

**BREEDING COWPEA [*Vigna unguiculata* (L.) Walp.] FOR STRIGA
[*Striga gesnerioides* (Wild.) Vatke] RESISTANCE USING MARKER
ASSISTED SELECTION IN NIGER**

By

SALIFOU MAHAMADOU

(10359704)

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

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

DECEMBER, 2015



ABSTRACT

Striga gesnerioides (Wild.) Vatke is one of the main biotic constraints restricting yield of cowpea in Sub-Saharan Africa (SSA) in general and particularly in Niger. The available resistant varieties developed so far often lack farmers' and end-users' desired traits and they also show levels of breakdown in resistance. Understanding farmers' knowledge regarding production constraints and varietal preferences is invaluable in breeding for cowpea improvement. The development of cowpea varieties by introgressing multiple *Striga* resistance into adapted genotypes will facilitate the achievement of food security in the country. This study aims to (i) assess farmers' preferred traits and perceptions of *Striga* on cowpea production (ii) identify new sources of *Striga* resistance in national and other available germplasm (iii) determine the presence of other *Striga* races besides SG3 in Niger (iv) introgress the resistance gene '*Rsg1*' into farmers' preferred varieties using marker assisted selection (MAS) (v) assess MAS for selection of *Striga* resistant genotypes.

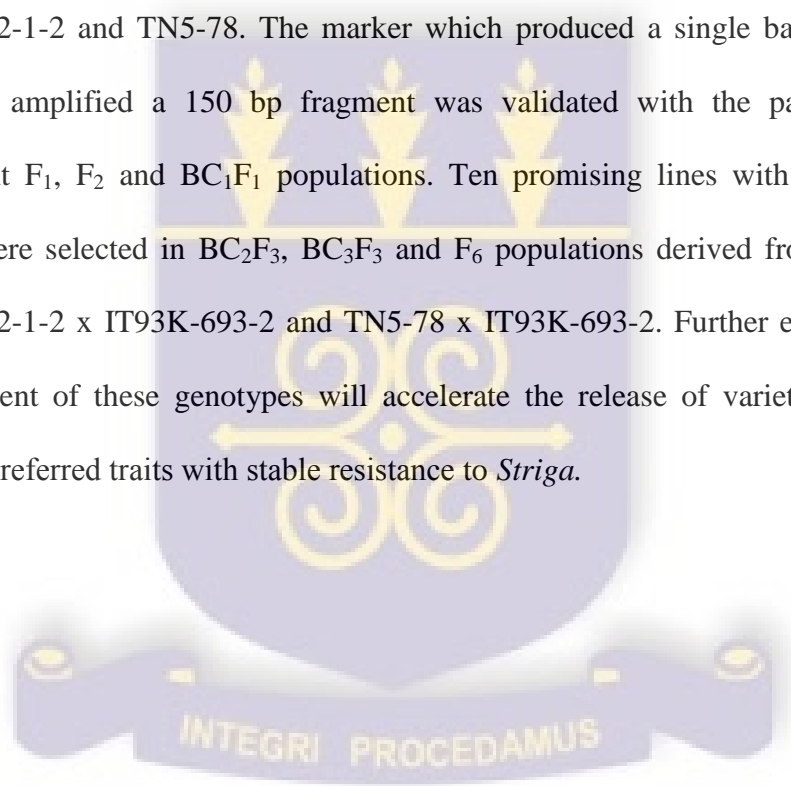
A participatory rural appraisal technique was used to identify the importance of cowpea in the farming system and its production constraints as well as farmers' knowledge on *Striga* and their cowpea preferred traits. Farmers ranked cowpea as the second most important crop after millet in Niger. Cowpea was grown for both food (56 %) and cash (44 %). The most important variety selection criteria used by farmers were high yielding potential, early maturity, white-colored grain and good taste. The major constraints to production were insects, *Striga*, drought and low soil fertility. Farmers had good knowledge on *Striga* and their preferred grain characteristics were large size and white seeded cowpea varieties.

Eighty cowpea genotypes were screened to evaluate their response to natural *Striga* infestation in the field. There was significant variation in the resistance of cowpea lines to *Striga*. The cowpea lines IT93K-693-2, IT99K-573-1-1 and IT98K-205-8 were free of *Striga* emerged shoots m^{-2} , while the lines 2491-171, 2472-154 and Suvita-2 supported only 0.43 to 0.87 *Striga* shoots m^{-2} . The other lines supported higher numbers ranging from 3.52 to 9.13 of emerged *Striga* shoots m^{-2} . *Striga* infestation resulted in 66% and 79% of yield losses of the susceptible genotypes as compared to the resistant and tolerant lines respectively. The screening did not reveal new sources of *Striga* resistance; however, the varieties IT93K-693-2, IT99K-573-1-1 and IT98K-205-8 were confirmed as potential sources of resistance and good donor parents to incorporate *Striga* resistance into well adapted genotypes. The cultivars B2/16/2378, B1/18/2542 and B1/12/2525-234 were tolerant to *Striga* with a significant yield potential compared to that of the resistant and the susceptible lines. Therefore, they could be used as donor parents in breeding cowpea for yield improvement in Niger.

Field and pot experiments were conducted during years 2013 and 2014 in order to identify the different *Striga* races that infest cowpea in Niger. Fifteen genotypes were screened under natural infestation as well as inoculated with three samples of the parasitic weed collected in three hot spot regions in Niger. The test revealed *Striga* attack on three multiracial *Striga* resistant varieties, B301, IT97K-499-35 and IT98K-205-8 suggesting the presence of other races other than SG3 in the studied area; or the breakdown of the resistance to *Striga* race SG3 in these cowpea varieties. Also, the resistance of varieties HTR and Suvita 2 observed, respectively in the field at Kollo and in pots with a sample of inoculum from Magaria suggested the prevalence of

Striga race SG1 in these sites. The geographical distribution of *Striga* races needs to be clarified in Niger.

Marker assisted selection with backcross breeding was used to transfer '*Rsg1*' *Striga* resistance gene from the breeding line IT93K-693-2 into three farmers' preferred varieties, IT90K-372-1-2, KVx30-309-6G and TN5-78. The microsatellite marker SSR₁ was used to track and introgress the resistance gene *Rsg1* into the varieties IT90K-372-1-2 and TN5-78. The marker which produced a single band in resistant lines and amplified a 150 bp fragment was validated with the parents and the subsequent F₁, F₂ and BC₁F₁ populations. Ten promising lines with the resistance marker were selected in BC₂F₃, BC₃F₃ and F₆ populations derived from the crosses IT90K-372-1-2 x IT93K-693-2 and TN5-78 x IT93K-693-2. Further evaluations and improvement of these genotypes will accelerate the release of varieties combining farmers' preferred traits with stable resistance to *Striga*.



ACKNOWLEDGEMENTS

All praises and glory be to ALMIGHTY ALLAH who has made the completion of this work possible for me.

I am grateful to the Alliance for a Green Revolution in Africa (AGRA) who provided funds for this study.

I am indebted to the West Africa Centre for Crop Improvement (WACCI) for giving me an excellent training in Plant Breeding. Special thanks to WACCI Director, Professor. Eric Y. Danquah, the lecturers and the administration staff of WACCI for the facilitation.

I would like to thank my supervisory committee composed of Professor Pangirayi Tongoona, Professor Samuel Offei and Professor Kwadwo Offori for their guidance in this research.

I am indebted to my In-country supervisor Doctor Jean Baptiste De La Salle Tignere for his advises and experience that guided me to the success of this work. I also acknowledge his support and facilitation for the genotyping conducted at INERA, Burkina Faso (Laboratoire de Biotechnologies Végétales de Kamboinsé).

I hereby thank INERA staff, particularly the technicians Léandre Poda and Serge Zida who helped me in the genotyping.

My sincere gratitude to INRAN, my institution for the facilities during my field research and to my technicians namely Illia Habou, Tassiou Moussa, Ousmane

Adamou, Garba Ali (Maradi station), Mahamadou Bizo (Kollo station) and Siradji Moumouni (Magaria station) for their assistance in field works.

A part of germplasm used in this study was introduced from INERA in Burkina Faso and IITA Kano; these institutions are sincerely acknowledged.

I thank all those who are not named here but contributed directly or indirectly to the success of this work.

Finally I am deeply indebted to my Family, especially my wife and my children for their support and patience during my absence.

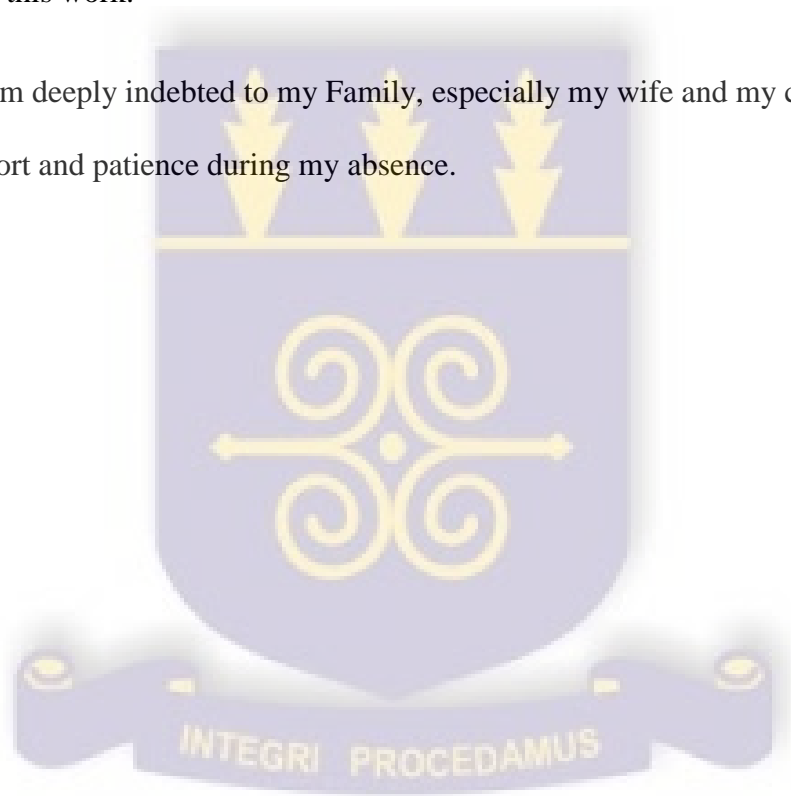


TABLE OF CONTENTS

DECLARATION	i
ABSTRACT.....	ii
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS.....	vii
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xviii
CHAPTER ONE	1
1.0 INTRODUCTION	1
CHAPTER TWO	8
2.0 Literature review	8
2.1 Cowpea	8
2.1.1 Origin and taxonomy.....	8
2.1.2 Reproductive biology	8
2.1.3 Crossing or artificial hybridization techniques	10
2.1.4 Cultivation, production and utilization.....	11
2.1.5 Farmer preferred traits.....	13
2.2 <i>Striga gesnerioides</i>	14
2.2.1 Taxonomy and geographical distribution.....	14
2.2.2 Host species and damage to crops.....	15
2.2.3 Life cycle of <i>Striga gesnerioides</i>	15
2.2.4 Available control measures	16

2.2.5 Mechanisms involved in the resistance to <i>Striga</i>	17
2.2.5.1 Resistance at germination	17
2.2.5.2 Resistance at attachment level.....	18
2.2.5.3 Resistance at and after the penetration of host vascular cells	18
2.2.6 Available sources of resistance to <i>Striga gesnerioides</i>	19
2.2.7 Biotypes of <i>Striga gesnerioides</i>	20
2.2.8 Genetics of resistance.....	21
2.2.9 Techniques of screening for <i>Striga</i> resistance in cowpea	22
2.2.9.1 Field and pot screening for resistance to <i>Striga gesnerioides</i>	22
2.2.9.2 “ <i>In-vitro</i> ” screening techniques.....	24
2.2.9.3 DNA screening techniques: use of marker assisted selection in breeding cowpea for <i>Striga</i> resistance	25
2.2.10 Correlation studies.....	26
2.2.11 Breeding procedures in cowpea	27
CHAPTER THREE	30
3.0 Farmers’ preferred traits and perceptions of cowpea production and constraints in Niger	30
3.1 Introduction	30
3.2 Materials and methods	31
3.2.1 Study area	31
3.2.2 PRA data collection and analysis	34

3.3.1 Cowpea farming system	35
3.3.2 Cowpea varieties currently grown and production constraints	36
3.3.3 Farmers’ knowledge about <i>Striga</i>	38
3.3.4 Farmers’ criteria for adopting varieties	38
3.3.5 Study of grain quality	39
3.3.6 Farmers’ preferred variety ranking based on grain characteristics.....	41
3.4 Discussion	45
3.4.1 Cowpea farming systems.....	45
3.4.2 Production constraints	47
3.4.3 Knowledge about <i>Striga</i>	48
3.4.5 Farmers’ preferred traits	49
3.5 Conclusion.....	51
CHAPTER FOUR.....	52
4.0 Screening of cowpea germplasm for resistance to <i>Striga gesnerioides</i>	52
4.1 Introduction	52
4.2 Materials and methods	54
4.2.1 Germplasm.....	54
4.2.2 Cultural practices and field evaluations.....	54
4.2.3 Data collection and analysis	54
4.3 Results	59

4.3.1 Correlation studies	62
4.3.2 Principal component analysis	62
4.3.3 Cluster analysis.....	64
4.4 Discussion	66
4.5 Conclusions	70
CHAPTER FIVE	71
5.0 Differential responses of 15 cowpea genotypes to three <i>Striga</i> hot spots in Niger	71
5.1 Introduction	71
5.2 Materials and methods	73
5.2.1 Location	73
5.2.2 Field and pot evaluations of cowpea germplasm under <i>Striga</i> infestation...	74
5.2.3 Data collection and analysis	75
5.3 Results	76
5.3.1 Field experiment	76
5.2.4.2 Pot experiment.....	81
5.4 Discussion	84
5.5 Conclusion.....	88
CHAPTER SIX.....	89
6.0 Backcrossing of <i>Striga</i> resistance gene into farmers’ preferred varieties.....	89
6.1 Introduction	89
6.2. Materials and methods	91

6.2.1 Plant Materials	91
6.2.2 Plant culture and DNA extraction	96
6.2.3 Preparation of samples for PCR Analysis	96
6.2.4 PCR analysis.....	97
6.2.5 Electrophoresis	98
6.2.6 Marker assisted selection for <i>Striga gesnerioides</i> resistance in segregating populations of cowpea.....	98
6.2.7 Marker validation	99
6.3 Results	99
6.3.1 Parental genotyping	99
6.3.2 BC ₁ F ₁ population.....	100
6.3.3 BC ₂ F ₁ populations	101
6.3.4 BC ₂ F ₂ derived populations genotyping	103
6.3.5 BC ₃ F ₁ derived populations genotyping	104
6.3.6 BC ₂ F ₃ , BC ₃ F ₃ and F ₆ derived populations genotyping.....	105
6.3.7 Marker validation	107
6.3.8 Pots screening of BC ₂ F ₃ , BC ₃ F ₃ and F ₆ derived populations	107
6.4 Discussion	111
6.5 Conclusions	114
CHAPTER SEVEN	116

7.0 General Discussion and Recommendations.....	116
7.1 Introduction.....	116
7.2 Main findings and their implications in agricultural research.....	117
7.3 Recommendations.....	121
REFERENCES.....	123
APPENDICES.....	143

LIST OF TABLES

Table 3. 1: Sites and dates of PRA sessions	33
Table 3. 2: Main crops grown by farmers and their rank order	35
Table 3. 3: Main cowpea production constraints in Kollo, Aguié and Magaria.....	37
Table 4. 1: Origin and seed color of the 80 cowpea germplasm.screened.....	57
Table 4. 2: Mean squares of the traits	59
Table 4. 3: <i>Striga</i> density, <i>Striga</i> dry biomass, yield and yield components (100-seed weight and pod weight) of cowpea germplasm screened at Maradi.	61
Table 4. 4: Correlation coefficients of <i>Striga</i> density and dry biomass with yield and yield components	62
Table 4. 5: Eigenvalues of the Correlation Matrix	63
Table 4. 6: Eigenvectors from the two principal component axes used to classify cowpea accessions	64
Table 5. 1: Cowpea germplasm screened at Kollo, Maradi and Magaria in 2013 with reported differential responses.....	75
Table 5. 2: Mean squares of <i>Striga</i> emerged shoots/plot of 15 cowpea varieties screened at Kollo, Maradi and Magaria in 2013 and 2014.	77
Table 5. 3: Mean numbers of emerged <i>S. gesnerioides</i> shoots per plot on 15 cowpea cultivars infested in field at Kollo, Maradi and Magaria in 2013 and 2014.	78
Table 5. 4: Differential responses to <i>S. gesnerioides</i> of 15 cowpea cultivars infested in field at Kollo, Maradi and Magaria in 2013 and 2014.....	80

Table 5. 5: Mean numbers of emerged <i>S. gesnerioides</i> shoots per plot on 15 cowpea cultivars infested in pots with samples from Kollo, Maradi and Magaria in 2013 and 2014.....	82
Table 5. 6: Differential responses to <i>S. gesnerioides</i> of 15 cowpea cultivars infested in pots with samples from Kollo, Maradi and Magaria in 2013 and 2014	83
Table 6. 1: Cowpea varieties used as parents in backcross breeding with their pedigree information.....	92
Table 6. 2: Development of recombinant lines	94
Table 6. 3: Structure of SSR1 primer used in MAS procedures.....	97
Table 6. 4: Reaction of cowpea lines: BC2F3, BC3F3 and F6 populations derived from crosses IT90K-372-1-2 x IT93K-693-2 and TN5-78 x IT93K-693 -2 with their parents to <i>Striga gesnerioides</i> in pots	108

LIST OF FIGURES

Figure 1.1 Cowpea area, production and yield trend over last twenty years in Niger...2

Figure 2. 1: *Fabaceae* family.....9

Figure 2. 2: Mature bud with corolla removed to expose the ten stamens, 9 with fused filaments and 1 free.....10

Figure 3. 1: PRA sites.....32

Figure 3. 2: Female (b) and male (a) farmers in discussion.....34

Figure 3. 3: Farmers’ criteria for adopting varieties.....39

Figure 3. 4: Farmers’ preferences for grain size.....40

Figure 3. 5: Farmers’ preferences for grain color.....41

Figure 3. 6: Male (a) and female (b) farmers ranking cowpea varieties.....42

Figure 3. 7: Farmers’ preferred varieties by rank order based on grain characteristics and according to the sex and age at Kollo, 2013.....43

Figure 3. 8: Farmers’ preferred varieties by rank order based on grain characteristics and according to sex and age at Aguié, 2013.....43

Figure 3. 9: Farmers’ preferred varieties by rank order based on grain characteristics and according to sex and age at Magaria, 2013.....44

Figure 3. 10: Farmers’ preferred varieties by rank order based on grain characteristics and according to sex and age in 2013 (multi-sites combined data).....44

Figure 4. 1: Dendrogram constructed based on yield and <i>Striga</i> resistance parameters of cowpea.....	65
Figure 5.1: Infested B301 plants in field at Kollo, Maradi and Magaria in 2013.....	79
Figure 5. 2: Mean monthly rainfall at Kollo, Maradi and Magaria in 2013 and 2014..	81
Figure 5. 3: Susceptible varieties infested in pots.....	83
Figure 6.1: Backcross breeding scheme for <i>Striga gesnerioides</i> resistance gene introgression.....	95
Figure 6. 2: Results from PCR amplification of genomic DNA by SSR ₁ for the parents.....	100
Figure 6. 3: Results from PCR amplification of genomic DNA by SSR ₁ for the BC ₁ F ₁ progenies derived from IT90K-372-1-2 x IT93K-693-2.....	100
Figure 6. 4: Results from PCR amplification of genomic DNA by SSR ₁ for the BC ₁ F ₁ progenies derived from KVx30-309-6G x IT93K-693-2 and TN5-78 x IT93K-693 - 2.....	101
Figure 6. 5: Results from PCR amplification of genomic DNA by SSR ₁ for the BC ₂ F ₁ progenies derived from IT90K-372-1-2 x IT93K-693-2.....	102
Figure 6. 6: Results from PCR amplification of genomic DNA by SSR ₁ for the BC ₂ F ₁ progenies derived from TN5-78 x IT93K-693-2.....	102
Figure 6. 7: Results from PCR amplification of genomic DNA by SSR ₁ for the BC ₂ F ₂ progenies derived from IT90K-372-1-2 x IT93K-693-2.....	103
Figure 6. 8: Results from PCR amplification of genomic DNA by SSR ₁ for the BC ₂ F ₂	

progenies derived from KVx30-309-6G x IT93K-693-2 and TN5-78 x IT93K-693.....104

Figure 6. 9: Results from PCR amplification of genomic DNA by SSR₁ for the BC₃F₁ progenies derived from IT90K-372-1-2 x IT93K-693-2, KVx30-309-6G x IT93K-693-2 and TN5-78 x IT93K-693 -2.....105

Figure 6. 10: Results from PCR amplification of genomic DNA by SSR₁ for the BC₂F₃ and F₆ progenies derived from IT90K-372-1-2 x IT93K-693-2.....106

Figure 6. 11: Results from PCR amplification of genomic DNA by SSR₁ for the BC₂F₃ and BC₃F₃ progenies derived from TN5-78 x IT93K-693 -2.....106

Figure 6. 12: Results from PCR amplification of genomic DNA by SSR₁ for marker validation.....107

Figure 6. 13: Phenotyping of cowpea lines in pots: parents and one selected F₆ line.110

Figure 6. 14: Phenotyping of cowpea lines: one selected F₆ line and two selected BC₂F₃ lines.....111

LIST OF ABBREVIATIONS

AFLP: Amplified Fragment Length Polymorphism

AGRA: Alliance for a Green Revolution in Africa

FAO: Food and Agriculture Organization of the United Nations

FAOSTAT: Statistical Database of the Food and Agriculture Organization of the United Nations

FTA: Fast Technology for Analysis

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

IITA: International Institute of Tropical Agriculture

INERA: Institut National de l'Environnement et de la Recherche Agronomique

INRAN: Institut National de la Recherche Agronomique du Niger (National Agricultural Research Institute of Niger)

INS : Institut National de la Statistique

IRAT : Institut des Recherches Agricoles Tropicales

MAS: Marker Assisted Selection

MDA: Ministère du Développement Agricole

PRA: Participatory Rural Appraisal

PCR: Polymerase Chain Reaction

QTL: Quantitative Trait Loci

RAPD: Random Amplified Polymorphic DNA

SCAR: Sequence Confirmed Amplified Region

SSA: Sub-Saharan Africa

SSR: Single Sequence Repeat

CHAPTER ONE

1.0 INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] ($2n = 2x = 22$) is one of the most important food legume crops in the world. It is the most important grain legume grown in Sub-Saharan Africa (Ehlers and Hall, 1997b; Timko *et al.*, 2007; Timko and Singh, 2008), and an important source of income for millions of people in West and Central Africa. Cowpea seed contains protein amounts varying from 23 to 32% of its weight which is rich in lysine and tryptophan, and a substantial amount of minerals and vitamins (folic acid and vitamin B) necessary for preventing birth defects during pregnancy (Nielson *et al.*, 1993; Hall *et al.*, 2003). Phillips *et al.* (2003) stated that cowpea contains fat and fiber in amounts required for preventing heart disease. All parts of the plant such as dried or fresh seeds, leaves, fresh immature pods and roots are consumed by humans and animals. Cowpea is a drought tolerant crop that helps to increase soil fertility through nitrogen fixation when grown in rotation with cereals (Elawad and Hall, 1987; Sanginga *et al.*, 2003).

Cowpea annual worldwide production was estimated at 6.2 million metric tons from 11.9 million hectares in 2013 with Africa accounting for 95% of the total production (FAOSTAT, 2015). Niger is the second largest producer of the crop in the world after Nigeria. About 1,300 MT of dried seeds were produced in 2013 in Niger versus 2,950 MT in Nigeria (FAOSTAT, 2015). Cowpea is the main food legume in Niger; it is the second most widely grown crop after millet (MDA, 2015) and the second most important cash crop after onion. Niger, though it is the second largest producer of cowpea, is the

largest exporter in the world with Nigeria being the largest importer. Cowpea plays a strategic role in the food security of the country because it is the earliest crop to be harvested thus complementing staple low-protein cereals and generates income for farmers. The availability of early varieties can shorten the period of food crisis occurring just before the harvest of the rainy season's crop (Timko *et al.*, 2007). In Niger, cowpea production has increased from 262.7 MT in 2000 to 1,586 MT in 2014 (MDA, 2015) with fluctuations due to climatic changes. The area harvested followed the same trend from 3,846,000.3 ha to 5,320,728 ha (Figure 1.1); this means the increase in total production was due to the increase in the area cultivated.

Average yield of 7000 kg ha⁻¹ was recorded under good conditions in the US (Sanden, 1993) however the average yield in Africa is less than 300 kg ha⁻¹ (Ehlers and Hall, 1997a, b). Cowpea grain yields in Niger are the lowest compared to those of the neighboring countries.

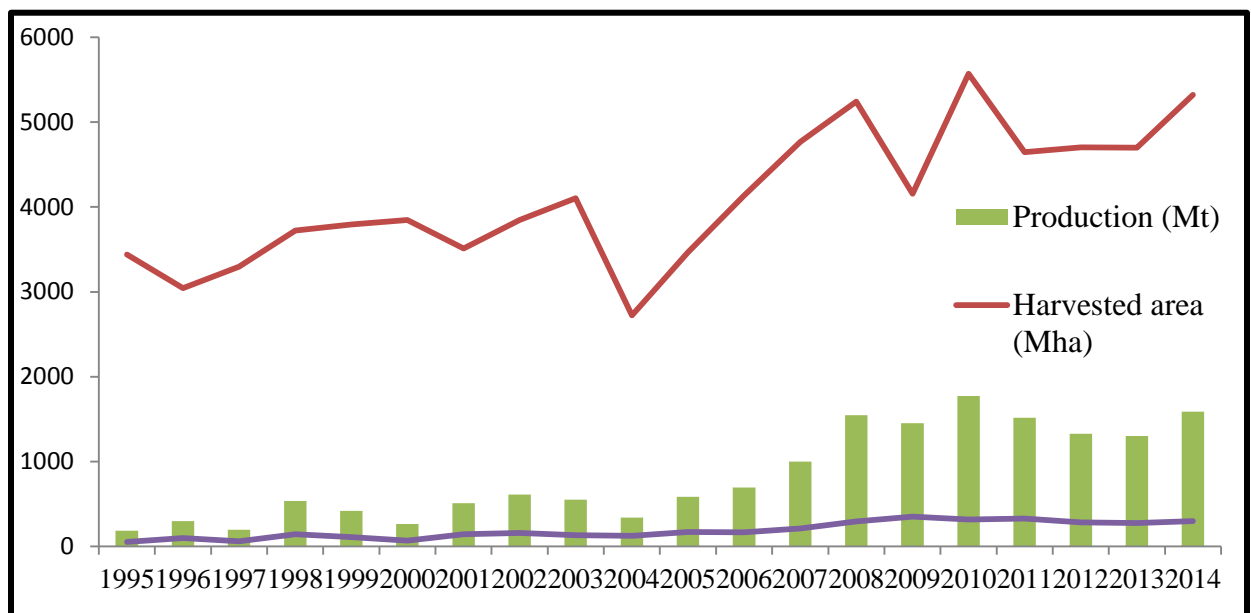


Figure 1.1 Cowpea area, production and yield trend over last twenty years in Niger

(INS, 2010; MDA, 2015).

For instance, in 2013, the average cowpea yield of 276.5 Kg/ha was recorded in Niger while 776.3 Kg/ha and 483.1 Kg/ha were obtained respectively in Nigeria and Burkina Faso under the same growing conditions (FAOSTAT, 2015).

Cowpea productivity in Niger is challenged by many abiotic and biotic constraints. The abiotic stresses include drought, heat and low soil fertility. The biotic stresses comprise insect pests (aphid, flower thrips, pod sucking bugs, maruca, and bruchids), diseases (bacterial and viral) and parasitic weeds (ICRISAT, 2013).

Striga, also called witchweed, is one of the major biological constraints to cereals and leguminous crops production in Sub-Saharan Africa. In Africa, the yield reduction caused by *S. gesnerioides* is estimated at as high as 83–100% on severely infested fields (Emechebe *et al.*, 1991; Omoigui *et al.*, 2011). In Niger two species of *Striga* infest crops: *Striga hermonthica* parasitizes millet and sorghum while *Striga gesnerioides* attacks cowpea. All the agro-ecological zones are infested with more severity in the Sahelian and Sudan Sahelian zones (rainfall between 300 mm and 700 mm). There is no reliable data on infestation rate and yield loss; however, severe infestation by *Striga gesnerioides* on susceptible cowpea varieties results in complete yield loss. *Striga gesnerioides* race SG3 prevails in Niger (Lane *et al.*, 1996) and is associated with low-yielding and susceptible genotypes mostly grown by farmers.

As a result, cowpea production is far below the expectations of a population increasing at an annual rate of 3.9 % (INS, 2013). In 1959, a cowpea variety TN88-63 produced and diffused by a breeding programme initiated under the French institute Institut de Recherches Agronomiques Tropicales (IRAT) was adopted by farmers (Mazzucato and Ly, 1994). Later on, in 1975, the National Institute of Agricultural Research (INRAN)

cowpea breeding developed and released TN5-78 that replaced TN88-63 because of its undesirable cooking and digestibility characteristics. Currently, Niger cowpea breeding focuses on collection of germplasm, hybridization, selection and maintenance of released elite varieties. Considerable research has also been done on plant protection, including trials for resistance to the parasitic weed *S. gesnerioides*, and the fungus *Macrophomia* (Mazzucato and Ly, 1994). Unfortunately, to date limited progress has been made in either pest or disease control. Past efforts on *Striga* resistance were essentially focused on mass selection that have led to the identification and purification of some local materials with a good level of resistance such as: TN121-80, TN93-80 and HTR. Also a number of resistant cultivars and lines including: B301 (Botswana landrace), IT97K-499-38, IT97K-499-35, IT82D-849 (lines from International Institute of Tropical Agriculture) were introduced in collaboration with IITA. All these lines have been tested in Niger and are adapted to local conditions (Moutari, personal communication). Unfortunately, the farmers did not accept them because they lack their preferred traits. On the other hand the control of *Striga* is difficult because of the high number and long viability of its seeds in the soil. The farmers cannot afford highly sophisticated techniques such as the use of ethylene to trigger ineffective *Striga* seed germination or the use of soil solarization technique. Therefore host plant resistance is the easiest and most sustainable alternative to control this parasitic weed at farmer level.

At least five distinct races of *Striga gesnerioides* parasitizing cowpea have been identified in West Africa based on differential variety tests (Lane *et al.*, 1996). Race differentiation in cowpea/*S. gesnerioides* was largely due to host-driving selection because the parasite is autogamous with very low outcrossings due to its floral biology

(Botanga and Timko, 2005). The races were designated as follows: SG1 (Burkina Faso), SG2 (Mali), SG3 (Nigeria and Niger), SG4 and SG4z (Benin), SG5 (Cameroon) (Lane *et al.*, 1996). In 2006, Botanga and Timko (2006) identified another race, SG6 in Senegal. In cowpea, *Striga* resistance is complex and depends on *Striga* strains and results from the combination of recognized mechanisms that influence the development of the parasite (Parker and Polniaszek, 1990; Lane *et al.*, 1996; Touré *et al.*, 1997; Reiss and Bailey, 1998). In Niger, some resistant varieties may be susceptible when grown in some areas (HTR is resistant in West and susceptible in East). On the other hand, the resistance to *Striga gesnerioides* race SG3 is conferred by a single dominant gene in B301 (Touré *et al.*, 1997) whereas it is conferred by a recessive gene in IT82D-489 (Atokple *et al.*, 1995). These results imply that either there is more than one race of *Striga* in Niger or that the response to the strain SG3 depends on the level of parasite inoculum or is due to the environment (Li and Timko, 2009). A study of the genetic diversity of *Striga gesnerioides* (Botanga and Timko, 2006) revealed Amplified Fragment Length Polymorphism (AFLP) markers associated with each of the *Striga* races. But, in a recent study Dube (2009), used AFLP, ISSR and cpSSR markers and could not differentiate the various races as described by Lane *et al.* (1996). To date, the easiest way to discriminate between the various *S. gesnerioides* races parasitizing cowpea is the differential variety test in which genotypes with known responses to the different *Striga* races are screened to determine the races prevailing in the study area. In order to find out whether there are some other races to this parasitic weed in the country, scientific investigations would be needed.

In addition, the break-down of resistance in introduced varieties and the ability of *Striga* to spread rapidly require an urgent need for varieties with multiple resistance genes (Boukar *et al.*, 2004). The current focus in applied breeding has been the application of molecular tools to speed up the process of crop delivery to farmers. The use of marker assisted selection (MAS) facilitates the selection of plant traits and shortens the time needed to develop new varieties. Molecular markers linked with resistance genes to races SG1, SG2 and SG3 have been identified, and several sequence-confirmed amplified regions (SCARs) have been developed for use in MAS (Li *et al.*, 2009).

A recently identified cowpea breeding line, IT93K-693-2, has resistance to all known *Striga* races (Singh, 2002) but lacks farmers' traits. It has a single dominant gene *Rsg1* that confers the resistance to the strain SG3 inherited from the cultivar B301 (Boukar *et al.*, 2004). Tignegre (2010) found that the gene *Rsg1* present in IT93K-693-2 and B301 was a dominant gene and conferred resistance to all the three *Striga* races (SG1, SG5 and SGKp) in Burkina Faso. He suggested that the presence of a single dominant gene in cowpea which is a self-pollinating crop could contribute to improving *Striga* resistant genotypes in pedigree breeding and backcross breeding. In order to meet the demand of producers, there is therefore the need to introgress this source of resistance into farmer-preferred varieties using marker assisted selection.

The overall objective of this research was to develop locally-adapted multi race *Striga* resistant cowpea varieties with farmer-preferred traits by introgressing existing sources of resistance.

Specific objectives were to:

- Assess farmers' preferred traits and perceptions of *Striga* on cowpea production;
- Identify new sources of *Striga* resistance in national and other available germplasm;
- Determine the presence of other *Striga* races besides SG3 in Niger;
- Introgress the resistance gene 'Rsg1' into farmers' preferred varieties using marker assisted selection; and
- Assess MAS for selection of *Striga* resistant genotypes.

CHAPTER TWO

2.0 Literature review

2.1 Cowpea

2.1.1 Origin and taxonomy

The origin of cultivated cowpea has been discussed with a lot of speculations for a long time. Studies (Rachie and Roberts, 1974; Ng and Maréchal, 1985 and Fery, 1990) showed that cowpea originated from Africa since the continent has the highest diversity of *Vigna* genera (Raynal-Roques, 1993). West Africa is thought to be the main center of diversity of cultivated cowpea (Ng and Padulosi, 1988) and of its domestication by farmers (Ba *et al.*, 2004). South-Eastern Africa is believed to be the center of diversity for wild *Vigna* (Ng and Padulosi, 1988; Padulosi and Ng, 1997).

Cowpea (*Vigna unguiculata* L. Walp.) belongs to the Phaseoleae tribe (Figure 2.1) of the Leguminosae family (Timko *et al.*, 2007). It comprises five sub-species namely *Vigna unguiculata*, *V. cylindrica*, *V. sesquipedalis*, *V. dekindtiana*, and *V. mensensis* (Ng, 1985). Domesticated cowpeas are grouped in the genera *Vigna* and the species *unguiculata* while *V. unguiculata* ssp *dekindtiana* or *V. unguiculata* ssp *spontanea* are assigned to wild forms (Pasquet, 1999).

