisor)

.....

Prof. Pangirayi B. Tongoona (Supervisor)

.....

Dr. Agyemang Danquah (Supervisor)

.....

Dr. Beatrice E. Ifie (Supervisor)

.....

Dr. Daniel Fonceka (Supervisor)

ABSTRACT

Groundnut (*Arachis hypogaea* L.) is a legume widely cultivated in tropical and sub-tropical regions. Although Senegal is among the largest African producers, yield has declined over the years. The narrow genetic diversity of the cultivated groundnut due to its monophyletic origin coupled with polyploidization has slowed down its genetic improvement using molecular tools. Wild species of groundnut are important sources of useful alleles that can be used to broaden the genetic base of the cultivated groundnut. However because of their inferior agronomic value, groundnut wild species have been little used to improve agronomic traits. Thus the present study was undertaken to enlarge the genetic diversity of cultivated groundnut and to estimate the relative contribution of the wild species to improve agronomic traits in cultivated groundnut. Specifically the objectives were to: i) identify the main constraints that farmers are facing in groundnut production and their varietal traits preferences; ii) identify promising breeding lines; iii) identify genomic regions that underlie the variation of the observed phenotypes and iv) evaluate the increase of the phenotypic value associated with the alleles of the wild species. A participatory rural appraisal (PRA) was conducted using focus group discussions (FGD) and questionnaires in three regions (Kaolack, Kaffrine and Tambacounda). Furthermore, an advanced backcross $BC_2F_{4:6}$ mapping population was developed at CERAAS (centre d'etudes regional pour l'amelioration de l'adaptation a la secheresse) from a cross between Fleur11 used as recurrent parent and a synthetic allotetraploid donor ISATGR52B (*A. duranensis (AA)* x *A. valida (*BB)) ^{4x}. Two hundred BC_2F_4 families from this cross were genotyped with 128 microsatellite markers (SSRs) at the genotyping lab (CERAAS) and the $BC_2F_{4:6}$ progenies plus Fleur11 were evaluated in four different environments. The PRA study revealed that groundnut remained the main cash crop cultivated by farmers. Farmers were ready to adopt varieties that

have high pod and haulm yield and large pod and seed size regardless of oil content. The main constraints highlighted by farmers were marketability and access to land. However drought was the constraint that farmers identified as the most important and that could be addressed by breeders. A large phenotypic variation was observed between the advanced backcross (AB) lines. The interaction between genotypes and environments was significant. The analysis performed in each site allowed selecting fifty lines based on the weight attributed to each target trait using the selection index. Among the fifty lines, eleven were constantly present across sites. The GGE biplot identified **B7_32_8_1** and **Fleur11** as the best performing lines in terms of pod yield. The genotyping analysis conducted on the BC_1 progenies permitted the construction of a genetic linkage map which spanned a cumulative length of 1792 cM with an average marker density of 6.4 cM. Three hundred and thirty loci were mapped onto 20 linkage groups (LGs). The quantitative trait loci (QTL) analysis conducted with the genotyping and phenotyping data of the $BC_2F_{4:6}$ families revealed 38 QTL related to agronomical traits. Most of the identified QTL for seed related traits were located on LG B3. For 50% of the identified QTL, the wild alleles contributed positively to the variation of the trait. Five QTL (5) were newly mapped. For 4 out of the 5 QTL, increase of the phenotypic value was associated with the alleles of the wild parent. The QTL for undesirable traits from the wild species (deep pod constriction, pronounced pod beak and spread growth habit) were also mapped and markers linked to them identified. This study has demonstrated the potential of alleles from wild relatives to enrich the genetic base of cultivated groundnut and to improve agronomic traits.

DEDICATION

To my parents Elhadji Ousmane Boute Sambou and Bineta Ndiaye Sambou

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LIST OF ABBREVIATIONS

AB: Advanced Backcross

AEA: Average Environment Coordinates

AFLP: Amplified Fragment Length Polymorphism

ANOVA: Analysis of Variance

ANSD: Agence National de la Statistique et de la Démographie

BIL: Backcross Inbred Line

CNRA: Centre National de Recherches Agronomiques

CERAAS: Centre d'Etudes Régional pour l'Amélioration de l'Adaptation a la Secheresse

CIRAD: Centre International de la Recherche Agricole et du Développement

CSSL: Chromosome Segment Substitution Line

DAS: Days After Sowing

DNA: Deoxyribonucleic Acid

EMBRAPA: Empresa Brasileira de Pesquisa Agropecuária

ICRISAT: International Crops Research Institute for the Semi-Arid Tropics

ISRA: Institut Sénégalais de Recherches Agricoles

LG: Linkage Group

MARS: Markers Assisted Recurrent Selection

MAS: Markers Assisted-Selection

NIL: Near Isogenic Line

PCR: Polymerase Chain Reaction

PRA: Participatory Rural Appraisal

PV: Phenotypic Variation

QTL: Quantitative Trait Loci

RFLP: Restriction Fragment Length Polymorphism

RIL: Recombinant Inbreed Line

SNP: Single Nucleotide Polymorphism

SSR: Simple Sequence Repeat

WAAPP: West Africa Agricultural Productivity Programme

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Groundnut or peanut (*Arachis hypogaea* L.*)* is an important legume crop grown mostly in tropical and subtropical regions of the world. It is an oilseed crop cultivated in all five continents in about 120 countries. The world production reached 41.3 million tons cultivated on 24.6 million ha in 2015 (FAOSTAT, 2015). The major producing continents are Asia (26 million tons) and Africa (14 million tons) which together account for 23.3 million ha representing 94.71% of the global groundnut cultivated areas in the world. About 60% of the total production is used for oil extraction, while 40% is consumed as food and other utilizations such as self-production of seed (Birthal *et al.*, 2010). The Senegalese groundnut sector is one of the most profitable agricultural sectors in the country. Groundnut is the most important crop in terms of production and harvested areas and it is mainly cultivated under rainfall conditions in the central part of the country called the groundnut basin. Groundnut is a cash crop providing incomes and improving livelihoods of farmers. It represents a strategic crop and its cultivation contributes to the sustainability of the integrated crop-livestock production system, the most predominant one in the semi-arid areas. Although Senegal is among the top five producing countries in Africa, yield has declined over the years. In 2015, the harvested area was 878 659 ha with a total production of 669 329 representing over 50% of the total production of all crops mixed (FAOSTAT, 2015). Nevertheless, the increase of the production has predominantly been caused by an increase of the cultivated area rather than an increase of yield. For instance, groundnut' acreage increased by 9% in 2013/14, but the yield was lower than the previous season (922 kg/ha versus 943 kg/ha) (FAOSTAT, 2015). The national average yield is approximately 900 kg/ha (ANSD, 2015), which is below the crop yield potential estimated at 5.4 t/ha (Nigam, 2005). With the liberalization of the export market and the ever-growing population, producers have the

challenge to produce more on smaller land areas. To meet the increasing demand and to enable farmers to make higher profits from groundnut, more effort is needed in breeding programmes; effort which take into account farmers' opinions and participation. According to Sanou *et al*. (2014), breeders have an incomplete understanding of why farmers choose the varieties they grow. Participatory rural appraisal (PRA) is one of the most widely used methods by breeders for identifying farmers' preferences and integrating their inputs in the varietal development process. According to Witcombe *et al*. (2005), a successful PRA should provide useful information needed to specify the characteristics in a new variety. In view of some shortcomings in some varieties experienced by farmers, breeders and researchers have to prioritize traits for breeding based on farmers' inputs. These shortcomings range from economic aspects to demand for more adapted groundnut varieties.

Efforts have been made by the national groundnut breeding programme of Senegalese Institute of Agricultural Research (ISRA) towards solving some of the challenges of the past 30 years. These efforts have been rewarded by the release of several varieties (55-437, 73-33, 756-A, 69-101, 73-28, 78-937, 28-206, Fleur 11). Recently (2016), seven newly developed varieties were released. However, breeders and researchers are facing the narrow genetic variability in the cultivated species which hampers the use of genomic tools to speed up the process of varietal creation. Cultivated groundnut is a self-pollinated crop with an allotetraploid genome structure (AABB) derived from a single natural hybridization between two diploid *Arachis* species with A and B genomes (Kochert *et al*., 1996; Seijo *et al*., 2004). The hybridization process was followed by chromosome doubling, a phenomenon which induced differences in ploidy level and thus isolated cultivated groundnut (tetraploid) from its related wild species which are diploids (Dwivedi & Upadhyaya, 2008; Feng *et al*., 2012). In addition to ploidy differences,

domestication and breeding ultimately resulted in narrowing the genetic variability of groundnut. Selection has resulted in crops that are able to feed the world but at the price of reducing their genetic diversity compared to their wild relatives (Ross-Ibarra *et al*., 2007; van Heerwaarden *et al*., 2011). This phenomenon is often called "domestication bottleneck" (Tanksley & McCouch, 1997). Fortunately, groundnut wild species are rich in valuable alleles that could be used to enhance the genetic base of the cultivated groundnut. Many sources of disease resistance have been reported in wild *Arachis* species (Abdou *et al*., 1971; Subrahmanyam *et al*., 1983; Garcia *et al*., 1996; Pande *et al*., 2001; Leal-Bertioli *et al*., 2015). While groundnut wild species are being extensively used as sources of biotic resistance (Leal-Bertioli *et al*., 2009, 2010, 2015), they have been little utilized to improve agronomic traits like yield related traits. The inferior phenotypic appearance of wild species associated with their long growth cycle could explain to some extend the low rate of utilization of wild species to improve complex traits (Pandey *et al*., 2012). Nevertheless, many studies conducted on several crops have demonstrated the potential of wild species in improving complex traits with the help of molecular markers (Fulton *et al*., 1997; Grandillo & Tanksley, 2003; Huang *et al*., 2004). In groundnut, Fonceka *et al*. (2012) showed that the positive effect of half of the detected QTL for drought and yield related traits were brought by the alleles from wild relatives.

In this dynamic process of varietal creation using wild species, the Centre d'Etudes Regional pour l'Amélioration de l'Adaptation à la Sécheresse (CERAAS) in collaboration with other research institutes such as the Centre National de Recherche Agricole (CNRA), the Centre de cooperation Internationale en Recherche Agronomique pour le Développement (CIRAD), the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) have developed a research programme

to widen the genetic basis of domesticated groundnut. Various types of interspecific genetic material ranging from a library of chromosome segment substitution lines (CSSLs) to the advanced backcross QTL (AB-QTL) populations, pyramiding populations and Near Isogenic Lines (NILS) (Fonceka *et al*., 2012; CERAAS, 2014 not published data) have been developed or are under development.

This present work aimed at exploring and exploiting the potential of wild groundnut species to improve yield related traits in cultivated groundnut *Arachis hypogaea*.

The objectives used to:

- identify farmers' groundnut trait preferences and their major production constraints,
- diversify the primary gene pool through introgression of beneficial alleles from wild species to cultivated groundnut,
- identify lines that could be used as breeding lines in the breeding programme,
- identify genomic regions (QTL) associated with the variation of useful pod yield related traits,
- determine the genetic contribution of the wild genome in the variation of yield related traits; and
- pyramid two wild fragments underlying QTL for pod and seed size, and biomass.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Origin and distribution

The history of the origin of groundnut traces a path back over several continents and finally ends up in America. The discovery of 7,600 years old fossilized groundnut hulls in the Ñanchoc Valley of Peru by archaeologists indicated that *Arachis hypogea* plant originated from South America in a region covering southern Bolivia - northern Argentina - northern Paraguay and western region of Matto Grosso Brazil (Hammons, 1994). Moreover, most of the wild species are found in that region. According to Hammons. (1994), groundnut distribution throughout the world is believed to has occurred in 15th century through Portuguese explorers when they arrived in Brazil and carried groundnuts from South America to Western Africa. The crop was then quickly accepted by African growers because of its similarity to the native Bambara groundnut (*Voandzeia subterranea* (L.). The Spanish then took them to west coast of South America, Philippines, China and various islands in the western Pacific. Later in 1700s the crop was taken to Europe. Introductions of groundnut in North America are presumed to have been from northern Brazil and the West Indies, where they are believed to have been loaded onto ships carrying slaves from Africa to the New World.

2.2. Botany and reproduction features

Arachis hypogaea is an annual and self-fertilized crop. Its flowers are perfect / hermaphroditic containing both male and female reproductive organs. Although appearing as and referred to as a nut, it is the underground pod of a legume rather than a true nut. As a legume, it is a member of the family *Fabaceae*. The leaves are opposite and pinnate with four leaflets (two opposite pairs; no terminal leaflet). Groundnut displays a geocarpy feature (Rao, 1994). The inflorescences are

born in the axils of leaves of primary and secondary branches. The inflorescences are simple or compound and each comprises up to 3 flowers. One flower per inflorescence opens per day and last for just one day. The colour of the flowers varies from orange to yellow depending on the cultivar. Groundnut flowers contain 10 anthers containing stamens. The stigmas are generally the same size or slightly smaller than the anthers. Pollen matures approximately 6 - 8 h before anthesis, which occurs within a few hours after sunrise. Generally, only one flower at an axil reaches anthesis on any given day. Pollination occurs at the same time that anthesis occurs. Enzymes expressed on the surface of the stigma to facilitate the adhesion of pollen degrade within 8 hours after anthesis (Rao, 1994). That is why it is important for practical aspects and success in crosses, to make the pollination in early hours of the morning. After pollination, a pedicel elongates to form a peg which will bear the pod.

2.3. Groundnut production

Groundnut is an important legume crop grown in more than 100 countries under different agro-ecological environments in the world. It was cultivated on 25.4 million ha worldwide with a production of 45.6 million tons in 2013 (FAOSTAT, 2015). For the periods 2003–2013, the total groundnut production increased with an annual production increasing from 0.4% in 2010 to 2.05% in 2013, and this could be attributed to increase in both yield (1%) and area under cultivation (1.04%) (FAOSTAT, 2015). To meet the demands of the ever growing world population, the production has to increase considerably. Asia and Africa account for more than 90% of global groundnut area where it is cultivated mostly under rainfed conditions by small scale farmers. China is the largest producer (16.9 million tons) followed by India (9.4 million), Nigeria (3 million tons) and USA (1.9 million tons) (FAOSTAT, 2015). Africa accounts for

25.5% (12 million hectares) of the total world production and ranks as the second most important groundnut producing continent in terms of acreage and production after Asia.

Groundnut production in Africa has grown significantly since 1990 and West African countries have contributed greatly (Revoredo & Fletcher, 2002). The high production attributed to groundnut in Africa is predominantly due to increase in cultivated area (12 million ha, 11.6 million tons) rather than the yield. Africa, although is the second largest producer has the lowest average yields per hectare (1 t/ha) compared to Asia (3 t/ha) and America (4 t/ha). When comparing the average yield obtained between 1972 and 1975 to that obtained between 1996 and 2000, Asia with 74.9% had the highest yield increase. For the same periods, the increase was 24.9% for America and 15.2% for Africa (Revoredo & Fletcher, 2002). The yields in major producing countries vary from just over 1 t/ha in Africa to 3 t/ha in Asia; 4 t/ha in America and can attain 6 t/ha in Israel and even 8 t/ha in Cyprus in Europe (FAOSTAT, 2015). The differences between developed and developing countries in terms of yield reflect the differences in the production systems of the crop. Groundnut cultivation is mechanized and very intensive with high level of inputs in developed countries compared to developing countries like Senegal where it is grown with low inputs under rainfed conditions by poor farmers.

2.4. Nutritional value and utilization of cultivated Groundnut

Groundnut provides income to farmers. It is also used as food through consumption of the kernels and provides nutritious haulms to livestock. It is a crop qualified as “gold” in Senegal. Groundnut is a rich source of energy composed of oil (48 - 50%) and protein (25 - 28%) in the kernels providing 564 kcal of energy from 100 g of seeds (Jambunathan, 1991). Groundnut is mainly used for oil production but 40% of the global production is consumed as food or other uses (Birthal *et al.*, 2010). In addition, groundnut seeds contain many nutrients such as minerals,

antioxidants, and vitamins and are rich in mono-unsaturated fatty acids (Jambunathan, 1991; Savage, 1994). Jiang *et al*. (2002) demonstrated in their clinical study that nuts consumption in general and more particularly groundnut butter reduces the incidence of type 2 diabetes in women. This can be explained by the richness in unsaturated fatty acids of groundnut which may be beneficial for glucose and insulin homeostasis. Moreover, the same authors found gain in regular nut consumption to avoid increasing caloric intake and therefore can be recommended as a replacement for red meats consumption. Being a legume, groundnut is used in rotation with mainly cereals in Africa to help improve soil fertility by fixing nitrogen (N) and leaving behind organic matter.

Despite its high economical, nutritional and therapeutic values, groundnut consumption is no longer without risk in human health. Several clinical studies reported the side effects of groundnut. Food allergy is a common problem and groundnut has been cited among the eight foods that cause more than 90% of food allergies (Metcalfe, 1996). These reactions are caused by the presence of allergenic peptides Ara h 1, 2, 3 and 6 in groundnut seeds (Burks *et al*., 1997; Rabjohn *et al*., 1999; Koppelman *et al*., 2010). However it has been reported by clinical and epidemiology studies that high levels of environmental (cutaneous) exposure to groundnut during infancy may promote sensitization (Fox, 2009). Another continuing challenge in groundnut production and marketing is the occurrence of the aflatoxin produced by the mold *Aspergillus flavus*. However, the research community is working to solve the problem and a sensitization is being done at producers' level to reduce the occurrence of the fungal infestation by good drying and storage practices.

2.5. Systematic and taxonomy

Groundnut belongs to the tribe *Aeschynomeneae*, subtribe *Stylosantinae*, family *Leguminosae* which is divided into 3 sub-families: *Mimosoideae*, *Caesalpinoideae* and *Papilionoideae*. The later includes groundnut and most of legume crops of high economic value. In 1994, Krapovickas & Gregory published a revised taxonomic of the genus *Arachis* which recognizes 80 species divided into 9 sections: *Caulorrhizae*, *Erectoides*, *Extranervosae*, *Heteranthae*, *Procumbentes*, *Trierectodes, Triseminatae, Rhizomatosae* and *Arachis*. The distinctions of the various sections are based on morphological characters, life cycle attributes, eco-geographic distribution, cross compatibility, cytological study as well as chromatographic and antigenic reactions (Krapovickas & Gregory, 1994; Stalker, 1997; Valls, 2005). Section *Arachis* is the most widely distributed. It includes the cultivated groundnut (*A. hypogaea*) species and 30 wild species. In this section, 26 species are diploid (2n = 2x = 20), three (03) are aneuploid or dysploid (2n = 2x = 18) and two (02) species, *A. hypogaea* and *A. monticola* are tetraploid (2n = 4x = 40) (Smartt & Stalker, 1982). On the basis of morphological features, *Arachis hypogaea* was classified into two (02) subspecies *hypogaea* and *fastigiata*. Subspecies *hypogaea* have a long life cycle, no flowers on the central stem, and regularly alternating vegetative and reproductive side stems. Subspecies *fastigiata* have a shorter life cycle, flowers on the central stem, and reproductive and vegetative stems distributed in a disorganized way. *Arachis hypogaea ssp hypogaea* is classified into two botanical varieties (var. *hypogaea* and var. *hirsuta*) while *ssp fastigiata* is further divided into four botanical varieties (var. *vulgaris*, var. *fastigiata*, var. *peruviana*, and var. *aequatoriana*) (Krapovickas & Gregory, 1994). These botanical varieties correspond to different commercial types and differ from each other by plant characteristics and

their growth habit; spreading in *hypogaea* and erect in *fastigiata* (Krapovickas & Gregory, 1994).

2.6. Cytology and genomic relationships in the Arachis genus

Arachis hypogaea is an amphidiploid (2n = 4x = 40) with a large and complex genome (2.8 Gb) (Berthioli *et al*., 2016). Chromosome morphology was reported by Husted. (1936) and Stalker & Dalmacio. (1986). Mendes. (1947) reported the number of chromosome of the diploid species (2n = 2x = 20). It is only in 2005 that Peñaloza & Valls. (2005) discovered the dysploid species (2n = 2x = 18) in some *Arachis* species. The genus is composed mostly of diploid species, but tetraploids exist in sections *Arachis* and *Rhizomatosae*, and aneuploid species in sections *Arachis* and *Erectoides*. Polyploidy evolved independently in sections *Arachis* and *Rhizomatosae* (Smartt & Stalker, 1982). In the genus *Arachis*, the largest section is the *Arachis* section which includes the cultivated species, one other tetraploid (*A. monticola*), 26 diploid (2n = 2x = 20), and three aneuploid species (2n = 2x = 18). These species are annual or perennial and are highly variable especially within the annual species.

The first published interspecific hybridization attempt in the genus was between the tetraploids *A. hypogaea* (section *Arachis*) and *A. glabrata* (section *Rhizomatosae*) (Hull & Carver, 1938). No hybrids were obtained from the crosses. Following this first experiment, an extensive hybridization programme was undertaken by Gregory & Gregory. (1979). These authors used a collection of 91 *Arachis* and reported the cross-compatibility among the species of the genus. Their findings indicated that hybridization between species within the same section was more successful than crosses between sections. Based on their results, the membership of a given species to a section was performed by analysis of the fertility of the interspecific hybrids (Gregory & Gregory, 1979). However, reproductive isolation mechanisms and pollen infertility

developed by a number of species and accessions within the same species have hindered classification efforts. Several attempts were then performed using *A. hypogaea* and various species of *Arachis* section. However, because of differences in ploidy level, most of the crosses failed.

Besides the classification based on chromosome number and hybrids fertility analysis, there is a classification based on karyotype morphology. Husted. (1936) identified one pair of smaller chromosome ('A' chromosome) in species of *Arachis* and one chromosome pair harbouring a secondary constriction ('B' chromosome) in the same section. Later studies reported on one hand that hybrids between species having the small chromosome pair (A chromosome) were partially to fully fertile and will produce F_2 seeds; on the other hand, hybrids between the species with A genome and the species harbouring a secondary constriction (B chromosome) were sterile (Stalker & Simpson, 1995). Thus, the terminology 'A' and 'B' genome has been used to describe the two cytological groups in section *Arachis*. Because the cultivated groundnut has one pair of smaller chromosomes and one pair of chromosomes with a constriction, it was described as an allotetraploid species with AABB genome (Husted, 1936).

In order to better understand and clarify the phylogenetic relationships among groundnut species, molecular studies have been undertaken using isozymes (Lu & Pickersgill, 1993; Stalker *et al*., 1994), Restriction Fragment Length Polymorphisms (RFLPs) (Kochert *et al*., 1991; Paik-Ro *et al*., 1992; Gimenes *et al*., 2002; Burow *et al*., 2009); Randomly Amplified Polymorphic DNA (RAPDS) (Lanham *et al*., 1992; Hilu & Stalker, 1995); *in situ* hybridization (Raina & Mukai, 1999; Seijo *et al*., 2004; Custódio *et al*., 2013) and Simple Sequence Repeats (SSRs) (Hopkins *et al*., 1998; Nagy *et al*., 2012). Most of these studies have involved species in section *Arachis* because of their relatedness with *A. hypogaea*. Using isozymes, Lu & Pickersgill. (1993)

differentiated the only B genome species described (*A. batizocoi*) from those of A genome and grouped species with A genome according to their life cycle (annual / perennial). Milla *et al*. (2005) confirmed using AFLP markers the differences between A and B genome species and figured out the close genetic relationship between B and D genomes.

