University of Ghana http://ugspace.ug.edu.gh

GENETIC AND GENOMIC RESOURCES TO IMPROVE RESILIENCE TO STRIGA [*Striga gesnerioides* (Wild.) Vatke] AND DROUGHT IN COWPEA

By

MOUHAMADOU MOUSSA DIANGAR

(10512767)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHYLOSOPHY DEGREE IN PLANT BREEDING

WEST AFRICA CENTRE FOR CROP IMPROVEMENT
COLLEGE OF BASIC AND APPLIED SCIENCES
UNIVERSITY OF GHANA
LEGON

DECEMBER 2018

University of Ghana http://ugspace.ug.edu.gh

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.

……………………………………

Mouhamadou Moussa Diangar

(Student)

…………………………………..

Prof. Kwadwo Ofori

(Supervisor)

…………………………………

Prof. F.K. Kumaga

(Supervisor)

…………………………………

Dr. Beatrice Elohor Ifie

(Supervisor)

…………………………………

Dr. Ndiaga Cisse

(Supervisor)

University of Ghana http://ugspace.ug.edu.gh

ABSTRACT

Cowpea (*Vigna unguiculatan* (L.) Walp.) is a grain legume cultivated worldwide in over 14 million ha but its productivity in Senegal is seriously affected by the infestation of *Striga gesnerioides* (Wild.) Vatke, a parasitic weed. Striga resistance is an important trait that is missing to most cultivated varieties in Senegal. Its negative effect on cowpea is exacerbated by drought which is a threat to agriculture. The present dissertation describes various options taken on the improvement of cowpea for Striga resistance and drought tolerance using microsatellite markers and drought tolerance indices and cultivar superiority coefficients. The first breeding activity describes methods and results obtained in identifying farmer concerns and interest on profile of their desired ideotype in Louga, Kebemer and Tivavouane involving 109 farmers. The second breeding activity was conducted in response to Participatory Rural Appraisal (PRA) indication on major constraints to anticipate on pre-breeding in order to identify drought tolerant lines that can be used as parental lines in developing new varieties. This study used phenotypic data recorded from well-watered and water-stressed experimental fields in Bambey (ISRA, CNRA de Bambey) and involved 112 accessions from the Senegalese cowpea breeding programme. The third breeding activity focused on cowpea resistance to *Striga gesnerioides* using Marker-assisted selection (MAS). The different options deployed involved in MAS were the creation of bi-parental lines of hybrids, RIL populations and advanced backcross populations using Marker-assisted backcrossing (MABC), the validation of resistance of the developed backcross lines in field in natural infestation and the identification of loci associated to Striga resistance in cowpea using Genome-Wide Association Analysis (GWAS) on a wide population composed of 367 unrelated accessions from diverse origins in the word. PRA revealed that drought was the major constraint to cowpea production and farmers were interested in large and brown seeded cowpea grain type in Senegal. Striga was important only in some divisions. Preliminary field and pot screening identified several lines more tolerant to

University of Ghana http://ugspace.ug.edu.gh

drought compared to best tolerant check Mouride, reliability of markers used in MABC were weak, resistant BC_4 lines performed unexpectedly in field in regards to SNP screening predictions. Significant markers were identified in 2 environments out of 4. Six candidate genes were identified in regions neighbouring identified markers and annotated. SNPs identified were not reported before in any article to best of our knowledge. The identified genetic and genomic resources could be used in population development for drought tolerance and *S. gesnerioides* resistance in cowpea.

University of Ghana http://ugspace.ug.edu.gh

DEDICATION

This thesis is dedicated to my wife Fattah and my daughter Bintou Rassoul. I love you more than anything.

University of Ghana http://ugspace.ug.edu.gh

ACKNOWLEDGMENTS

I acknowledge sincerely the Director of CERAAS, Dr. Ndiaga Cisse for the trust he has put in me and my work. Lord knows words cannot express how I feel about you. Dr. Ndiaga Cisse taught me courage, relevance, discernment, humbleness and patience, traits that I wish one day to entirely inherit in spite of all environmental noises. I pray on behalf of all his students and admirers to have a safe retirement and devoted ambassadors all around the world to solve issues relating to global food security.

I express my gratitude to the Director of ISRA, Dr. Alioune Fall, the Executive Director Dr. El Hadj Traoré, the General Secretary Dr. El Hadj Mbaye Babacar Diop as well as all ISRA workers for giving training opportunities to young scientists for the success of the research institute. I thank Dr. Nathalie Diagne Director of ISRA CNRA de Bambey, researchers and workers in Bambey for their support. There were many people who helped in the activities carried out in the studies. They included Mr. Sene, Mme. Keita, Mr. Diop, Petit, Badji, Diagne, Mass, Diouf, Diegane, Demba, and Lamp Mbacké. There were other people who worked in the background since 2012 who together with me initiated Striga resistance using markers. Thank you Mr. Moctar Wade, Mme Tacko Sall and Mr. Ndiogou Diouf for teaching and supervising my pod screening trials; Dr. Sassoum and Dr. Ardaly supported by the cowpea breeding program team for carrying out the preliminary marker screening tests for Striga resistance in CERASS lab. At CERAAS, I deeply thank Dr. Daniel Fonceka and Dr. Bassirou Sine for their valuable help, providing expertise in Marker-Assisted Selection and drought tolerance assessment. I thank Dr. Elisabeth Diop, Dr. Rachelle and Dr. Jodelle for doing the genotyping test. I also want to thank my sisters Mariama Senghor, Mme. Faye, Marie Sane, Mme. Diallo, Dr. Amy Bodian, Dr. Awa Sarr, Dr. Belko and Dr. Elisabeth Diatta for the good collaboration and love.

University of Ghana http://ugspace.ug.edu.gh

I will also take this opportunity to congratulate the Director of West Africa Centre for Crop Improvement (WACCI), Prof. Eric Danquah for winning the 2018 GCHERA World Agriculture Prize. I also thank him for his time and efforts to make us comfortable candidates and competent plant breeders and among the best in the world. I thank all teaching and non-teaching staff of WACCI for their support.

I thank my supervisors, Prof. Ofori, Prof. Kumaga, Dr. Beatrice Ifie and Dr. Ndiaga Cisse for the valuable inputs and expertise they have provided to complete this dissertation.

My special gratitude for The West Africa Agricultural Productivity Program (WAAPP) fellowship and Feed the Future Innovation Lab for Climate Resilient Cowpea project for financial support for my thesis. Special thanks also to the Professors of the University of California: Prof. Phil Roberts, Prof. Tim Close, Prof Jeff. Ehlers and Prof. Maria Muñoz.

I thank my sisters Maria, Lena, Cole and Rosalie and relatives for their prayers. My late farther Salim inspired me to become a plant breeder thank you for introducing me to life. May Allah forgive him and grant him paradise. Mom did not cry Hamdlah. I could not thank that great lady enough, Mom AichaMay thank you very much.

I thank all the cohort 8 students of WACCI for being supportive in words and in actions.

In the authentic hadith recorded by Abu Da'ood, Ahmad and others, the Prophet Muhammad (peace and blessings be upon him) said: "Do not thank Allah for those who do not thank people" so after thanking you, let me say Alhamdulillah: All praise belongs to Allah Subhanahu wa Ta'ala.

University of Ghana http://ugspace.ug.edu.gh

Table des matières

University of Ghana http://ugspace.ug.edu.gh

University of Ghana http://ugspace.ug.edu.gh

University of Ghana http://ugspace.ug.edu.gh

University of Ghana http://ugspace.ug.edu.gh

LIST OF TABLES

University of Ghana http://ugspace.ug.edu.gh

University of Ghana http://ugspace.ug.edu.gh

LIST OF FIGURES

University of Ghana http://ugspace.ug.edu.gh

LIST OF ABREVIATIONS

ABA:	Abscisic Acid
AFLP:	Amplified Fragment Length Polymorphism
cDNA:	Complementary Deoxyribonucleic Acid
cM:	Centimorgan
CRSP:	Collaborative Research Support Program
DLS:	Delayed leaf senescence
DNA:	DeoxyriboNucleic Acid
DE:	Drought escape
DS:	Drought stress
DT:	Drought tolerance
DP:	Drought period
EST:	Expressed Sequence Tag
EUW:	Efficient Use of Water
FAO:	Food and Agriculture Organization of the United Nations
FGD:	Focus Group Discussion
GBS:	Genotyping By Sequencing
GCP:	Generation Challenge Programme
GMP:	Geometric Mean Productivity
HTTP:	High-Throughput
IITA:	International Institute of Tropical Agriculture
Indel:	Insertion deletion
KASP/KASPAR:	Kompetitive Allele Specific PCR
MAB:	Marker-Assisted Breeding
MABC:	Marker-Assisted Backcrossing

University of Ghana http://ugspace.ug.edu.gh

MAF:	Major Allele Frequency
MAS:	Marker-Assisted Selection
MB:	Molecular Breeding
MP:	Mean Productivity
mRNA:	messenger Ribonucleic Acid
OA:	Osmotic Adjustment
PCA:	Principal Component Analysis
PCR:	Polymerase Chain Reaction
PIC:	Polymorphic Information Content
PRA:	Participatory Rural Appraisal
QTL:	Quantitative Trait Loci
RAPD:	Random Amplified Polymorphism DNA
REML:	Residual Maximum Likelihood
RFLP:	Restricted Fragment Length Polymorphism
SCARs:	Sequence Characterized Amplified Regions
SI:	Stress Intensity
SNP:	Single Nucleotide Polymorphism
SSI:	Stress Susceptibility Index
SSR:	Simple Sequence Repeat
STI:	Stress tolerance index
TE:	Transpiration Efficiency
TOL:	Tolerance index
UCR:	University of California Riverside
USA:	United State of America
WUE:	Water Use Efficiency

University of Ghana http://ugspace.ug.edu.gh

CHAPTER ONE

1. GENERAL INTRODUCTION

Cowpea (*Vigna unguiculata*) is among the most important grain legume crops (Marcenaro and Valkonen, 2016; Muchero *et al.*, 2009). The crop serves as high-quality food for the world's growing population. It is utilized for both food and animal feed (Barrett, 2006; Singh, 1997) particularly in West and Central Africa (Boukar *et al.*, 2018).

Cowpea is produced predominantly under rain-fed conditions in regions of sub-Saharan Africa (SSA) with late, erratic and short rainfall (Fatokun *et al.*, 2012). Consistently, in Senegal, cowpea production is mainly in the rainy season, which lasts 2 to 3 months per year (Cissé, 2016). Major producing areas are where drought mostly occurs in the Senegalese Sahelian zone. Annual rainfall since 1980 has decreased, thereby lowering yield of cowpea cultivars despite the inherent resilience of cowpea to cope with water deficit. Compared to traditional crops such as millet (*Pennisetum glaucum* L.) and sorghum (*Sorghum bicolor* (L.) Moench), cowpea is the best alternative for agriculture under drought situations (Cissé, 2016).

Constraints to cowpea production are numerous including infestation by *Striga gesnerioides* (Atokple *et al.*, 1995; Dubé and Olivier, 2001; Omoigui *et al.*, 2017a). In Senegal, *Striga gesnerioides* is one of the major constraints to cowpea production (Tonessia *et al.*, 2009). Cowpea grain yield in heavily infested field can be nil (Omoigui, 2012; Omoigui *et al.*, 2007, 2017b). Best control measure is varietal resistance (Ouedraogo *et al.*, 2001a).

Genetic and genomic resources that can be used to fast-track development of high yielding varieties adapted to Striga prone areas have been identified (Boukar *et al.*, 2004; Muñoz-Amatriaín *et al.*, 2017; Ouedraogo *et al.*, 2001b; Tignegre *et al.*, 2013). Genetic resources include multi-parent advanced generation inter-cross MAGIC population (> 300), the West African Partner favourite (>100) and the Mini-core (> 360). Genomic resources include SSR

University of Ghana http://ugspace.ug.edu.gh

markers linked to *S. gesnerioides* resistance (Asare *et al.*, 2013a,b; Ouedraogo *et al.*, 2002), consensus genetic map and the Illumina golden gate assay for high throughput genotyping (Muñoz-Amatriaín *et al.*, 2017). However, there has been little success in the use of Striga markers in Senegal. The most plausible reason seems to be the specificity of *S. gesnerioides* prevalent in Senegal (Botanga and Timko, 2006; Tonessia *et al.*, 2014). Recent attempts at using Marker-Assisted Selection (MAS) in Senegal towards solving the Striga problem in cowpea has shown some promise (Tonessia *et al.*, 2009; Tonessia *et al.*, 2014). These attempts must be sustained to achieve the desired results to increase cowpea yields. However, as cowpea is grown in erratic areas in Senegal and *S. gesnerioides* negative effects on cowpea is exacerbated by drought, attention on drought tolerance is as well critical.

The main objective of the project was to convert a highly elite susceptible line into a *S. gesnerioides* resistant line using MAS.

The specific objectives of the study were to:

i) identify constraints to cowpea production and farmers preferred grain quality traits in the Sahelian and Sahelo-Soudanian zone in Senegal;

ii) identify drought tolerant lines among 112 accessions;

iii) validate resistance of advanced backcross lines and discover new SNP markers and candidate genes aligned to *S. gesnerioides* resistance.

University of Ghana http://ugspace.ug.edu.gh

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Cowpea

Cowpea *Vigna unguiculata* L. Walp., is a grain legume of great importance for resource poor communities in sub-Saharan Africa (Timko and Singh, 2008). Large genetic diversity of the crop exists, over 1500 cowpea cultivated accessions are conserved at the International Institute of Tropical Agriculture (IITA), 75 % of them are from the West Africa sub-region (Fatokun *et al.*, 2018). Cowpea has a narrow genetic base of 620 million base pairs; it is a diploid crop with 22 pairs of chromosomes (2n = 22) and is closely related to mung bean (*Vigna radiata*) (Lucas *et al.*, 2013). Despite the existence of several sources of resistance to *Striga gesnerioides*, little progress has been made in improvement of the Senegalese germplasm which relied more on conventional breeding. Statistics have shown a low total production of 64.0 tons obtained from over 150 000 ha and an average productivity of 400 kg/ha (Boukar *et al.*, 2018). This gives room for crop improvement because genomic resources are now available that one can use to fast-track selection (Muñoz-Amatriaín *et al.*, 2017).

2.2. Origin, domestication, taxonomy and genetic diversity

Cowpea also called niebe in Francophone Africa is a herbaceous annual legume mostly grown in the dry agro-ecologies of the tropics in Latin America, Africa and south Asia (Boukar *et al.*, 2018). Cowpea origin has been discussed for many years and remains to be fully elucidated (D’Andrea, *et al.*, 2007). It has been described by Linne as *Dolichos unguiculata*, *Vigna sinensis* and then *Vigna unguiculata* (Faris, 1963; Pasquet and Baudoin, 1997). Ba *et al.*, (2004) reported different centres of origin however, because of diversity observed in *Vigna* genera in the African continent (Pernes, 1984) it has been concluded by authors that cowpea originated from Africa (Raynal-Roques, 1993). Also, it has been reported based on linguistic evidence and phylogenetic studies that cowpea originated in tropical West Africa precisely in Nigeria

University of Ghana http://ugspace.ug.edu.gh

(Faris 1965), with Botswana being probably a second centre of origin (Panella *et al.* 1993). Rawal (1975) and Vaillancourt *et al.* (1993) reported that Africa region around the equator and South Africa were cowpea centres of origin. Cisse (2016) supported cowpea originated from South East Africa.

Huynh *et al.*, (2013a) have reported the lack of knowledge about cowpea domestication origins and patterns of genetic variation compared to other crops. In a study where Coulibaly *et al.*, (2002) cited by D'Andrea *et al.*, (2007) used amplified fragment length polymorphism analysis (AFLP), indicated West or north-eastern Africa as the area of domestication. Ba *et al.*, (2004) have demonstrated the ambiguity of possible domestication of cowpea in Southern and Eastern Africa. Considering these statements, cowpea domestication occurred in Africa based on the noticeable absence of wild progenitor outside this continent (Murdock 1959; Faris 1965; Vaillancourt and Weeden 1992 and Padulosil and Ng, 1997). Cowpea may have been domesticated alongside sorghum and pearl millet (Steele, 1976) from eastern Africa to the Arabian Peninsula, the Asian subcontinent (Faris, 1965) and to East Asia. Evidence of two major gene pools in cultivated cowpea in Africa have been reported (Huynh *et al.*, 2013a). Landraces from gene pool 1 are more located in western Africa while landraces of gene pool 2 are mostly located in eastern Africa. In the same study of Huynh *et al.*, (2013a), it has been reported that accessions from Asia and Europe were closer (related) to those belonging to gene pool 1 while accessions from the Americas were more related to those of gene pool 2.

Cowpea belongs to the tribe Phaseoleae of the Fabaceae family, the genus Vigna, the species *unguiculata* and the subspecies *unguiculata* (Timko *et al.*, 2007). According to Ng and Marechal (1985), *Vigna unguiculata* is divided into 5 subspecies namely *Vigna unguiculata*, *V. cylindrica*, *V. sesquipedalis*, *V. dekindtiana*, and *V. mensensis*. According to Pasquet (1999), variable forms within cowpea species include cultivated species, wild annual and wild perennial species. Wild types are named *V. unguiculata ssp dekindtiana* or *V. unguiculata ssp*

University of Ghana http://ugspace.ug.edu.gh

spontanea. *Vigna unguiculata unguiculata* is divided into 5 groups of cultivars: *Unguiculata*, *Biflora*, *Sesquipedalis*, *Textilis* and *Melanophthalmus* (Padulosil *et al.*, 1997; Boukar *et al.*, 2018). West and Central Africa are the area where maximum diversity and cultivated cowpeas are found (Boukar *et al.*, 2018).

2.3. Production and major constraints

On the African continent, cowpea is the most widely grown grain legumes (Timko *et al.*, 2012) and also widely grown in Asia, Latin America, the south-eastern United States and California (Dadson *et al.*, 2005). FAO estimates that the annual production of cowpea is around 4 million tons on about 10 million hectares of which two thirds come from Africa (FAO, 2008). Most of the production occurs in West Africa (68%), Brazil (17 %), Asia (3%) and USA (2%) while the remaining 10 % is produced by the rest of the world. Important producers are Nigeria, Niger, Burkina Faso, Senegal, Ghana, Cameroon and Mali. Nigeria is the largest cowpea producer and consumer, the country produces 56% of the world production (FAO, 2008).

Major constraints of production are drought, parasitic weeds, insect pests, diseases and nematodes attacking cowpea during every stage of its life cycle. The difficulty of production input procurement is also a major constraint (Singh and Tarawali, 1997).

2.3.1. Breeding for drought tolerance

Crop plants at one stage or another in their life cycle are exposed to drought (Boyer and McPherson, 1975). Drought is among the most important environmental stress in agriculture affecting crop growth and development (Basu *et al.*, 2016).

2.3.1.1. Plant and trait response to water stress

Plant when subjected to drought stress react in different ways depending on species, varieties and the type of economic product (Kramer, 1980). Leafy crops such as lettuce are very sensitive

University of Ghana http://ugspace.ug.edu.gh

to drought while hay crops such as alfalfa are more drought resistant due to their deeper roots allowing them to get access to deep soil moisture. Drought is more severe at early flowering or fruit filling than at vegetative time. Indeterminate plants such as cowpea are more tolerant to mid drought than determinate plants such as sorghum, pearl millet and rice. This can be explained by the fact that indeterminate plants have the ability when first flowers have dropped due to water stress effect, to compensate by producing other flushes of flowers and pods after drought is experienced (Hall, 2012) while determinate plants are unable to produce any more fruits (Gwathmey *et al.*, 1992). This shows that mechanisms of drought sensitivity differ from species to species. An apposite example is responses to drought at the early flowering stage in wheat and maize. According to Wilson et al. (1980), drought happening 7 days prior to anthesis might reduce number of seeds per spike in wheat and number of kernels per ear in maize. In terms of drought sensitivity, there is a major difference between the two crops. In wheat floret sterility is associated to pollen sterility (Jones, 1993) while in maize pollen will be still viable when plant undergo drought during flowering (Hall *et al.*, 1982). Likewise, seed number and seed size is also affected in different ways depending on if an indeterminate or determinate crop is involved. Also seed size injuries from drought depends on the type of drought involved, whether late or mid-season. Usually, mid-season coincides with anthesis while late drought occurs after or during late stages of fruit formation. Viewed in this aspect, late drought only affects seed size. The selective effect can be explained further by an asynchronous occurrence of drought and drought sensitive stage defined in this example as flowering time. Obviously, damaging effect of drought on leaves leads to a reduction of photosynthetic sources (Wang *et al.*, 2016) due to leaf senescence. In sorghum, low seed size has been observed in modern cultivars subjected to late season drought, definitely, determinate crops are more sensitive than indeterminate crops to terminal water stress. The size of photosynthetic source affected by late

University of Ghana http://ugspace.ug.edu.gh

season drought leads to non-vigorous seeds then an ample reduction of seed size (Turk *et al.*, 1980).

2.3.1.2. Drought induced morphological and physiological changes in plants

Plant responses to drought stress can be classified as morphological and physiological aiming at reducing water losses through transpiration (Basu *et al.*, 2016). Various physiological responses to drought are deployed via molecular, cellular and whole plant pathways (Farooq *et al.*, 2009).

Modifications in plants constitute a way of preventing damages from drought stress. This include reduction of leaf area, leaf angle changes, tillering, root growth (Farooq *et al.*, 2009). It has been reported in tepary bean (*Phaseolus acutifolius*) and common bean (*P. vulgaris*), that when stressed, plants rotated their leaflets and reduced their sizes in order to make them parallel to incoming radiation. This modification allows leaves to cool down temperature. (Farooq *et al.*, 2009) have reported drought escape (DE) where the plant matches its phenological development to duration of water available in the soil; avoidance where plant shapes root system and leaves to respectively improve water uptake and stomatal control of transpiration and phenotypic flexibility; where plant produces smaller or shedding leaves, enhance roots quality such as distribution and structure or root quantity such as density at the expense of up-ground growth and biomass.

Physiological modifications concern leaf enlargement, osmotic adjustment *(I)*, stomatal behaviour *(II)*, photosynthesis *(III)*, leaf cuticular wax *(IV)*, translocation *(V)* and proline accumulation *(VI)*. ***I)*** Osmotic adjustment (AO) has been referred to as a biochemical process where various osmotically active substances are accumulated in the cell aiming at helping plants to acclimatize to drought stress (DS) and maintain metabolic processes (Turner, 1978), Accumulated solutes are for protective purposes, they prevent dehydration in cellular proteins,

University of Ghana http://ugspace.ug.edu.gh

enzymes, and cellular membranes. Substances contributing to AO include amino acids, organic acids, inorganic cations and anions (Iljin, 1957; Hsiao and Acevedo, 1974, Begg and Turner, 1974). This mechanism is important in the sense that it promotes the potential of solute to move from a hypotonic to a hypertonic solution, hence a more negative osmotic potential creating favourable condition into the cell (cell hydration) and maintaining turgor (Sanders and Arndt, 2012). Turgor is critical for cell enlargement and growth. In stressed cotton, Cutler *et al.*, (1977) reported maintenance of turgor happened because of involvement of sugar and malate accumulated and high cell wall elasticity. OA was reported to happen in sorghum more after flowering than before (Brevedon and Hodges, 1973). Plants when undergoing drought stress suffer less when maintenance is granted by thicker cell walls and smaller cell. This allows more water in the cell wall and less solvent in the protoplast. A suggestion of Morgan (1977) to plant breeders was to breed for OA to improve water stress tolerance of wheat (Jones and Turner, 1978). ***II)*** There is no constant threshold water potential at which plant stomatal comportment is triggered when plants are subjected to drought (Blum, 1996; Jordan *et al.*, 1975). However, a strong relationship between stomatal closer and water potential has been reported (Turner, 1974) (Wang *et al.*, 2016). Stomatal behaviour changes with water potential which is dependent on factors including stress history, growing conditions and age of the plant (Begg and Turner, 1976, Saranga *et al.*, 1991). In a comparative study where cotton plants were preconditioned and not, it was observed that in preconditioned plants, abaxial stomata closing occurred at a lower water potential than non-preconditioned plants. Klar *et al.*, (1978) illustrated this with guinea grass. Growing condition has been shown to affect stomatal behaviour. Threshold triggering closing of stomata were different in sorghum genotypes grown in condition of water stress and in unfavourable condition for root development. In watered-stressed condition, sorghum genotypes had their stomata still open at -20 bars while genotypes grown in unfavourable condition for root growth closed their stomata at -16 bars (Fereres *et*

University of Ghana http://ugspace.ug.edu.gh

al., 1978). It has been observed in aged plants a drop of CO2 exchange rate and leaf photosynthetic capacity (Davis and McCree, 1878). In wheat flag, leaf potential at which plant closes its stomata (-13 bars at tillering) decline gradually with age (-31 bars at grain filling) (Frank *et al.*, 1973). ***III)*** Efficiency of photosynthesis rely on total leaf area and photochemical activity of various leaves (Boggess and Stewart, 1976). When plants are subjected to drought, in response they close their stomata in order to minimize water losses however, that defensive reaction reduce transpiration and photosynthesis as well (Farooq *et al.*, 2009). The latter is also reduced in prolonged drought stress by depression of chloroplast and enzyme activity and non-stomatal factors (Begg and Turner, 1974; Toole *et al.*, 1976; O' Toole, 1977). Leaf area index (LAI) is reduced by water stress. ***IV)*** Mild water stress is of significant effect on leaf enlargement but does not affect plants as prolonged drought does, this refer to recovery. Plants subjected to mild water stress have reduced leaf elongation rate. This phenomenon has been observed in corn genotypes that reacted favourably for leaf elongation at -1.5 to -.2.5 bars leaf water potential, while at -4 bars leaf elongation rate dropped by 25%. A threshold has been assigned as -2 bars for this species, in other words, corn plants subjected to mild drought will grow slowly (Acevedo *et al.*, 1979) and recover when water becomes available if their leaf potential does not exceed -2 bars. In fact, cell division is less sensitive to drought compared to cell enlargement, cells that were developed during mild drought period, when water becomes available tend to expand and stimulate re-growth. In opposite, plant subjected to long period drought may not totally recover when re-watered (Boyer, 1970). ***V)*** Plants have vascular network comprising of two separated cellular translocation pathways namely xylem and phloem. Water and nutrients taken by roots are transported to shoot by xylem. Xylem delivered solutes are redistributed to heterotrophic organs such as roots, seeds and flowers. By the same way assimilates move in these pathways, plant hormones and signalling molecules moves with them by mass flow. Among other roles, phloem provides a conduit for trafficking macromolecules (nucleic acids and proteins), some of which may regulate gene expression as a consequence of their translocation. Xylem is believed to be the conduit in which root signalling elements move (Atkins and Smith, 2007). When drought occurs, translocation shows less sensitivity compared to photosynthesis (Boyer, 1970, McPherson and Boyer, 1977, Sung and Krieg, 1979). In such condition of drought tolerance (DT), it is understood that assimilates movement from the photosynthetic cells into the conducting system is reduced, however, translocation pathways may not be affected by drought (Wardlaw, 1969). This means the conducting system still operate efficiently when the plant is grown under water stress but sink is altered (Begg and Turner, 1974). It has been suggested that plants can mobilize photosynthate produced prior to grain filling period and use it in grain filling (Boyer, 1970). ***VI)*** It has been indicated that proline accumulate during severe drought stress in water stress tissues (McMichael and Elmore, 1977). Correlation between proline accumulated and yield stability of barley under drought stress were found (Singh *et al.*, 1973). However, Blum and Ebercon (1976) found in sorghum that proline used as a marker was indication of ability to recover after drought period (DP). In fact, it was noticed that proline accumulated in wilted leaf tip were reduced after plant recovered from drought stress. Breeding for high proline were reported to lead on stress susceptible cultivars. This means proline will not be of practical value to specifically address drought tolerance (Hanson *et al.*, 1977, Hanson *et al.*, 1979).

2.3.1.3. Measurement of drought tolerance among genotypes

Phenotyping of assembled lines with different genomic background and their utilization in breeding is limited by the identification of stress responsive traits allowing quick discernment of tolerant / resistant to susceptible lines (Fatokun *et al.*, 2012). Various methods of measurements have been proposed to quantify drought tolerance in crops including C isotope discrimination, root phenotyping, wooden boxes screening using morphological markers (Hall *et al.*, 2003) or screening in rain out shelter. However, such methods are very complex,

University of Ghana http://ugspace.ug.edu.gh

sophisticated or laborious and thus might not be most suited for highly skilled plant breeders with limited resources. A consistent and efficient way to address drought tolerance would be the consideration of traits correlated with grain yield in contrasting environments, associated with high heritability and suitable genetic variability easy to record at a reasonable budget (Araus, 2002; Beebe *et al.*, 2013; Jackson *et al.*, 1996; Polania *et al.*, 2016). This presupposes high skills in stability and adaptation analysis to allow reliable prediction of cultivars' performances (Malosetti *et al.*, 2013).

Several models have been used to predict response of crop species in contrasting environments. Among others are the regression on the mean model (Finlay and Wilkinson, 1963; Eberhart and Russel 1966), the additive main effects and multiplicative interactions (AMMI) (Gauch, 1988) and GGE analysis (Yan *et al.*, 2000). Additionally, stability coefficients including Superiority performance (Linn and Binns, 1988), Static stability coefficient, Wricke's ecovalence (Wricke, 1962) and several quantitative indices of stress tolerance such as Mean Productivity (MP) and Stress Tolerance index (TOL) (Rossielle and Hamblin, 1981), Drought Resistance index (DRI) (Bidinger *et al.*, 1987), Geometric mean productivity (GMP) (Fernandez, 1992), Stress Susceptibility index (SSI) (Fischer and Maurer, 1978) have been used in plant breeding for drought tolerance assessment (Mohammadi, 2010; 2011). In cowpea, few studies such as (Yasir *et al.*, 2013) have used stability coefficients and quantitative indices of tolerance to drought. Most of these although are straightforward methods that allow breeders to make reliable inferences.

University of Ghana http://ugspace.ug.edu.gh

2.3.2. Breeding for resistance to Striga

From an agricultural point of view, drought-prone areas of Africa are the stronghold of most economically important Striga species (Mohamed *et al.*, 2003). In 25 countries, occurrence of Striga in 2005 led to economic damages estimated to 1 billion $US per year (Spallek *et al.*, 2013). *Striga* species are holoparasitic or hemiparasitic with regards to their capacity to perform photosynthesis activities once they have emerged from the soil (Wolfe and Pamphilis, 1998; Matusova *et al.*, 2005). Plants with adequate irrigation suffer less from Striga parasitism (Dembélé, 1988). Control measures include the use of stimulants, bacterium *Pseudomonas seryngae* and trap crops to allow suicidal germination of Striga (Berner and Williams, 1998; Berner *et al.*, 1997). Farming system can be improved by crop rotation involving cultivation of cereals and legume but also by mixed cropping agriculture. These techniques have been found to reduce at least three times the stock of Striga seeds present in the soil (Cardwell and Lane, 1995). Most of these approaches are however, expensive for farmers or non-relevant, the use of resistant genotypes is the best way of controlling Striga (Alonge *et al.*, 2005). Some useful pieces of information for breeding cowpea for Striga resistance are presented in the following subsections.