2.1.2 Reproductive biology

Cowpea is a self-pollinated crop but cross-pollination rates up to 5% have been reported (Timko *et al.*, 2007). The inflorescences are racemes on which flowers are borne at the

end of peduncles arising from the leaf axils. Each inflorescence comprises four to six units of flowers. A flower contains ten stamens, nine fused and one free; an ovary and a bearded style ending in an oblique stigma (figure 2.2). In a particular cowpea flower, the dehiscence of the anther and the pollination happens normally early in the morning the day when the flower opens (Ehlers and Hall, 1997). Artificial hybridization is favored by the fact that stigmas become receptive 12 hours before anther dehiscence.

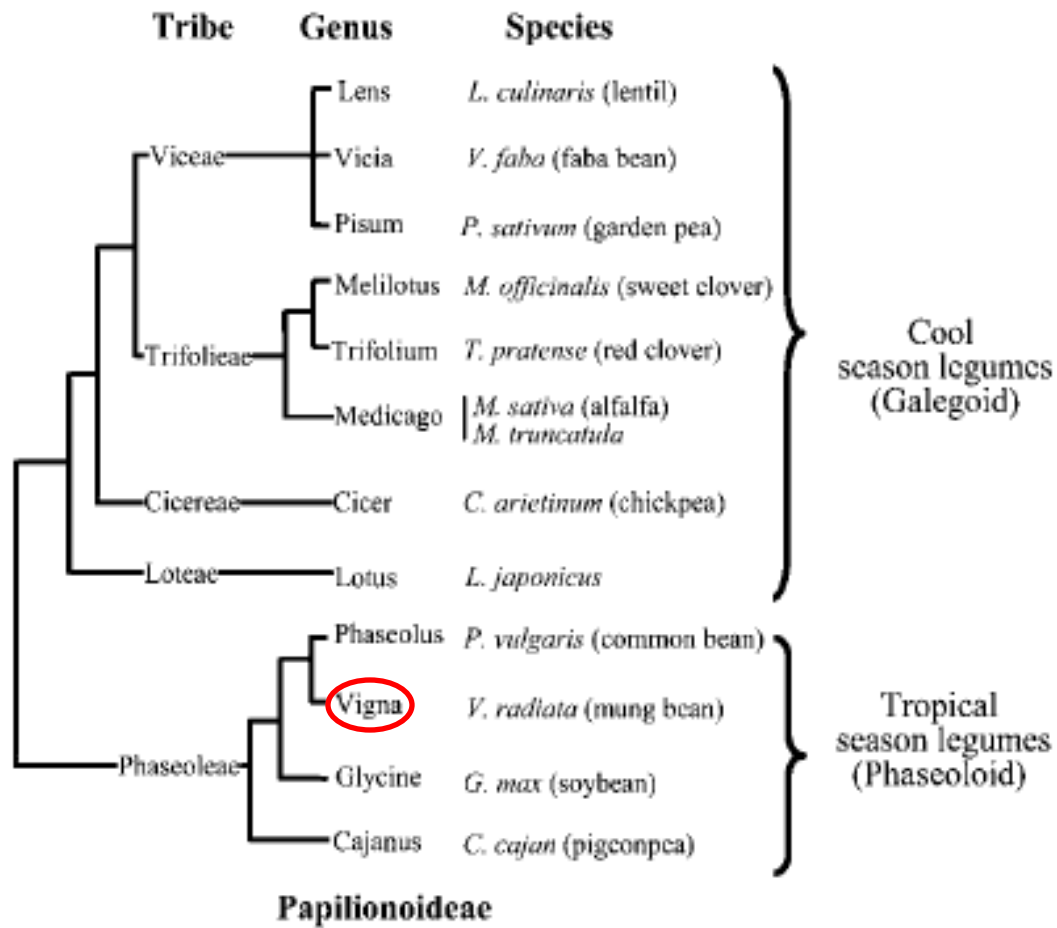


Figure 2. 1: *Fabaceae* family (adapted from Choi *et al.*, 2004).

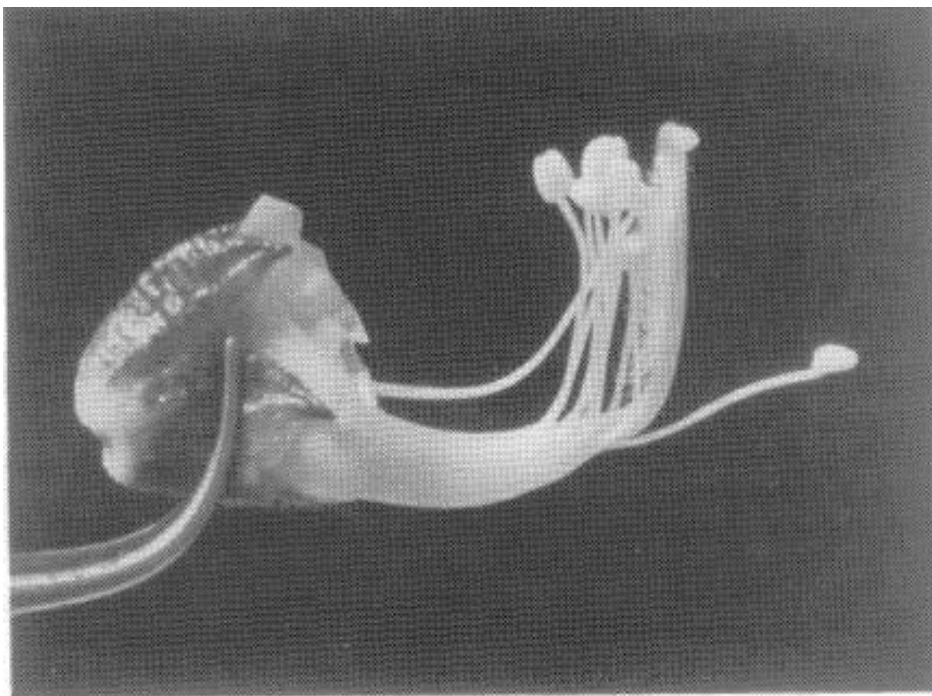


Figure 2. 2: Mature bud with corolla removed to expose the ten stamens, 9 with fused filaments and 1 free. (Blackhurst and Miller, 1980)

2.1.3 Crossing or artificial hybridization techniques

The relative size of its floral parts renders cowpea crossings easier compared to the other grain legumes, but under natural conditions the rate of success is 10-20% (Myers, 1996). The floral buds that will open the following day are identified and emasculated in the late afternoon. The emasculated flowers are tagged and left uncovered during the night. The emasculating is done by opening the sepals and then the keels to remove the anthers. It is a delicate operation performed with tweezers. The anthers should not be broken to avoid self-pollination. The pollination is done early or in the mid-morning (Ehlers and Hall, 1997) by brushing pollen from the burst anthers of the desired opened male flowers onto the emasculated female flowers. An alternative way of performing pollination is to

refrigerate collected open flowers of the male parent at 4 to 10 °C until late afternoon. Mature buds of the female flowers are emasculated and pollinated with the conserved pollen at the same moment. According to Myers (1996) trigger planting in order to synchronize flowering time under cool temperature and high humidity increase the success of hand crossing to 50%. The success of a cross is apparent two to three days following pollination. A successful cross yields a pod containing 8-12 seeds (Myers, 1996).

2.1.4 Cultivation, production and utilization

Cowpea is an important grain legume grown in tropical and subtropical regions including Asia, Africa, Central and South America, the United States and parts of Southern Europe. The estimated area cultivated under cowpea in the world was around 11.9 million hectares in 2013 (FAOSTAT, 2015). The drier savanna and the Sahelian region of West and Central Africa produce about 70% of worldwide cowpea production (Singh, 2002). The major cowpea-producing countries in West Africa are Nigeria, Niger, Mali, Senegal, Burkina Faso and Ghana (Singh *et al.*, 2003). The other producing countries in Africa include Uganda, Mozambique, Tanzania and Ethiopia. Nigeria is the largest producer and consumer of cowpea grain, with about 5 million ha and over 2 million MT production annually, followed by Niger (650,000 MT) and Brazil (490,000 MT) (Singh *et al.*, 2002). According to the Food and Agriculture Organization of the United Nations (FAO), as of 2012, the average cowpea yield in Western Africa was estimated at 483 kg/ha (FAOSTAT, 2012) which is still 50% below the estimated potential production yield. In

some traditional cropping methods, the yield can be as low as 100 kg/ha (Kormawa *et al.*, 2000).

Cowpeas are drought-tolerant and warm-weather crops that are well-adapted to the drier regions of the tropics, where other food legumes do not perform well. It also has the ability to fix nitrogen through its root nodules, and it grows well in poor soils with more than 85% sand and with less than 0.2% organic matter and low levels of phosphorus (Singh *et al.*, 2003). In addition, it is shade tolerant, so it is compatible as an intercrop with maize, pearl millet, sorghum, sugarcane, and cotton. This makes cowpeas an important component of traditional intercropping systems especially in the complex subsistence farming systems of the dry savanna in Sub-Saharan Africa. In these systems the haulm (dried stalks) of cowpea is a valuable by-product, used as animal feed. Cowpea seed contains 23.1% of proteins, 1.4% of lipids and 61.4% of sugars (Louis, 2006). Cowpea is especially appreciated because of its protein content.

The major cowpea production areas in Niger are Dosso (Sudano Savanna) and Maradi and Zinder in the Sahel, where the annual rainfall is 400-600 mm. These regions account for about 80% of cowpea production in the country. Cowpea enhances the food security of the country's 66% poor farmers (ICRISAT, 2013). It is widely consumed by both rural and urban people. In general, the grain can be cooked alone or mixed with rice and consumed as the main meal. The grain can also be processed into flour which is used for a variety of recipes such as "Kossai", a popular breakfast meal in West and Central Africa which contributes significantly to women's incomes (Ibro *et al.*, 2006).

2.1.5 Farmer preferred traits

Breeding efforts have made available many improved cowpea varieties in Sub-Saharan Africa in general and in Niger in particular but the adoption is slow or in some cases they may not be adopted due to non-compatibility or relevancy with farmers' conditions and perceptions (Saidou *et al.*, 2011). There are a number of visual characteristics of cowpeas that have been shown, at least anecdotally, to be preferred by consumers. For example, the main varieties available in the open markets in West Africa are white cowpea seeds with black eye (Lambot, 2000), but in some areas red or black speckled cowpeas are preferred.

New cowpea varieties must have features desired by consumers and farmers, including grain appearance and desirable cooking and processing qualities for specific products. Large white grains with rough seed coat are preferred throughout West Africa and can be marketed over a wide area (Langyintuo *et al.*, 2003). They are also amenable to direct dry milling for use in value-added foods (*akara*, *moin-moin*) and prototype value-added products. There is also considerable demand for large rough-brown seed types, especially in urban centers in Nigeria, but current varieties are susceptible to pests and diseases (Langyintuo *et al.*, 2003).

In a participatory study conducted in Uganda, Orawu (2007), indicated that farmers' preferred traits in choosing cowpea varieties were white seed color, earliness, yield potential, good taste, and tolerance to insect pests and diseases. In a participatory evaluation of improved cowpea lines and cropping systems in Maradi and Zinder regions in Niger, Saidou *et al.* (2011) found that farmers were interested in early maturing varieties with good seed germination and high pod load as well as the white seed coat

color and yield potential. The grain characteristics preferred by farmers and consumers are white colored and large sized grain (Langyintuo *et al.*, 2003; Tignegre, 2010 and Egbadzor *et al.*, 2013).

Farmers' participatory evaluation of crop varieties and cropping systems are a better option in improving the adoption of new varieties and technologies.

2.2 *Striga gesnerioides*

2.2.1 Taxonomy and geographical distribution

The genus *Striga* which comprises approximately 50 species belongs to *Scrophulariaceae* family (Botanga and Timko, 2005). *Striga hermonthica* (Del.) Benth, *S. asiatica* (L.) Kuntz, and *S. gesnerioides* are the most agronomically important species (Botanga, 2005); that constitute a serious challenge to food crop production in Africa. *Striga spp.* are considered as hemiparasitic plants because of the aerial photosynthesis observed after *Striga* emergence (Matusova *et al.*, 2005). For some authors who considered this photosynthetic activity as insignificant, *Striga* species are holoparasitic plants (Wolfe, 1998).

Unlike *Striga hermonthica* (Del.) Bent. which is essentially an African weed, *Striga gesnerioides* occurs in India (Ozenda and Capdepon, 1972), Arabia, and in the United States (Wunderlin *et al.*, 1979; Dembélé, 1988; Lane and Bailey, 1992). According to Mohamed *et al.* (2001) *S. gesnerioides* is the most wide spread *Striga* species in the world.

2.2.2 Host species and damage to crops

Striga gesnerioides occurs spontaneously throughout drought-prone areas in Africa where it parasitizes genera within the *Leguminosae* (e.g. *Indigofera* and *Tephrosia*) and *Convolvulaceae* families (Mohamed *et al.*, 2003). The most economically important hosts of *Striga gesnerioides* include cowpea, tobacco and sweet potato (Vasudeva Rao and Musselman, 1987; Berner and Williams, 1998). As a result of a strong host specificity which evolved within its populations, the *Vigna* strain causes severe damage to cowpea in the Sudano-Sahelian region of West Africa. Yield losses of about 30% or more and even total crop losses particularly in Nigeria have been reported (Aggarwal and Ouédraogo, 1989; Emechebe *et al.*, 1991).

Striga gesnerioides is entirely dependent on the host for its carbon nutrition during the early stages of its life cycle. Even after emergence, which occurs 4 to 5 weeks after vascular connection (Berner and Williams, 1998), *S. gesnerioides* is more dependent on its host than *S. asiatica* and *S. hermonthica* because of its rudimentary photosynthetic system and the high rate of haustorium respiration (Thalouarn *et al.*, 1991; Graves, 1992; Alonge *et al.*, 2005).

2.2.3 Life cycle of *Striga gesnerioides*

Striga life cycle is connected to the alternating humid and dry periods in the semiarid zones of Tropics. The major phases in *Striga* life cycle are successively germination, haustorial induction, attachment to and penetration into host tissues (Botanga and Timko, 2005). To germinate, *Striga* seeds require an after-ripening period of 2 to 3 months (Botanga and Timko, 2005) followed by a preconditioning period during which seeds

imbibe water at a temperature ranging from 25 to 35 °C (Worsham and Musselman, 1987; Kuiper *et al.*, 1996). The germination is achieved only when preconditioned seeds are exposed to root exudates from a host plant (Worsham and Musselman, 1987) in close proximity of 2 mm. *Striga* seedlings can survive for 3 to 7 days in the absence of a host (Pieterse and Pesch, 1983; Worsham and Musselman, 1987). After the seeds are germinated, a second chemical signal produced by host roots called haustoria initiation factor (HIF) induces the differentiation of radicles into the haustorium that connects *Striga* to the host tissues (Butler, 1995; Darr, 1997; Riopel and Timko, 1995). Once the vascular link is established between the parasite and its host, *Striga* seedlings increase in size, forming structures called tubercles (Botanga and Timko, 2005). The *Striga* plant feeds during its underground development by means of the haustorium which drains nutrients and water from the host plant (Lane and Bailey, 1992). At this stage *Striga* behaves as an obligate parasite and the damage to the host plant is high (Lane and Bailey, 1992). The emergence of *Striga* on susceptible cowpea plant is observed within four to six weeks (Tignegre, 1988).

2.2.4 Available control measures

There are many methods of controlling parasitic plants that infest crops including hand pulling, cultural practices using rotations and trap crops, biological method with *Fusarium* (Zahran *et al.*, 2008) and chemical control by use of ethylene or diverse herbicides (Kabambe *et al.*, 2007; De Groote *et al.*, 2008). However some of these methods are expensive for small-scale farmers of Sub-Saharan Africa. Fertilization with high rates of nitrogen coupled with suitable sowing dates and appropriate cowpea

irrigation proved effective in controlling *Striga* (Dembele, 1988). Field solarization, in which plastic films are used to heat the soil, is effective in controlling *Striga* only for the first 2 cm of the soil layer (Parker, 1991). The use of resistant varieties combined with adequate cultural practices is however the best, most affordable and simplest method of controlling *Striga* at farmer level. According to Lane and Bailey (1992), complete resistance is the most desirable since it reduces the amount of seeds in the ground. Horizontal resistance (polygenic resistance) would be more stable than vertical resistance (monogenic resistance) since the latter is easily overcome by the parasite (Rubiales *et al.*, 2006). However, most of the work done in cowpea is related to the vertical resistance since this type of resistance is easier and faster to transfer into elite varieties (Parker, 1991; Rubiales *et al.*, 2006).

2.2.5 Mechanisms involved in the resistance to *Striga*

Because *Striga* is an obligate parasite, its survival is entirely dependent on the host plant and their life cycles are tightly connected with a series of interactions. Resistance to *Striga* can be achieved only by disrupting these interactions following the crucial stages of its life cycle (Ejeta and Butler, 1993).

2.2.5.1 Resistance at germination

The production of low *Striga* germination stimulant by the host plant is the most understood mechanism of resistance. It was described in Sorghum variety N13 that induces reduced number of *Striga* shoots (Ramaiah, 1987; Lane and Bailey, 1992). This form of resistance is controlled by a single recessive gene (Ejeta *et al.*, 1991; Ramaiah *et*

al., 1990). This mechanism of resistance has not been observed in cowpea which stimulates always *Striga* seeds germination by its root exudates.

2.2.5.2 Resistance at attachment level

Lane *et al.* (1993b) studied the mechanisms of *Striga* resistance in cowpea using “*in-vitro*” methods. *Striga* attachment to the resistant host root is associated with necrosis of root cells around the attachment site. This type of resistance was observed in cultivar 58-57 in response to *Striga* race SG4 attacks (Lane *et al.*, 1993a). Few days (3 to 4) after the attachment, the radicle turns dark, indicating the death of the parasite. This type of resistance is comparable to the hypersensitive reaction exhibited by some plants following infection by a pathogen (Lane *et al.*, 1996) or other parasitic plants (Joel *et al.*, 1996). Biochemical reactions involved in this form of resistance have not yet been defined in cowpea (Berner *et al.*, 1995). Phytoalexins are sometimes associated with the hypersensitive reaction in plants response to infection, but Lane *et al.* (1996) could not isolate the phytoalexins from infected cowpea plant tissues.

2.2.5.3 Resistance at and after the penetration of host vascular cells

Maiti *et al.* (1984) reported that a number of *Striga* resistant Sorghum genotypes developed mechanical barriers to haustoria attachment and penetration into their root cortex. In some cases the haustorium is able to penetrate into host stele and form some tubercles that are visible on the surface of the root, but their further growth is inhibited. These tubercles are composed mostly of large cortical cells. At the interface between host tissues and the parasite, a browning is observed (Reiss *et al.*, 1995). A xylem disc is

formed, but does not fully develop (Reiss *et al.*, 1995). Elements of sieve tubes are present, but in smaller number than in normal tubercles found in susceptible cultivars (Reiss *et al.*, 1995). On the other hand, certain parasites die before forming a tubercle, without necrosis of host cells. Cowpea cultivar B301 showed this type of resistance “*in-vitro*” and in pots in response to *Striga* race SG1 and SG2 (Lane *et al.*, 1993a). Indeed no parasitic shoot was developed after tubercle formation. The same type of resistance was described in cultivar IT81D-994 using “*in-vitro*” study in response to the attack of *Striga* race SG4. In this case, a development of short parasitic shoot of up to 3 mm in length was observed. However, there was no subsequent development of the parasite after the transfer of the host in pot (Lane *et al.*, 1993a).

2.2.6 Available sources of resistance to *Striga gesnerioides*

Several *Striga* resistant cowpea cultivars have been identified (Lane *et al.*, 1991; Singh, 2002). Suvita-2, known as Gorom local from Burkina Faso and 58-57 from Senegal were identified through field screening under natural infestation (Aggarwal *et al.*, 1986; Singh and Emechebe, 1990). Many other genotypes were found to be resistant to *S. gesnerioides* including B301 (Botswana landrace), IITA lines such as IT82D-849, IT81D-994, IT90K-82-2, IT97K-497-2 (Berner *et al.*, 1995; Carsky *et al.*, 2003). Among these cultivars some combine resistance to both *Striga* and *Alectra* which is another parasitic plant that attacks cowpea, as well as to various viruses and diseases of importance (Singh, 2006; Ajeigbe *et al.*, 2008). IT93K-693-2, a recently identified breeding line was found to be resistant to all known *Striga* races in Africa. In Niger, a number of cultivars with good levels of resistance to *Striga gesnerioides* including TN121-80, TN93-80, and HTR were developed through mass selection. Up to date, none

of the landraces grown mostly by farmers was found to be resistant to *Striga* and introduced resistant varieties often lack producers' and consumers' preferred traits.

2.2.7 Biotypes of *Striga gesnerioides*

The existence of distinct races or biotypes of *Striga gesnerioides* parasitizing cowpea was demonstrated by the differential responses of cowpea cultivars to the various geographic *Striga* samples in Africa. Five races were firstly recognized in West and Central Africa (Lane *et al.*, 1996). Later on, Botanga and Timko (2006) distinguished six distinct races after molecular analysis of a wide collection of *Striga* isolates from the same region. The different *Striga* races were named as follows: SG1 (Burkina Faso), SG2 (Mali), SG3 (Niger and Nigeria), SG4 (Benin), SG4z (Zakpota in Benin), SG5 (Cameroon) and SG6 (Senegal). The race SG1 is mostly prevalent in Burkina Faso, but it is also found in Mali, Togo and Nigeria. The Race SG2 is exclusively found in Mali while race SG3 is prevalent only in Niger and Nigeria (Parker and Polniaszek, 1990; Lane *et al.*, 1996). The Race SG4 is specific to South Benin whereas the race SG5 is found in Benin, Burkina Faso, Cameroon and Nigeria (Moore *et al.*, 1995; Lane *et al.*, 1996). The race SG6 is prevalent only in Senegal (Botanga and Timko, 2006).

The existence of these different *S. gesnerioides* races in Africa render breeding cowpea for resistance to this parasitic weed very challenging. Indeed, all these races have not been characterized morphologically in order to distinguish between two different isolates and the risk of propagating their seeds from an area to another is very high. Therefore, breeding for only one *Striga* race may not be durable in a breeding programme. This is

why in this study, investigation about the different possible *Striga* races in Niger and breeding cowpea for multiracial resistance are necessary.

2.2.8 Genetics of resistance

The genetics of resistance of some cowpea cultivars to *Striga* infestation has been studied. The cultivars B301 and IT81D-849 have been shown to be resistant to races SG1, SG2, SG3 and SG5 (Singh and Emechebe, 1997; Berner *et al.*, 1995; Lane *et al.*, 1996). Suvita-2 was found to be resistant to races SG1, SG2 and SG4 (Parker and Polniaszek, 1990; Berner *et al.*, 1995), while the cultivar 58-57 showed resistance to races SG1, SG4 and SG5 (Parker and Polniaszek, 1990; Lane *et al.*, 1994, 1996). The mode of inheritance of *Striga* resistance in these cowpea cultivars has also been studied. In B301, the resistance was found to be conferred by a single dominant gene for SG1 (Burkina Faso) (Lane and Bailey, 1992; Atokple *et al.*, 1995), SG2 (Mali) and SG3 (Niger, Nigeria) (Touré *et al.*, 1997; Singh and Emechebe, 1990; Atokple *et al.*, 1995) and by a recessive gene in IT82D-489 (Atokple *et al.*, 1995). Touré *et al.* (1997) and Singh *et al.* (2002) proposed that the resistance to these different races would be controlled by the same gene. In the cultivar IT82D-849, the resistance to SG1 is conferred by a dominant gene (Atokple *et al.*, 1995) as well the resistance to SG2 (Touré *et al.*, 1997). However, its resistance to SG3 is controlled by a recessive gene (Touré *et al.*, 1997). According to Atokple *et al.* (1995), the gene controlling *Striga* resistance in IT82D-849 is different from that in B301, but these genes could be alleles at the same locus or tightly linked. The resistance to SG1 in Suvita-2 is conferred by a single dominant gene (Atokple *et al.*, 1995), as well as the resistance to SG2 (Touré *et al.*,

1997). In IT81D-994, the resistance to SG1 is also conferred by a single dominant gene. The symbols *Rsg1*, *Rsg2* and *Rsg3* were proposed by Atokple *et al.* (1995) to designate the resistance to *S. gesnerioides* in B301 (race SG3), IT82D-849 (race SG3) and Suvita-2 (race SG1).

These findings showed that breeding cowpea for resistance to *S. gesnerioides* can be achieved using pedigree selection and backcross breeding.

2.2.9 Techniques of screening for *Striga* resistance in cowpea

A number of screening methods have been used in order to find sources of *Striga* resistance in cowpea (Lane and Bailey, 1992; Ouédraogo *et al.*, 2002a; Boukar *et al.*, 2004). The most common techniques include (i) field and pots screening, with more reliability from pots, (ii) “*in-vitro*” screening techniques, through which *Striga* resistance mechanisms were studied (Lane *et al.*, 1991), and (iii) the molecular screening techniques based on DNA markers associated with the resistance of cowpea to *S. gesnerioides* (Ouédraogo *et al.*, 2002a; Boukar *et al.*, 2004).

2.2.9.1 Field and pot screening for resistance to *Striga gesnerioides*

Field experiments are still necessary for yield evaluation and the performance under field conditions reflects the true varietal performance. Pot and field screenings are required in studying post-emergence *Striga* development such as *Striga* emergence date, number of *Striga* shoots per host and vigor of *Striga*. Field screening for *Striga* resistance is hampered by the following challenges: heterogeneity of infestation in naturally infested

fields, environmental effects on *Striga* emergence, complex interactions between host, parasite and environment that influence the parasite's establishment and reproduction (Hausmann *et al.*, 2000). An improved field screening methodology should include several appropriate practices such as field inoculation with *Striga* seeds, suitable experimental design allowing high replication and specific field layout, use of appropriate susceptible and resistant checks. Also, it is very important that susceptible and resistant checks should be planted at frequent intervals, including adjacent *Striga*-free and *Striga* sick-plots (Hausmann *et al.*, 2000). According to Botanga and Timko (2005) the accuracy of a *Striga*-infested field experiment would be increased if TVx3236, a universal susceptible cowpea genotype is included. Pot screening techniques are simulations to *Striga*-infested field conditions. Pot screening has, compared to field screening, the advantage of higher control over the environmental conditions. However, Hausmann *et al.* (2000) and Omanyia *et al.* (2004) reported low heritability estimates and moderate to low correlations to *Striga* resistance when identified resistances in pot screening are tested under field conditions. An adequate screening for *S. gesnerioides* can be achieved in pots by implementing the method described by Musselman and Ayensu (1984) who recommended 1000 *Striga* seeds per pot (8 to 10 liter-content). However, pots and their sandy-content are sterilized at 100°C for 24 hours using humid heating with an autoclave before conducting the screening. While studying *Striga* resistance in sorghum, Hausmann *et al.* (2000) developed a *Striga* vigor score with a scale from 1 to 9 which could be adapted for studying *S. gesnerioides* infesting cowpea. On the other hand, Atokple *et al.* (1995) defined a scale with two classes (resistant or susceptible) but they considered individuals or genotypes with intermediate resistance as susceptible. Singh

and Emechebe (1997) used the number of emerged *Striga* plants and the symptoms on cowpea plants to describe a scale in which cultivars were classified as resistant, moderately resistant, or tolerant:

1. Resistant = 100% plants resistant; no *Striga* emergence in the plot and no *Striga* symptom observed on plants.
2. Susceptible = 5 to several *Striga* plants emerged per plot and plants show severe *Striga* symptoms.
3. Moderately resistant = few *Striga* emerged (2-3) per plot but no *Striga* symptom observed.
4. Tolerant: Several *Striga* emerged but no significant yield reduction.

2.2.9.2 “*In-vitro*” screening techniques

“*In-vitro*” screenings enable hosts to be screened for resistance to *Striga* establishment at all critical stages of its life cycle (Lane and Bailey, 1992). In addition, “*in-vitro*” techniques allow the evaluation of much more materials in time than it would be possible with limited field space (Berner *et al.*, 1997). Different mechanisms of *Striga* resistance can be evaluated with “*in-vitro*” screening using petri dishes. The culture media proposed by Berner *et al.* (1997) is agar-based and its composition depends on *Striga* species to be studied.

2.2.9.3 DNA screening techniques: use of marker assisted selection in breeding cowpea for *Striga* resistance

Striga gesnerioides resistance genes can be exploited easily through Marker Assisted Selection (MAS). Some DNA markers identify quickly and easily individuals with resistance genes. The use of markers also facilitates the cloning of these genes and their introgression into elite varieties (Rispaill *et al.*, 2007). Molecular markers for identification and selection of *Striga*-resistant genotypes have been developed for most of the races of the parasite prevalent in West Africa. Ouédraogo *et al.* (2001) identified three AFLP markers closely linked to the resistance gene associated with race SG1 present in IT82D-849 (*Rsg2-1*), and six markers tightly linked to the resistance gene associated with race SG3 present in Tvu 14676 (*Rsg4-3*). Two of these AFLP markers were located in the same region of the first linkage group on the current cowpea genetic map developed by Menéndez *et al.* (1997) and improved by Ouédraogo *et al.* (2002b). Similarly, they also identified seven markers linked to the gene conferring resistance to the race SG1 in Gorom (*Rsg3*) and five markers linked to the resistance gene associated with the same race in IT81D-994 (994*Rsg*). These findings suggest the existence of a complex of *Striga* resistance genes in cowpea. Another marker linked to *Rsg1*, a gene in IT93K-693-2 conferring resistance to the race SG3 was sequenced and converted into SCAR (*Sequence Characterized Amplified Region*) marker, which is codominant and easier for use in marker assisted selection (Boukar *et al.*, 2004). This SCAR marker is associated with resistance to *Striga* race SG3, which is one of the most wide spread races in West Africa.

In MAS, access to simple and low cost tools for molecular study is crucial to get the knowledge required. The ability to obtain a high quality DNA from plant tissues is a frequent challenge in studying DNA at molecular level. Several methods of extracting DNA from leaf tissues have been developed. Previous isolation methods required grinding individual plant tissue to a fine powder under liquid nitrogen before DNA extraction in a buffer (Dellaporta *et al.*, 1983; Mohapatra *et al.*, 1992). Liquid nitrogen can be difficult to procure in regions where laboratory facilities are limited; thus, a new method not requiring its use would be helpful (Omoigui *et al.*, 2012). The use of FTA cards (Whatman) is a simple alternative method for collection, storage and retrieval of genomic DNA for molecular study.

2.2.10 Correlation studies

Omanya *et al.* (2004) found that “*in-vitro*” germination distance was positively correlated with field resistance to *Striga* in sorghum. However, they reported that *Striga* resistance parameters in the pot trials were weak and not consistently correlated to field resistance. According to Omanya *et al.* (2004) the low correlation observed between pot and field trials could be partly attributed to the artificial and highly limited root environment in pots. *Striga* resistance and grain yield are genetically positively correlated (Omanya *et al.*, 2004). This should facilitate selection of materials with low *Striga* emergence and high grain yield for *Striga*-infested areas. Recent studies, Kamara *et al.* (2008); Tignegre (2010); Omoigui *et al.* (2012) and Ekeleme *et al.* (2013) reported significant negative correlation between yield components and number of *Striga* count in the field.

2.2.11 Breeding procedures in cowpea

While reviewing methodologies for breeding *Striga*-resistant sorghum varieties, Haussmann *et al.* (2000) proposed a number of strategies which could be applied to cowpea: (i) the characterization of germplasm for *Striga* resistance, (ii) the improvement of available sources of resistance for better agronomic traits, (iii) the transfer of resistance genes into adapted farmer preferred cultivars, (iv) the pyramiding of resistance genes into these adapted cultivars. The development of molecular markers could also facilitate marker-assisted selection (Boukar *et al.*, 2004; Haussmann *et al.*, 2000; Ouédraogo *et al.*, 2002). In addition, multilocation experiments allow the identification of stable resistance across different environments (Haussmann *et al.*, 2000).

Breeding procedures in cowpea include conventional and innovative breeding methods. The following methods are used in intervarietal breeding: pedigree, bulk, single seed descent, and doubled haploid method (Singh, 1993). Developing pure lines is achieved by backcross and pedigree methods which are applied to genetically segregating generations (Singh, 1993). The pedigree method is based on a detailed record keeping; so that each offspring can be traced back to the F₂ generation from which it originated at any generation of concern (Singh, 1993).

The classical pedigree breeding method was used successfully in breeding for characteristics that are governed by a single or a few genes, such as *Striga* specific resistance genes in cowpea (Aggarwal and Ouedraogo, 1989; Muleba *et al.*, 1997). In the bulk selection method, population improvement methods such as recurrent selection can be exploited to incorporate multiple traits, resistance to insects and resistance to diseases, if only low to moderate levels of resistance are identified (Ehlers and Hall, 1997). This

method is useful for achieving long-term objectives (Ehlers and Hall, 1997). Ehlers and Hall (1997) described an efficient recurrent selection procedure for combining multiple desirable traits in cowpea consisting of:

- developing sets of early F₃ or F₄ generations inbred lines from different biparental crosses,
- screening them to identify individuals or lines with desirable new combinations of traits,
- crossing selected individuals from different biparental crosses, and
- selecting progeny individuals with desirable traits common to all four parents.

The characteristics of grain types preferred are varied and depend on locality, but in most regions, cowpea consumers prefer large seeded types which can be difficult to produce because most cowpea accessions have small seed (Redden *et al.*, 1984; Ehlers and Foster, 1993). It has been shown that individual seed weight has high heritability (Fery and Singh, 1997); also a dominant gene controls the large seed trait in cowpea (Karkannavar *et al.*, 1991). According to Redden *et al.* (1984), seed size and quality can be easily selected in early generations such as F₂ using visual characters. Recurrent selection methods can be used to enhance grain and fodder yield traits because of the additive gene effects involved in these characters.

In interspecific hybridizations, backcross methods are used in order to transfer important and simply inherited characters from an exotic cowpea variety into an elite variety. For instance, Fatokun (2002) used backcross technique to transfer the pubescence character which is a physical pod barrier for pod sucking bugs (*Clavigralla tomentosicolis*) and borers (*Maruca vitrata*) from wild species to cultivated cowpea.

At the same time, efforts made to hybridize cowpea (*Vigna unguiculata*) with *V. vexillata* have been unsuccessful due to incompatibility (Fatokun, 2002).

CHAPTER THREE

3.0 Farmers' preferred traits and perceptions of cowpea production and constraints in Niger

3.1 Introduction

Cowpea is an important and strategic crop for small-scale farmers in Niger because of its high protein content and market value. Its production has significantly increased from 515 MT to 1,700 MT during the last decade (Anon., 2013). Cowpea is grown at all agricultural zones in Niger but its production is severely affected by *Striga gesnerioides* (Aggarwal and Ouédraogo, 1989). Several control methods such as cultural practices, biological, physical and chemical methods have been recommended, however, *S. gesnerioides* is still a biological constraint to cowpea production in the country. In Niger, the adoption of new agricultural technologies has been slow by farmers (Charlick, 1992; Mazzucato and Ly, 1994). According to Mazzucato and Ly (1994) only 5% of improved millet seed and less than 1% of improved cowpea seed were grown by farmers in 1990. In 2013, the improved seeds rate of adoption ranges from 8 to 10 % for the same crops (MDA, 2013). Due to the low adoption rates of improved varieties, it has become necessary to obtain information on the end-users' perceptions and preferences, and constraints affecting the crop in the development of varieties for any successful and sustainable breeding programme.