2.7. Genetic diversity of Arachis

Most of the variability in the genus was observed in South America, the centre of origin of groundnut. The genetic diversity of the genus is classified into four gene pools according to Singh & Simpson. (1994). The primary genepool consisting of *A. hypogaea* and its wild form *A. monticola*; both are AABB genome structure; the secondary genepool consisting of diploid species from section *Arachis* that are cross compatible with *A. hypogaea*; they are either A, B, D, F or K genome; the tertiary genepool consisting of species of section *Procumbentes* that are weakly cross-compatible with *A. hypogaea* and the quaternary genepool consisting of the remaining wild *Arachis* species classified into seven other sections. According to the distribution of the two subspecies of *Arachis* (*A. hypogaea ssp. hypogaea* and *A. hypogaea ssp.fastigiata*) and their respective botanical varieties described, seven secondary centers of diversity have been reported (Gregory & Gregory, 1976). The first is the Guarani region (between Paraná basin in Paraguay and southern Brazil), the second in Goias and Minas Gerais regions in Brazil, the third in the Rondônia region and the northeastern Mato Grosso in Brazil, the fourth in Bolivia (eastern slope of the Andes), the fifth from the Peruvian region to the west coast, the sixth in the northeast of Brazil and the seventh at the Ecuadorian region. West Africa and Southeast Asia were described as tertiary centres of diversity. The variability observed in these latter centres results from the combined effects of natural hybridization and selection.

In total contrast to what have been observed in terms of phenotypic diversity, little variation has been reported at molecular level in cultivated groundnut (Gregory & Gregory, 1976). In fact several studies reported low levels of polymorphism even between the most distantly related cultivars in cultivated groundnut whatever the marker types used (Halward *et al*., 1991; Kochert *et al*., 1996; Gimenes *et al*., 2002; Tseng *et al*., 2016). The narrow diversity at the molecular level has been attributed to three major causes. Firstly the recent monophyletic origin of cultivated groundnut greatly reduced the genetic diversity in the gene pools. In fact, studies involving molecular markers suggested that *A. hypogaea* evolved from a natural single hybridization between two wild species, *A. duranensis* and *A. ipaensis* reported to be the progenitors of the cultivated form (Kochert *et al*., 1996). Thus cultivated groundnut has a single origin (Kochert *et al*., 1996; Bechara *et al*., 2010).

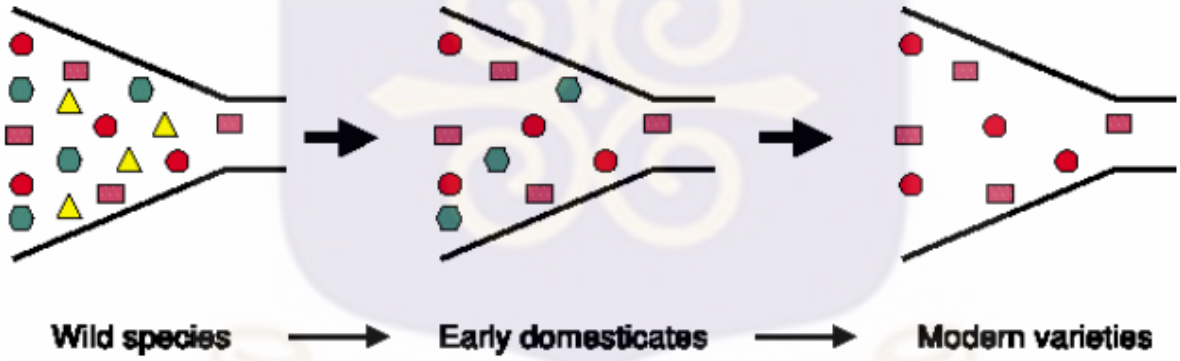


Figure 2.1: Genetic bottlenecks on crop plants during domestication and plant breeding

Different geometric figures represent allelic variations of genes originally found in wild species, but gradually lost through domestication and breeding. Such lost alleles can be recovered only by going back to the wild ancestors of our crop species: from (Tanksley & McCouch, 1997).

Secondly, hybridization of the two genomes paved the way to polyploidization, an event that has led to the separation of the cultivated tetraploid from the rest of the diploid species (Hopkins *et al*., 1999). Barriers to gene flow from related diploid species to domesticated groundnut due to ploidy differences combined with the self-pollinating nature of the crop have blocked genetic

exchange between cultivated groundnut and the wild species. The third factor that contributed to reduce the genetic base of cultivated groundnut is the domestication process. Tanksley & McCouch. (1997) clearly illustrated diagrammatically the reduction of the allelic diversity imposed on crop plants during domestication and plant breeding (Figure 2.1).

2.8. Targeted traits for groundnut improvement

The targeted traits for improvement are country dependent and are determined by the level of productivity achieved and consumers' and industry preferences. If productivity is no longer a challenge in developed countries where yields can reach 4 tons per ha, it remains a critical objective in developing countries. Yield and related traits are the most widely targeted traits of groundnut breeding programmes worldwide (Janila *et al*., 2013; 2016). The most important groundnut traits that contribute to yield are: number of pods per plant, shelling outturn, pod and kernel size and 100-seed weight. Recently, traits associated with yield without direct correlation such as ease in shelling, ease in harvesting (peg strength), reticulation, beak and constriction of pod are also important considerations (Janila *et al*., 2013). With the use of exotic germplasm to broaden the cultivated genepool, growth habit (erect, spread) is becoming an important breeding trait. In the Sahel context where groundnut plant is also used for animals feeding, the development of varieties with high haulm yield becomes important. In areas where groundnut is grown exclusively under rainfall, the crop cycle should match with the length of the rainy season. This is particularly important with the climate change and the shortening of the rainy season in the Sahel. Thus early maturity is an important trait in breeding programmes mostly in the semi-arid regions. Resistance to a specific groundnut insect pest and or disease is a target trait to reduce yield losses.

2.9. Potential of wild species in groundnut improvement

Cultivated groundnut breeding has suffered from the lack of genetic diversity. Conversely, groundnut wild relatives have high diversity (Moretzsohn *et al*., 2004) and possess desirable alleles that can be used to improve resistance for several biotic and abiotic traits as well as quality traits such as protein and oil content (Nautiyal *et al*., 2008; Rao *et al*., 2003). While the exploitation of exotic germplasm is now becoming a routine in some crops notably tomato, rice, wheat and barley (Concibido *et al*., 2003; Feuillet *et al*., 2008; Bernardo, 2009; Jordan *et al*., 2011), it remains difficult for other crops like groundnut due mostly to cross incompatibility. Nevertheless, efforts have been deployed by the groundnut scientific community to ease the use of the wild species in breeding programmes. The first wide hybridizations between wild groundnuts with *A. hypogaea* L were in 1940 by Gregory and Krapovickas (Simpson, 2001). Their first attempts were unsuccessful, but with continued effort, their success rate improved over the years (Gregory & Gregory, 1979). Hammons. (1970) and Simpson & Smith. (1975) released respectively 'Spancross' and 'Tamnut 74' the first groundnut cultivars derived from interspecific hybridizations.

Concerning the hybridization strategies, Simpson. (2001) described two major pathways namely the hexaploid and the amphidiploid. In the hexaploid way, the tetraploid cultivated species is crossed with a wild diploid. This results in a sterile triploid hybrid which, after chromosome doubling gives fertile hexaploid plants. The 4x ploidy level is recovered through successive backcross with the cultivated parent. North Carolina State University and ICRISAT have had success with this pathway (Stalker *et al*., 1979). However, high sterility, instability of the hybrids and the possibility of loss of chromosomes or chromosomal fragments are the main disadvantages of this method. In the amphidiploid way, two diploid wild species are crossed to

produce a diploid hybrid. To obtain the tetraploid species, the obtained F_1 interspecific hybrids are treated with colchicine-mediated chromosome doubling. The synthetic tetraploid is then crossed with the tetraploid cultivated parent. This technique has the advantage of directly generating individuals with the same level of ploidy as the cultivated species. The amphidiploid method is actually the pathway mostly used in transferring genes from wild species (Simpson, 1991; Simpson & Starr, 2001; Fonceka *et al*., 2012). A nematode resistance variety 'NemaTAM' was developed using this technique (Simpson *et al*., 2003).

Table 2.1: Trait-related QTL/genes in groundnut characterized using wild relatives

Trait/s	Population Strategy	Genotyping/Mapping	QTL/Gene	Reference
Root-knot nematodes resistance, drought-related traits and agronomic/domestication traits	RILs (wild × wild)	SNP markers and integrated consensus map from Shirasawa *et al*. 2013	QTL	(Leal-Bertioli *et al*., 2016)
Water availability, flowering precocity, seed and pod number, length and size, and pod maturity	87 BC_3F_1 and 55 BC_2F_2 [cultivated × wild amphidiploid (*A. ipaensis* × *A. duranensis*)]	SSR	QTL	(Fonceka *et al*., 2012)
Root-knot nematode resistance	BC_4F_2 population (cultivated × wilds)	RAPD	Resistance associated to markers	(Burow *et al*., 1996)
Plant growth habit, height of the main stem, plant spread	CSSLs [wild synthetic (*A. ipaensis* × *A. duranensis*) × cultivated]	SSR	QTL	(Fonceka *et al*., 2012)
Late leaf spot resistance	F_2 (*A. duranensis* × *A. stenosperma*)	SSR, AFLP	QTL	(Leal-Bertioli *et al*., 2009)

Research teams in Brazil, USA and India are currently developing several artificial amphidiploids using different combination of A and B genome species. Nowadays, tremendous progress have been accomplished in using wild species in breeding programmes, thanks to the advance of genomic and biotechnological tools. These tools enable tackling differences in

chromosome numbers, identifying wild chromosome segments that contribute significant improvement of traits of agronomical interest as well as dealing with linkage drag and deleterious alleles associated with wild x cultivated crosses. Table 2.1 gives an update of the interspecific mapping populations developed in groundnut and the target traits for which QTL have been identified.

2.10. Genomic resources in groundnut applicable in marker assisted selection

2.10.1 Molecular markers

Isozymes were among the earliest genetic markers used in groundnut to study the genetic diversity of cultivated groundnut and its wild diploid relatives. Little variation was found among *Arachis hypogaea* (Grieshammer & Wynne, 1990). Three out of the 25 isozymes used in their study were polymorphic among 71 lines. However, isozyme polymorphisms were reported to be frequent among wild species compared to cultivated groundnut (Lanham *et al*., 1992; Lu & Pickersgill, 1993; Stalker *et al*., 1994). Isozymes have been used in other crops but, as in groundnut, low polymorphism have been generated (Weeden, 1989). The development of genetic markers evolved from the more complex and less informative protein-based molecular marker (isozyme) to the simplest and most informative DNA markers namely randomly amplified polymorphic DNA (RAPD), restriction fragment-length polymorphism (RFLP), amplified fragment-length polymorphism (AFLP), sequence-characterized amplified region (SCAR), simple sequence repeat (SSR) and single nucleotide polymorphism (SNP). RFLP have been used in diversity studies in groundnut and the results were the same as other markers: low polymorphism in *Arachis hypogaea* (Halward *et al*., 1991). RAPDs are PCR-based markers and are easy and rapid to perform. The disadvantage of RAPD markers is that only dominant markers are detected. However, they have been used in various studies. SSRs are based on PCR primers

designed to flank a set of tandem repeats of a short DNA sequence (Liang *et al*., 2009). SSRs are highly polymorphic, multiallelic, codominant, abundant in the genome, simple to analyse, and transferable between species (He *et al*., 2006). Currently, more than 6000 SSRs have been developed and published for groundnut in PeanutBase (http://www.peanutbase.org). Until now, SSRs are being used for genetics and breeding studies in cultivated groundnut.

With the genomes sequenced of the wild progenitors of groundnut (Bertioli *et al*., 2016) SNP markers are being widely used. Significant effort has been made at ICRISAT in collaboration with the University of Georgia in developing SNPs markers (Pandey *et al*., 2017). Pandey and collaborators developed a high density genotyping 'Axiom_*Arachis*' array with 58 K SNPs. This new technology will contribute to accelerate the process of molecular breeding in cultivated groundnut. Moreover, Bertioli *et al*. (2016) published the sequences of the genome of the progenitors of cultivated groundnut (*A. duranensis* and *A. ipaensis*). This opens new perspectives for the more efficient use of groundnut genomic resources and constitutes a starting point for the whole groundnut genome sequencing which has been initiated by the Groundnut Genome Project (PGP) in collaboration with BGI-Shenzhen (China).

2.10.2 Genetic linkage map

A major application of molecular markers is the construction of linkage maps required for QTL mapping and marker-assisted breeding. Linkage maps were constructed using diploid species (either AA or BB genome) as well as tetraploid species with different marker types. Using RFLP, Halward *et al*. (1993) developed the first linkage map in groundnut. This map was constructed with an F_2 population derived from the hybridization between *A. stenosperma* and *A. cardenasii* both AA genome species. The first SSR based linkage map in *Arachis* consisted of 170 markers mapped on 11 LGs (Moretzsohn *et al*., 2005). A linkage map was also constructed

with an F_2 population which involved BB genome species *A. ipaensis* and *A. magna* (Moretzsohn *et al*., 2009). The same year, Foncéka *et al*. (2009) published a SSR-based interspecific tetraploid map using a BC_1F_1 population ["Fleur11" × (*A. ipaënsis* × *A. duranensis*)^{4x}], and 298 loci were mapped in 21 LGs. Recently, Nguepjop *et al*. (2016) constructed a genetic map of 357 loci using an F_2 population ["Fleur11" × (*A. ipaënsis* × *A. batizocoi*)^{4x}] where the authors reported homeologous recombination between the A and the K genomes. Cultivated groundnut was also used to construct linkage maps. Two intra-specific maps were first published (Varshney *et al*., 2009; Hong *et al*., 2010). These two maps were developed using SSR markers. A linkage map spanning a total distance of 1,446.7 cM with 1685 markers including 1621 SNPs and 64 SSRs was recently published by Zhou *et al*. (2014). Two consensus map based on 11 mapping populations and 3 recombinant inbred line populations were constructed (Gautami *et al*., 2012; Shirasawa *et al*., 2013).

2.11. Exploitation of groundnut wild allelic potential for varietal creation

2.11.1 Interests of integrating molecular breeding approaches

For harnessing the beneficial alleles from wild species, particular types of mapping population such as AB-QTL, CSSLs, and NILs have been developed for different crops (Concibido *et al*., 2003; Feuillet *et al*., 2008; Bernardo, 2009; Jordan *et al*., 2011). Figure 2.2 gives a comparison of the different types of population. The development of such interspecific populations has been made possible by the use of molecular markers. DNA markers come as a tool not only to speed up the process of developing new varieties but to increase the efficiency too. The major benefits of integrating molecular markers in breeding approaches are to:

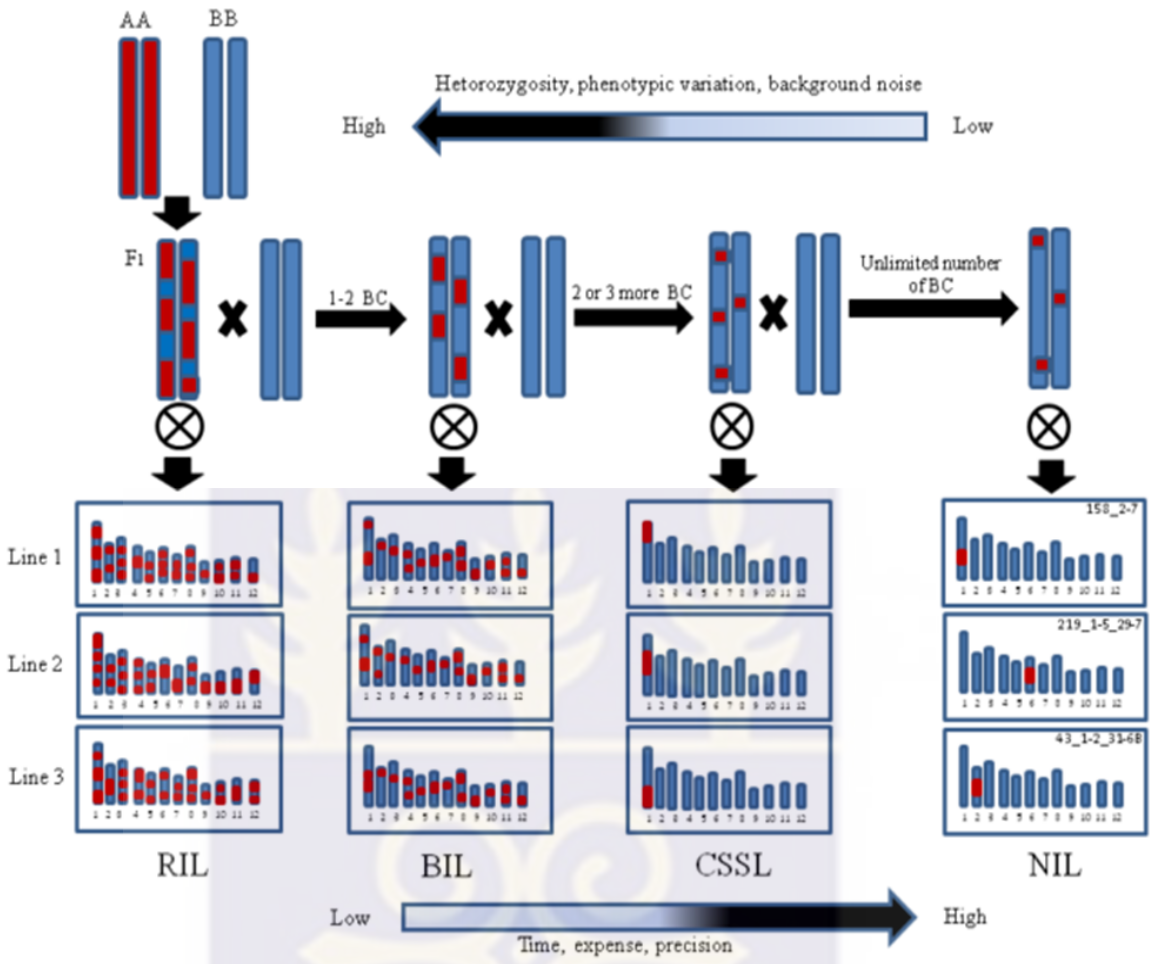


Figure 2.2: Comparison of the methods for creating primary and advanced bi-parental mapping populations

As shown are the number of backcrosses required and the genotypes of the lines obtained by each of the following methods recombinant inbred lines (RILs), backcross inbred lines (BILs), chromosomes segment substitution lines (CSSLs) and near isogenic lines (NILs). Also shown are Karyotypes of the three CSSLs illustrate how chromosome 1 of the donor can be introgressed into the recurrent parent genetic background (Shakiba & Eizenga, 2014).

- ✓ Handle complex traits that may not be amenable by conventional breeding' methods due to their polygenic nature and the large G x E interaction.
- ✓ Transfer desirable traits from wild species into improved varieties while getting rid of the unfavourable traits.

- ✓ Introgress part or the entire genome of wild specie into cultivated background which enable tapping alleles from wild species and varieties development.
- ✓ Select plants on the basis of their genotype and thus optimize time, resources and the cost of phenotyping.
- ✓ Avoid linkage drag and limit the failure of phenotype screen, a recurrent problem with field evaluation.
- ✓ Pyramid genes or QTL through molecular breeding.

Marker-assisted backcrossing (MABC) is extensively used in molecular breeding to improve existing varieties and to pyramid few genes / QTL. It is also used to develop near-isogenic lines (NILs) or chromosome segment substitution lines (CSSLs). The most important limitation of MABC is its inability to deal with several traits simultaneously because of the difficulty of managing large population size (Ribaut & Hoisington, 1998; Varshney *et al*., 2012). For such traits, marker-assisted recurrent selection (MARS) has been suggested. MARS can target simultaneously several minor and major QTL (Ribaut & Ragot, 2007). Actually in groundnut breeding, MABC is used for improving simple traits such as oil quality traits and resistance to biotic constraints while for more complex traits like drought tolerance where 100 main and epistatic effects QTL were mapped, MARS was proposed (Ravi *et al*., 2011).

2.11.2 The concept of advanced Backcross (AB) methodology

According to Tanksley & Nelson. (1996), the less expected impact of marker-based QTL analysis on the development of varieties may be attributed to two factors: (1) QTL discovery and variety development are separate processes; (2) Most breeding-related QTL studies are targeted toward manipulating quantitative trait variation within elite germplasm. Advanced Backcross QTL (AB-QTL) population was first described by Tanksley *et al*. (1996) in tomato for

overcoming these two factors. It refers to a methodology that allows mapping and introgressing QTL from an un-adapted donor into the background of an elite variety. The creation of an AB mapping population follows generally the steps described by Tanksley *et al*. (1996): a wild specie is used as the donor parent and is crossed with the recurrent parent, usually an elite cultivar. The hybrid is then crossed with the recurrent parent in subsequent crosses. To avoid the cytoplasmic male sterility, in most cases the donor parent is a male and the recurrent parent is the female. The first generation of backcross results from a cross between the F_1 hybrid (s) and the recurrent parent. The resulting BC_1 may be backcrossed again with the recurrent parent to generate a BC_2 population. Since the publication of Tanksley *et al*. (1996), the AB-QTL methodology has been used in various crops to tap the variability that exists in crop wild relatives (Huang *et al*., 2003; Korff *et al*., 2006).

The advantages of the AB populations are that one may develop AB-QTL population (Tanksley *et al*., 1996), BIL population (Keurentjes *et al*., 2007), CSSL population (Fonceka *et al*., 2012) or NIL population (Monforte & Tanksley, 2000). In the process of developing AB-QTL populations, lines with unwanted genes from the donor parents are often removed from the population after phenotypic and genotypic evaluation. The selection in favour of lines with desirable alleles and the genetic background of the recurrent parent can skew the allele's distribution toward the recurrent parent. Therefore, in more advanced backcrossing generations for example after the BC_3 generation, the power of the statistical analysis to detect QTL decreases. Since sequential backcrossing in AB-QTL removes epistatic interactions, the chance of detecting QTL with epistatic interactions among alleles from the donor parent decreases due to the overall lower frequency of donor alleles. Nevertheless the ability to detect additive, dominant, partially dominant or over dominant QTL increases (Tanksley & Nelson, 1996) and

because of the homozygosity status of the lines developed, multi-location trials may be done on interesting individual(s) for varietal release. Research to broaden the gene pool of cultivated groundnut using its wild relatives has been undertaken in Senegal resulting in the development of two AB-QTL populations (unpublished data). Moreover a pyramiding programme to increase seed size and biomass is ongoing using the CSSLs lines developed (Fonceka *et al*., 2012). Such populations would be useful in creating new varieties with desired preferences.