2.3.2.1. Taxonomy and geographical distribution

The word Striga comes from Latin which mean is witch. The term witchweed is appropriate for Striga in the sense that before emerging, the parasite feed entirely from nutrients and water from the host plant (Thalouarn *et al.*, 1991). The monophyletic group including Striga genus were earlier grouped in *Scrophulariaceae family* (Botanga and Timko, 2005). Later, studies placed Striga in the family Orobanchaceae (Spallek et al., 2013), the family that contains majority of parasitic weeds (Bennett and Mathews, 2006).

University of Ghana http://ugspace.ug.edu.gh

In the genus Striga, some species are serious threat to crop production namely *Striga gesnerioides*, *S. hermonthica* and *S. asiatica*. *S. gesnerioides* is found in West and Southern Africa, USA and India. Many articles have investigated the pathogenicity of *S. gesnerioides* in West Africa. It is prevalent in Nigeria, Benin, Niger, Togo, Senegal, Burkina Faso and Cameroon (Dembélé, 1988; Botanga and Timko, 2006; Tonessia *et al.*, 2014). *S. hermonthica* and *S. asiatica* have similar host range and are found in East, West and Southern Africa and USA. It has been reported that majority (80%) of described Striga are prevalent in Africa. The rest is distributed across other parts of the word including Australia continent namely *S. curvilflora* Benth., *S. multiflora* Benth. and *S. parviflora* Benth (Spallek *et al.*, 2013). *Striga* species are found in the semi-arid tropical regions on open grass lands and savannahs.

2.3.2.2. Races of *Striga gesnerioides* and cowpea differential set of lines

Five races of *Striga gesnerioides* have been well characterized across Africa (Timko *et al.*, 2012; Timko and Singh, 2008) using molecular tools and differential host response. To date, at least 7 races exist namely SG1 (Burkina Faso), SG2 (Mali), SG3 (Nigeria and Niger), SG4 (Benin), SG4z (localized to the Zakpota region of Benin), SG5 (Cameroon), and SG6 (Senegal). Most divergence have been found between SG3 and SG4, relatedness have been reported to be higher between SG1 and SG5 (Timko *et al.*, 2007). Evidence of existence of different races is based on non-consistent reaction of cultivars to different biotypes of Striga collected. For instance, Suvita 2 was reported to be resistant to all races except SG3 (Nigeria) and SG5 (Cameroon), IT97K-499-35 is resistant to SG1 (Burkina Faso, SG2 (Mali) and SG3 (Nigeria). An exhaustive list of these germplasm can be found in the literature (Ouedraogo *et al.*, 2001a; Salifou *et al.*, 2017).

University of Ghana http://ugspace.ug.edu.gh

2.3.2.3. Host, non-host and cowpea cultivar mechanism of resistance

Unfortunately, most parasitized plants in Africa are crops that are the most importantly produced in subsistence agriculture (Spallek *et al.*, 2013). *Striga gesnerioides* parasitize genera within Leguminosae and Convolvulaceae (Mohamed *et al.*, 2003) and is strictly dependent of its host due to the fact that it has an inefficient photosynthesis system (Berner and Williams, 1998; Alonge *et al.*, 2005). Most important hosts comprise *Vigna Alysicarpus, Indigofera,* and *Tephrosia*, and non-legumes such as *Ipomea, Jaquemontia, Merremia, Euphorbia,* and *Nicotiana*, (Berner and Williams, 1998; Botanga and Timko, 2005, Timko and Singh 2008). *S. Asiatic* and *S. hermonthica* host range are similar and comprises cereals such as sorghum, sugarcane, maize, finger and pearl millets. Preference of host by *S. gesnerioides* is highly variable, Timko *et al.* (2007) reported that strains collected from *Indigofer*a spp., *Tephrosia* spp. and *Jacquemontia* spp attacked only the hosts from which they were collected. Similarly, strains from tabaco from Zimbabwe are only able to complete their life cycle on tabaco, although, their germination can be stimulated by other crops.

Resistance of cowpea cultivars to *S. gesnerioides* exists in two forms: the first is called hypersensitive response and the second describe a situation where Striga tubercles do not exceed 0.5 mm in diameter or expansion of Striga cotyledons fails. A landrace from Senegal (58-57) showed hypersensitive response to Striga by killing all cells that were around the region of infection. Cultivars from Botswana (B301) and IITA (IT81D-994) exhibited failure of expansion of parasite’s tubercle and cotyledons. It is important to highlight that resistance can occur at different development stages since underground to upground life cycle of Striga. Parasite life cycle involves germination, haustorial induction, host root attachment and vascular connection. Host plants induce germination of Striga by producing germination stimulants particularly strigolactones (Cardoso *et al.*, 2011).

University of Ghana http://ugspace.ug.edu.gh

There is a lack of knowledge about the factors controlling association of parasites and plant hosts. Non-host plants are adverse or incapable to support parasitic weed growth (Press and Graves 1995) while in host plants, parasite have adapted their life cycle hence can grow and reproduce. Non-host plant is a theatrical concept in the sense that in case of strong selective pressure to survive, plant parasite always finds ways to destroy complex multifactorial barriers to resistance. Besides, resistant genotypes exist in variety of parasite host species. Resistance can occur in two levels: before attachment or after attachment. The latter is defined as successfulness of establishment of vascular connection linking the two species. Resistance occurring before attachment involve mechanical barriers such as thickened cell-walls in point of attachment, reduction of signals stimulating development of a specialized invasive organ called haustorium and programmed cell death so called hypersensitive reaction. In cellular level, many articles have reported that parasite evolve protein effectors to enter host cells. Effectors are detected either directly or indirectly (Greenshield and Jones 2008; van der Hoorn and Kamoun 2008) by anti-virulence genes involving complex signalling scenarios resulting to inhibition of parasite growth (Timko *et al.*, 2012).

2.3.2.4. Inheritance of Striga resistance

Knowledge on inheritance of a trait resistance is very important in breeding. In the case of prevalence of multiple races, inheritance study should define clearly regions where study was conducted and source of the strain used in inoculation, in addition to the description of the vegetal material used. Numerous studies have given valuable indications on the genetic of Striga resistance. Genotypes Suvita 2 and B301 were reported to confer monogenic dominant inheritance to SG1, SG2 and SG3. All breeders accepted that resistance to *S. gesnerioides* is governed by single genes with major effects (Boukar *et al.*, 2004; Ouedraogo *et al.*, 2002; Timko *et al.*, 2007).

University of Ghana http://ugspace.ug.edu.gh

2.3.3. Evidence of molecular breeding gaps in cowpea

Cowpea is a diploid species with 2n = 22 chromosomes and a genome size of about 620 million base pairs (Boukar *et al.*, 2018). Unlike in other crops, the development of genomic resources has been very slow (Muñoz-Amatriaín *et al.*, 2017). Majority of studies involving marker technologies are oriented in QTL mapping or taxonomic relationships (Boukar*et al.*, 2013). Several QTLs have been identified however, their validation never succeeded due to multiple reasons among which are lack of population purity and reliability of phenotypic data recorded during phenotyping. Very few studies have used genomic resources to conduct an effective marker assisted selection program. Marker-assisted backcrossing (MABC) was used by plant breeders in various crop improvement programmes (Ribaut and Ragot, 2006). Identification of markers is a primary step prior to MABC. Markers exist for cowpea resistance to *S. gesnerioides* (Ouedraogo *et al.*, 2002; Timko *et al.*, 2007) but were not effective for races prevalent in Senegal. No study to date has been conducted using high throughput genotyping on a diverse population likely to have abundant resistant alleles. Such study is necessary to generate genomic resources that breeder can use directly to undergo limitations during phenotyping and linkage drag when developing populations.

University of Ghana http://ugspace.ug.edu.gh

CHAPTER THREE

3. CONSTRAINTS TO COWPEA PRODUCTION AND FARMER PREFERRED GRAIN QUALITY TRAITS IN THE SAHELIAN AND SAHELO-SUDANIAN ZONE IN SENEGAL

3.1. Introduction

Cowpea is a staple crop cultivated for its leaves, fresh and dry grains, fresh pods, fodder, protein and vitamin content and its adaptation to stressful environments. Cowpea admirably generates additional revenues and fresh pods over a long period of time during the hunger period called "soudure" for resource-poor communities in sub-Saharan Africa (Cissé, 2016; Ehlers and Hall, 1997). Cowpea is grown in almost all ecologies in Senegal. The main cowpea production areas are Louga, Thies and Diourbel. Louga in the northern part of the country is the largest producing area (Cissé, 2016).

In the interest of cowpea growers, great deal of efforts, in this case the funding of United States Agency for International Development (USAID) under the Bean/Cowpea Collaborative Research Support Program (CRSP), have enabled the development of several improved lines based on farmers' need which were at that time earliness and photo sensitivity (Cissé *et al.*, 2005). More recently in 2015, five large seeded varieties were released. All these varieties when grown under well-watered fertile conditions and appropriate agronomic practices produce at least 3 tons per hectare. However, despite all efforts made in the creation of varieties, the development and popularization of techniques of good agricultural practices and the conservation of harvested grains, yields in farmers' field reach barely 350 kg.ha.$^{-1}$ (Kamara *et al.*, 2018; Magen *et al.*, 2012). Causes of the considerable yield reduction can be attributed to many biotic and abiotic stresses as well as breeding objectives being out of sync with farmers' real concerns.

University of Ghana http://ugspace.ug.edu.gh

In many breeding programmes, farmers are only involved at later stages (Egbadzor *et al.*, 2015), as a result, released varieties tend to lack farmers' preferred traits while many of the eliminated lines including parental and bi-parental lines during filtering might have been of interest to end-users. Studies have reported low adoption of improved varieties when compared to traditional and unknown varieties in Senegal (Magen *et al.*, 2012). It is urgent that breeders prior to population development identify farmers' needs for reasonable demand-led breeding programs (PAEPARD, 2018).

The objectives of this study were to identify constraints affecting cowpea production and to investigate grain traits preferred by farmers in order to take them into account in future breeding activities.

3.2. Materials and Methods

3.2.1. Study sites

The study was carried out in May, July and September 2017 in the Sahelian and Sahelo-Sudanian zone of Senegal which is the major area for cowpea production. The two main cowpea growing regions, namely Louga and Thiès were selected. A distance of 124.9 km separates the two localities. Average yearly precipitation from July to October is less than 500 mm starting. Two districts in the region of Louga namely Louga and Kebemer and one in the region of Thiès called Tivavouane were used for this study.

3.2.2. Sampling procedures

Stratified sampling method was used to allow random sampling of participants in each of the two main cowpea growing sites selected. Population sampled for the focus group discussion (FGD) involved 30 individuals including 16 women and 14 men. In Semi-structured survey (SSS), 109 participants were questioned including 54 females and 55 males. Assistance of development agents facilitated linkage with contact persons at each village. Interviews were

University of Ghana http://ugspace.ug.edu.gh

done with experienced farmers known to have grown cowpea the previous five rainy seasons (years).

3.2.3. Data collection

Data were recorded on famers' background information to allow possibility to go back to information easily in case of need. During FGD session, issues discussed were related to the importance of feedback from farmers to breeders about cowpea production, the importance of involving farmer's varietal development and the constraints to cowpea production. Farmers were also asked about their preferred traits and willingness to participate in semi-structured survey (SSS). In the SSS, questionnaire included farmers background information, the importance of cowpea, factors that affect cowpea production, the knowledge of farmers about *S. gesnerioides* and farmers' preferred traits with regard to grain quality.

3.2.4. Data analysis

Quantitative data collected in FGD and SSS were arranged to fit in meaningful class intervals. All data including binary data were analysed by calculating their frequencies and cumulative contribution in row (designations) and column (districts and gender). Further analysis of the SSS involved Fisher exact test and t-test respectively for binary data and count data to allow assessment of significance in regard to districts and gender. Data regarding constraints to cowpea production and farmers' preferred traits were ranked based on yes rating (total yes of a variable divided by total yes of all variables times 100). The R. software was used to compute table contingency and calculate p-value of variables according to division and gender using appropriate test of statistical significance.

University of Ghana http://ugspace.ug.edu.gh

3.3. Results

3.3.1. Demographic characteristics of respondents

Of the 30 individuals in the FGD session 27 % were illiterate, 43 % attended primary school and 30 % attended secondary school. Respondents in the FGD who were more than 40 years constituted 83% and the rest of the respondents were between 30 and 39 years old. The SSS involved 54 females and 55 males. Among the SSS sample, 20 % were illiterate, 60 % attended primary school, and 20 % attended secondary school. Majority (61 %) of respondents were between 40 and 61 years old, 16 % were between 18 and 39 years old, 19 % had between 62 to 83 years old and 4 % were not able to communicate their age.

3.3.2. Importance of cowpea

Variables studied in SSS for importance of cowpea were all significantly different in respect to divisions however, only plot size for cowpea production were significantly affected by gender (Table 3.1). Majority of women had plot size between 1 and 3 ha while plot size greater than 4 ha were reserved to men (Figure 3.1). More than 90 % of respondents in Louga and Tivavouane declared utilizing cowpea for multi-purposes.

University of Ghana http://ugspace.ug.edu.gh

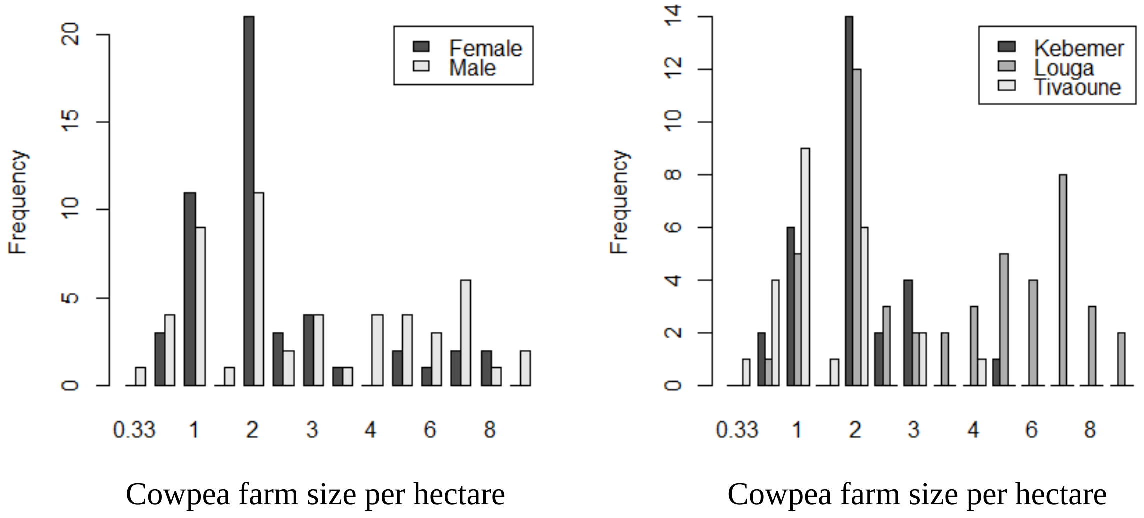


Figure 3. 1: Plot sizes assigned to gender and geographic division

At Kebemer, 84.38 % of respondents utilized cowpea only for human food and livestock feed (Table 3.1). About 60 to 94 % of the farmers indicated that cowpea was grown as a main crop in the three divisions of Senegal, with 94% of respondents from Louga. Very few respondents in Louga (5.77 %) grew cowpea in association cropping while in Kebemer and Tivavouane 40.63 % and 24 % respectively grew cowpea in association with other crops. In all three divisions cowpea production provided a lucrative business opportunity (Figure 3.2).

Table 3. 1: Importance of cowpea in Louga, Kebemer and Tivavouane

Variable	**Geographic division**			**Fisher Exact / Students' t.test**	
Importance of cowpea	**Louga**	**Kebemer**	**Tivavouane**	**Division**	**Gender**
Size of cowpea plots (ha)	2.29	1.66	1.36	1.67^{-13}**	2.68^{-05}**
Human and livestock %	3.85	84.38	8.00	2.20^{-16}**	0.14
Multiple purposes %	96.15	15.63	92.00	2.88^{-16}*	0.14
As main crop %	94.23	59.38	76.00	3.05^{-04}*	0.48
Intercrop %	5.77	40.63	24.00	3.39^{-03}*	0.12
A lucrative business %	100.00	90.63	88.00	0.07	0.36

Figures with ** indicates high significance at $p < 0.01$; * indicates significance at $p < 0.05$

University of Ghana http://ugspace.ug.edu.gh

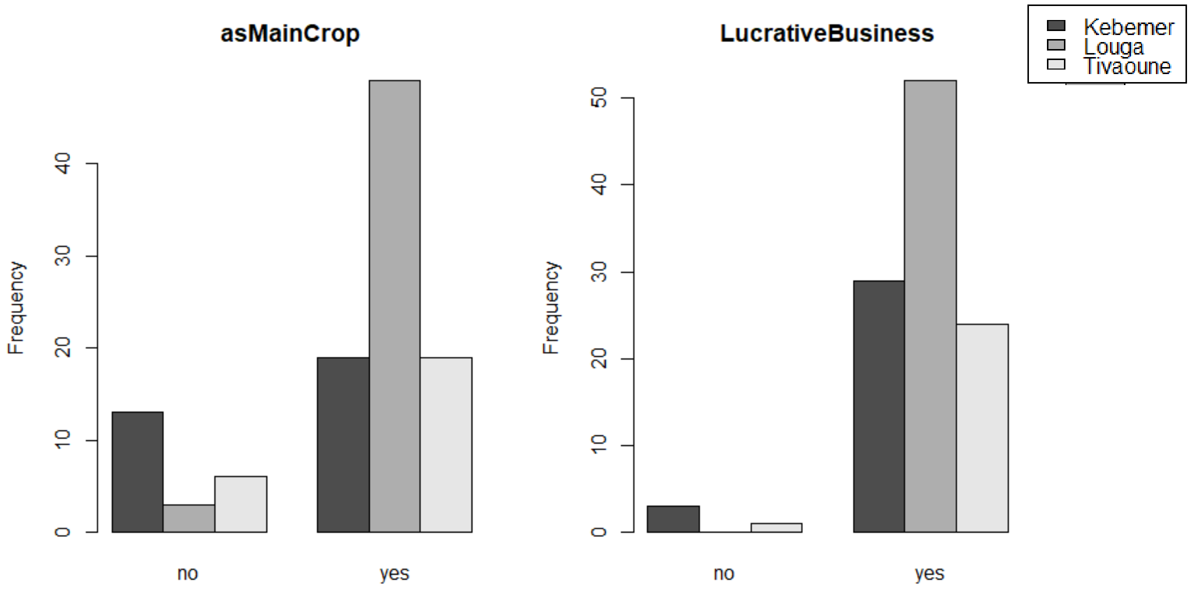


Figure 3. 2: Frequency of counted yes-no of cowpea cropping as a main crop and a lucrative business in Louga, Kebemer and Tivavouane.

3.3.3. Constraints to cowpea production

In division level, farmers’ perception of constraints limiting cowpea production were consistent for all variables except for aphid, Maruca, Bruchid, soil fertility and cultural practices. No significant differences were observed in gender level for all variables. In general, the five most important constraints to cowpea production were drought, aphid, Macrophomina, maruca and Striga (Table 3.2). The relative rankings of these five constraints differed among the divisions.

University of Ghana http://ugspace.ug.edu.gh

Table 3. 2: Main constraints to cowpea production in the Sahelian and Sahelo-Sudanian zone in Senegal

Constraint	**Division respondents %**			**Gender respondents %**		**Fisher's Exact Test** **p. value**		**Rank**
	Louga	**Kebemer**	**Tivavouane**	**Female**	**Male**	**Divsion**	**Gender**	
Drought	11.03	9.70	9.13	9.80	10.35	0.43290	0.74250	1
Aphid	8.58	6.02	9.13	8.16	6.61	**0.00311**	0.10410	2
Macrophomina	7.84	9.70	9.57	10.20	9.69	0.01913	1.00000	3
Maruca	6.86	10.37	5.65	6.73	8.59	**0.00003**	0.09819	4
Striga	10.05	9.70	8.70	9.18	9.91	0.52350	0.58100	5
Bruchid	6.62	8.70	10.87	8.78	7.71	**0.00004**	0.26450	6
Pod sucking bug	8.58	8.36	7.39	7.96	8.37	0.71920	0.82830	7
Seed quality	0.98	0.00	0.87	1.02	0.22	0.26640	0.20630	8
Soil ferlility	9.80	10.37	5.22	8.37	9.25	**0.00003**	0.47750	9
Amsacta	8.33	7.02	9.57	8.16	8.15	0.13070	1.00000	10
Weeds	0.25	0.33	0.43	0.20	0.44	1.00000	0.61070	11
Nematode	0.00	0.33	0.00	0.00	0.22	0.54290	0.48570	12
Thrips	9.56	9.70	10.87	10.61	9.03	0.04150	0.01345	13
Cultural practicies	8.33	9.03	10.87	8.57	9.69	**0.00296**	0.31520	14
Shattering	0.49	0.00	0.00	0.41	0.00	0.49890	0.49560	15
Heat	0.25	0.33	0.00	0.20	0.22	1.00000	1.00000	16
Market	0.00	0.00	0.43	0.00	0.22	0.23810	0.48570	17
Sowing dates	2.21	0.33	1.30	1.63	1.10	0.09739	0.55740	18
Pesticides	0.25	0.00	0.00	0.00	0.22	1.00000	0.48570	19
Total	100	100	100	100	100			

Figures in bold indicate high significance at $p < 0.01$

University of Ghana http://ugspace.ug.edu.gh

3.3.4. Knowledge of farmers on *Striga gesnerioides*

Majority of farmers in Louga, Kebemer and Tivavouane (Table 3.3) were able to describe Striga as a parasitic weed affecting crops productivity. In Louga 50 % of the people sampled were able to make clear differentiation between *S. gesnerioides* and *S. hermonthica* while very few were able to differentiate the two parasitic species in Kebemer (18.75%) and Tivavouane (18.75%). Striga was not considered as a major stress across divisions, only 42.15% considered it as a major constraint to cowpea production. However, 57.69 % of respondents in Louga considered it as a major stress factor. Overall, few farmers declared the existence or availability of resistance cultivars (Table 3.3)., In Tivavouane however, farmers indicated resistant cultivars among the local germplasm. Farmers indicated that *S. gesnerioides* was visible in infested fields at 44 days after sowing. Farmers in Louga indicted *S. gesnerioides* emergence at 40 days after sowing. To verify famers answers relating to their knowledge on *S. gesnerioides* a picture of *S. gesnerioides* was presented to them. Test indicated that most of them had come across a plant of *S. gesnerioides*. Louga (94.23 %) had the highest number of farmers who correctly recognized the parasitic weed, while Kebemer and Tivavouane recorded 84.38 % and 64 % respectively.

Table 3. 3: Knowledge of farmers about *S. gesnerioides* in the Sahelian and Sahelo-Sudanian zone in Senegal

Variable	**Geographic division**			**Fisher Exact / Students' t.test**	
Knowledge on *Striga gesnerioides*	**Louga**	**Kebem**	**Tivav**	**Division**	**Gender**
Ability to describe *S. gesnerioides*	92.31	68.75	76.00	0.02*	0.63
Ability to differentiate Striga spp	50.00	18.75	18.75	0.01*	0.84
S. gesnerioides as a major stress	57.69	46.88	21.88	0.05	0.57
Existence of resistant cultivars	32.69	3.13	68.00	3.31E-07**	0.68
Days to *S. gesnerioides* emergence	40.21	47.05	46.11	1.34E-05	2.92E-06
Picture recognition	94.23	84.38	64.00	4.22E-03*	0.29

*Figures with ** indicates high significance at $p < 0.01$; * indicates significance at $p < 0.05$. Kebem= Kebemer; Tivav= Tivavouane.*

University of Ghana http://ugspace.ug.edu.gh

Figure 3.3 presents opinions of farmers on yield losses due to *S. gesnerioides*. Majority of farmers in Louga and Kebemer have declared high yield losses in cowpea fields infested with *S. gesnerioides* whereas, very few people in Tivavouane considered high yield due to *S. gesnerioides*. The farmers in Louga and Tivavouane however, indicated that the negative effect of *S. gesnerioides* infestation on yield were not of major importance.

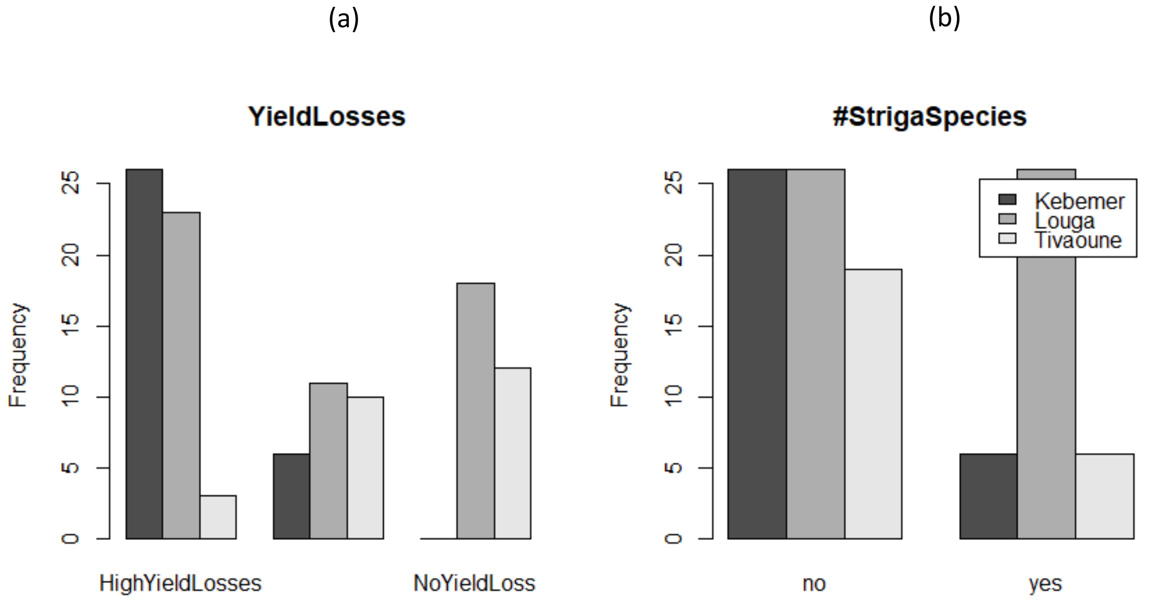


Figure 3. 3: Yield losses due to *S. gesnerioides* (a) and ability of farmers to differentiate *S. gesnerioides* and *S. hermonthica* (b)

3.3.5. Farmers' preferred grain quality traits

Division and gender had a significant effect on preferences for all grain quality traits. In other words, farmers' answers were not consistent across locations and gender indicating grain trait preferences were specific (Table 3.4, Figure 3.4). However, large seed size and brown seed

University of Ghana http://ugspace.ug.edu.gh

coat colour were ranked first in all the three divisions based on frequency counts. Variation in seed testa colour is presented in Table 3.5. In Louga, prostrate and climbing cultivars (prostrate-climbing) were ranked first while strictly climbing and strictly prostrate were ranked first respectively in Kebemer and Tivavouane. Extra early cultivars were ranked first in Louga and Tivavouane while early cultivars were ranked first in Kebemer.

Table 3. 4: Preferences of farmers for cowpea grain size, growth habit and maturity rating in the Sahelian and Sahelo-Sudanian zone of Senegal

Grain quality traits	**Geographic division**						**Students' t.test**	
	Louga		**Kebemer**		**Tivavouane**			
	Contrib.	**Rank**	**Contrib.**	**Rank**	**Contrib.**	**Rank**	**Division**	**Gender**
Seed size								
Large	86.54	1	50.00	1	96.00	1	**0.043**	0.078
Medium	0.00	4	9.38	3	0.00	3.5		
Small	1.92	3	0.00	4	4.00	2		
Irrelevant	11.54	2	40.63	2	0.00	3.5		
Seed coat colour								
Black	1.92	5.5	0.00	5.5	8.00	3.5	**0.015**	0.040
Brown	59.62	1	93.75	1	52.00	1		
Red	11.54	3	3.13	2.66	24.00	2		
White	15.38	2	3.13	2.66	8.00	3.5		
White-brown	9.62	4	0.00	5.5	4.00	5.5		
Irrelevant	1.92	5.5	3.13	2.66	4.00	5.5		
Growth habit								
Climbing	23.08	3	81.25	1	4.00	5	**0.002**	**0.001**
Erect	25.00	2	15.63	4	24.00	2		
Prostrate	13.46	4	21.88	3	44.00	1		
Prostrate-climbing	36.54	1	59.38	2	20.00	3		
Semi-erect	1.92	5	3.13	5	8.00	4		
Maturity rating								
Early	9.62	2	87.50	1	32.00	2	**0.046**	**0.026**
Extra early	88.46	1	12.50	2	56.00	1		
Late	1.92	3	0.00	3	12.00	3		

Contrib.: Contribution; figures in bold indicate significance at $p < 0.05$

University of Ghana http://ugspace.ug.edu.gh

Table 3. 5: Variability for cowpea seed testa colour according to famers in the Sahelian and Sahelo-Sudanian zone of Senegal

Sample	Seed colour	Example
	Brown seed testa color	Suvita 2
	Red seed testa color	TVu-8673
	White seed testa color	Melakh
	White.brown seed testa color	TVu-7362
	Black seed testa color	TVu-3552

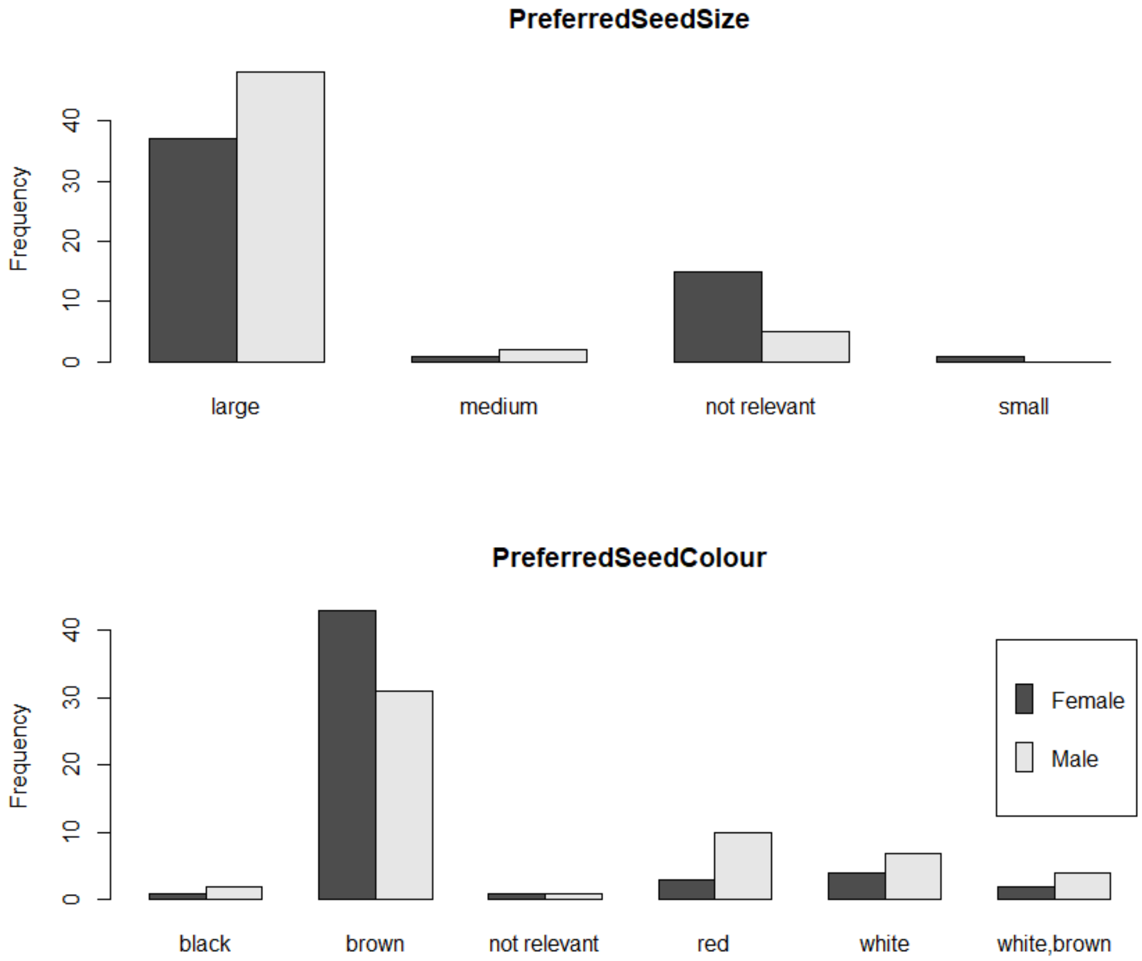


Figure 3. 4: Preferences of seed size and colour by farmers in the Sahelian and Sahelo-Sudanian zone of Senegal as affected by gender

University of Ghana http://ugspace.ug.edu.gh

3.4. Discussion

Farmers participated massively in Semi-Structure Surveys as committed during focus group discussion. Majority of them attended school, only 20 % were illiterate. A clear distinction between Koranic education and formal school enrolment was not assessed in this survey. However, a study in Senegal (André and Demonsant, 2009) on a dataset covering 1,800 households has shown that there was a relationship between Koranic and formal education because people who attended Koranic education were more likely to attend formal education than people who did not. Additionally, the paper stated that competition between the two instances in favour of Koranic education were mainly due to the poor quality of formal schools in rural area.