Participatory methods have been used in developing countries to make use of rural communities knowledge in agricultural research. A participatory rural appraisal (PRA) enables local communities to analyze their own conditions, plan and make decisions (Chambers, 1992).

In 2008, a participatory study was conducted by the Netherland “Development Organization” in Niger (SNV) to analyze cowpea production systems and development opportunities in Maradi region. In another study funded by the Generation Challenge Programme between 2008 and 2009, to identify potential sources of *Striga* resistance, farmers were engaged in order to understand their problems and preferences (Muranaka *et al.*, 2010). Participating farmers in Niger acknowledged that *Striga* was a serious and increasing challenge to cowpea production (Muranaka *et al.*, 2010). This study focuses on other cowpea production districts.

The objective of this research was to obtain information on cowpea production constraints, farmers’ knowledge about *Striga gesnerioides* and their preferred cowpea traits.

3.2 Materials and methods

3.2.1 Study area

The PRA was conducted in Kollo (Western Niger), Aguié (Central Niger) and in Magaria (South-Eastern Niger) (Figure 3.1) from the 3rd to 12th February 2013. At

Kollo the PRA was carried out in Sakay Koira Tegui, Sakay Koira Zeno and Winde Day Tegui. At Aguié the PRA was undertaken in Dan Saga, Nakikarfi and Debi while at Magaria the PRA was conducted in the villages of Getshi, Angoual Gamji and Incharoua peulh. A total of 126 farmers attended the PRA sessions with equity of gender (Table 3.1).

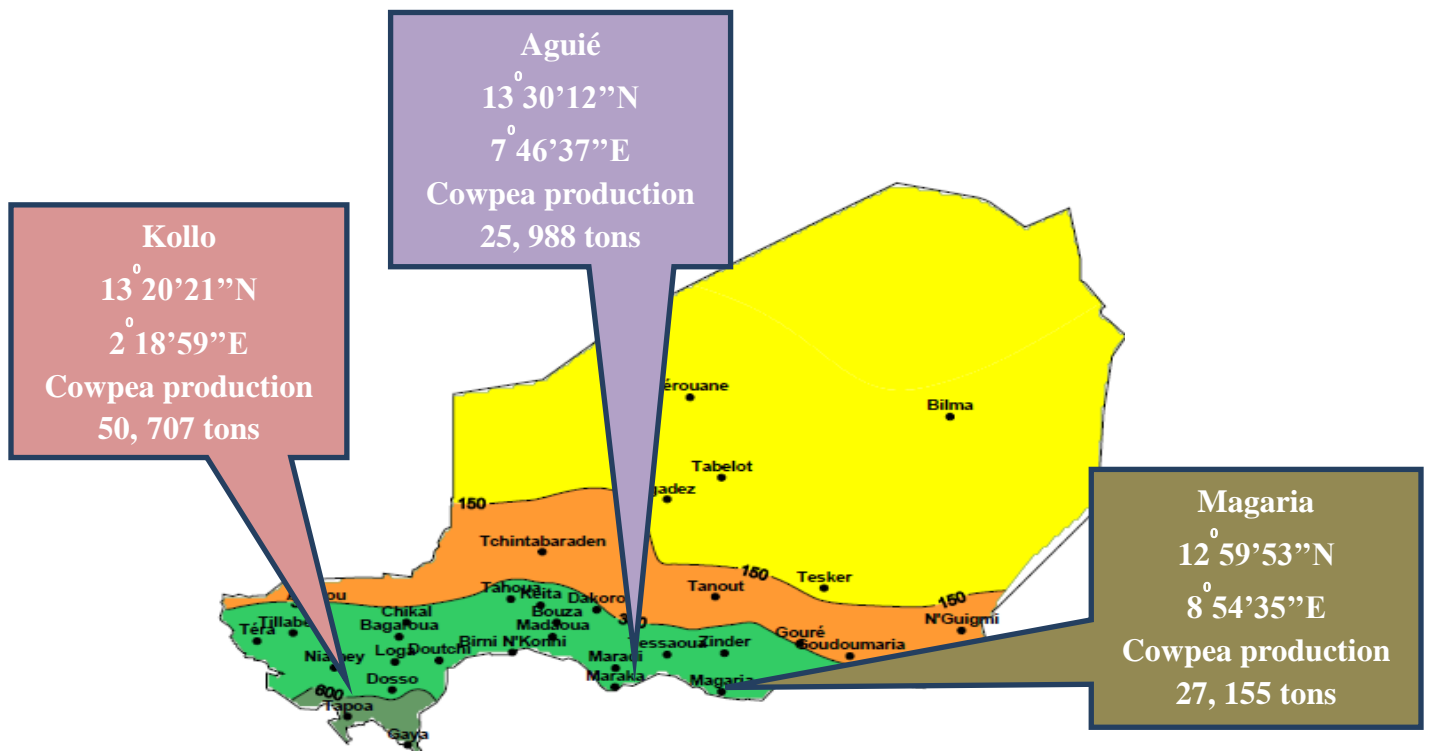


Figure 3. 1: Selected PRA sites in Niger

Table 3. 1: Sites, number of farmers and dates of PRA sessions

Villages	Districts	Regions	No of farmers		Dates for PRA
			Females	Males	
Sakay K. Tegui.	Kollo	Tillabéri	7	7	03/02/2013
Sakay K. Zeno	Kollo	Tillabéri	7	7	03/02/2013
Winde D. Tegui	Kollo	Tillabéri	7	7	04/02/2013
Dan Saga	Aguié	Maradi	7	7	09/02/2013
Nakikarfi	Aguié	Maradi	7	7	09/02/2013
Debi	Aguié	Maradi	7	7	10/02/2013
Getshi	Magaria	Zinder	7	7	11/02/2013
Angoual Gamji	Magaria	Zinder	7	7	11/02/2013
Incharoua peulh	Magaria	Zinder	7	7	12/02/2013

Cowpea traders (13) and processors (14) were interviewed in addition to the group discussions to gather information on market and consumers' requirements. Most of the traders found in the market were males while most of the food processors were females.

A preparatory visit was conducted two weeks before the PRA sessions in each district agricultural office to discuss the questionnaires with a team of extension officers and identify the villages and farmer groups. The team sensitized the farmers about the PRA sessions.

3.2.2 PRA data collection and analysis

The PRA implementation team included a facilitator, a technician, and in each district an extension officer. At each site, the local extension officer facilitated the discussion in the local dialect. The focus group discussion was composed of fourteen farmers, seven of each gender (Figure 3.2). The discussions were conducted separately; women were taken before men wherever it was possible. The preferences of farmers were assessed using priority ranking and scoring. The different farmer groups were given 100 stones to rank cowpea varieties so that any given number to a variety was converted in percentage. The results obtained were recorded in Excel and analyzed using non parametric statistics in SAS 9.2.



Figure 3. 2: Female (b) and male (a) farmers in discussion.

3.3 Results

3.3.1 Cowpea farming system

The predominant crops grown in the three districts were pearl millet (*Pennisetum glaucum*), cowpea (*Vigna unguiculata*), sorghum (*Sorghum bicolor*), groundnut (*Arachis hypogaea*), false sesame (*Ceratotheca sesamoides*), roselle (*Hibiscus sabdarifa*), Tiger nut (*Cyperus esculentus*) and Bambara groundnut (*Voandzea subterranea*). At all sites, farmers ranked cowpea as the second most important crop after pearl millet (Table 3.2).

Table 3. 2: Main crops grown by farmers and their rank order

Crop grown	Ranking	Kollo %	Aguié %	Magaria %
Pearl millet	1	41	41	41.16
Cowpea	2	30.5	25.83	28.33
Sorghum	3	7.83	20.83	12.33
Groundnut	4	3.16	3.66	14.16
Roselle	5	13.66	1.66	
False sesame	6	3.5	5.16	3.66
Tiger nut	7		1.86	
Bambara groundnut	8	0.35		0.36

Cowpea was mostly intercropped with pearl millet or sorghum; however some farmers grew it in pure stand culture for seed multiplication purposes and for testing new varieties. Across the sites, 56 % of the farmers grew cowpea for food and 44 % for cash. About 83 % of the farmers in Kollo district grew cowpea for food, while 67 % of the farmers in Aguié grew it as a cash crop. For participating farmers in the Magaria district,

50% of them grew cowpea for food and 50% for cash. Farmers in all districts preferred early maturing cowpeas, as a supplement for major staple foods like pearl millet, and grew cowpea because of its protein content. Some also indicated that financial need was the reason for growing cowpea as a cash crop. Farmers in the three districts used all parts of cowpea plant. However, dried seeds were ranked number one in importance followed by leaves, stems and roots.

At all sites, women had poor access to land which is mostly men's or family property. However, at Aguié and Magaria districts, some women had their own farms aside from or within the family property. Farm size was generally small across all the sites. The average farm size ranged between 0.5 and 2 hectares. Farmers principally managed soil fertility by adding manure. There was very poor access to inputs. Most farmers indicated that they used crop rotation in their farming systems where cowpea was rotated with pearl millet or sorghum every one to two years. However, the rotation was not practiced fully because of limited land sizes.

Most farmers obtained their seeds from the market as they were available when required. Some improved varieties have been adopted though most farmers still grew their low-yielding and photoperiod sensitive landraces.

3.3.2 Cowpea varieties currently grown and production constraints

The main cowpea varieties grown at the three districts included both the landraces such as DOUNGOURI KWARE, DAN BAUSHI, DAN MALAN IDI, DAN MOUSSA, BANJARASS, OLOCA and the improved varieties IT90K-372-1-2, KVx30-309-6G and TN5-78. The landraces were

grown only at specific districts of the study area, while the improved varieties were grown in at least two districts. Doungouri Kware was grown only at Kollo; Dan Baushi, Dan Malam Idi and Dan Moussa were grown at Aguié; and Oloca was grown at Magaria district. The improved KVx30-309-6G was grown in all the districts while IT90K-372-1-2 was grown at Aguié and Magaria; and TN5-78 was grown at Kollo and Magaria.

Farmers identified a number of constraints to cowpea production. Their importance varied across sites. Insect pests were the most important constraints reported by farmers (33.64%; 38.69% and 40.83%) followed by *Striga* (33.5%; 35.16% and 35%). Drought was scored 18.5%; 16.66% and 24.17% in Kollo, Aguié and Magaria, respectively. Low soil fertility, land scarcity, late sowing, poor access to inputs and diseases were identified as minor constraints by farmers in Kollo and Aguié (Table 3.3).

Table 3. 3: Main cowpea production constraints in Kollo, Aguié and Magaria

Constraints to production	Ranking	Kollo %	Aguié %	Magaria %
Insect	1	31.16	38.69	40.83
<i>Striga</i>	2	31.00	35.16	35.00
Drought	3	18.50	16.66	24.17
Low soil fertility	4	12.60	7.66	Not listed
Late sowing	5	3.33	1.83	Not listed
Lack of land	6	1.83	Not listed	Not listed
Poor access to inputs	7	1.75	Not listed	Not listed
Disease	8	1.66	Not listed	Not listed

3.3.3 Farmers' knowledge about *Striga*

Across all the sites, farmers acknowledged having knowledge about *Striga*. They used a number of terms such as cowpea disease, weed that attacks cowpea, weed that emerges during rainy season, a scourge and a witch.. Farmers mentioned several uses of *Striga* plants such as a cure for constipation, application to cut body surfaces; snake bite treatment.. Despite these beneficial uses of the *Striga* plant, farmers supported the eradication of this parasitic weed by any possible means. Farmers associated low soil fertility to *Striga* infestation. They were able to describe the major symptoms associated with *Striga* attack on the cowpea plant. Farmers did not know any landrace that was resistant to *Striga*; and most of the improved varieties that were *Striga* resistant lacked their preferred traits such as white-colored seed and big sized grain. They mentioned hand-pulling followed by burning the dried biomass and fertilization as preventive measures but these are not always affordable to small-scale farmers.

3.3.4 Farmers' criteria for adopting varieties

Farmers rated high yield potential, early maturity, white-colored seed coat and good taste as the most important selection criteria in choosing cowpea varieties (Figure 3.3). Although insects, *Striga* and drought were the three major constraints to cowpea production identified, farmers considered resistance and/or tolerance to these stresses as additional selection criteria.

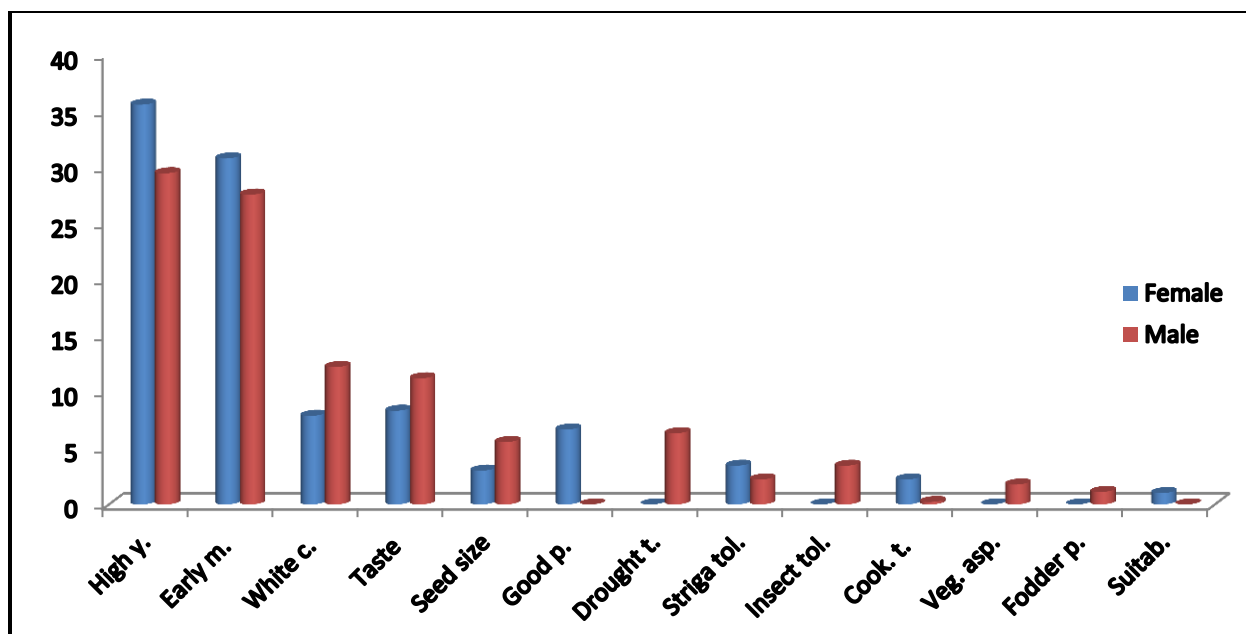


Figure 3. 3: Farmers' criteria for adopting varieties.

High y.: high yield; **White c.:** white color; **Good p.:** good price; **Drought t.:** drought tolerance; **Striga, insect tol.:** *Striga*, insect tolerance; **Cook. t.:** cooking time; **Veg. asp.:** vegetative aspect; **Fodder p.:** fodder production; **Suitab.:** suitability for cooking local meal

3.3.5 Study of grain quality

Farmers at all sites agreed that seed size and color were the most important features of grain quality. The results of their choices are presented in Figures 3.4 and 3.5. Most farmers (100% at Aguié and 75% at Magaria) preferred large seeded cowpea for consumption as well as for the market (83% at Aguié and 75% at Magaria). At Kollo district 58% of the farmers preferred the small seeded cowpea for their consumption and 55% of farmers chose it for the market. White colored grain was preferred for consumption by all farmers in Kollo and Aguié, and 50% of the farmers in Magaria. About 99.96% and 100% preferred the white grained cowpea for market in Kollo and

Aguié respectively. At Magaria district, 50% of the farmers preferred brown colored grain for the market, while only 25% of the farmers chose the white colored grain.

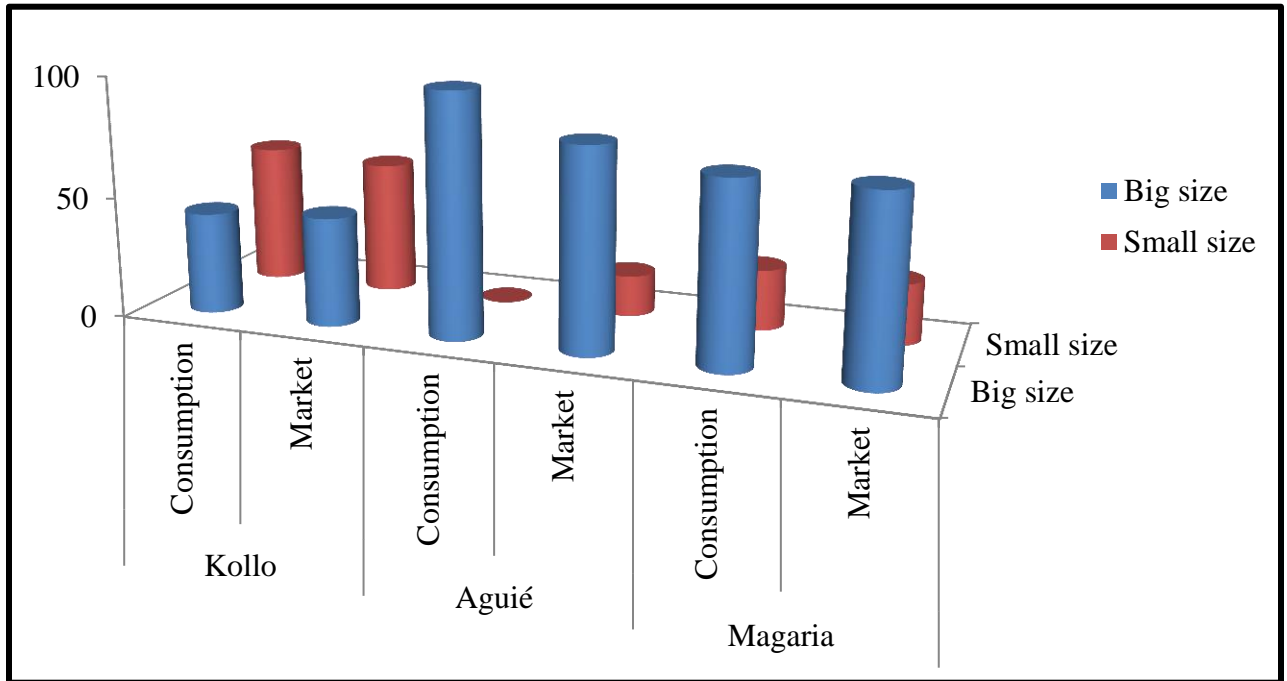


Figure 3. 4: Farmers’ preferences for grain size at Kollo, Aguié and Magaria districts.

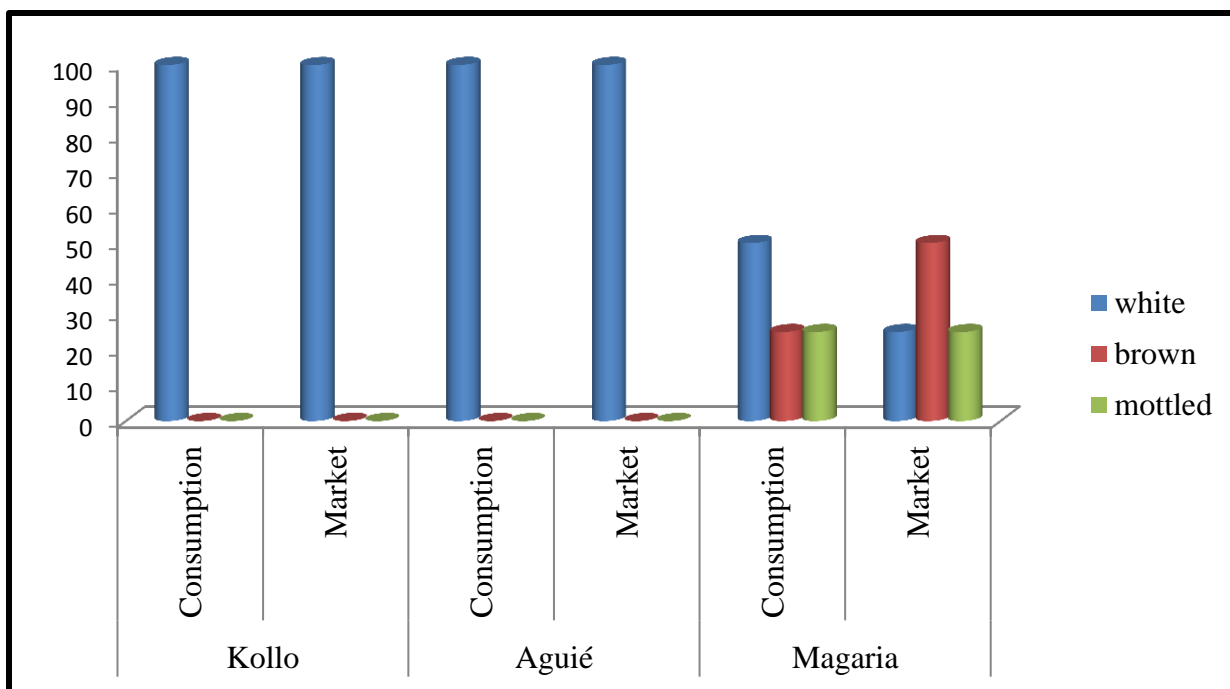


Figure 3. 5: Farmers’ preferences for grain color at Kollo, Aguié and Magaria districts.

3.3.6 Farmers’ preferred variety ranking based on grain characteristics

In this section, only the top preferred varieties grown by farmers were used for the priority ranking (Figure 3.6).

Farmers’ choice by sex and age (youth: below 40 years; elders: above 40 years old) is shown in Figures 3.7, 3.8, 3.9 and 3.10.

At Kollo district “Kware” (Figure 3.7) was rated higher than KVx30-309-6G and TN5-78 by all the groups except by the elders (45% by females; 43% by males and youths). KVx30-309-6G was ranked higher by elders (36% versus 35% for Kware).

At Aguié, KVx30-309-6G (Figure 3.8) was preferred to Dan Mallam Moussa, Dan Mallam Idi, Dan Baushi and IT90K-372-1-2 by all the groups except by the youth, 28%, 27% and 30% of females, males and elders respectively. Dan Baushi was rated higher by youths (34% versus 20% for KVx30-309-6G).



Figure 3. 6: Male (a) and female (b) farmers ranking cowpea varieties.

At Magaria, 26% of females and 28% of youths preferred Banjaras (Figure 3.9) to the other four varieties while 28% of males rated IT90K-372-1-2 higher and 32% of elders preferred Oloca. The combined data across the three districts (Figure 3.10) show that three varieties (KVx30-309-6G, IT90K-372-1-2 and TN5-78) were the top farmers' preferred varieties. However, farmers ranked Kware in Kollo, KVx30-309-6G in Aguié and IT90K-372-1-2 in Magaria as most popular varieties for their grain characteristics and yield potential.

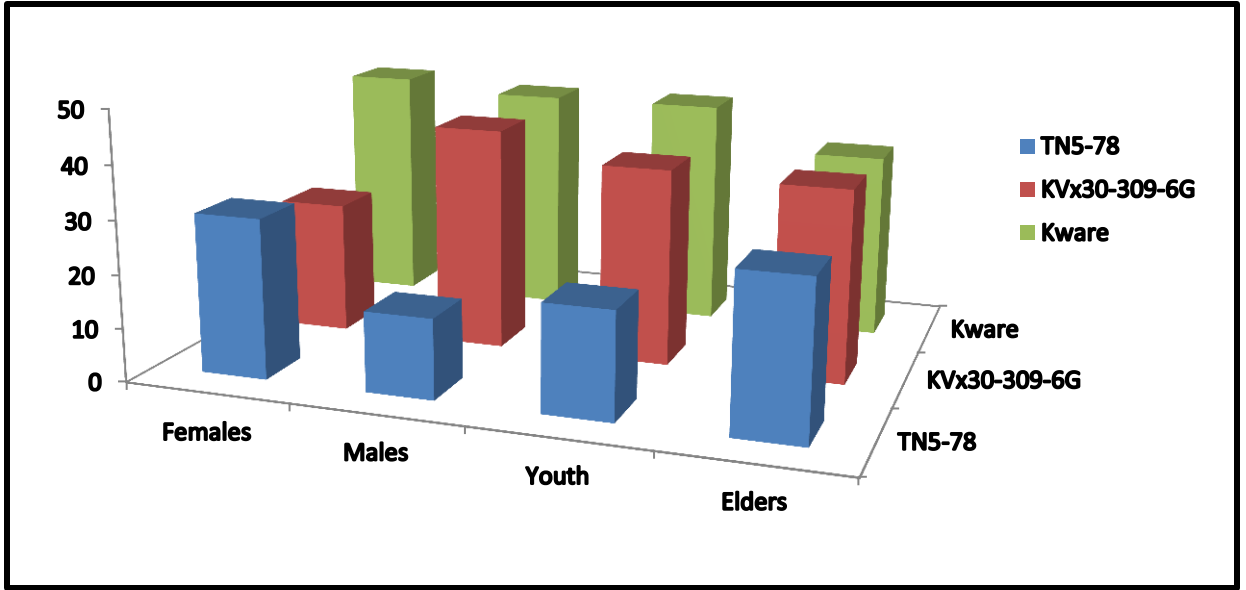


Figure 3. 7: Farmers' preferred varieties by rank order based on grain characteristics and according to sex and age at Kollo, 2013.

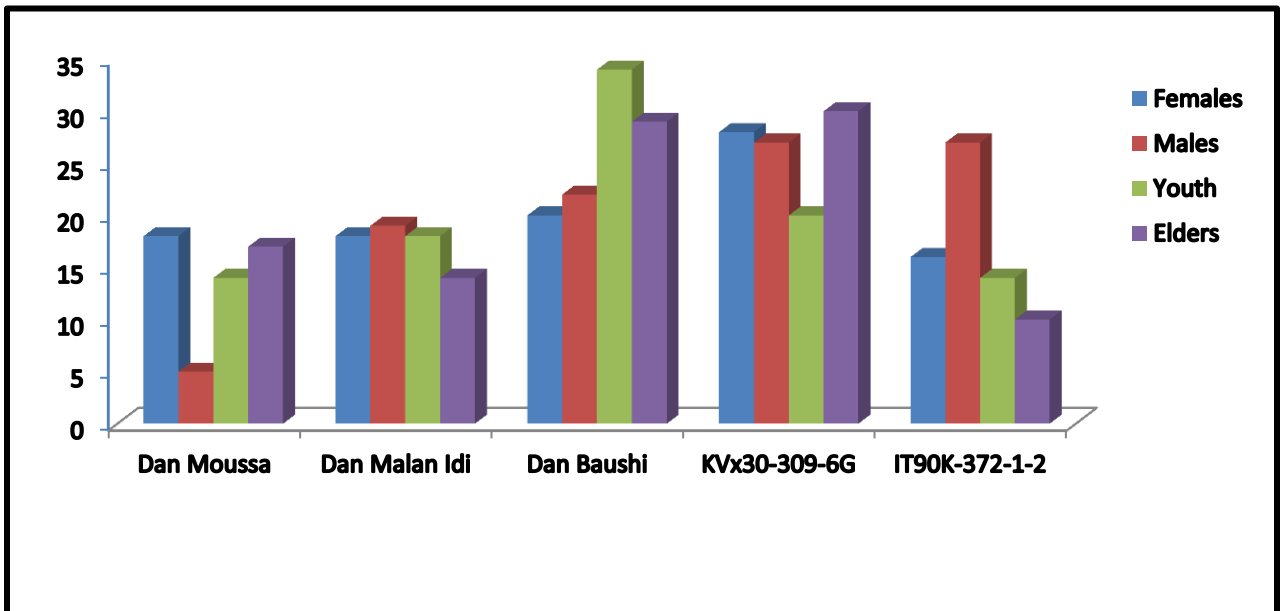


Figure 3. 8: Farmers' preferred varieties by rank order based on grain characteristics and according to sex and age at Aguié, 2013.

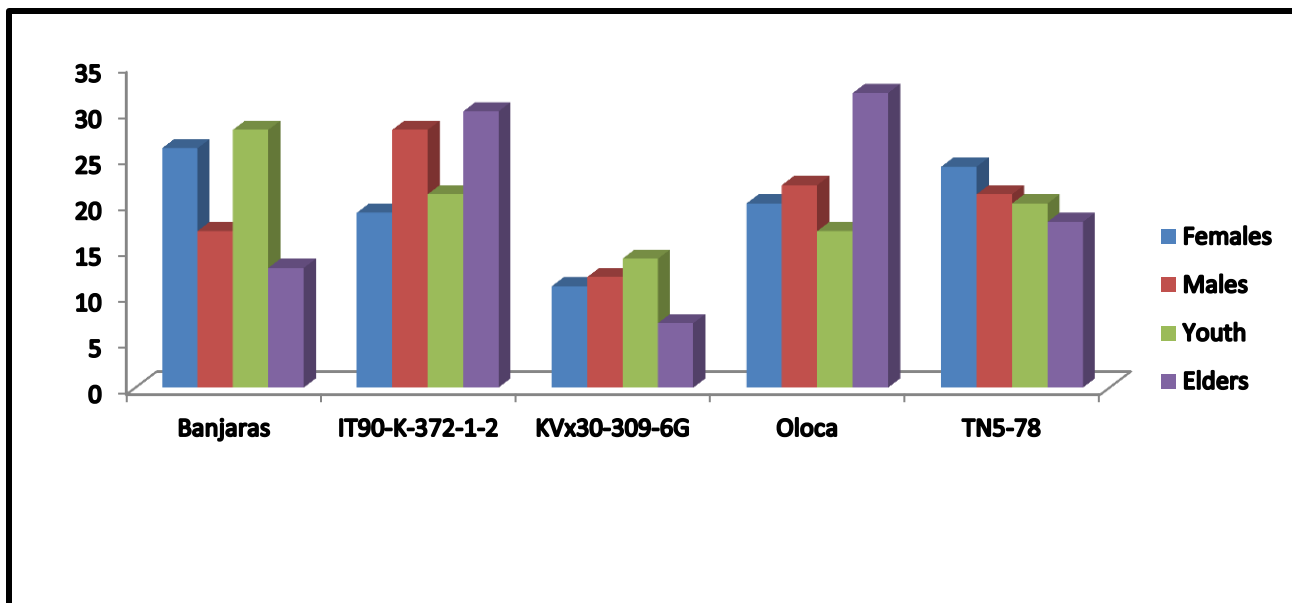


Figure 3. 9: Farmers' preferred varieties by rank order based on grain characteristics and according to sex and age at Magaria, 2013.

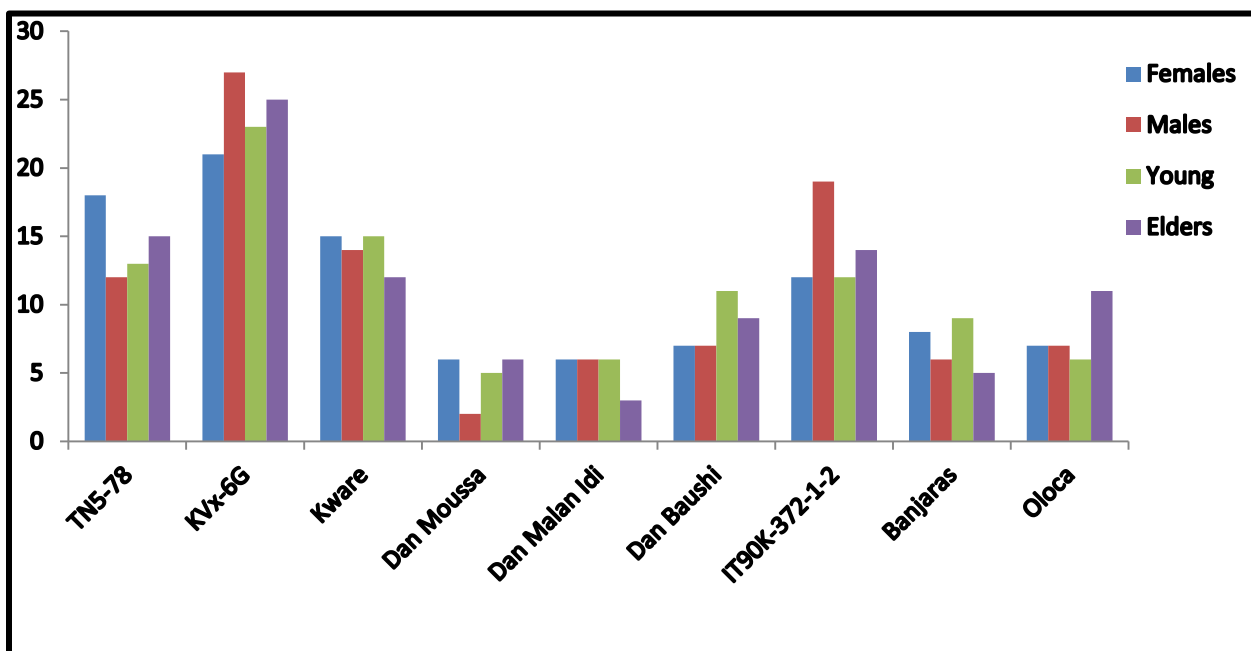


Figure 3. 10: Farmers' preferred varieties by rank order based on grain characteristics and according to sex and age in 2013 (multi-sites combined data).

In addition to the focus group discussions, 13 traders and 14 food processors were interviewed about grain quality. Most traders (90%) sold large seeded and white-colored cowpea at the market. They also mentioned that the same characteristics were suitable for consumers. For 75% of traders at Magaria district, the small seeded and mottled cowpea was preferred by consumers. Most food processors (80%) used white-colored and large seeded cowpea in processing. They indicated that these had the best taste and were suitable for preparing local meals.

3.4 Discussion

3.4.1 Cowpea farming systems

The results showed that cowpea plays an important role in the cropping system in the three districts. It was mainly intercropped with pearl millet or sorghum. This confirms the findings of Langyintuo *et al.* (2003) who reported that in the drier savanna and sahelian zones of West and Central Africa cowpea is usually grown as an intercrop with pearl millet or sorghum and, less frequently as a sole crop or intercropped with maize, cassava or cotton. Cowpea was grown for food and cash but mostly for household consumption where farmers (56%) grew it for food. This is contrary to an earlier report which indicated that 80% of cowpea production in Niger was exported (Anon., 2013). This may be due to the contribution of seed enterprises whose production is essentially exported or due to some unexpected circumstances that lead farmers to sell crops originally meant for home consumption. Farmers were interested in adopting improved varieties that combine their preferred traits and were adapted to the local conditions. However, they were more accustomed to their widely grown landraces. This is similar to

a study conducted in Zinder region (SNV, 2009) where 85% of seeds grown by farmers were from landraces versus only 15% of improved seeds. Similarly, Tignegre (2010) in Burkina Faso, reported that farmers had access to improved varieties but were still growing landraces under mixed-cropping. Despite the availability of improved varieties, adoption rate is low due to seed shortage or cost and the lack of information on their availability. Cowpea productivity can be increased by promoting a wide range of productive varieties and crop management options. Access to and availability of seed of preferred varieties can be improved by undertaking sustainable seed production and delivery schemes (ICRISAT, 2013). Farmers managed soil fertility by use of manure in their farms. Therefore, fertilizers were used at sub-optimal levels with very little or no crop rotation practiced by the farmers due to land insufficiency. This probably restricts the productivity of the landraces they grew and even some improved varieties with high yield potential. The importance of fertilizers as key inputs in accelerating food production in West African Semi-Arid Tropics is well recognized by researchers and policy makers (Bationo *et al.*, 1991). It was demonstrated that improved cowpea varieties can give higher grain yield in farmers' fields under optimum management, especially in sole cropping (Saidou *et al.*, 2011). Hence, appropriate and intensive extension work needs to be done towards improvement of cultural practices and new varieties' adoption. At all districts visited, the results showed that land belonged only to males, except in the case of families where a widow possessed land. However, in general females can get a piece of land within the family property for farming. This is in line with the findings of a study (SNV, 2009) in Zinder region where it was reported that 70.33% of land was owned by

males only versus the 8.33% owned solely by females while 21.33% were owned by both.