CHAPTER THREE

3.0 ASSESSMENT OF FARMERS' PRODUCTION CONSTRAINTS AND VARIETAL TRAIT PREFERENCES OF GROUNDNUT IN SENEGAL

3.1 Introduction

Despite its leading position in terms of production and acreage in the country, groundnut production in Senegal (669329 tons in 2014) is low compared to China (6 million tons), India (5.6 million tons) and Nigeria 1 to 1.5 million tons (FAOSTAT, 2015). Many efforts have been made since 1966 by the National breeding programme in developing varieties that are adapted to Senegalese environments. However, the average national yield is still low and is around 1t/ha on farmers' fields, which is below the yielding potential of the varieties that have been released. A number of questions related to the adoption of the varieties that have been released include; i) the awareness of farmers of the existence of newly developed varieties that are more adapted to climatic variations; and ii) the characteristics of such varieties in terms of targeted traits preferences of farmers. These questions may help to have a better understanding of the problem and could help the breeder to be more relevant in his/her breeding objectives.

Plant breeders, to improve the level of adoption of newly developed varieties, should work closely with farmers through participatory plant breeding. This is where farmers' knowledge and preferences are integrated during the cultivar development (Bidinger, 1998). Oppong-Sekyere *et al*. (2015) conducted a Participatory Rural Appraisal (PRA) in Upper East, Upper West and Northern Ghana to identify the major characteristics of groundnut production. They found that drought was the major constraint in Northern Ghana. Egbadzor *et al*. (2013) assessed cowpea production constraints using matrix scoring, pair-wise ranking and individual interviews in Ghana. Nowadays, such studies are widely conducted by plant breeders to facilitate adoption of newly bred cultivars by farmers (Ceccarelli *et al*., 2007). In fact, this approach has been

demonstrated to enhance adoption and may help plant breeders to identify farmers' production constraints and preferred traits (Mulatu & Belete, 2001). This approach is also more effective in terms of cost and transferring the most suitable varieties and packages to farmers (Mangione *et al*., 2006).

While such studies have been conducted on groundnut in other countries in Africa (Ntare *et al*., 2007; Monyo *et al*., 2012; Oppong-Sekyere *et al*., 2015), limited information on the main groundnut production constraints and farmers' preferred traits is available in Senegal where groundnut is one of the most important crops. Most surveys made on groundnut sector in Senegal are on the economic aspect of the crop. Assessment of constraints and farmers' preferred traits for a specific crop does not have a global application. According to Omanya *et al*. (2007) plant breeders should develop, based on survey studies, cultivars adapted to a specific location rather than aiming at a wide range of environments. Knowledge of the groundnut production constraints and farmers' preferred traits would guide the breeding programme to set breeding objectives to meet farmers' needs. The objectives of this study were to:

- identify farmers' groundnut production constraints; and
- identify varietal trait preferences in the groundnut basin of Senegal

3.2 Methodology

3.2.1 Study area

The study was conducted between March and April 2015 in two agroecological zones in the groundnut basin in Senegal. These included three representative groundnut growing regions namely Kaolack, Kaffrine and Tambacounda (Figure 3.1). These areas were chosen because they produce groundnut in large quantities and on large areas (ANSD, 2015). There is only one rainy season from July to October and the rainfall amount ranges from 600 to 1200 mm per year. One department was selected in each region. Within each rural commune, three villages were selected providing a total of 9 study sites.

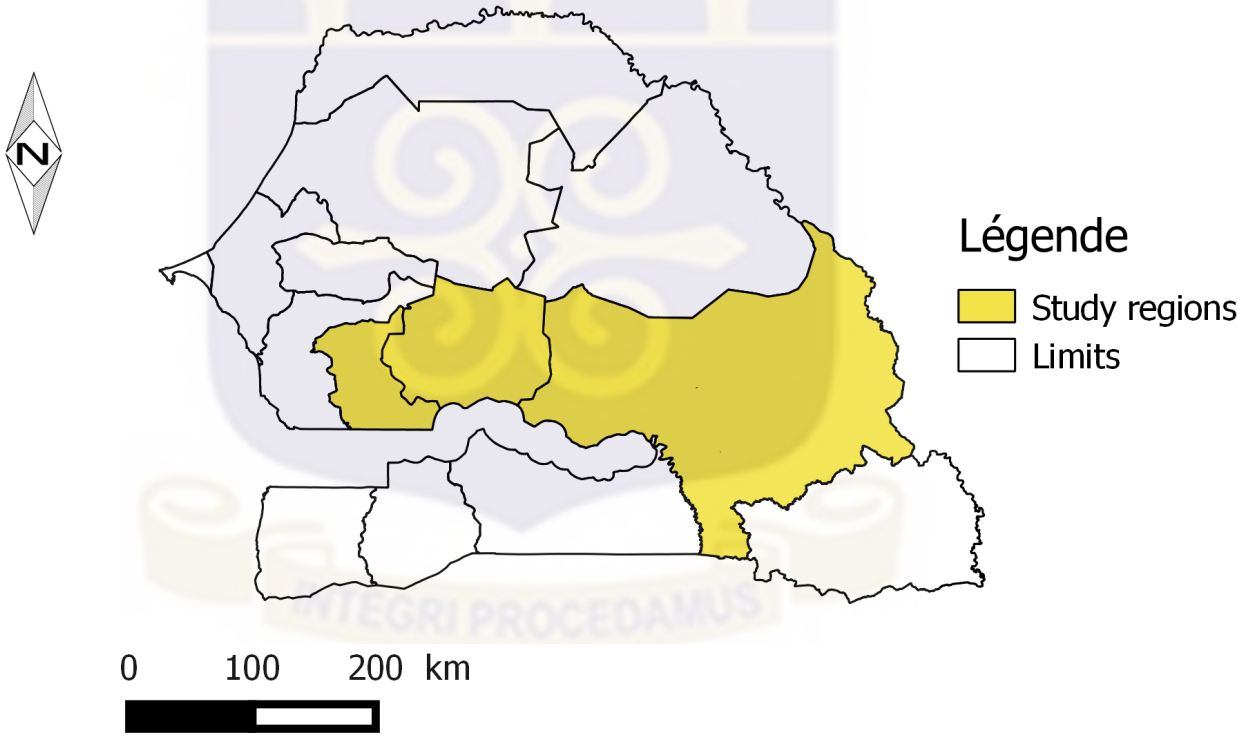


Figure 3.1: Location of the study sites on the Senegal map

3.2.2 Sampling procedure

The sampling procedure was done with the assistance of the regional agriculture extension officers. A multi-stage sampling procedure was used to select the three most important growing regions (Kaolack, Kaffrine and Tambacounda) and then the biggest producer department in each region. At the department level, three villages were chosen. In Kaffrine and Tambacounda, the chosen villages are in two rural communes and in Kaolack the three villages are in three different rural communes. Seven rural communes were involved in the survey. Nine (9) FGDs comprising of ten to seventeen farmers were organized (one in each village). Local extension staff and sub-district managers at the respective localities facilitated the PRA process by mobilizing farmers to participate in the FGD. In addition within each village, 10 farmers were randomly selected providing a total of 90 producers for the semi-structured interviews.

3.2.3 Data collection

Focus group discussions were standardized using a checklist of topics to be covered. The topics discussed included: farm characteristics and farmers' agronomic practices, farmers' varietal preferences, marketing of their produce and the perceived constraints affecting the production. The group discussions were followed by formal interviews using semi-structured questionnaires to explore more specific issues. During the survey pictures and drawings were frequently used to illustrate difficult points. Ten important traits including pod and haulm yield, morphometric and morphological descriptors for pod, seed and growth habit were used to assess farmers' preferred plant characteristics for improved groundnut varieties. Prioritization of the constraints was based on frequency of occurrence and severity. These constraints to groundnut production were identified and listed during the focus group discussions. They were further ranked by each interviewed farmer.

3.2.4 Data analysis

Data were coded and entered in Microsoft Excel. The analysis was performed using Statistical Package for the Social Scientists (SPSS version 19). Data were analysed per rural commune. Variables were subjected to descriptive statistics, cross tabulation, one-sample t (two-sided) and chi-square tests. Simple scoring and ranking techniques were used to identify farmers' production constraints and preferences. Standard errors were used to separate means where necessary.

3.3 Results

3.3.1 Demographic and socio-economic characteristics

Of the total interviewed farmers, 88.9% were males while 11.1% were females Table 3.1. For 72.2% of the respondents farming is their only source of income while 27.8% had additional income-generating activities. Most of the interviewed farmers 65.6% and 16.7% have been respectively in Koranic school and local language schools. A small percentage (8.9%) was educated up to primary level, 5.6% secondary level and 3.3% without any education. The most representative range of age in the study areas was between 35 and 60. There was significant difference (p= .004) in terms of age in the selected rural communes. The proportion of the active population involved in groundnut production was 71.1%. About 63% did not have any form of support or training and only 36% of the farmers belonged to farmer association.

3.3.2 Groundnut importance and production level

Although the selected rural communes were predominantly groundnut producing areas, other crops like cereals played a key role in the cropping systems. A ranking of the importance of groundnut compared to other crops was done on the basis of the cultivated areas allocated to each crop and the production levels. The average land holding varied from a minimum of 1 to a maximum of 30 ha and significant difference of the importance of the crops grown was observed between locations. In almost all rural communes groundnut occupied the most important piece of land and was ranked number one with an average mean rank of 1.3.

Table 3.1: Demographic and socio-economic characteristics of the respondents across locations

Variables	Modalities	Rural commune								Chi-*x2*
		G Kaye	Kahi	Kathiot	Kaymor	Koussanar	Maka	Porokhane	Total	%	P-Value
Gender											0.239
	female	0	3	0	3	3	1	0	10	11	
	male	10	17	10	7	17	9	10	80	89	
Age											0.004
	<15	0	0	0	0	1	0	0	1	1	
	15-35	0	2	3	1	0	5	2	13	14	
	35-60	3	10	5	7	16	2	8	51	57	
	>60	7	8	2	2	3	3	0	25	28	
Education											0.066
	koranic school	9	13	7	9	7	5	9	59	66	
	local language	0	6	2	1	5	1	0	15	17	
	none	1	1	0	0	1	0	0	3	3	
	primary	0	0	1	0	5	2	0	8	9	
	secondary	0	0	0	0	2	2	1	5	6	
MOA											0.006
	no	10	11	6	7	7	4	9	54	60	
	yes	0	9	4	3	13	6	1	36	40	
	yes	5	16	7	7	16	6	8	65	72	
Support											0.115
	no	10	12	7	6	11	8	9	63	70	
	yes	0	8	3	4	9	2	1	27	30	
Training											0.009
	no	10	16	8	10	11	9	10	74	82	
	yes	0	4	2	0	9	1	0	16	18	

MOA: Member of association

Groundnut was followed by pearl millet or corn or sorghum depending on the locality. In the central part of the groundnut basin (Kaolack, Kaffrine), pearl millet was the second most important crop. Elsewhere in the eastern part of the country (Tambacounda region), corn or sorghum was ranked the second most important crop. The comparison of the mean yield revealed significant differences among localities (p=.042) (Table 3.2).

Table 3.2: Groundnut pod yield estimated as a function of area and NPK fertilizer applied

	Guin Kaye	Kahi	Kathiot	Kaymor	Koussanar	Maka	Porokh	Total	F
Yield (kg/ha)	1500.25	960.19	967.40	1200.00	1110.79	1289.44	912.50	1112.01	.042
NPK (kg/ha)	116.00	53.50	85.00	45.00	90.20	72.50	28.00	70.43	.05
Area (ha)	4.90	5.81	8.90	5.70	5.61	5.56	12.78	6.76	

The average groundnut yield was estimated at 1112 kg/ha. The highest (1500.25 kg/ha) was recorded in Guinth Kaye in the groundnut basin while the lowest value 912.5 was recorded in Porokhane (Table 3.2). Both localities are in the same region (Kaolack). This result showed differences within the same region. Similar trend was observed for the application of fertilizer. On average, 116 kg/ha of NPK (6 20 10) fertilizer was recorded at Guinth Kaye while 28 kg/ha was recorded at Porokhane. The higher the quantity of NPK applied the higher the yield was. The regression analysis showed that the amount of NPK applied was one of the factors determining the yield (P =.001) and also the area allocated to groundnut did not have any impact on the yield obtained (Table 3.3).

Table 3.3: Regression analysis between estimated yield, rate of fertilizer and acreage

Model	Mean Square	F	Sig.	R^2
Regression	962984.662	5.474	.006	
Residual	175906.986			
Total				
dose		3.296	.001	0.127
area		-.859	.393	

3.3.3 Varieties grown and their provenance

Six varieties were cultivated in the surveyed zones as shown Figure 3.2. Some varieties were more popular than others depending on the locality. Out of the six varieties, '73-33' was the most cultivated and was ranked number one in almost all rural communes except in Guinth Kaye, Porokhane and Kaymor. The variety '28-206' was predominantly grown in Guinth Kaye and Porokhane while 'GH119-20' was the most cultivated in Kamor.

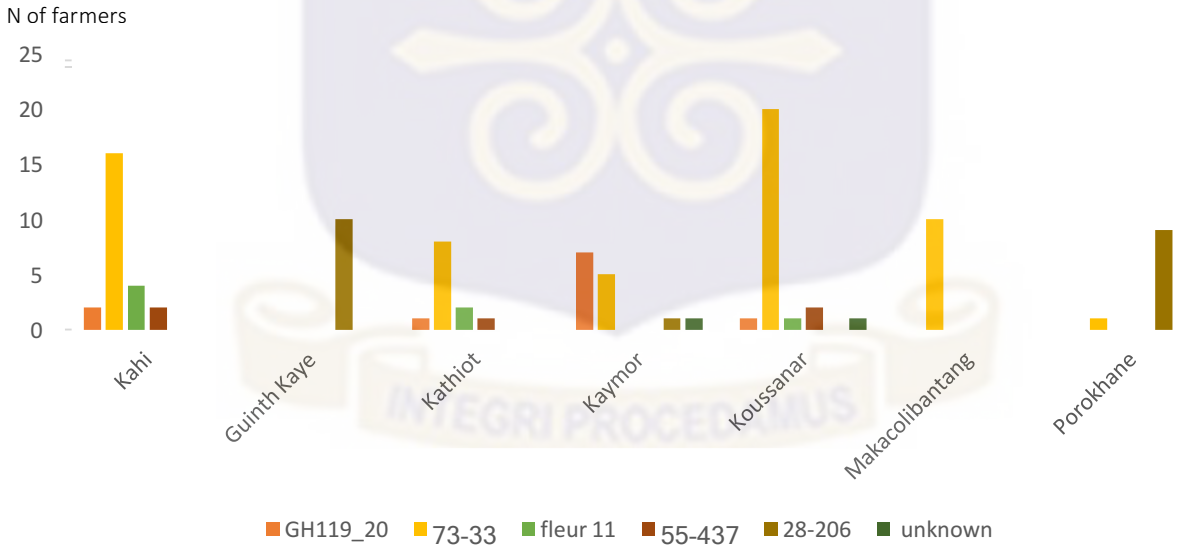


Figure 3.2: Cultivated groundnut varieties by community

A total of 60 respondents representing 66.67% of the farmers cultivated 73-33. Fleur11 was grown in few communes (Kahi & Kathiot). Two percent of the respondents did not have any idea

about the type of variety they grew. Most of the farmers around 83.33% used saved seeds from the previous harvest. Twenty percent of the respondents had previously obtained government-subsidized seeds or procured them from the local market. In Koussanar, farmers belonged to a farmers' organization and made seed' multiplications for the next growing season.

3.3.4 Crop management

Ninety nine percent of groundnut farmers did not plough their fields. Land preparation consisted mainly of cleaning the field manually using home-made craft tools. The overall results from both focus group discussions and formal surveys demonstrated that farmers sowed groundnut seeds at the onset of the rains in humid soil. All known Senegalese' types of soil (clayey, sandy-clay and sandy) were used to cultivate groundnut with, however, a predominance of sandy soil (42%). Thirty six percent of the farmers cultivated groundnut on sandy-clay soils. The most common row spacing used was 50 cm (51% of the total respondents). However, 30% of farmers used 40 cm row spacing. While the difference in row spacing did not vary significantly ($F = .61$), significant differences ($F < .05$) between rural communes was observed in the distance between plants in a row. Mainly two seed-sowing discs were used 30 cm and 20 cm. In Koussanar and Makacolibatang (Tambacounda region), 30 cm spacing between plants in a row was used while in Kahi, Guinth Kaye and the remaining rural communes 30 and 20 cm were used. Approximately 94% of the farmers treated their seeds against insect pests and other pathogens using fungicides before sowing. Around 66% of farmers used mineral fertilizer (NPK) at different doses. Groundnut was mainly cultivated in rotation with different crops mainly cereals in all localities (98%). In the surveyed zones of Tambacounda, it was cultivated in rotation with maize or sorghum while in Kaffrine and Kaolack pearl millet was the main crop used in rotation.

Weeding was done three times in a cropping season and there was no chemical weed control in all study zones.

3.3.5 Farmers trait preferences for groundnut variety

Except for tolerance to biotic stresses (P=.007) where highly significant difference was observed among communities, all other preferences ranked by farmers were non-significant (Table 3.4). Most of the farmers identified haulm yield as the second most important character after pod yield, except in Koussanar where good pod filling was ranked number one. The same locality (Koussanar) identified erect plant habit as an important groundnut criterion for variety adoption. Farmers in Kahi, Kathio, Kaymor and Makacolibatang preferred varieties that had more pod weight than those which were light. Farmers preferred intermediate season varieties to very short season varieties. Almost all farmers in the study areas did not want extra-early maturing varieties (less than 80 days) as they were prone to additional rainfalls and animal' damage. Marketability was ranked among farmer-preferred traits.

Table 3.4: Farmers preferences for groundnut traits in different communities

Traits	Rural communes							Total		
	Guinth	Kahi	Kathiot	Kaymor	Koussanar	Maka	Porokhane	Mean	Rank	F
Pod yield	1.00	1.00	1.00	1.00	1.19	1.00	1.00	1.04	1	0.783
biotic toleran	5.00	3.67	1.67		3.33	3.25	4.00	3.31	8	0.531
drought toler	2.75	3.00	3.50	4.00	3.38	2.33	3.00	3.08	7	0.007***
Short cycle	4.00	3.00	3.13	1.00	3.33	2.83		3.06	6	0.413
Haulm yield	2.00	2.19	2.43	2.17	2.36	2.00	2.00	2.19	2	0.756
Pod weight		2.00	2.00	2.00		2.50		2.20	3	0.521
Oil content	3.75	4.00	4.00	3.50	3.67	.	3.00	3.80	9	0.919
Erect plant	.	.	.	.	2.00	.	3.00	2.25	4	0.276
Good pod fillin	3.00	2.56	2.00	3.00	1.00	2.50	3.50	2.65	5	0.827
Good taste	4.00	5.00	.	4.00	5.00	.	.	4.60	10	0.764

*Characteristic with smallest mean rank is perceived to be the most important

Tolebiotic: tolerance to biotic, toledrought: tolerance to drought

During group discussions, farmers unanimously mentioned that a variety with long and large pod and seed characteristics commanded better acceptability in local markets when sold and produced higher yield. Farmers correlated big pod and seed size with high yielding and marketability. In general, farmers were not concerned much about storability and animal' feed quality in groundnut haulm and ranked them low. Farmers argued that they had not seen groundnut variety with resistance to storage pests nor with special haulm qualities as feed for animals.

3.3.6 Groundnut production constraints

The top five constraints to production across the commune were availability of seed, land degradation, insects in storage, land availability and drought (Table 3.5).

Table 3.5: Relative ranking of major groundnut production constraints by community

constraints	Rural communes							Total		
	Guinth	Kahi	Kathiot	Kaymor	Koussanar	Maka	Porokhane	Mean	F	Rank
Land degradation	2.31	2.33	2.2	2.06	1	1	4.66	2	0.47	2
Insects at storage	4.01	2	1	3	2.40	1.56	4	2.04	0.06	3
Leaf diseases	3.89	2.78	1	1	2	3.21	3.41	2.56	0.41	7
Drought	4	2.67	3	3.67	1.75	1.75	4	2.36	0.02	5
Land availability	2.17	1	3	2	3.71	4.18	3	2.13	0.60	4
Seeds availability	2.82	1.63	2	1.6	1.67	2.23	2	1.91	0.14	1
Commercialization	3.44	2	2.5	3	1.75	2	2.45	2.50	0.02	6
Lack of tools	5	3	2	2.40	1.67	2.56	2	2.58	0.047	8
Cattle damage	5.07	4.22	4.34	3	3.50	4	5	3.33	0.667	9

*Characteristic with the smallest value is perceived to be the most important

Marketing and foliar diseases were ranked sixth and seventh respectively. These constraints were common to groundnut growing areas but the order mostly varied from one commune to another. Insects mainly *Caryedon serratus* was identified as an important constraint to groundnut storage

and ranked as main constraint in Kathiot with foliar diseases. Lack of agricultural tools was ranked in Koussanar, Kaymor and Kathiot as an important constraint. Significant differences were observed in the ranking of drought, commercialization and lack of tools.

3.4 Discussion

A small number of women (11%) were represented in this study. This reflects the limited access they have to land. The major explanation of this situation is socio-cultural. In Senegal, land' acquisition is by inheritance, transmitted from father to son. As a result, few women have their own land. Diatta. (2017) reported the low involvement of women in sorghum and pearl millet cultivation due to their limited access to land. They found that women were more involved in vegetable cultivation which required meticulous techniques. Groundnut was the most important crop grown by farmers in the areas of the study. Production for the market was the major objective followed by animal feeding (production of biomass). These results suggested that the importance of groundnut in farmers' livelihood is strongly associated with its use as a source of income. Besides groundnut, farmers cultivated mainly in rotation a wide range of crops such as pearl millet, sorghum, maize, rice, fonio, cowpea etc. The option of growing several crops is a strategy to improve resilience (Lin, 2011). According to Traoré *et al*. (2015) it may prevent farmers from focusing sufficiently on adequate production of one particular crop. Moreover, it allows farmers to have a staple food in addition to cash crop like groundnut.