Sampled farmers were higher in Louga (47.70 %). Tivavouane recorded the lowest number of participants (22.93 %). All divisions where surveys were conducted belonged to the same agro-ecological domain however Louga is the major area where cowpea production occurs hence justifying its superiority in terms of participants compared to other localities. Additionally, it is a strategic division in Senegal attractive to various projects and NGOs promoting investments on the use of technologies generated by ISRA. Very few youths contributed to the study with a participation rate of 16.51%. The low participation is explained by adaptation strategies implemented by populations allowing young people to migrate to the regions of Dakar in order to increase the income by participating in non-agricultural activities not requiring professional qualifications. This is the consequence of traditional agriculture that can no longer feed households because of the joint occurrence of several biotic and abiotic factors.

Men were more concerned in cultivating cowpea in large plots than women. Household tasks, such as buying market condiments, preparing meals, cleaning the house and caring for children, are entrusted to women. Hence, they do not have much time to devote to field work. However

University of Ghana http://ugspace.ug.edu.gh

as indicated in this study, they cultivate cowpea on about 2 ha farms. More profound reasons have been raised that could explain why female farmers do not cultivate large plots such as pregnancy which reduces their productivity and that of other fellow female workers (Mianda, 1998). Men in rural areas generally have more responsibilities than women in terms of feeding people primarily because of polygamy, but also because of the extended family typical of Senegalese in particular or African in general, a family that is not limited to the man, his own wives and children. In most cases, distant relatives tend to be included in the family thus, making its composition extremely large.

It was found in this study that cowpea was utilized for dual or multiple purposes. In fact in Senegal, as in many African countries, consumers utilize cowpea leaves, fresh pods and dry grains (Cissé, 2016). Dual purpose is defined here as food and feed. This indicates that grain yield alone does not appeal to farmers in rural areas; they prefer grain yield and additional traits such as fodder. Obviously, grain yield alone does not mean much in plant breeding because it is underlined by many factors (Plant *et al.*, 1999) that interact resulting in stability and or higher performances of a genotype in specific environments. Our results indicated that in Kebemer, people were using cowpea as human food and animal feed while in Louga and Tivavouane cowpea in addition to the dual-purpose was also used in trading during “the hanger period” as an alternative to have available food for the family (Cissé, 2016; Ehlers and Hall, 1997). In fact, during that period, cultivar with fresh long pods are very valuable in the market and along roads sides where travellers stop to bye. Farmers informed informally that cowpea was being sold in the market in order to generate cash to buy new seeds from seed companies when cropping season were approaching (Hallensleben *et al.*, 2009). Majority of the people in Senegal grow cowpea as a main crop. Respondents also recorded that cowpea trading was a very lucrative business. This is in agreement with the statement of Cisse (2016).

University of Ghana http://ugspace.ug.edu.gh

Across divisions, drought, aphid, Macrophomina, maruca and Striga were the most important threat to cowpea production. Among the constraints which famers considered as of great importance, drought was rated first. Farmers clearly stated that water availability was the first most necessary input for plants to develop. Unambiguously, insufficient water is the most precarious threat to world food production (Farooq *et al.*, 2009). Some pre-breeding studies in Senegal at ISRA identified sources of tolerance to drought (Belko *et al.*, 2012; Halime, *et al.*, 2014) but to date, except Mouride having adaptation to terminal drought, no variety has been released. Ongoing work aiming at identifying and using genetic and genomic resources in the filtering process will make varietal development in Senegal faster and more efficient. In this study, it seemed odd that some farmers did not know about *Striga gesnerioides*. They were referring to *Striga hermonthica* when answering questions on *S. gesnerioides*. Most of the farmers claimed to be able to differentiate the two species and in general they argued that fields were more affected by *S. hermonthica* than by *S. gesnerioides*. Controversially, when the picture of *S. gesnerioides* were brought to them, they responded that they had seen the parasitic weed in cowpea fields and specially, in their own field, too. However, 36 % against 64 % said damages due to *S. gesnerioides* were not affecting cowpea grain yield in a significant way. Various articles reported the negative effects of *S. gesnerioides* on cowpea grain yield (Boukar *et al.*, 2004; Omoigui *et al.*, 2007; Omoigui *et al.*, 2017a,b,c) ranging from 30% to 100 % yield losses. Tonessia *et al.*, (2009 and 2014) reported its prevalence in Senegal in different forms constituting biotypes or races different to all races identified before (Botanga and Timko, 2006). Striga propagate very quickly and to date, none of the released varieties has resistance to races in Senegal. Breeding program on *S. gesnerioides* resistance are ongoing. However, *S. gesnerioides* does not cause economic damage in some specific areas hence, it might not be a priority for some communities. Nevertheless, resistance to *S. gesnerioides* is a valuable additional trait that must be introgressed into farmers' preferred varieties.

University of Ghana http://ugspace.ug.edu.gh

Consumers actually do not buy products per se but rather their characteristics that provide utility. Therefore, it is imperative to identify grain quality trait perceived by growers and consumers as of first choice. Grain quality influences not only the choice of the utilizer but also its pricing. In this study, farmers surveyed in Louga, Kebemer and Tivavouane opted for large-seeded cowpea varieties as first choice. The evidence was corroborated with the results in surveys conducted previously in West Africa (Faye *et al.*, 2004; Langyintuo *et al.*, 2003). The authors indicated that adoption of cowpea varieties would be easier if they were large seeded. In Senegal, it was reported that buyers were willing to pay a premium for larger-seeded cowpea varieties (Faye *et al.*, 2004). Consequently, it is of great interest that cowpea breeders emphasize on grain size, fortunately in the WAAPP program and USAID funding through cowpea climate change ISRA has released 5 varieties for large seed size.

Other important traits were seed coat colour, growth habit and earliness. Farmers perceived brown seed coat colour as very attractive and rated it as first choice in Louga, Kebemer and Tivavouane. In fact, brown seed coated cultivars has more weight in the market than other seed coat colours. Farmers during FGD mentioned that demand for Yacine, a variety developed by Cisse (Cisse *et al.*, 2005) was priced higher in the market. Similar grain colour preferences were reported in south-western parts of Nigeria where farmers preferred brown-seeded grains (Boukar *et al.*, 2013). The same author argued that black colour is not desired in Africa whereas in Cuba and some other Latin American communities it is preferred to other seed coat colours. Dissimilar results were reported in Ghana where farmers were more enthusiastic to cream and white seed coated genotypes (Egbadzor *et al.*, 2015). Growth habit was rated differently in the three divisions. Some farmers were able to distinguish between prostrate and climbing while others did not but took the two as one trait. In Louga farmers opted for prostrate-climbing, in Kebemer their first choice was strictly climbing and in Tivavouane strictly prostrate was of interest. They argued that prostrate varieties were more suited in fields infested to weeds.

University of Ghana http://ugspace.ug.edu.gh

However, women were more interested in erect genotypes probably because they are easier to harvest. This is probably because harvesting, the most difficult task in cowpea cultivation is handled mostly by women. Farmers were interested in extra early varieties than late maturing genotypes. It is noted in Senegal that harvesting of traditional food crops such as peanut and late maturing cowpea genotypes occur between October and December resulting in exhaustion of previous year harvested product. But then if harvest of the previous year were of small quantity, the only alternative is the extra early cowpea which is available for harvesting from August (Cissé, 2016). Extra early varieties were most preferred. Indirectly this confirm the occurrence of drought in these area specially in Louga and Kebemer which were reported earlier (Hall *et al.*, 2003). By developing extra early varieties, we simultaneously assess drought tolerance as early varieties escape terminal drought. It were reported widely that drought escape is an efficient mechanism of plants to cope with drought (Basu *et al.*, 2016; Farooq *et al.*, 2009).

3.5. Conclusion

Drought was the major constraint to cowpea production based on farmers' assessment; The farmers indicated their preference for large seeded and brown testa cowpea seeds; *Striga gesnerioides* is a constraint to cowpea production in certain districts and should be considered in breeding varieties for specific adaptations. Majority of the farmers could identify *Striga gesnerioides* and even some could distinguish it from *S. hermonthica*.

University of Ghana http://ugspace.ug.edu.gh

CHAPTER FOUR

4. COMPARATIVE AGRONOMIC AND MORPHO-PHYSIOLOGICAL RESPONSES OF COWPEA CULTIVARS TO TERMINAL DROUGHT

4.1. Introduction

Cowpea is of valuable importance in arid and semi-arid regions for its seeds, leaves and fodder (Ehlers and Hall, 1997). It has broad adaptation to harsh environments and is widely grown by poor communities in the semi-arid tropics where adverse heat and drought effects are of major significance (Singh, 1997). Cowpea is produced predominantly under rain-fed conditions in regions of sub-Saharan Africa (SSA) where rainfall comes late, is erratic and of short duration (Fatokun *et al.*, 2012). Despite its inherent resilience ability to survive under stress conditions, when cultivated under rain fed conditions, cowpeas suffer the negative effects of heat and the scarcity of rains that result in high seedling mortality, flower abortion and weak pod and grain filling substantially reducing number of pods per plant, 100 seed weight and thus grain yield. In a survey conducted in Senegal in major cowpea producing regions, drought came out as the major constraint in cowpea production. Unfortunately, few pre-breeding studies have been conducted on the improvement of cowpea for its tolerance to water stress in Senegal (Belko *et al.*, 2012; Halime *et al.*, 2014).The need for better adaptation of cowpea released lines is an increasingly urgent issue.

Large variations in cowpea yield under severe drought stress ranging from 30 % to 100 % have been reported (Mai-Kodomi *et al.*, 1999). Nevertheless, phenotyping of assembled lines with different genomic background and their utilization in breeding is limited by the identification of stress responsive traits allowing quick discernment of tolerant / resistant to susceptible lines (Fatokun *et al.*, 2012). Various methods of measurements have been proposed to address drought tolerance of crops such as C isotope discrimination, root phenotyping, wooden boxes

University of Ghana http://ugspace.ug.edu.gh

screening using morphological markers (Hall *et al.*, 2003) or screening under rain out shelter. However, such methods are very complex, sophisticated or laborious and thus might not be most suited methods for highly skilled plant breeders with limited resources. In the meantime, ISRA's breeding activities focused on cowpea's resistance to biotic stresses, with great interest in extra-earliness as an alternative for drought tolerance (Hall *et al.*, 2003). However, the use of early or extra early varieties may not be the appropriate solution to mitigate terminal drought, as these varieties may lack farmers' preferred traits and be very sensitive to intermittent drought.

A consistent and efficient way to address drought tolerance would be the consideration of traits correlated with grain yield in contrasting environments, associated with high heritability and suitable genetic variability and easy to record at a reasonable budget (Araus, 2002; Beebe *et al.*, 2013; Jackson *et al.*, 1996; Polania *et al.*, 2016). This presupposes high skills on stability and adaptation analysis to allow reliable prediction of cultivars' performances (Malosetti *et al.*, 2013).

The objective of this study was to determine terminal drought tolerance of cowpea genotypes by means of morpho-physiological markers and indices involving stability coefficients and drought tolerance indices.

4.2. Materials and methods

4.2.1. Experimental site

Screening cowpea for drought tolerance was done at ISRA research station in Bambey in the offseason from 7 February (sowing) to 22 April (harvest) 2018. Bambey is located in the peanut belt of the Sahelo-Sudan zone. This area is characterized by a 3-month rainy season with mean annual rainfall ranging from 500 to 700 mm during late July, August, September and early October and 9 months of dry season. The soil is characterized by a low clay content, a

University of Ghana http://ugspace.ug.edu.gh

deficiency in phosphorus ($P_2O_5 < 2‰$), a low organic matter content (<1%) and is sandy (Badiane, 1993; Badiane *et al.*, 2000). Soil pH is between 6.18 and 6.6 at 0-20 and 20-40 cm horizon. Between February and April, minimum temperature of 13.5 C° were recorded February 2nd 2018 and maximum temperature of 42.6 C° recorded on March 14th 2018.

4.2.2. Plant material and field management

A set of 112 cowpea genotypes from the collection of ISRA cowpea breeding program were tested in the field for their responses to two different water regimes. Of the germplasm 7 originated from UCR, 48 from ISRA and 57 from IITA. In addition, three checks included drought tolerant genotypes namely Suvita 2, Mouride and IT93K-503-1 and three drought susceptible genotypes Bambey 21, CB46, KVx525 and IT97K-556-6 were included in the study as checks. The population derived from a collection of the ISRA breeding program composed of initially 800 lines on which selfing was done to obtain a core pure collection.

Before sowing, field was ploughed, fertilized with 150 kg / ha of NPK 6:20:10 and watered to the field capacity. Two days after pre-sowing irrigation, genotypes were planted and cultivated under sprinkler irrigation until the initiation of flower buds. Forty-two days after planting (42 DAP), water supply was withheld up to maturity in the water stress condition (WSC) while irrigation was continued regularly in the second condition called well-watered condition (WWC). The fields were kept free of weeds and insects throughout the experiment. Water treatments or environments were separated by 15 meters to avoid undesired runoff.

4.2.3. Experimental design and data collection

Genotypes were planted in plots of 2 rows of 3 m long where observations were taken. Plants intra-row spacing was 0.25 m. Plots were separated by 0.75 m. Accessions were arranged in a resolvable incomplete block design with 3 replications of 14 blocks composed of 8 plots each.

University of Ghana http://ugspace.ug.edu.gh

Data collected included grain yield, 100 seed weight, number of pods per plant, days to flowering and days to maturity, Stem greenness, wilting, SPAD and leaf area index LAI. Stem greenness and wilting were scored as suggested by (Muchero *et al.*, 2008) with slight modification in scoring scale. In this study, we used a scale of 0 to 4 for the two morphological markers. Scoring of 0 was given to plants with no stem greenness and no sign of wilting while a score of 4 was given to plants completely green (stem) and wilted. Scoring observations for stem greenness was taken at 56 DAP while wilting scores were taken at 59 DAP. SPAD observations were recorded from April 3 to 8th coinciding at 56-61 DAP. LAI observations were taken from April 10th to April 13th which coincided at 63-66 DAP. Canopy density was measured using a ceptometer Decagon Device referenced as AccuPAR LP-80. Two measures were taken in each plot under the canopy to optimize quality of measurements. LAI measurements were made on clear sunny days between 11am to 3pm. Greenness of plants were measured using a chlorophyll meter referenced as SPAD 502. Before measuring, 3 healthy representative plants were fixed as samples in order to allow consistency.

4.2.4. Data analysis

Residual maximum likelihood (REML) procedure was used to analyse data with GenStat 12.0 software (Payne *et al.*, 2009) following the linear mixed model $Y = \mu + Gi + Wj + (G\times W)ij + e$ where Y is the variable effects, μ the overall mean, Gi the genotypic main effects, W the water regime effect, $(GW)ij$ the interaction effect and e the experimental error. Terms considered as fixed were μ and W while Gi, $GWij$ and e were random. Significance test in fixed effects were done using Wald Statistics. Post hoc analysis was done using R Version 1.1.442 – © 2009-2018 RStudio, Inc. Various way used for the ranking of genotypes were: based on drought tolerance indices combining stability and performance, based on stability of genotypes accounting for performance of the best line in each environment and based on the overall mean.

University of Ghana http://ugspace.ug.edu.gh

4.3. Results

4.3.1. Summary of results and combined ANOVA on agro-morpho-physiological traits

Evidence of intense stress on plants was the level of reduction observed on agronomic and morpho-physiological traits. Traits with high rate of reduction were grain yield, number of pods per plant, wilting and leaf area index. Flowering and maturity parameters had very low reduction rate. For maturity, wilting and SPAD, negative mean reduction % were observed during the trial indicating higher mean values obtained under water stress condition (WSC) than under well-watered condition (WWC) (Table 4.1).

Table 4. 1: Relative mean performance of agronomic traits of 112 cowpea genotypes evaluated under well-watered (WWC) and water stress (WSC) conditions at ISRA CNRA de Bambey, 2018

Trait	**Mean in WWC**	**Mean in WSC**	**Mean Reduction** (%)	**Reduction range** (%)
Grain yield (Kg/ha)	1127.83	131.70	87.74	71.09 – 98.32
Seed weight (g)	15.01	13.72	3.44	-79.19 – 43.70
Pods per plant	19.65	8.72	55.01	22.56 – 77.79
Flowering	51.65	51.46	0.28	-15.44 – 21.04
First pod mature	63.98	63.05	1.41	-10.71 – 9.33
Maturity	77.89	78.41	-0.68	-5.27 – 12.16
Stem greenness	3.64	3.23	12.17	-9.57 – 77.10
Wilting	2.114	2.81	-43.85	-319.46 – 31.51
Chlorophyll content (SPAD)	69.20	69.89	-1.12	-16.32 – 8.19
Leaf area index (LAI)	2.23	0.65	69.05	45.09 – 86.48

WWC= well-watered condition; WSC= water stress condition

Grain yield, Wilting and Leaf area index (LAI): Results of the combined ANOVA (Table 4.2) showed evidence of a statistically significant interaction effect between main factors Genotype and Environment over Genotype (based on magnitude of components) on grain yield,

University of Ghana http://ugspace.ug.edu.gh

wilting and leaf area index (LAI). This implies inferences on differences in mean treatment responses for genotypes should be made separately for WWC and WSC. Low correlation between environments were observed for grain yield (r = 0.2591), wilting (r = 0.3516) and LAI (r = 0.2544). Grain yield, wilting and LAI were highly heritable under WWC (respectively 0.7091, 0.6986, 0.6754) while under WSC heritability was 0.5586, 0.8650, 0.3377 for the traits, respectively. The results indicated an improvement of wilting scoring under WSC.

Table 4. 2: Combined ANOVA for grain yield, wilting and leaf area index of 112 cowpea genotypes evaluated under well-watered and water stress conditions at ISRA, 2018.

Source of variation		**Grain yield**		**Wilting**		**Leaf area index**	
Random	**d.f.**	**Variance**	**s.e.**	**Variance**	**s.e.**	**Variance**	**s.e.**
Genotype	111	9068	8899	0.1739	0.0512	0.0492	0.0378
Interaction	111	56657	11494	0.2145	0.0458	0.2091	0.0475
Fixed	**d.f.**	**Wald (DF)**	**Pvalue**	**Wald (DF)**	**Pvalue**	**Wald (DF)**	**Pvalue**
Environment	1	295.2	<0.001	55.25	<0.001	200.97	<0.001
Replication	2	10.8	0.038	3.34	0.508	3.41	0.496

d.f= degree of freedom; s.e= standard error

Seed weight, number of pods per plant and stem greenness: There was no evidence of a statistically significant interaction effect between main factors Genotype and Watering regime over Genotype (based on magnitude of components) on seed weight, number of pods per plant and stem greenness. Watering regime main effect was highly significant ($P < 0.001$) for the three traits. Results suggest a post-hoc analysis to determine either an inference based on genotype mean in environments, or an inference based on genotype average in each environment separately. High correlation between water conditions was observed for seed weight (r = 0.6974) and stem greenness (r = 0.7438) while number of pods per plant under WWC and WSC were moderately correlated (r = 0.4248). Seed weight, number of pods per plant and stem greenness were highly heritable under WWC (respectively 0.9386, 0.5485and

University of Ghana http://ugspace.ug.edu.gh

0.659) while under WSC respective trait's heritability was 0.6911, 0.6337, 0.8376. Data indicated an improvement of stem greenness scoring under WSC.

Table 4. 3: Combined ANOVA for seed weight, number of pods per plant and stem greenness of 112 cowpea genotypes evaluated under well-watered and water stress conditions at ISRA CNRA de Bambey, 2018.

Source of variation		**Seed weight**		**Number of pods per plant**		**Stem greenness**	
Random	**d.f.**	**Variance**	**s.e.**	**Variance**	**s.e.**	**Variance**	**s.e.**
Genotype	111	14.035	2.338	11.34	3.17	0.3292	0.0598
Interaction	111	3.121	0.857	6.57	2.84	0.1003	0.0288
Fixed	**d.f.**	**Wald (DF)**	**Pvalue**	**Wald (DF)**	**Pvalue**	**Wald (DF)**	**Pvalue**
Environment	1	13.41	<0.001	159.35	<0.001	38.68	<0.001
Replication	2	1.12	0.89	4.74	0.325	13.98	0.013

d.f = degree of freedom; s.e. = standard error

Days to flowering, days to first pod mature and days to maturity: Interaction effect between main factors Genotype and Environment over Genotype (based on magnitude of components) on days to flowering, days to first pod mature and days to maturity were not significant. Environment main effect on each of the three parameters was also not significant (Table 4.4). Results suggest a single inference based on average of genotype mean across environments. High correlation between environments were observed for days to flowering (r = 0.6888) and days to first pod maturity (r = 0.7738) while low correlation were observed for days to maturity (r = 0.4446). Seed weight, number of pods per plant and stem greenness were highly heritable under WWC (respectively 0.8218, 0.5485, 0.659) and under WSC. Respective trait's heritability was 0.8189, 0.792, 0.6947.

University of Ghana http://ugspace.ug.edu.gh

Table 4. 4: Combined ANOVA for Days to flowering, days to first pod mature and days to maturity of 112 cowpea genotypes evaluated under well-watered and water stress conditions at ISRA CNRA de Bambey, 2018.

Source of variation		**Days to flowering**		**Days to first pod mature**		**Days to maturity**	
Random	**d.f.**	**Variance**	**s.e.**	**Variance**	**s.e.**	**Variance**	**s.e.**
Genotype	111	10.665	1.635	11.87	1.862	5.32	1.11
Interaction	111	0.338	0.401	0.547	0.478	0.48	0.69
Fixed	**d.f.**	**Wald (DF)**	**Pvalue**	**Wald (DF)**	**Pvalue**	**Wald (DF)**	**Pvalue**
Environment	1	0.06	0.809	9.2	0.004	2.63	0.11
Replication	2	7.7	0.117	2.72	0.608	7.2	0.139

d.f= degree of freedom; s.e = standard error

Chlorophyll content: Interaction effect between main factors Genotype and Environment over Genotype (based on magnitude of components) on chlorophyll content (SPAD) were not significant. Environment main effect was not significant (Table 4.5) suggesting poling average of each genotype to make inferences. Low correlation between environments was observed (r = 0.3590). High heritable under WWC (0.5819) and WSC (0.4961).

Table 4. 5: Combined ANOVA for chlorophyll content (SPAD) and leaf area index (LAI) of 112 cowpea genotypes evaluated under well-watered and water stress conditions at ISRA CNRA de Bambey, 2018.

Source of variation		**SPAD_3**	
Random	**d.f.**	**Variance**	**s.e.**
Genotype	111	11.11	3.11
Interaction	111	5.55	2.81
Fixed	**d.f.**	**Wald (DF)**	**Pvalue**
Environment	1	1.08	0.303
Replication	2	1.19	0.879

d.f= degree of freedom; s.e = standard error

University of Ghana http://ugspace.ug.edu.gh

4.3.2. Performance of genotypes in water regimes

Grain yield: Stress intensity based on grain yields in the two environments was 88.32 % with a minimum and maximum yield reduction of 71.09 % and 98.32 % respectively. All genotypes had greater yield under well-watered condition (1127.83 kg/ha) than in stressed environment (131.70 kg/ha). Grain yield of genotypes grown under WWC ranged from 446.11 to 1956.81 kg/ha. Top highest grain yield predicted means were obtained with 78-8 (1956.81 kg/ha), TVu 4622 (1914.24 kg/ha), 59-13 (1771.45 kg/ha), BC4str-11 (1727.97 kg/ha) and 58-162 (1674.23 kg/ha). In stressed plants grain yield ranged from 12 to 262.74 kg/ha. Genotypes 59-39, 67-219, TVu 9556, TVu 1391 and IT93K-693-2 were the highest performing lines under WSC with 262.74 kg/ha, 259.69 kg/ha, 248.48 kg/ha, 244.03 kg/ha and 239.50 kg/ha respectively. Twenty-two genotypes recorded better yield compared to the best performing check Suvita 2 which yielded 157.53 kg/ha under WSC. Forty-eight genotypes outperformed the lowest performing check CB 46 which recorded 114.04 kg/ha. In general, genotypes performed better under well-watered condition (1127.8 kg/ha) than in water-stressed (131.7 kg/ha).

Wilting: Wilting average scores across environments was 2.46 with a range of 0.49 and 3.89. Genotypes under WWC recorded a mean wilting score of 2.11 with a range between 0.49 and 3.48 while genotypes under WSC recorded a mean wilting score of 2.81 with a range between 1.53 to 3.89. Under WWC, best performing genotype scores for wilting were obtained with 58-57 (0.49), ACC-006-1 (0.84), IT97K-499-39 (0.63), Mouride (10.0) and N'diambour (0.89) while highest scores synonymous of wilted plants were obtained with genotypes TVx 3236 (3.01), TVu 1037 (2.99), BC4str-5 (3.04), 66.64 (3.48) and 3301 (2.96). Under WSC, best performing genotype scores for wilting were obtained with 58-74 (1.81), 78-39 (1.80), IT95K-1095-4 (1.53), IT97K-499-39 (1.80) and TVu 10100 (1.79) while highest scores synonymous of wilted plants were obtained with genotypes TVu 9556 (3.89), TVu 15220 (3.81), Marfo Tuya (3.86), 60.6 (3.83) and 524B (3.81). Drought sensitive checks namely Bambey 21, CB

University of Ghana http://ugspace.ug.edu.gh

46, KVx 525 and IT97K-556-6 respectively had wilting mean score of 3.55, 3.54, 3.29 and 2.69 under WSC. Eighteen (18) genotypes outperformed Mouride which was the check with the lowest wilting score while twelve (12) genotypes underperformed the check with the highest wilting score namely Bambey 21.

Leaf area index (LAI): Leaf area index (LAI) average value were 1.44 across environments with a range between 1.04 and 3.88. Mean LAI value for genotypes under WWC were 2.23 and ranged between recorded 1.04 and 3.88 while mean of 0.65 ranging between 0.51 to 0.90 were observed under WSC. Under WWC, best performing genotypes were UCR 707 (3.88), TVu 4984 (3.49), TVu 1536 (3.41), 78-8 (3.37) and TVu 1036 (3.33) while lowest LAI were recorded for TVu 15639 (1.04), SH50 (1.07), IT98K-555-1 (1.25), IT00K-1263 (1.27) and Marfo Tuya (1.35). Under WSC, highest LAI mean scores recorded were for genotypes N'diambour (0.90), TVu 4622 (0.87), IT83D-442 (0.83), 60-3 (0.81) and 78-40 (0.80) while lowest LAI scores were recorded for IT97K-819-132 (0.510), TVu 2736 (0.515), 58-55 (0.524), UCR 707 (0.524) and IT82E-18 (0.527). Drought sensitive checks namely Bambey 21, CB 46, KVx 525 and IT97K-556-6 respectively had LAI mean score of 0.544, 0.611, 0.599 and 0.614 under WSC. Thirty (30) genotypes outperformed Suvita 2 (0.68) which were the check with the highest LAI score while seven (7) genotypes underperformed the check with the smallest LAI value namely Bambey 21 (0.54).

Seed weight: Weight of 14.36 g and a range between 5.34 g and 25.90 g were recorded for 100 seeds across water conditions. Best 100 seed weight were recorded for genotypes 3178 (25.90 g), 3211 (25.37 g), 524B (24.64 g), SH50 (23.79 g) and Diongoma (17.70 g). Drought sensitive checks namely Bambey 21, CB 46, KVx 525 and IT97K-556-6 recorded respectively 16.35 g, 19.55 g, 17.56 g and 16.62 g. Genotypes in well irrigated condition showed greater 100 seed weight (15.01 g) than those grown under water stress (13.73 g). Average weight of 100 seeds varied between 5.34 to 25.90 g. Genotypes with smallest (<10 g), small (10-15 g), medium

University of Ghana http://ugspace.ug.edu.gh

(>15<20 g) and large seed size (>20) were respectively 3.6 %, 60.7 %, 27.7 % and 8 % of the total assembled germplasm.

Stem greenness: Overall stem greenness score across environments was 3.44 ranging between 1.44 to 3.91. Genotypes with lowest stem greenness were 60-6 (1.44), TVu 15220 (1.90), 67-30 (1.93), TVu 12710 (2.00) and 524B (2.18). Drought sensitive checks namely Bambey 21, CB 46, KVx 525 and IT97K-556-6 respectively recorded stem greenness scores of 3.74, 2.94, 3.47 and 3.86. Mean scores were 3.86 in irrigated condition and 3.24 under water-stressed plants. In non-stressed environment, best performing scores were recorded with genotypes BC4str-11 (3.94), 58-74F (3.92), TVu 1536 (3.92), N'diambour (3.92), and IT97K-499-35 (3.92) while genotypes 60-6 (2.34), TVu 15220 (2.72), 67-30 (2.79), TVu 9556 (2.94) and 524B (2.99). In stressed environment, best genotypes recorded highest stem greenness :78-43 (3.90), IT97K-499-39 (3.89), 58-74F (3.89), IT83D-442 (3.89), IT97K-499-35 (3.89), and genotypes with lowest stem greenness were 60.6 (0.53), TVu 12710 (0.80), 67-30 (1.07), TVu 15220 (1.08), TVu 15114 (1.35).