3.4.2 Production constraints

Striga was among the top two constraints to cowpea production listed by farmers. Tignegre (2010) found similar results in Burkina Faso where *Striga* was ranked among the top three constraints to cowpea production. Insect pests were ranked first, while drought was ranked third at all sites. Previous studies (Adipala *et al.*, 2000; Asante *et al.*, 2001; Makoi *et al.*, 2010) reported similar findings and indicated that insect pests were the top constraints to cowpea production in Africa. Recently, Egbadzor *et al.* (2013) found that insects, drought and weeds were the most important limiting factors to cowpea production in Ghana. The consistency in ranking of these constraints by farmers at all the districts suggested that they were the major constraints for which priority should be given in breeding objectives. The other constraints such as low soil fertility, late planting, insufficient land, poor access to inputs and disease pressure were ranked low. This may be due to the limited knowledge of farmers about consequences of some of these constraints like diseases and low soil fertility. Meanwhile, in a participatory study (SNV, 2008) the main constraints identified were low soil fertility, low yield, and high cost of inputs. In that particular study the surveyed population was larger and the whole cowpea production value chain was considered in Maradi region where Aguié district is located. Experiments have shown that by using improved seeds and adequate doses of fertilizers the average yields can be raised 3 to 4 times (Singh *et al.*, 1997). However, in practice

only limited quantities of these are available and are also not easily accessed by small-scale farmers due to distance and cost (Saidou *et al.*, 2011).

3.4.3 Knowledge about *Striga*

Surveyed farmers recognized *Striga* as a major problem in cowpea production. Each ethnic group designated *Striga* with a precise name, with most of the names reflecting the yield losses induced by *Striga* to the cowpea plant. This showed that farmers were aware of this pest. They even highlighted the symptoms of infested cowpea plants and linked *Striga* infestation to low soil fertility. All the landraces and the preferred improved varieties they grew were susceptible to *Striga*. Farmers had no means to control *Striga* except through hand weeding which is tedious and laborious. Also, by the time weeding is done most of the damage may have been done to the crop, and in some cases the *Striga* plants would have set seeds therefore increasing the *Striga* seed bank in the soil. Preventive control measures such as fertilization were mentioned by farmers, but the implementation was not affordable for them. This may be why farmers supported the idea of breeding for resistant varieties even if it would eradicate the *Striga* despite its use for medicinal purposes. This study revealed that farmers have a deep knowledge about *Striga* but effort should be made to educate them on integrated *Striga* control to minimize its damage to cowpea productivity. A study on integrated *Striga* control conducted in 2005-2006 in Niger (FAO, 2007), showed that the number of emerged *Striga* shoots was reduced by 56% and 83% in Dan Gao and Kala-paté respectively while cowpea yield increased by 88% at Dan Gao.

3.4.5 Farmers' preferred traits

Farmers stated high yield potential, early maturity, white-colored seed coat and good taste as the most important traits in preferred cowpea varieties. Similarly, in Uganda, Orawu (2007) indicated that preferred traits were white seed color, earliness, high yield potential, good taste, and tolerance to insect pests and diseases. In a participatory evaluation of improved cowpea lines and cropping systems in Maradi and Zinder regions in Niger, Saidou *et al.* (2011) found that farmers were interested in early maturing varieties with good seed germination and high pod load as well as white seed coat color and yield potential as an important considerations for cowpea variety adoption.

Resistance to pests and diseases as well as tolerance to drought were not considered by farmers as important criteria of adopting varieties though pests and drought were major constraints identified. This may be due to the fact that these constraints are difficult to control at their level and all the varieties they grow are susceptible. Although farmers did not consider *Striga* resistance as a major criterion for their preferred varieties it is necessary in breeding to consider resistance to *Striga* for any new variety targeted for these communities while also taking into account the preferred quality traits.

Grain characteristics preferred by most farmers were white colored and large grain for their own consumption as well as for sale. This also was confirmed by cowpea processors and traders. Similar results were reported by Langyintuo *et al.* (2003; Tignegre (2010) and Egbadzor *et al.* (2013). However, in Magaria district only 25% of farmers preferred the white colored grain for the market while 50% of farmers chose brown seeds for sale. This may be due to the proximity of that district to Nigeria where Kormawa *et al.* (2000)

reported that generally consumers preferred brown colored cowpea grains over white colored grains in the Nigerian markets they studied.

The top three farmers' preferred varieties in this study were KVx30-309-6G, IT90K-372-1-2 and TN5-78. Similarly, in 2008, the results of a study conducted by SNV in Maradi region revealed that farmers' top preferred varieties were KVx30-309-6G and IT90K-372-1-2 because of their seed characteristics (big sized, white colored grain and good taste). This is consistent with the fact that the three varieties are the top commercial cowpea varieties in Niger to date. These are the recommended varieties in multiplication at INRAN stations and seed companies. In another participatory farmer evaluation, IT90K-372-1-2 was identified at the top of varieties having higher grain and fodder yields on-farm (Saidou *et al.*, 2011). This variety was also recommended to be grown on fields with less *Striga* infestation and to be improved urgently by incorporating *Striga* resistance genes in it. Though these improved varieties were not highly adopted by farmers, they were recognized as superior to their local landraces. This demonstrated again farmers' willingness to adopting new technology when it is consistent with their perceptions and affordable to them. In some cases significant differences were observed between males' and females' choices of varieties. For example, at Aguié district where Dan Moussa was grown, females preferred it three times over males. Similarly, the variety TN5-78 was ranked two times higher by females over males at Kollo district. This may be due to some characteristics like cooking time or suitability to local meals these varieties had and that were more or less women concern.

3.5 Conclusion

In this study, a participatory rural appraisal was very useful in gathering important information that will help to make decisions beneficial to farmers. Breeding cowpea for *Striga* resistance is a priority objective. *Striga* is a particularly serious challenge to cowpea production in Niger. Development of new cowpea varieties should include criteria such as high yield potential, earliness, white color and good taste. Most farmers preferred white colored and large sized cowpea grain. However, small sized cowpea grain was preferred by farmers at Kollo district and brown colored cowpea seed coat was considered for market at Magaria district. There is an urgent need to incorporate *Striga* resistance genes into the top three farmers' preferred varieties identified: KVx30-309-6G, IT90K-372-1-2 and TN5-78 in order to meet farmers demand and improve food security.

CHAPTER FOUR

4.0 Screening of cowpea germplasm for resistance to *Striga gesnerioides*

4.1 Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important source of protein for millions of people worldwide. In Sub-Saharan Africa (SSA) cowpea is the most widely grown food legume (Ehlers and Hall, 1997; Timko *et al.*, 2007; Timko and Singh, 2008). All parts of the plant are consumed by humans and animals. In Niger, cowpea is the major food legume and the second most widely grown crop after pearl millet. It has adaptation to a wide range of environments, but various biotic and abiotic constraints reduce its productivity in SSA. These constraints include insect pests, diseases, parasitic plants and drought. *Striga gesnerioides* (Wild.) Vatke, a parasitic flowering plant is one of the main biotic stresses that challenges cowpea production in drought-prone areas. In Africa, the yield reduction caused by *S. gesnerioides* is estimated to be high (83–100%) on susceptible cultivars (Emechebe *et al.*, 1991; Cardwell and Lane, 1995). Five to seven races of *Striga* have been identified in Africa (Lane *et al.*, 1996; Botanga and Timko, 2006).

The variability in *Striga* virulence due to different races renders the development of resistant varieties very difficult (Tignegre, 2010). However, a number of control methods that can lessen *Striga* damage are available. These methods include cultural practices, chemical and biological methods. But the most feasible and affordable alternative control method for small-scale farmers seems to be the host plant resistance. Many sources of

resistance to the various *Striga* strains were identified in Africa (Singh and Emechebe, 1990; Atokple *et al.*, 1995a; Atokple *et al.*, 1993).

In Niger, selected local cultivars with good levels of resistance to the race 3 including TN121-80, TN93-80, HTR and introduced resistant varieties such as B301 (Botswana landrace), IT97K-499-38, IT97K-499-35, IT82D-849 are being tested. These resistant cultivars often lack farmers' preferred traits and are not adapted to some local conditions (Moutari, personal communication). This requires breeding cowpea for specific areas and environments. To achieve that goal, it is necessary to find new sources of resistance in the local germplasm or to incorporate resistance genes into farmer-preferred varieties. The national cowpea gene bank has not been screened for *Striga* resistance and no diversity studies have been conducted on this collection so far in Niger. Research work has been mainly focused on testing some improved local materials and introduced varieties in collaboration with international research institutes such as International Institute of Tropical Agriculture (IITA) and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Understanding the diversity among accessions contributes to successful conservation of germplasm (Karuri *et al.*, 2010). A wide genetic base in a germplasm collection provides breeders with important sources to address climatic and environmental challenges, thus preserving food security. In this study, 80 genotypes mostly from the national cowpea gene bank and some introductions were evaluated in order to identify new sources of *Striga gesnerioides* resistance and suitable parents for cowpea improvement.

4.2 Materials and methods

4.2.1 Germplasm

The germplasm used comprised of 80 genotypes that included 68 landraces and 12 improved genotypes from IITA, Burkina Faso and INRAN (Table 4.1). The varieties IT93K-693-2, IT99K-573-1-1 and IT98K-205-8 were used as resistant checks, while the varieties TN88-63, TN27-80 and IT90K-372-1-2 were included as susceptible checks.

4.2.2 Cultural practices and field evaluations

The trial was conducted under natural infestation in fields that are severely infested with *S. gesnerioides*. The experiment was carried out under rainfed conditions in 2012 at Maradi station (13° 28'N latitude and 7° 10'E longitude) in the Sudano Sahelian zone.

The experimental set up was a 4x20 α lattice design with three replications. Each plot comprised two rows of 3 m long with inter-row spacing of 0.80 m, and intra-row spacing of 0.50 m. Three seeds were planted per hill and were thinned to one per hill two weeks after seedling emergence, resulting in a total of 14 plants per plot. Super single phosphate (SSP) fertilizer was applied at a rate of 100 kg per hectare one week before sowing cowpea. Two hoe-weedings were done before *Striga* emergence. The pesticide applied was dimethoate (C₅H₁₂NO₃PS₂) at pre-flowering, flowering and after pods formation to control insects at a rate of 1 l/ha.

4.2.3 Data collection and analysis

Data was collected on the following ten quantitative parameters:

- Number of days from planting to flowering (**DFL**)

- Number of days from planting to 50% flowering (**50% FL**)
- *Striga* shoots per plot (**SSP**). *Striga* shoots were counted for each plot nine weeks after planting (WAP).
- *Striga* density (**DS**). This is expressed as the number of emerged *Striga* shoots per plot over the surface area of the plot (SSP/5.4 m²)
- *Striga* dry biomass (**SDB**) measured by weighing all the dried *Striga* shoots from each plot.
- The pod weight (**PW**) measured by weighing the harvested pods at maturity followed by a drying process using an oven at 100°C for 8 hours.
- Grain yield (**GY**) was estimated to Kg ha⁻¹ from the weight of the seeds obtained per plot.
- One hundred seed weight (**100-SW**) was obtained as dry weight of 100 seeds taken randomly from each plot.
- Fodder yield (**FY**) was obtained by drying and weighing stems and leaves left after pods were harvested in each plot.

The genotypes were classified as resistant or susceptible using the scale described by Singh and Emechebe (1997) as follows:

- 1. Resistant = no *Striga* emergence in a plot and no *Striga* symptoms observed on cowpea plants.
- 2. Susceptible = 5 to several *Striga* plants emerged per plot and plants show severe *Striga* symptoms.
- 3. Moderately resistant = few *Striga* emerged (2-3) per plot and no *Striga* symptoms observed.

- 4. Tolerant = Several *Striga* emergence but no significant yield reduction.

All the data obtained from the trial were subjected to analysis of variance (ANOVA) using PROC GLM in SAS 9.3. The linear model of observations in alpha lattice design used is as follows:

$$y_{ijk} = \mu + \tau_i + \rho_j + \beta_{jk} + e_{ijk}$$

Where: y_{ijk} is the value of the observed trait for i^{th} treatment received in the k^{th} block within j^{th} replicate (superblock),

τ_i is the fixed effect of the i^{th} treatment ($i = 1, 2 \dots t$);

ρ_j is the effect of the j^{th} replicate (superblock) ($j = 1, 2 \dots r$);

β_{jk} is the effect of the k^{th} incomplete block within the j^{th} replicate ($k = 1, 2 \dots s$)

e_{ijk} is an experimental error associated with the observation of the i^{th} treatment in the k^{th} incomplete block within the j^{th} complete replicate.

Pearson correlation coefficients were used to test correlations among the following measured traits 50%FL, DS, SDB, GY, 100-SW and PW using SAS (SAS 9.3).

Principal component analysis (PCA) and cluster analysis using the same software were also performed.

Table 4. 1: Origin and seed color of the 80 cowpea germplasm screened for resistance to *Striga gesnerioides*.

N ^o	Name	Origin	Color	N ^o	Name	Origin	Color
1	TN 121-80	Niger	Wh	26	B6/15/2367-58	Niger	Mo
2	IT99K-573-1-1	IITA	Wh	27	B5/11/2492	Niger	Wh
3	TN5-78	Niger	Br	28	2450-132 (sac)	Niger	Wh
4	TN88-63	Niger	Wh	29	B5/13/2374-156	Niger	Wh
5	KVx30-309-6G	B. Faso	Wh	30	B3/9/2526-200	Niger	Br
6	Suvita-2	B. Faso	Br	31	2409 (Etq)	Niger	Wh
7	IT93K-693-2	IITA	Br	32	B1/16/2470-152	Niger	Br
8	HTR	Niger	Wh	33	IT90K-372-1-2	IITA	Wh
9	KVx771-10G	B. Faso	Wh	34	TN27-80	Niger	Wh
10	2354 (Etq)	Niger	Wh	35	IT98K-205-8	Niger	Wh
11	B3/13/2399-81 A (1)	Niger	Wh	36	2472-154 (Sac)	Niger	Wh
12	B5/15/2627 (2eR)	Niger	Wh	37	B1/1/2409-91 (1)	Niger	Wh
13	2505 (sac)	Niger	Wh	38	B1/6/2356-38	Niger	Wh
14	2326 (sac)	Niger	Wh	39	2432 (Etq)	Niger	Mo
15	2462-144 (sac)	Niger	Wh	40	B4/1/2381-63 (2eR)	Niger	Wh
16	B4/9/2610 (2eR)	Niger	Wh	41	B3/18/2381-63	Niger	Wh
17	B3/17/2458-140 (2)	Niger	Br	42	B2/16/2378 (1ereR)	Niger	Wh
18	2458-140 (sac)	Niger	Br	43	2491-171 (Etq)	Niger	Br
19	B6/3/392-74	Niger	Wh	44	2510-192 (Etq)	Niger	Wh
20	2367-58 (sac)	Niger	Wh	45	2649-151 (Etq)	Niger	Wh

Wh: white; Br: brown; Mo: mottle; B. Faso: Burkina Faso

Table 4.1 continued

N ^o	Name	Origin	Color	N ^o	Name	Origin	Color
21	B4/2/2491-171	Niger	Br	46	B3/20/2323 2R	Niger	Wh
22	2374-56 (sac)	Niger	Wh	47	B1/12/2525-234	Niger	Wh
23	2429-111 (sac)	Niger	Wh	48	2372-54 (sac)	Niger	Wh
24	2598 (Etq)	Niger	Wh	49	B5/19/2410-92	Niger	Wh
25	B1/9/2320-02	Niger	Wh	50	B1/5/2354 2R	Niger	Wh
51	2383 (Etq)	Niger	Wh	66	B1/4/2413-95	Niger	Wh
52	B6/14/2472-154	Niger	Wh	67	B2/12/2472-150	Niger	Mo
53	2610 (Etq)	Niger	Wh	68	2432-144 (Etq)	Niger	Wh
54	B4/8/2436-118	Niger	Wh	69	B3/4/2507 (2eR)	Niger	Wh
55	B4/7/2338-20 (2eR)	Niger	Wh	70	B6/2/2516	Niger	Wh
56	2504-186 (Etq)	Niger	Wh	71	2491-191 (Sac)	Niger	Br
57	2420-102 (sac)	Niger	Wh	72	B1/18/2542 (2e R)	Niger	Wh
58	2431-113 (Etq)	Niger	Wh	73	2400-82 2R	Niger	Wh
59	2390-72 (Sac)	Niger	Wh	74	2326 (Etq)	Niger	Wh
60	2392-74 (Etq)	Niger	Wh	75	B3/3/2350-32 (1)	Niger	Wh
61	B1/13/2614-296 (2e R)	Niger	Wh	76	B4/13/2563-245 1R	Niger	Wh
62	B5/12/2462-144 (2eR)	Niger	Wh	77	B2/19/2405-87 (2e R)	Niger	Wh
63	B3/18/2525-30	Niger	Wh	78	B1/14/2473-155	Niger	Wh
64	B4/14/2343-25 (1)	Niger	Wh	79	2477-152 (Etq)	Niger	Br
65	B2/10/2457-119	Niger	Wh	80	2427 (Etq)	Niger	Br

Wh: white; Br: brown; Mo: mottled

4.3 Results

There were significant differences ($p < 0.0001$) among the genotypes for all the traits measured (Table 4.2).

Table 4. 2: Mean squares of the traits

Sources	DF	50%	DS	SDB	GY	PW	100-SW
Blocks (Rep)	9	13.67	9.68	86.82	22893.85	13072.69	0.78
Treatments	79	62.42***	13.01***	332.44***	59224.66***	33497.39***	16.90***
Error	149	10.90	5.37	145.95	14718.74	10242.81	0.79
Total	239						

(***): Significant at 0.0001 probability level

The means of flowering dates, *Striga* density (DS), *Striga* dry biomass (SDB), grain yield (GY), 100-seed weight (100-SW) and pod weight (PW) for 27 genotypes out of 80 are provided in Table 4.3.

The number of days to 50% flowering varied from 49 to 73 days with a mean of 59 days. The number of days to 50% flowering was highest in B5/15/2627 2R (73 days) and was lowest in IT98K-205-8 (49 days).

The three resistant checks (IT93K-693-2, IT99K-573-1-1 and IT98K-205-8) had no emerged *Striga* shoots m^{-2} . The *Striga* shoots m^{-2} was low in lines 2491-171, 2472-154 and Suvita-2 with values of 0.43, 0.74 and 0.87 respectively and these values were significantly different from that of the susceptible check IT90K-372-1-2 (7.84 *Striga* shoots m^{-2}). Varieties 2491-171, 2472-154 and Suvita-2 were identified as moderately resistant to *Striga*. Eight lines (B2/16/2378, B1/13/2614-296, B1/4/2413-95, B2/19/2405-

87, B1/18/2542, B4/7/2338-20, B1/12/2525-234, and B3/13/2399-81A) described as tolerant had high DS (2.53 to 8.77 *Striga* shoots m⁻²) but no significant yield loss was observed. Ten susceptible lines (B4/13/2563-245 1R, IT90K-372-1-2, B5/15/2627 2R, B1/14/2473-155, B6/2/2516, B2/10/2457-119, B3/3/2350-32, B3/18/2381-63, 2505 and 2510-192) had low to high number of emerged parasite shoots m⁻² (3.52 to 9.13).

Cowpea grain yield varied from 54.88 kg ha⁻¹ for line 2510-192 to 691.67 kg ha⁻¹ for line B1/18/2542 with an average yield of 246.12 kg ha⁻¹. Only five genotypes (6%) had yields exceeding 500 kg ha⁻¹ while 35 (44%) had yield less than 200 kg ha⁻¹. Yield reductions were significantly high (79% and 66%) in the susceptible cowpea lines as compared to the tolerant and resistant lines. In contrast, it was observed that the yield of tolerant cultivars (441.17 Kg ha⁻¹ to 691.67 Kg ha⁻¹) was higher than those of the resistant lines (276.63 Kg ha⁻¹ to 380 Kg ha⁻¹). *Striga* dry biomass (SDB) varied from 0.00 (IT93K-693-2, IT99K-573-1-1 and IT98K-205-8) to 52.88 g (B6/2/2516). The overall genotype mean for SDB was 17.91 g per plot.

One hundred seed weight (100-SW) varied from 7.3 g (2512-192) to 22.25 g (B4/13/2563-245 1R) with a mean of 14.91 g.

Cowpea pod weight ranged from 50.93 g (2429-111) to 548.07 g (B1/18/2542 2R) with an average of 195.52 g per plot.

Table 4. 3: Fifty percent flowering, *Striga* density, *Striga* dry biomass, yield and yield components of cowpea germplasm screened at Maradi (rainy season 2012).

Genotypes	50%FL (days)	DS (shoot m ⁻²)	SDB (g)	GY (Kg/ha)	100-SW (g)	PW (g)
Resistant checks						
IT93K-693-2	54.00	0.00	0.00	380.00	15.55	268.40
IT99K-573-1-1	50.33	0.00	0.00	340.74	17.70	170.27
IT98K-205-8	49.00	0.00	0.00	276.63	15.10	248.20
Moderately resistant						
2491-171	58.67	0.43	4.00	258.15	16.80	182.67
2472-154	57.00	0.74	4.45	141.73	16.35	158.67
Suvita-2	63.67	0.87	6.70	244.75	16.75	178.17
Tolerant to <i>Striga</i>						
B2/16/2378	61.33	8.77	49.73	502.78	13.80	393.10
B1/13/2614-296	60.00	5.37	23.30	477.16	15.55	263.27
B1/4/2413-95	59.00	5.06	21.60	441.17	14.75	347.73
B2/19/2405-87	58.00	4.5	28.53	476.79	15.45	333.07
B1/18/2542 (2eR)	62.67	3.52	16.70	691.67	17.65	548.07
B4/7/2338-20 (2eR)	61.67	3.46	20.40	561.79	14.50	462.43
B1/12/2525-234	60.00	2.84	28.60	671.05	15.10	521.47
B3/13/2399-81 A (1)	63.00	2.53	11.47	573.21	17.10	458.97
Susceptible checks						
IT90K-372-1-2	57.33	7.84	25.83	150.37	15.55	111.70
TN88-63	61.00	3.89	18.83	230.00	12.75	131.03
TN27-80	59.00	1.67	19.87	170.19	15.75	170.60
Ten most susceptible						
B4/1/2381-63(2eR)	59.66	9.13	52.96	287.16	15.15	177.97
B4/13/2563-245 1R	65.33	8.27	34.70	98.27	22.25	95.47
B5/15/2627 2R	73.00	7.84	42.50	105.62	17.85	91.17
B1/14/2473-155	61.00	7.53	34.13	194.26	14.40	158.17
B6/2/2516	71.33	6.42	31.00	80.93	17.60	68.70
B2/10/2457-119	56.00	6.17	29.56	190.43	11.95	169.77
B3/3/2350-32	67.67	6.05	29.167	163.4	14.1	151.8
B3/18/2381-63	61.33	5.43	30.16	82.90	14.95	79.43
2505	63.33	5.37	20.73	72.41	11.5	56.90
2510-192	58.67	3.52	19.1	54.88	7.3	62.67
Grand Mean	59.4	3.37	17.91	246.12	14.91	195.52
Total LSD (p≤ 0.05)	5.34	3.75	19.39	193.68	1.44	156.65
Total CV %	5.58	69.04	67.10	50.06	6.01	51.46

CV: Coefficient of variation; LSD: Least Significant Difference at p = 0.05;

50% FL: days to 50% flowering; **DS:** *Striga* density; **PW:** pod weight; **SDB:** *Striga* dry biomass; **GY:** cowpea grain yield; **100-SW:** one hundred seeds weight.

(Break Table 4.3 into two in order not to get into the margin space: Table 4.3 and Table 4.4)

4.3.1 Correlation studies

Table 4.4 shows the correlation coefficients of *Striga* density and dry biomass on yield and yield components. In this study *Striga* density (DS) and SDB were not correlated ($P > 0.05$) to yield and yield components. However, *Striga* density was positively significantly ($P < 0.05$) correlated ($r = 0.78$) to SDB. Also, yield was positively significantly ($p < 0.05$) correlated to PW ($r = 0.91$) (Table 4.4).

Table 4. 4: Correlation coefficients of *Striga* density and dry biomass on yield and yield components

	50%FL	DS	PW	SDB	GY	100-SW
50%FL						
DS	0.17 NS					
PW	-0.05 NS	-0.01 NS				
SDB	0.18 NS	0.78*	0.01 NS			
GY	-0.08 NS	-0.03 NS	0.91*	-0.01 NS		
100-SW	0.03 NS	0.006 NS	0.19 NS	-0.04 NS	0.23 NS	

50%FL: days to 50% flowering; DS: *Striga* density; PW: pod weight; SDB: *Striga* dry biomass; GY: cowpea grain yield; 100-SW: one hundred seeds weight; (*) the correlation coefficients were significant at $p = 0.05$; NS: not significant.

4.3.2 Principal component analysis

The first two principal components (PCs) or latent correlation matrix with coefficient values (Eigenvalues) greater than 1.0 are given in Table 4.5, together with the percentage of total variability accounted for by each component, and the cumulative percentages.

The first two components accounted for 69.68% of the total variance in the present data set (Table 4.5). The first PC accounted for 35.48 % whereas the second accounted for 34.19%.

Table 4. 5: Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
PC1	2.12896842	0.07734040	0.3548	0.3548
PC2	2.05162802	1.14255976	0.3419	0.6968
PC3	0.90906826	0.15078337	0.1515	0.8483
PC4	0.75828490	0.64364438	0.1264	0.9747
PC5	0.11464052	0.07723063	0.0191	0.9938
PC6	0.03740988		0.0062	1.0000

The identification of the components may be achieved by examination of the latent vectors (eigenvectors) for these principal components but with emphasis on the first two principal components. The first PC with reference to its high value (Table 4.6) was positively associated with pod weight (PW) and grain yield. The second PC was associated with traits such as *Striga* dry biomass (SDB), *Striga* density (DS) and grain yield (GY).

Table 4. 6: Eigenvectors from the two principal component axes used to classify cowpea accessions

	Prin1	Prin2
50% days to flowering	-.247130	0.310818
Pod weight	0.531040	0.396442
<i>Striga</i> dry biomass	-.380179	0.524134
100 seed weight	0.268934	0.198453
<i>Striga</i> density	-.398485	0.514312
Grain yield	0.530350	0.409400

4.3.3 Cluster analysis

The agglomerative hierarchical clustering dendrogram illustrated the relationship among the 80 accessions (Figure 4.1) based on the traits that contributed most to the first two (2) principal components (Table 4.6). Three main clusters were identified. The cluster A had 4 accessions while cluster B had 20 and cluster C had 56 accessions. Cluster A included high yielding and tolerant varieties to *Striga*. The cluster B included intermediate yielding and resistant varieties to *Striga* while cluster C had the low-yielding and *Striga* susceptible lines.

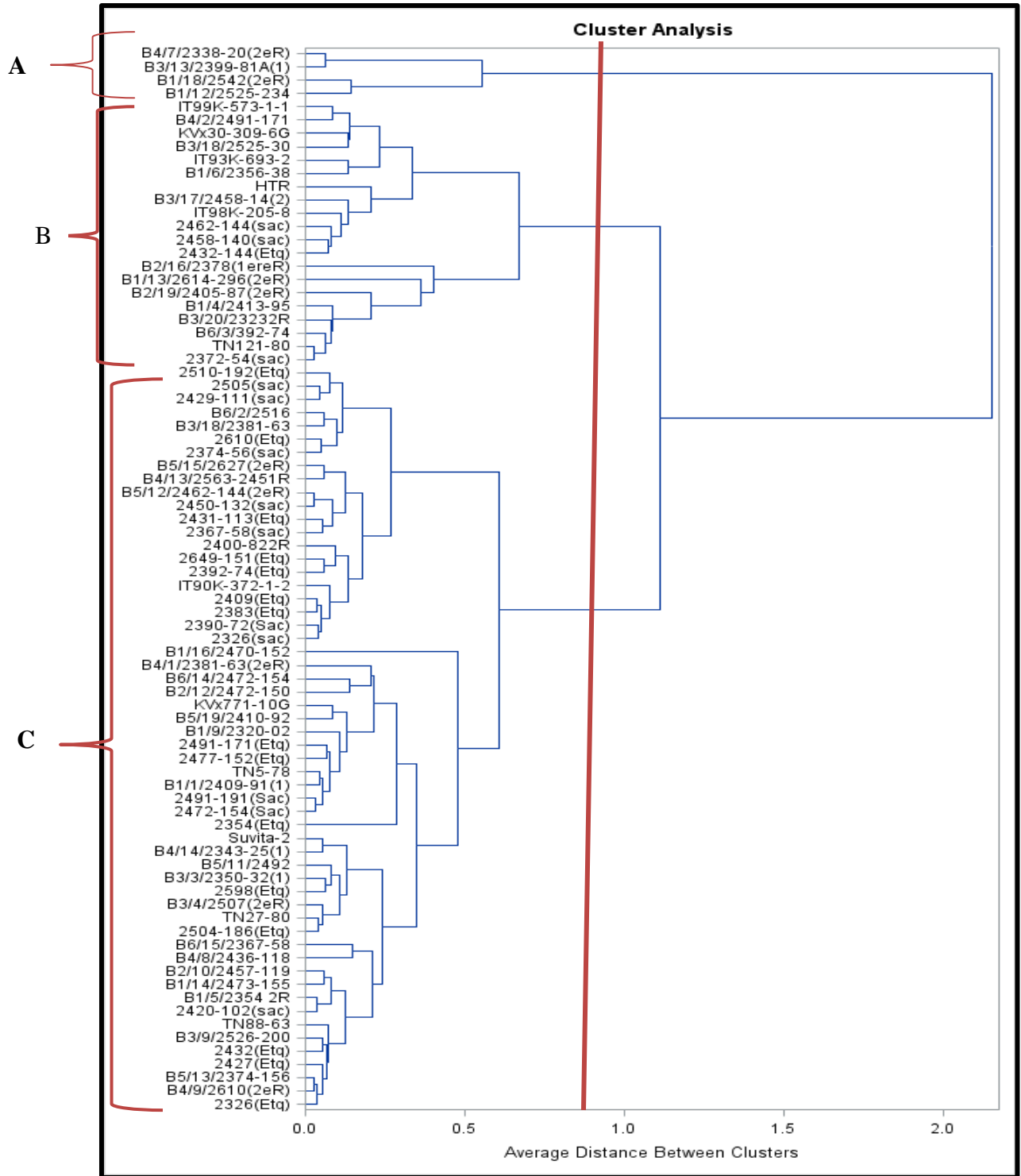


Figure 4. 1: Dendrogram constructed based on yield and *Striga* resistance parameters of cowpea

4.4 Discussion

There was variability in the number of days to 50% flowering among the genotypes. This is in line with the findings of Egbe *et al.* (2010) and Ekpo *et al.* (2012). This variability may be due to climatic conditions or the genetic background of the varieties. In this study the number of days to 50% flowering ranged from 49 to 73 days after planting with a mean of 59.4 while it varied from 32.72 to 54.84 days after planting (DAP) with a mean of 44.31 DAP in the study by Egbe *et al.* (2010). This showed that there were no extra early maturing varieties in the germplasm screened. Early maturity is one of the major criteria for choosing cowpea varieties highlighted by farmers during the PRA. Therefore, it is an important breeding goal in the country. Developing early maturing cowpea varieties in Niger requires introduction of exotic material or the identification of the character in the germplasm that has not been screened.

Only three genotypes (IT93K-693-2, IT99K-573-1-1 and IT98K-205-8) included as resistant checks were free of *Striga* emergence in this study. The resistance in IT93K-693-2 confirmed the results of Singh (2002) and Boukar *et al.* (2004) who reported that this genotype was resistant to all the five known *Striga* races in Africa. Recently, Tignegre (2010) found similar results with the races prevailing in Burkina Faso. The resistance observed in IT99K-573-1-1 and IT98K-205-8 is in line with the findings of Tchiagam *et al.* (2010) who observed no *Striga* emergence from the two genotypes screened for resistance to race 5 in Cameroon. Though, these genotypes were highly resistant to *Striga* in this study, their grain yields were very low as compared to their yields obtained from studies in Cameroon and in Burkina Faso. IT99K-573-1-1 and IT98K-205-8 yielded 1042.75 kg ha⁻¹ and 871.10 kg ha⁻¹ respectively in Cameroon

versus 340.74 kg ha⁻¹ and 276.63 kg ha⁻¹ in Niger, respectively. The line IT93K-693-2 yielded 911.60 kg ha⁻¹ in Burkina Faso versus only 380 kg ha⁻¹ in this study. These differences observed may be attributed to genotypes response to climatic conditions and soil types. The genotypes are well adapted to the conditions in Cameroon. In Cameroon, the study was conducted in the Sudano-Sahelian belt with ferruginous vertisols and an average annual rainfall ranging from 800-900 mm. The soil is sandy clay with 8.2 mg kg⁻¹ of organic matter content and pH 5.65 (Guissai, 2010). In Burkina Faso, the experiment was conducted in a station where the average annual rainfall was 1131 mm, while, it was about 480 mm at Maradi and the soil is ferruginous tropical with a pH of 6.5 and contains 12% clay, 5% loam, 4% coarse silt, 77% sand and 2% organic matter (Raynaut *et al.*, 1984). This probably could explain the differences in yield observed in the different experiments with the same varieties.

However, these resistant lines, although they showed no emerged *Striga* shoots, had lower yields as compared to the tolerant lines. This suggests that these lines have low yield potential but can be exploited in breeding cowpea as donor parents for resistance to *Striga*. In contrast, some genotypes such as B2/16/2378, B1/18/2542 and B1/12/2525-234 supported high numbers of *Striga* shoots but performed well for yield indicating that they are tolerant to *Striga*. These lines are potential sources for breeding for yield improvement in cowpea. In this study the effect of *Striga* attack was more pronounced on the susceptible lines than the moderately resistant or the tolerant lines. Out of the susceptible lines, six genotypes gave significantly lower yields as compared to the weakest susceptible control (IT90K-372-1-2) suggesting that they were highly susceptible to *Striga*. The high number of genotypes for which the yield was less than

200 kg ha⁻¹ indicates the inherent low yield of landraces in Niger coupled with poor conditions of germplasm conservation. In this study about 120 accessions were supposed to be screened but 40 were discarded because of low germination rates due to the poor conservation conditions.