Crop rotation plays an important role in groundnut management and farmers have understood this. In most studied sites, pearl millet was the main crop cultivated in rotation with groundnut. It is known that crop rotation is beneficial for restoring soil fertility and for preventing crop pests and soil borne diseases. This may partly explain why farmers did not apply pesticides or insecticides in their fields. These products are beyond the reach of farmers who also think that such products degrade the soil. The average yield estimated (1.1 t/ha) in the studied sites was not so different from the national average of 0.9 t/ha reported by Sylla. (2016). However, variability was noted among sites regarding yield. This variability was related to the quantity of mineral

fertilizer applied as shown by the results. In general, as the dose of NPK applied increased, the yield increased in most of the study sites. But exceptions were observed for example at Koussanar where the yield obtained with fertilizer did not reach the yield in Makacolibantang whereas higher quantities of NPK were applied. These results suggested that besides fertilizer application there were other factors that determined the yield obtained. This difference may due to adequate rainfall, the type of soil or different constraints that farmers faced in groundnut cultivation.

Difficulties faced by farmers in groundnut cultivation could be classified in two groups: the first group included the most important constraints ranked by priority by farmers namely seed availability, land degradation, storage pests, land availability and commercialization. The consistency in ranking of these constraints over the communities suggested that these were major constraints affecting groundnut production in the groundnut basin. The first group of constraints involved the whole value chain and could not be addressed by research only except for insects during storage.

One of the major insects that plague groundnut production is *Caryedon serratus.* This insect is a coleopteran which can cause within four months up to 80% of production losses in storage in Senegal (Diome *et al*., 2013). Entomologists have made huge efforts in determining the mechanisms involved in the infestation process and how to minimize them. They have discovered that the *Caryedon* species that infest groundnut pods are genetically close to those that infest the pods of a widely distributed legume in most areas of Senegal, Ngigis (*Piliostigma reticulatum*). Noting that the infestation is almost absent when this wild legume is absent, recommendations have been formulated to reduce the incidence of the *Caryedon* species on groundnut (Sembene *et al*., 2006, 2008).

The second group included drought and leaf diseases. Senegal is located in the Sahelian zone and is subjected to different scenarios of drought. As a result production of many crops is threatened yearly by the inter annual and across location rainfall variability. Drought is characterized by an unequal distribution of rainfall in both time and space (Upadhyaya, 2005). Results showed significant differences in drought ranking between the communities. In the localities of the eastern part of the groundnut basin (Tambacounda region) where the annual average rainfall is 800 mm, drought is perceived to be more important than in the central zone where the annual rainfall is between 500 - 600 mm. This paradoxical situation could be explained by the type of cultivated variety and the distribution of the rains. In the Eastern zone where intermediate and long cycle varieties are grown, two drought periods are generally experienced. One is at seedling stage immediately after the first rainfall events; drought at seedling stage is manageable because of the plasticity of the crop (Reddy *et al*., 2005). The second occurs during the pod filling stage. Varieties 73-33 and 28-206 are long cycle varieties that are mostly cultivated in the eastern zone while in the central areas a mixture of short and intermediate cycle varieties was grown (55-437, Fleur11 with 90 days). Seed availability and drought were identified as the major challenging constraints in Mali (Ndjeunga *et al*., 2010; Ntare *et al*., 2012). Non-availability of seed was also identified as a constraint in Karnataka State in India (Varman *et al*., 2012). Oppong-Sekyere *et al*. (2015) reported drought as a major constraint to groundnut in northern Ghana. In Mozambique, drought was the main constraint to cowpea production (Chiulele *et al*., 2011). The years 2002 and 2011 were the most challenging years in groundnut cultivation in Senegal due to low and erratic rainfall patterns. The impact of such events is not only extremely damaging, but it can also be lasting. Development of varieties better adapted to cope with the impact of drought is required as drought is unpredictable and erratic. In Senegal, Clavel *et al*. (2006, 2008) worked

to identify drought tolerant germplasm and breeding lines but they experienced difficulties due to the limited genetic resources available in *Arachis hypogaea* to handle complex traits like drought. In this context, expanding the genetic base of cultivated groundnut could play an important role in finding new sources of variability.

In Kathiot and Kaymor (Nioro zone), leaf diseases were the major constraints that affected groundnut production while in the other localities, leaf diseases were not perceived by farmers as important. Environmental conditions such as weather, susceptibility of the host plants related to the type of varieties grown could explain such disease occurrence. However, good agricultural practices may help in the management of such diseases. Groundnut is cultivated in rotation with pearl millet or with other cereal species mostly as it was the case in most of the studied zones. For farmers, crop rotation is a strategy which can restore soil fertility, avoid severe foliar diseases and prevent soil borne diseases. Farmers did not apply any fungicides for leaf diseases. These results indicate that breeding for improved drought tolerance and leaf diseases resistance should constitute major breeding objectives to ensure high yield and varietal acceptance by farmers in specific zones.

Farmers indicated pod and haulm yield as the most important criteria for selecting groundnut varieties. High pod yield was the most highly ranked criterion in all surveyed sites; followed by biomass yield. Farmers gave importance to seed and pod characteristics. Farmers correlated pod characteristics to pod yield. However, scientific studies have shown that there was no correlation between pod yield and pod and seed size characteristics (Fonceka *et al*., 2012). Another important consideration for farmers is the attractiveness and the business side of large pods and seeds. The fact that groundnut is a cash crop could justify why farmers paid much attention to pod and seed size. Similar consideration was given to big seeds and pods by farmers in Mali. In a

study conducted in Mali by Sanogo. (2017), pod yield, pod and seed size played an important role in farmers' choices in addition to pod yield. Oil content and good taste received little attention. This result could be different if the survey had taken into account the industrial processors and end users. The agreement between farmers and breeders in some of the criteria used for selecting groundnut varieties suggests the need for collaborative work to improve the efficiency of selection.

3.5 Conclusion

The most important constraints across sites were seed availability, land degradation, insects at storage and land availability. Drought was the fifth constraint with differences between sites. Marketing and foliar diseases were important constraints to farmers. Insects mainly *Caryedon serratus* was identified as an important constraint to groundnut storage and was ranked as main constraint in Kathiot with leaf diseases. Pod and haulm yield were the most preferred traits in groundnut variety. Large pod and seed were also important traits in groundnut variety.

CHAPTER FOUR

4.0 ASSESSMENT OF AGRONOMIC POTENTIAL OF AN ADVANCED BACKCROSS QTL POPULATION AND IDENTIFICATION OF PROMISING BREEDING LINES

4.1 Introduction

Exploiting the genetic potential of wild species has been of great interest in several plants mostly for crops with narrow genetic base (Tanksley & McCouch, 1997; Zamir, 2001; Zhang *et al*., 2017). Cultivated groundnut has a narrow genetic base (Kochert *et al*., 1991) and wild groundnut species have been reported to be rich in alleles that can be used to enrich the cultigen genetic background. For a long time, wild groundnut species have been used as donors to transfer useful characters, mainly biotic resistance traits, in cultivated varieties (Mallikarjuna *et al*., 2004). Because of the polyploidy differences between the tetraploid *Arachis hypogaea* and the diploid species of the section *Arachis*, two methods have been mainly used to move genes from wild species to cultivated species: the tri-hexaploïd technique and the amphidiploid technique (Simpson, 2001; Fávero *et al*., 2006). The success of the first method has been limited due to the high rate of sterility and the instability of the hybrids. The latter method has the advantage of generating tetraploid hybrids with the same level of ploidy as the cultivated species. This method has been used to transfer nematode resistance genes from *A. cardenasii* into *A. hypogaea* (Simpson & Starr, 2001; Simpson *et al*., 2003). In terms of resistance to biotic stresses, wild species have been greatly exploited. However, for traits related to yield, wild species have not been of much interest in groundnut improvement. Nevertheless, in crops such as tomato, barley and rice, many studies have demonstrated that genomic regions from wild species can be used to improve complex traits including yield (Gur & Zamir, 2004; Fu *et al*., 2010; Nevo & Chen, 2010). The under exploitation of wild species to improve complex traits may be partly attributed to their inferior phenotypic value associated often with undesirable characters.

In fact the wild species' alleles have no agronomical interest in a wild genetic background. However what would be of interest in terms of yield components is a small proportion of the wild genome in a cultivated genetic background. Fonceka *et al*. (2012) developed an interspecific genetic material using a synthetic allotetraploid created from the progenitors of the cultivated groundnut (*Arachis duranensis* and *Arachis ipaensis*). They found a valuable benefit of using wild species in improving agronomic traits in groundnut. In the present work, a different synthetic allotetraploid donor ISATR52B was used to:

- develop an interspecific mapping population,
- assess the agronomic potential of the introgressed groundnut lines; and
- identify superior and stable genotypes for agronomic traits from the introgressed lines.

4.2 Materials and Methods

4.2.1 Population development

A synthetic allotetraploid ISATR52B derived from a cross between [*A. duranensis* (AA genome) X *A. valida* (BB genome)] ^{4x} was developed and provided by ICRISAT. The allotetraploid was then used to develop a population of 200 $BC_2F_{4:6}$ progenies at CERAAS (Figure 4.1). The synthetic wild allotetraploid ISATR52B was used as the male donor and Fleur11 a cultivated Spanish type variety of *A. hypogaea* used as the recurrent female parent. Fleur11 is popular moderate yielding variety introduced from China in 1990. The advanced backcross population $BC_2F_{4:6}$ was developed using two successive generations of backcrossing of selected F_1 individuals to Fleur11 followed by four generations of self-fertilization (from BC_2F_1 to BC_2F_4) using single seed descent (SSD). Then seed multiplication was done in bulk up to $BC_2F_{4:6}$. Crosses were performed under greenhouse at the Centre d'Etudes Regional pour l'Amelioration de l'Adaptation a la Secheresse (CERAAS, Senegal) between 2012 and 2015. The Fleur11 was used as female parent to produce the F_1, BC_1F_1 and BC_2F_1 generations. The process of the crossing was as follows: Fleur11 plants used as female were manually emasculated the afternoon of the day before each crossing step. The pollinations were performed early in the morning the following day between 7 am and 10 am by capping the pollen sacs of the flowers of the male parent on the pistils of the female parent. Flowers that had not been pollinated were removed manually. F_1 hybrids were selected based on phenotypic appearance. For the BC_1 and BC_2, microsatellite markers were applied to select the true BC_1 and BC_2 individuals. Eight SSRs were used to select the BC_1 and 20 markers for the BC_2. Figure 4.1 gives a summary of the crossing scheme and the number of selected plants advanced at each generation.

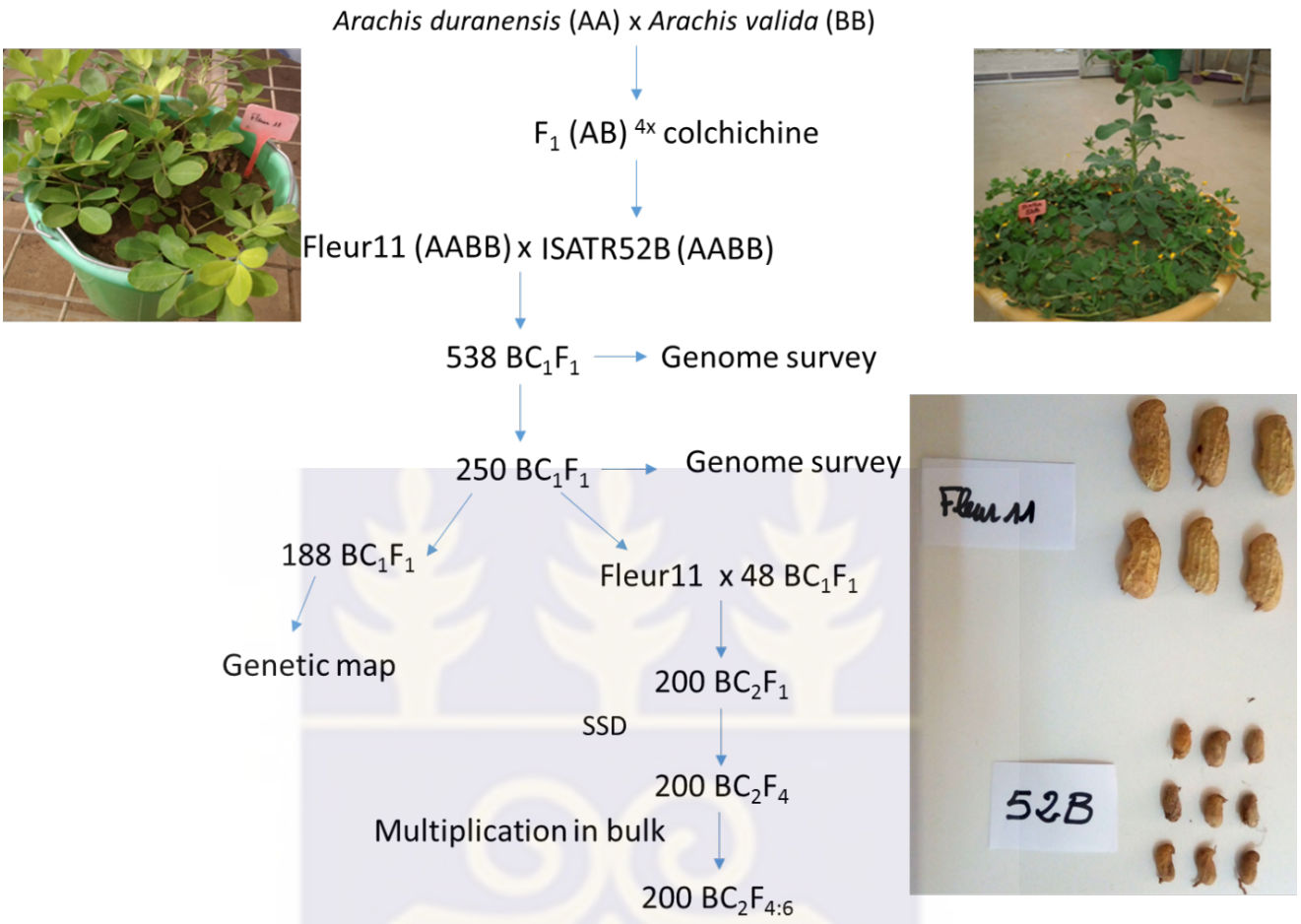


Figure 4.1: Description of the development of the advanced backcross QTL (AB-QTL) population

4.2.2 Study sites and field design

The 200 lines along with the recurrent parental line Fleur11 were evaluated in five environments. Two water regimes were imposed in 2015, well-watered (WW) and water limited (WS) (pod filling), during the off season (October - January) at ISRA's station of Nioro du rip (13°43'N; 15°46'W) located in South Groundnut basin zone. The two experimental conditions received the same quantity of water from sowing up to 65 days after sowing (DAS). A total of 30 mm of water per week divided in two irrigations /week was applied. From 66 DAS, irrigations were stopped in the WS until harvest (53 days of stress). The WW received in total 331 mm and the WS received 157 mm of water. The lines were also evaluated in 2016 during the rainy season (July - October) in three locations: Nioro du rip, Darou located both in south- eastern part of the Groundnut basin and the third location was ISRA's station of Sinthiou Maleme (13°49'N; 13°55' W) (Figure 4.2).

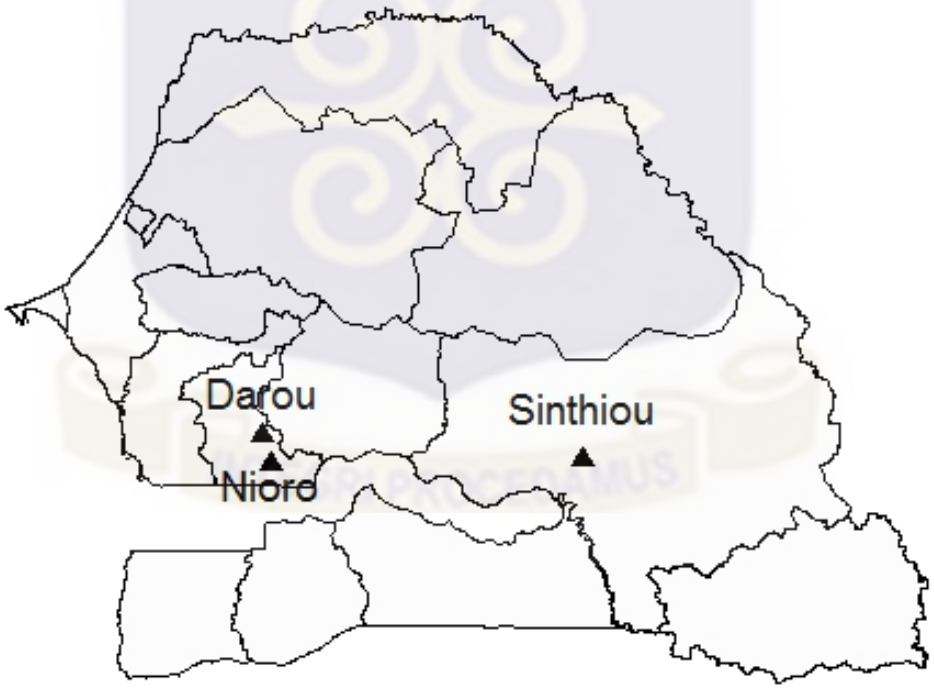


Figure 4.2: Location of the study sites

Each experiment was arranged in an augmented incomplete block design made of 25 blocks of 8 families with 5 checks including Fleur11. The block size was 13 entries. The plot size was 1 row of 3 m length with 10 plants, 30 cm between plants and 50 cm inter-row spacing. Standard field management for groundnut was applied in all experiments.

4.2.3 Data collection

Thirteen quantitative and three qualitative traits were recorded in one or more environments. Except for 50% days to flowering, the plant growth habit and the main stem height, all traits were recorded after harvest. The mean phenotypic value of each trait was expressed on a per plant basis by averaging the values of a minimum of 6 to a maximum of 10 plants.

Days to flowering (Dflo):

- Number of days from sowing to the first flower appearance date on 50% of the plants in a row

Plant architecture:

- The plant height (PH) was measured from the base of cotyledonary axil up to the terminal bud. Groundnut growth habit (GH) is usually recorded on a 1–6 scale based on the IBPGR of ICRISAT (1992) descriptors where, 1 = procumbent 1, 2 = procumbent 2, 3 = decumbent 1, 4 = decumbent 2, 5 = decumbent 3, and 6 = erect. However, this method yields qualitative data that may not be suitable for QTL mapping. Therefore, in this study, the method applied was that one described by Fonceka *et al*. (2012) where GH trait was represented by the ratio of the length of the creeping part of a given lateral branch to the total length of the lateral branch. This technique generates quantitative data. No data transformation was performed.

Pod and seed morphology:

- Pod length (PL) and width (PW), seed length (SL) and width (SW) were evaluated on 30 pods and 30 seeds respectively using a caliper with a digital display. These traits were measured in all environments. Data on pod beak and constriction were recorded in one site on 30 pods by using a 0-9 scale according to the descriptors of groundnut. For Pod constriction, only pods with at least two seeds were considered. The data obtained were transformed into frequencies by multiplying the number of pods of a given class by the value of the class (0, 3, 5, 7 or 9). The sum of the frequencies divided by the total number of pods (30) yielded an average that was used for QTL analysis.

Yield components:

- The yield components were determined based on the whole plant, the pod and haulm dry mass. The total plant biomass was first weighed to determine the total biomass per plant (TPW). Then pods were removed and weighed to determine the total pod weight per plant (PPW). The haulm weight per plant (HW) was calculated as the difference between the total biomass and the total pod weight. One hundred (100) pods (HPW) were randomly sampled and weighed. The 100 pods were then shelled and the percentage at maturity (PMAT) was determined by counting mature pods characterized by a dark black internal pericarp colour. All seeds contained in the 100 pods were weighed and mature seeds were separated from immature seeds and counted. The weight of 100 seeds (HSW) was calculated as the weight of mature seeds divided by the number of mature seeds multiplied by 100.

4.2.4 Statistical analysis

Data collected at Darou in 2016 were removed from the analysis due to the high number of $BC_2F_{4:6}$ families with missing data. Data normality was checked with the Shapiro test for normality. Basic statistical analyses (mean and standard deviation) were calculated for each trait. The combination

location and year was considered as an environment. Since the environment x genotype (GxE) interaction was significant, data from each environment were subjected to independent analysis of variance (ANOVA) using the general linear model (GLM) procedure implemented in SAS for the analysis of data issued from augmented block designs. The macros of Parsad *et al*. (2011) were used to estimate the block effects within the experiments, the environments, the genotypes and the genotype-by-environment interactions. The linear effect model for such ANOVA was as follow:

$$\boldsymbol{Y = \mu + E + B\,(E) + G + G\,x\,E + \varepsilon}$$

Where Y is the trait, $\boldsymbol{\mu}$ is the overall population mean, E is the environmental effect, B (E) the block within the environmental effect, G is genotype effect, G x E is the environment x genotype interaction, and $\boldsymbol{\varepsilon}$ is the experimental error. All effects were treated as fixed. Pearson correlation coefficients were computed for combined localities using PROC CORR procedure of SAS 9.4 (SAS Institute Inc, 2013). Broad-sense heritability was estimated in each location based on the formula:

$$\boldsymbol{H^2 = ((MSG - MSE)/r)/\,(((MSG - MSE)/r) + (MSE/r))}$$

Where, MSG and MSE are the genetic and residual mean squares and r the number of block in the experiment. Statistical analyses were done in SAS/STAT version 9.4 (SAS Institute Inc 2013). A composite selection index called index of agronomic stability (IAS) was used to select the best AB families based on pod and seed size traits in each environment. IAS combined four variables namely hundred pod weight (HPW), hundred seed weight (HSW), seed length (SL) and seed width (SW). These four traits were selected on the basis of the PRA results reported in this research and correlation patterns. IAS was computed according to Trouche *et al*. (2011):

$$\boldsymbol{IASi = \sum_{j} aj * [x_{ij} - m_j \div s_j]}$$

where xij is the phenotypic value of the line i for trait j, mj the population mean performance and sj the standard deviation of all lines for trait j, aj is the relative weighting of trait j in the index, where j varied from 1 to 3 with: 1 = HPW; 2 = SL; 2 = SW; 3 = HSW. Stability analysis was performed using the pod and biomass yield data of the selected genotypes. Genotype main effects plus genotype x environment interaction (GGE) biplot analysis were computed in GenStat (12th edition) to evaluate the yield stability following the model described by Yan *et al*. (2007); Alwala *et al*. (2010); Rao *et al*. (2011):

$$\boldsymbol{Yij - Yj = \lambda 1 \xi i 1 \eta j 1 + \lambda 2 \xi j 2 \eta j 2 + \Sigma ij}$$

Yij is the mean yield of genotype i in environment j, Yj is the mean yield across all genotypes in environment j, λ1 and λ2 are the values for principal component PC1 and PC2, ξi1 and ξj2 are the PC1 and PC2 scores for genotype i, ηj1and ηj2 are the PC1 and PC2 scores for environment j and Σij is the residual of the model associated with the genotype i in environment j.

4.3 Results

4.3.1 Traits variation and correlations

Except for plant growth habit (GH), all other traits showed continuous normal or almost-normal distribution in all studied environments (Figure 4.3). Plant growth habit (GH) showed a wide range of morphologies, ranging from completely prostrate to totally erect. Except for growth habit (GH), pod beak (PB) and pod constriction (PC), the mean population values for all other quantitative traits tended to be skewed towards the phenotypic value of the recurrent Fleur11 parent (Table 4.1).