Number of pods per plant: Number of pods per plant across environments was 14.19 with a range between 9.08 and 23.80. Genotypes under WWC recorded a mean of 19.65 with a range between 12.30 and 33.01 while genotypes under WSC recorded a mean of 8.72 with a range between 4.73 and 20.77. Under WWC, best performing genotype scores were obtained with 60-6 (26.83), 67-30 (26.79), KVx525 (33.01), TVu 1036 (30.09) and TVu 3236 (27.11) while lowest scores were obtained with genotypes 3217 (14.17), 58-86 (13.74), IT93K-693-2 (14.37), IT93K-93-10 (12.30) and UCR707 (14.34). Under WSC, best performing genotypes were 524B (14.47), 60-6 (20.77), TVu15220 (17.17), TVu 3236 (15.78) and UCR 162 (14.89) while lowest performing genotypes were 58-1 (5.44), TVu 4535 (5.49), TVu 6641 (5.62), UCR707 (5.06) and Yacine (4.73). Drought sensitive checks namely Bambey 21, CB 46, KVx 525 and IT97K-556-6 respectively had number of pods per plant of, respectively, 8.85, 8.85, 7.17 and

University of Ghana http://ugspace.ug.edu.gh

9.49 under WSC. Twenty-nine (29) genotypes outperformed KVX525 which were the check with the highest number of pods per plant while twenty-four (24) genotypes underperformed IT97K-556-6 the check with the lowest number of pods per plant.

Days to flowering: Genotype flowered at 51.56 days with a range between 46.33 and 62.75. Genotypes under WWC recorded a mean of 51.65 days with a range between 46.38 and 65.18 while genotypes under WSC recorded a mean of 51.46 days with a range between 45.56 and 67.25. Under WWC, best performing genotype scores were obtained with 524B (46.85), 60-6 (47.46), 67-30 (46.44), TVu 3346 (47.07) and UCR707 (46.38) while latest flowering genotypes were 58-57 (58.25), ACC-006-1 (65.18), IT97K-499-35 (57.1), IT97K-499-39 (57.44) and N'diambour (57.07). Under WSC, best performing genotype were 524B (46.21), 60-6 (45.90), 78-8 (53.22), CB5 (47.67) and TVu 3346 (47.07) while lowest performing genotypes were 58-57 (58.25), Diongoma (56.02), TVu 10100 (54.02), TVu 4535 (54.67) and Yacine (54.51).

Days to first pod maturuty: Mean of genotypes across environment was 63.52 days with a range between 56.67 and 77.13 days. Mean of genotypes under WWC and WSC were 63.98 and 63.05 days. Ranges were respectively [56.68-77.70] and [56.47-76.45]. Best genotypes under WWC were 60-6 (56.68), 67-30 (57.21), TVu 12710 (57.73), TVu 15220 (57.15) and TVu 1656 (57.68). Under WSC best genotypes were 60-6 (58-68), 67-30 (57.21), TVu 15220 (57.15), TVu 1656 (57.68) and TVu 2736 (58.37).

SPAD: Chlorophyll content of genotypes across environments was 69.55 with a range of [63.35-76.52]. best genotypes under WWC were CB46 (77.66), IT81D-994 (77.33), IT84S-2246 (78.91), IT93K-693-2 (75.83) and TVu 15114 (75.01). Among all checks, CB46 was the best one under WWC with SPAD value of 77.66 and outperformed 109 genotypes. Best SPAD values under WSC were obtained with 58-57 (77.51), IT93K-693-2 (77.21), Bambey 21

University of Ghana http://ugspace.ug.edu.gh

(76.49), TVu 14190 (75.31) and TVu 1656 (75.22). Only 2 genotypes outperformed Bambey 21.

Days to maturity: Mean phenotypic value across environments was 78 days. Days to maturity ranged between 71 to 81 DAP. Mean of genotypes was 77.90 days with a range between 74 and 85 days while under water stress environment, their mean were 78.41 days with a range between 66 and 82 days. Late maturing genotypes were IT93K-503-1, ACC-006-1, Yacine, 58-4 and N'diambour with recorded days to maturity between 81 and 82 DAP. Genotypes 60-6, 67-30, 524B, TVu 12710 and TVu 15720 were the earliest matured lines at 71 to 75 DAP.

Below is presented normality and homoscedasticity test for traits for which no interaction effect was observed while main factors were of significant effects hence suggesting post-hoc tests. In normality test, Ho were rejected for all the three traits namely stem greenness, seed weight and pods per plant. This is a clear indication that the data were not normally distributed. In homoscedasticity test ratio of variances below the critical value of the standard deviation except for seed weight however, kurtosis confirmed leptokurtic, mesokurtic and platikurtic distribution of estimate of variances. Normal distribution is assumed for seed weight pods per plant.

Table 4. 6: Normality and homoscedasticity tests for stem greenness, seed weight and pods per plant of 112 cowpea genotypes evaluated under well-watered and water stress conditions at ISRA CNRA de Bambey, 2018.

Trait	Shapiro-Wilk Test			Significance of variances			
	Calculated W	**p-value**	**Critical W**	**sd^2**	**sd^2**	**sd Ratio**	**Kurtosis**
1	0.726347	2.68E-11	0.98764	0.408	1.112	1.6509059	5.573
2	0.964646	2.28E-05	0.98764	23.74	26.08	1.0481259	0.333
3	0.928691	5.98E-09	0.98764	87.38	28.61	1.7476203	-0.935

1: stem greenness; **2**: seed weight; **3**: pods per plant

University of Ghana http://ugspace.ug.edu.gh

Results of Welch's t-test showed that only seed weight confirmed its consistence across environments. Ranking of data as a consequent will be done separately according to table.

Table 4. 7: Welch's t-test of statistical significance for stem greenness, seed weight and pods per plant of 112 cowpea genotypes evaluated under well-watered and water stress conditions at ISRA CNRA de Bambey, 2018.

Trait	Welch's t-test	Implication
Stem greenness	< 2.52E-09***	Ranking of genotypes for each environment
Seed weight	0.1223 ns	Ranking of genotypes across environments
Pods per plant	< 2.2e-16 ***	Ranking of genotypes for each environment

Figures with *** indicates high significance at $p < 0.01$; ns indicates no significance at $p < 0.05$

4.3.3. Relationship among plant traits using correlation and principal component analyses

Tables 4.8 and 4.9 indicate respectively correlation between traits observed under WWC and WSC. Under WWC, grain yield was moderately correlated to SPAD_3 (r = -0.30), an indication that high chlorophyll content adversely affected grain yield. On the other hand, LAI_4 had a positive correlation with grain yield (r = 0.59) indicating that higher yield resulted from higher LAI values. High correlations were also found between days to flowering and first pod maturity (r= 0.67), days to flowering and days to maturity (r=0.54), days to flowering and stem greenness (r=0.50), days to flowering and wilting] (r=-0.54) and stem greenness and wilting (r=-0.60).

University of Ghana http://ugspace.ug.edu.gh

Table 4. 8: Correlation coefficients among traits of 112 cowpea genotypes evaluated under well-watered conditions at ISRA CNRA de Bambey, 2018.

Codes	Variables	1	2	3	4	5	6	7	8	9
1	**GrainYield**	**1**								
2	**SeedWeight**	-0.20	**1**							
3	**PodsPlant**	0.18	-0.43	**1**						
4	**Flowering**	0.21	-0.08	0.07	**1**					
5	**FirstPodMat**	0.11	0.27	-0.02	0.67	**1**				
6	**Maturity**	0.04	0.09	0.00	0.54	0.36	**1**			
7	**Stemgreeness**	0.11	0.16	-0.14	0.50	0.52	0.45	**1**		
8	**Wiltering**	0.10	-0.12	0.18	-0.54	-0.43	-0.53	-0.60	**1**	
9	**SPAD_3**	-0.30	0.35	-0.31	-0.21	-0.04	-0.10	-0.18	0.02	**1**
10	**LAI_4**	0.59	-0.25	0.17	0.43	0.23	0.32	0.37	-0.32	-0.52

Figures highlighted in yellow are significant correlations at $p < 0.05$

Grain yield of plants grown under water stress was also moderately correlated to LAI_4 (r = 0.35) (Table 4.9). Higher correlations coefficients were found for days to flowering and first pod matured (r= 0.67), days to maturity and stem greenness (r= 0.75) and stem greenness and wilting (r=-0.74), number of pods per plant and maturity (r = -0.52), number of pods per plant and stem greenness (r = -0.54), days to flowering and stem greenness (r = 0.58), days to flowering and wilting (r = -0.58) and maturity and wilting (r = -0.52).

University of Ghana http://ugspace.ug.edu.gh

Table 4. 9: Correlation coefficients among traits of 112 cowpea genotypes evaluated under water stress conditions at ISRA CNRA de Bambey, 2018.

Codes	Variables	1	2	3	4	5	6	7	8	9
1	**GrainYield**	**1**								
2	**SeedWeight**	-0.02	**1**							
3	**PodsPlant**	0.29	-0.04	**1**						
4	**Flowering**	-0.19	-0.07	-0.26	**1**					
5	**FirstPodMat**	-0.17	0.19	-0.24	0.67	**1**				
6	**Maturity**	-0.20	0.07	-0.52	0.46	0.43	**1**			
7	**Stemgreeness**	-0.25	-0.04	-0.54	0.58	0.49	0.75	**1**		
8	**Wiltering**	0.30	0.17	0.41	-0.58	-0.43	-0.52	-0.74	**1**	
9	**SPAD_3**	0.20	0.14	0.14	0.06	0.08	-0.14	-0.14	0.23	**1**
10	**LAI_4**	0.35	-0.10	-0.06	0.33	0.16	0.28	0.26	-0.26	-0.02

Figures highlighted in yellow are significant correlations at $p < 0.05$

First two axes of the principal component biplot explained 98.51 % of the variation observed in the dataset composed of yield under irrigated and water stress conditions and drought tolerance indices. The biplot displays a first cluster composed of correlated variables namely geometric mean productivity, drought tolerant index, grain yield under stressed condition, grain yield under well-watered condition and mean productivity and a second cluster composed of stress susceptibility index and reduction rate. Prior to trait contribution, very high correlations were observed with reduction rate and stress susceptibility index ($r = 1$) and geometric mean productivity and drought tolerance index ($r = 0.983$) indicating redundancy of variables (Table 4.10).

University of Ghana http://ugspace.ug.edu.gh

Table 4. 10: Correlation coefficients between grain yield stress tolerant indices for 112 cowpea genotypes evaluated under well-watered and water stress conditions at ISRA CNRA de Bambey, 2018.

Variables	Gyield_ETM	Gyield _STR	Reduction	MP	SSI	GMP	DTI
Gyield_ETM	**1**						
Gyield _STR	0.254	**1**					
Reduction	0.446	-0.703	**1**				
MP	0.989	0.392	0.318	**1**			
SSI	0.446	-0.703	1.000	0.318	**1**		
GMP	0.714	0.851	-0.252	0.807	-0.252	**1**	
DTI	0.699	0.844	-0.242	0.792	-0.242	0.983	**1**

MP= Mean productivity; SSI= Stress Susceptibility Index; GMP= Geometric Mean Productivity; DTI= Drought tolerance Index. Figures highlighted in yellow and blue are the highest correlations observed; **Gyield_ETM**= *Grain yield in Well water Condition and* **Gyield_STR**= *Grain yield in Well stress Condition.*

In the first dimension, GMP and DTI contributed to 24.697 % and 24.286 % respectively while the residual contribution was accounted for by grain yield under water stress (17.624), mean productivity (16.980), grain yield under water stress (13.445). Reduction rate and stress susceptible index were the least contributing traits with 1.484 % and 1.484 % respectively. Rationally, drought tolerance index (DTI) can be substituted for GMP to allow for reduction of duplicated variables. In the second cluster reduction rate and stress susceptibility index contributed more than other traits with respectively 32.213% and 32.213 %. These variables were earlier identified as redundant variables and thus emphasis was put on SSI to the detriment of reduction rate for the rest of the analysis. Other minor contribution in the second dimension included grain yield of irrigated plants (15.310), mean productivity (10.569), grain yield under water stress plants (9.680), geometric mean productivity (0.008) and drought tolerance index (0.007). Additionally, negative correlation was observed in susceptibility parameters (reduction rate and stress susceptibility index) and stability and drought tolerance parameters (drought tolerance index and geometric mean productivity). Based on these results, first

University of Ghana http://ugspace.ug.edu.gh

dimension can be associated to stability and performance or tolerance of genotypes to terminal water deficit defined by geometric mean productivity while the second dimension is associated with cultivar sensitivity defined as stress susceptibility index.

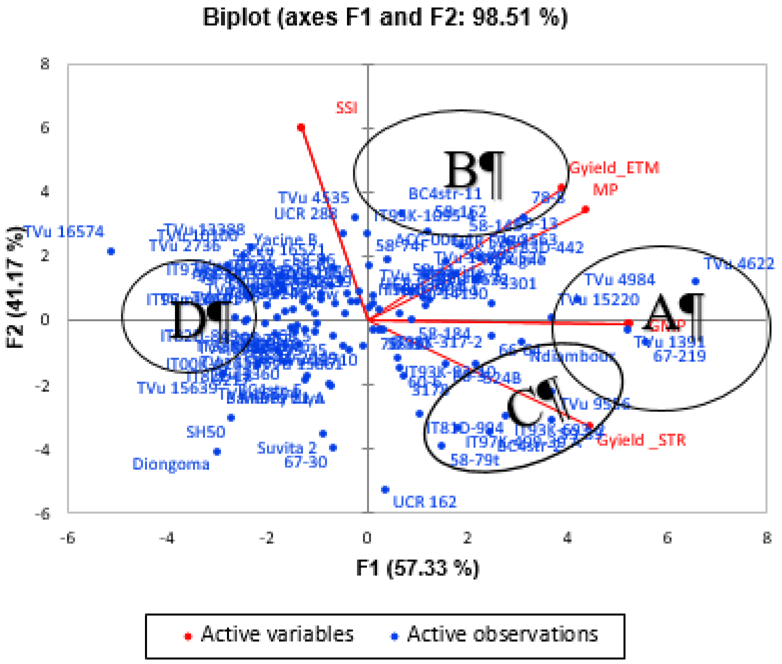


Figure 4. 1: Biplot display of drought tolerance indices and yields

Stress susceptibility index (SSI), grain yield under well-watered condition (Gyield_ETM), mean productivity (MP), geometric mean productivity (GMP), grain yield under water stress condition (Gyield_STR) and active observations.

According to information obtained from the biplot, genotypes can be grouped into 4 groups: A, B, C and D. Group A is high yielding genotypes expressing higher superiority in the two contrasting environments, Group B is high yielding genotypes favoured by the WWC, Group C is high yielding genotypes favoured under WSC and Group D low yielding genotypes under both well-watered and water stress conditions.

4.3.4. Ranking of genotypes based on stability and high performance

Based on GMP which has been shown as an indicator of most stable and high yielding genotypes across environments, the best 15 genotypes are listed in Table 4.11. Genotypes having the highest score were ranked as most stable and high yielding. Table 4.11 also includes additional information on grain yield under water stress and well-watered condition of best genotypes using GMP-based ranking.

Table 4. 11: Ranking of genotypes based on geometric mean productivity

Genotype	**GMP**	**Grain yield in WWC**	**Grain yield in WSC**
TVu 4622	679.085	1925.852	239.456
67-219	639.632	1575.410	259.697
TVu 1391	623.448	1592.782	244.031
TVu 4984	578.653	1625.040	206.050
TVu 15220	557.125	1496.630	207.392
TVu 9556	553.918	1234.774	248.487
59-39	549.238	1148.118	262.745
N'diambour	531.380	1348.010	209.468
IT93K-693-2	508.944	1081.513	239.502
78-8	506.695	1963.260	130.772
66-64	503.299	1313.140	192.904
3301	503.127	1444.107	175.289
IT83D-442	502.895	1624.803	155.652
59-13	502.815	1781.447	141.920
BC4str-2	490.146	1007.218	238.521

GMP= Geometric mean Productivity; WWC= Well-watered Condition; WSC= Water-stressed Condition

4.3.5. Phenotypic stability and adaptation analysis on grain yield

4.3.5.1.Ranking of genotypes based on cultivar Superiority performance

Genotypes were ranked in figure 4.2. Based on stability superiority measures (Lin and Binns (1988), Figure 4.2 is a visual representation of the most stable and high yielding along with least stable genotypes compared with the performance of the check genotypes.

University of Ghana http://ugspace.ug.edu.gh

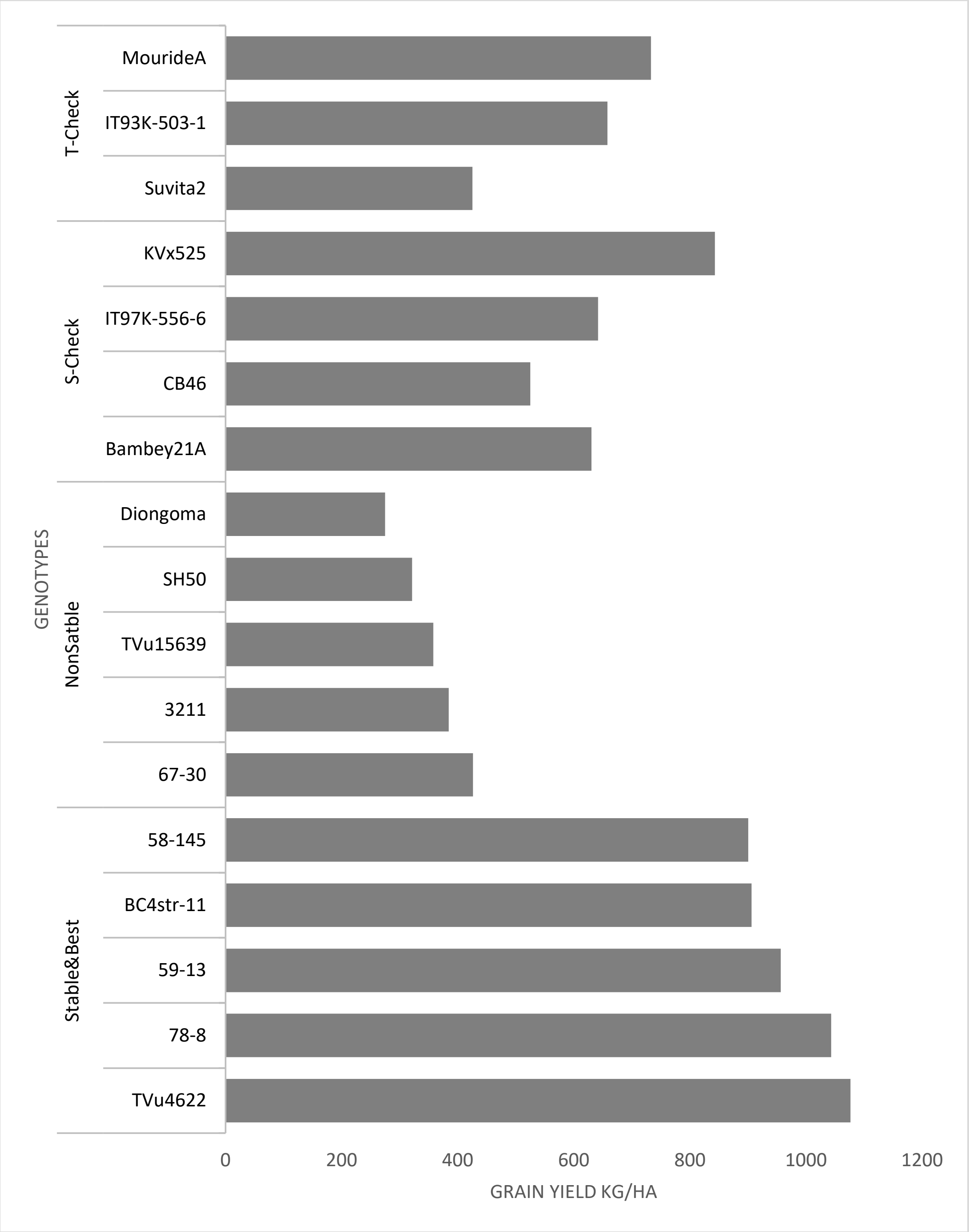


Figure 4. 2: Stability superiority ranking of the most stable and high yielding cowpea varieties, along with least stable genotypes compared with the performance of the check genotypes.

University of Ghana http://ugspace.ug.edu.gh

Table 4. 12: Rank of genotypes based on cultivar superiority index.

Attribute	Genotypes	Cultivar Superiority	Ranking
Best stable	TVu4622	589	1
	78-8	4354	2
	59-13	12240	3
	BC4str-11	20991	4
	58-145	24583	5
Worse Stable	67-30	405750	108
	3211	419505	109
	TVu15639	452453	110
	SH50	510770	111
	Diongoma	576896	112
Susceptible Checks	Bambey21A	180558	52
	CB46	266119	82
	IT97K-556-6	156953	43
	KVx525	48058	14
Tolerant Check	Suvita2	401920	107
	IT93K-503-1	154307	42
	Mouride	102471	26

4.3.5.2.Specific adaptation of cultivars for grain yield

Genotypes tested in the contrasting environments were well ranked according to their specific performance under both well-watered and water stress conditions. Some genotypes were high yielding such as 78-8 and BC4str11 while Suvita 2 yield was low under irrigated condition. Under WSC, the best genotypes were 58-39, 67.219, TVu9556, TVu1391, IT93K-693-2. The five best and lowest performing genotypes under each of WWC and WSC are presented in Figure 4.3.

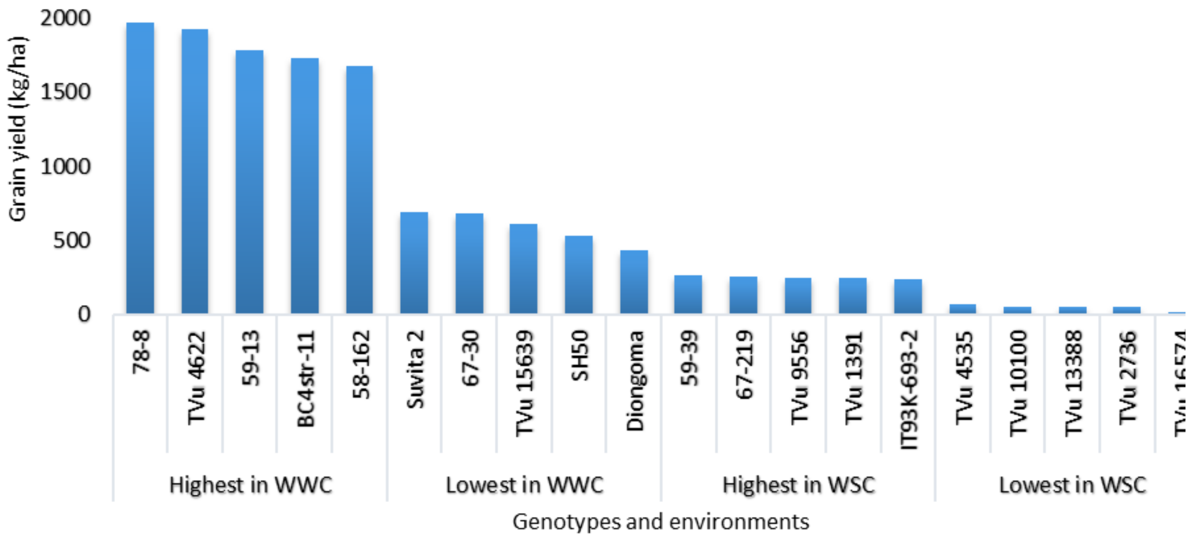


Figure 4. 3: Grain yield of highest and lowest performing genotypes under well-watered and water-stressed conditions at ISRA CNRA de Bambey, 2018.

4.3.6. Covariance and correlation between stability indices

Evident covariance was between Wricke's Ecovalence and Static Stability (cov =5.25) (Table 4.13). A weaker linear relationship between Wricke's Ecovalence and Cultivar Superiority were observed. It was found that Geometric mean productivity do not covariate with cultivar superiority and, Static stability and Wricke's Ecovalence.

Table 4. 13: Similarity between indices for ranking of genotypes

	Cultivar Superiority	**Static Stability**	**Wricke's Ecovalence**	**GMP**
Cultivar Superiority	1054.666667	-1031.540541	-20.91891892	778.801802
Static Stability	-0.97807257	1054.666667	5.252252252	-632.594595
Wricke's Ecovalence	-0.01983463	0.0049800	1054.666667	-89.4954955
GMP	0.73843407	-0.59980524	-0.08485666	1054.66667

University of Ghana http://ugspace.ug.edu.gh

4.4. Discussion

Water stress intensity highly affected grain yield, LAI, wilting and number of pods per plant of the cowpea genotypes evaluated in this study. Similar inferences were made by (Souza *et al.*, 2017) in whose study drought reduced yield by 72% and LAI by 42%. Our results were also in agreement with (Singh and Malik, 1983) who reported that seed weight decreased as stress intensity increased. Insensitivity of grain yield to changes in environment can only be predicted from very low-performing genotypes that do not take advantage of favourable environments. This phenomenon can be understood easily by considering resurrection plants that had been exposed to long term drought periods (years) forcing them to optimize their growth to survive at the expense of seed production. Consequently, plants with similar responses when grown under water stress environments produce subsistence grain yield which is way far lower than that obtained under normal watering (Basu *et al.*, 2016). The observed genetic variation indicates that the population used is suitable to address drought tolerance.

There were minor reductions in seed weight, flowering, first pod maturity, maturity and chlorophyll content (SPAD), with no statistically significant genotype x water regime interaction effects. Therefore, differences among the genotypes for these traits were independent of water availability, unlike stem greenness and number of pods per plant for which water regime effect was highly significant. In lentil plants where it was found that flowering, podding and maturity in stressed treatment occurred much earlier than plant in the control treatment (Sehgal *et al.*, 2017). Some earlier studies have reported an escape strategy which allow plants to fit their cycle to available water (Farooq *et al.*, 2009). Similarly, it was reported that drought tolerance capacity of transgenic crops depended on developmental stage and intensity of the stress (Fahad *et al.*, 2017). In this study, water withholding was initially planned when flowering was observed at least on 15% of the plants screened. However, abortion can occur due to sprinkler water pressure on plants. We might not notice presence of

University of Ghana http://ugspace.ug.edu.gh

a flowers on the grown indicating occurrence of flowering. As it appeared that plants delayed to show flowers, consequently drought stress was imposed at 44 days after sowing. Nevertheless, this population were initially 800 lines and underwent selfing activity to ensure purity of lines. 400 lines resulted from filtering and among them 112 accessions were chosen for the trial based on similar maturity time. Population used might not segregate for phenological traits such as maturity. All differences found will be environmental injuries. In fact, Number of days to maturity of genotypes grown under water-stress condition is expected to be accelerated. Counter to what has been observed in the present study, several reviews have also reported speed up of flowering and maturity of plants under environmental stress. (Samarah, 2005) indicated that drought stress intensity in soybean were negatively correlated with duration of grain filling. (Agbicodo *et al.*, 2009) reported escape strategy of plants to drought by modification of its phenological development. Also, changes in phenology have been observed in sorghum genotypes exposed to low temperature which speeded up their flower initiation (Tarumoto *et al.*, 2003). In this study no difference was observed between environments regarding plant cycle parameters. In addition to earlier hypothesis given, genotypes would have suffered as well rather from the effect of temperature than the effect of water stress regarding maturity parameters. Vernalisation has been reported in wheat consisting in postponing time to flowering when cold occurs for a period of 4 to 8 weeks (Zikhali *et al.*, 2014). That epigenic silencing action has been observed in Arabidopsis and said to involve the suppression of genes expression that encode repressors of flowering (Amasino, 2005). The trial was established in early February coinciding with lower temperatures in Senegal. The delay of maturity parameters can be as well attributed to a similar phenomenon to vernalisation or the thermosensitivity of cowpea causing prolonging genotype cycle despite water stress. Susceptible checks flowered and matured before tolerant checks, Bambey 21 and CB46

University of Ghana http://ugspace.ug.edu.gh

flowered earlier compared to Mouride and IT93K-503. (Halime *et al.*, 2014) *et al.*, reported as well susceptible genotypes matured earlier compared to tolerant genotypes.

Higher correlations under well-watered condition were obtained between flowering and days to first pod maturity (r= 0.67) and wilting and stem greenness (r = -0.6). Higher correlations in water-stressed condition were obtained between stem greenness and maturity (r = 0.75) and stem greenness and wilting (r = - 0.74). Relationship between flowering and maturity parameters was not affected by drought in this study (Tables 2 and 3). However, a difference of 1 day was observed between water conditions, non-stressed genotypes flowered at 52 while stressed genotyped flowered at 51 DAP.

In a study conducted by (Muchero *et al.*, 2008), it was reported that delayed leaf senescence and stem greenness are important traits in the improvement of cowpea response to water stress. However, highest correlation was observed between grain and leaf area index, r = 0.59 and 0.35 under WWC and WSC respectively. It was reported that secondary traits were often not correlated to grain yield (Basu *et al.*, 2016). For breeder to consider indirect selection, substitution parameter should be highly correlated to grain yield, easier to record compared to substituted parameter with a high heritability in optionally morphologically distinct.

Genotypes SPAD values were insensitive to GEI and were not significantly different from environment to environment. SPAD mean value of genotypes in irrigated conditions were lower compared to watered-stressed SPAD values. The increase of SPAD mean value under water stress condition is consistent with results reported by (Hirut *et al.*, 2017) who obtained a significant increase of chlorophyll content from well-watered to water stress genotypes of potato. Other studies in potato have shown similar results (Ramírez *et al.*, 2014; Rolando *et al.*, 2015). According to Hirut *et al.*, (2017), the increase in chlorophyll content results from loss of turgor hence more concentration on leaf chlorophyll. Turgor maintenance can result from

University of Ghana http://ugspace.ug.edu.gh

osmotic adjustment. It has happened in sorghum genotypes grown under stress and non-stress condition to maintain their turgor resulting in no difference in leaves. This is in contradiction with work carried out on the chlorophyll concentration response of plants under water deficit conditions. In a study carried out by Ings *et al.* (2013) on a mutant of *Miscanthus*, it has been reported that by withholding water, stomatal conductance decreased first, followed by decreases in chlorophyll fluorescence and chlorophyll content. They have clearly stated that SPAD value declines under water stress (Ings *et al.*, 2013). In a different angle, it has been shown that photosynthesis can be limited by other factors such as the canopy of leaves. In fact, a canopy of light green leaves could have a more uniform distribution of light compared to a canopy of dark leaves. Uniformity is determinant in the sense that intra plot variability can affect SPAD measurements and leads to outliers or false predictions.

In this study, parameters considered as secondary traits were drought related morphological markers namely wilting, stem greenness, LAI and SPAD at a certain level. Traits can be relevant when researchers are interested most to survival of plants under WSC. However, survival of plant under WSC is not necessarily correlated to grain yield as mentioned in previous sentences.

4.5. Conclusion

In this study, most emphasis was put on grain yield, however it was interesting to investigate as well the relationship between yield and yield traits. The population segregated for grain yield. Four cultivars outperformed yield of the positive control Mouride by more than 30%. Candidates that have outperformed the best check were identified for future bi-parental population development.