There were no significant correlations between yield components and *Striga* emergence parameters. This result is not consistent with the findings of Kamara *et al.* (2008); Tignegre (2010); Omoigui *et al.* (2012) and Ekeleme *et al.* (2013) who reported significant negative correlations between these characters. This may be because the number of *Striga* shoots in a plot does not always indicate the incidence on the individual plants which depends on the number of cowpea plants attacked. This corroborates the observation that susceptible genotypes with the smallest *Striga* population recorded some of the lowest yields and tolerant genotypes with highest *Striga* population gave some of the highest yields in this study. Also, Ekeleme *et al.* (2013), in a biophysical *Striga* survey found that the number of *Striga* shoots was negatively correlated with available Fe and Cu, clay, organic C and total N in the soil. The correlation between yield and pod weight was reported by Ajibade and Morakinyo (1999) and Attah (2002) who showed that yield of cowpea was largely a function of pod length, number of pods per plant and number of seeds per pod.

Information from principal component analyses may guide plant breeders in making selected crosses in a selection programme. The results in this study reveal that out of six parameters used, only four contributed significantly to the variability observed among the genotypes. These variables were highly positively correlated in pairs, *Striga* density with *Striga* dry biomass and yield with pod weight.

This is not consistent with the results obtained by Manggoel and Uguru (2011), and Sulnathi *et al.* (2007). These authors found that days to 50% flowering, days to 50% maturity and 100 seed weight contributed to the differences observed among the genotypes. They concluded that these three characters should be considered while selecting parents for hybridization programme in yield improvement of cowpea. But, in this study, the parameters days to 50% flowering and 100-SW were not correlated to yield. This may be attributed to the differences of the conditions under which the experiments were conducted. This study was conducted under *Striga* infestation while the former was carried out under free infestation conditions.

Cluster analysis grouped the genotypes into three main clusters A, B and C. The cluster A was composed of four accessions: B4/7/2338-20 (2eR), B3/13/2399-81 A (1), B1/18/2542 (2eR) and B1/12/2525-234 that were the top yielding and tolerant to *Striga*. These accessions had yields exceeding 550 kg/ha. This cluster can be further divided into two sub-clusters of 2 accessions each according to the yield importance. The sub-cluster A1 includes the genotypes B1/18/2542 (2eR) and B1/12/2525-234 with a yield exceeding 670 kg/ha. These can be used as donor parents in breeding for yield improvement in environments where *Striga* infestation is not a constraint to cowpea production because it has been demonstrated that the use of tolerant genotypes increase *Striga* seed bank in the soil. The cluster B has genotypes that are moderate yielding and the resistant to *Striga*. This cluster too can be divided into two sub-clusters. Five genotypes classified as tolerant with high yield and some *Striga* susceptible varieties were grouped in this cluster meaning that yield parameter contributed greatly in discriminating the genotypes. This was revealed by its high contribution to the first two principal components. The cluster C

was composed of the low-yielding and most of the susceptible genotypes. Also the cluster C can be partitioned into three sub-clusters according to the importance of yield. The moderately resistant genotypes, 2491-171, 2472-154 and Suvita-2 are grouped in this cluster because of their low yield. This classification implies that genotypic variability exists among the accessions. Therefore yield improvement and *Striga* resistance can be achieved with this germplasm using recurrent selection or gene pyramiding.

4.5 Conclusions

In conclusion, new sources of *Striga* resistance were not found in this screening. However, the genotypes IT93K-693-2, IT99K-573-1-1 and IT98K-205-8 were confirmed as potential donor sources for incorporating resistance genes into adapted and farmer-preferred varieties. The cultivars B2/16/2378, B1/18/2542 and B1/12/2525-234 were identified as donor parents in breeding cowpea for yield improvement in Niger. The best combination of crosses to incorporate *Striga* resistance into adapted lines would be IT93K-693-2 as donor parent and the top three farmers' preferred varieties identified in PRA (KVx30-309-6G, IT90K-372-1-2 and TN5-78) as recurrent parents. The clustering of the genotypes was predominantly based on their response to *Striga* and grain yield.

CHAPTER FIVE

5.0 Differential responses of 15 cowpea genotypes to three *Striga* hot spots in Niger

5.1 Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most important grain legumes, fodder and cover crops in the sub-Saharan African countries. Its grain contains between 20-25% of protein, about twice the protein content of most cereals (Kay, 1979) and complements nutritionally low-protein staple cereals and tuber crops. In addition to this nutritional value, cowpea is a cash crop that generates income to farmers. Also, the crop provides good fertility restoration for cropping systems in rotation with cereals. However, despite the high adaptability of cowpea to drought-prone areas, its productivity is challenged by several biotic and abiotic stresses. *Striga gesnerioides* is a serious constraint to cowpea production in Sub-Saharan Africa. Yield losses ranging from 83-100% due to the infestation of this parasitic plant were recorded on susceptible cowpea varieties (Emechebe *et al.*, 1991; Cardwell and Lane, 1995). *Striga* control is very difficult because of the long viability of its seed stock in the soil. Several control strategies including cultural practices, chemical and biological methods and host-plant resistance have been proposed (Dube and Olivier, 2001; Boukar *et al.*, 2004) but no single method on its own is effective in field. The most feasible and affordable control method for small scale farmers is the use of resistant varieties coupled with adequate cultural practices. At least six different *Striga* races have been identified in West Africa (Lane *et al.*, 1996; Botanga and Timko, 2006) based on differential test. The different *Striga* races were categorized as follows: SG1 (Burkina Faso), SG2 (Mali), SG3 (Nigeria

and Niger), SG4 and SG4z (Benin), SG5 (Cameroon) (Lane *et al.*, 1996). Botanga and Timko (2006) identified another race, SG6 in Senegal. Resistant cowpea varieties with specific responses to various *Striga* strains were also identified (Lane *et al.*, 1996). B301, a landrace originating from Botswana and IT81D-849 were found to be resistant to *Striga* races SG1, SG2, SG3 and SG5 (Singh and Emechebe, 1990; Berner *et al.* 1995; Lane *et al.*, 1996). The variety Gorom local showed resistance to races SG1, SG2 and SG4 (Parker and Polniaszek, 1990; Berner *et al.*, 1995), while the cultivar 58-57 was resistant to races SG1, SG4 and SG5 (Parker and Polniaszek, 1990; Lane *et al.*, 1996). Several resistance mechanisms are involved in cowpea resistance to *Striga* (Parker and Polniaszek, 1990; Lane *et al.*, 1996; Reiss and Bailey, 1998; Touré *et al.*, 1997). According to Botanga and Timko (2005), the race differentiation in *Striga gesnerioides* was largely due to host-driven selection. In Niger, the resistance to the race SG3 was reported to be conferred by a single dominant gene in B301 (Touré *et al.*, 1997) and by a recessive gene in IT82D-489 (Atokple *et al.*, 1995). On the other hand, in some hot-spots of the country, previously reported resistant cultivars, HTR, for instance showed symptoms of *Striga* infestation; suggesting either the existence of more than one race of *Striga* in Niger or the breakdown of this resistance (Li *et al.*, 2009). The lack of sufficient information on the different *Striga* races prevailing in Niger and the ability of this parasitic weed to spread need to be addressed by conducting a differential variety test in the hot spots of the country.

Field screening with *Striga* inoculum have been effective in assessing the response of genotypes to *Striga gesnerioides*. However, field screening with artificial *Striga* infestation may not always be feasible because of parasite dissemination risks; and it may

not always be reliable because weed pressure and distribution cannot be controlled by the researcher (Hausmann *et al.*, 2000). Pots screening is considered as an alternative method that ensures more uniform infestation by *Striga* inoculum. Thus, combining hot-spots and pots screening could help in identifying differential responses of cowpea genotypes to *Striga*.

The objective of the present research was to determine if other *Striga* races aside from SG3 are present in Niger using differential variety test.

5.2 Materials and methods

5.2.1 Location

The experiments were carried out in the field (natural infestation) at three locations: Kollo (13°20' N, 2°18' E); Maradi (13° 28'N, 7° 10'E) and Magaria (12°59'N, 8°54'E) in 2013 and 2014 in Niger. Kollo is 30 Km South-East of Niamey and received 573 mm in 2013 and 595.9 mm in 2014. Maradi is 700 Km East of Niamey and received 591.7 mm in 2013 and 435.1 mm in 2014. Magaria is 990 Km East of Niamey and received 509.6 mm in 2013 and 491.3 mm in 2014 (Figure 5.1). The three sites chosen are the hot spots of *Striga gesnerioides* in the country. The pots experiment (artificial infestation) was conducted at Maradi station during the same periods.

5.2.2 Field and pot evaluations of cowpea germplasm under *Striga* infestation

Fifteen genotypes were evaluated for both field and pot experiments. The genotypes used in the test included ten race specific resistant varieties (B301, IT97K-449-35, Suvita 2, IT93K-693-2, 58-57, HTR, KVx771-10G, IT99K-573-1-1, IT98K-205-8 and TN121-80), three susceptible farmers' preferred varieties (IT90K-372-1-2, KVx30-309-6G and TN5-78) and two other susceptible genotypes (Dan Illa and IN92E-26) to SG3 (Table 5.1). The genotype IT93K-693-2 is resistant to all the races while B301 is resistant to all the races except SG4z. The experimental set up was a randomized complete block design with three replications. The plots were composed of two rows of 3 m long each with a spacing of 0.80 m between rows, and 0.50 m within the rows. Three seeds were planted per hill and were thinned to one per hill, resulting in a total of 14 plants per plot. One hundred kg ha⁻¹ of Single Super Phosphate fertilizer was applied before planting cowpea. Two hoe-weedings were done before *Striga* emergence. The pesticide applied was dimethoate (C₅H₁₂NO₃PS₂) at pre-flowering, flowering and after pod formation to control insect pests at a rate of 1 l ha⁻¹.

For the pot experiments, the same genotypes were screened using a completely randomized design replicated three times. The pots were the experimental units consisting of a single cowpea genotype. Each pot had a volume of seven liters filled with 5 kg of a mixture of sandy soil, clay and farmyard manure at a ratio of 2:1:1, respectively. The mixture had been previously sterilized. After soil infestation with about 1000 seeds per pot of one year-old *Striga gesnerioides*, the pots were watered for two weeks to precondition *Striga* seeds in order to break their dormancy and ensure optimum germination. *Striga* seeds used in this experiment were collected from the three locations

(Kollo, Maradi and Magaria) where field experiments were established. Three seeds of cowpea were sown per pot in three replications. The seedlings were thinned to one per pot at 2 weeks after germination. The pots were watered every two days or when necessary in order to keep them moist. Three sets of the trials were carried out; each one infested by one of the three *Striga* samples.

Table 5. 1: Cowpea germplasm screened at Kollo, Maradi and Magaria in 2013 and 2014 with reported differential responses to *Striga* races.

N ⁰	Cowpea cultivar	Race of <i>Striga gesnerioides</i> , country of origin						
		SG1 B. Faso	SG2 Mali	SG3 Niger	SG4 Benin	SG4z Zakpota	SG5 Cameroon	SG6 Senegal
1	B301	R	R	R	R	S	R	R
2	IT97K-449-35	R	R	R	S	S	S	S
3	Suvita 2	R	R	S	R	R	S	R
4	IT93K-693-2	R	R	R	R	R	R	R
5	58-57	R	S	S	R	R	R	S
6	HTR	R	S	S	S	S	S	S
7	KVx771-10 G	R	S	S	S	S	R	S
8	Dan Illa	S	S	S	S	S	S	S
9	TN5-78	S	S	S	S	S	S	S
10	KVx30-309-6G	S	S	S	S	S	S	S
11	IT90K-372-1-2	S	S	S	S	S	S	S
12	IT99K-573-1-1	S	S	R	S	S	R	S
13	IT98K-205-8	R	S	R	S	S	R	S
14	TN121-80	S	S	R	S	S	S	S
15	IN92E-26	S	S	S	S	S	S	S

Source: (Lane *et al.*, 1996; Li *et al.*, 2009; Omoigui *et al.*, 2012), B. Faso: Burkina Faso

5.2.3 Data collection and analysis

In this test, the term resistance to *Striga* means that the cowpea lines did not support any emerged *Striga*, although the cowpea lines may stimulate germination and permit attachment of the parasite's radicles to their roots, the development of the haustorium is

inhibited and there will be no visible *Striga* shoots. To ease the comparison between genotypes with differential responses a two class scale: absence (R – an indication of resistance), presence (S – an indication of susceptibility) of *Striga* plants was used to assess the responses of cowpea cultivars. In pots, destructive sampling was carried out 9 weeks after planting. The plant-soil mass from each pot was removed and immersed into a bucket of water. After separation of the soil mass, the roots were thoroughly washed and examined for presence of necrotic hypersensitive lesions and *Striga* attachments. The plants that allowed attachment, normal development and emergence of *Striga gesnerioides* were classified as susceptible and those free of infection were considered as resistant. The numbers of emerged *Striga* shoots were recorded for each cowpea genotype from the field and pots. The data from field and pots experiments were subjected to analysis of variance using SAS GLM. The differences between mean values were assessed using the least significant difference (LSD) at 5% level of significance.

5.3 Results

5.3.1 Field experiment

The analysis of variance for location, year and varietal effect were significant for emerged *Striga* shoots (Table 5.2). The number of emerged *Striga* shoots and details of cowpea responses as influenced by location and year are presented in Table 5.3. The differential responses of the genotypes in the three locations are presented in Table 5. 4. Only the genotype IT93K-693-2 was completely free of emerged *Striga* shoots in all locations and years thus, it was completely resistant across the three locations. The

varieties IT99K-573-1-1, IT97K-499-35, B301, IT98K-205-8 and TN121-80 are the resistant checks to the race SG3 present in Niger that supported *Striga* shoots in at least two sites. B301 was infested in all sites in 2013 (Figure 5.1) and in 2 sites in 2014 (Table 5.3). All the susceptible checks to the race SG3 supported *Striga* shoots in the three sites, except HTR that was free of *Striga* emerged shoots at Kollo in 2014. The rainfall pattern of the period of the experiments is shown in Figure 5.2.

Table 5. 2: Mean squares of *Striga* emerged shoots/plot of 15 cowpea varieties screened at Kollo, Maradi and Magaria in 2013 and 2014.

Source	DF	Mean Square	F Value	Pr > F
Year	1	21422.31481	136.50	<.0001
Location/year	6	5648.34938	107.97	<.0001
Rep/location/year	12	168.52654	6.44	0.0020
Entry	14	2608.54656	16.62	<.0001
Year*Location	2	4123.02593	26.27	<.0001
Location*Entry	28	887.98862	5.66	<.0001
Year*Location*Rep	10	352.03481	2.24	0.0176
Year*Location*Entry	42	510.27302	3.25	<.0001

Table 5. 3: Mean numbers of emerged *S. gesnerioides* shoots per plot on 15 cowpea cultivars infested in field at Kollo, Maradi and Magaria in 2013 and 2014.

Differential cultivars	<i>Striga</i> samples					
	Kollo		Maradi		Magaria	
	2013	2014	2013	2014	2013	2014
B301	2.00	0.33	10.00	0.00	57.00	2.67
IT97K-499-35	0.00	0.67	1.33	0.33	0.33	2.33
Suvita-2	4.67	1.00	49.33	5.00	111.00	21.67
IT93K-693-2	0.00	0.00	0.00	0.00	0.00	0.00
58-57	7.00	4.33	66.33	4.67	50.00	26.33
HTR	2.00	0.00	47.67	3.33	55.00	12.67
KVx771-10G	8.67	4.33	38.00	3.33	69.00	35.67
Dan Illa	5.33	2.33	15.20	5.00	99.67	50.00
TN5-78	7.00	4.00	54.50	6.67	71.67	35.67
KVx30-309-6G	5.33	2.33	40.67	5.67	38.00	24.00
IT90K-372-1-2	9.00	1.00	68.33	5.67	66.33	28.33
IT99K-573-1-1	0.00	0.00	2.00	0.00	0.33	0.00
IT98K-205-8	0.00	0.33	2.00	0.00	0.00	3.00
TN121-80	0.00	0.00	33.67	1.33	5.00	14.33
IN92E-26	1.00	0.67	26.33	2.67	28.00	22.67
LSD ($p \leq 0.05$)	4.75	3.90	27.24	4.40	40.66	28.11

Striga infestation was higher in 2013 than in 2014 for each site. Magaria was the site with highest number of *Striga* emerged shoots while Kollo was the site with lowest number of parasite emergence for the two years. The genotype IT93K-693-2 was free of *Striga* infestation across all the sites and locations. The multiracial resistant variety B301 supported emerged *Striga* shoots at all locations and years except at Maradi in year 2014. The genotype IT97K-499-35 showed *Striga* infestation at all locations and years except at Kollo in 2013. The line IT99K-573-1-1 was infested by *Striga* emerged shoots at Maradi and Magaria in year 2013 while IT98K-205-8 supported emerged *Striga* shoots at Kollo and Magaria in 2014 and at Maradi in 2013. The variety TN121-80 showed *Striga*

infestation at Maradi and Magaria in the two years while it was free of infection at Kollo. The genotype HTR showed no *Striga* emerged shoots at Kollo in 2014. All the other genotypes supported high numbers of *Striga* emerged shoots in all locations and years.

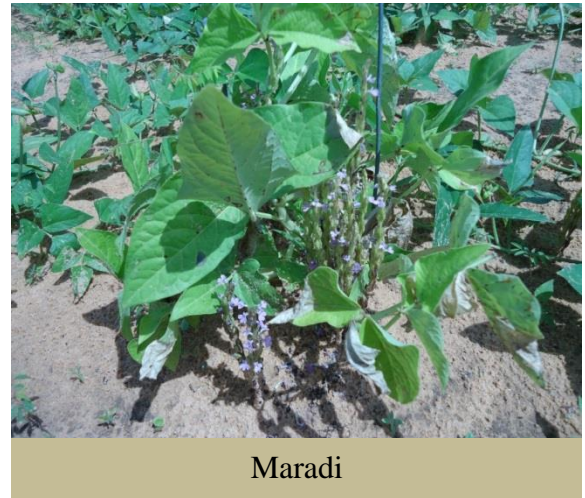


Figure 5. 1: Infested B301 plants in field at Kollo, Maradi and Magaria in 2013.

Table 5. 4: Differential responses to *S. gesnerioides* of 15 cowpea cultivars infested in field at Kollo, Maradi and Magaria in 2013 and 2014.

Differential cultivars	<i>Striga</i> samples					
	Kollo		Maradi		Magaria	
	2013	2014	2013	2014	2013	2014
B301	S	S	S	R	S	S
IT97K-499-35	R	S	S	S	R	S
Suvita 2	S	S	S	S	S	S
IT93K-693-2	R	R	R	R	R	R
58-57	S	S	S	S	S	S
HTR	S	R	S	S	S	S
KVx771-10G	S	S	S	S	S	S
Dan Illa	S	S	S	S	S	S
TN5-78	S	S	S	S	S	S
KVx30-309-6G	S	S	S	S	S	S
IT90K-372-1-2	S	S	S	S	S	S
IT99K-573-1-1	R	R	S	R	S	R
IT98K-205-8	R	S	S	R	R	S
TN121-80	R	R	S	S	S	S
IN92E-26	S	S	S	S	S	S

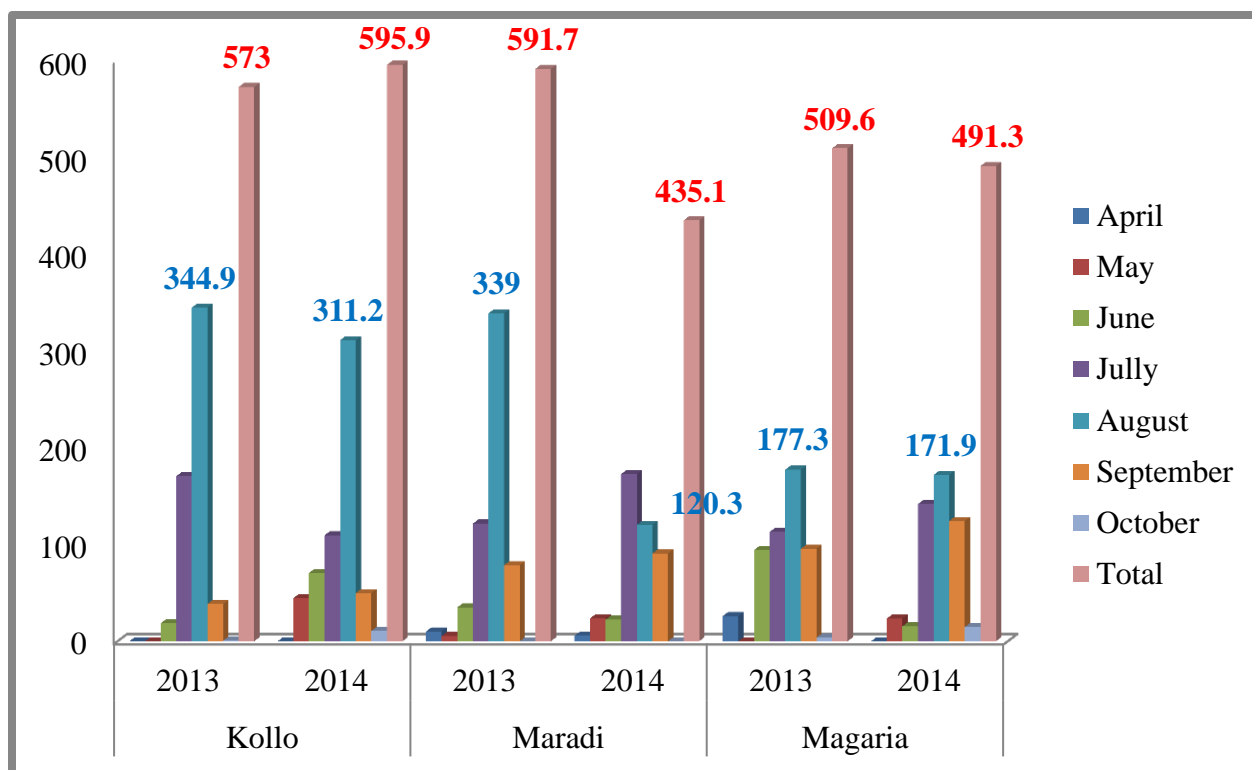


Figure 5. 2: Mean monthly rainfall at Kollo, Maradi and Magaria in 2013 and 2014.

5.2.4.2 Pot experiment

Tables 5.5 and 5.6 show the differential responses of cowpea genotypes to *S. gesnerioides* in pots. The resistant varieties B301, IT93K-693-2 and IT99K-573-1-1 were completely free of *Striga* shoots in the two years, while IT97K-499-35 (3 and 1 shoot) and IT98K-205-8 (4.33 shoots) supported significantly high numbers of *Striga* emerged shoots in 2014. The variety IT97K-499-35 was infested with Kollo and Maradi sample while the variety IT98K-205-8 was attacked by Magaria sample. HTR and Suvita 2 were free of *Striga* with Magaria sample in 2014. TN5-78 was the only susceptible variety that was free of *Striga* emergence at all sites in 2014. The genotypes 58-57 and TN5-78 (Figure 5.3) had the highest numbers of emerged *Striga* shoots. *Striga* infestation was

higher in the two years in the pots infested with *Striga* from Kollo than those infested with *Striga* from Maradi and Magaria.

Table 5. 5: Mean numbers of emerged *S. gesnerioides* shoots per plot on 15 cowpea cultivars infested in pots with samples from Kollo, Maradi and Magaria in 2013 and 2014.

Differential cultivars	<i>Striga</i> samples					
	Kollo		Maradi		Magaria	
	2013	2014	2013	2014	2013	2014
B301	0.00	0.00	0.00	0.00	0.00	0.00
IT97K-499-35	0.00	3.00	0.00	1.00	0.00	0.00
Suvita 2	1.33	2.33	2.33	0.67	1.00	0.00
IT93K-693-2	0.00	0.00	0.00	0.00	0.00	0.00
58-57	0.00	7.00	0.33	3.00	1.33	1.67
HTR	3.00	2.67	1.66	1.33	0.00	0.00
KVx771-10G	1.00	7.00	0.33	3.00	0.33	1.33
Dan Illa	5.00	0.00	2.33	1.00	0.00	0.33
TN5-78	6.67	0.00	1.33	0.00	0.00	0.00
KVx30-309-6G	1.33	1.67	0.00	1.33	0.00	0.67
IT90K-372-1-2	3.50	5.00	1.33	2.33	0.00	1.00
IT99K-573-1-1	0.00	0.00	0.00	0.00	0.00	0.00
IT98K-205-8	0.00	0.00	0.00	0.00	0.00	4.33
TN121-80	1.00	0.00	2.33	0.00	1.33	0.00
IN92E-26	1.00	0.00	0.66	1.00	3.50	0.00
LSD ($p \leq 0.05$)	5.79	5.80	1.79	3.66	1.96	3.56



Figure 5. 3: Susceptible varieties infested in pots.

Table 5. 6: Differential responses to *S. gesnerioides* of 15 cowpea cultivars infested in pots with samples from Kollo, Maradi and Magaria in 2013 and 2014.

Differential cultivars	<i>Striga</i> samples					
	Kollo		Maradi		Magaria	
	2013	2014	2013	2014	2013	2014
B301	R	R	R	R	R	R
IT97K-499-35	R	S	R	S	R	R
Suvita 2	S	S	S	S	S	R
IT93K-693-2	R	R	R	R	R	R
58-57	S	S	S	S	S	S
HTR	S	S	S	S	R	R
KVx771-10G	S	S	S	S	S	S
Dan Illa	S	R	S	S	R	S
TN5-78	S	R	S	R	R	R
KVx30-309-6G	S	S	R	S	R	S
IT90K-372-1-2	S	S	S	S	R	S
IT99K-573-1-1	R	R	R	R	R	R
IT98K-205-8	R	R	R	R	R	S
TN121-80	S	R	S	R	S	R
IN92E-26	S	R	S	S	S	R

5.4 Discussion

Three hot-spots of *Striga gesnerioides* in Niger were characterized using differential reactions of cowpea cultivars that were previously identified as having race specific resistance. Under natural infestation, the number of emerged *Striga* shoots was more across locations in 2013 compared to 2014 where emerged *Striga* shoots were decreased for most of the genotypes. This may be due to the rainfall amount that was higher in 2013 for most locations particularly in August, the period that coincided with *Striga* emergence. Magaria had the highest infestation rate probably because the soils are less fertile in this station compared to the two other sites.

In pot experiments, *Striga* infestation was low. The numbers of *Striga* emerged shoots were higher with Kollo samples and in year 2014 for all the sites. This could be attributed to the quality of Kollo inoculum in one side and the moderate amount of rainfall in 2014 at Maradi station where pot experiment was laid out in ambient temperature. Indeed, in 2013 the high amount of rainfall recorded at Maradi station compared to that of 2014 might have affected *Striga* emergence in pots for all the samples.

Out of the six genotypes previously reported as resistant to the race SG3, only IT93K-693-2 was completely free of *Striga* infestation across sites and years. The resistance observed in IT93K-693-2 confirmed the findings of Singh (2002); Boukar *et al.* (2004) and Tignegre (2010) who reported that this genotype was resistant to all known *Striga* races in Africa. IT99K-573-1-1, another resistant genotype to SG3 supported few *Striga* shoots in 2013 at Maradi and Magaria. This is not in line with the findings of Tchiagam *et al.* (2010) who observed no *Striga* emergence on IT99K-573-1-1 when it was screened to race SG5 in Cameroon. The multi-race *Striga* resistant B301 showed severe *Striga*

infestation in the field at all sites in 2013 and at Kollo and Magaria in 2014, but it supported no *Striga* emergence in pots. Previously, Touré *et al.* (1998) found similar results with Cinzana race in Mali. Although, B301 was considered as a stable resistant genotype across four *Striga* races in Sub-Saharan Africa, these results suggest that Zakpota race SG4_Z or its variants exist within *S. gesnerioides* populations in the study area. The resistance of B301 observed at Maradi in 2014 could be partly attributed to the rainfall pattern which was low that year compared to the previous year during which B301 showed *Striga* infestation at the same site. The resistance of B301 observed in pots might be due to the quality of inoculum or the presence of *Striga* race SG3 seeds to which B301 is resistant in the different collected samples. Another cultivar, IT97K-499-35, reported previously as resistant to *Striga* races 1, 2 and 3 (Atokple *et al.*, 1995; Singh, 2006) showed *Striga* emergence at all sites in the field during the two years except in 2013 at Kollo as well as with Kollo and Maradi samples in pots in 2014. This result revealed that IT97K-499-35 may not be completely resistant to race SG3 confirming early reports by Kamara *et al.* (2008) and Omoigui *et al.* (2011) suggesting the presence of new *Striga* races or variants in the different sites. However, further studies would be required to confirm this hypothesis.

The variety IT98K-205-8 which was previously reported to be resistant to races SG1, SG3 and SG5 showed *Striga* infestation in the field at Maradi in 2013 and at Kollo and Magaria in 2014 as well as with Magaria sample in pots in 2014. This result is not in line with the findings of Ajeigbe *et al.* (2008) who observed during two years of field evaluation under natural infestation that IT98K-205-8 and four other cultivars were completely resistant to *Striga* thus supported no *Striga* emergence in northern Nigeria

where *S. gesnerioides* race SG3 is widely distributed. The relatively high numbers of emerged *Striga* shoots recorded in the field (3.33) and in pots (4.33) at Magaria suggests the breakdown of the resistance in IT98K-205-8 or the presence of another *S. gesnerioides* race.

TN121-80 is another *Striga* race SG3 resistant cultivar that supported *Striga* emerged shoots in the field at Maradi and Magaria in the two years and with all the samples in pot in 2013 suggesting the breakdown of its resistance to this race or the prevalence of new races in the two localities.

In the present study all the susceptible checks to *Striga* race SG3 showed infestation at all sites in the field during 2013 and 2014 except the cultivar HTR that showed no *Striga* infestation at Kollo in 2014. This may be due to the presence of *Striga* race SG1 seeds for which HTR is resistant or to the escapism of this genotype from race SG3 attack, therefore corroborating previous reports (Moutari and Adam, unpublished data) who observed resistance of HTR in Western Niger and high susceptibility in East, especially at Magaria. The *Striga* emergence observed with Kollo sample in pots on HTR in 2013 and 2014 may be due to the mixture of the seeds from the two races together in the same field where the sample was collected. But further investigations need to be conducted to confirm this hypothesis.

Five *Striga* susceptible cultivars that showed infestation in field supported no emerged *Striga* shoots in pots in 2014. These cultivars include HTR and Suvita 2 for Magaria sample, Dan Illa for Kollo, TN5-78 for samples from all sites and IN92E-26 for Kollo and Magaria samples.

The differences observed between field and pots results for some cultivars could be partly attributed to the artificial and highly limited root environment in pots and other factors like humidity or inoculum quality that may affect *Striga* emergence. HTR and Suvita 2 are recognized resistant cultivars to *Striga* race SG1; therefore Magaria sample used as inoculum in pots experiment may contain *Striga* race SG1 seeds or that the conditions required for a good *Striga* emergence were not fulfilled.

Assuming that most of the results from pots that were not concordant with field observed susceptibility were escape. The combined results of differential responses revealed the following status of the three hot-spots about *S. gesnerioides* race distribution:

At Kollo, *Striga* emergence observed on cowpea genotypes B301, IT97K-499-35 and IT98K-205-8 would have confirmed the prevalence of *Striga* race SG4z if only the varieties Suvita 2 and 58-57 were not susceptible. Indeed these varieties were reported to be resistant to race SG4 (Lane and Bailey, 1992). This suggests the presence of *Striga* race SG3 with SG4z or its variant. Also, the resistance of HTR observed could be explained by the prevalence of race SG1 with SG3 in the same field. This hypothesis of compendium i.e. the mixture of more than one *Striga* race seeds in the same field requires further investigations.

At Maradi, the resistance of cowpea varieties B301 and IT98K-205-8 observed in 2014 coupled with the susceptibility of Suvita-2, 58-57 and HTR may be justified by the presence of *Striga* SG3. However, *Striga* infestation on IT97K-499-35 and TN121-80 in the two years may be due to the breakdown of SG3 resistance or the presence of a new race.

At Magaria, *Striga* attack observed on cowpea varieties B301, IT97K-499-35 and IT98K-205-8 coupled with the resistance of Suvita-2 observed in pots may be due to the presence of *Striga* race SG4z. The emergence of *Striga* on 58-57 and Suvita-2 in field suggests the prevalence of *Striga* race SG3. The presence of *Striga* race SG1 with the other two races was probably indicated by the resistance of cowpea variety HTR observed in pots in both years.

5.5 Conclusion

This study confirmed the potential of some cowpea cultivars to control *Striga gesnerioides* and revealed race specificity and ability of this parasitic weed to overcome recognized resistance. The breeding line IT93K-693-2 is a potential parent for use in transferring *Striga* resistance into adapted and farmer preferred varieties. The varieties B301, IT97K-499-35, IT99-573-1-1 and IT98K-205-8 were found not completely resistant, thus indicating either the breakdown of their resistance to *Striga* race SG3 or the prevalence of *Striga* race SG4z or variants in Niger particularly at Magaria and Kollo. Also the resistance of HTR and Suvita-2 in the two localities would not exclude the prevalence of *Striga* race SG1. The use of sophisticated screening techniques such as molecular studies of a representative collection of *Striga* samples throughout the country would be needed to ascertain the geographical distribution of *Striga* races in Niger. Breeding for multi-racial *Striga* resistant cowpea varieties by gene pyramiding or transferring a gene that confers the resistance to all known *Striga* races in the locally adapted cultivars is required urgently.

CHAPTER SIX

6.0 Backcrossing of *Striga* resistance gene into farmers' preferred varieties

6.1 Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most important grain legumes in Africa. It is now emerging as one of the most important food legumes in the 21st century because of its early maturity and ability to fix nitrogen and fits in a wide range of cropping systems (IITA, 2010). Cowpea is an essential supplement to diet due to its relatively high protein content. It is also, a valuable commodity that generates income to farmers. There has been a significant increase of cowpea production worldwide in the last few decades. However, *Striga gesnerioides* has become a serious biological constraint to the increase of production in smallholders' farms. *S. gesnerioides* is difficult to control and no single method can counter its effect on yield. Indeed, yield losses ranging from 83-100 % have been reported on susceptible cultivars (Emechebe *et al.*, 1991; Cardwell and Lane, 1995). Thus, host plant resistance appears to be the most economically and environmentally sound strategy to control *Striga* effectively since it is affordable to small-scale farmers (Omoigui *et al.*, 2015). Unfortunately, resistant cowpea varieties identified or bred so far have mostly poor agronomic traits hardly accepted by farmers or end-users. The current focus in cowpea breeding and genetic improvement around the world is combining desirable agronomic characteristics such as: time to maturity, photoperiod sensitivity, plant type, and seed quality with resistance to the major diseases, insect pests or parasites that agronomically afflict adapted cowpea cultivars (Timko *et al.*, 2007; Timko and Singh, 2008). Currently, depending on the type of trait being

introgressed a decade would be required to breed a superior improved line through conventional breeding methods. Developing molecular marker-based tools for tracking single genes and quantitatively inherited traits linked to major disease and pest resistance, as well as the establishment of an array of protocols for marker assisted selection (MAS) can shorten the time frame. Indeed molecular markers used in the identification and selection of *Striga* resistant genotypes have been developed for most of the races of the parasite prevalent in West Africa. However, the differential virulence of races of *S. gesnerioides* on cowpea genotypes (Lane *et al.*, 1994; Singh, 2002) has serious impact on breeding and selection procedures. Therefore, the need of using race specific markers to complement conventional breeding methods for identification of *Striga* resistant genotypes is essential. To date, at least seven races of *S. gesnerioides* have been identified based on host differential response and genetic diversity analysis within the cowpea growing regions of West Africa (Lane *et al.*, 1996, Botanga and Timko, 2006). These races were designated as SG1 (Burkina Faso), SG2 (Mali), SG3 (Nigeria and Niger), SG4 and SG4z (Benin), SG5 (Cameroon) and SG6 (Senegal). Recent studies conducted by Ouédraogo *et al.* (2001) using amplified fragment length polymorphism (AFLP) and bulk segregant analysis (BSA) identified three markers tightly linked to the resistance gene *Rsg2*, effective against *S. gesnerioides* race SG1 from Burkina Faso, and present in IT82D-849; and six AFLP markers associated with the resistance gene *Rsg4*, effective against *S. gesnerioides* race SG3 from Niger, and present in Tvu 14676. Molecular markers linked with resistance genes to races SG1, SG2 and SG3 have been identified, and several sequence-confirmed amplified regions (SCARs) have been developed for use in MAS (Li *et al.*, 2009).