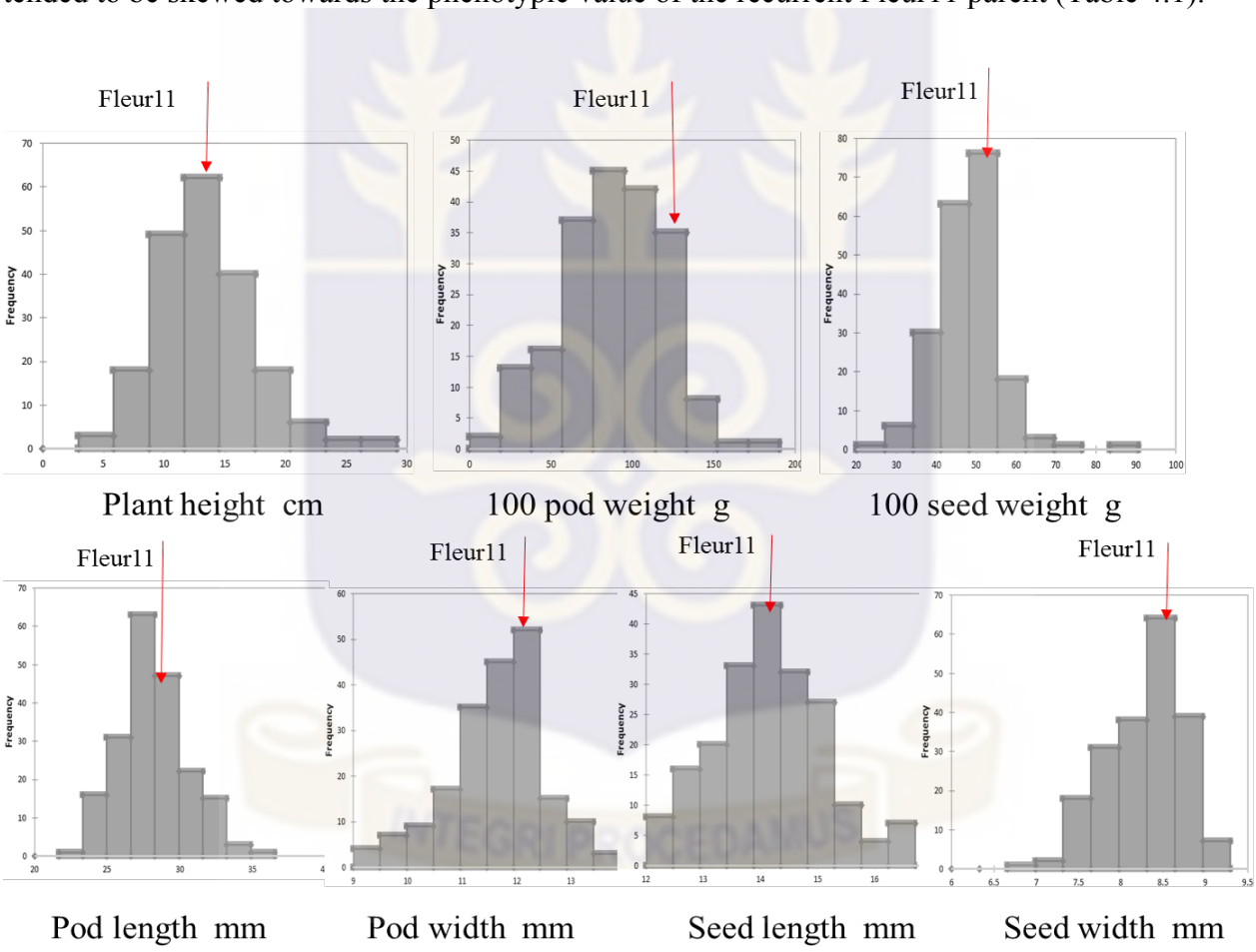


Figure 4.3: Distribution and variation of studied traits among the $BC_2F_{4:6}$ progenies

Excluding the date to flowering (Dflo) trait that displayed little variation ranging from 17 to 22 days, all traits studied showed extreme phenotypes indicating transgressive segregation for these

traits. The analysis of variance was highly significant P<0.01 for all traits in all four environments except in Nioro and Sinthiou 2016 where TPW and HW did not display significant differences among tested genotypes (Table 4.1). The trend of the estimated broad sense heritability values (H^2) was quite similar in the studied sites for characters such as pod and seed size. PL, PW, SL, SW and PB displayed high broad sense heritability ranging from 0.6 to 0.9 in all environments except in Nioro where SL showed a low value of 0.3. A similar pattern was observed for yield related traits HPW and HSW in all sites with H^2 ranging from 0.5 to 0.8. For yield traits such as TPW, PPW and HW, moderate to low heritability estimates were detected respectively in the off-season and rainy season environments.

Table 4.1: Summary statistics of traits in the studied environments

Symbol	Well-watered		Water-stressed		Nioro16		Sinthiou16	
	F	H^2	F	H^2	F	H^2	F	H^2
Dflo	2.1**	0.5	1.5**	0.3	5.8**	0.8	_	_
PH	3.2**	0.7	3.3**	0.7	1.7**	0.4	1.5**	0.3
GH	<001	0.9	<001	_	_	_	_	_
PB	3.3**	0.7	_	_	_	_	_	_
PC	2.2**	0.5	_	_	_	_	_	_
PL	7.9**	0.9	4.9**	0.8	13.8**	0.9	9.1**	0.9
PW	3.0**	0.7	2.5**	0.6	2.5**	0.6	3.3**	0.7
SL	5.0**	0.8	6.7**	0.9	1.4**	0.3	9.1**	0.9
SW	6.2**	0.8	2.6**	0.6	5.9**	0.8	3.7**	0.7
TPW	3.2**	0.7	1.9**	0.5	1.3ns	0.2	1.3ns	0.2
PPW	3.0**	0.7	2.8**	0.6	1.4**	0.3	2.0**	0.5
HW	4.7**	0.8	2.7**	0.6	1.3ns	0.2	1.3ns	0.2
HPW	5.0**	0.8	4.1**	0.8	4.1**	0.8	2.3**	0.6
HSW	2.1**	0.5	6.6**	0.8	3.8**	0.7	2.5**	0.6
PMAT	2.0**	0.5	2.6**	0.6	3.7**	0.7	1.8**	0.4

Dflo: flowering, PH: plant height, GH: growth habit, PB: pod beak, PC: pod constriction, PL: pod length, PW: pod width, SL: seed length, SW: seed width, TPW: total plant weight, PPW: plant pod weight, HW: haulm weight, HPW: hundred pod weight, HSW: hundred seed weight, PMAT: Percentage maturity.

Significant correlations were found between most of the traits (Table 4.2). The values in bold were highly significant at P = 0.001. Pod and seed morphology related traits namely PL, PW, SL, and SW showed significant positive correlations with HPW and HSW. PL, PW, SL and SW displayed also significant positive correlations between them. The correlation values ranged from 0.45 to 0.65. High positive correlations were observed among pod and seed morphometric traits. PMAT was not correlated to any of the studied traits except for PPW (0.36). The highest correlation coefficients were obtained between TPW and HW (0.93), HPW and PL (0.65), HSW and SW (0.58) and between PPW and HPW (0.55). HPW was highly correlated with PPW (0.55). The main stem height (PH) was positively correlated with almost all traits except for PMAT and negatively correlated with GH. Plant growth habit (GH) was negatively correlated with all studied traits. The days to flowering (Dflo) was not correlated to any of the studied traits.

Table 4.2: Correlation between studied traits in one site

Traits	Dflo	PH	GH	TPW	PPW	HW	HPW	PMAT	HSW	PL	PW	SL	SW
Dflo	1												
PH	-0.26	1											
	0.0001												
GH	-0.00589	-0.22789	1										
	0.934	0.0012											
TPW	0.07174	**0.46782**	-0.0931	1									
	0.3127	<.0001	0.1898										
PPW	-0.16632	0.1892	-0.03521	**0.2859**	1								
	0.0192	0.0076	0.6224	<.0001									
HW	0.12367	**0.43526**	-0.08195	**0.9375**	-0.06531	1							
	0.0826	<.0001	0.251	<.0001	0.3606								
HPW	-0.2099	**0.40292**	**-0.29652**	**0.3051**	**0.55287**	0.13026	1						
	0.0029	<.0001	<.0001	<.0001	<.0001	0.0674							
PMAT	-0.11057	0.07267	0.02787	0.0164	**0.36612**	-0.10885	0.2213	1					
	0.1191	0.3065	0.6953	0.8174	<.0001	0.1269	0.0016						
HSW	-0.17989	**0.40729**	-0.21968	0.2009	0.15773	0.1602	**0.5346**	0.05976	1				
	0.011	<.0001	0.0018	0.0044	0.0269	0.0245	<.0001	0.4018					
PL	-0.11685	**0.29179**	-0.17233	0.2638	**0.41467**	0.13366	**0.6505**	0.12179	**0.4509**	1			
	0.1003	<.0001	0.0149	0.0002	<.0001	0.0611	<.0001	0.0866	<.0001				
PW	-0.16886	**0.29979**	-0.08719	0.2072	0.1412	0.18422	**0.5481**	-0.00005	**0.4707**	**0.5679**	1		
	0.0168	<.0001	0.2196	0.0032	0.0472	0.0094	<.0001	0.9995	<.0001	<.0001			
SL	-0.13421	**0.32244**	-0.19221	0.1595	0.15666	0.12264	**0.5124**	-0.09721	**0.5329**	**0.6945**	**0.5002**	1	
	0.0588	<.0001	0.0065	0.0244	0.0279	0.086	<.0001	0.172	<.0001	<.0001	<.0001		
SW	-0.21845	**0.41327**	-0.16499	0.2663	0.24198	0.17743	**0.5842**	0.02304	**0.5885**	**0.6134**	**0.5511**	**0.8101**	1
	0.0019	<.0001	0.0196	0.0001	0.0006	0.0124	<.0001	0.7461	<.0001	<.0001	<.0001	<.0001	

Values in bold are highly significantly correlated at 0.1

4.3.2 Performance of the population in the studied environments

The characterization of the 200 progenies showed significant variation among sites for plant pod weight (PPW) and for plant biomass weight or haulm weight (BW=HW) (Figure 4.4). For HW, the population performed better at Sinthiou and in the well-watered condition than in the other environments. For PPW, the population showed best performance at Nioro 2016 and in the well-watered condition. On the other hand traits such as pod and seed size were more stable and displayed little variation between tested environments. Within environment, the population displayed important variation except for seed width in Sinthiou 2016.

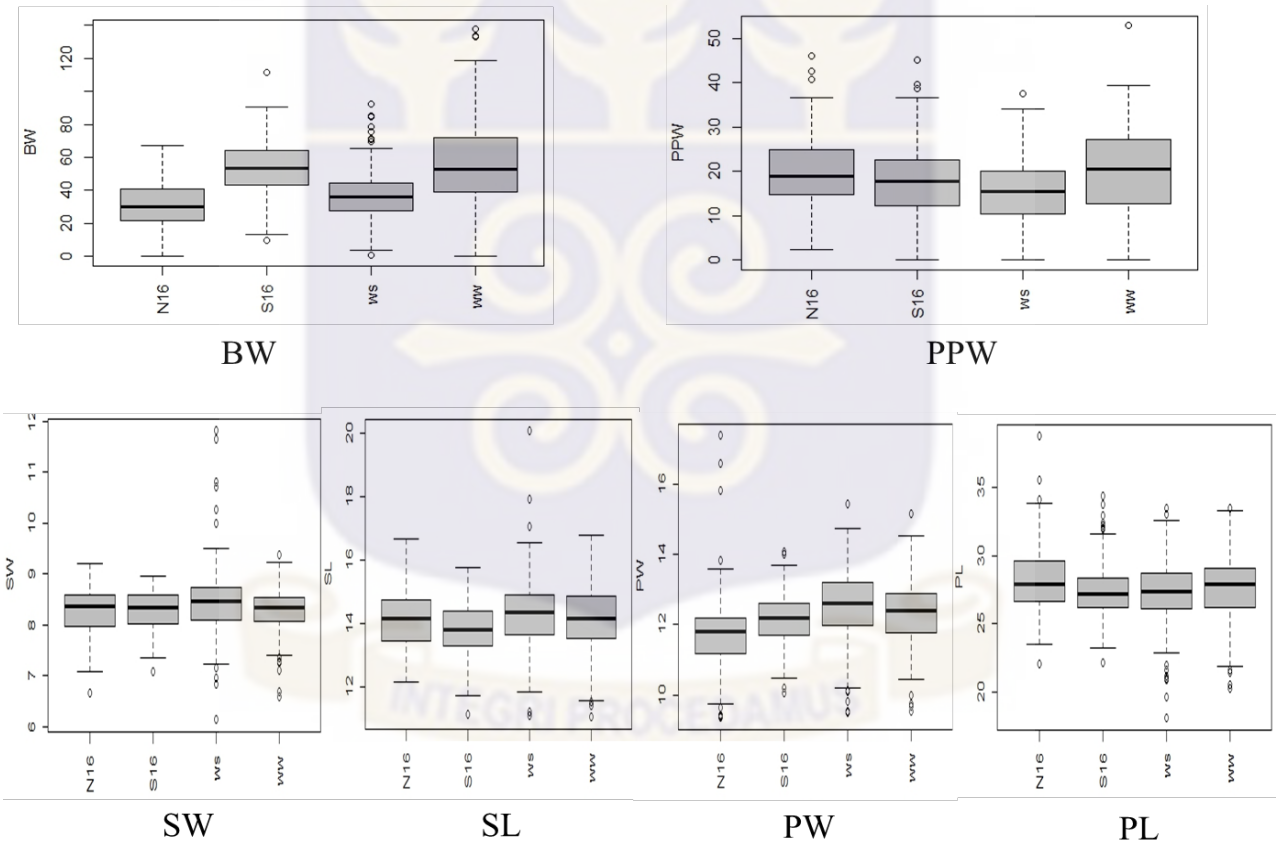


Figure 4.4: Traits variation among studied sites

Traits: BW or HW (biomass weight or haulm weight), PPW (plant pod weight), SW (seed width), SL (seed length), PW (pod width), PL (pod length); Sites: N16 (Nioro 2016), S16 (Sinthiou 2016), WW (well-watered), WS (water stressed).

4.3.3 Selection of the promising lines based on IAS

Using the selection index IAS, the top 50 genotypes were selected in each of the four environments (Table 4.1 in appendix). In the well-watered environment, 106 genotypes exhibited an IAS score between 0.45 and 13.09. These families outperformed the local recurrent parent Fleur11 which scored 0.43. The water limited environment ranked second with 88 lines that outperformed the recurrent parent Fleur11 for HSW, pod and seed size traits, followed by Sinthiou (49 genotypes) and Nioro (44 genotypes). Considering all four environments, 11 $BC_2F_{4:6}$ genotypes listed in Table 4.3 were constantly present and exhibited IAS scores varying between 3.83 and 15.38.

4.3.4 Pod yield performance and stability of the selected genotypes

The top 11 genotypes selected for HPW, HSW, and pod and seed sizes plus Fleur11 were subjected to GxE biplot analysis in order to identify the best performing genotype for a specific environment and to identify the more stable genotype across environments based on pod yield. The GGE biplot explained 86.67% of the total variation for pod yield with PC1 and PC2 accounting for 56.41% and 30.26%, respectively (Figure 4.5). The lines **B7_32_8_1** (243), **B7_23_10_3** (66) and **Fleur11** (0) appeared as the highest yielding genotypes as they are present on the far right-hand side of the biplot towards the pointing arrow of the AEC abscissa (Figure 4.5). Moreover, these three lines were also stable as they are positioned very near to the AEC abscissa. **B7_28_14_1** (189) and **Fleur11** (0) were the most stable lines across environments as these two lines had a very low PC2 score near zero.

Table 4.3: Top selected genotypes across environments based on IAS indices

Genotypes	IAS			
	WW	**WS**	**N16**	**S16**
B12_14_2_2	5.66	14.40	15.13	6.58
B12_14_2_5	11.48	5.37	8.04	13.09
B7_20_3_1	8.39	11.79	12.17	7.60
B7_21_2_8	7.69	3.83	7.09	6.31
B7_23_10_3	11.18	12.37	15.38	7.61
B7_23_10_8	6.67	4.26	9.10	5.94
B7_27_5_7	11.36	5.73	4.63	8.76
B7_28_14_1	6.96	7.11	9.05	7.65
B7_30_6_5	6.97	6.64	5.61	9.74
B7_32_7_5	11.15	12.27	7.83	13.11
B7_32_8_1	9.36	11.21	8.43	14.46

IAS: Index of Agronomic Stability; environments: WW, WS, N16, S16

B7_23_10_8 (70), **B7_21_2_8** (34) and **B7_30_6_5** (217) were regarded as consistently the lowest yield genotypes as they are on the far left-hand side of Figure 4.4. The **B7_30_6_5** (217) was the more unstable line as it is away from AEC abscissa.

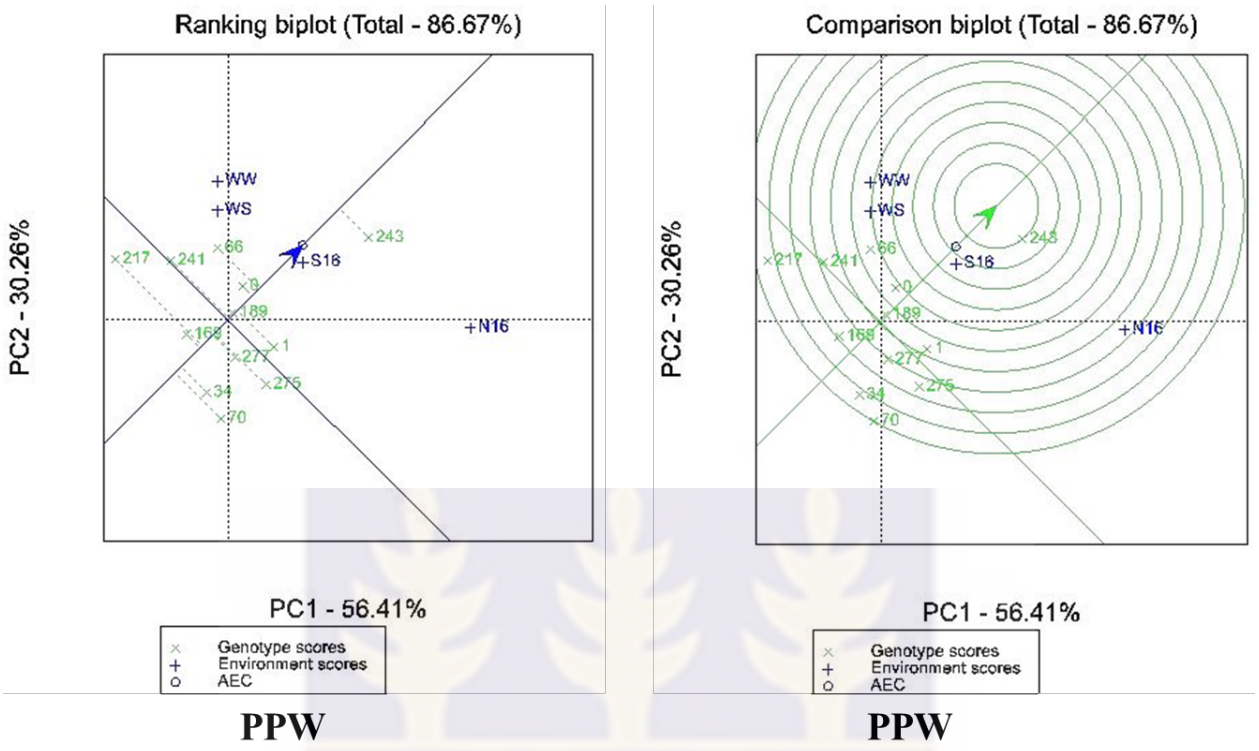


Figure 4.5: Ranking and stability of selected genotypes based on mean pod yield

AEC: average environment coordinate; the single arrowed horizontal line represents the AEC abscissa and the double arrowed vertical line represents the AEC ordinate. The direction of AEC arrowhead indicates increasing yield. The vertical projection on the AEC indicates stability. Greater the projection, higher is the instability.

The "which won where" pattern (Figure 4.6) displayed five sectors based on genotypes performance for pod yield. Sinthiou 2016 (S16) and Nioro 2016 (N16) were clustered together in one sector and well-watered (WW) and water stressed (WS) clustered together in a separate sector. The best performer for a given site is located on the vertex of each corresponding sector at which the site (s) is represented. Hence, **B7_32_8_1** (243) was the best performer at S16 and N16.