University of Ghana http://ugspace.ug.edu.gh

CHAPTER FIVE

5. MARKER ASSISTED SELECTION AND GENOME-WIDE ASSOCIATION FOR COWPEA RESISTANCE TO *STRIGA GESNERIOIDES* PREVALENT IN SENEGAL

5.1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is a grain legume cultivated worldwide over 14 million ha (Fatokun *et al.*, 2018; Timko and Singh, 2008). Three-thirds of the grown area (10.5 ha) being in Africa produce 1, 710 thousand tons out of an estimated world production of 2,565,000 (Ehlers and Hall, 1997). It is a highly appreciated staple food (Omoigui *et al.*, 2015) because of its adaptation to low soil fertility and low rainfall environments and its significant economic importance to the livelihood of millions of poor people (Baudoin, 2001; Bressani, 1985) in sub-Saharan African (SSA) countries. In addition to providing food for human and fodder for livestock, cowpea has a smart ability to tolerate drought and contribute to soil fertility by fixing 300 kg nitrogen per ha (Fatokun *et al.*, 2018) that can be used by other crops such as cereals.

Because cowpea production is constrained by various factors, yield in developing countries has drastically lessened way far below its known yield potential (Muñoz-Amatriaín *et al.*, 2017). Major reasons are the use of low yielding or unimproved cultivars, harsh and erratic conditions characterized by irregularly distributed rainfall and high parasitic pressure including infestation by *Striga gesnerioides*, an obligate root hemiparasitic (Botanga and Timko, 2005; Hibberd *et al.*, 1996) flowering plant belonging to the family Orobanchaceae that is parasitic on C_3 dicotyledonous hosts from (Hibberd *et al.*, 1996) Leguminosea, Convolvulaceae, Euphorbiaceae and Solanaceae families (Dubé and Olivier, 2001). It has become a serious threat to cowpea production because of its pathogenicity causing yield losses exceeding 15 % in infected fields (Boukar *et al.*, 2004) and even more, Emechebe *et al.* (1991) for example reported 100 % on susceptible cultivars in the northern Guinea Savannah of Nigeria.

University of Ghana http://ugspace.ug.edu.gh

Suggested control measures including biological methods, chemical control and cultural practices (Dubé and Olivier, 2001) are inefficient or expensive (Omoigui *et al.*, 2017a) or complex and hard to put into practice in developing countries. Actually, none of these means taken individually is capable of controlling the parasitic plant. A successful means of control to mitigate the effects of Striga on yield is host plant resistance (Omoigui *et al.*, 2007). Unambiguously, the use of improved resistant cultivars is the most effective (Jeremy T. Ouedraogo *et al.*, 2001b), suitable and affordable alternative (Lane *et al.*, 1996) for resource-poor communities.

Several sources of resistance to Striga have been identified including 58-57 and Melakh (Senegal), Suvita-2 (Burkina Faso), B301 (Botswana), IT82D-849 (Nigeria) and TN121-80 (Niger) (Atokple *et al.*, 1995; Lane *et al.*, 1996; Ouedraogo *et al.*, 2001a,b; Tignegre *et al.*, 2013). But actually, these genotypes lack mostly farmer preferred traits such as yield and earliness or race specific resistance to a particular Striga biotype.

In Senegal released varieties for resistance to insects or diseases have been widely adopted by farmers. However, all of them are sensitive to the prevalent Striga which limits their use in Striga prone areas. Recent studies conducted by Tonessia *et al.* (2009) have identified sources of resistance to Striga prevailing in Senegal. This significant development has yielded to the initiation of improvement of Melakh using IT97K-499-39 as donor to create a fourth filial generation backcross population by conventional breeding (Boukar *et al.*, 2016) by conventional breeding. Although this method has been used for a long time by breeders, it remains obsolescent, time-consuming, laborius and requires more phenotypic evaluations, more crosses and creates more undesirable genotypes. Fortunately, over the last decade, rapid progress has been made in the field of genomics-assisted breeding (Varshney *et al.*, 2014) giving rise to approaches combining conventional breeding and genomic tools to effectively fast-track crop plant improvement in selecting for multifactorial and monogenic traits (Ribaut

University of Ghana http://ugspace.ug.edu.gh

and Ragot, 2006). Marker-assisted selection (MAS), the most widely used type of genomic-assisted crop improvement has allowed to identify genes and genomic segments responsible for expression of quantitative trait loci (Ribaut *et al.*, 1996). The usefulness of marker-assisted backcrossing is no longer just to be demonstrated, evidence of its effectiveness has been reported in many studies including resistance of cowpea to *Striga gesnerioides* (Ouedraogo *et al.*, 2001b; Boukar *et al.*, 2004; Omoigui *et al.*, 2017b). However, some advance lines developed using the resulting markers showed susceptibility in field and therefore, failed to validate effectiveness of such markers in regards to *S. gesnerioides* prevailing in Senegal. A smart approach that takes advantage of allele variation in unrelated lines is Genome-Wide Association Study. This approach has been used to identify many genomic elements publicly available to breeders to use in their breeding programs (Fatokun *et al.*, 2018; Huynh *et al.*, 2013b; Lo *et al.*, 2017; Muñoz-Amatriaín *et al.*, 2017).

The present study presents major results of four years of research on various populations with emphasis on marker identification and its use in molecular breeding. Objectives of the research were to: 1) assess conversion of a highly desired Striga susceptible line Melakh for Striga resistance using microsatellite markers, 2) validate field resistance of developed Melakh lines in Striga-prone areas in Senegal and 3) identify significant SNP markers and candidate genes associated to *S. gesnerioides* resistance prevailing in Senegal using high throughput data recorded at ISRA in CNRA de Bambey, Senegal.

5.2. Materials and Methods

5.2.1. Plants, inoculum DNA markers materials

Cowpea genotypes used in this study were obtained from the breeding collection of ISRA, CNRA de Bambey, Senegal and from the University of California Riverside (mini-core).

University of Ghana http://ugspace.ug.edu.gh

5.2.1.1. Marker-assisted backcrossing

Progenies of the different filial generations used in this study to develop current B4F8 lines were generated from IT97K-499-39 *1 / Melakh. As indicated in background information, until BC_1F_4, lines development relied entirely on conventional breeding. The female parent Melakh is a Striga susceptible breeding line popularly grown and very much appreciated by farmers for notably its extra earliness and resistance to aphids (Cisse *et al.*, 1997) while donor parent IT97K-499-39 is resistant to Striga in Senegal. The reaction of the resistant and susceptible lines was determined from previous studies in pot trials.

Seventy-five BC_1F_7 lines were used to perform background selection prior to backcrossing-based markers. Resulting 6 BC_4F_3 lines from marker-assisted backcrossing were used for SNP screening to validate their resistance. The lines were named STR-1. STR-2, STR-5, STR-8, STR-11, and OFF.

One hundred and twenty-eight SNP from KASP assay were used in the background selection. Microsatellite markers used in foreground selection were named C42-2B, SSR1 and 7548_1327 which corresponds to SNP 1_0958 (Huynh, Ehlers, *et al.*, 2013). SNP 1_0958 co-localize with previous identified markers for Striga resistance (Tim close, personal communication). Marker SSR1 was reported to be linked to resistance of *S. gesnerioides* race 1 (SG1), SG2, SG3 and SG5 (Asare, *et al.*, 2013a; Asare, *et al.*, 2013b).

Striga seeds used in breeding involving conventional techniques were collected from Bambey Serere, Senegal in 2012 which is not part of this dissertation. Striga inoculum used from BC_2 lines to BC_4 lines were collected from Louga in November 2014, 2015 and 2016 in the same locality in farmers' fields that were distant to less than 10 km.

University of Ghana http://ugspace.ug.edu.gh

5.2.1.2. Multi-environment Striga resistance and grain yield trials

BC_4F_6 lines namely STR-1, STR-2, STR-5, STR-8 and STR-11 were used in multi-environmental Striga trials in Louga and Bambey. The trials were conducted in 4 localities namely Ndatt Fall, Ndagour Ndiaye, Nderep and CNRA de Bambey. For simplicity, the trials are referred to as METstr in this report.

Grain yield trials were conducted in 6 localities namely Malicounda, Sine Dieng, Maka Bira Gueye, Ndangour Ndiaye, Cherif Ka and CNRA de Bambey. The trials are referred to as METyld hereinafter.

5.2.1.3. Striga resistance SNP linked markers using wide scanning

A set of 367 worldwide diverse accessions were used in pot phenotyping to assess genome-wide association with the aim to identifying significant SNPs that could be used in Marker-Assisted Selection and candidate genes identification.

The Cowpea iSelect Consortium array containing 51,128 SNPs (Muñoz-Amatriaín *et al.*, 2017) were used to genotype the 367 accessions. Genotyping was performed and genotypic data were formatted for TASSEL.

5.2.2. Methods

5.2.2.1. Method used in Marker-assisted backcrossing

Activities started in 2014 in background selection until where multi-locational trials were conducted in the rainy season 2017. The multiplication trials continued in 2018 off-season and purity test were assessed to ensure purity of candidate lines for release in November 2018. Backcross (BC_1) lines were developed prior to this work (not presented here) using Pedigree breeding method and pot test until the BC_1F_5 lines were produced. Background selection was performed once on BC_1F_7 plants to allow selection against the donor genome IT97K-499-39 using 128 markers from the KASP assay in 2014. The identified candidate was crossed to the

University of Ghana http://ugspace.ug.edu.gh

recurrent parent Melakh to obtain BC_2F_1 plants in 2014. Hybrids were field planted and screened with marker SSR1 to obtain BC_3F_1 plants in 2015. The resulting 10 individual plants were raised in pots and phenotyped. The remnant seeds were sown at the same time in the field where their development was optimal to allow early leaf sampling for SSR1 screening at 21 days after sowing. Among the 9, four plants were chosen to cross back to Melakh in 2016 to obtain BC_4F_1 plants. The few seeds obtained from the BC_4F_1 were bulked for field multiplication. The BC_4F_3 plants obtained were screened using 1_0958 in 2016. Resulting homozygous resistant lines were tested in pots in Bambey in 2016. The remnant seeds were planted in the field for multiplication in the off-season of 2017. Flapjack software was used to compute percentage of recovery of lines in reference to the recurrent parent Melakh.

All genotypes involving pot screening experiments were grown in 10 litre plastic pots filled with soil at CNRA, Senegal. Pots were 45 cm deep with 25 cm diameter. Pots were filled using soil collected at horizon 0-15 cm as described by Tonessia *et al.* (2009). To make sure that no Striga infestation occurred on the soil prior to testing, susceptible variety Melakh was grown in 12 pots filled with the same soil used in inoculation and no Striga presence was observed at 50 days after removal of cowpea plants. Screening tests were conducted according to the method of phenotyping used by Musselman and Ayensu (1984) with slight differences in inoculum dosage. About the triple of 5000 Striga seeds (Omoigui *et al.*, 2017a) equivalent to 0.3 g (Tonessia *et al.*, 2009) of the inoculum was used to infested the soil in pots 2 days before sowing cowpea lines. Except the parental lines replicated 2 times in each new experiment as a control measure, each genotype was sown without replication because a susceptible genotype remained susceptible and diagnostic markers added values to accuracy of results. Pots were thinned to 1 plant 10 days after cowpea emergence. Water supply started 2 days before inoculation and continued on a daily basis until pod formation when the plants were irrigated

University of Ghana http://ugspace.ug.edu.gh

with 300 ml of water every 3 days. Phenotypic data were collected by visual scoring, R= no Striga attachment, S= Striga emergence and attachment.

Total genomic DNA was extracted from fresh leaves of each genotype, the bi-parental population and separately the 12 accessions following a modified MATAB protocol described by Fonceka *et al.* (2009). Quality of DNA was assessed using 0.8% (w/v) agarose gel, and the concentrations were estimated by comparing the fluorescent-band-intensities to the fluorescent-band intensities of known concentrations of the standard Smart ladder (Eurogentec, Seraing, Belgium). PCR amplification was performed in an Eppendorf Mastercycler in 10 µl reaction mixture containing 5 µl of 5 ng/µl of the DNA template and 0.1 µM of each primer, 0.2 mM of each dNTP, 1X PCR buffer, 2.5 mM MgCl2, 0.1U/µl of Taq polymerase and 0.1 mM of IR700 or IR800-labeled M13 primer (MWG Germany) for fluorescence detection of the amplicons. The labelled PCR products were separated on 6% polyacrylamide gel by electrophoresis run for 1.5h at 1500V using a DNA Sequencer (LI-COR 4300 DNA Analyzer, Lincoln, NE, USA). The laboratory experimental design used for screening BC_4F_3 plants involved all the 6 BC_4F_3 genotypes replicated thrice (three sample collected in a same row representing a single genotype). The checks Melakh and IT97K-499-39 were replicated twice. During field sampling, one genotype had 2 different phenotypes thus were collected by the name OFF in case it be an off-type. Genotyping involving background selection, done at LGC and data formatted to flapjack genotyping input were performed in UCR and sent to us. Genotyping involving foreground selection was done at CERAAS, Senegal and output data were sent to us for analysis and interpretation.

Trials in MABC did not follow a specific experimental design as in multi-environmental trials or as in Chapter 4 where drought tolerance was addressed using a different panel. Main goal was more of discarding susceptible lines along the process rather than using the population as a mapping population to performing QTL analysis.

University of Ghana http://ugspace.ug.edu.gh

5.2.2.2. Method used in multi-environment field trials

Resulting genotypes from MABC and their parents were tested in 4 localities (for Striga resistance) and in 6 localities (for grain yield) to assess their validation for being resistant to Striga and their yield performance. The parental lines described earlier were used as checks namely Melakh and IT97K-499-39. The BC_4F_7 lines were STR-1, STR-2, STR-5, STR-8 and STR-11. Experiments were conducted at the same time. Line OFF was not used in field performance because resembled as an off-type.

On Striga experiment, data collected were presence of Striga emergence and number of days to first Striga emergence. On yield experiment, only grain yield was taken. Genotypes were replicated 3 times. Data were analysed using following bilinear models $\boldsymbol{Yij} = \mu + \boldsymbol{Gi} + \boldsymbol{Ej} + \sum_{k=1}^{k} \boldsymbol{\lambda_k y_{ik} \delta_k}$ and $\boldsymbol{Yij} = \mu + \boldsymbol{Ej} + \sum_{k=1}^{k} \boldsymbol{\lambda_k y_{ik} \delta_k}$ to respectively assess *i)* genotype by environment (GxE) and *ii)* genotype by environment and performance. The following is an explanation of terms in the models: Y is the variable observed, μ is the mean effect, G_i is the deviation of the genotype i from the overall mean, e_i is the deviation of environment term from the overall mean, λ_k is the singular value in the interaction principal component (IPC) axis, k, y_{ik} and δ_k are IPC scores of genotype and environment. Ranking of genotypes were assessed at mega-environment level identified using indications from the GGE biplot. An additional ranking on grain yield were performed using Cultivar Superiority coefficients.

5.2.2.3. Method used in Genome-wide association

Genome-Wide Association analysis (GWAS) was conducted to identify significant SNPs and potential candidate genes. Two phenotypes were observed: absence of Striga or presence of Striga. Absence was free of Striga - root attachment while presence meant the opposite. Phenotypic data associated with genotypic data were obtained from pot screening in Bambey

University of Ghana http://ugspace.ug.edu.gh

at the end of July 2018 in an experiment involving 4 locations. The same design used in previous pot test was used.

Raw genotypic data were filtered using SNPs with MAF > 0.05. GWAS was performed using the *Q* + *K* method implemented in TASSEL v.5.2 as a mixed linear model (MLM) function (Zhang *et al.*, 2010). Population structure (*Q* matrix) was accounted for using the result of a Principal Component Analysis (5 PCs) while a kinship matrix (*K*) was used to correct for the relationships between accessions. Manhattan plots were generated using p-values obtained from the association analysis. Significance thresholds were assessed using False Discovery Rate (FDR) correction method in R at $\alpha = 0.05$. No specific package was needed for FDR computation. SNPs over the threshold were identified as significant. The significant SNPs were localized on the cowpea reference genome (Lonardi *et al.* submitted; www.phytozome.net) to assess their physical positions and determine the underlying candidate genes (Lonardi, 2019).

5.3. Results

5.3.1. Marker-assisted backcrossing

Marker C42-2B was 15 cM far apart from resistance to *S. gesnerioides* (background information). Thirty-two lines carrying the allele from the resistant parent namely IT97K-499-39 identified using C42-2B were screened using SSR1 for comparison. The screening resulted to 20 out of 32 lines with the allele coming from the donor parent (Figure 5.1). As SSR1 was able to show susceptible lines that C42-2B did not show, it was used in the development of subsequent backcross lines.

University of Ghana http://ugspace.ug.edu.gh

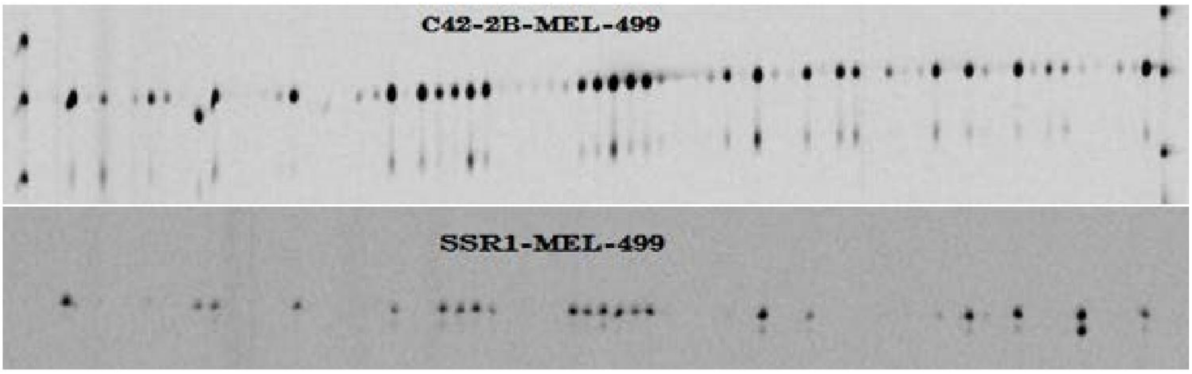


Figure 5. 1: Gel image of resistant lines using C42-2B against SSR1

Background selection: Percentage of recovered Melakh background among the BC_1F_6 lines ranged from 0 % (IT97K-499-39) to 100 % (Melakh). After the donor parent, line L114 was mostly distant to Melakh background (36 %) while line L21 was the closest to Melakh by (96 %). Among the resistant BC_1F_6 lines, 5 genotypes that recovered higher percentage were L127 (84 %), L137 (83 %), L139 (81 %), L60 (80 %) and L134 (79 %) (Figure 5.2).

University of Ghana http://ugspace.ug.edu.gh

Melakh	1.00
21	0.96
MELAKH	0.95
2	0.91
92	0.91
52	0.90
64	0.89
69	0.88
46	0.86
111	0.84
127	0.84
130	0.84
89	0.83
119	0.83
71	0.83
137	0.83
120	0.83
59	0.82
125	0.82
139	0.81
60	0.80
100	0.80
94	0.80
131	0.79
134	0.79
123	0.78
61	0.77

Figure 5. 2: Cowpea genotype similarities based on Melakh background

The first column in the left side of the plot represent the names of BC_1F_6 line from the cross IT97K-499-39 *1 / Melakh. Figures in the right side of genotype designation are percentage recovery in reference to Melakh. A second version of the parental lines was added to the panel to check how distant they were from the parents used in developing the BC_1F_6 lines. Those two lines namely MELAKH and 499-39 were obtained from UC Riverside.

University of Ghana http://ugspace.ug.edu.gh

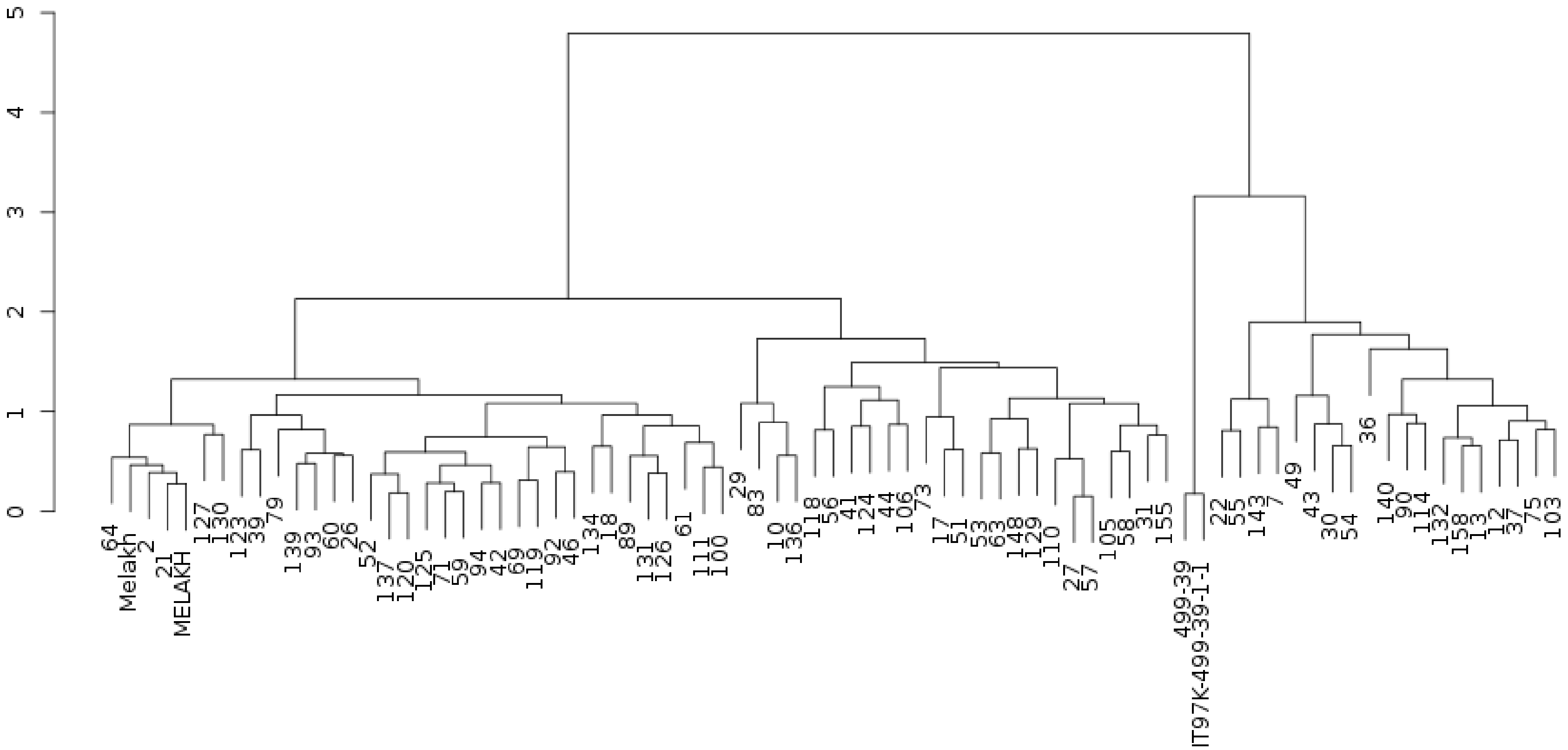


Figure 5. 3: Clustering dendrogram of cowpea BC_1F_6 lines and their parents

The vertical line represents the dissimilarities between clusters. The horizontal line represents the genotypes and clusters. Three clusters were found between levels 1 and 2. In the cluster in the left are lines with high similarity to Melakh, in the middle cluster are lines between the 2 parents and the third cluster in the right are lines with high similarity to IT97K-499-39.

University of Ghana http://ugspace.ug.edu.gh

Three of the lines namely L127, L137 and L139 were sown in the field to be backcrossed to the recurrent parent. Succeeded cross involved L137 in IT97K-499-39 *2 / Melakh. The few seeds obtained allowed to grow 3 BC_2F_1 plants namely L134-1, L134-2 and L134-3. Plants were genotyped using SSR1 to identify resistant allele in L134-1 and in L134-3, we failed to harvest seeds from L134-2. In IT97K-499-39 *3 / Melakh, 10 plants were obtained in crosses involving L134-1 and L134-3. Pot screening revealed in the first cross that all lines were resistant to *S. gesnerioides* while in the second cross 1 out of 10 allowed attachment of *S. gesnerioides*. Simultaneously, backcross lines BC_3F_1 plants were also genotyped to allow premature identification of a donor. From the genotyping using SSR1, out of 9, four had the resistant allele and crosses were made using 3 donors in IT97K-499-39 *4 / Melakh. BC_4F_1 plants were advanced in heavily infested pots to get rid of plants allowing *S. gesnerioides* attachment. The resistant plants were harvested and the same screening method was repeated for the BC_4F_2 plants. Five BC_4F_3 plants were harvested and planted on field for marker genotyping using SSR1 and C42-2B. For each of the BC_4F_3 plants, 3 individuals were screened. All plants carried the positive allele. Marker SSR1 and C42-2B were consistent.

University of Ghana http://ugspace.ug.edu.gh

Table 5. 1: Reaction of BC_4F_4 and their parents to markers SSR-1 and C42-2B

Genotype	Replication	Marker SSR-1	Marker C42-2B
BC4F3_1	1	+	+
	2	+	+
	3	+	+
BC4F3_2	1	+	+
	2	+	+
	3	+	+
BC4F3_5	1	+	+
	2	+	+
	3	+	+
BC4F3_8	1	+	+
	2	+	+
	3	+	+
BC4F3_11	1	+	+
	2	+	+
	3	+	+

Results of lines advanced in BC_4F_4 using SNP 1_0958 were not consistent with the previous results obtained using marker SSR1 and C42-2B. Genotypes STR-1, STR-8 were susceptible while STR-2, STR-5, STR-11 and OFF were resistant. Parents were the same as in screening with SSR1 and CB42-2B, Melakh was susceptible and IT97K-499-39 was resistant as expected. Markers indicated different results however, further analysis in field in different Striga prone locations and as well using a wider population with unrelated individuals (small co-ancestry) to take advantage of historical allelic variations accumulated in order to find SNP markers associated to *S. gesnerioides* resistance that can be deployed in selection using markers as an alternative to phenotyping.

University of Ghana http://ugspace.ug.edu.gh

Table 5. 2: Reaction of BC_4F_4 and their parents to SNP 1_0958

Genotype	Results	SNP allele
STR_1	S	G-G
STR_2	R	T-T
STR_5	R	T-T
STR_8	S	G-G
STR_11	R	T-T
OFF	R	T-T
Melakh	S	G-G
IT97K-499-39	R	T-T

5.3.2. Multi-environment trials

Striga resistance: Results of combined analysis of variance for presence / absence of Striga showed that environment effect was highly significant ($p < 0.001$) while no significant effect occurred for genotype source of variation (Table 5.3). Environments explained 86.04 % of the variation observed while interaction effect accounted for only 9.89 %. This is an indication that genotypes did not segregate differentially for Striga resistance, but there was distinctiveness of locations used for evaluation.

University of Ghana http://ugspace.ug.edu.gh

Table 5. 3: Striga presence / absence analysis of variance for AMMI model

Source	d.f.	s.s.	%	m.s.	v.r.	F pr
Genotypes	6	1037	4.07	173	1.23E+00	0.3352
Environments	3	21936	86.04	7312	5.22E+01	<0.001
Interactions	18	2521	9.89	140		
IPCA 1	8	1727	68.50	216	3.34E+11	<0.001
IPCA 2	6	794	31.50	132	2.04E+11	<0.001
Residuals	4	0		0		

d.f = degree of freedom; s.s. = sum of squares; m.s. = mean squares; v.r. = variance. Figure highlighted in yellow are significant P < 0.001

Presence of Striga was observed in all sites studied. Mean scores ranged from 24.57 to 95.28. Environments namely Sine Dieng, Ndagour Ndiaye, Nderep and Ndatt Fall recorded 95.28, 90.57, 67 and 24.57. Figures indicated that Ndatt Fall was the most parasitized site. Best performing genotypes were STR-11 and IT97K-499-39 with a score of 76.05 based on BLUPs overall means. Two genotypes underperformed the susceptible parent Melakh which scored 65.63.

Table 5. 4: Ranking of genotypes based on BLUPs performance for resistance to Striga

Genotypes	Resistance	Ndangour Ndiaye	Ndatt Fall	Rank	Nderepp
STR-11	76.05	90.57	51.36	1	67
IT97K-499-39	76.05	90.57	51.36	2	67
STR-5	74.38	90.57	30.52	3	81.14
STR-2	69.17	90.57	9.68	4	81.14
Melakh	65.63	90.57	9.68	5	67
STR-8	65.63	90.57	9.68	6	67
STR-1	58.56	90.57	9.68	7	38.71

In the AMMI model, interaction accounted for 9.89 % of the variation observed among the genotypes in environments. The first principal component in AMMI and GGE biplot explained 68.50 % and 68.26 % respectively (Figure 5.4). According to AMMI the 4 sites can be reduced

University of Ghana http://ugspace.ug.edu.gh

to 3. Similar results were obtained when the data were subjected to GGE biplot. On the right side of the GGE biplot are environments free of Striga infestation while those on the left side were more infested environments. Environment effects were -45.1 %, -1.5 %, 25.53 %, 21.12 % for respectively Ndatt Fall, Nderepp, Ndangour Ndiaye and Sine Dieng, respectively. Despite Striga presence in Ndatt Fall, it had a good adaptation of genotypes STR-11 and IT97K-499-39. Based on GGE information, validation of the resistance of BC_4F_7 lines should be oriented to sites with higher Striga prevalence to avoid misleading inferences. Presence – absence scoring is reliable for a trait characterized by Mendelian inheritance. However, ranking is tricky in the sense that genotypes that have same scores are ranked differently as seen in Ndangour Ndiaye. At this step, breeders should be careful in order to take profit of the simplicity of method in identifying site adapted genotypes.