A recently identified cowpea breeding line, IT93K-693-2, has resistance to all known *Striga* races (Singh, 2002) but lacks farmers' preferred traits. It has a single dominant gene *Rsg1* that confers the resistance to the strain SG3 inherited from the cultivar B301 (Boukar *et al.*, 2004). This gene *Rsg1* present in the breeding line IT93K-693-2 and Botswana landrace B301 was found to be a dominant gene that could be introgressed into adapted cowpea genotypes through pedigree and backcross breeding (Tignegre, 2010).

In Niger, none of the landraces grown by farmers was found to be resistant to *Striga* and most of the introduced resistant varieties have poor agronomic traits. On the other hand, some of the resistant genotypes show levels of breakdown of the resistance. The virulence of this parasitic weed and its rapid spread require an urgent need of varieties with multiple resistance (Boukar *et al.*, 2004).

The objective of this research was to introgress the resistance gene *Rsg1* into three farmers' preferred varieties using backcross breeding and select resistant lines as basis for developing well adapted varieties.

6.2. Materials and methods

6.2.1 Plant Materials

Three farmer-preferred varieties (recurrent parents): IT90-K-372-1-2; KVx30-309-6G; TN5-78, susceptible to *Striga gesnerioides* were selected through a PRA study as parents for improvement to *Striga* resistance. The breeding line IT93K-693-2 was selected as the donor line. The choice of IT93K-693-2 was because it was resistant in the germplasm screening in Niger and it has been reported also to be resistant to all known *Striga* races

(Table 6.1). The genotype IT93K-693-2 is a three way cross hybrid: [(IT88D-867-11 x IT90K-76) x IT89KD-374] that inherited the resistance gene *Rsg1* from B301 and the resistance to *Striga* race SG4z from the line IT88D-867-11.

Table 6. 1: Cowpea varieties used as parents in backcross breeding with their pedigree information

Lines or cultivars	Pedigree	Resistance to <i>S. gesnerioides</i>
IT93K-693-2	(IT88D-867-11 x IT90K-76) x IT89KD-374.	<i>Rsg1</i> from B301 and resistance to SG4 from Benin
IT90K-76	(B301 x IT90K-2246-4) x IT90K-2246-4	
IT88D-867-11		SG4 from Benin
IT90K-2246-4		Susceptible to all races
IT89KD-374		Susceptible to all races
TN5-78	Selected and improved landrace	Susceptible to all races
IT90K-372-1-2	(IT87F-1784-2 x IT84S-2246-4) x IT87F-1784-2	Susceptible to all races
KVx30-309-6G	Not found	Susceptible to all races

Crosses were made between IT90-K-372-1-2 and IT93K-693-2; KVx30-309-6G and IT93K-693-2; and TN5-78 and IT93K-693-2 at Maradi INRAN station in 2013. The F₁ generations were backcrossed to the recurrent parents (IT90-K-372-1-2; KVx30-309-6G and TN5-78) and also self-pollinated to generate both BC₁F₁ and F₂ populations as follows:

- BC₁F₁ (A) derived by backcrossing F₁ between IT90-K-372-1-2 and IT93K-693-2 to recurrent parent, IT90-K-372-1-2 = (IT90-K-372-1-2 x IT93K-693-2) / IT90-K-372-1-2;

- BC₁F₁ (B) derived by backcrossing F₁ between KVx30-309-6G and IT93K-693-2 to recurrent parent, KVx30-309-6G = (KVx30-309-6G x IT93K-693-2) / KVx30-309-6G;
- BC₁F₁ (C) derived by backcrossing F₁ between TN5-78 and IT93K-693-2 to recurrent parent, TN5-78 = (TN5-78 x IT93K-693-2) / TN5-78.
- F₂ (A) derived by self-pollinating F₁ of IT90-K-372-1-2 x IT93K-693-2;
- F₂ (B) derived by self-pollinating F₁ of KVx30-309-6G x IT93K-693-2;
- F₂ (C) derived by self-pollinating F₁ of TN5-78 x IT93K-693-2.

BC₁F₁ and F₂ generations were screened for *Striga* resistance in pots and the selected plants from BC₁F₁ were again backcrossed to the recurrent parents (IT90-K-372-1-2; KVx30-309-6G and TN5-78) to produce BC₂F₁ and F₂ plants were self-pollinated to produce F₃ generations as follows:

- BC₁F₁ (A) was backcrossed to IT90-K-372-1-2 to generate BC₂F₁ (A);
- BC₁F₁ (B) was backcrossed to KVx30-309-6G to generate BC₂F₁ (B);
- BC₁F₁ (C) was backcrossed to TN5-78 to generate BC₂F₁ (C);
- F₂ (A) was self-pollinated to generate F₃ (A);
- F₂ (B) was self-pollinated to generate F₃ (B);
- F₂ (C) was self-pollinated to generate F₃ (C);

The same procedure was used to generate BC₃F₁ and F₄ generations. BC₂F₁, BC₃F₁ and F₄ were advanced by successive self-pollination to BC₂F₃, BC₃F₃ and F₆ generations. Marker assisted selection was applied from BC₁F₁ to BC₃F₃ and at F₆ generation. The lines advancement was done by combining genotypic and phenotypic data for the backcross populations while the selfed progenies (F₂ to F₆) were advanced based on the

the absence of *Striga* infestation in pots. BC₃F₃ generations were obtained only in the family: (TN5-78 x IT93K-693-2) while F₆ generation was produced by the family IT90K-372-1-2 x IT93K-693-2). The progenies developed from each cross are presented in Table 6. 2. The backcross scheme used is shown in Figure 6.1.

Table 6. 2: Development of recombinant lines

Crosses	Progenies composition
IT90-K-372-1-2 x IT93K-693-2	BC ₁ F ₁ , BC ₂ F ₁ , BC ₂ F ₂ , BC ₂ F ₃ , BC ₃ F ₁ , F ₂ -F ₆
KVx30-309-6G x IT93K-693-2	BC ₁ F ₁ , BC ₂ F ₁ , BC ₂ F ₂ , BC ₂ F ₃ , BC ₃ F ₁ , F ₂ -F ₅
TN5-78 x IT93K-693-2	BC ₁ F ₁ , BC ₂ F ₁ , BC ₂ F ₂ , BC ₂ F ₃ , BC ₃ F ₁ , BC ₃ F ₂ , BC ₃ F ₃ , F ₂ -F ₅

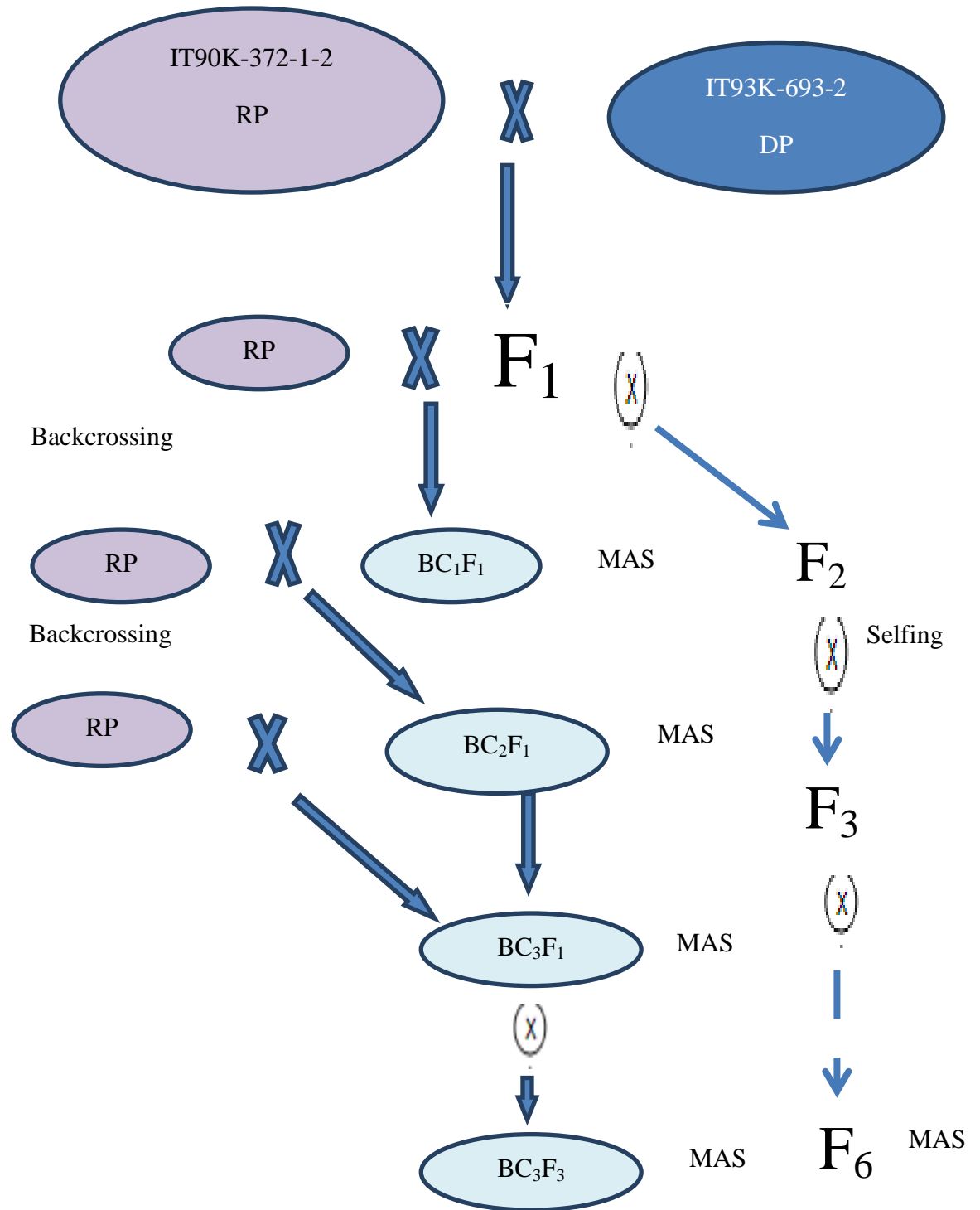


Figure 6. 1: Backcross breeding scheme for *Striga gesnerioides* resistance gene introgression.

RP: recurrent parent; DP: donor parent; MAS: marker assisted selection.

6.2.2 Plant culture and DNA extraction

Cowpea parental lines and the derived populations were grown in pots in a greenhouse at INRAN Maradi station from 2013- 2015. Each pot had a volume of seven liters filled with 5 kg of a mixture of sandy soil, clay and farmyard manure in a ratio of 2:1:1, respectively. The mixture was previously sterilized. After soil infestation with about 1000 seeds per pot of one year-old *Striga gesnerioides*, the pots were watered for two weeks to precondition *Striga* seeds in order to break their dormancy and ensure optimum germination. Three seeds of cowpea were sown per pot. The seedlings were thinned to one per pot at 2 weeks after germination. The pots were watered every two days or when necessary in order to keep them moist.

Genomic DNA was extracted from leaf tissues of 2 weeks old plants using the Fast Technology for Analysis (FTA) cards as described by Omoigui *et al.* (2012). The young leaf was placed on the FTA Plantsaver card covered with parafilm paper, pressure was applied with a pestle briefly until the plant material was sufficiently transferred to the card. After air drying for about 1 hour, FTA cards were placed in a paper punch and stored at ambient temperature in a dry location. The samples were taken to Institut de l'Environnement et de Recherches Agricoles (INERA), Genetics and Plant Biotechnology laboratory at Kamboinse in Burkina Faso for the genotyping.

6.2.3 Preparation of samples for PCR Analysis

The samples were prepared as described by Omoigui *et al.* (2012). A disc from the dried FTA card was removed using a clean Haris micro punch and placed directly into a 1.5 mL Eppendorf tube. Precautions were taken in order to prevent cross contamination, by

cleaning the Haris micro punch with a tissue dampened with 70% ethanol in between samples. The disc was washed twice with 200 μ L of FTA reagent incubating for five minutes for each wash followed by a repeated wash with 200 μ L of 70% ethanol, incubating for 5 minutes at room temperature and the liquid was discarded. The tubes were inverted and drained on a paper towel and air dried for close to 1 h. After drying, the tubes were transferred for PCR analysis (Omoigui *et al.*, 2012).

6.2.4 PCR analysis

One primer SSR₁ was used for the PCR analysis. Each PCR mixture (25 μ L final volume) contained, besides the purified 2 mm FTA DNA disc containing the DNA sample, 18 μ L of sterilized water, 2.5 mM each of DNTPs mix and 10 x PCR buffer, 0.05 μ L of Taq polymerase, and 1 μ L of each of the forward and reverse primers. PCR reactions were performed on a heated lid thermo-cycler (Biometra) using the thermo-cycler (Biometra) operated at the following conditions: 35 cycles of denaturation at 94 °C for 30 s, followed by annealing at 57.5 °C for 30 s and extension at 72 °C for 2 min. The repeat sequences of the primers linked to the resistance gene *RsgI* are as shown below (Table 6.3).

Table 6. 3: Structure of SSR₁ primers used in MAS procedures.

Name	Repeat sequence
SSR ₁ CP3 (F)	CAAGAAGGAGGCGAAGACTG
SSR ₁ CP3 (R)	CCTAAGCTTTTCTCCA ACTCC

6.2.5 Electrophoresis

PCR product was electrophoresed on a 2% agarose gel stained with ethidium bromide. The gels were run for approximately 1 hour 30 minutes at 120 volt in 1 X TAE buffer (45 mmol l⁻¹ glacial acetic acid, 0.5 mmol l⁻¹ EDTA, pH, 8.4). A 1 kb DNA standard ladder was loaded in the first well for band size determination of PCR products. The ethidium bromide-stained gel was visualized on an UV transilluminator and images photographed using a Polaroid camera.

6.2.6 Marker assisted selection for *Striga gesnerioides* resistance in segregating populations of cowpea

Plant genotyping was done using SSR₁ marker at BC₁F₁, BC₂F₁, BC₂F₂, BC₂F₃, BC₃F₁, BC₃F₃ and F₆ generations. For all these generations, plant sampling, DNA extraction, PCR analysis and electrophoresis were done as described above. Data was scored by observing gels under UV light and recording the number of samples showing marker's single band. SSR₁ marker produced single bands of 150bp PCR product with amplification only in resistant genotypes. Selection for advancement was done based on the presence of marker allele. No screening was done in the field in all the stages; however, in order to confirm the effectiveness of marker assisted selection in introgressing *Striga* resistance gene, data on *Striga* emergence and dates to flowering were taken in pots for the genotyped BC₃F₃, BC₂F₃, F₆ populations and their parents included as checks. The numbers of plants per generation were 3, 10 and 7 for BC₃F₃, BC₂F₃ and F₆ respectively.

6.2.7 Marker validation

SSR₁ marker validation was done by genotyping the resistant parent, one susceptible parent, and the progenies derived from the cross between the two contrasting parents: F₁ individual, a susceptible F₂ individual plant, a resistant homozygous F₂ individual plant, a susceptible BC₁F₁ individual plant and a resistant BC₁F₁ individual plant. The homozygous individual F₂ plants were identified after a progeny test following the phenotypic selection of a number of F₂ plants. DNA from these F₂ individual plants was extracted before the progeny test. After selfing these F₂ plants, the ones for which all the F₃ progenies were resistant were identified as homozygous resistant.

6.3 Results

DNA was successfully extracted from leaf tissues using FTA cards. One primer SSR₁ linked to *Striga* resistance gene *Rsg1* was used to discriminate between resistant and susceptible lines in the populations.

6.3.1 Parental genotyping

The resistant parent IT93K-693-2 and the three farmers' preferred varieties were first genotyped in order to confirm the polymorphism of SSR₁ marker. The results showed the existence of a unique band with the resistant parent at 150bp of PCR products while the susceptible varieties had no such bands (Figure 6.2)

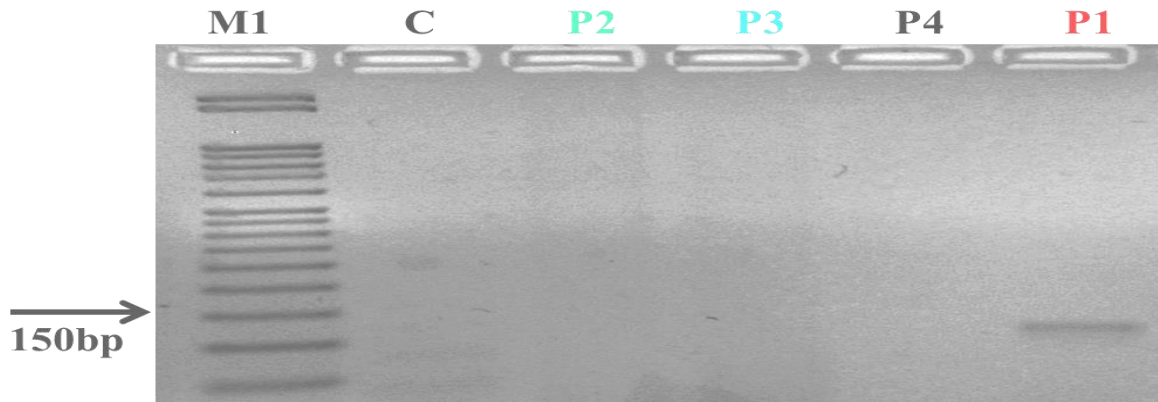


Figure 6. 2: Results from PCR amplification of genomic DNA by SSR₁ for the parents. M1=Ladder; C=control; P₁=IT93K-693-2; P₂=IT90K-372-1-2; P₃= KVx30-309-6G; P₄=TN5-78.

6.3.2 BC₁F₁ population

A total of 34 DNA samples from young leaves of BC₁F₁ plants and their parents were genotyped. Eleven BC₁F₁ individuals revealed the presence of the resistance gene (Figures 6.3 and 6.4) and were therefore selected for the next backcross.

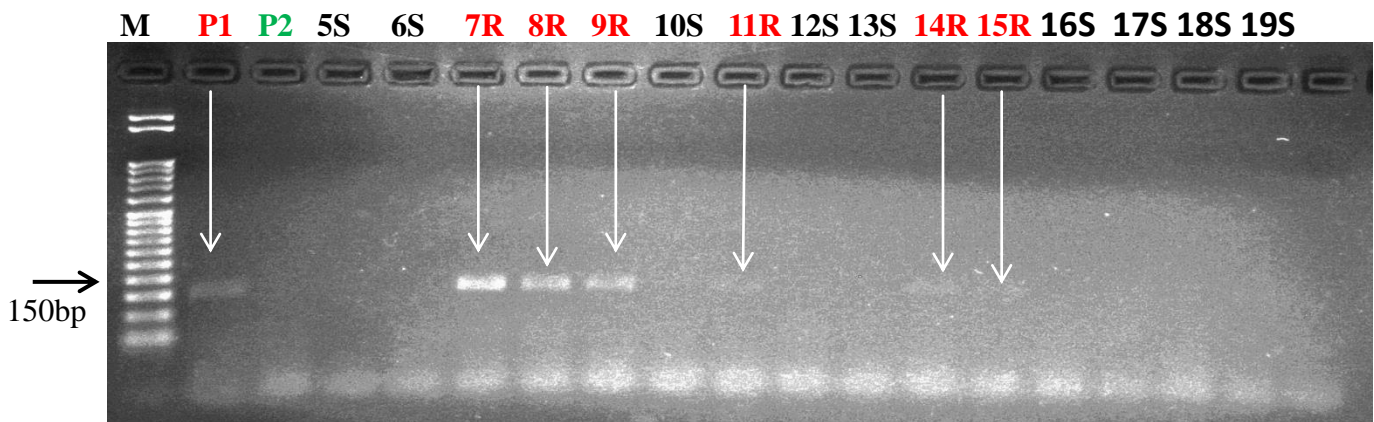


Figure 6. 3: Results from PCR amplification of genomic DNA by SSR₁ for the BC₁F₁ progenies derived from IT90K-372-1-2 x IT93K-693-2. M = 1 kb ladder, P₁ = IT93K-693-2, P₂ = IT90K-372-1-2, R and S indicate resistant and susceptible, respectively.

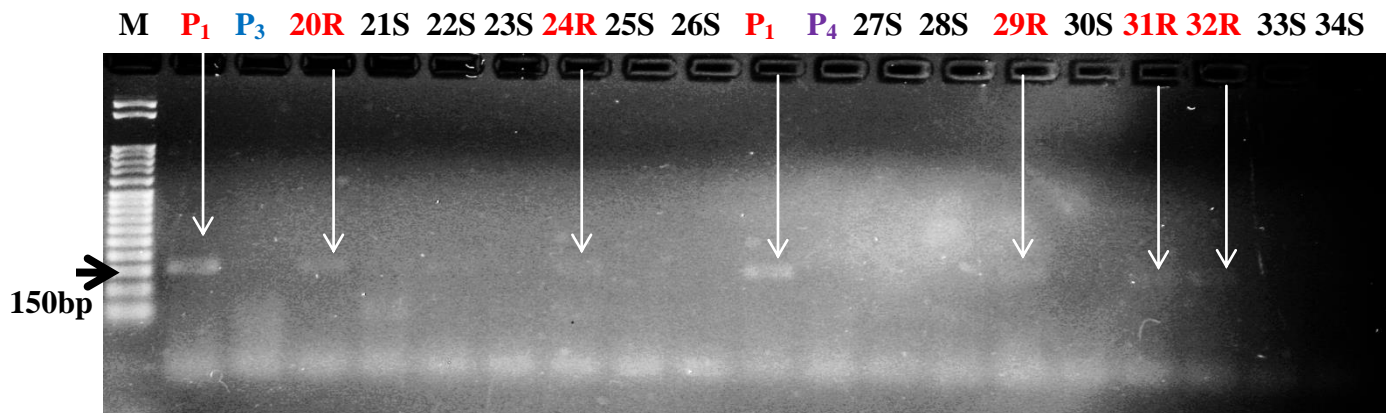


Figure 6. 4: Results from PCR amplification of genomic DNA by SSR₁ for the BC₁F₁ progenies derived from KVx30-309-6G x IT93K-693-2 and TN5-78 x IT93K-693 -2. M = 1 kb ladder, P1 = IT93K-693-2, P3 = KVx30-309-6G, P4 = TN5-78, R and S indicate resistant and susceptible, respectively.

6.3.3 BC₂F₁ populations

DNA samples from nineteen (19) progeny were taken from BC₂F₁ population and their parents for genotyping. Only Four (4) BC₂F₁ individuals carried the resistance marker shown by the presence of single band at 150bp of PCR products (Figures 6.5 and 6.6).

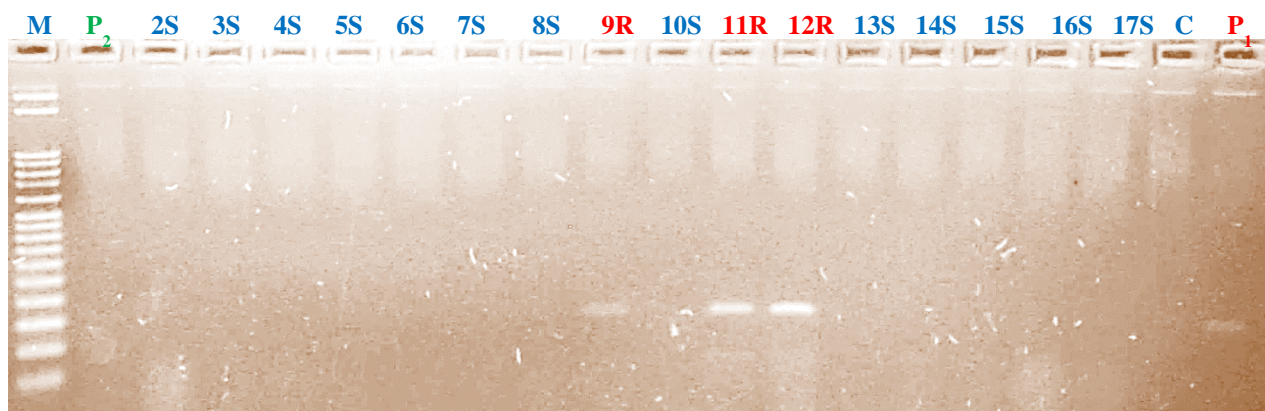


Figure 6. 5: Results from PCR amplification of genomic DNA by SSR₁ for the BC₂F₁ progenies derived from IT90K-372-1-2 x IT93K-693-2. M = 1 kb ladder, C = control without genomic DNA template, P₁ = IT93K-693-2, P₂ = IT90K-372-1-2, R and S indicate resistant and susceptible, respectively.

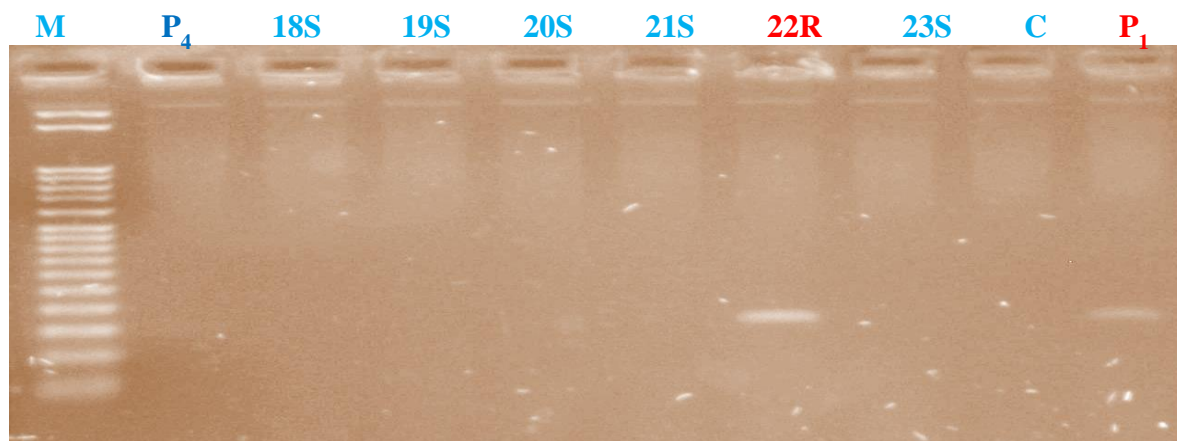


Figure 6. 6: Results from PCR amplification of genomic DNA by SSR₁ for the BC₂F₁ progenies derived from TN5-78 x IT93K-693-2. M = 1 kb ladder, C = control without genomic DNA template, P₁ = IT93K-693-2, P₄ = TN5-78, R and S indicate resistant and susceptible, respectively.

6.3.4 BC₂F₂ derived populations genotyping

A total of twenty two samples of genomic DNA from BC₂F₂ derived progeny with their parents were taken for genotyping. Seven individual plants revealed the presence of the resistance marker as shown by the single band of 150bp of PCR products (Figures 6.7 and 6.8).

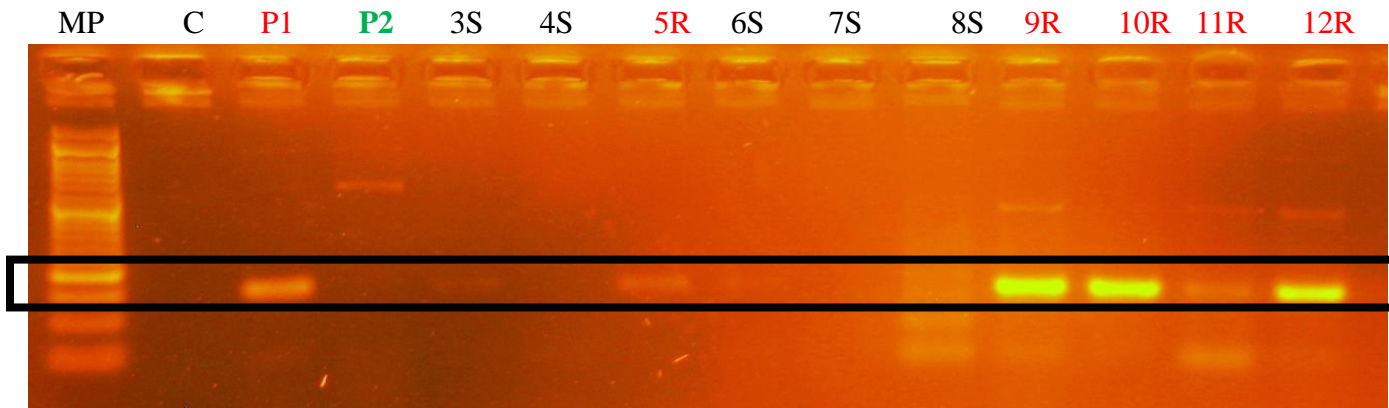


Figure 6. 7: Results from PCR amplification of genomic DNA by SSR₁ for the BC₂F₂ progenies derived from IT90K-372-1-2 x IT93K-693-2. M = 1 kb ladder, C = control without genomic DNA template, P₁ = IT93K-693-2, P₂ = IT90K-372-1-2, R and S indicate resistant and susceptible, respectively.

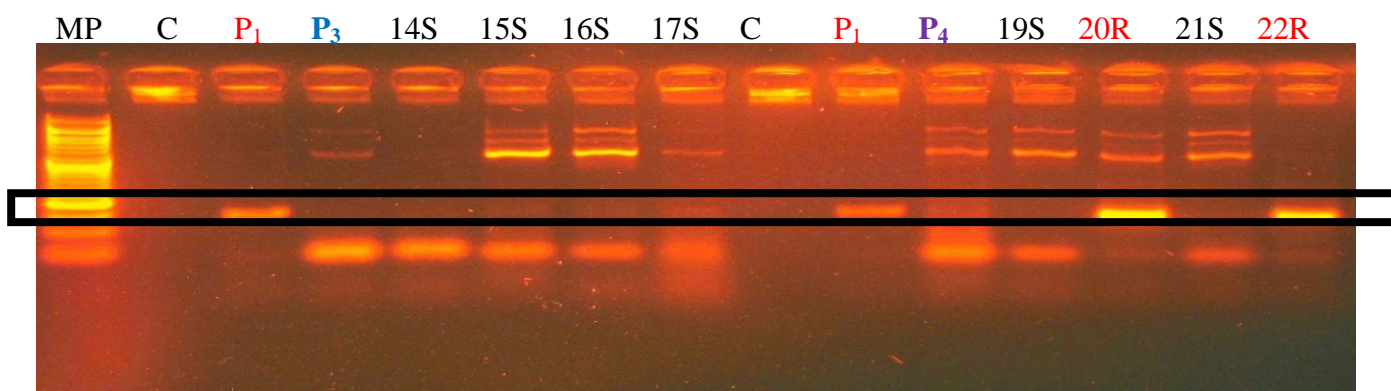


Figure 6. 8: Results from PCR amplification of genomic DNA by SSR₁ for the BC₂F₂ progenies derived from KVx30-309-6G x IT93K-693-2 and TN5-78 x IT93K-693 -2. M = 1 kb ladder, C = control without genomic DNA template, P₁ = IT93K-693-2, P₃ = KVx30-309-6G, P₄ = TN5-78, R and S indicate resistant and susceptible, respectively.

6.3.5 BC₃F₁ derived populations genotyping

DNA samples from eight BC₃F₁ progeny derived from 3 different crosses (IT90K-372-1-2 x IT93K-693-2, KVx30-309-6G x IT93K-693-2 and TN5-78 x IT93K-693 -2) with their parents were genotyped. Two individual plants in BC₃F₁ populations carried the resistance marker as indicated by single bands of 150bp of PCR products (Figure 6.9).

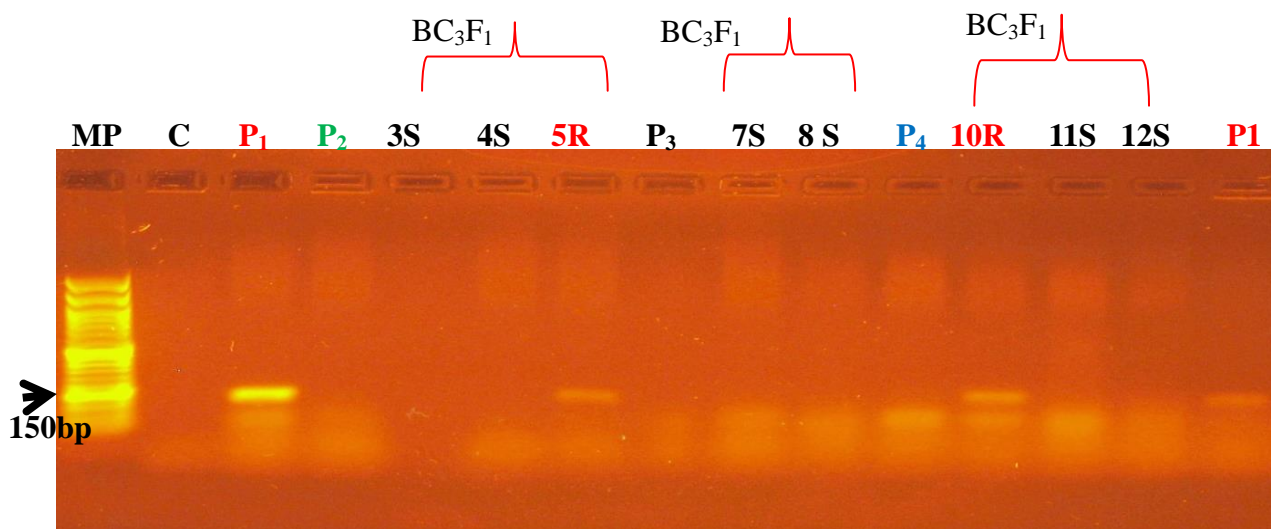


Figure 6. 9: Results from PCR amplification of genomic DNA by SSR₁ for the BC₃F₁ progenies derived from IT90K-372-1-2 x IT93K-693-2, KVx30-309-6G x IT93K-693-2 and TN5-78 x IT93K-693 -2. MP = 1 kb ladder, C = control without genomic DNA template, P₁ = IT93K-693-2, P₂ = IT90K-372-1-2, P₃ = KVx30-309-6G, P₄ = TN5-78, R and S indicate resistant and susceptible, respectively.