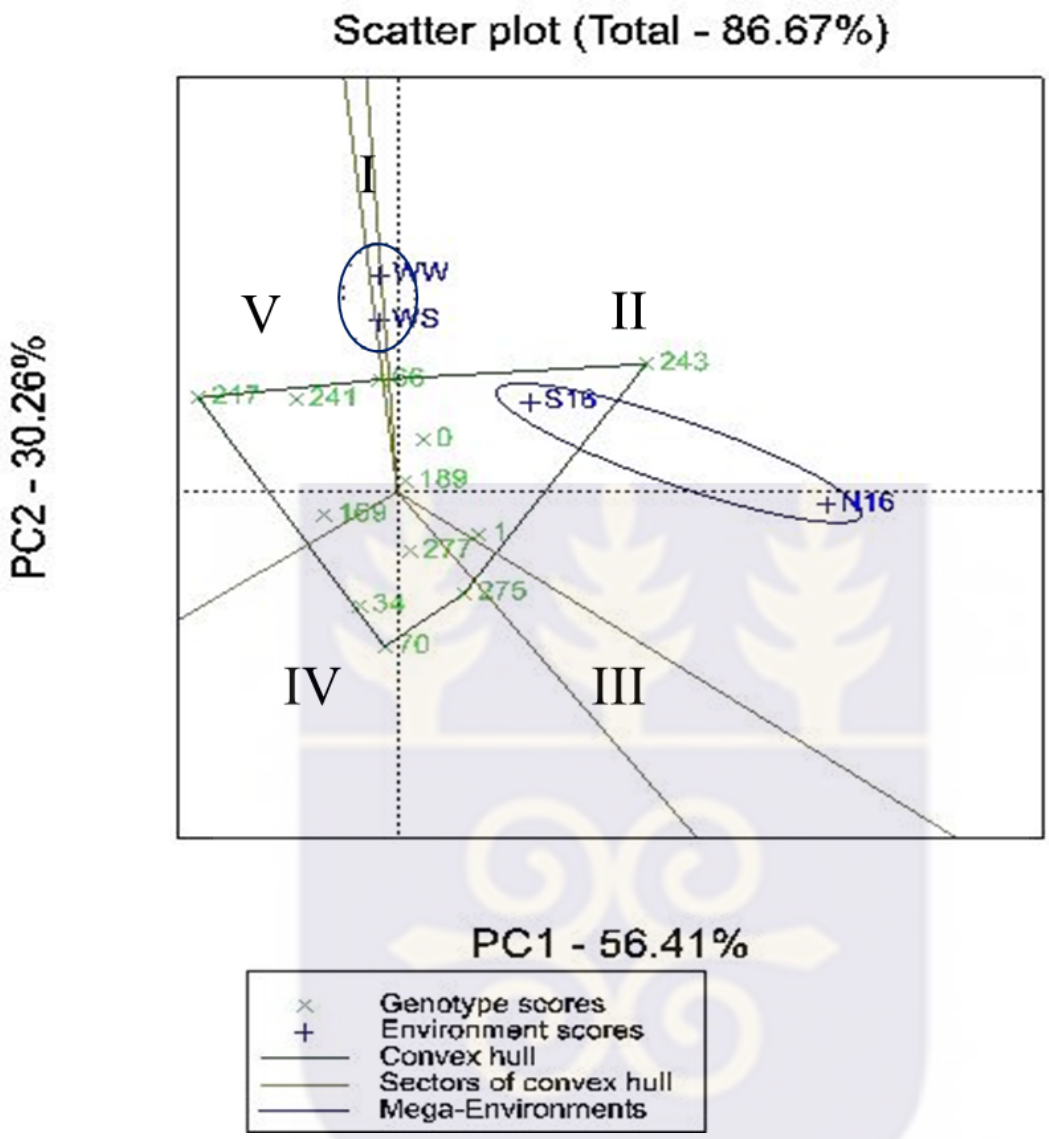


Figure 4.6: GGE biplot for the "which won where" pattern of genotypes and environments

4.4 Discussion

Although the stress imposed in 2015 was not sufficient to discriminate the genotypes for drought stress tolerance in terms of pod yield probably because of the late application of the stress and the weather conditions, this study showed significant genetic differences for various traits among the 200 genotypes of the interspecific $BC_2F_{4:6}$ population. The higher variations were observed for TPW, PPW and HW traits in the different environments which displayed low broad sense heritability in Nioro16 and Sinthiou16 and moderate heritability under water stress and well-watered environments. However, high heritability for pod and biomass yield was reported in groundnut (Songsri *et al*., 2008). This result reflects the complex and polygenic nature of these traits and the large part of the environmental component on the expression of these characters. This is part of the reasons why breeding for such traits is difficult and time consuming because small genetic gain can be achieved (Tester & Langridge, 2010; Sinclair, 2011). An alternate way is to breed for surrogate traits.

HPW and HSW have been reported to be positively correlated to pod yield in groundnut (Gomes & Lopes, 2005; Luo *et al*., 2017). High and moderate positive correlations between HPW and PPW (0.74) and between HSW and PPW (0.34) were observed, indicating that such traits may be used as alternate traits to improve pod yield. Pod and seed' sizes were found to be highly correlated with HPW and HSW (Gomes & Lopes, 2005; Luo *et al*., 2017); the values ranged in the present study from 0.51 to 0.76. Additionally, pod and seed size displayed high heritability and were less influenced by the environments. These results suggest that important genetic gains can be achieved in a rapid manner for pod and seed size and therefore indirectly on pod and seed weight. Earlier studies have demonstrated that improvement in seed size, seed weight, and number of pods per plant largely contributes to enhanced pod yield (Rathnakumar *et al*., 2012).

When several traits are being simultaneously improved, a selection index has been suggested as a good approach (Lin, 1978; Bänziger & Lafitte, 1997; Yan & Frégeau-Reid, 2008). In the present study, a two step selection was carried out to select genotypes with desired traits based on the economic weights given to each trait using selection index and subsequently, the top performer and stable genotypes were identified. It is worth noting that the weights attributed to each of the traits were somewhat subjective and depended on the breeding objectives. Siddique *et al*. (2006) reported a path analysis approach for improving groundnut yield. For both strategies the main objective was to perform indirect selection with secondary traits to improve more complex traits. Safari *et al*. (2013) reported that selection index for increasing oil yield, grain pod ratio and 100 grain weight of groundnut genotypes would be between 5 and 62% more efficient than direct selection, depending on the trait and selection method.

GGE biplot analysis applied in this study helped to identify the best performing and most stable genotypes **B7_32_8_1** and **B7_23_10_3**. It was used to select five high oil content and stable genotypes (ICGV 05155, ICGV 06049, ICGV 06041, ICGV 06420 and ICGV 03043) promoted to multilocation trials for their release for cultivation and use as parents in breeding programmes (Janila *et al*., 2016). Both lines **B7_32_8_1** and **B7_23_10_3** were erect plants. However, **B7_32_8_1** exhibited deep pod constriction value (6.69) while **B7_23_10_3** exhibited pronounced pod beak (6.60). These are some unwanted traits associated with the use of wild species in groundnut. Nevertheless, the identification of molecular markers linked to such traits may help to counteract such effects. The rainy season environments (S16 and N16) clustered together and were different from off season environments (Well-watered and Water-limited). These findings were reported earlier by Janila *et al*. (2016) and may be attributed to the regular moisture available through irrigations in post rainy seasons.

4.5 Conclusion

An advanced backcross QTL population was developed. The important genetic variation and the transgressive segregation observed attest of the potential of the wild donor to enlarge the genetic base of the cultigen. The variation and low heritability observed for pod and biomass yield across environments confirm the complex nature for such traits and the need to use secondary traits to improve them. Hundred pod weight, hundred seed weight, pod and seed size were less influenced by the environment and exhibited high heritability and were correlated to pod yield. The use of the selection index (IAS) allowed the identification the best fifty genotypes in each environment among which eleven were constantly present in all environments. Among the 11 genotypes selected, **B7_32_8_1** and **B7_23_10_3** were identified as the best performing genotype for pod yield.

CHAPTER FIVE

5.0 QUANTITATIVE TRAIT LOCI (QTL) MAPPING FOR POD YIELD RELATED TRAITS AND USE IN GROUNDNUT

5.1 Introduction

Cultivated groundnut is a useful legume widely grown in most tropical and subtropical areas of the world. One of the most important groundnut breeding objectives in developing countries is to increase pods and biomass yields because of the double use of this crop in agro-pastoral systems. Such traits are complex and polygenic and are therefore difficult to improve using direct selection. However the use of yield surrogate traits seems to have wide application in groundnut breeding schemes. Yield related traits such as the height of main stem (HMS), pod and seed characteristics may contribute to yield in groundnut (Holbrook & Stalker, 2003; Shirasawa *et al*., 2012). Indices like 100 pod weight (HPW), weight of 100 seed (HSW) quantified by pod and seed size and percentage of maturity are important and related to yield of groundnut. Such indices have showed high correlation with pod and seed size (Shirasawa *et al*., 2012).

Marker-assisted selection (MAS) has been described as an efficient way to improve complex traits. The use of molecular markers appears to be a powerful tool in accelerating and increasing selection efficiency especially in the case of traits with low heritability. The construction of a genetic map and the identification of major genes or regions of the genome (QTL delimited by markers) involved in the variation of the traits of interest are prerequisites for the application of MAS (Francia *et al*., 2005). To identify genomic regions underlying important agronomic traits, quantitative trait locus (QTL) analysis has been widely conducted. This method allows the identification of tightly linked markers to the QTL that can be further deployed in marker-assisted breeding.

While MAS application has been a routine procedure for various crops (Buerstmayr *et al*., 2002; Zhang *et al*., 2003; Fan *et al*., 2006; Foiada *et al*., 2015; Jiao *et al*., 2015), in cultivated groundnut, because of the low genetic polymorphism (Moretzsohn *et al*., 2004), QTL mapping has been slow in the past. In recent years, progress has been achieved in the creation of new sources of genetic variation using wild groundnut species and in the generation of new genomic resources. Many QTL for various characters have been mapped either in cultivated groundnut and/or in interspecific genetic material. Successes in MAS' application have been reported on oil quality, rust, nematode and late leaf spot improvement (Khedikar *et al*., 2010; Chu *et al*., 2011; Shoba *et al*., 2012; Varshney *et al*., 2014).

To date there is no documented report on the deployment of MAS for yield related traits in groundnut. Nevertheless, an important number of QTL for yield components have been detected and studies are still in progress (Selvaraj *et al*., 2009; Fonceka *et al*., 2012; Shirasawa *et al*., 2012; Jiang *et al*., 2014; Pandey *et al*., 2014; Faye *et al*., 2015; Huang *et al*., 2015; Chen *et al*., 2016; Chen *et al*., 2017). Among these QTL studies, only two reported QTL analysis were conducted on interspecific genetic material using either the advanced backcross QTL (AB-QTL) QTL approach (AB-QTL) (Fonceka *et al*., 2012) or QTL mapped using chromosome segment substitution lines (CSSLs) (Fonceka *et al*., 2012). About half of the QTL positive effects were associated with alleles of the wild parent. Seventeen QTL for pod and seed size were mapped into 7 linkage groups (LGs) and explained 8 to 23.7% of the observed phenotypic variation in the AB-QTL genetic material. Some of these QTL related to biomass, pod and seed characteristics were also mapped in the CSSLs thus allowing to validate some of them and to identify interesting chromosome segment lines bearing major and constant QTL associated with these traits. Such interesting material (CSSLs) have been used in tomato (Lecomte *et al*., 2004;

Vidavski *et al*., 2008; Hanson *et al*., 2016) and in rice (Ando *et al*., 2008; Wang *et al*., 2012) to pyramid useful QTL. Pyramiding QTL has been conducted in groundnut for nematode resistance and high oleic acid traits (Chu *et al*., 2011) but to date there are no reports on its deployment for yield related traits.

The objectives of the present work were to:

- identify QTL associated with yield related traits in groundnut using an advanced backcross (AB) population,
- estimate the introgression rate of the selected genotypes; and
- pyramid identified and validated fragments from wild relatives bearing QTL for increased pod and seed sizes.

5.2 Materials and Methods

5.2.1 QTL mapping

5.2.1.1 Population development and phenotypic characterization

The plant material used for the QTL study consisted of the advanced backcross QTL (AB-QTL) population of 200 $BC_2F_{4:6}$ progenies described in the previous section. Field evaluation was described in the previous section (chapter four).

5.2.1.2 Map construction and molecular analysis

The genetic linkage map was constructed using the BC_1 population of 150 progenies derived from the cross between the cultivated variety Fleur11 and the synthetic allotetraploid ISATGR52B ((*A. valida* (BB) x *A. duranensis* (AA)) ^{4x}) hereafter called AvAd. The synthetic allotetraploid AvAd combines the AA genome of *A. duranensis* (2n =2x =20), a close wild relative and one of the most probable ancestors of *A. hypogaea*, and the BB genome of *A. valida* (2n =2x =20), a wild relative taxa that was reported to pair with the B genome of the cultivated species during meiosis. AvAd was used as male donor parent and Fleur11 was as female and recurrent parent. Genomic DNA of both parents and BC_1 progenies were extracted from young leaves according to the MATAB protocol as described by Fonceka *et al*. (2009). Polymorphism between parents was assessed using 602 primer pairs of SSR, mainly selected from a previous study (Nguepjop *et al*., 2016). Out of the 602 SSRs 330 polymorphic markers were used to genotype the parents and the 150 progenies according to PCR amplification as described by Nguepjop *et al*. (2016).

PCR products were separated by electrophoresis run at a constant 95 W for 1 - 2h in a DNA-Sequencer (LI-COR 4300 DNA Analyzer, Lincoln, USA). Segregation for parental alleles was examined visually in the gels and data were manually scored using the type codes employed for

BC_1 population in Jelly 2.017b application (Rami, unpublished). For the BC_1 population, each polymorphic allele was scored as a locus and then tested for goodness-of-fit to the expected segregation ratio of 1:1 using the chi-square test ($P<0.05$).

On the basis of the genetic map produced in BC_1, 128 polymorphic SSR markers were selected in order to cover the genetic map with a distance of about 10 cM between adjacent markers. The genotyping of the BC_2F_4 generation was done using a LI-COR 4200 dual-dye DNA analyzer (LI-COR Bio-sciences, Lincoln, Nebraska USA). Segregation for parental alleles was examined visually in the gels and data were manually scored using the type codes employed for BC_2F_4 population in Jelly 2.017b application (Rami, unpublished) where A= AA homozygous for the cultivated parent, B= BB homozygous for the alleles of the wild parent and H= AB heterozygous.

5.2.1.3 Genetic map and QTL statistical analysis

The Fleur11xAvAd map was computed using Mapdisto software (Lorieux, 2012). Markers were grouped into LGs applying the following MapDisto parameters: LOD of 3 - 7, maximum recombination frequency of 0.3 and Kosambi mapping function. Marker order within LG was estimated using the "order" and "ripple" commands. LGs were drawn using SpiderMap 1.6 (Rami, unpublished). Chi-square test was used to test possible segregation distortion for each marker by comparing for each locus the frequency of the genotypic classes obtained with that expected in a BC_2F_4 segregating population. QTL identification was performed with R/qtl package (version 1.41 - 6) (Broman & Sen, 2009). The trait mean values from each individual environment were used. The association between trait and marker data was calculated by single-marker regression (SMR) and the locations of the detected QTL were estimated using simple interval mapping (SIM) (Haley & Knott, 1992). The confidence interval estimates of the QTL

location were obtained using the 1.5-LOD support interval method (Broman & Sen, 2009). The critical LOD threshold value at a type I error rate of 5% was determined to indicate a significant QTL effect for each trait using 1000 permutations. The percentage of the phenotypic variation (PV%) explained by a QTL was estimated using an additive multi-QTL model which involved all detected QTL for a given trait using the function "fitqtl" implemented in R/qtl (Broman & Sen, 2009). For a QTL to be declared, two conditions were set: 1) the threshold value should be significant that is, higher than the threshold at 5% and 2) the number of individuals with BB genotypes at that locus should be ≥ to 5. QTL were declared major if the phenotypic variance explained was higher than 10% and minor when it accounted less than 10% (Collard *et al*., 2005). QTL for different traits were considered to be co-located when their positions with highest LOD scores (peak) were located in the same marker intervals. The contribution of wild alleles was determined by computing the additive effect of the QTL following the formula:

$$\boldsymbol{a = (\mu BB - \mu AA)/2}$$

Where μBB and μAA represent the phenotypic values of the BB and AA individuals respectively. The QTL were illustrated graphically on the linkage groups using Spidermap software version 1.5.7b (Rami, unpublished). The percentage of introgression of the wild genome into the genetic background of the selected genotypes was estimated using GGT graphical genotypes software version 2.0. The genotype of the selected line **B7_32_8_1** was visualized graphically using the GGT software.

5.2.2 Pyramiding of QTL using CSSLs

5.2.2.1 Material

Six chromosome segment substitution lines (CSSLs) developed from a previous study (Fonceka *et al*., 2012) were used. Each of the CSSL carries a particular chromosome fragment derived from the wild donor that governs the variation of a particular character. Differences between the CSSLs reside in the length of the wild target chromosomic segment that has been introgressed and the location of the insert fragment. Details of the selected lines involved in the crosses and the molecular markers (SSRs) used to follow the introgression are given in Table 5.1.

Table 5.1: Details of the CCSLs involved in the development of the pyramiding populations

Lines	Traits	Segment size (cM)	Linkage group	% donor genome	Crosses
12CS_037	seed length and biomass	25.5	B5	4.0%	12CS_031*12CS_037
12CS_031	Hundred seed weight and biomass	89.0	A9	6.5%	12CS_031*12CS_039
12CS_069	hundred seed weight	9.5	B6	1.2%	12CS_069*12CS_037
12CS_039	pod and seed length	15.9	A8	2.3%	12CS_037*12CS_039
12CS_028	yield stability and seed length	42.3	A9	2.7%	12CS_069*12CS_028
12CS_120	yield stability	31.3	A1	1.7%	12CS_028*12CS_120

5.2.2.2 DNA extraction from seeds

A modified MATAB protocol used for DNA extraction from leaves was applied to extract DNA from seeds. The different steps involved in the extraction are described in (Figure 5.1):

The end of the seed (1/3) about 100 mg from the opposite side of the embryo was cut and put in 2 ml Eppendorf tube. The rest of the seed was kept in a 15ml conical tube and stored in the fridge at 4 °C. The tubes containing the 100 mg of the seed and the rest of the seed was correctly identified. The same process was applied to the total number of seeds to be extracted.

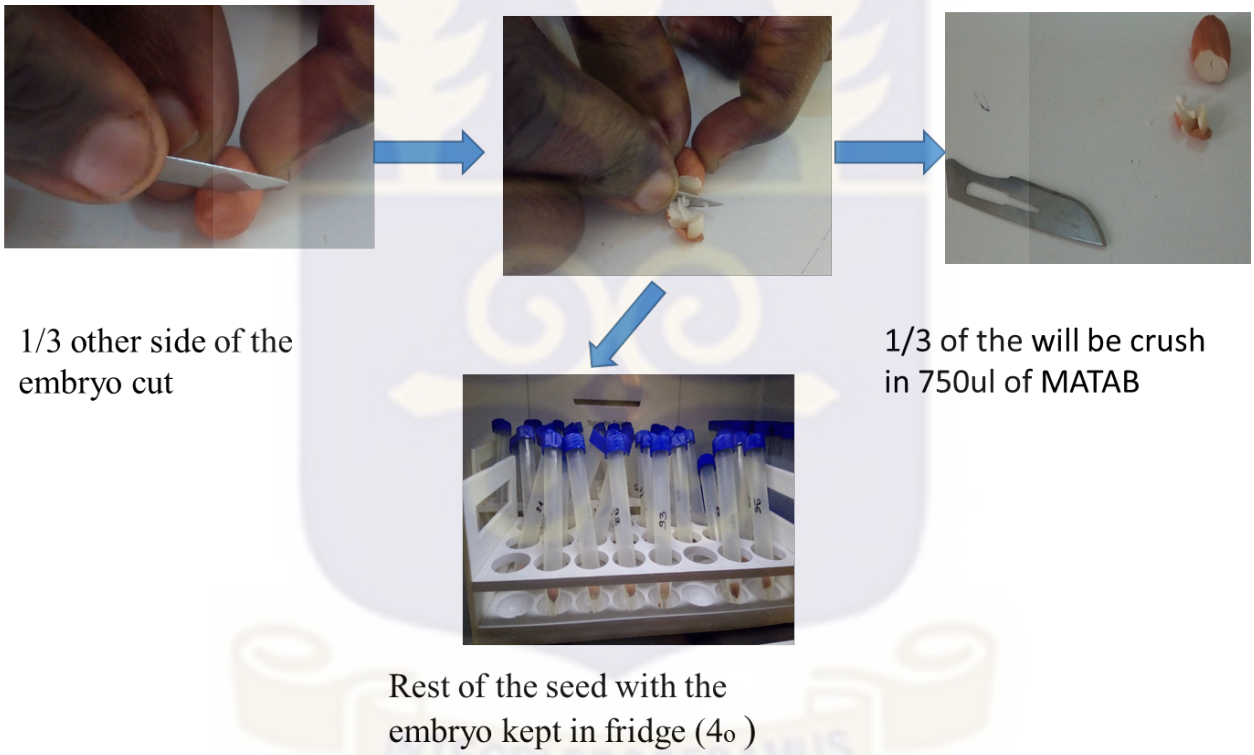


Figure 5.1: Description of the way to cut the seed for DNA extraction

Briefly, the 100mg of seed were ground in a mortar and pestle and dissolved in 750µL of MATAB buffer at 74°C. The samples were incubated 20 minutes at 74°C and cooled for 5 minutes at room temperature. A volume of 750µL of CIA (24:1) was added in each sample and all samples were shaken gently until homogenization before centrifugation at 12000 rpm for 20

minutes. 300 µl of supernatant was harvested and 300 µl of phenol-chloroform added. Homogenize and centrifuge. After centrifugation, 200 µl of supernatant was transferred to a tube of 1.5 µl. Then 200ul of cold isopropanol was added. This was homogenized gently and then centrifuge for 20 minutes. Pellets were washed with 500 µL of 70% ethanol; air dried and dissolved in 100 µL of TE 1X.

5.2.2.3 F_1 control and selection of the F_2 lines

For the quality control of the F_1 plants of each of the crosses, one SSR marker mapped in a wild introgression segment from one parent was used. Heterozygous individuals were selected and the corresponding stored seeds were sown to obtain the F_2 seeds. Among the F_2 seeds, desired events which mean F_2 individuals homozygous at both loci for the two corresponding fragments from both parents were selected. For that two SSRs each located on the wild fragment associated with the QTL of each of the parents involved in the cross were used. This is like a foreground selection. A background selection was not needed because the parents differed almost exclusively on the introgressed wild chromosome segment meaning that except the introgressed fragment, their genetic background remains similar. PCR amplifications were performed as described in Nguepjop *et al*. (2016).

5.3 Results

5.3.1 Marker segregation and polymorphism

5.3.1.1 Linkage map

A set of 250 polymorphic primers providing a strong amplification was selected to construct the Fleur11xAvAd map. A total of 341 loci were obtained out of which 79 (23.2%) deviated from the expected Mendelian segregation ratio of 1:1 ($P<0.05$). Except for one locus (PM032), all distorted loci were skewed toward the cultivated allele suggesting an occurrence of gametic or unintentional selection toward the recurrent parent. In total, 330 loci were mapped onto 20 LGs that spanned a cumulative length of 1792 cM with an average marker density of 6.4 cM. LGs were classified into 10 pairs according to homeologous loci (Figure 5.2). In A-genome, 172 loci were distributed in 10 LGs with a mean number of 17.2 (6 - 24) markers per LG. Each LG spanned an average length of 96.4 cM (45 - 111.2 cM) with inter-marker spacing of 6.1 cM. In B-genome, 158 loci were mapped in 10 LGs with a mean number of 16 markers per LG (9 - 23). Each LG spanned 88.6 cM on average (59.4 - 137 cM) with inter-marker spacing of 6.4 cM. The number of bridge markers within each pair of homoeologous LG ranged from 2 (LG1) to 11 (LG5) with an average of 8 (Figure 5.2). A good collinearity was found among homeologous pairs with the exception of the pairs 1 and 6. Likewise, a quadrivalent relationship was found between the pairs 7 and 8 thought to be bridges loci between the LGs B7 and A8 (Figure 5.2). A total of 29 (11.9%) primer pairs detected duplicated loci within the genome and the proportion of duplicated loci was slightly higher on the B-genome than the A (54% versus 46%). Most duplicated loci were clustered onto LGs and some duplicated blocks were found in the LGs B10, B2, B7, A6, B6, A1 and A10.