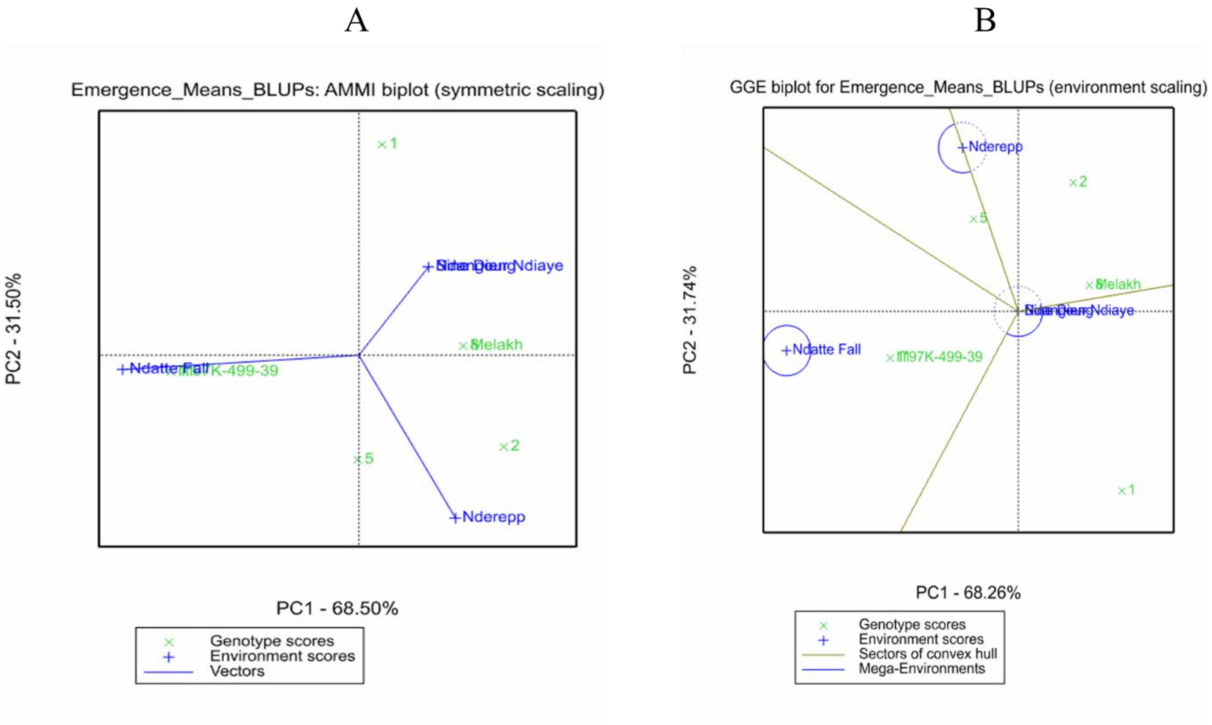


Figure 5. 4: (A) Performances in relation with test environments and (B) genotype-by-environment interaction

University of Ghana http://ugspace.ug.edu.gh

Plot displays levels of correlations using colours (Figure 5.5). High correlations were dark blue or dark red representing respectively a negative correlation of -1 and a positive correlation of +1. High correlations were found between Sine Dieng and Ndangour Ndiaye and Sine Dieng and Nderepp (Figure 5.5).

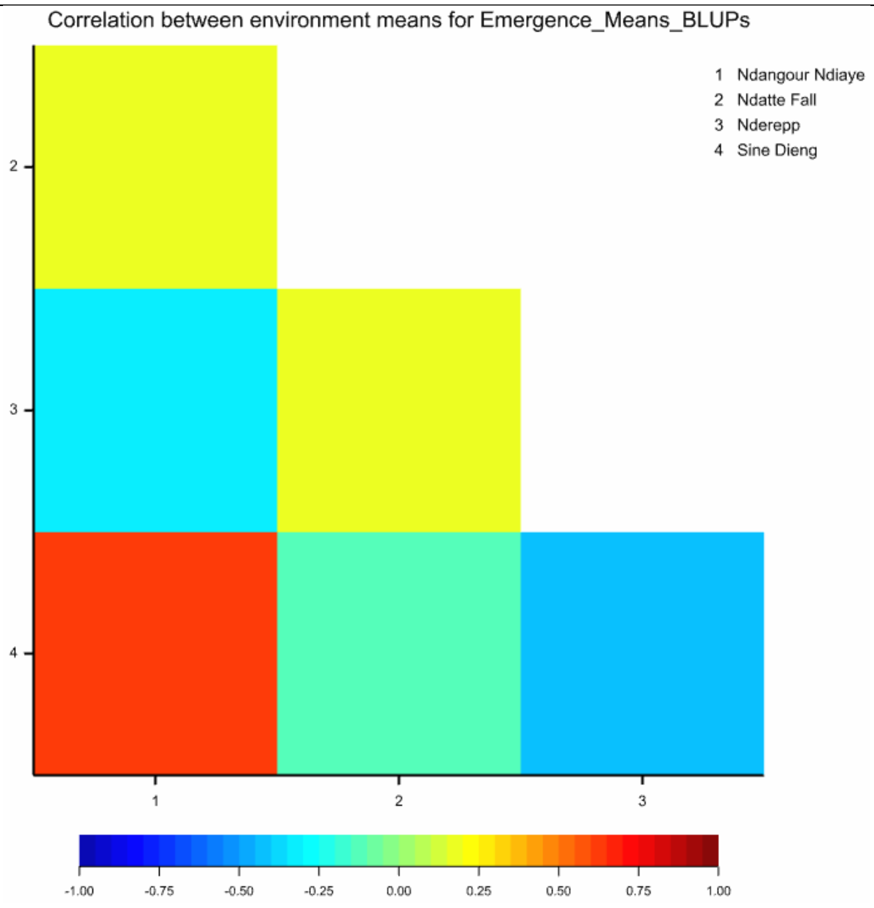


Figure 5. 5: Correlation of environments for Striga prevalence

Grain yield: Here also, there were highly significant differences among environments but not among genotype. Most variation was found in environment (98.69 %) while genotype and GxE accounted respectively for only 0.28 % and 1.03 % respectively (Table 5.5).

University of Ghana http://ugspace.ug.edu.gh

Table 5. 5: Grain yield analysis of variance for AMMI model

Source	**d.f.**	**s.s.**	**%**	**m.s.**	**v.r.**	**F pr**
Genotypes	6	4289	0.28	715	1.35	0.2647
Environments	5	1514819	98.69	302964	574.1	<0.001
Interactions	30	15832	1.03	528		
IPCA 1	10	14889	94.04	1489	2375.67	<0.001
IPCA 2	8	935	5.91	117	186.43	<0.001
Residuals	12	8	0.05	1		

d.f = degree of freedom; s.s. = sum of squares; m.s. = mean squares; v.r. = variance. Figure highlighted in yellow are significant P < 0.001

Mean scores ranged from 10.78 kg/ha to 531.89 Kg/ ha. Environments namely CNRA, Malicounda, Cherif Ka, Ndangour Ndiaye, Maka Bira Gueye and Sine Dieng recorded respectively 531.89 kg / ha, 239.05 kg / ha, 46.10 kg / ha, 30.03 kg / ha, 15.13 kg / ha and 10.78 kg / ha (Table 5.6). This indicated that best yield was obtained in CNRA de Bambey and Malicounda. Best genotypes that outperformed the best check IT97K-499-39 were genotypes STR-1 (592.63 kg/ha), STR-8 (580.52 kg/ha) and STR-2 (578.35 kg/ha) by respectively 9.09 %, 6.86 % and 6.46 %. In reference to Melakh the recurrent parent, these genotypes were higher by 17.90 %, 15.49 %, 15.06 %. The genotype IT97K-499-39 outperformed Melakh by 8.08 %. with a score of 76.05 based on BLUPs overall means. Two genotypes underperformed the susceptible parent Melakh whose yield was 502.65 Kg/ha.

University of Ghana http://ugspace.ug.edu.gh

Table 5. 6: Ranking of genotypes based on BLUPs performance for grain yield

Genotypes	**CNRA**		**Malicounda**		**Ndangour Ndiaye**	
	Yield	**Rank**	**Yield**	**Rank**	**Yield**	**Rank**
STR-1	592.6331198	1	242.723011	1	33.74253828	2
STR-8	580.5283181	2	238.4982929	4	31.03528305	4
STR-2	578.3564039	3	239.5016316	3	18.58190898	6
IT97K-499-39	543.2582707	4	240.5177031	2	60.16534934	1
Melakh	502.6579548	5	238.4931998	5	16.55949729	7
STR-5	463.4476641	6	238.218173	6	18.63605409	5
STR-11	462.3182687	7	235.3813219	7	31.52258899	3
Mean	531.8857143		239.047619		30.03474572	

Three homogeneous groups of locations were observed (Figure 5.3). Group 1 (G1) was CNRA, Group 2 (G2) was Ndagour Ndiaye and Group 3 (G3) was Malicounda, Cherif Ka, Maka Bira Gueye and Sine Dieng. Two highest recommended genotypes for G1 were STR-1 and STR-8. In G2 the best genotypes for recommendation were STR-8 and STR-1. In G3 the best genotypes to recommend were STR-1 and STR-2. Environment effects were ranked as follow: CNRA (387.0), Malicounda (92.1), Cherif Ka (-99.5), Ndangour Ndiaye (-114.9), Maka Bira Gueye (-130.2) and Sine Dieng (-134.5). CNRA de Bambey, which had the longest vector along the first axis, accounted for most of the environmental variation (Figure 5.3).

University of Ghana http://ugspace.ug.edu.gh

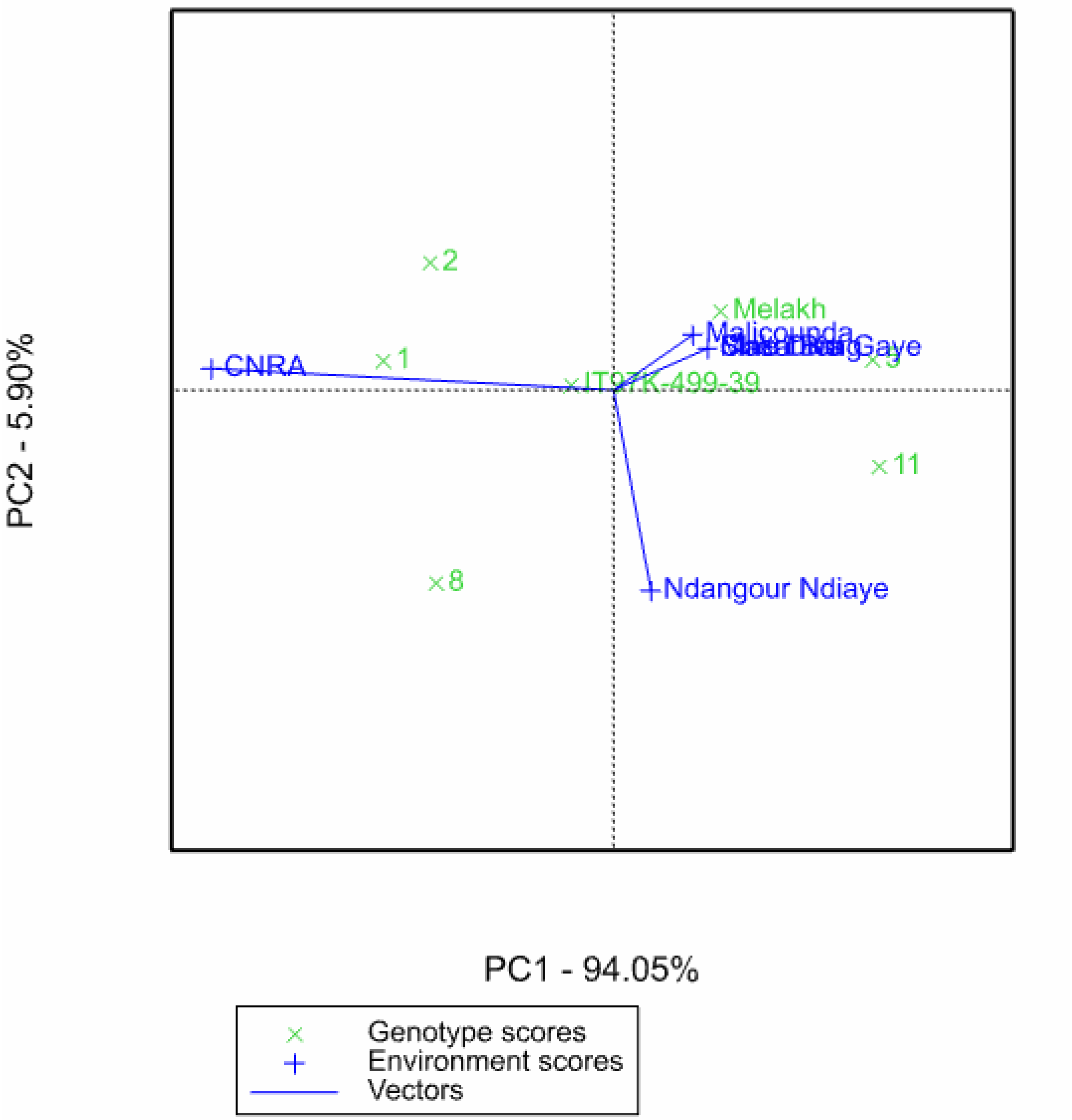


Figure 5. 6: Performances in relationship among test environments

Similar results were obtained also in the GGE biplot (Figure 5.7). The 4 sites were grouped into 3: Group 1 (CNRA), Group 2 (Ndangour Ndiaye) and Group 3 (Malicounda, Cherif Ka, Maka Bira Gueye and Sine Dieng). The largest mean was recorded for Cluster 1 (CNRA) with 531.88 kg/ha. Cluster 2 and 3 recorded respectively 30.03 kg/ha and 77.76 kg/ha. Genotype recommendation for mega-environment are presented in Table 5.6.

University of Ghana http://ugspace.ug.edu.gh

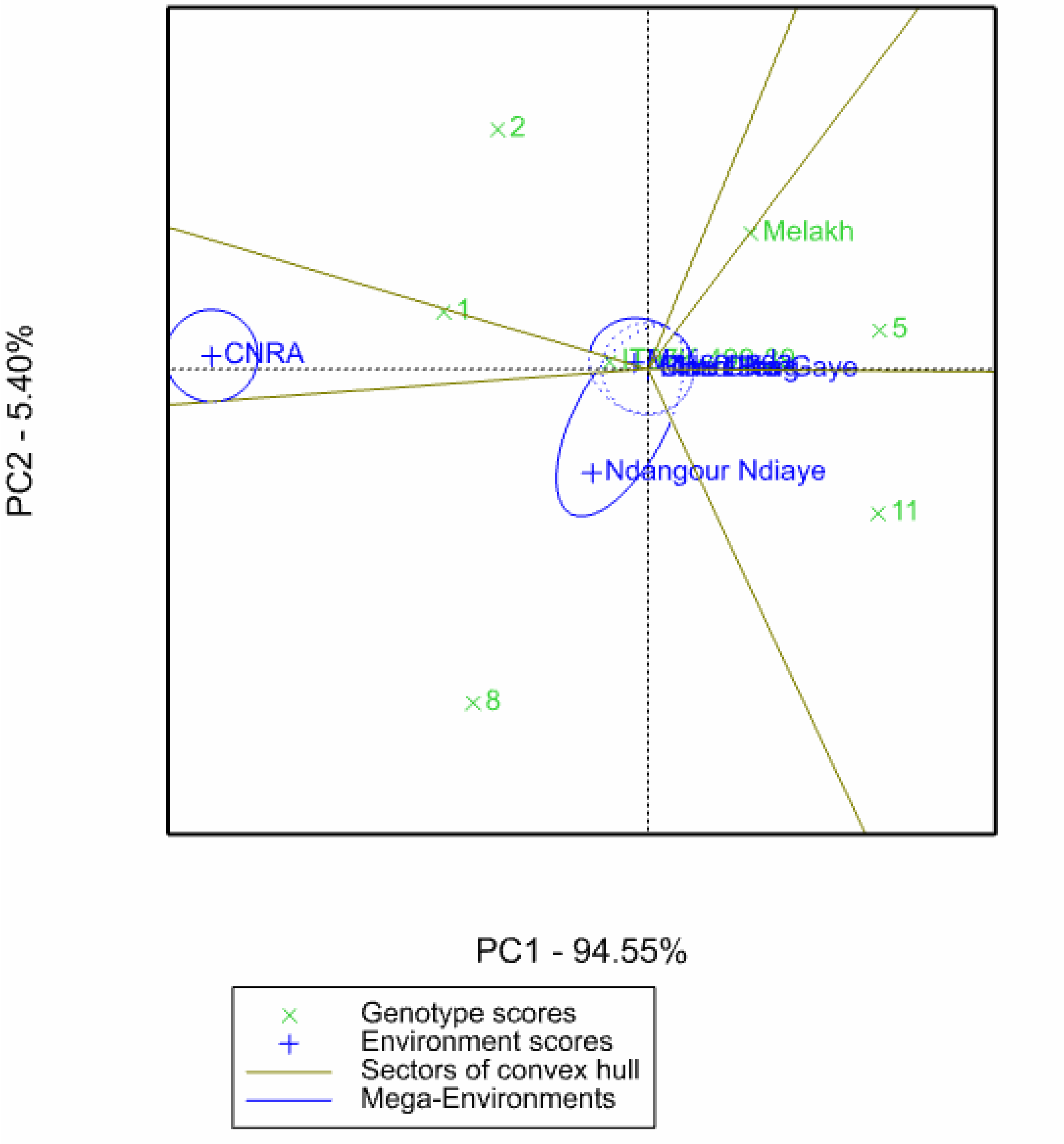


Figure 5. 7: Genotype-by-environment interaction for grain yield

High correlation coefficients were observed (Figure 5.8) between Sine Dieng and CNRA (0.86) and Sine Dieng and Malicounda (0.79). Malicounda was highly correlated with CNRA (0.74) and Maka Bira Gueye (0.74).

University of Ghana http://ugspace.ug.edu.gh

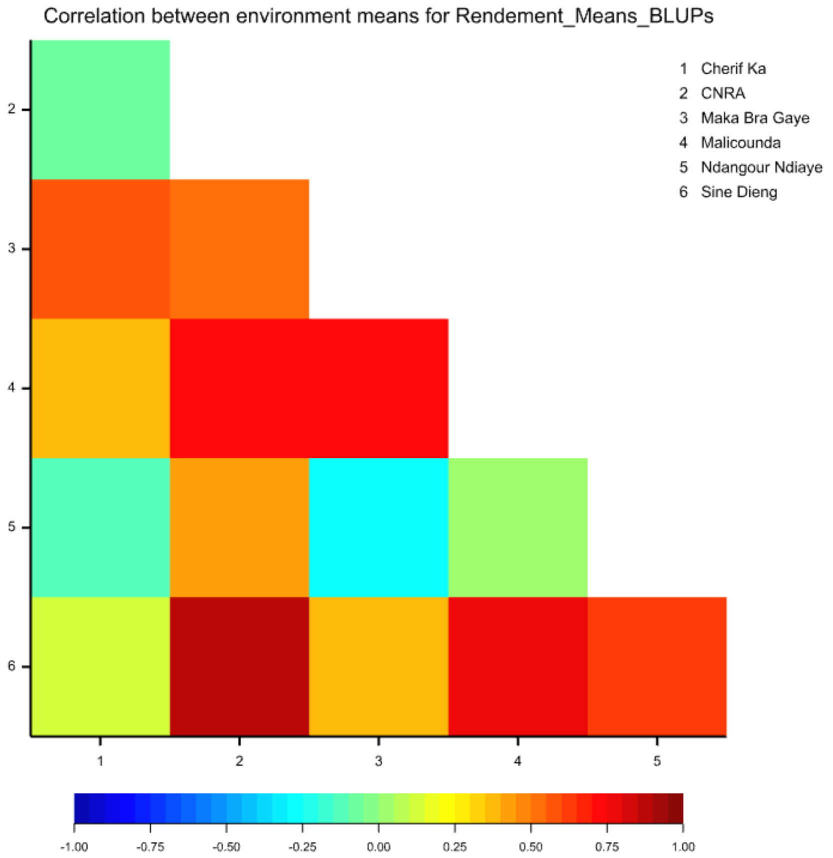


Figure 5. 8: Correlation between environment means for grain yield

5.3.3. Genome-wide association study (GWAS)

After removing SNPs with minor allele frequency <0.05, 41658 SNP marker were used to perform GWAS. No significant SNP were found for days to emergence of *S. gesnerioides* on pots for environments 1, 2 and 3. No significant SNP marker for Striga fixation were found in environments 2 and 3. However, 3 significant SNPs (Figure 5.9) were found: 2_10935 for days to Striga emergence and 2_44813 and 2_21670 for Striga fixation. The significant threshold for Striga emergence calculated based on False Discovery Rate were: 3.901 for environment 1; 3.902 for environment 2; 3.899 for environment 3; and 3.903 for environment 4 (Table 5.7). For Striga fixation FDR thresholds were: 3.898 for environment 1; 3.895 for environment 2; 3.897 for environment 3; and 3.881 for environment 4.

University of Ghana http://ugspace.ug.edu.gh

Table 5. 7: Significant SNPs resulting from high throughput data analysis for resistance to *Striga gesnerioides*

Trait	SNP	Allele	Chromosome	Position (bp)	–log10(p)	Effect	Threshold
Days to emergence 1	none						3.90
Days to emergence 2	none						3.90
Days to emergence 3	none						3.90
Days to emergence 4	**2_10935**	**G**	Vu10	39,307,602	5.30	58.26	3.90
Fixation Striga 1	**2_44813**	**A**	Vu01	7,025,288	4.95	0.39	3.90
Fixation Striga 2	none						3.89
Fixation Striga 3	none						3.90
Fixation Striga 4	**2_21670**	**C**	Vu10	851,864	4.14	-0.30	3.88

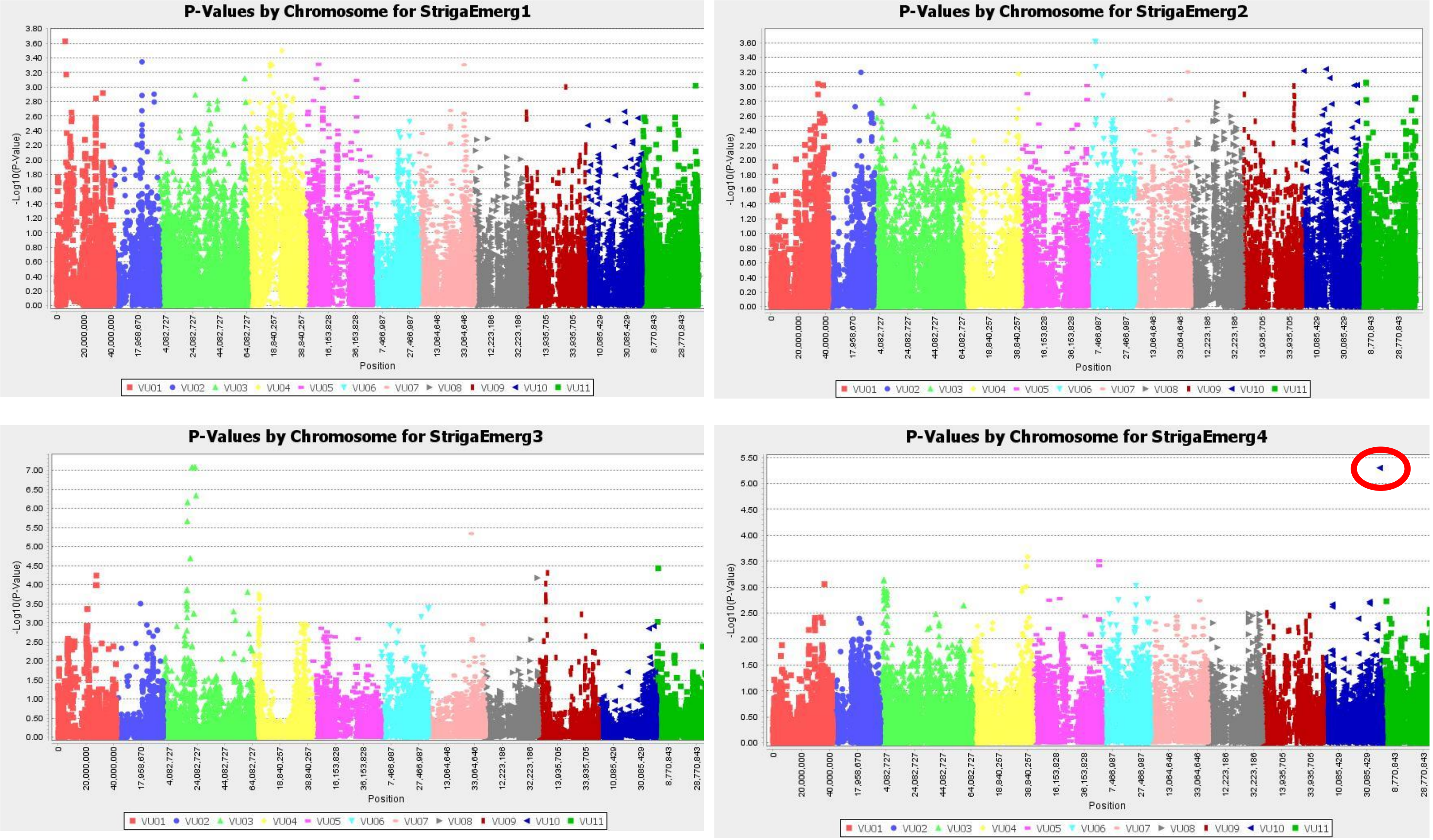
The position of the three markers identified in Genome-wide Association were used for identification of candidate genes. SNPs marker position were 851,864.00, 7,025,288.00 and 39,307,602.00. Considering those positions, 6 proteins or candidate genes involved in plant defence were identified. In reference to candidate gene positions in the physical map, 3 candidate genes were very close to SNP 2_44813 whose position is 7,025,288.00 (Table 5.8).

University of Ghana http://ugspace.ug.edu.gh

Table 5. 8: Candidate gene annotation using the new chromosome numbering

Locus Name	chromosome	Start (bp)	End (bp)	Human-Readable-Description
Vigun01g010700	Vu01	1,215,524	1,223,327	PREDICTED: E3 ubiquitin protein ligase RIN2-like isoform X3 [Glycine max]
Vigun01g013300	Vu01	1,479,417	1,482,363	Protein kinase superfamily protein LENGTH=669
Vigun01g015700	Vu01	1,682,331	1,686,584	disease resistance family protein / LRR family protein LENGTH=905
Vigun01g016500	Vu01	1,801,653	1,806,619	Disease resistance protein (TIR-NBS-LRR class) family LENGTH=1191
Vigun01g041400	Vu01	5,772,383	5,777,283	Disease resistance protein (TIR-NBS-LRR class) family LENGTH=1188
Vigun01g224500	Vu01	39,748,885	39,753,594	PREDICTED: protein TRANSPORT INHIBITOR RESPONSE 1-like isoform X1 [Glycine max]

University of Ghana http://ugspace.ug.edu.gh


P-Values by Chromosome for StrigaEmerg1

P-Values by Chromosome for StrigaEmerg2

P-Values by Chromosome for StrigaEmerg3

P-Values by Chromosome for StrigaEmerg4

Figure 5. 9: Manhattan plot for number of days to first Striga emergence

University of Ghana http://ugspace.ug.edu.gh

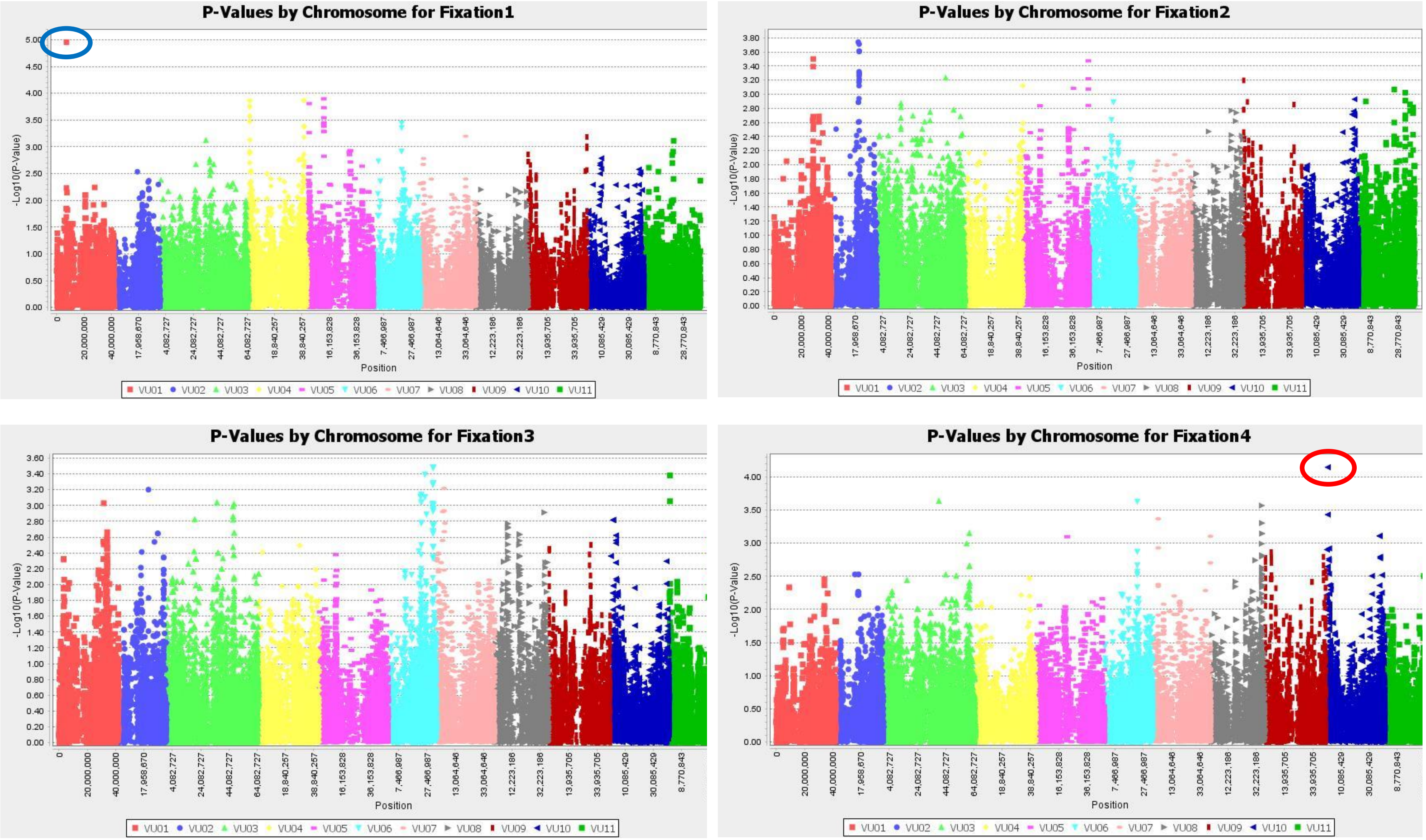


Figure 5. 10: Manhattan plot for presence / absence (fixation of Striga)

University of Ghana http://ugspace.ug.edu.gh

5.4. Discussions

Genomic resources have been developed to include molecular markers in the breeding pipeline (Muñoz-Amatriaín *et al.*, 2017), to shorten the selection duration for a trait and increase precision on records (Ribaut and Ragot, 2006). We took advantage of these resources to develop backcross lines in order to convert a highly desirable line Melakh to a Striga resistant line. Marker-Assisted Backcrossing started with background selection. The highest background recovered by an R line was 84%. Our results corroborate the results of a study involving rice which aimed at analysis of recurrent parent genome recovery. In the elater study, recovery of BC_1F_1 plants ranged from 75.40% to 91.3% (Miah *et al.*, 2015). Theoretically, at the first backcross cycle, progenies are expected to recover 75 % of the recurrent genome (Collard *et al.*, 2005).

By comparing SSR1 and C42-2B, it was found that SSR1 was more robust in the sense that it identified the same lines as C42-2B but in addition, identified some susceptible lines that were classified as resistance using C42-2B. Similar results were obtained in Ghana where SSR1 was 8 cM far apart from the resistance locus while C42-2B was 15 cM far away (Asare *et al.*, 2013a). Previous studies in Senegal also resulted in about the same efficiency of C42-2B as found in Ghana using bulked-segregant analysis (not published). Subsequent backcross population until the attainment of IT97K-499-39 *4 / Melakh lines were assessed using the presence or absence marker SSR1. Contradictory results were obtained in IT97K-499-39 *4 / Melakh population when screened with SSR1 and Marker C42-2B. Strangely, both markers gave the same results. In fact, conventional breeding cannot ensure purity of lines hence leading to type 2 error in F1 hybrids. To the best of our knowledge, the use of SNP 1_0958 has not been reported for such studies. If marker linkage with the desirable locus is loose, recombination can occur leading to type 1 error (Zhang *et al.*, 2012). These findings were confirmed by the results obtained in the field trials conducted in 4 Striga prone environment.