6.3.6 BC₂F₃, BC₃F₃ and F₆ derived populations genotyping

DNA samples were taken from twenty-three BC₂F₃, BC₃F₃, F₆ progenies and their parents for genotyping. Ten individual plants had the resistance marker as shown by the presence of single band at 150bp of PCR products (Figures 6.10 and 6.11). The different individual plants showing the marker selected per population were as follows:

- 2 individuals from IT90K-372-1-2 x IT93K-693-2 BC₂F₃ population
- 2 individuals from IT90K-372-1-2 x IT93K-693-2 F₆ population
- 1 individual from TN5-78 x IT93K-693-2 BC₃F₃ population
- 5 individuals from TN5-78 x IT93K-693-2 BC₂F₃ population

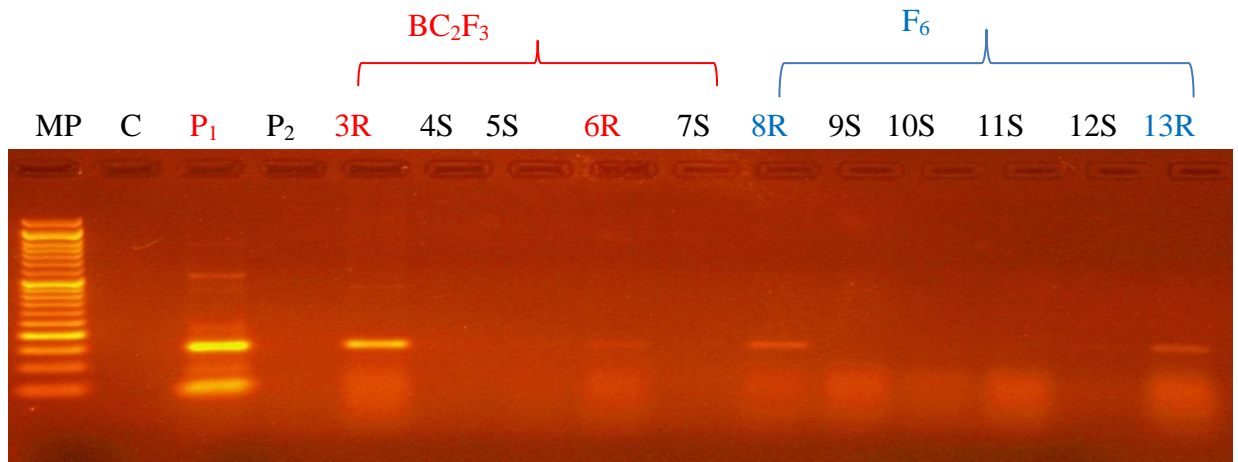


Figure 6. 10: Results from PCR amplification of genomic DNA by SSR_1 for the BC_2F_3 and F_6 progenies derived from IT90K-372-1-2 x IT93K-693-2. M = 1 kb ladder, C = control without genomic DNA template, P_1 = IT93K-693-2, P_2 = IT90K-372-1-2, R and S indicate resistant and susceptible, respectively.

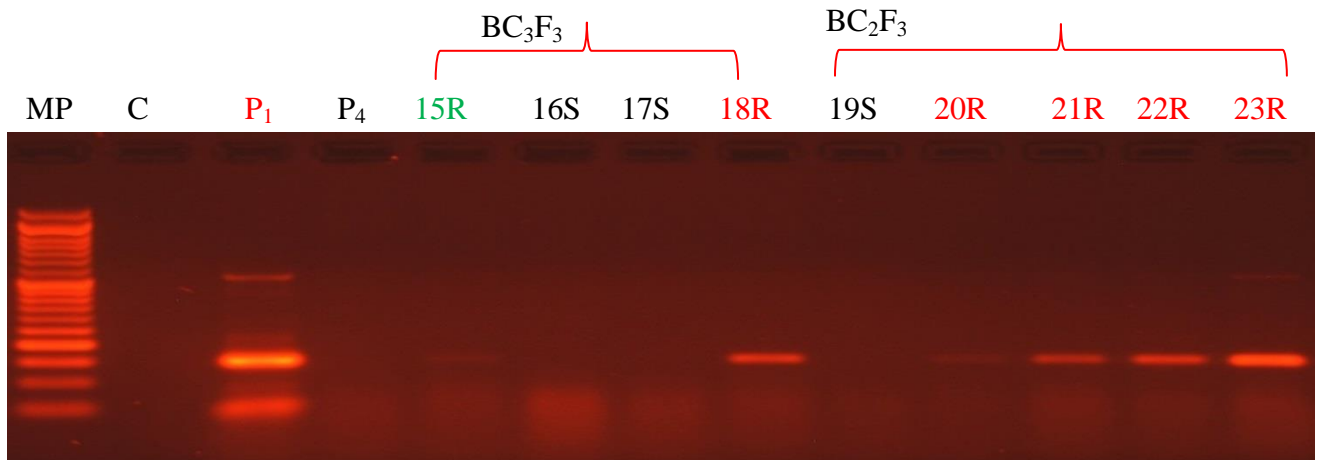


Figure 6. 11: Results from PCR amplification of genomic DNA by SSR_1 for the BC_2F_3 and BC_3F_3 progenies derived from TN5-78 x IT93K-693 -2. MP = 1 kb ladder, C = control without genomic DNA template, P_1 = IT93K-693-2, P_4 = TN5-78, R and S indicate resistant and susceptible, respectively.

6.3.7 Marker validation

The marker SSR_1 produced monomorphic banding pattern that is scoreable, readable and reproducible. The single band at 150bp of PCR product was observed with the resistant parent, the F_1 plants, the homozygous resistant F_2 plants and the resistant BC_1F_1 plants while the band was absent with the susceptible parent, the susceptible F_2 plants and the susceptible BC_1F_1 plants (Figure 6.12)

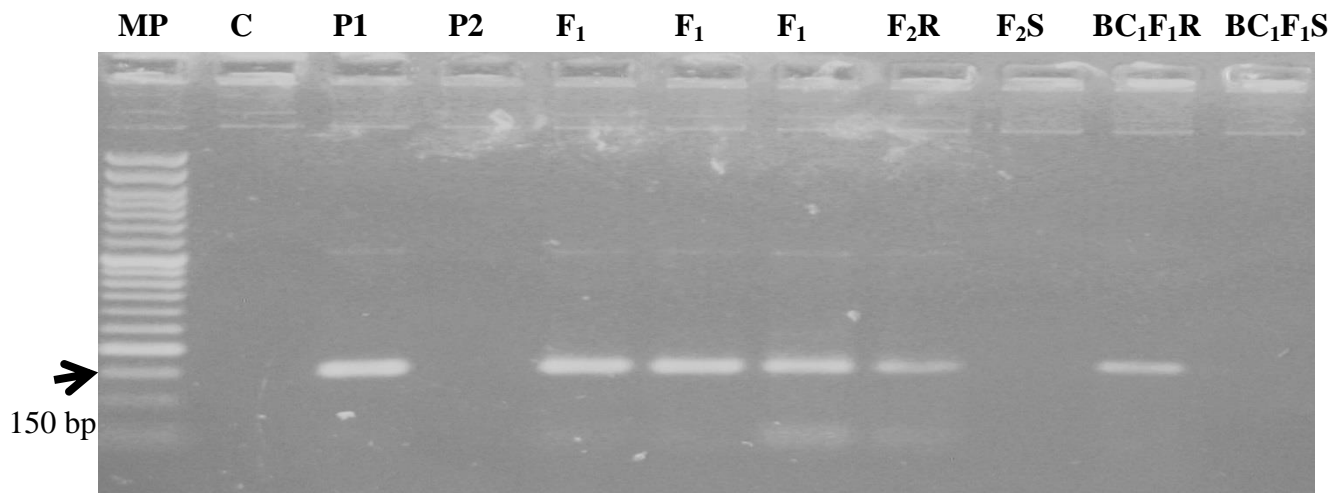


Figure 6. 12: Results from PCR amplification of genomic DNA by SSR_1 for marker validation. P_1 = IT93K-693-2; P_2 = TN5-78; R and S indicate resistant and susceptible, respectively.

6.3.8 Pops screening of BC_2F_3 , BC_3F_3 and F_6 derived populations

Table 6.4 presents the results from the phenotyping (Figures 6.13 and 6.14) of the 23 genotyped individuals. Dates to flowering varied from 34 days after planting (DAP) for the line $BC_2F_3A1-1219-4$ to 57 days for F_6A1-21 . All the selected resistant progenies: $BC_2F_3A1-1219-4$, $BC_2F_3A1-1219-1$ and F_6A1-12 derived from the cross IT90K-372-1-2

x IT93K-693-2 with 34, 36 and 39 days, respectively, flowered before the recurrent parent IT90K-372-1-2 (40 days). The line F₆A1-24 with 45 DAP was the only resistant progeny derived from the same cross that flowered after the recurrent parent. The progeny BC₂F₃A1-1219-4 flowered before both parents. In the second cross, TN5-78 x IT93K-693-2, the progeny BC₃F₃C1-1 with 38 DAP was the only one that flowered before the recurrent parent TN5-78 which flowered at 42 DAP. The number of emerged *Striga* shoots varied from 0 to 19 shoots. The resistant individuals that carried SSR₁ marker were free of emerged *Striga* shoots. However, 50% of the susceptible individuals without the resistant marker did not support *Striga* emergence. The susceptible line F₆A1-21 had the highest number (19) of emerged *Striga* shoots.

Table 6. 4: Reaction of cowpea lines: BC₂F₃, BC₃F₃ and F₆ populations derived from crosses IT90K-372-1-2 x IT93K-693-2 and TN5-78 x IT93K-693-2 with their parents to *Striga gesnerioides* in pots

N ⁰	Genotypes	Phenotypic reaction			Genotypic reaction
		Days to flowering	Pot trial	<i>Striga</i> emergence /pot	SSR ₁ marker 150bp
1	IT93K-693-2	36	R	0	+
2	IT90K-372-1-2	40	S	6	-
3	BC ₂ F ₃ A1-1219-1	36	R	0	+
4	BC ₂ F ₃ A1-1219-2	45	R	0	-
5	BC ₂ F ₃ A1-1219-3	39	R	0	-
6	BC ₂ F ₃ A1-1219-4	34	R	0	+
7	F ₆ A1-11	40	R	0	-
8	F ₆ A1-12	39	R	0	+
9	F ₆ A1-13	41	R	0	-
10	F ₆ A1-21	57	S	19	-
11	F ₆ A1-22	37	S	1	-
12	F ₆ A1-23	37	R	0	-
13	F ₆ A1-24	45	R	0	+
14	TN5-78	42	S	4	-
15	BC ₃ F ₃ C1-1	38	R	0	+
16	BC ₃ F ₃ C1-2	39	S	1	-
17	BC ₃ F ₃ C1-3	40	S	17	-
18	BC ₂ F ₃ C1-220-1	52	R	0	+
19	BC ₂ F ₃ C1-220-2	53	R	0	-
20	BC ₂ F ₃ C1-220-3	54	R	0	+
21	BC ₂ F ₃ C1-220-4	52	R	0	+
22	BC ₂ F ₃ C1-220-5	44	R	0	+
23	BC ₂ F ₃ C1-220-6	55	R	0	+



Resistant parent, P₁: IT93K-693-2



Susceptible parent, P₂: IT90K-372-1-2



Susceptible parent, P₃: TN5-78

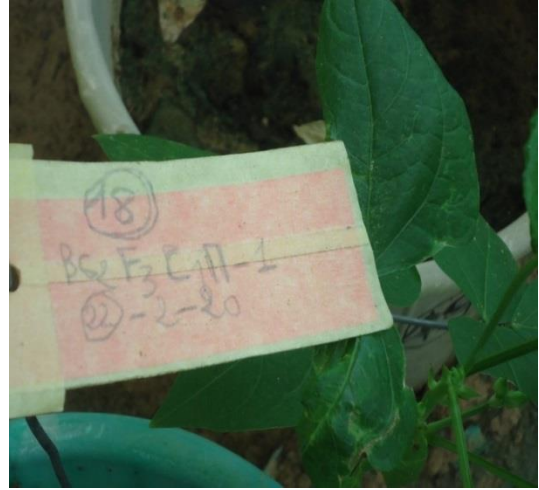


Resistant line F₆ from P₂ x P₁

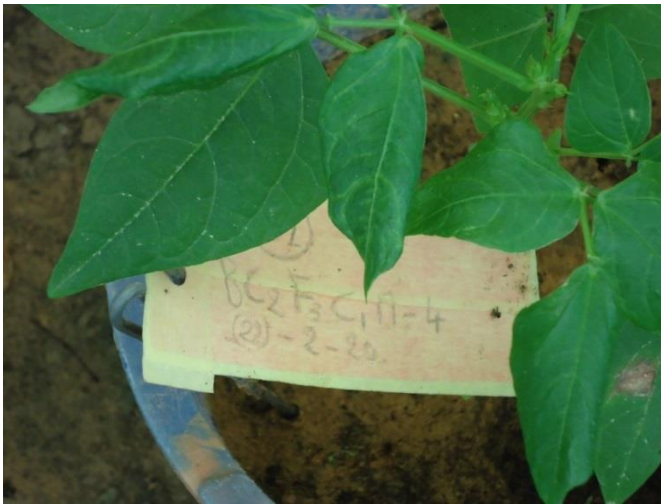
Figure 6. 13: Phenotyping of cowpea lines in pots: parents and one selected F₆ line



Resistant line F₆ from P₂ x P₁



Resistant line BC₂F₃ from P₃ x P₁



Resistant line BC₂F₃ from P₃ x P₁

Figure 6. 14: Phenotyping of cowpea lines: one selected F₆ line and two selected BC₂F₃ lines

6.4 Discussion

Transferring resistance genes into well adapted cowpea cultivars is the most reliable and acceptable method to develop resistant varieties that would help *Striga* control for small-

scale farmers. However, breeding cowpea varieties with stable *Striga* resistance has been slowed by lack of reliable screening techniques. Field screening is affected by a number of complex environmental factors. Today, tracking the genes that govern traits of interest using genetic maps on which QTLs and markers are localized is used for most crops. In cowpea, molecular markers linked to the major genes conferring resistance to the various *Striga* races prevalent in Africa have been identified and their MAS protocols developed. In the present study, FTA cards were successfully used in DNA extraction from leaf tissues. As previously reported by Omoigui *et al.* (2012), this method is suitable for molecular analysis by PCR-based techniques similar to that obtained by classical methods using liquid nitrogen extraction. SSR₁ marker was used to discriminate between resistant and susceptible cowpea genotypes. This marker was found to co-segregate with *Striga gesnerioides* race 3 or SG3 resistance gene (Li and Timko, 2009; Omoigui *et al.*, 2009). SSR₁ primer identified resistant lines by amplification of the 150 bp bands in only resistant genotypes as found by Asare *et al.* (2013). The marker was reliable since all the genotypes with SSR₁ did not support any *Striga* emergence in pots during the gene introgression process. Moreover, the resistant progenies could be traced back to their first parental resistant generations. The reproducibility of all the genotyping results and the consistency of the expected results for marker validation confirmed this reliability. In an earlier study (Asare *et al.*, 2013), the 150bp SSR₁ marker was found to be efficient at 92.6% in discriminating ability. This implies the closeness of the marker to the resistance gene *Rsg1*. Pot *Striga* screening results revealed that IT93K-693-2 has the resistance to *Striga* race SG3 prevalent in Niger. Its resistance was characterized by neither emergence nor attachment of the parasite to the roots. The line IT93K-693-2 is a derivative of

Botswana landrace B301. Earlier inheritance studies indicated the monogenic dominance of the resistance to *Striga* races SG1, SG2, SG3 and SG4 in some cowpea cultivars (Singh and Emechebe, 1990; Atokple *et al.*, 1993; Moore *et al.*, 1995; Tignegre, 2010; Asare *et al.*, 2013; Omoigui *et al.*, 2015). In this study, the segregation ratios were not analyzed in order to confirm previous work because the population size was low (10-20) for efficient handling of pots culture in limited facilities of a common screen house. The three farmers' preferred varieties used in this study showed high levels of *Striga* susceptibility during the gene introgression process, demonstrated by the high number of *Striga* emergence or attachments. All the three crosses between IT90-K-372-1-2 and IT93K-693-2; KVx30-309-6G and IT93K-693-2 and TN5-78 and IT93K-693-2 were compatible and able to generate progeny populations. The resistance gene *Rsg1* was tracked and introgressed into 2 different adapted varieties IT90-K-372-1-2 and TN5-78 using marker assisted selection. The cross between KVx30-309-6G and IT93K-693-2 yielded poor seeds at BC₂F₂ and BC₃F₁ generations resulting in the absence of resistant lines from these stages of gene introgression process. This could be attributed to the low compatibility of this particular cross. However, the combination of FTA technique of DNA extraction, a good source of *Striga* resistance, IT93K-693-2 having a single dominant gene and the use of a reliable marker contributed to transferring *Striga* resistance into farmer-preferred cowpea varieties. Marker assisted selection was used from early generations of backcrossing to BC₃F₃ generations while it was delayed to F₆ generations for the recombinant inbred lines. Phenotypic selection was applied in developing F₆ populations, but the precision of MAS enabled the identification of two individual resistant F₆ plants out of a total of seven genotyped. This result confirmed that conventional methods of breeding and DNA marker technology are complementary. In

this study, some individual plants selected as resistant based on their phenotype were identified later on as susceptible after a progeny test. This can be explained by the escape from *Striga* infection which may be due to the growing conditions in pots. This could be why 50% of individuals without the marker were free of *Striga* emergence in the phenotyping in pots. It can also be explained by crossing over during meiosis where the gene separates from the marker. Those progenies without the marker but carrying the resistance gene will be resistant while the progenies without both marker and gene will be susceptible. Meanwhile, all the genotypes with SSR₁ marker were *Striga* resistant because they were free of *Striga* infestation. This confirmed the efficiency of MAS over the conventional method which is time consuming, laborious and lacks precision. The earliness of some progenies derived from the different crosses over the parents can be explained by the transgressive segregation of flowering characters from these parents. The donor parent IT93K-693-2 is an early maturing breeding line as compared to farmers' preferred varieties.

6.5 Conclusions

In this study, marker assisted selection with backcross breeding coupled with successive selfing was used to introgress *Striga* resistance gene *Rsg1* into two adapted and farmer preferred cowpea varieties. The resistant genotypes were identified by the presence of single DNA band of 150bp by SSR₁ marker which was absent in the susceptible lines. The resistant BC₂F₃ and BC₃F₃ cowpea lines identified in the current study will have to be further advanced using marker assisted background selection to recover entirely the genome of the farmers' preferred varieties. After the 4th backcross, the lines will be selfed

before further evaluation and released to farmers. Also, the resistant F_6 lines will be advanced to F_8 before evaluation and release to farmers. Pyramiding of *Striga* resistance genes and other preferred traits identified as breeding priorities into elite cowpea varieties is becoming possible through MAS breeding which saves the breeder progeny testing. This will speed up the system of creating and delivery of new adapted varieties to farmers.

CHAPTER SEVEN

7.0 General Discussion and Recommendations

7.1 Introduction

Cowpea is an important food security crop in Africa because it complements the late-maturity and the low-protein content of the major grown cereals and tuber crops (Kay, 1979). Its relative drought tolerance and adaptability to a wide range of environments and cropping systems coupled with a high market value render cowpea a competitive crop in the global climate change. Cowpeas replaced groundnuts as Niger's principal cash crop since the 1960s. This was due to better performance of cowpea under deteriorating climatic conditions and the strong demand from Nigeria (Mazzucato and Ly, 1994).

Although cowpea production is increasing in Sub-Saharan Africa, many constraints threaten its production potential. *Striga gesnerioides* has become one of the main factors of yield loss in low-input farming systems of Niger. The resistant varieties developed so far are mostly introduced and lack farmers' preferred traits. Breeding efforts in the past used mainly mass selection and testing exotic varieties took very little account of stakeholders' perceptions. Therefore, a pre-requisite to increase cowpea production potential in the country is to understand farmers' preferences in adopting new varieties before breeding for superior varieties combining resistance to *Striga* and their preferred traits. This study was undertaken to screen cowpea germplasm for *Striga* resistance, conduct a differential variety test and introgress a multiracial *Striga* resistant gene into farmers' preferred varieties. The objectives were: (i) to interact with cowpea growers to assess their preferred traits and perceptions of *Striga* on cowpea production (ii) to identify

new sources of *Striga* resistance in the national and other available germplasm (iii) determine the presence of other *Striga* races besides SG3 in Niger (iv) to introgress the multiracial *Striga* resistance gene *Rsg1* into adapted cultivars and (v) to assess MAS for selection of *Striga* resistant genotypes.

7.2 Main findings and their implications in agricultural research

The major findings are summarized and discussed in the following paragraphs.

- In order to investigate cowpea breeding priorities in Niger a participatory rural appraisal (PRA) was conducted. **Cowpea was ranked the second most widely grown crop after millet and it was mostly intercropped with cereals.** The study revealed the strategic role of cowpea in the cropping system of the country characterized by poor management practices including low fertilization levels, lack of fallow and crop rotations. It was grown for food by 56% of farmers while 44% grew cowpea for cash. This is not in line with an earlier report which indicated that 80% of cowpea production was exported (Anon., 2013). This could be attributed to the contribution of seed companies' production which is essentially exported or to some unexpected circumstances that lead farmers to sell their production originally meant for home consumption.
- **Cowpea production constraints highlighted by farmers included insect pests followed by *Striga* and drought. The other constraints listed were low soil fertility, late planting, land insufficiency, poor access to inputs and disease pressure. Farmers had deep knowledge about *Striga*, its symptoms on**

cowpea and some of the control measures. Farmers' awareness of *Striga* as a serious challenge to cowpea production was indicated by the fact that this parasitic weed was ranked second among the constraints listed. They recommended that eradication of *Striga* was needed because of the ineffective control methods coupled with the high susceptibility of their landraces.

- **The most important traits for adopting varieties were yield potential, earliness, white-colored seed coat and taste.** Most farmers preferred big sized and white seeded cowpea for their consumption and the market as confirmed by traders and processors. Nonetheless, at Kollo district, 58% of farmers preferred small seeded cowpea for consumption while 55% chose it for market. This was probably due to the availability of this type of grain in the local market at the time of the survey. At Magaria district, 50% of farmers were more comfortable with brown colored cowpea for sale. The influence of Nigerian demand for brown cowpea could be the reason of such an option in that district.
- **Globally, the top three farmer preferred varieties across all the sites were KVx30-309-6G, IT90K-372-1-2 and TN5-78.** The first two are white-colored and large-sized cowpea varieties while the last variety is a large-sized and brown-colored variety. These varieties have become the recommended commercial varieties of cowpea maintained by the national agricultural research institute and produced by seed companies in Niger. The PRA showed therefore, that an ideal cowpea variety should combine many traits such as yield potential, earliness, white color, good taste and large seeds. The adoption of improved varieties could

be enhanced when breeders integrate farmers' preferences in their breeding objectives.

- Field screening was conducted in 2012 under natural *Striga* hot-spots at Maradi station to identify new sources of *Striga* resistance in the national germplasm and to select the best parents for yield improvement. **Genotypic variation exists among the genotypes screened. New sources of *Striga* resistance were not found;** however the varieties **IT93K-693-2, IT99K-573-1-1 and IT98K-205-8 were confirmed as potential sources of resistance and good donor parents to incorporate *Striga* resistance into well adapted genotypes. The cultivars B2/16/2378, B1/18/2542 and B1/12/2525-234 were tolerant to *Striga* with high yield potential compared to the resistant and the susceptible lines. Therefore, they could be used as donor parents in breeding cowpea for yield improvement in Niger.** In this study, the relative low yields of the resistant varieties observed coupled with the high susceptibility of the top three farmer-preferred varieties confirmed the need for transferring sources of *Striga* resistance into well adapted genotypes. To do that, the best combination of crosses are IT93K-693-2 that is resistant to all known *Striga* races with each of the top three farmers' preferred varieties: KVx30-309-6G, IT90K-372-1-2 and TN5-78.
- A differential variety test was conducted to find out whether there are some other *Striga gesnerioides* races in Niger. **The test revealed *Striga* attack on three multiracial *Striga* resistant varieties: B301, IT97K-499-35 and IT98K-205-8**

suggesting the presence of other races like SG4_z or its variants in the studied area, or the breakdown of the resistance to *Striga* race SG3 in these cowpea varieties. Also, the resistance of the varieties HTR and Suvita 2 observed respectively in the field at Kollo and in pots with Magaria sample suggested the prevalence of *Striga* race SG1 or its variants in these sites. This result is in line with previous reports that indicated *Striga* emergence on B301 with SG2 in Mali (Touré *et al.*, 1998) and on IT97K-499-35 with race SG3 in Nigeria (Kamara *et al.*, 2008; Omoigui *et al.*, 2012). From these observations, the geographical distribution of *Striga* races needs to be clarified. Further investigations would be needed to find out whether the mixture of seeds from two or more races could have infested the same field or the races were distinct within a unit area. On the other hand, the breakdown of resistance in some cultivars may be due to the recombination of *Striga* genes that overcome host resistance or by a combination of environmental factors that influence cowpea response.

- In order to combine stable and multiple *Striga* resistance with farmer preferred traits, marker assisted backcross breeding was undertaken to introgress *RsgI* resistance gene into adapted cowpea cultivars. The genetic populations were derived from three different crosses between IT93K-693-2, a line resistant to all known *Striga* races with poor agronomic traits and three farmers' preferred varieties identified in the PRA: KVx30-309-6G, IT90K-372-1-2 and TN5-78. One pair of primers SSR₁ was used to discriminate genomic DNA samples extracted with FTA cards. The marker had a monomorphic banding pattern and was

consistent in identifying resistant lines with a single band while the susceptible lines had no band. **The resistance gene *Rsg1* was successfully introgressed into IT90K-372-1-2 and TN5-78 using MAS. Ten individual plants including two individuals from BC₂F₃ population derived from the cross IT90K-372-1-2 x IT93K-693-2, two individuals from F₆ population derived from the cross IT90K-372-1-2 x IT93K-693-2, one individual from BC₃F₃ population derived from the cross TN5-78 x IT93K-693-2 and five individuals from BC₂F₃ population derived from the cross TN5-78 x IT93K-693-2 which contained the resistance marker were selected.** The marker was validated and recommended for use in marker assisted selection of cowpea varieties to *Striga* resistance. The resistant lines from BC₂F₃ and BC₃F₃ populations can be advanced to BC₄ generation followed by selfing before evaluation and release to farmers. The F₆ lines can be advanced to F₈ before evaluation and release.

7.3 Recommendations

- There is need for sustaining interaction between breeders and farmers as well as follow-up by extension services to increase adoption of improved varieties with recommended cultural practices.
- Breeding objectives should take into account yield potential, early maturity, white-colored and large-sized grain, good taste, resistance to pests and diseases and tolerance to drought. Focus should also be on brown-colored grain for the market, especially for Magaria district.

- Molecular screening of a representative collection of *Striga* samples throughout the country is an urgent need to characterize the races prevailing in Niger with their geographical distribution.
- The resistant lines selected through marker assisted selection would have to be advanced and evaluated before their release to farmers.
- There is need for confirmation of resistance under *Striga* infestation of the selected lines in the field.
- Gene pyramiding by introgression of traits such as insect resistance, drought tolerance and high yield potential combined with *Striga* resistance into elite cowpea varieties are recommended.

REFERENCES

- Adipala, E., Nampala, P., Karungi, J. & Isubikalu, P. (2000) A review on options for management of cowpea pests: experiences from Uganda. *Integrated Pest Management Reviews*, 5, 185-196.
- Aggarwal, V. & Ouedraogo, J. (1989) Estimation of cowpea yield loss from *Striga* infestation. *Tropical agriculture*, 66, 91-92.
- Aggarwal, V. D., Haley, S. D. & Brockman, F. E. (1986) Present status of breeding cowpea for resistance to *Striga* at IITA. *Proceedings of a workshop on biology and control of Orobanche, Wageningen (Netherlands), Jan 1986*. pp. 13-17.
- Ajeigbe, H., Ihedioha, D. & Chikoye, D. (2008). Variation in physico-chemical properties of seed of selected improved varieties of Cowpea as it relates to industrial utilization of the crop. *African Journal of Biotechnology*, pp. 7.
- Ajibade, S. R. & Morakinyo, J. A. (1999). Heritability and correlation studies in cowpea (*Vigna unguiculata* (L.) Walp). *Nigerian Journal of Genetics*, pp. 14.
- Alonge, S., Lagoke, S. & Ajakaiye, C. (2005) Cowpea reactions to *Striga gesnerioides* II. Effect on grain yield and nutrient composition. *Crop Protection*, 24, 575-580.
- Anonymous (2013). Amélioration de la production du niébé au Niger. *PROVIGNA*, 1, 14.
- Asante, S., Tamo, M. & Jackai, L. (2001) Integrated management of cowpea insect pests using elite cultivars, date of planting and minimum insecticide application. *African Crop Science Journal*, 9, 655-665.

- Asare, A. T., Galyuon, I. K., Padi, F. K., Otwe, E. P. & Takrama, J. (2013). Responses of recombinant inbred lines of cowpea [*Vigna unguiculata* (L.) Walp] to *Striga gesnerioides* infestation in Ghana. *European Scientific Journal*, 9, 11 p.
- Atokple, I., Singh, B. & Emechebe, A. (1993) Independent Inheritance of *Striga* and *Alectra* Resistance in Cowpea Genotype B30. *Crop Science*, 33, 714-715.
- Atokple, I., Singh, B. & Emechebe, A. (1995). Genetics of resistance to *Striga* and *Alectra* in cowpea. *Journal of Heredity*, 86, 45-49.
- Attah, E. S. (2002) Evaluation of maize-cowpea intercrop with selected cowpea (*Vigna unguiculata* (L.) Walp) cultivars, plant density and row arrangement. Ph.D thesis, University of Agriculture, Makurdi. 135pp.
- Ba, F. S., Pasquet, R. S. & Gepts, P. (2004) Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] as revealed by RAPD markers. *Genetic Resources and Crop Evolution*, 51, 539-550.
- Bationo, A., Ndaguru, B. J., Ntare, B. R., Christianson, C. B. & Vlokwunye, A. U. (1991) Fertilizer management strategies for legume-based cropping systems in the West African Semi-Arid Tropic. In: Johansen, C., Lee, K. K. and Sahrawat, K. I. (eds) Phosphorus Nutrition of Grain Legumes in the Semi-Arid Tropic. . ICRISAT, 1991, Pantacheru, A. P. India, 216-236.
- Berner, D., Kling, J. & Singh, B. (1995) *Striga* research and control. A perspective from Africa. *Plant Disease*, 79, 652-660.
- Berner, D. K., Winslow, M. D., Awad, A. E., Cardwell, K. F., Mohan Raj, D. R. and Kim S.K. (1997). *Striga research methods*. The Pan African *Striga* Control Network

- (PASCON)/ International Institute for Tropical Agriculture (IITA). Ibadan, Nigeria 80 pp.
- Berner, D. & Williams, O. (1998) Germination stimulation of *Striga gesnerioides* seeds by hosts and nonhosts. *Plant Disease*, 82, 1242-1247.
- Blackhurst, H. T. & Miller, J. C. (1980) Cowpea. *Hybridization of Crop Plants*, 327-337.
- Botanga, C. J. (2005) Genetic and molecular analyses of factors defining host-specificity and virulence in *Striga* species (Scrophulariaceae), Ph.D. thesis, University of Virginia, Charlottesville, VA. 215 p.
- Botanga, C. J. & Timko, M. P. (2005) Genetic structure and analysis of host and nonhost interactions of *Striga gesnerioides* (witchweed) from central Florida. *Phytopathology*, 95, 1166-1173.
- Botanga, C. J. & 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., Murdock, L. & Ohm, H. (2004a) AFLP and AFLP-Derived SCAR Markers Associated with Resistance in Cowpea. *Crop Science*, 44, 1259-1264.
- Butler, L. G. (1995) Chemical communication between the parasitic weed *Striga* and its crop host: A new dimension in allelochemistry. In *Allelopathy: Organism, Processes and Application*. Edited by Inderjit, K., Dakshini, M. M, Einhellig F. A., Washington, DC: *ACS Symposium Series*; 1995:158-168
- Cardwell, K. & Lane, J. (1995) Effect of soils, cropping system and host phenotype on incidence and severity of *Striga gesnerioides* on cowpea in West Africa. *Agriculture, ecosystems & environment*, 53, 253-262.

- Carsky, R., Akakpo, C., Singh, B. & Detongnon, J. (2003) Cowpea yield gain from resistance to *Striga gesnerioides* parasitism in Southern Benin. *Experimental Agriculture*, 39, 327-333.
- Chambers, R. (1992) Rural appraisal: rapid, relaxed and participatory. Discussion Paper 311. *Institute of Development Studies*, University of Sussex (UK), 68p.
- Charlick, R. B. (1992) Niger: *Personal rule and survival in the Sahel*, 91, 307-308.
- Choi, H.-K., Mun, J.-H., Kim, D.-J., Zhu, H., Baek, J.-M., Mudge, J., Roe, B., Ellis, N., Doyle, J. & Kiss, G. B. (2004) Estimating genome conservation between crop and model legume species. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 15289-15294.
- Darr, I. (1997) How *Striga* parasitizes its host: a TEM and SEM study. *Annals of Botany*, 79, 463-472.
- De Groote, H., Wangare, L., Kanampiu, F., Odoendo, M., Diallo, A., Karaya, H. & Friesen, D. (2008) The potential of a herbicide resistant maize technology for *Striga* control in Africa. *Agricultural Systems*, 97, 83-94.
- Dellaporta, S. L., Wood, J. & Hicks, J. B. (1983) A plant DNA miniprep: version II. *Plant molecular biology reporter*, 1, 19-21.
- Dembele, B. (1988) Aspects biologiques et agronomiques de deux scrophulariaceae parasites tropicales: *Striga hermonthica* (Del.) Benth. et *Striga gesnerioides* (Willd.) Vatke. Ph. D. Thesis, Montpellier, France, 100p.
- Dube, M. P. (2009) L'étude de la diversité génétique au sein des génomes nucléaires et chloroplastiques chez les cinq races connues du *Striga gesnerioides*, une plante parasite d'importance mondiale. Thèse de Doctorat, Université de Laval, 167p.