Figure 5.2: Genetic map derived from Fleur11 x AvAd population

LGs were grouped into homoeologous pairs according to homeologous loci among the A and B genomes connected by blue dashed lines. The LGs deriving from the A genome, named from A1 to A10 and the B genome named from B1 to B10. The map distances in Kosambi map units (cM) of each LG are shown on the left, and the loci marker names are on the right. Duplicated loci are identified by the number 1, 2 or 3 after the suffix A or B. Distorted Loci are identified by asterisk after the locus name. The colour and number of asterisk indicate the direction and the intensity of the segregation distortion respectively. Blue: markers skewed toward the alleles of the cultivated parent. Red: markers skewed toward the alleles of the wild parent.

Map location of distorted markers was also analysed. Except for 2 loci, distorted loci (78) were clustered across LGs where 21 (26.9%) were mapped onto A genome (LGs A4, A7 and A9) while 57 (73.1%) were mapped onto B genome (LGs B3, B4, B6, B7 and B10).

5.3.1.2 QTL detection

The framework map of 250 SSR markers was used for QTL mapping. Out of the 128 markers selected for the genotyping, 26 SSRs were removed due to bad genotyping quality. Hundred and two markers that amplified 119 loci were used for the QTL analysis. LOD significance thresholds estimated by 1000 permutation tests for each trait ranged from a minimum of 2.49 to a maximum of 9.93 (Table 5.2). At least one QTL was detected for each of the 10 traits analysed except for pod beak. A total of 38 QTL were mapped on 17 LGs and mostly on LGs belonging to the B genome with a percentage of 63.2 (Figure 5.3). A summary of QTL is provided in Table 5.2.

Days to flowering

- Four QTL for Dflo were mapped on 4 LGs (A4, A8, B1 and B10). For the QTL on LGs A4 and B10, the flowering precocity was associated with the alleles of the wild parent. Together they explained 17.09% of the phenotypic variance. The detected QTL on LG B10 represents alone 12% of the observed phenotypic variance and consequently can be considered as a major QTL.

Table 5.2: Summary of the detected QTL

Trait	LG	Conf1	Peak	Conf2	R^2	Add	Lod	Increase parent
SL_ww	A1	36.0	55.0	67.0	1.9	0.1	4.7	ISATR52B
SW_ww	A1	36.0	54.0	62.0	2.2	0.1	4.6	ISATR52B
PH_ww	A10	79.5	86.6	93.0	0.8	-1.4	4.1	Fleur11
PC_ww	A2	44.6	85.8	85.8	3.0	1.1	7.9	ISATR52B
SL_WS	A2	14.0	42.8	98.0	1.4	0.1	4.6	ISATR52B
PH_WS	A3	-3.5	2.5	12.5	0.7	-1.7	4.3	Fleur11
PH_ww	A3	-0.5	4.6	11.5	3.8	-1.5	5.0	Fleur11
Dflo_N16	A4	92.0	99.0	108.0	5.1	-1.2	2.5	Fleur11
HSW_S16	A4	98.0	106.6	112.0	1.0	1.8	4.8	ISATR52B
HPW_S16	A5	18.6	31.1	46.6	1.1	-16.1	3.4	Fleur11
HPW_WS	A5	13.6	24.6	44.6	0.6	-2.7	4.7	Fleur11
PW_S16	A6	36.0	51.9	83.0	2.2	0.3	4.2	ISATR52B
SW_N16	A7	0.0	25.0	57.0	1.3	-0.2	4.1	Fleur11
Dflo_WS	A8	0.0	7.0	20.0	6.4	0.4	3.4	ISATR52B
Dflo_N16	B1	0.0	40.0	53.0	5.4	0.4	2.6	ISATR52B
PC_ww	B1	75.0	77.0	77.0	0.3	1.0	4.3	ISATR52B
PH_ww	B1	38.0	40.0	57.0	2.2	0.4	3.7	ISATR52B
Dflo_ww	B10	33.0	36.2	40.0	12.0	-0.2	5.6	Fleur11
PC_ww	B2	23.0	68.0	70.0	1.3	1.3	3.5	ISATR52B
HPW_WS	B3	94.0	130.5	135.0	1.6	-5.5	4.2	Fleur11
HPW_ww	B3	91.0	112.0	134.0	1.7	-6.2	3.9	Fleur11
HSW_WS	B3	93.0	114.5	132.0	2.2	-3.0	5.0	Fleur11
HSW_ww	B3	112.0	119.0	130.0	1.6	-3.1	6.9	Fleur11
PC_ww	B3	26.5	63.5	137.0	0.6	1.4	4.3	ISATR52B
PW_S16	B3	109.0	119.5	132.0	1.7	-0.1	4.7	Fleur11
PW_WS	B3	111.0	121.0	129.0	0.3	-0.7	4.6	Fleur11
PW_ww	B3	105.0	120.5	129.0	1.3	-0.5	5.2	Fleur11
SL_WS	B3	114.0	121.5	129.0	0.3	-0.4	9.9	Fleur11
SL_ww	B3	96.0	108.5	117.0	1.4	0.2	4.6	ISATR52B
SW_N16	B3	110.0	121.5	131.0	0.7	-0.2	3.6	Fleur11
SW_S16	B3	110.0	129.0	134.0	1.7	-0.1	3.8	Fleur11
SW_WS	B3	114.0	115.5	119.0	1.5	-0.3	5.4	Fleur11
SW_ww	B3	96.0	111.0	121.0	1.4	-0.1	4.8	Fleur11
HPW_WS	B4	18	28.0	52.0	0.8	-2.5	3.5	Fleur11
GH_WS	B5	0	4.0	13.0	1.0	1.9	8.6	ISATR52B
GH_ww	B5	0	6.0	18.0	0.8	0.3	4.8	ISATR52B
PL_WS	B8	29.1	29.1	49.5	0.8	0.4	4.4	ISATR52B
PL_ww	B8	44.5	49.6	54.5	2.7	-0.3	4.7	Fleur11

Dflo: days to flowering, PC: pod constriction, SW: seed width, SL: seed length, PW: pod width, PL: pod length, GH: growth habit, HPW: 100 pod weight, HSW: 100 seed weight, PH: plant height, WW, WS, S16 and N16: environments

Plant architecture

- Four QTL located on LGs A3, A10 and B1 were detected for PH. Two of the QTL PH_ww and PH_ws were consistent across the two environments. Both QTL were located on the same LG A3 and almost at the same position with an R^2 of 4.5%. Increase in plant height phenotypic value was associated with the cultivated parent Fleur11 alleles

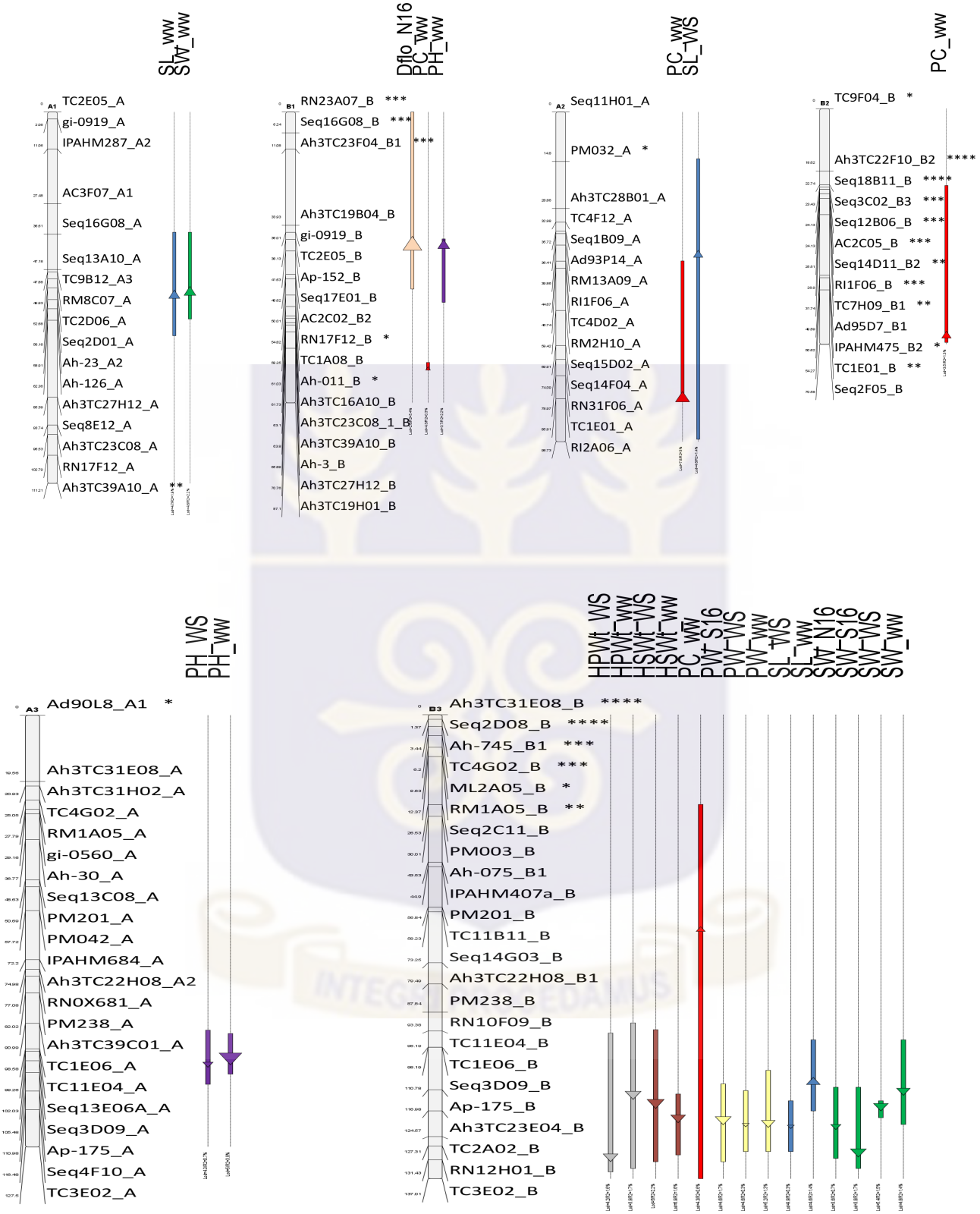
at QTL on LGs A3 and A10. On LG B1 the PH increase was associated with alleles from the synthetic amphidiploid donor parent. Two QTL were associated with plant growth habit (GH) and both were located on the same LG B5. At the mapped QTL, the spreading growth habit was linked to the wild parental alleles. A total of six QTL involved in the plant architecture was detected.

Yield related traits

- Hundred pod weight (HPW) and hundred seed weight (HSW) are the most direct yield related traits in groundnut. A total of 5 QTL for HPW have been identified in three out of the four environments with phenotypic variation (PVE) ranging from 0.6 to 1.7%. Two QTL were detected on LG A5, two on B3 and one QTL on LG B4. The increase in HPW at the identified QTL was all associated with the alleles of the cultivated parent Fleur11. QTL for HPW on LGs A5 and B3 were consistent across two environments. Three QTL for HSW have been mapped on LGs A4 and B3. The two QTL detected on LG B3 colocalized with QTL for HPW and at those QTL, Fleur11' alleles contributed positively to increase HSW. But at the QTL identified on LG A4, increase in hundred seed weight was associated with the amphidiploid alleles.

Pod and seed morphology

- A total of 10 QTL were detected for traits related to **pod morphology**: four QTL for pod constriction (PC) spread over 4 different linkage groups (A2, B1, B2 and B3). These QTL were detected in only one environment and individually explained 0.3% to 3% of the phenotypic variance (Table 5.2). At all detected QTL the amphidiploid wild alleles tended to confer deep constriction.



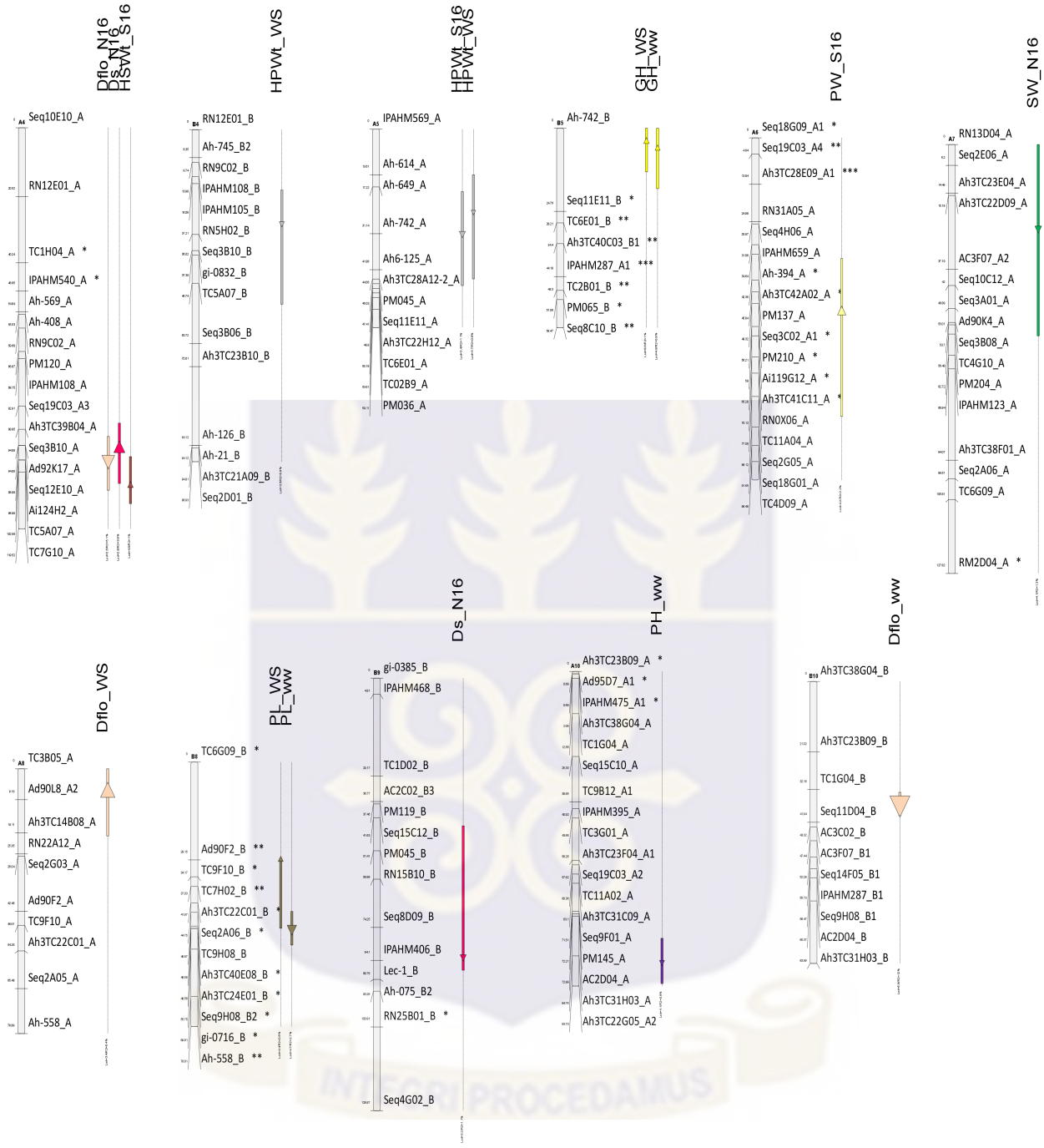


Figure 5.3: Location on the genetic map of detected QTL

Each QTL is represented by a triangle located at QTL peak and indicating the sign of the additive effect (upward: positive effect from the wild parent, downward: positive effect from the cultivated parent), and by a box representing the confidence interval. The size of triangle is proportional to the part of phenotypic variance explained by the QTL (R^2). QTL identified for the same trait have same colour. The name of the QTL is the trait followed by the location where the QTL was detected.

Two QTL for PC were located on homeologous regions on LGs A2 and B2 and at both loci the wild alleles conferred deep constriction. Six QTL were identified for pod size: two for PL localized on LG B8 and four for PW localized on LG A6 and B3. QTL for PL on LG B8 were common to two environments and their positions on the LG overlapped. But the two QTL were in repulsion meaning that phenotypic contribution of the cultivated and wild parents differed. At all QTL (3 in total) identified for PW on LG B3, increase in pod width was associated with the cultivated parent Fleur11. However at the QTL on LG A6, the wild parent brought the increase in pod width.

The QTL analysis for **seed morphology** revealed four QTL for SL and six QTL for SW. The QTL mapped on LGs A1, A2, A7 and B3 taken individually, explained 0.3% to 2.2% of the observed phenotypic variance. Increase in seed length was associated with the synthetic amphidiploid alleles at all detected QTL except for one of the two QTL (SL_ws) where the positive alleles were associated with Fleur11. The QTL detected on LGs A1 and A2 were specific to one environment. Four out of the six QTL identified for SW were mapped across the four locations on LG B3 at proximal positions with the favourable alleles coming from Fleur11. The only QTL identified for SW with the positive effect brought by the wild parent was SW_N16 specific to an environment localized on LG A1. A co-localization of QTL for SL and SW for the same environment was found on LG A1 and at both QTL, the alleles of the synthetic donor parent conferred superior seed size. Most of the detected QTL for pod and seed size (PW, SW and SL) were distributed on LG B3 and co-localized with QTL for yield related traits (HPW and HSW) in a region between 93.4 and 130.1cM.

5.3.1.3 Wild alleles' introgression rate and examination of the genotype of the selected line

From the genotyping data, the quantity of wild donor alleles present in the genome of the top 11 genotypes selected from chapter four was estimated (Table 5.3).

Table 5.3: Percentage of introgression of the wild donor into the selected genotypes

Genotypes	AA	AB	BB	NA
B12_14_2_2	86.9	11	1.8	0.4
B12_14_2_5	96.6	2.4	1	0
B7_20_3_1	89.4	3.4	7.3	0
B7_21_2_8	97.1	0	2.9	0
B7_23_10_3	97.6	0	2.4	0
B7_23_10_8	89	5.9	4.6	0.5
B7_27_5_7	89.3	4	7.2	0.5
B7_28_14_1	95.6	2.8	1.6	0
B7_30_6_5	83.6	12.4	1.6	2.4
B7_32_7_5	88.8	1.3	9.5	0.3
B7_32_8_1	82.1	1.7	14.5	1.7

AA: cultivated allele, AB: heterozygous, BB: wild allele, NA: missing data

The range of the introgressed genome from the synthetic amphidiploid varied from 1% (**B12_14_2_5)** to 14.5% (**B7_32_8_1**). The best genotype identified **B7_32_8_1** had the highest introgression rate of 14.5% of BB which would correspond roughly to the rate of introgression expected in a BC_2 generation. The graphical visualization of the genome of the line B7_32_8_1 (Figure 5.4) shows introgressions of the wild chromosomic segments on LGs A1, B1, A2, A3, B3, A6, A9 and B9.

Figure 5.4: Graphical representation of the genotype of the introgressed LGs of B7_32_1_8. QTL with positive effect associated with the wild alleles have been located on LGs A1 (1), B1 (11), A2 (2), B3 (13) and A6 (6) close to the positions of introgressed regions of the selected genotype. On LGs A1 (1) and A6, QTL associated with pod and seed characteristics (SL, SW and PW) were mapped. QTL associated with an increase in seed length and seed width co-localized on LG A1 between 35 and 56 cM. On LG A6, QTL for pod width was located between 31.86 and 70.12 cM. On LG B1, QTL associated with the shortening of the flowering time was located between 11.08 and 41.63 in a position comprised within the introgressed region of the line B7_32_8_1 (Figure 5.4). QTL associated with deep constriction have also been mapped on LGs A2 and B3 and at these QTL, the effect was associated with the wild alleles. These regions corresponded also to the introgressed regions on LGs A2 and B3 of the line B7_32_8_1.

5.3.2 Selection of the F_2 individuals

For each of the cross, five F_2 individuals that accumulated both target fragments each deriving from one parent involved in the cross were selected based on genotyping data. Three of the five selected F_2 seeds from the cross 12CS_37 x 12CS_39 named 037039_30, 037039_75 and 037039_78 are shown (Figure 5.5). For each F_2, the remaining part of the seed kept in the fridge was sown to produce the F_3 generation. The number of F_3 seeds harvested from each F_2 plant varied from 12 to 30. A picture of the F_3 pods from the cross between 12CS_37 and 12CS_39 is given in Figure 5.5. The F_3 families will be evaluated in different locations in 2018. The effect of the pyramiding process will be evaluated and their additive effect estimated.

Cross **12CS_37*12CS_39;** desired event: homozygous at **TC6E01** and homozygous at **TC1E05**

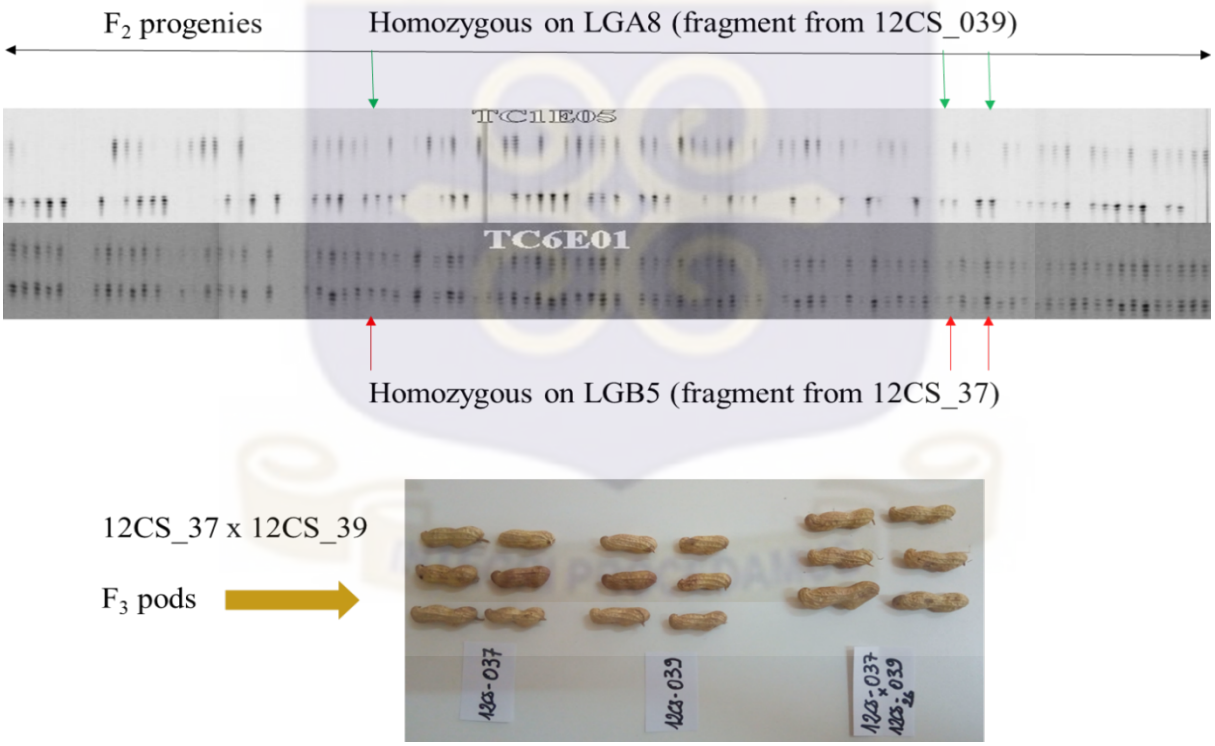


Figure 5.5: Selection of the desired F_2 individuals based on marker genotype.