University of Ghana http://ugspace.ug.edu.gh

It was recorded that STR-11 and STR-5 were more adapted to those environments while screening using markers did not reveal any susceptible lines.

Surprisingly, field validation indicated that among lines that were inferenced as resistant some allowed Striga emergence. Results indicated that all markers used so far were not reliable for resistance screening of Striga prevailing in Senegal. However, among developed lines, STR-11 had greater resistance to the resistant line IT97K-499-39 used as donor in the creation of backcross populations. Another aspect is race septicity, if not taken into account can lead to controversial results. At least 7 races of *Striga gesnerioides* are known (Botanga and Timko, 2006; Li *et al.*,, 2009). Additionally, it has been reported that two different clusters of *Striga gesnerioides* populations prevalent in Senegal based on Nei's unbiased genetic distance (Tonessia *et al.*, 2014). It becomes obvious that markers used were not unlinked to loci for resistance of cowpea to *S. gesnerioides* but probably were linked to resistance for a specific race in Senegal. In this study, we also found that susceptible lines had higher grain yield performance than the resistant. In fact, grain yield trial was carried out in field free of Striga.

Consequently, a wider population hypothesized to have less co-ancestry history was used in order to identify SNP markers linked to R phenotype and find candidate genes around that region. The population size was suitable for genome wide association study compared to other where fewer size were used (Raman *et al.*, 2016; Varshney *et al.*, 2012; Xu *et al.*, 2012). GWAS revealed 3 SNP markers located on Vu01 and Vu10 in reference genome (new chromosome numbering). Resistant loci were reported on chromosomes 1 and 6 in reference to the old chromosome numbering (Li *et al.*, 2009). This is probably an indication that SNPs found in the current study have not been reported so far in any study.

Manhattan plots indicated some pics in Vu01, Vu06 and Vu03 for days to emergence respectively for environment 1, 2 and 3. Other pics were obtained on Vu02 and Vu06 for Striga

University of Ghana http://ugspace.ug.edu.gh

fixation respectively in environment 2 and 3. However, only three markers were over the threshold computed. GWAS can be run using different approach including the full optimization method and the 'population parameters previously determined' (P3D). In this experiment, the p-values used to compute the FDR that allowed the calculation of the threshold for each trait in each environment resulted from the algorithm of P3D in which re-computation of variance components are eliminated. This method is faster and suitable for computers with limited features. Resulting SNP markers are not expected to deviate significantly from P3D to 'compressed Linear Mixed Model' (Zhang *et al.*, 2010) hence markers identified are significant markers and can be validated in well characterized lines in regards to their resistance to known *S. gesnerioides* races.

In addition to identification of significant SNP markers, 6 candidate genes nearby to the SNPs positions were identified. The distance between candidate genes and SNPs varied among traits. There is no clear indication of the range to look for candidate genes based on the best of our knowledge. In this study, we focused on candidate genes that are known to be involved in gene-for-gene hypothesis. The following is a general explanation of how these candidate gene expression fits in plant-pathogen interaction including *S. gesnerioides* and host plant cowpea. In fact, most abnormal peptides and also short-lived cellular regulators are under control of ubiquitin–proteasome system (UPS) whose role is to delete or reshape them. This system is found in yeast and in animals. Recently in plants, stress responsive defense was found to be influenced by the UPS. Because of parasite aggressions, plant have developed a bundle of defense mechanisms in order to counter infection. The bundle includes basal responses, triggered by recognition of conserved pathogen-associated molecular patterns, and pathogen-specific responses, mediated via pathogen- and plant-specific gene-for-gene recognition events (Craig *et al.*, 2009). Most accepted model describing how plants interact with pathogens is the zig-zag model (Jones and Dangl, 2006) describing innate immune responses pathways

University of Ghana http://ugspace.ug.edu.gh

deployed by host plants to allow an unsuccessful parasitizing. In zig-zag model, resistance is at two levels where pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs) (Boller and He 2009) developed gradually by the parasite are recognized by receptors proteins from the host (kinases) which allow deployment of defensive responses known as pathogen triggered immunity (PTI). To overcome this first barrier, phytopathogens develop virulence factors that will destroy the first shield of protection at plant cellular level. As parasite effectors were able to overcome PTI, plants evolve a second shield of protection known as effector triggered immunity (ETI). In ETI, new receptors with NBS and LRR domain are evolved, those proteins are referred as resistant (R) proteins (Timko *et al.*, 2012). The six candidate genes identified and annotated fits perfectly in this model where effectors from parasites and receptors from plants are involved. In *Arabidopsis*, defense is evolved when mutations occur in the ubiquitin–proteasome pathway. These changes block hormone responses, senescence, and pathogen invasion (Craig *et al.*, 2009).

In conclusion, pathogen-delivered effectors evolved by parasites are recognized by disease resistance (R) proteins which evolve a protein-mediated reaction called gene-for-gene resistance. The latter were reported to be involved in *S. gesnerioides* and cowpea interaction.

5.5. Conclusion

In MABC, 3 lines were adapted to Striga prone area. In field validation we succeeded partially in the conversion of Melakh to a Striga resistant line. Novel SNPs were identified and candidate genes surrounding identified SNPs regions were identified. These SNPs will be designed to PCR markers to allow their validation in Bulked Segregant Analysis.

University of Ghana http://ugspace.ug.edu.gh

CHAPTER SIX

6. GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1. General conclusions

Main results in the PRA were that farmers ranked drought as the most limiting factor to cowpea production in Senegal. This was not expected because ISRA most breeding effort were focused on earliness to indirectly breed for drought tolerant lines. Feedback from farmers exhibited that mid-season drought was also a major constraint in which early varieties are very susceptible. The survey revealed also that *Striga gesnerioides* was of importance but not to the point of being the major limiting factor to cowpea production in Senegal. In addition, farmers informed that *S. hermonthica* was very abundant in the localities surveyed.

It was found in this study that farmers were interested in large seeds and brown seed colour as in Yacine a variety developed by Dr. Ndiaga Cisse. The PRA conducted increased the chances that our varieties could be widely adopted by producers. Another important aspect was that farmers showed enthusiasm in participating in breeder-farmer activities. As a matter of fact, the pet peeve of all breeders is adoption of released varieties. Time required for adoption can be effectively reduced if development of varieties is based on farmers' needs and their involvement in early stages of breeding such as during parent selection. Smallholder farmers expect drought tolerant lines with specific grain quality traits.

Suitable genotypic variation for drought tolerance was found in the 112 accessions from Senegalese cowpea breeding programme which can be used for improvement of cowpea tolerance to drought in low rainfall environments. Among the varieties released by ISRA, Mouride showed good adaptability when drought occurred. In water-stressed conditions, 17 cultivars produced more than 30% higher grain yield than Mouride.

University of Ghana http://ugspace.ug.edu.gh

Among the 5 lines identified as having the allele for resistance to Striga using SSR markers, only 3 lines were confirmed to be resistant under natural Striga infestation in the field. Therefore, this study showed that DNA markers were partially effective in developing Striga resistant lines.

GWAS for Striga resistance in the mini core resulted in identification of 3 SNP markers namely **2_10935, 2_44813 and 2_21670** not previously reported to be associated with resistance to Striga. In addition, 6 candidate genes formerly reported to be involved in gene-for-gene interaction were identified.

6.2. Recommendations

More profit from this work could be made if further studies could be carried out. Those studies include:

(i) Characterization of *Striga gesnerioides* biotypes in Senegal using genetic resources;

(ii) Revalidation of markers used in MABC in a different population;

(iii) Validation of markers identified in GWAS using a different population;

(iv) Development of Striga resistant lines and drought tolerant lines using new identified SNPs.

University of Ghana http://ugspace.ug.edu.gh

REFERENCES

Acevedo, E., Fereres, E., Hsiao, T.C., Henderson, D.W. (1979). Diurnal Growth Trends, Water Potential, and Osmotic Adjustment of Maize and Sorghum Leaves in the Field. *Plant Physiology* 64, 476–480. https://doi.org/10.1104/pp.64.3.476.

Agbicodo, E. M., Fatokun, C. A., Muranaka, S., Visser, R. G. F., and Linden van der, C. G. (2009). Breeding drought tolerant cowpea: constraints, accomplishments, and future prospects. *Euphytica*, *167*(3), 353–370.

Alonge, S.O., Lagoke, S.T.O., Ajakaiye, C.O. (2005). Cowpea reactions to *Striga gesnerioides* II. Effect on grain yield and nutrient composition. *Crop Protection* 24, 575–580. https://doi.org/10.1016/j.cropro.2004.08.015

Amasino, R. M. (2005). Vernalization and flowering time. *Current Opinion in Biotechnology*, *16*(2), 154–158.

André, P., and Demonsant, J.-L. (2009). Koranic Schools in Senegal : A real barrier to formal education ? Retrieved from https://www.parisschoolofeconomics.eu/IMG/pdf/JobMarket-2ndpaper-ANDRE-PSE.pdf

Araus, J. L. (2002). Plant Breeding and Drought in C3 Cereals: What Should We Breed For? *Annals of Botany*, *89*(7), 925–940.

Asare, A. T., Galyuon, I. K., Padi, F. K., Otwe, E. P., and Takrama, J. F. (2013a). Responses of recombinant inbred lines of cowpea [(*vigna unguiculata* (l.) Walp] to *Striga gesnerioides* infestation in Ghana. *European Scientific Journal*, *9*(21). Retrieved from http://eujournal.org/index.php/esj/article/view/1487.

University of Ghana http://ugspace.ug.edu.gh

Asare, A. T., Gowda, B. S., Galyuon, I. K. A., Aboagye, L. M., Takrama, J. F., Padi, F. K., and Timko, M. P. (2013b). Identification of potential sources of striga resistance in cowpea [*Vigna Unguiculata* (L.) walp.] accessions from Ghana. *Journal of Microbiology and Biotechnology Research*, 10.

Atkins, C. A., and Smith, P. M. C. (2007). Translocation in Legumes: Assimilates, Nutrients, and Signaling Molecules. *Plant Physiology*, *144*(2), 550–561.

Atokple, I. D. K., Singh, B. B., and Emechebe, A. M. (1995). Genetics of Resistance to Striga and Alectra in Cowpea. *Journal of Heredity*, *86*(1), 45–49.

Ba, F. S., Pasquet, R. S. and Gepts, P. (2004) Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] as revealed by RAPD markers. *Genetic Resources and Crop Evolution*, 51**,** 539-550.

Badiane AN (1993) Le statut organique d'un sol sableux de la zone Centre-Nord du Sénégal. *Thèse doctorat*, INPL/ENSAIA, Nancy, 200 pp.

Badiane, A. N., Khouma, M., and Séne, M. (2000), Région de Diourbel: Gestion des sols, Drylands Research Working Paper 15, Drylands Research, Somerset, England, p. 25.

Collard B. C. Y., Jahufer M. Z. Z., Brouwer J. B., and Pang. E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, *142*(1–2), 169–196.

Barrett, J. R. (2006). The Science of Soy: What Do We Really Know? *Environmental Health Perspectives*, *114*(6), A352–A358.

Basu, S., Ramegowda, V., Kumar, A., and Pereira, A. (2016). Plant adaptation to drought stress. *F1000Research*, *5*, 1554.

University of Ghana http://ugspace.ug.edu.gh

Baudoin, J.-P. (2001). Contribution des ressources phytogénétiques à la sélection variétale de légumineuses alimentaires tropicales. *Biotechnology, Agronomy, Society and Environment*. 5 (4), 11.

Beebe, S. E., Rao, I. M., Blair, M. W., and Acosta-Gallegos, J. A. (2013). Phenotyping common beans for adaptation to drought. *Frontiers in Physiology, 4(35)*.

Begg, J. E., and B. W. R. Torsell. (1974). *In Mechanism of regulation of plant growth*, R. L. Bieleski, A. R. Ferguson, M. M. Cresswell (Eds.) Royal Society of New Zealand Bull. 12:277-283.

Begg, J.E. and Turner, N.C. (1976). Crop water deficits. *Advances in Agronomy*, 28: 161-217.

Belko, N., Zaman-Allah, M., Cisse, N., Diop, N. N., Zombre, G., Ehlers, J. D., and Vadez, V. (2012). Lower soil moisture threshold for transpiration decline under water deficit correlates with lower canopy conductance and higher transpiration efficiency in drought-tolerant cowpea. *Functional Plant Biology*, *39*(4), 306.

Bennett, J.R. and Mathews, S. (2006) Phylogeny of the parasitic plant family Orobanchaceae inferred from phytochrome A. *American Journal of Botany*, 93, 1039–1051

Berner, D.K. & O.A. Williams. (1998). Germination of Striga gesnerioides seeds stimulated by hosts and non-hosts. *Plant Disease* 82(11): 1242–1247.

Berner DK, Winslow MD, Awad AE, Cardwell KF, Mohan Raj DR, Kim SK. (1997). Striga research methods – A manual. 2nd edition. Ibadan (Nigeria): International Institute of Tropical Agriculture (IITA). http://iita.titaninternet.co.uk/cms/details/Striga.pdf

University of Ghana http://ugspace.ug.edu.gh

Bidinger, F. R., Mahalakshmi, Y., and Rao, G. D. P. (1987). Assessment of drought resistance in pearl millet. II. Estimation of genotype response to stress. *Australian Journal of Agricultural Research* 38, 49-59.

Blum, A. (1996). Crop responses to drought and the interpretation of adaptation. *Plant Growth Regulation*, *20*(2), 135–148.

Blum A, Ebercon A. (1976). Genotypic responses in sorghum to drought stress. III. Free proline accumulation and drought resistance. *Crop Science,* 16: 428–431.

Boggess, S.F., and Stewart, C.R. (1976). Contribution of arginine to proline accumulation in water-stressed barley leaves. *Plant Physiology,* 58:796–797.

Boller, T., He, S.Y. (2009). Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science,* 324, 742–744. https://doi.org/10.1126/science.1171647

Botanga, C. J., and Timko, M. P. (2005). Genetic Structure and Analysis of Host and Nonhost Interactions of*Striga gesnerioides* (Witchweed) from Central Florida. *Phytopathology*, *95*(10), 1166–1173.

Botanga, C. J., and Timko, M. P. (2006). Phenetic relationships among different races of *Striga gesnerioides* (Willd.) Vatke from West Africa. *Genome*, *49*, 1351–1365.

Boukar, O., Kong, L., Singh, B. B., Murdock, L., and Ohm, H. W. (2004). AFLP and AFLP-Derived SCAR Markers Associated with Resistance in Cowpea. *Crop Science*, *44*(4), 1259–1264.

University of Ghana http://ugspace.ug.edu.gh

Boukar, O., Belko, N., Chamarthi, S., Togola, A., Batieno, J., Owusu, E., Haruna, M., Diallo, S., Umar, M.L., Olufajo, O., Fatokun, C., 2018. Cowpea (*Vigna unguiculata*): Genetics, genomics and breeding. *Plant Breeding*. https://doi.org/10.1111/pbr.12589

Boukar, Ousmane, Bhattacharjee, R., Fatokun, C., Kumar, P. L., and Gueye, B. (2013). Cowpea. In *Genetic and Genomic Resources of Grain Legume Improvement*, 137–156). Elsevier.

Boukar, Ousmane, Fatokun, C. A., Huynh, B.-L., Roberts, P. A., and Close, T. J. (2016). Genomic Tools in Cowpea Breeding Programs: Status and Perspectives. *Frontiers in Plant Science*, *7*.

Boyer, J.S. (1970). Leaf Enlargement and Metabolic Rates in Corn, Soybean, and Sunflower at Various Leaf Water Potentials. *Plant Physiology*, 46, 233–235. https://doi.org/10.1104/pp.46.2.233

Boyer, J. S. (1971). Resistance to water transport in soybean, bean and sunflower. *Crop Science*, 11: 403–407.

Boyer, J. S. and McPherson H. G. (1975). Physiology of water deficits in cereal crops. *Advances in Agronomy*, 27: 1-23.

Bressani R (1985). Nutritive value of cowpea. In Singh SR, Rachie, KO (eds) Cowpea Research, Production and Utilization. Wiley, Winchetser, UK. pp.353-359.

Brevedon, E.R. and Hodges, H.P. (1973). Effect of moisture deficit on C^{11} translocation in Corn (Zea mays. L.). *Plant Physiology*, 52: 436 - 439

University of Ghana http://ugspace.ug.edu.gh

Cardoso, C., Ruyter-Spira, C., and Bouwmeester, H. J. (2011). Strigolactones and root infestation by plant-parasitic Striga, Orobanche and Phelipanche spp. *Plant Science, 180* (3), 414–420.

Cardwell, K.F. and Lane, J. A. (1995). Effects of soils, cropping system and host phenotype on incidence and severity of *Striga gesnerioides* on cowpea in West Africa. *Agriculture, Ecosystems & Environment,* Environ. 53: 253-262.

Cisse, N., Ndiaye, M., and Sene, A. (2005). Registration of "Yacine" cowpea. *Crop Science,* Retrieved from http://agris.fao.org/agris-search/search.do;jsessionid=41FAAB4CC088A5BCEACACD0BEC4513D6?request_locale=ru&recordID=US201300981876&query=&sourceQuery=&sortField=&sortOrder=&agrovocString=&advQuery=&centerString=&enableField=

Cissé, N., Wey, J., Seck, D., Gueye, M. T., and Gueye, M. (2005). *Les légumineuses à graines* (pp. 257–261). ISRA, ITA, CIRAD. Retrieved from www.bameinfopol.info/IMG/pdf/Bilan-Senegal

Cissé, Ndiaga. (2016, June). La culture traditionnelle du niébé au Sénégal - IED afrique | Innovations Environnement Développement. Retrieved November 17, 2018, from http://www.iedafrique.org/La-culture-traditionnelle-du-niebe-au-Senegal.html

Cisse, Ndiaga, Ndiaye, M., Thiaw, S., and Hall, A. E. (1997). Registration of 'Melakh' Cowpea. *Crop Science, 37*(6), 1978.

Coulibaly, O. and Lowenberg-Deboer, J. (2002). The economics of cowpea in west africa *In:* Fatokun, C. A., S.A. Tarawali, B.B. Singh, P.M. Kormawa, and M. Tamò (ed.) *Challenges and opportunities for enhancing sustainable cowpea production*

University of Ghana http://ugspace.ug.edu.gh

International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria IITA, Ibadan, Nigeria 351-366.

Coulibaly, S.; Pasquet, R. S.; Papa, R. and Gepts, P. (2002). Aflp analysis of the phenetic organization and genetic diversity of *Vigna unguiculata* (L.) Walp Reveals extensive gene flow between wild and domesticated types. *Theoretical and Applied Genetics,* 104: 358–366.

Craig, A., Ewan, R., Mesmar, J., Gudipati, V., and Sadanandom, A. (2009). E3 ubiquitin ligases and plant innate immunity. *Journal of Experimental Botany*, *60*(4), 1123–1132.

Culter, J. H., Rains, D. W. and Loomis, R. S. 1977. The importance of cell size in the water relations of plants. *Physiologia Plantarum*, 40: 255-260.

Dadson, R.B., Hashem, F.M., Javaid, I., Joshi, J., Allen, A.L., Devine, T.E. (2005). Effect of Water Stress on the Yield of Cowpea (*Vigna unguiculata* L. Walp.) Genotypes in the Delmarva Region of the United States: Water Stress in Cowpea. *Journal of Agronomy and Crop Science*, 191, 210–217. https://doi.org/10.1111/j.1439-037X.2005.00155.x

Davis S. D., McCree K. J. (1978) Photosynthetic rate and diffusion conductance as a function of age in leaves of bean plants. *Crop Science,* 18:280–282

D'Andrea, A. C., Kahlheber, S., Logan, A. L., and Watson, D. J. (2007). Early domesticated cowpea (*Vigna unguiculata*) from Central Ghana. *Antiquity*, *81*(313), 686–698.

Dembele, B. (1988). Aspects biologiques et agronomiques de deux scrophulariacees parasites tropicales : Striga hermonthica (Del.) Benth. et *Striga gesnerioides* (Willd.) Vatke.

University of Ghana http://ugspace.ug.edu.gh

These de doctorat, Ecole nationale superieure agronomique de Montpellier, Montpellier.

Dubé, M. P., and Olivier, A. (2001). Le *Striga gesnerioides* et son hote, le niebe : interaction et methodes de lutte. *Canadian Journal of Plant Botany*, *79*, 1225–1240.

Eberhart, s. A., and Russell, w. A. (1966). Stability parameters for comparing varieties. *Crop Science*, 6, 36-40.

Egbadzor, K.F., Offei, S.K., Danquah, E.Y., Kotey, D.A., Gamedoagbao, D.K., Dadoza, M., Yeboah, M., Ofori, K. (2015). Farmer participation in selection within segregating populations of cowpea in Volta Region, Ghana. *Agriculture & Food Security*, 4. https://doi.org/10.1186/s40066-015-0037-1

Ehlers, J. D., and Hall, A. E. (1997). Cowpea (*Vigna unguiculata* L. Walp.). *Field Crops Research*, (53), 187–204.

Emechebe A. M., Singh B. B., Leleji O., Atopklee I. D. K., and Adu J. K. (1991). Cowpea Striga problems and research in Nigeria. In: Kim, S.K. (ed): Combating Striga in Africa. Proceedings of International Workshop organised by IITA, ICRISAT and IDRC: 22 – 24 August, 1988, IITA, Ibadan, Nigeria, pp. 18 – 28.

Fahad, S., Bajwa, A.A., Nazir, U., Anjum, S.A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M.Z., Alharby, H., Wu, C., Wang, D., Huang, J., 2017. Crop Production under Drought and Heat Stress: Plant Responses and Management Options. *Frontiers in Plant Science*. 8. https://doi.org/10.3389/fpls.2017.01147.

University of Ghana http://ugspace.ug.edu.gh

FAO. (2008). Search results | FAO | Food and Agriculture Organization of the United Nations. Retrieved November 29, 2018, from http://www.fao.org/search/en/?cx=018170620143701104933%3Aqq82jsfba7w&q=cowpea+2008&cof=FORID%3A9&siteurl=www.fao.org%2Ffaostat%2Fen%2F%23home&ref=www.fao.org%2Ffaostat%2F&ss=2726j852680j11.

Faris, D. G. (1963). *Evidence for West African origin of Vigna sinensis* (L.) Savi. Doctor of Philosophy University of California 89p.

Faris, D.G. 1965. The Origin and Evolution of the Cultivated Forms of Vigna sinensis. Canadian Journal of Genetics and Cytology 7: 433-52.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., and Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development*, *29*(1), 185–212.

Fatokun, C. A., Boukar, O., and Muranaka, S. (2012). Evaluation of cowpea (*Vigna unguiculata* (L.) Walp.) germplasm lines for tolerance to drought. *Plant Genetic Resources*, *10*(03), 171–176.

Fatokun, C., Girma, G., Abberton, M., Gedil, M., Unachukwu, N., Oyatomi, O., Yusuf, M., Rabbi, I., Boukar, O., (2018). Genetic diversity and population structure of a mini-core subset from the world cowpea (*Vigna unguiculata* (L.) Walp.) germplasm collection. Scientific Reports 8. https://doi.org/10.1038/s41598-018-34555-9

Faye, M., Jooste, A., Lowenberg-DeBoer, J., and Fulton, J. (2004). The influence of cowpea characteristics on cowpea prices in senegal. *Agrekon*, *43*(4), 418–429.

University of Ghana http://ugspace.ug.edu.gh

Fernandez, G.C.J. (1992). Effective selection criteria for assessing plant stress tolerance. Proceedings of the International Symposium on Adaptation of Vegetables and other Food Crops in Temperature and Water Stress, August 13-16, 1992, Shanhua, Taiwan, 257-270.

Fereres, E., E. Acevedo, D. W. Henderson, and T. C. Hsiao. (1978). Seasonal changes in water potential and turgor maintenance in sorghum and maize under water stress. *Physiologia. Plantarum.* 44:261:267.

Finlay, K. W. and Wilkinson, G. N. (1963). The analysis of adaptation in plant breeding programme. *Australian Journal of Agricultural Research*, 14: 742-754.

Fischer, R.A. and Maurer R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. *Australian Journal of Agricultural Research*, 29: 897-912.

Fischer, R.A. and Turner, N.C. (1978). Plant productivity in the arid and semi-arid zones. *Annual Review of Plant Biology*, 29: 277--317.

Frank A.B., Power J.F., Willis W.O. (1973). Effect of temperature and plant water stress on photosynthesis, diffusion resistance, and leaf water potential in spring wheat. *Agronomy Journal* 65, 777-780. http://dx.doi.org/10.2134/agronj1973.00021962006500050031x

Gauch, H.G. (1988) Model Selection and Validation for Yield Trials with Interaction. *Biometrics*, 44, 705-715. http://dx.doi.org/10.2307/2531585

Greenshields, D. L., and Jones, J. D. (2008). Plant pathogen effectors: Getting mixed messages. *Current Biology* 18: R128-130.

Gwathmey, C. O., Hall, A. E., and Madore, M. A. (1992). Adaptive Attributes of Cowpea Genotypes with Delayed Monocarpic Leaf Senescence. *Crop Science*, *32*(3), 765.

University of Ghana http://ugspace.ug.edu.gh

Halime, M. H., Belko, N., Cisse, N., Sine, B., and Ndoye, I. (2014). Amélioration de l'adaptation à la sécheresse chez le niébé (*Vigna unguiculata* L. Walpers). *Journal of Applied Biosciences*, *77*(1), 6550.

Hall A. E. (2012). Phenotyping Cowpeas for Adaptation to Drought. *Frontiers in Physiology*, *3*.

Hall, A.E., Cisse, N., Thiaw, S., Elawad, H.O.A., Ehlers, J.D., Ismail, A.M., Fery, R.L., Roberts, P.A., Kitch, L.W., Murdock, L.L., Boukar, O., Phillips, R.D., McWatters, K.H., 2003. Development of cowpea cultivars and germplasm by the Bean/Cowpea CRSP. *Field Crops Research* 82, 103–134. https://doi.org/10.1016/S0378-4290(03)00033-9

Hallensleben, M., Polreich, S., Heller, J., and Maass, B. L. (2009). *Resource Management and Rural Development*. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.627.7812&rep=rep1&type=pdf

Hanson AD, Nelsen CE, Everson EH (1977) Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. *Crop Science*, 17: 720–726

Hanson, A.D., Nelson, C.E., Pedarson, A.R., Everson, E.H.: Capacity for free proline accumulation during water stress in barley and its implication for drought resistance. *Crop Science*, 19: 489–493, 1979.

Hibberd, J. M., Quick, W. P., Press, M. C., and Scholes, J. D. (1996). The influence of the parasitic angiosperm *Striga gesnerioides* on the growth and photosynthesis of its host, *Vigna unguiculata*. *Journal of Experimental Botany*, *47*(4), 507–512.

University of Ghana http://ugspace.ug.edu.gh

Hirut, B., Shimelis, H., Fentahun, M., Bonierbale, M., Gastelo, M., and Asfaw, A. (2017). Combining ability of highland tropic adapted potato for tuber yield and yield components under drought. *PLOS ONE*, *12*(7), e0181541.

Hsiao, T.C. and Acevedo, E., 1974. Plant responses to water deficits, water-use efficiency and drought, resistance. *Agricultural Meteorology*, 14 : 59—84.

Huynh, B.-L., Close, T.J., Roberts, P.A., Hu, Z., Wanamaker, S., Lucas, M.R., Chiulele, R., Cissé, N., David, A., Hearne, S., Fatokun, C., Diop, N.N., Ehlers, J.D., 2013. Gene Pools and the Genetic Architecture of Domesticated Cowpea. The Plant Genome 6, 0. https://doi.org/10.3835/plantgenome2013.03.0005

Huynh, B.-L., Ehlers, J.D., Close, T.J., Cissé, N., Drabo, I., Boukar, O., Lucas, M.R., Wanamaker, S., Pottorff, M., Roberts, P.A., 2013b. Enabling tools for modern breeding of cowpea for biotic stress resistance. *Translational Genomics for Crop Breeding* 1, 183–200.

IBP. 2015. https://www.integratedbreeding.net/579/breeding-management-system/tutorials/maize-multi-site-gxe-analysis

Ings, J., Mur, L. A. J., Robson, P. R. H., and Bosch, M. (2013). Physiological and growth responses to water deficit in the bioenergy crop Miscanthus x giganteus. *Frontiers in Plant Science*, *4*.

Iljin, W.S., (1957). Drought Resistance in Plants and Physiological Processes. Annu. Rev. Plant. Physiol. 8, 257–274. https://doi.org/10.1146/annurev.pp.08.060157.001353

University of Ghana http://ugspace.ug.edu.gh

Jackson, P., Robertson, M., Cooper, M., and Hammer, G. (1996). The role of physiological understanding in plant breeding; from a breeding perspective. *Field Crops Research*, *49*(1), 11–37.

Jones, M.M., and N.C. Turner. 1978. Osmotic adjustment in leaves of sorghum in response to water deficits. *Plant Physiology*, 61:122–126.

Jones, H. G. (1993). Drought tolerance and water use efficiency in water deficits. *In:* Smith, J. A. C. and Griffiths, H. (eds.) *Plant responses from cell to community*. Oxford (UK): Bios Scientific Publishers, 193-203.

Jones, J.D.G., Dangl, J.L., (2006). The plant immune system. *Nature*, 444, 323–329. https://doi.org/10.1038/nature05286

Jordan, W. R. Brown, K. W., and Thomas, 1. C. (1975) Leaf age as a determinant in stomatal control of water loss from cotton, during water stress. *PIant Physiology*, 56 : 595 - 599.

Kamara, A. Y., Omoigui, L. O., Kamai, N., Ewansiha, S. U., and Ajeigbe, H. A. (2018). Improving cultivation of cowpea in West Africa. In D. Bergvinson, P. Gaur, S. Kumar Agrawal, S. Beebe, and M. Tamò (Eds.), *Burleigh Dodds Series in Agricultural Science* (pp. 235–252). Burleigh Dodds Science Publishing.

Klar, A. E., J. A. Usberti, and D. W. Henderson. (1978): Differential responses of guinea-grass population to drought stress. *Crop Science*, 18, 853–857.

Kramer P. J. (1980) Drought stress and origin of adaptation. In Adaptation of Plants to Water and High Temperature Stress. Eds. NC Turner and PJ Kramer, pp 6-20. John Wiley & Sons, New York, NY.

University of Ghana http://ugspace.ug.edu.gh

Lane, J. A., Moore, T. H. M., and Child, D. V. (1996). Characterization of virulence and geographic distribution of *Striga gesnerioides* on cowpea in West Africa. *Plant Disease*, *80*, 299–301.