- Dube, M. P. & Olivier, A. (2001) *Striga gesnerioides* and its hosts, the cowpea: Interaction and methods of control. *Canadian journal of botany-revue canadienne de botanique*, 79: 1225-1240.
- Egbadzor, K., Yeboah, M., Offei, S., Ofori, K. & Danquah, E. (2013) Farmers' key production constraints and traits desired in cowpea in Ghana. *Journal of Agricultural Extension and Rural Development*, 5, 14-20.
- Egbe, O., Alibo, S. & Nwueze, I. (2010) Evaluation of some extra-early-and early maturing cowpea varieties for intercropping with maize in southern Guinea Savanna of Nigeria. *Agricultural and Biology Journal of North America*, 1, 845-858.
- Ehlers, J. & Foster, K. (1993) Introgression of agronomic characters from exotic cowpea germplasm into blackeye bean. *Field Crops Research*, 35, 43-50.
- Ehlers, J. D. & Hall, A. E. (1997) Cowpea (*Vigna unguiculata* L. Walp.). *Field Crops Research*, 53, 187-204.
- Ejeta, G. & Butler, L. (1993) Host-parasite interactions throughout the *Striga* life cycle, and their contributions to *Striga* resistance. *African Crop Science Journal*, 1, 75-80.
- Ejeta, G., Butler, L., Hess, D. & Vogler, R. (1991) Genetic and breeding strategies for *Striga* resistance in sorghum. *5th. International Symposium of Parasitic Weeds, Nairobi (Kenya), 24-30 Jun 1991*.
- Ekeleme, F., Jibrin, J., Kamara, A., Oluoch, M., Tofa, I., Solomon, R. & Kadafur, M (2013). Level and extension of *Striga* infestation of maize and cowpea crops in

- Bauchi and Kano states, Nigeria. *Biophysical Baseline Survey, International Institute of Tropical Agriculture*, Ibadan, Nigeria 49p.
- Ekpo, I. A., Agbor, R. B., Osuagwu, A. N., Okpako, E. C. & Ekanem, B. E. (2012) Evaluation of Eight Cowpea (*Vigna unguiculata* (L.) Walp) species for yield and associated traits. *Journal of Pure and Applied Sciences and Technology*, 12, 1-7.
- Elawad, H. O. A. & Hall, A. E. (1987) Influences of early and late nitrogen fertilization on yield and nitrogen fixation of cowpea under well-watered and dry field conditions. *Field Crops Research* 15, 229-244.
- Emechebe, A., Singh, B., Leleji, O., Atokple, I. & Adu, J. (1991) Cowpea Striga problems and research in Nigeria. *Combating Striga in Africa, edited by SK Kim. IITA, Ibadan, Nigeria*, 18-28.
- FAO (2007) Progress on farmers training on parasitic weed management Food and Agriculture Organization of the United States.
- Faostat (2012). *Statistics of cowpea [Online] Available* [<http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567>](http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567) [Accessed on 24/01/2015].
- FAOSTAT (2015). Statistics of cowpea production [Online] Available [<http://faostat3.fao.org/download/Q/QC/E>](http://faostat3.fao.org/download/Q/QC/E) [Accessed on 02/08/2014].
- Fatokun, C. (2002). Breeding cowpea for resistance to insect pests: Attempted crosses between cowpea and *Vigna vexillata*. In: Challenges and opportunities for enhancing sustainable cowpea production. C. A. Fatokun, Tarawali, S. A., Singh, 27 B. B., Kormawa, P.M., Tamo M. (ed.) International Institute of Tropical Agriculture (IITA). Ibadan, Nigeria 62-80.

- Fery, R. (1990). The cowpea: production, utilization, and research in the United States. *Horticultural Review* 12:197–222.
- Fery, R. & Singh, B. (1997) Cowpea genetics: a review of the recent literature. *Advances in Cowpea Research*, 29.
- Graves, J. D. (1992) The carbon canopy economy of the association between cowpea and the parasitic angiosperm *Striga gesnerioides*. Press MC Smith S. Stewart GR. *Plant, Cell & Environment*, 15, 283-288.
- Guissai, B. S. (2010) Screening of cowpea (*Vigna unguiculata* (L.) Walp.) varieties resistant to *Striga gesnerioides* (Willd.) Vatke at Maroua, Sudano-Sahelian zone of Cameroon. Master thesis, University of Ngaoundéré, Cameroon (In French).
- 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.
- Hausmann, B. I., Hess, D. E., Welz, H.-G. & Geiger, H. H. (2000) Improved methodologies for breeding *Striga* resistant sorghums. *Field Crops Research*, 66, 195-211.
- Ibro, G., Fulton, J. & Lowenberg-Deboer, J. (2006) Factors affecting success for women entrepreneurs in West Africa: the case of *Kossai*, a value added cowpea product. . *AAEA Selected Paper, Long Beach, California*, 19p.
- ICRISAT (2013) Tropical legume farming in Niger. *Bulletin of Tropical Legumes*, 22, 7p.

- Igbinnosa, I. & Okonkwo, S. (1991) Screening of tropical legumes for the production of active germination stimulants and for resistance to Nigerian cowpea witchweed (*Striga gesnerioides*). *Nigerian Journal of Weed Science*, 4, 1-9.
- INS (2010). Annuaire statistique des cinquante ans d'indépendance du Niger, 262-266
- INS (2013) Rapport National de Synthèse, Evaluation des Récoltes de la Campagne Agricole d'Hivernage 2012 et Résultats Définitifs 2012-2013. 39 p.
- IITA (2010). The quiet revolution. R4D, Review, Edition, 5, September 2010, p58.
- Joel, D., Losner-Goshen, D., Hershenhorn, J., Goldwasser, Y. & Assayag, M. (1996) The haustorium and its development in compatible and resistant hosts. *Congresos y Jornadas-Junta de Andalucia (Espana)*. JA, DGIA.
- Kabambe, V., Kanampiu, F., Nambuzi, S. & Kauwa, A. (2007) Evaluation of the use of herbicide (Imazapyr) and fertilizer application in integrated management of *Striga asiatica* in maize in Malawi. *African Journal of Agricultural Research*, 12, pp. 687-691.
- Kamara, A. Y., Chikoye, D., Ekeleme, F., Omoigui, L. O. & Dugje, I. Y. (2008) Field performance of improved cowpea varieties under conditions of natural infestation by the parasitic weed *Striga gesnerioides*. *International Journal of Pest Management*, 54, 189-195.
- Karkannavar, J., Venugopal, R. & Goud, J. (1991) Inheritance and linkage studies in cowpea (*Vigna unguiculata* (L.) Walp.) *Indian Journal of Genetics and Plant Breeding*, 51, 203-207.
- Karuri, H. W., Ateka, E. M., Amata, R., Nyende, A. B., Tmuigai, A. W., Mwasame, E. & Gichuki, S. T. (2010) Evaluating diversity among Kenyan sweet potato genotypes

- using morphological and SSR markers. *International Journal of Agricultural Biology*, 12, 33-38.
- Kay, D. E. (1979) Food legumes, crop and product digest, No.3. Natural Resources Institute, Chatten, UK. p. 214.
- Kormawa, P. M., Chianu, J. N. & Manyong, V. M. (2000) Cowpea Demand and Supply Pattern in West Africa: The case of Nigeria,” Proceedings of the World Cowpea Conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, 4–8 September 2000. IITA, Ibadan, Nigeria. pp 376 – 386.
- Kuiper, E., Verkleij, J. & Pieterse, A. (1996) Differences in the primary dormancy pattern of *Striga* species: an ongoing study. *Congresos y Jornadas-Junta de Andalucia (Espana)*.
- Lambot, C. (2000) Industrial Potential of Cowpea. *Proceedings of the World Cowpea Conference III* held at the International Institute of Tropical Agriculture (IITA), Ibadan,. 367 - 375.
- Lane, J., Bailey, J. & Terry, P. (1991). An "*in vitro*" growth system for studying the parasitism of cowpea (*Vigna unguiculata*) by *Striga gesnerioides*. *Weed Research*, 31, 211-217.
- Lane, J. & Bailey, J. (1992) Resistance of cowpea and cereals to the parasitic angiosperm *Striga*. *Euphytica*, 63, 85-93.
- Lane, J., Moore, T., Child, D. & Cardwell, K. (1996). Characterization of virulence and geographic distribution of *Striga gesnerioides* on cowpea in West Africa. *Plant Disease*, 80, 299-301.

- Lane, J., Moore, T., Child, D., Cardwell, K., Singh, B. & Bailey, J. (1993a). Virulence characteristics of a new race of the parasitic angiosperm, *Striga gesnerioides*, from southern Benin on cowpea (*Vigna unguiculata*). *Euphytica*, 72, 183-188.
- Lane, J. A., Bailey, J. A., Butler, R. C. & Terry, P. J. (1993b). Resistance of cowpea [*Vigna unguiculata* (L.) Walp.] to *Striga gesnerioides* (Willd.) Vatke, a parasitic angiosperm. *New Phytologist*, 405-412.
- Lane, J. A., Moore, T. H. M., Child, D. V., Cardwell, K. F., Singh, B. B. & Bailey, J. A. (1994) Virulence characteristics of a new race of the parasitic angiosperm, *Striga gesnerioides*, from southern Benin on cowpea (*Vigna unguiculata*). *Euphytica*, 72, 183-188.
- Langyintuo, A., 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*, 82, 215-231.
- Li, J., Lis, K. E. & Timko, M. P. (2009) Molecular genetics of race-specific resistance of cowpea to *Striga gesnerioides* (Willd.). *Pest Management Science*, 65, 520-527.
- Li, J. & Timko, M. P. (2009) Gene-for-gene resistance in *Striga*-cowpea associations. *Science*, 325, 1094.
- Louis, J. G. (2006). Le niébé, La plante [Online] Available <http://ntec.free.fr/spip.php?rubrique5> [Accessed on 12/10/2008].
- Maiti, R., Ramaiah, K., Bisen, S. & Chidley, V. L. (1984). A comparative study of the haustorial development of *Striga asiatica* (L.) Kuntze on sorghum cultivars. *Annals of Botany*, 54, 447-457.

- Makoi, J. H., Belane, A. K., Chimphango, S. B. & Dakora, F. D (2010). Seed flavonoids and anthocyanins as markers of enhanced plant defence in nodulated cowpea (*Vigna unguiculata* L. Walp.). *Field Crops Research*, 118, 21-27.
- Manggoel, W. & Uguru, M. I. (2011) Comparative study on the phenology and yield components of two photoperiodic groups of cowpea (*Vigna unguiculata* (L.) Walp.) in two cropping seasons. *African Journal of Agricultural Research*, 23, 5232-5241.
- Matusova, R., Rani, K., Verstappen, F. W., Franssen, M. C., Beale, M. H. & Bouwmeester, H. J. (2005) The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. *Plant physiology*, 139, 920-934.
- Mazzucato, V. & Ly, S. (1994) An economic analysis of research and technology transfer of Millet, Sorghum and Cowpea in Niger. ISNAR, The Hague, Netherlands. *MSU International Development Working Papers*, 121p.
- MDA (2013). Direction des statistiques/Ministère du développement agricole du Niger, pp. 39.
- MDA (2015). Direction des statistiques/Ministère du développement agricole du Niger, pp. 32.
- Menéndez, C. M., Hall, A. E. & Gepts, P. (1997) A genetic linkage map of cowpea (*Vigna unguiculata*) developed from a cross between two inbred, domesticated lines. *Theoretical and Applied Genetics*, 95, 1210-1217.
- Mohamed, A., Ellicott, A., Housley, T. & Ejeta, G. (2003). Hypersensitive response to *Striga* infection in sorghum. *Crop Science*, 43, 1320-1324.

- Mohamed, K. I., Musselman, L. J. & Riches, C. R. (2001). The genus *Striga* (Scrophulariaceae) in Africa. *Annals of the Missouri Botanical Garden*, 60-103.
- Mohapatra, T., Sharma, R. & Chopra, V. (1992). Cloning and use of low copy sequence genomic DNA for RFLP analysis of somaclones in mustard (*Brassica juncea* (L.) Czren and Coss). *Current Science*, 62, 482-484.
- Moore, T., Lane, J., Child, D., Arnold, G., Bailey, J. & Hoffmann, G. (1995) New sources of resistance of cowpea (*Vigna unguiculata*) to *Striga gesnerioides*, a parasitic angiosperm. *Euphytica*, 84, 165-174.
- Muleba, N., Ouedraogo, J. T. & Tignegre, J. B. (1997) Cowpea yield losses attributed to *Striga* infestations. *Journal of Agricultural Science* 129, 43-48.
- Muranaka, S., Boukar, O., Abdoulaye, A. & Fatokun, C. A. (2010) Cowpea improvement via farmer participatory varietal selection activity in the Republic of Niger. *Proceedings of the Fith World Cowpea Conference. IITA*, 46-55.
- Musselman, L. J. & Ayensu, E. S. (1984). Taxonomy and biosystematics of *Striga*. In: E.S. Ayensu, H. Doggett, H.D. Keynes, J. Marton-Lefevre, L.J. Musselman, C. Parker et A. Pickering (Éds). *Striga*, Biology and Control. Workshop on the Biology and Control of *Striga* . ICSU Press, Dakar, Sénégal. p. 37-45.
- Musselman, L. J. & Parker, C. (1981) Studies on indigo witchweed, the American strain of *Striga gesnerioides* (Scrophulariaceae). *Weed Science*, 594-596.
- Myers, G. O. (1996) Hand crossing of cowpeas. *IITA Research Guide 42 (IITA)*, 21p.
- Ng, N. & Maréchal, R. (1985) Cowpea taxonomy origin and germplasm. *Cowpea research production and distribution. Jonh Wiley, New York*, pp. 11-21.

- Ng, N. & Padulosi, S. (1988) Cowpea genepool distribution and crop improvement. In: Ng NQ, Perrino P, Attere F, Zedan H (eds.), *Crop Genetic Resources of Africa*, Vol II. IBPGR, Rome, pp. 161–174
- Nielson, S. S., Brandt, W. E. & Singh, B. B. (1993) Genetic variability for nutritional composition and cooking time of improved cowpea lines. *Crop Science*, 33, 469-472.
- Omanya, G. O., Haussmann, B. I. G., Hess, D. E., Reddy, B. V. S., Kayentao, M., Welz, H. G. & Geiger, H. H. (2004) Utility of indirect selection traits for improving *Striga* resistance in two Sorghum recombinant inbred populations. *Field Crops Research*, 89, 237-252.
- Omoigui, I. O., Timko, M. P., Ishiyaku, F. S., Ousmane, B., Muranaka, B. S., Kamara, A. Y. & Yeye, M. Y. (2009) Molecular characterization of cowpea breeding line for *Striga* Resistance using FTA technology. *African Crop Science Conference, Uganda*, 9, 527-530.
- Omoigui, L., Kamara, A., Ishiyaku, M. & Boukar, O. (2011) Comparative responses of cowpea breeding lines to *Striga* and *Alectra* in the dry savanna of northeast Nigeria. *African Journal of Agricultural Research*, 7, 747-754.
- Omoigui, L. O., Yeye, M., Ousmane, B., Gowda, B. S. & Timko, M. P. (2012) Molecular characterization of cowpea breeding lines for *Striga* resistance using SCAR markers. *Journal of Agricultural Science and Technological Biology*, 2, 362-367.
- Omoigui, L. O., Ishiyaku, F. S., Gowda, B. S., Kamara, A. Y. & Timko, M. P. (2015) Suitability and use of two molecular markers to track race-specific resistance

- Striga gesnerioides* in cowpea (*Vigna unguiculata* (L.) Walp.). *African Journal of Biotechnology*, 14, 2179-2190.
- Orawu, M. (2007) Occurrence of cowpea aphid-borne mosaic virus and prospects of improving resistance in local cowpea landraces in Uganda. Ph.D thesis. School of Biochemistry, Genetics, Plant pathology and Microbiology. University of Kwazulu-Natal, South Africa., University of Kwazulu-Natal, 171p.
- Ouédraogo, J. T., Maheshwari, V., Berner, D. K., St-Pierre, C. A., Belzile, F. & Timko, M. P. (2001) Identification of AFLP markers linked to resistance of cowpea (*Vigna unguiculata* L.) to parasitism by *Striga gesnerioides*. *Theoretical and Applied Genetics*, 102, 1029-1036.
- Ouédraogo, J. T., Gowda, B. S., Jean, M., Close, T. J., Ehlers, J. D., Hall, A. E., Gillaspie, A. G., Roberts, P. A., Ismail, A. M., Bruening, G., Gepts, P., Timko, M. P. & Belzile, F. J. (2002a) An improved genetic linkage map for cowpea (*Vigna unguiculata* L.) Combining AFLP, RFLP, RAPD, biochemical markers, and biological resistance traits. *Genome*, 45, 175-188.
- Ouédraogo, J. T., Tignegre, J.-B., Timko, M. P. & Belzile, F. O. J. (2002b) AFLP markers linked to resistance against *Striga gesnerioides* race 1 in cowpea (*Vigna unguiculata*). *Genome*, 45, 787-793.
- Ozenda, P. & Capdepon, M. (1972) Recherches sur les phanerogames parasites: 2. Une Scrophulariaceae holoparasite meconnue, *Striga gesnerioides*. *Phytomorphology*, 22, 314-324.
- Padulosi, S. & Ng, N. (1997) Origin, taxonomy, and morphology of *Vigna unguiculata* (L.) Walp. *Advances in Cowpea Research*, 1-12.

- Parker, C. (1991) Protection of crops against parasitic weeds. *Crop Protection*, 10, 6-22.
- Parker, C. & Polniaszek, T. I. (1990) Parasitism of cowpea by *Striga gesnerioides*: variation in virulence and discovery of a new source of host resistance. *Annals of Applied Biology*, 116, 305-311.
- Pasquet, R. (1999) Genetic relationships among subspecies of *Vigna unguiculata* (L.) Walp. based on allozyme variation. *Theoretical and Applied Genetics*, 98, 1104-1119.
- Phillips, R. D., Mcwatters, K. H., Chinannan, M. S., Hung, Y., Beuchat, L. R., Sefah-Dedeh, S., Saki-Dawson, E., Ngoddy, P., Nnanyelugo, D., Enwere, J., Komey, N. S., Liu, K., Mensa-Wilmot, Y., Nnanna, I., Okeke, C., Prinyawiwatkul, W. & Saalia, F. K. (2003) Utilization of cowpeas for human food. *Field Crops Research*, 82, 193-213.
- Pieterse, A. & Pesch, C. (1983) The witchweeds (*Striga* spp.). *Abstracts on Tropical Agriculture (Netherlands)*, 9, 9-34.
- Rachie, K. & Roberts, L. (1974) Grain legumes of the lowland tropics. *Advances in agronomy*, 132p.
- Ralston, D., Riches, C. & Musselman, L. (1987) Morphology and hosts of three *Striga* species (Scrophulariaceae) in Botswana. *Bulletin du Museum national d'histoire naturelle. Section B, Adansonia*, 9, 195-215.
- Ramaiah, K. V. (1987) Breeding cereal grains for resistance to witchweed. In: *Parasitic Weeds in Agriculture*. Musselman, L. J. (ed.) Boca Raton, Florida, USA 1: . 227-242.

- Ramaiah, K. V., Chidley, V. L. & House, L. R. (1990) Inheritance of *Striga* seed-germination stimulant in sorghum. *Euphytica*, 45, 33-38.
- 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.
- Raynaut, C., Koechlin, J., Basset, P., Cheung, C. & Stigliano, M. (1984) Le développement rural de la Région de Maradi : Analyser et comprendre la diversité. GRID, Université de Toulouse.
- Redden, R., Drabo, I. & Aggarwal, V. (1984) IITA programme of breeding cowpeas with acceptable seed types and disease resistance for West Africa. *Field Crops Research*, 8, 35-48.
- Reiss, G. C., Pring, R. J., Lane, J. A. & Bailey, J. A. (1995) *Striga gesnerioides*: mechanisms of infection and resistance. *Aspects of Applied Biology*, 42, 301-306.
- Reiss, G. & Bailey, J. (1998) *Striga gesnerioides* parasitising Cowpea: Development of Infection Structures and Mechanisms of Penetration. *Annals of Botany*, 81, 431-440.
- Riopel, J. & Timko, M. (1995) Haustorial initiation and differentiation. *Parasitic plants*, 39-79.
- Rispail, N., Dita, M. A., González-Verdejo, C., Pérez-De-Luque, A., Castillejo, M.-A., Prats, E., Román, B., Jorrín, J. V. & Rubiales, D. (2007) Plant resistance to parasitic plants: molecular approaches to an old foe. *New Phytologist* 173, 703-712.

- Rubiales, D., Perez-De-Luque, A., Fernandez-Aparico, M., Sillero, J. C., Romã;N, B., Kharrat, M., Khalil, S., Joel, D. M. & Riches, C. (2006) Screening techniques and sources of resistance against parasitic weeds in grain legumes. *Euphytica*, 147, 187-199.
- Saidou, A. K., Ajeigbe, H. A. & Singh, B. B. (2011) Participatory evaluation of improved cowpea lines and cropping systems for enhancing food security and income generation in Niger republic. *Journal of Agriculture and Environmental Science*, 11, 55-61.
- Sanden, B. (1993) Blackeye varietal and irrigation cutoff trial. In: University of California Dry Bean Research – 1993. Progress Report California Dry Bean Advisory Board, Dinuba, CA, pp 120–121
- Sanginga, N., Dashiell, K. E., Diels, J., Vanlauwe, B., Lyasse, O., Carsky, R. J., Tarawali, S., Asafo-Adjei, B., Menkir, A. & Schulz, S. (2003) Sustainable resource management coupled to resilient germplasm to provide new intensive cereal-grain-legume-livestock systems in the dry savanna. *Agriculture, ecosystems & environment*, 100, 305-314.
- Singh, B. & Emechebe, A. (1990) Inheritance of *Striga* resistance in cowpea genotype B301. *Crop Science*, 30, 879.
- Singh, B. D. (1993) Pedigree selection. In: *Plant breeding: Principles and methods*. Kalyani (ed). New Delhi, India 215-226.
- Singh, B. B. & Emechebe, A. M. (1997) Advances in research on cowpea *Striga* and *Alectra*. *Advances in Cowpea Research*, 215-224.

- Singh, B. B. (2002) Breeding cowpea varieties for resistance to *Striga gesnerioides* and *Alectra vogelii*. *Challenges and Opportunities for Enhancing Sustainable Cowpea Production*, 154-163.
- Singh, B.B, Ehlers, J.D., Sharma, B. & Freire Filho, F.R. (2002). Recent progress in cowpea breeding. In Fatokun, C.A., Tarawali, S.A., Singh, B.B., Kormawa, P.M. & Tamò, M. (Éds). *Challenges and opportunities for enhancing sustainable cowpea production*. Proceedings of the World Cowpea Conference III, p. 22-40. IITA, Ibadan, Nigeria.
- Singh, B. B., Ajeigbe, H. A., Tarawali, S. A., Fernandez-Rivera, S. & Abubakar, M. (2003) Improving the production and utilization of cowpea as food and fodder. *Field Crops Research*, 84, 169-177.
- Singh, B. (2006) Cowpea breeding at IITA: Highlights of advances impacts. *Congresso nacional de*, 4p.
- SNV (2008) *Etude de la filière niébé dans la région de Maradi, Juillet 2008.*, 59 p.
- SNV (2009) *Etude de référence sur la filière niébé dans la région de Zinder.* 80p.
- Sulnathi, G., Prasanthi, L. & M., R. S. (2007) Character contribution to diversity in cowpea. *Legume Research*, 30: 70-72.
- Tchiagam, J. B. N., Bell, J. M., Birwe, S. G., Gonne, S. & Youmbi, E. (2010) Varietal response of cowpea (*Vigna unguiculata* (L.) Walp.) to *Striga gesnerioides* (Willd.) Vatke race SG5 infestation. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 38: 33-41.
- 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.

Tignegre, J. B. S. (1988) Study of the resistance of selected cowpea lines to *Striga gesnerioides*. Institut du developement rural (IDR). B. Sc. University of Ouagadougou, Burkina Faso, 47p.

Tignegre, J. B. D. L. S. (2010) Genetic study of cowpea (*Vigna unguiculata* (L.) Walp) resistance to *Striga gesnerioides* (Willd.) Vatke in Burkina Faso. Ph.D thesis. School of Agricultural Sciences and Agribusiness. University of Kwazulu-Natal, South Africa, 167p.

Timko, M. P., Ehlers, J. D. & Roberts, P. A. (2007) Cowpea. *Pulses, Sugar and Tuber Crops*, 49-67.

Timko, M. P. & Singh, B. (2008) Cowpea, a multifunctional legume. *Genomics of Tropical Crop Plants*, 227-258.

Toure, M., Olivier, A., Ntare, B., Lane, J. & St-Pierre, C. A. (1997) Inheritance of resistance to *Striga gesnerioides* biotypes from Mali and Niger in cowpea (*Vigna unguiculata* (L.) Walp.). *Euphytica*, 94, 273-278.

Touré, M., Olivier, A., Ntare, B. R., Lane, J. A. & St-Pierre, C. A. (1998) Reaction of cowpea (*Vigna unguiculata*) cultivars to *Striga gesnerioides* races from Mali and Niger. *Canadian Journal of Plant Science*, 78: 477-480.

Vasudeva Rao, M. & Musselman, L. (1987) Host specificity in *Striga* spp. and physiological strains. In: Musselman L. J. (ed). *Parasitic Weeds in Agriculture*, Volume I *Striga*. CRC Press: Boca Raton, FL, pp. 13–15.

Wikipedia (2013) Regions and Departments of Niger: Available

<<http://en.wikipedia.org/wiki/Magaria-Department>> (Consulted 11 Nov. 2013)

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

Worsham, A. D. & Musselman, L. (1987) *Germination of witchweed seeds*, CRC Press Inc.

Wunderlin, R., Musselman, L. & Shuey, A. (1979) *Striga gesnerioides* (Scrophulariaceae), first record of the species in the New World [Weed of crop and ornamental plants, Florida, North Carolina, South Carolina]. *Plant Disease Reporter*.

Zahran, E., Sauerborn, J., Elmagid, A. A., Abbasher, A. & Muller-Stover, D. (2008) Granular formulations and seed coating: delivery options for two fungal biological control agents of *Striga hermonthica*. *Journal of Plant Diseases and Protection*, 115, 178-185.

APPENDICES

Appendix 3.1 Check list for PRA

1. General information on cowpea production

- 1.1 What is the rank of cowpea among the crops you grow?
- 1.2 Is it grown mostly pure or intercropped? What is the ratio intercropped: pure? In which season?
- 1.3 Is it used mostly as cash crop and /or food crop?
- 1.4 What are the different uses of leaves, seeds, stems, roots in terms of human, animal consumption and medical purposes? Classify them in order of importance?
- 1.5 What is your motivation in choosing the first or the second type of production?

2. Production systems used

- 2.1 Are you practicing rotation? Which crops do you use in rotation with cowpea and what is the planning in time?
- 2.2 Do you use manure or do you incorporate residues in your farm with cowpea?
- 2.3 Do you realize a decrease of fertility and rainfall?

3. Cowpea farming

- 3.1 What is the average size of your farms?
- 3.2 What varieties do you grow actually in your farm? The color? The cycle?
- 3.3 Which one do you prefer? Why?
- 3.4 Where do you get your seeds from?

3.5 Do you use fertilizers? Which types? At which stages of growth? Spreading or localized?

3.6 Please quote in order the different cultural practices from sowing to harvesting. Quote in order the most difficult in time spent and labor.

4. Choice of new varieties by farmers

What are your preferred traits used as criteria in adopting any new variety: **color, maturity cycle, size, taste, cooking time, yielding....**

5. Production constraints

5.1 What are cowpea production constraints?

5.2 Choose the major ones in order.

6. Local knowledge on *Striga* and control methods used

6.1 What do you know about *Striga*?

6.2 Is It useful ?

6.3 In your view what causes *Striga* infestation?

6.4 Why is it a problem?

6.5 Among the varieties you grow which ones are attacked by *Striga*? And in which order ?

6.6 How do you control it?

7. Level of adoption of new technologies by farmers

- 7.1 Do you inquire about the availability of improved seeds from agricultural offices, INRAN, from foreign countries (Nigeria) or from your colleagues (farmers)?
- 7.2 Have you ever been trained on fertilization, crop association, sowing density or any other training in agriculture?
- 7.3 Are you assisted by extension officers?
- 7.4 Who advise you in the choice and the application of chemicals and fertilizers?

(7) Which type is easier to cook?

.....

.....

(8) Which color is most suitable for preparing local meals?

.....

.....

Appendix 3.3 : Questionnaire to cowpea traders

Section 1: Information

Investigator' name -----

Questionnaire no.

Locality

Date of survey.....

Section 2: Respondent identity

(9) Name

(10) Gender: Male [] Female []

(11) Age: 11-20 [] 31-40 [] 51-60 []

21-30 [] 41-50 [] above 60 []

Section 3: Cowpea commercialization

(12) What type of cowpea grain do you sale?

White [] Black [] Red [] Brown [] Mottled []

Other []

(13) What type of color is most preferred at market?

White [] Black [] Red [] Brown [] Mottled []

Other []

(6) How prices fluctuate throughout the year?

.....
.....
.....

(7) What are the sources of the grains you sell?

.....
.....

(8) Which source is most important?

.....

(9) Cite the different storage

constraints.....

.....
.....
.....

(10) Which is most important?

Appendix 4.1: *Striga* density of cowpea germplasm tested in the field at Maradi in 2012.

Genotypes	<i>Striga</i> density (shoots.m ⁻²)
B4/1/2381-63 (2eR)	9.13
B2/16/2378 (1ereR)	8.77
B4/13/2563-245 1R	8.27
IT90K-372-1-2	7.84
B5/15/2627 (2eR)	7.84
B1/14/2473-155	7.53
KVx771-10G	7.04
B3/18/2525-30	6.48
B6/2/2516	6.42
HTR	6.23
B2/10/2457-119	6.17
B3/3/2350-32 (1)	6.05
B3/18/2381-63	5.43
2505 (sac)	5.37
B1/13/2614-296 (2e R)	5.37
B5/19/2410-92	5.31
B1/4/2413-95	5.06
2450-132 (sac)	4.57
2462-144 (sac)	4.57
B2/19/2405-87 (2e R)	4.50
2326 (Etq)	4.38
Mean	3.37
CV (5%)	3.75
LSD (5%)	69.04

CV: Coefficient of variation (5%); LSD: Least Significant Difference at p = 0.05

Appendix 4.1 continued

Genotypes	<i>Striga</i> density (shoots.m ⁻²)
2504-186 (Etq)	4.38
B4/9/2610 (2eR)	4.32
TN88-63	3.89
B4/14/2343-25 (1)	3.76
2420-102 (sac)	3.64
2510-192 (Etq)	3.52
B1/18/2542 (2e R)	3.52
B4/7/2338-20 (2eR)	3.46
B3/4/2507 (2eR)	3.46
B1/1/2409-91 (1)	3.46
B1/9/2320-02	3.46
B1/6/2356-38	3.46
2432-144 (Etq)	3.46
B6/3/392-74	3.40
2354 (Etq)	3.33
B5/11/2492	3.15
B3/17/2458-140 (2)	3.09
B5/13/2374-156	3.03
B6/14/2472-154	3.03
2427 (Etq)	3.02
B1/12/2525-234	2.84
Mean	3.37
CV (5%)	3.75
LSD (5%)	69.04

CV: Coefficient of variation (5%); LSD: Least Significant Difference at p = 0.05

Appendix 4.1 continued

Genotypes	<i>Striga</i> density (shoots.m ⁻²)
B1/5/2354 2R	2.84
2390-72 (Sac)	2.78
2409 (Etq)	2.78
2431-113 (Etq)	2.78
2374-56 (sac)	2.59
B3/13/2399-81 A (1)	2.53
2477-152 (Etq)	2.53
2598 (Etq)	2.53
B3/9/2526-200	2.47
2326 (sac)	2.28
2400-82 2R	2.22
2458-140 (sac)	2.22
2392-74 (Etq)	2.22
B2/12/2472-150	2.10
B3/20/2323 2R	2.10
2383 (Etq)	2.04
B6/15/2367-58	1.91
2610 (Etq)	1.85
B1/16/2470-152	1.85
2432 (Etq)	1.79
B4/8/2436-118	1.73
Mean	3.37
CV (5%)	3.75
LSD (5%)	69.04

CV: Coefficient of variation (5%); LSD: Least Significant Difference at p = 0.05

Appendix 4.1 continued

Genotypes	<i>Striga</i> density (shoots.m ⁻²)
B5/12/2462-144 (2eR)	1.73
TN27-80	1.67
B4/2/2491-171	1.61
TN5-78	1.54
2429-111 (sac)	1.36
KVx30-309-6G	1.24
2649-151 (Etq)	1.18
2367-58 (sac)	1.18
2372-54 (sac)	1.17
TN 121-80	1.11
2491-191 (Sac)	0.99
Suvita-2	0.87
2472-154 (Sac)	0.74
2491-171 (Etq)	0.43
IT98K-205-8	0.00
IT93K-693-2	0.00
IT99K-573-1-1	0.00
Mean	3.37
CV (5%)	3.75
LSD (5%)	69.04

CV: Coefficient of variation (5%); LSD: Least Significant Difference at p = 0.05

Appendix 4.2: Yield (Kg.ha⁻¹) of cowpea germplasm tested in the field at Maradi in 2012.

Genotypes	Yield (Kg.ha ⁻¹)
B1/18/2542 (2e R)	691.67
B1/12/2525-234	671.05
B3/13/2399-81 A (1)	573.21
B4/7/2338-20 (2eR)	561.79
B2/16/2378 (1ereR)	502.78
B1/13/2614-296 (2e R)	477.16
B2/19/2405-87 (2e R)	476.79
B1/4/2413-95	441.17
TN 121-80	432.72
2372-54 (sac)	430.93
B3/20/2323 2R	422.90
B6/3/392-74	421.91
B1/6/2356-38	389.63
IT93K-693-2	380.00
B3/18/2525-30	355.49
KVx30-309-6G	347.28
IT99K-573-1-1	340.74
B4/2/2491-171	335.99
B3/17/2458-140 (2)	312.16
B6/14/2472-154	299.01
B2/12/2472-150	294.51
Mean	246.12
CV (5%)	50.06
LSD (5%)	193.68

CV: Coefficient of variation (5%); LSD: Least Significant Difference at p = 0.05

Appendix 4.2 continued

Genotypes	Yield (Kg.ha ⁻¹)
2432-144 (Etq)	292.04
2462-144 (sac)	291.60
B4/1/2381-63 (2eR)	287.16
2458-140 (sac)	281.17
IT98K-205-8	276.73
B1/9/2320-02	268.09
HTR	266.67
2472-154 (Sac)	258.15
2491-191 (Sac)	255.12
B1/1/2409-91 (1)	254.01
2354 (Etq)	251.91
B5/19/2410-92	250.19
TN5-78	249.50
2491-171 (Etq)	244.75
KVx771-10G	238.95
2477-152 (Etq)	237.84
TN88-63	230.00
B3/9/2526-200	222.04
2432 (Etq)	219.13
B5/13/2374-156	219.01
B4/9/2610 (2eR)	217.04
Mean	246.12
CV (5%)	50.06
LSD (5%)	193.68

CV: Coefficient of variation (5%); LSD: Least Significant Difference at p = 0.05

Appendix 4.2 continued

Genotypes	Yield (Kg.ha ⁻¹)
2326 (Etq)	212.16
2427 (Etq)	210.31
B4/8/2436-118	200.49
B1/5/2354 2R	195.62
B1/14/2473-155	194.26
2420-102 (sac)	193.83
B2/10/2457-119	190.43
B6/15/2367-58	183.46
2598 (Etq)	173.52
B5/11/2492	170.37
TN27-80	170.19
2504-186 (Etq)	165.80
B3/3/2350-32 (1)	163.40
B3/4/2507 (2eR)	162.35
IT90K-372-1-2	150.37
B4/14/2343-25 (1)	146.30
2390-72 (Sac)	145.80
Suvita-2	141.73
2326 (sac)	141.73
2409 (Etq)	139.44
B1/16/2470-152	138.95
Mean	246.12
CV (5%)	50.06
LSD (5%)	193.68

CV: Coefficient of variation (5%); LSD: Least Significant Difference at p = 0.05

Appendix 4.2 continued

Genotypes	Yield (Kg.ha ⁻¹)
2383 (Etq)	133.89
2400-82 2R	116.05
2649-151 (Etq)	114.81
2392-74 (Etq)	108.27
2431-113 (Etq)	108.24
2450-132 (sac)	107.22
B5/12/2462-144 (2eR)	106.67
B5/15/2627 (2eR)	105.62
2367-58 (sac)	105.56
B4/13/2563-245 1R	98.27
2374-56 (sac)	87.78
B3/18/2381-63	82.90
B6/2/2516	80.93
2610 (Etq)	79.32
2505 (sac)	72.41
2429-111 (sac)	66.67
2510-192 (Etq)	54.88
Mean	246.12
CV (5%)	50.06
LSD (5%)	193.68

CV: Coefficient of variation (5%); LSD: Least Significant Difference at p = 0.05