The F_2 progenies are distributed from lane 1 to 96. Each primer pair amplified two bands. One band segregated for each primer. The green and red arrows indicate F_2 individuals that are fixed at the locus TC1E05 on LGA8 bearing the fragment from 12CS_39 and are fixed at TC6E01 on LGB5 bearing the fragment from 12CS_37 respectively.

5.4 **Discussion**

In the present study, a BC_1 mapping population was used to construct a linkage map containing 330 loci that spanned a total length of 1792 cM with an average marker density of 6.4 cM. This is the first SSR-based map reported on AB-genome tetraploid *Arachis* involving *Arachis valida* as a BB genome donor. When comparing the produced map to other published AB tetraploid involving wild species maps, the length and density of the produced map was relatively similar to that of Fonceka *et al*. (2009) which covered 1843.7 cM with a maker density of 6.1 cM between adjacent markers. The map from this present study was, however, smaller than the consensus map which integrated marker information of 16 linkage maps (Shirasawa *et al*., 2013), smaller than the map produced by Burow *et al*. (2001) using RFLP. The rate of distorted marker was similar to the one published by Burow *et al*. (2001) (24.2% versus 25%). However, this rate is higher compared to the one (10.7%) reported by Fonceka *et al*. (2009). Segregation distortion is a common feature exhibited by most linkage maps in most plants. Genetically, segregation distortion has been attributed to differences in chromosomes rearrangements, DNA content, abortion of male or female gametes, or the selective fertilization of particular gametic genotypes (Liang *et al*., 2006). In this particular case, the distorted alleles were skewed toward the cultivated parent. High level of segregation distortion were reported in rice mostly in interspecific crosses (Xu *et al*., 1997). Therefore, the relatively high proportion of distorted markers may likely imply a huge variability in the parental genotypes as it is often the case in interspecific crosses. The nearly good collinearity found among homeologous pairs suggested relatively conserved genomic sequences between A and B genomes.

Groundnut wild species have long been used as useful sources of disease resistance genes. Introgression of disease resistance into cultivated species has been successful and varieties have

been released and cultivated 'Coan' (Simpson & Starr, 2001), 'NemaTAM' (Simpson *et al*., 2003), 'Tifguard' (Holbrook *et al*., 2008) and Webb (Simpson *et al*., 2013). However, groundnut wild species have had limited application to decipher more complex traits such as yield surrogate traits due to their low phenotypic performance. In this study, an AB-QTL approach was utilized to evaluate the genetic potential of wild species to improve agronomic traits in cultivated groundnut. Thirty eight (38) QTL for 11 traits were mapped. For more than half of the detected QTL, the positive effects were associated with the wild alleles. There were 10 QTL involving HSW, pod and seed size (PW, PL, SL and WS) for which the wild parent contributed positively to increase the phenotypic value of the trait. These traits clustered on LGs A1, B8 and largely on LG B3. Moreover these traits showed high positive correlations suggesting pleiotropic effects or a number of linked genes responsible for the observed variations.

However, for most of the QTL mapped on LG B3, the positive effects were associated with the cultivated parent alleles. In relation to the findings of Huang *et al*. (2015) study, one could believe that LG B3 may harbour genes related to pod and seed traits which can be deployed in marker-assisted selection even though most QTL mapped on LG B3 in the present study, have positive effects associated with the cultivated parent. Furthermore, Zhang *et al*. (2017) found a region of 6.89 Mb on chromosome B3 harbouring 107 selective genes one of which codes for Gibberellin-related protein and three genes encoding flavonoid biogenesis and regulation and five nucleotide-binding site leucine-rich repeat NBLRR- encoding genes. As a conclusion, this region may be involved in different traits like plant resistance, plant type related traits and seed related traits. An unanswered question is: is it the same region that is involved in the variation of most of the detected QTL in the present study? Further research such as fine mapping may be

conducted to investigate the genes responsible for the QTL on LG B3 even if in the present study, increase of the traits is associated with the cultivated parent.

For all identified QTL for PC and GH, the wild alleles tended to increase the value of the trait: deep pod' constriction and spread growth habit. This result demonstrated in a way the undesirable effects associated with the use of wild species in breeding programmes. However, the detection of QTL and markers associated with these traits could be used to counter-select such regions during introgression processes. While QTL that affected pod and seed size appeared to cluster together, those affecting pod morphology seemed to be dispersed across the genome. Fonceka *et al*. (2012) highlighted a similar observation and attributed it to some extent to a process related to domestication.

A salient result which came out from the present study was the differences in QTL distribution between A and B genomes and the non-consistency of QTL between homeologous LGs while good collinearity between sub-genomes was reported in the present study and from others (Feng *et al*., 2012; Shirasawa *et al*., 2013; Nguepjop *et al*., 2016). Except for PC for which 2 QTL were mapped on LGs A2 and B2, QTL for all remaining traits were identified on non homeologous LGs. Fonceka *et al*. (2012) reported 96% of QTL cases mapped in non-homeologous regions. This fact could be explained partly by the segregation of alleles in one genome versus the other. No QTL has been identified on LGs A9, B6 and B7 probably due to the lack of wild chromosome introgressions on these particular LGs as a result of strong disequilibrium within the population and/or lack of polymorphism genes located in the cited LGs associated with the variations of studied characters. The examination of the genotype of the B7_32_8_1 line showed a correspondence between the localization of some of the QTL associated with seed and pod characteristics and the introgression regions of that particular line. This result indicates probably

an accumulation of useful QTL associated with the wild alleles which makes this particular line to be of interest. In addition, QTL linked to deep pod constriction have also been mapped on genomic regions corresponding to wild introgression regions in B7_32_8_1. This fact could explain the deep pod constriction that is observed on B7_32_8_1.

By comparing QTL detected in the present work to other QTL identified for the same trait in other studies, new QTL were mapped. QTL for PH were located on LGs A3, A4, A7, B4, B7, B8, and B10 (Fonceka *et al*., 2012; Huang *et al*., 2015). In addition to the QTL identified on A3, two new QTL have been mapped on A10 and B1 groups in this study; and increase in plant height on LG B1 was associated with the wild alleles. They explained moderate PV. This result confirms the findings reported on the polygenetic control of PH (Chen *et al*., 2017). For pod and seed related traits, previous studies reported QTL on A2, A3, A5, A7, A8, A9, A10, B2, B3, B5, B6, and B9 LGs (Fonceka *et al*., 2012; Shirasawa *et al*., 2012; Jiang *et al*., 2014; Pandey *et al*., 2014; Huang *et al*., 2015; Chen *et al*., 2016; Chen *et al*., 2016; Huang *et al*., 2016). Recently, QTL for pod and seed related traits have also been mapped on B1, B4, B7 and B8 (Chen *et al*., 2017). In this study, QTL for these traits have been mapped on A1, A4 and A6. For all the three newly mapped QTL, increase in the phenotypic value of the trait was associated with the alleles of the wild parent. The distribution of QTL for yield related traits throughout the genome confirms the complex genetic architecture of yield. Moreover, the fact that no QTL has been mapped for pod and haulm yield attest of the complex nature of those traits.

5.5 Conclusion

From the study 38 QTL were mapped among which 5 were considered as new. For four out of the five newly mapped QTL, increase of the phenotypic value was associated with the alleles of the wild parent. For about half of the detected QTL, increase of the phenotypic value of the trait was associated with the alleles from the wild relatives. Most of the identified QTL for seed related traits were co-localized on LG B3 and for most of them increase of the trait was brought by the cultivated parent. QTL for traits associated with the negative effects from the wild species were also mapped and markers linked to them identified. This result will be useful to counter-select such non desirable traits during introgression processes. The success of the DNA extraction performed from seeds has permitted selection of desired F_2 lines namely 037039_30, 037039_75 and 037039_78 bearing the two wild fragments in the pyramiding process.

CHAPTER SIX

6.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 General conclusions

The Senegalese groundnut sector is one of the most fruitful and important sectors in the country. With the liberalization of the export market and the overgrowing population, producers have more challenges: Produce more on smaller surfaces. Although Senegal is among the top five African producers, yield has declined over the years. The increase in the production is attributed more to cultivation of larger acreage. Many studies have reported the opinions, ideas and participation of farmers regarding the economic aspect but to the best of my knowledge none related to agronomic traits. In this study, a participatory rural appraisal was conducted to assess farmers' preferences and production constraints and see how the information obtained could help in related researches. The findings imply that during the selection process for developing new varieties attention should be paid to high pod yield, high biomass yield, big pod and seed' size as well. After the commercialization, drought is the most important abiotic constraint highlighted by farmers.

One of the most important limitations that researchers and breeders are facing in groundnut improvement is the low genetic variation available in the cultivated groundnut. In the present work a mapping based population was developed using wild groundnut species. The material developed displayed large phenotypic variation which attests of the potential of the wild species in widening the genetic base of the cultivated groundnut. The variation and low heritability observed for pod and biomass' yield across environments confirm the complex nature for these traits and the need to use related traits to improve more complex traits. The use of the selection index (IAS) permitted the identification of the best fifty genotypes in each environment. The

phenotypic values of the selected genotypes were higher than those of the cultivated parent Fleur11. Among the eleven lines, **B7_32_8_1** was identified as the best performing genotype.

The genotyping of the BC_1 population using 250 polymorphic SSRs allowed production of a genetic map of 330 loci on 20 LGs spanning a cumulative length of 1792 cM with an average marker density of 6.4 cM. LGs were classified into 10 pairs according to homeologous loci. In A-genome, 172 loci were distributed in 10 LGs with a mean number of 17.2 (6 - 24) markers per LG. Each LG spanned on average length of 96.4 cM (45 - 111.2 cM) with inter-marker spacing of 6.1 cM. In B-genome, 158 loci were mapped in 10 LGs with a mean number of 16 markers per LG (9 - 23). Each LG spanned on average of 88.6 cM (59.4 - 137 cM) with inter-marker spacing of 6.4 cM. The QTL analysis mapped 38 QTL for 15 agronomic traits. For yield traits (TPW, PPW and HW) no QTL were identified. Among the 38 QTL, 5 were considered as new loci. For half of the detected QTL, increase of the phenotype value of the trait was associated with the wild alleles. The shortening of the flowering time QTL was associated with the wild alleles. QTL for traits associated with the negative effects from wild species (deep pod constriction, pronounced pod beak and spread growth habit) were also mapped and markers linked to them identified. This result will be useful to counteract such undesirable effects during introgression processes. The use of the genomic DNA extracted from seed to pyramiding useful fragments was a good approach to save time, space and therefore money. It has allowed selecting efficiently the desired F_2 individuals which are homozygous at the target loci.

The obtained results demonstrate the positive contribution of the wild species to groundnut improvement and offer novel perspectives for exploiting the unused useful alleles available in groundnut wild species.

6.2 Recommendations

- Senegalese' groundnut breeding programme has to pay more attention to farmers' priorities and engage farmers through participatory varietal selection for them to identify and select varieties that meet their preferences before release.
- Breeding for varieties that are drought tolerant and that carry preferred traits would be welcomed by the farmers. Lastly farmers need to have access to land and to enough seeds of quality.
- As wild species are known to be tolerant to several biotic and abiotic stresses, it could be interesting to characterize the developed material for drought and leaf spot diseases to identify sources of resistance which may be further used in breeding programmes.
- Something that could be interesting is to look at the quality of the haulm to see whether there is an improvement in terms of quality related to the use of wild species
- The identified lines should be subjected to multi-locations and multi-years testing for release.
- The QTL analysis may be run with more abundant molecular markers (SNP) to confirm the QTL identified in the present study.
- The obtained F_3 seeds derived from the pyramiding process should be subjected to multi-location and multi-year evaluations to estimate the effect(s) of the accumulation of the two wild segments on the traits. Their pod yield performances should be evaluated as well and compared to Fleur11.
- It could be interesting to look at the number of pods per plant of the selected lines to evaluate the impact of an increase in pod size.

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APPENDICES

Table 4.1: Selected genotypes based on IAS in each site

WW				WS			
genotypes	PPW	HW	IAS	genotypes	PPW	HW	IAS
B7_25_7_7	9.5	70.5	13.10	B12_14_2_2	12.4	39.2	14.40
B7_26_6_3	39.4	51.1	12.56	B7_32_6_6	18.8	43.5	13.72
B12_14_2_5	18.4	106.6	11.48	B7_23_10_3	20.3	52.5	12.37
B7_30_9_1	13.1	89.6	11.37	B7_32_7_5	17.0	36.3	12.27
B7_27_5_7	14.3	76.1	11.36	B7_20_3_1	17.4	47.7	11.79
B7_23_10_3	26.1	64.7	11.18	B7_32_8_1	22.7	17.2	11.21
B7_32_7_5	27.3	72.9	11.15	B7_27_5_1	11.4	32.1	10.60
B7_32_6_3	20.6	45.6	10.73	B7_29_7_7	16.4	36.3	10.24
B7_21_14_3	18.9	34.3	10.34	B7_22_7_2	19.8	33.8	9.39
B7_26_4_8	26.9	37.6	9.95	B7_30_6_4	31.1	41.4	9.33
B7_32_8_1	25.2	32.4	9.36	B7_24_13_9	18.4	38.5	9.27
B7_27_13_3	11.9	76.7	9.25	B7_33_7_2	17.3	15.1	8.81
B7_23_16_2	20.2	68.3	8.80	B7_30_9_3	18.0	32.8	8.47
B7_20_3_1	16.9	65.3	8.39	B12_14_2_7	21.5	53.9	8.46
B12_11_18_6	9.6	79.1	8.21	B7_23_10_1	9.9	32.4	8.04
B7_30_3_4	25.7	55.8	8.17	B7_26_4_9	5.2	35.9	7.92
B7_27_16_6	20.6	55.4	8.15	B7_26_6_3	13.8	40.8	7.73
B7_21_17_1	39.0	80.6	7.87	B7_22_12_1	22.8	39.5	7.58
B7_24_13_9	25.5	43.5	7.81	B7_30_9_1	8.7	69.9	7.51
B7_21_2_8	13.9	42.9	7.69	B12_11_18_3	15.0	37.5	7.46
B7_21_2_1	23.6	82.9	7.53	B7_26_16_1	24.9	44.3	7.42
B7_24_5_4	21.9	11.8	7.26	B7_25_22_5	19.7	32.3	7.23
B7_33_7_1	11.8	133.6	7.21	B7_28_14_1	15.9	43.6	7.11
B7_30_6_5	24.4	4.8	6.97	B7_30_3_5	14.5	52.3	6.92
B7_28_14_1	20.4	42.6	6.96	B7_27_10_5	11.9	40.2	6.84
B7_25_4_8	27.5	23.4	6.74	B12_11_18_6	12.9	49.4	6.67
B7_23_10_8	12.8	89.7	6.67	B7_30_6_5	25.7	22.0	6.64
B7_32_8_2	34.9	42.6	6.32	B7_30_9_8	3.9	60.8	6.50
B7_33_7_2	27.0	28.0	6.19	B7_21_17_1	29.2	44.4	6.35
B7_21_14_6	15.1	34.3	6.15	B7_33_7_4	8.3	19.9	6.00
B7_32_2_1	16.3	49.9	6.01	B7_26_16_4	26.5	40.7	5.97
B7_24_17_3	30.9	71.9	5.99	B12_14_9_5	18.3	47.0	5.75
B12_11_18_5	24.8	24.7	5.90	B7_27_5_7	18.2	31.2	5.73
B7_32_7_4	34.5	60.1	5.88	B7_32_6_3	10.9	26.0	5.63
B7_20_10_2	8.1	45.1	5.71	B12_14_2_5	12.0	58.3	5.37
B12_14_2_2	11.1	35.1	5.66	B7_27_13_3	10.5	44.7	5.05

B7_25_4_6	32.4	54.0	5.26	B7_25_22_6	20.4	36.9	4.85
B7_26_16_4	35.4	65.5	5.20	B7_24_17_3	27.9	42.7	4.85
B12_17_17_6	27.9	66.8	5.15	B7_25_7_6	13.9	64.3	4.85
B7_22_12_1	24.6	54.3	5.13	B7_33_7_1	13.4	50.6	4.84
B7_27_16_3	8.4	27.1	5.10	B12_14_9_1	15.3	34.0	4.79
B7_24_5_7	2.4	56.9	4.89	B7_23_16_3	19.0	20.0	4.74
B7_29_14_4	19.7	59.1	4.86	B7_24_8_1	16.2	41.4	4.67
B7_27_16_7	27.0	70.6	4.78	B7_27_16_9	34.2	29.6	4.60
B7_27_13_10	20.2	61.3	4.74	B7_32_8_3	15.0	52.4	4.52
B12_14_2_7	21.2	114.9	4.73	B12_14_9_4	14.5	30.7	4.27
B7_24_8_8	28.8	51.6	4.71	B7_23_10_8	9.2	47.4	4.26
B7_29_7_7	24.0	91.5	4.67	B7_20_5_5	28.4	25.1	4.16
B7_20_5_9	34.3	86.0	4.66	B12_17_17_3	20.6	28.8	4.12
B7_23_16_3	21.0	45.4	4.51	B7_21_2_8	11.4	43.3	3.83

PPW: plant pod weight; HW: haulm weight

Table 4.1: Selected genotypes based on IAS in each site (continued)

N16				S16			
genotypes	PPW	HW	IAS	genotypes	PPW	HW	IAS
B7_23_10_3	22.1	36.0	15.38	B7_32_8_1	30.6	74.6	14.46
B12_14_2_2	29.1	67.3	15.13	B7_32_7_5	23.8	57.5	13.11
B7_26_4_8	17.2	12.4	14.97	B12_14_2_5	19.5	63.6	13.09
B7_20_3_1	33.6	47.8	12.17	B7_23_16_4	16.2	53.6	12.26
B7_22_12_1	31.5	65.2	11.32	B7_26_4_8	26.9	59.0	12.04
B7_25_22_6	24.1	33.8	11.14	B7_20_5_5	24.4	47.5	11.41
B7_27_16_7	29.8	32.6	10.87	B7_21_14_3	21.9	61.9	11.17
B7_23_16_4	18.9	40.5	10.30	B7_32_2_3	27.6	64.0	10.98
B7_21_2_1	28.5	50.7	10.23	B7_27_16_3	21.3	68.5	10.11
B7_25_7_7	4.9	25.1	9.98	B12_17_17_6	38.7	64.2	10.03
B12_11_18_6	23.7	31.0	9.88	B7_21_2_1	18.4	58.7	9.82
B7_20_5_5	40.7	30.9	9.77	B7_30_6_5	14.0	30.3	9.74
B7_27_3_1	20.5	21.4	9.61	B7_20_10_10	10.1	52.7	9.56
B12_14_9_4	35.3	63.3	9.23	B7_25_7_7	10.7	30.4	9.46
B7_23_10_8	25.0	35.7	9.10	B7_24_8_8	24.1	52.5	9.42
B7_28_14_1	24.9	33.1	9.05	B7_30_6_6	36.0	83.1	8.98
B7_25_22_8	20.7	21.2	8.92	B7_27_5_7	25.3	43.6	8.76
B7_32_8_1	46.0	31.9	8.43	B7_33_7_2	29.1	51.0	8.43
B7_32_6_6	17.5	31.0	8.21	B7_30_9_3	21.4	60.4	8.42
B7_20_10_10	9.3	58.9	8.21	B7_21_2_6	16.8	45.6	8.37
B12_14_2_5	26.4	55.7	8.04	B7_21_17_1	22.6	50.3	8.33
B12_11_18_3	27.9	13.5	7.97	B12_14_9_4	23.6	49.5	7.95
B7_27_10_5	21.5	26.6	7.90	B7_29_7_7	16.6	45.9	7.77

B7_26_16_4	32.2	50.0	7.87	B7_24_17_3	25.2	69.4	7.72
B7_20_10_7	18.9	33.7	7.83	B7_28_14_1	23.9	73.0	7.65
B7_32_7_5	14.9	33.0	7.83	B7_23_10_3	26.0	61.3	7.61
B7_24_5_2	27.1	39.9	7.81	B7_20_3_1	17.9	73.9	7.60
B12_14_2_3	26.7	40.6	7.61	B7_30_3_5	24.3	71.0	7.31
B7_21_2_8	21.9	19.2	7.09	B7_33_7_1	12.1	86.2	7.03
B7_25_19_1	16.9	31.9	7.01	B7_27_10_2	15.2	63.2	6.97
B7_30_9_8	22.0	49.9	6.84	B7_20_10_2	12.4	64.3	6.91
B7_25_22_3	14.3	60.1	6.68	B7_21_14_1	27.5	53.1	6.85
B7_21_14_6	6.2	36.0	6.63	B7_22_7_2	23.4	46.3	6.69
B12_11_12_4	15.1	11.4	6.61	B7_32_8_2	21.0	64.0	6.65
B12_17_17_6	24.6	36.9	6.44	B7_27_3_1	16.3	38.4	6.60
B7_21_14_3	25.8	30.4	6.41	B12_14_2_2	26.6	53.2	6.58
B7_24_8_1	21.6	40.9	6.32	B7_32_8_3	10.3	72.8	6.51
B7_32_8_2	33.6	58.8	6.27	B7_25_4_6	26.6	34.3	6.43
B7_25_4_5	19.4	25.6	6.12	B7_21_2_8	18.0	57.7	6.31
B7_27_16_9	21.8	19.0	6.07	B12_14_2_7	19.4	61.4	6.25
B7_27_13_10	15.5	31.5	5.89	B12_11_18_5	31.3	57.1	6.03
B7_30_6_5	9.0	0.0	5.61	B7_23_10_8	15.1	50.0	5.94
B7_27_5_2	15.1	25.2	4.96	B12_11_18_2	18.8	46.9	5.78
B7_30_6_4	31.2	40.1	4.83	B7_32_7_1	23.6	44.1	5.73
Fleur11	26.9	34.8	4.77	B7_32_6_6	16.1	42.2	5.66
B7_25_7_6	15.1	22.0	4.72	B7_25_13_6	25.5	70.7	5.64
B12_14_2_1	21.6	27.7	4.71	B12_11_18_4	20.5	55.2	5.55
B7_27_5_7	16.7	65.5	4.63	B7_32_7_4	36.2	40.0	5.50
B7_20_5_9	35.8	40.3	4.54	B7_23_16_2	22.9	76.9	5.45
B12_11_18_2	42.7	8.2	4.46	Fleur11	23.5	56.7	5.30