Langyintuo, A.S., Lowenberg-DeBoer, J., Faye, M., Lambert, D., Ibro, G., Moussa, B., Kergna, A., Kushwaha, S., Musa, S., Ntoukam, G., 2003. Cowpea supply and demand in West and Central Africa. *Field Crops Research*, Research Highlights of the Bean/Cowpea Collaborative Research Support Program, 1981 - 2002 82, 215–231. https://doi.org/10.1016/S0378-4290(03)00039-X

Li, J., Lis, K. E., and Timko, M. P. (2009). Molecular genetics of race-specific resistance of cowpea to *Striga gesnerioides* (Willd.). *Pest Management Science*, *65*(5), 520–527.

Lin, C.S.; Binns, M.R.A. (1988) superiority measure of cultivar performance for cultivar x location data. *Canadian Journal of Plant Science*, 68, 193-198.

Lo, S., Munoz-Amatriain, M., Boukar, O., Herniter, I., Cisse, N., Guo, Y.-N., Roberts, P.A., Xu, S., Fatokun, C., Close, T.J., 2017. Identification of genetic factors controlling domestication-related traits in cowpea (*Vigna unguiculata* L. Walp). *bioRxiv* 202044.

Lonardi, S. (2019). Phytozome v12.1: Info. Retrieved December 11, 2018, from https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Vunguiculata_er

Lucas, M. R., Ehlers, J. D., Huynh, B.-L., Diop, N.-N., Roberts, P. A., and Close, T. J. (2013). Markers for breeding heat-tolerant cowpea. *Molecular Breeding*, *31*(3), 529–536.

University of Ghana http://ugspace.ug.edu.gh

Magen, B. B., Crawford, E., and Maredia, M. (2012). An Ex Post Economic Impact Assessment of Bean/Cowpea Crsp's Investment on Varietal Development in Senegal. Retrieved from https://ageconsearch.umn.edu/record/142739

Mai-Kodomi Y, Singh BB, Terao T, Myers O Jr, Yopp JH, Gibson PJ (1999b) Inheritance of drought tolerance in cowpea. *Indian Journal of Genetics and Plant Breeding*, 59:317–323

Malosetti, M., Ribaut, J.-M., van Eeuwijk, F.A. (2013). The statistical analysis of multi-environment data: modeling genotype-by-environment interaction and its genetic basis. Frontiers in Physiology 4. https://doi.org/10.3389/fphys.2013.00044

Marcenaro, D., and Valkonen, J. P. T. (2016). Seedborne Pathogenic Fungi in Common Bean (Phaseolus vulgaris cv. INTA Rojo) in Nicaragua. *Plos One*, *11*(12), e0168662.

Matusova, R. (2005). The Strigolactone Germination Stimulants of the Plant-Parasitic Striga and Orobanche spp. Are Derived from the Carotenoid Pathway. *Plant Physiology* 139, 920–934. https://doi.org/10.1104/pp.105.061382

McMichael, B.L. and Elmore, C.D. (1977). Proline accumulation in water stressed cotton leaves. *Crop Science,* 17,905-908.

McPherson, H.G. & Boyer, J.S. (1977). Regulation of grain yield by photosynthesis in maize subjected to water deficiency. (*Agronomy Journal*, 69: 714–718.

Miah, G., Rafii, M. Y., Ismail, M. R., Puteh, A. B., Rahim, H. A., and Latif, M. A. (2015). Recurrent parent genome recovery analysis in a marker-assisted backcrossing program of rice (Oryza sativa L.). *Comptes Rendus Biologies*, *338*(2), 83–94.

University of Ghana http://ugspace.ug.edu.gh

Mianda, G. (1998). Deborah Fahy BRYCESON (dir.). Women Wielding the Hoe : Lessons from Rural Africa for Feminist Theory and Development practice. Oxford, Berg Publisher, 1995, xi + 282 p. fig., index. *Anthropologie et Sociétés*, *22*(1), 206.

Mohamed A, Ellicott A, Housley TL, Ejeta G. (2003). Hypersensitive response to Striga infection in Sorghum. *Crop Science,* 43: 1320–1324.

Mohammadi, M., Karimizadeh, R., Abdipour, M. (2011). Evaluation of drought tolerance in bread wheat genotypes under dryland and supplemental irrigation conditions. Australian Journal of *Crop Science*, 5(4), 487–493.

Mohammadi R, Armion M, Kahrizi D, Amri A (2010) Efficiency of screening techniques for evaluating durum wheat genotypes under mild drought conditions. *International Journal of Plant Production*, 4: 11-24.

Morgan, J.M. (1977). Differences in osmoregulation between wheat cultivars. *Nature* (London) 270:234–235.

Muchero, W., Diop, N.N., Bhat, P.R., Fenton, R.D., Wanamaker, S., Pottorff, M., Hearne, S., Cisse, N., Fatokun, C., Ehlers, J.D., others, 2009. A consensus genetic map of cowpea [*Vigna unguiculata* (L) Walp.] and synteny based on EST-derived SNPs. *Proceedings of the national academy of sciences* 106, 18159–18164.

Muchero, W., Ehlers, J. D., and Roberts, P. A. (2008). Seedling Stage Drought-Induced Phenotypes and Drought-Responsive Genes in Diverse Cowpea Genotypes. *Crop Science*, *48*(2), 541.

University of Ghana http://ugspace.ug.edu.gh

Murdock, G.P. (1959). Africa, its peoples and their culture history. McGraw Hill Book Company, New York, Toronto, London.

Musselman L J and Ayensu E S (1984) Taxonomy and biosystematics of Striga. In Striga: Biology and Control. Eds. E S Ayensu, H Dogget, R D Keynes, J Marton-Lefevre, L J Musselman, C Parker and A Pickering, pp 37-45. ICSU Press/IR DC Pari

Muñoz-Amatriaín, M., Mirebrahim, H., Xu, P., Wanamaker, S.I., Luo, M., Alhakami, H., Alpert, M., Atokple, I., Batieno, B.J., Boukar, O., others, 2017. Genome resources for climate-resilient cowpea, an essential crop for food security. *The Plant Journal* 89, 1042–1054.

Ng, N.Q. and R. Maréchal. (1985). Cowpea taxonomy, origin and germplasm. Pages 11–21 in Cowpea Research, Production, and Utilization, edited by S.R. Singh and K.O. Rachie. John Wiley & Sons, Chichester.

Ng, N.Q. (1995). Cowpea, in J. Smartt and N.W. Simmonds (ed.) Evolution of crop plants: 326-32. New York: Wiley.

Omoigui, L. O., Kamara, A. Y., Masswe, F. S., Ishiyaku, M. F., Boukar, O., Alabi, S. O., and Ekeleme, F. (2007). Evaluation of cowpea genotypes for their reactions to *Striga gesnerioides* in the dry Savannah of Northeast Nigeria. *African Crop Science Conference Proceedings*, *8*, 273–278.

Omoigui. (2012). Comparative responses of cowpea breeding lines to Striga and Alectra in the dry savanna of northeast Nigeria. *African Journal of Agricultural Research*, *7*(5).

Omoigui, L. O., Ishiyaku, M. F., Gowda, B. S., Kamara, A. Y., and Timko, M. P. (2015). Suitability and use of two molecular markers to track race-specific resistance *striga*

University of Ghana http://ugspace.ug.edu.gh

gesnerioides in cowpea (*Vigna unguiculata* (L.) Walp.). *African Journal of Biotechnology*, *14*(27), 2179–2190.

Omoigui, L. O., Kamara, A. Y., Alunyo, G. I., Bello, L. L., Oluoch, M., Timko, M. P., and Boukar, O. (2017a). Identification of new sources of resistance to *Striga gesnerioides* in cowpea *Vigna unguiculata* accessions. *Genetic Resources and Crop Evolution*, *64*(5), 901–911.

Omoigui, Lucky O., Kamara, A. Y., Ajeigbe, H. A., Akinwale, R. O., Timko, M. P., Oyekunle, M., and Bello, L. L. (2017b). Performance of cowpea varieties under *Striga gesnerioides* (Willd.) Vatke infestation using biplot analysis. *Euphytica*, *213*(11).

Omoigui, Lucky O., Kamara, A. Y., Moukoumbi, Y. D., Ogunkanmi, L. A., and Timko, M. P. (2017c). Breeding cowpea for resistance to *Striga gesnerioides* in the Nigerian dry savannas using marker-assisted selection. *Plant Breeding*, *136*(3), 393–399.

Ouedraogo, J. T., Maheshwari, V., Berner, D. K., St-Pierre, C.-A., Belzile, F., and Timko, M. P. (2001a). Identification of AFLP markers linked to resistance of cowpea (*Vigna unguiculata* L.) to parasitism by *Striga gesnerioides*. *Theoretical and Applied Genetics*, *102*(6–7), 1029–1036.

Ouedraogo, Jeremy T., St-Pierre, C.-A., Olivier, A., Timko, M. P., Dubé, M. P., Dabiréi, C., and Belzile, F. J. (2001b). Utilisation des marqueurs moléculaires en sélection, 372–386.

Ouedraogo, J.T., Tignegre, J. B., Timko, M. P., and Belzile, F. J. (2002). AFLP markers linked to resistance against *Striga gesnerioides* race 1 in cowpea (*Vigna unguiculata*). *Genome*, *45*(5), 787–793.

University of Ghana http://ugspace.ug.edu.gh

O'Toole, J.C., Crookston, R.K., Treharne, K.J. and Ozbun, J.L. (1976). Mesophyll resistance and carboxylase activity. A comparison under water stress conditions. *(*. 57: 465–468.

O'Toole JC, Ozbun JL, Wallace DH (1977) Photosynthetic response to water stress in Phaseolus vulgaris. *Physiologia Plantarum*, 40:111–114

Padulosi, S. and N.Q. Ng. (1997). Origin, taxonomy, and morphology of *Vigna unguiculata* (L.) Walp. Pages 1–12 in Advances in Cowpea Research, edited by B.B Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. IITA-JIRCAS, Ibadan.

Padulosi, S., Ng, Q.N. and Perrino, P., (1997). Origin, taxonomy, and morphology of *Vigna unguiculata* (L.) Walp. In: eds. B.B.

PAEPARD: The business of plant breeding. (2018, January 16). Retrieved November 18, 2018, from http://paepard.blogspot.com/2018/01/the-business-of-plant-breeding.html

Panella, L., J. Kani and P. Gepts. (1993). Vignin Diversity in Wild and Cultivated Taxa of *Vigna unguiculata* (L.) Walp. (Fabaceae). *Economic Botany* 47(4): 371-86.

Pasquet, R., S., and Baudoin, J. P. (1997). Le niébé. *L'amélioration Des Plantes Tropicales*. Retrieved from http://www.documentation.ird.fr/hor/fdi:010012937.

Pasquet, R. (1999) Genetic relationships among subspecies of *Vigna unguiculata* (L.) Walp. based on allozyme variation. *Theoretical and Applied Genetics*, 98**,** 1104- 1119.

Plant, R.E., Mermer, A., Pettygrove, G.S., Vayssieres, M.P., Young, J.A., R. O. Miller, L. F. Jackson, R. F. Denison, K. Phelps, 1999. Factors underlying grain yield spatial variability in three irrigated wheat fields. *Transactions of the ASAE*. 42, 1187–1202. https://doi.org/10.13031/2013.13284

University of Ghana http://ugspace.ug.edu.gh

Pernes, J. (ed.) (1984). *Gestion des ressources génétiques des plantes*.346p

Polania, J. A., Poschenrieder, C., Beebe, S., and Rao, I. M. (2016). Effective Use of Water and Increased Dry Matter Partitioned to Grain Contribute to Yield of Common Bean Improved for Drought Resistance. *Frontiers in Plant Science, 7*.

Press M. C. and Graves J. D. Eds. 1995. Parasitic Plants. London, Chapman and Hall.

Raman, H., Raman, R., Coombes, N., Song, J., Prangnell, R., Bandaranayake, C., Tahira, R., Sundaramoorthi, V., Killian, A., Meng, J., Dennis, E.S., Balasubramanian, S., 2016. Genome-wide association analyses reveal complex genetic architecture underlying natural variation for flowering time in canola: Genetic analysis for natural variation of flowering time. *Plant, Cell and Environment*, 39, 1228–1239. https://doi.org/10.1111/pce.12644

Ramírez, D. A., Yactayo, W., Gutiérrez, R., Mares, V., De Mendiburu, F., Posadas, A., and Quiroz, R. (2014). Chlorophyll concentration in leaves is an indicator of potato tuber yield in water-shortage conditions. *Scientia Horticulturae*, *168*, 202–209.

Raynal-Roques, A. (1993) Contribution à la connaissance de la biologie des *Striga* (Scrophulariaceae): types biologiques et phenologie. *Bulletin du Museum national d'histoire naturelle*. *Section B*, *Adansonia*, 15**,** 3-21.

Rawal, K.M. (1975). Natural Hybridization Among Wild,Weedy, and Cultivated *Vigna unguiculata* (L.) Walp. *Euphytica* 24(3): 699-707.

University of Ghana http://ugspace.ug.edu.gh

Ribaut, J.-M., Hoisington, D. ., and Deutsch, J. A. (1996). Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval. *Theoretical and Applied Genetics*, *96*, 905–914.

Ribaut, J.-M., and Ragot, M. (2006). Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives. *Journal of Experimental Botany*, *58*(2), 351–360.

Rolando, J. L., Ramírez, D. A., Yactayo, W., Monneveux, P., and Quiroz, R. (2015). Leaf greenness as a drought tolerance related trait in potato (Solanum tuberosum L.). *Environmental and Experimental Botany*, *110*, 27–35.

Rosielle AA, Hamblin J (1981) Theoretical aspects of selection for yield in stress and non-stress environments. *Crop Science,* 21:943-946 SAS Institute (2001) The SAS system for Windows. Release

Salifou, M., Tignegre, J. B. L. S., Tongoona, P., Offei, S., Ofori, K., and Danquah, E. (2017). Differential responses of 15 cowpea genotypes to three Striga hot spots in Niger. *International Journal of Biological and Chemical Sciences*, *11*(4), 1413.

Samarah, N. H. (2005). Effects of drought stress on growth and yield of barley. *Agronomy for Sustainable Development*, *25*(1), 145–149.

Sanders, G.J., Arndt, S.K. (2012). Osmotic Adjustment Under Drought Conditions, in: Aroca, R. (Ed.), Plant Responses to Drought Stress. *Springer Berlin Heidelberg*, Berlin, Heidelberg, pp. 199–229. https://doi.org/10.1007/978-3-642-32653-0_8

University of Ghana http://ugspace.ug.edu.gh

Saranga, Y., Rudich, J., and Marani, A. (1991). The relations between leaf water potential of cotton plants and environmental and plant factors. *Field Crops Research*, *28*(1–2), 39–46.

Sehgal, A., Sita, K., Kumar, J., Kumar, S., Singh, S., Siddique, K. H. M., and Nayyar, H. (2017). Effects of Drought, Heat and Their Interaction on the Growth, Yield and Photosynthetic Function of Lentil (Lens culinaris Medikus) Genotypes Varying in Heat and Drought Sensitivity. *Frontiers in Plant Science*, *8*.

Singh, B. B. (1997). *Advances in Cowpea Research*. IITA.

Singh, T.N., Aspinall, D., Paleg, L.g., Boggess, S.F. (1973): Stress metabolism. II. Changes in proline concentration in excised plant tissues. *Australian Journal of Biological Sciences*, 26, 57–63.

Singh, T., and Malik, D. S. (1983). Effect of water stress at three growth stages on the yield and water-use efficiency of dwarf wheat. *Irrigation Science*, *4*(4), 239–245.

Singh SR, Allen DT (1980). Pests, Diseases Resistance and Protection in Cowpeas. Advanced Legume Sci. Ed. Summerfield, England, pp: 419-443.

Singh, B. B. and Tarawali, S. A. (1997). Cowpea and its improvement: Key to sustainable mixed crop/livestock farming systems in west africa *In:* Renard, C. (ed.) *Crop residues in sustainable mixed crop/livestock farming system.* Wallingford, UK: ICRISAT/ILRI/CABI 79-100.

Singh, B. B. (1997). Breeding cowpea varieties for resistance to *Striga gesnerioides* and *Alectra vogelii*. *Cowpea Integrated Pest Management,* 2: 154-163.

University of Ghana http://ugspace.ug.edu.gh

Singh, B. B.; Chambliss, O. L. and Sharma, B. (1997). Recent advances in cowpea breeding. *In:* Singh, B. B., Mohan Raj, D. R., Dashiell, K. E. and Jackai, L. E. N. (eds.) *Advances in cowpea breeding.* IITA, Ibadan, Nigeria: International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), 30-49.

Singh SR, Taylor TA. (1978). Pests of grain legumes and their control in Nigeria. In: Singh HF, Van Emden, Taylor TA, editors. Pests of grain legumes: ecology and control. New York: Academic Press. p. 99–111.

Souza, P. J. D. O. P. D., Farias, V. D. D. S., Lima, M. J. A. D., Ramos, T. F., and Sousa, A. M. L. D. (2017). Cowpea leaf area, biomass production and productivity under different water regimes in castanhal, pará, brazil. *Revista Caatinga*, *30*(3), 748–759.

Spallek, T., Mutuku, M., and Shirasu, K. (2013). The genus *Striga*: a witch profile: Profile of *Striga*, the witchweed. *Molecular Plant Pathology*, *14*(9), 861–869.

Steele, W.M. (1976). Cowpeas, *Vigna unguiculata* (Leguminosae-Papilionatae). Pages 183–185 in *Evolution of Crop Plants*, edited by N.W. Simmonds. Longman, London.

Sung, F. J. M., and D. R. Krieg. (1979). *Plant Physiology*, 64:852-856.

Thalouarn, P., Theodet, C. & Renaudin, S. (1991) Evidence of plasmid and nuclear genes for the large and small subunits of Rubisco in the Scrophulaceae holoparasite Lathraea-clandestina L-comparision with the autoriauph Digitalis-purpurea L. and hemiparasite Melampytium-pratense L. Comptes Rendus de l' Academie des Sciences serie III-Sciences de la VIE-Life Sciences, 312, 1-6.

University of Ghana http://ugspace.ug.edu.gh

Tarumoto, I., Yanase, M., Iwahara, Y., Kuzumi, Y., Morikawa, T., and Kasuga, S. (2003). Inheritance of a Thermo-sensitivity Gene Controlling Flower Initiation in Sorghum. *Breeding Science*, *53*(4), 353–357.

Tignegre, J.-B. S., Ouedraogo, J. T., Melis, R., Tongoona, P., Sibiya, J., Makanda, I., and Drabo, I. (2013). Identification of new sources of resistance to *Striga gesnerioides* in cowpea germplasm. *Plant Breeding*, *132*(3), 330–336.

Timko, M. P., Gowda, B. S., Ouedraogo, J., Ousmane, B., Ejeta, G., and Gressel, J. (2007). Molecular markers for analysis of resistance to *Striga gesnerioides* in cowpea. *Integrating New Technologies for Striga Control: Towards Ending the Witch-Hunt., World Scientific Publishing Co. Pte Ltd, Singapore*, 115–128.

Timko, M.P., and B.B. Singh. (2008). Cowpea, a multifunctional legume. In: Moore, P.H., and Ming, R., editors, *Genomics of tropical crop plants. Springer*, New York. p.227–258. doi:10.1007/978-0-387-71219-2_10

Timko, Michael P., Huang, K., and Lis, K. E. (2012). Host Resistance and Parasite Virulence in Striga–Host Plant Interactions: A Shifting Balance of Power. *Weed Science*, *60*(2), 307–315.

Tonessia, C., Wade, M., Cissé, N., and Severin, A. (2009). Characterization of *Striga gesnerioides* from Senegal: reaction of various cowpeas (*Vigna unguiculata* (L.) Walp.) to *Striga gesnerioides* strains from Sénégal. *Journal of Applied Biosciences*, *24*, 1462–1476.

Tonessia, Charlotte, Moctar, W., Gowda, B., Cisse, N., Severin, A., and Timko, M. (2014). Genetic diversity of *Striga gesnerioides* sampled from cowpea production areas of

University of Ghana http://ugspace.ug.edu.gh

Senegal using AFLP markers. *Canadian Journal of Plant Protection (CJPP)*, *2*(2), 67–76.

Turner, N. C. (1974). *Stomatal behaviour and water status of maize, sorghum and tabaco under field condition. II.At low soil water potential. Plant physiology.*, 53: 3560-365.

Turner, N. C. (1978). Drought resistance and adaptation to water deficits in crop plants. *In Stress Physiology in Crop Plants*, ed. H. Mussell, R. C. Staples. New York: Wiley-Interscience. In press

Turner, N.C., Begg, J.E., Rawson, H.M., English, S.D., and Hearn, A.B. (1978). Agronomic and physiological responses of soybean and sorghum crops to water deficit. III. Components of leaf water potential, leaf conductance, -CO 2 photosynthesis, and adaption to water deficits. Aust. J. *Plant Physiology*, 5: 179-194.

Turk, K. J., A. E. Hall, and C. W. Asbell. (1980). Drought adaptation of cowpea I. Influence of drought on seed yield. *Agronomy Journal*,72:413-420.

Valllancourt, R.E. and Weeden, N.F. (1992). Chloroplast DNA polymorphism suggests Nigerian center of domestication for the cowpea, *Vigna anguiculata*, Leguminosae. *American Journal of Botany,* 79: 1194-1199.

Vaillancourt, R. E., Weeden, N. F. and Barnard, J. (1993). Isozyme diversity in cowpea species complex. *Crop Science*, 33: 606-613.

Van der Hoorn RAL, Kamoun S. (2008). From guard to decoy: a new model for perception of plant 120 pathogen effectors. *Plant Cell* 20: 2009–2017.

University of Ghana http://ugspace.ug.edu.gh

Varshney, R. K., Terauchi, R., and McCouch, S. R. (2014). Harvesting the Promising Fruits of Genomics: Applying Genome Sequencing Technologies to Crop Breeding. *PLoS Biology*, *12*(6), e1001883.

Varshney, R.K., Paulo, M.J., Grando, S., van Eeuwijk, F.A., Keizer, L.C.P., Guo, P., Ceccarelli, S., Kilian, A., Baum, M., Graner, A., 2012. Genome wide association analyses for drought tolerance related traits in barley (Hordeum vulgare L.). *Field Crops Research* 126, 171–180. https://doi.org/10.1016/j.fcr.2011.10.008

Wardlaw, I.F.: The effect of water stress on translocation in relation to photosynthesis and growth. II. Effect during leaf development in Lolium temulentum L. *Australian Journal of Biological Sciences*, 22, 1–16 (1969)

Wang, X., Cai, X., Xu, C., Wang, Q., and Dai, S. (2016). Drought-Responsive Mechanisms in Plant Leaves Revealed by Proteomics. *International Journal of Molecular Sciences*, *17*(10).

Wilson, J., Ludlow, M., Fisher, M., Schulze, E. (1980). Adaptation to Water Stress of the Leaf Water Relations of Four Tropical Forage Species. *Functional Plant Biology*, 7, 207. https://doi.org/10.1071/PP9800207

Wolfe, A.D., et dePamphilis, C.W. (1998). The effect of relaxed functional constraints on the photosynthetic gene *rbc*L in the photosynthetic and non-photosynthetic parasitic plants. *Molecular Biology and Evolution*, 15: 1243-1258.

Wricke, G. (1962). Uber eine method zur erfassung der okogischen streubreite in feldversuchen. Zeitschriff Fur Pflanzenzuchtung, 47, 92-96.

University of Ghana http://ugspace.ug.edu.gh

Xu, P., Wu, X., Wang, B., Luo, J., Liu, Y., Ehlers, J.D., Close, T.J., Roberts, P.A., Lu, Z., Wang, S., others, 2012. Genome wide linkage disequilibrium in Chinese asparagus bean (*Vigna. unguiculata ssp*. sesquipedialis) germplasm: implications for domestication history and genome wide association studies. *Heredity* 109, 34–40.

Yan, W., L.A. Hunt, Q. Sheng and Z. Szlavnics, (2000). Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Science*, 40: 597-605.

Yasir, T., Chen, X., Tian, L., Condon, A.G., Hu, Y., 2013. Screening of Chinese bread wheat genotypes under two water regimes by various drought tolerance indices. *Australian Journal of Crop Science*, 7, 2005–2013.

Zhang, X., Huang, S., Zhang, Z., and Wang, W. (2012). Chapter 10: Mining Genome-Wide Genetic Markers. *PLOS Computational Biology*, *8*(12), e1002828.

Zhang, Z., Ersoz, E., Lai, C.-Q., Todhunter, R.J., Tiwari, H.K., Gore, M.A., Bradbury, P.J., Yu, J., Arnett, D.K., Ordovas, J.M., Buckler, E.S., 2010. Mixed linear model approach adapted for genome-wide association studies. *Nature Genetics* 42, 355–360. https://doi.org/10.1038/ng.546.

Zikhali, M., Leverington-Waite, M., Fish, L., Simmonds, J., Orford, S., Wingen, L.U., Goram, R., Gosman, N., Bentley, A., Griffiths, S., 2014. Validation of a 1DL earliness per se (eps) flowering QTL in bread wheat (*Triticum aestivum*). *Molecular Breeding,* 34, 1023–1033. https://doi.org/10.1007/s11032-014-0094-3

University of Ghana http://ugspace.ug.edu.gh

APPENDICES

Appendix 1: Heritability of traits in chapter 4

Trait	Heritability Well-Watered	Heritability Water-stressed
GrainYeld	0.7950	0.5586
SeedWeight	0.9386	0.6911
PodsPlant	0.5526	0.6337
Flowering	0.8226	0.8189
FirstPodMature	0.8009	0.7920
Maturity	0.4848	0.6947
Stemgreeness	0.6590	0.8376
Wiltering	0.6764	0.8789
Chlorophyll content (SPAD_3)	0.5758	0.4961
Canopy density (LAI_4)	0.6676	0.3377

University of Ghana http://ugspace.ug.edu.gh

Appendix 2: Guide to asking questions in Focus Group Discussion and Participatory Rural Appraisal

Focus Group Discussion (FGD):

1. **Presentation of investigators running the Focus Group Discussion**

a.	Bineta Samba Keita,	cowpea breeding
b.	Mamadou Diop,	cowpea breeding
c.	Assane Sene,	cowpea breeding
d.	Ousseynou Fall,	cowpea breeding
e.	Mouhamadou Moussa Diangar,	cowpea breeder

2. **Goal of the meeting**
3. **What is focus group discussion and its importance**
4. **Benefits from focus group discussion for farmers' and researchers**
5. **Questions on willingness of farmers to participate on surveys**
    a. **Are you willing to participate on FGD and PRA?**
    b. **Will you inform and encourage your neighbors to participate?**
6. **Questions on interest that farmers have on growing cowpea**
    a. **Did you grow cowpea last two years?**
    b. **What is the purpose of growing cowpea?**
    c. **Is it a lucrative activity?**
7. **Questions on constraints to cowpea production**
    a. **What are the constraints to cowpea production?**
8. **Questions on cowpea cultivars that farmers' grow**
    a. **What are the cowpea cultivars that farmers grow?**

Participatory Rural Appraisal

University of Ghana http://ugspace.ug.edu.gh

Name of the researcher asking questions and date of interview:

Location:

Region	Departement
Commune	Village
Latitude	Longitude

Farmer's background information:

Name	ID or mobile
Gender	Age
Marital status	Highest school attended
#Household members	#Involved in agricultural activities
Primary occupation	Group affiliated
Own arable surface (m^2)	Cowpea plots (m^2)

Do you own livestock?
How many of each?
Do you have machine?
Who take care of labors? [1] Employee [2] Family [3] Others
How many years have you been growing cowpeas?
When do you plant?
Did you grow cowpea last 2 years?
How many crop cycle per year did you have?

Importance of Cowpea:

Purpose of growing cowpea? [1] Household consumption [2] Animal feeding [3] Trade
Is it lucrative to grow cowpea? [1] yes [2] no
Cropping system? [1] Major crop [2] Association (specify) [3] Rotation (specify)
Utilization? [1] leaves [2] seeds [3] pod residus [4] fresh pods [5] medical purposes? Classify them in order of importance?

Cultural practices:

Clearing before sowing [1] yes [2] no	Fertilizer before sowing [1] yes [2] no
Certified seeds [1] yes [2] no	Whatever Seeds [1] yes [2] no
Seed treatment chemical [1] yes [2] no	Specify chemicals and dosage

University of Ghana http://ugspace.ug.edu.gh

Deep ploughing [1] yes [2] no	Surface ploughing [1] yes [2] no
Weeding [1] Hiler [2] Houe Sine	Weeding frequency 1 2 3
Synthetic fertilizer [1] yes [2] no	Biological fertilizer [1] yes [2] no
Manure before sowing [1] yes [2] no	Type and dosage
Pesticide [1] yes [2] no	Specify used pesticide

Cultivated varieties:

Name varieties you know:
Name your favorites:
Why are they favorite?
What do they have that lacks on others?
Name most cultivated in your area:
Do you grow local cultivars?
Why?
Are you aware of new improved varieties? [1] yes [2] no
How? [1] Media [2] Extension officers [3] ISRA [4] Training [5] NGOs [6] Neighbors
Have you ever tried to cultivate new improved varieties? [1] yes [2] no

Buy seeds? [1] yes [2] no	Source?
Expensive? [1] yes [2] no	Price?
Do you keep seeds for next? [1] yes [2] no	How do you stock?

Constraints to cowpea production:

What are the major constraints?
List some minor constraints:
What stress affect most cowpea production?
Is drought a major stress?
Have you ever faced water deficit during cowpea production?
Can you estimate yield losses due to drought?
Any strategies against drought?

Sowing period? [1] yes [2] no	Climate? [1] yes [2] no
Water deficit? [1] yes [2] no	Days to maturity? [1] yes [2] no
Diseases and Insectes? [1] yes [2] no	Improved lines? [1] yes [2] no
Soil fertility [1] yes [2] no	Monoculture [1] yes [2] no
Market to sell [1] yes [2] no	Credit [1] yes [2] no
Educational level [1] yes [2] no	Lack of training? [1] yes [2] no

University of Ghana http://ugspace.ug.edu.gh

Constraints to production	**Rank**
	1
	2
	3
	4
	5
	6
	7

Constraints to production	**Rank**
	1
	2
	3
	4
	5
	6
	7

Knowledge on *Striga gesnerioides*:

Is Striga a major stress?
How do you describe a plant of *S. gesnerioides?*
When does it attack cowpea?
Where does it attack cowpea?
Can you differentiate a plant of *Striga gesnerioides* and *Striga hermonthica*?
Do you know any infested field?
Do you know how *S. gesnerioides* seeds multiply?
Can you estimate losses due to Striga?
Do you know some *S. gesnerioides* susceptible varieties?
Do you know some *S. gesnerioides* resistant varieties?
How do you fight against *S. gesnerioides*

Farmers' preferred traits:

Traits	**Rank**
Maruca resistance	
Aphid resistance (cotoote)	
Fodder	
Pod length	
Thrips resistance	
Taste	
Cooking time	

Traits	**Rank**
Bruchid resistance	
High yielding	
Dual purpose	
Drought tolerant	
Striga resistance	
Nutrition qualities	
Macrophomina	

What is your preferred seed size? [1] Large [2] Medium [3] Small [4] Not determinant
What is your preferred seed color? [1] Black [2] Red [3] White [4] Brown [5] Others
Best growth habit trait? [1] Erect [2] Semi erect [3] Prostrate [4] Climbing
Best phenological trait? [1] Extra early [2] Early [3] Late [4] Others