GENETIC STUDIES OF PEARL MILLET [*Pennisetum glaucum* (L.) R. Br.] FOR RESISTANCE TO *Striga hermonthica*

By

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON, IN PARTIAL FULFILMENT 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 2018

DECLARATION

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

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ABSTRACT

Pearl millet is a major food crop in the northern part of Nigeria. However, its production has been constrained due to several factors, among them is *Striga hermonthica*. To understand pearl millet farmers' varietal preference traits, production constraints and knowledge of *Striga* infestation, a participatory rural appraisal was conducted across six districts in three local government areas of Jigawa State in Nigeria. Data were sourced using a semi-structured questionnaire among 143 farmers that involved focus group discussion sessions were used to verify information gathered from the questionnaires. Results showed that resistance to *Striga* infestation, resistance to downy mildew, tolerance to shattering, lodging tolerance and good quality local beverage were the most preferred traits by farmers. Major production constraints identified by farmers across the study areas were poor soil fertility, *Striga* infestation, downy mildew, and high cost of labour. Farmers were well informed about *Striga* as a menace and emphasized that it is likely to become worse unless controlled. A germplasm collection composed of 240 accessions of pearl millet were screened under natural *Striga hermonthica* infestation in the field. The results showed significant variation in the resistance of pearl millet genotypes to *Striga*. Fifteen genotypes identified as the most resistant were free of emerged *Striga* shoots, 10 genotypes supported 1-4 *Striga* shoots but with appreciable yield and 15 genotypes with 1-4 *Striga* shoots and low grain yield. Principal Component and cluster analyses grouped these genotypes into three main clusters as medium yielding tolerant to *Striga*, low yielding susceptible to *Striga* and medium to high yielding resistant to *Striga*. The high level of resistance observed in some breeding lines enabled the selection of suitable parents for population development. Two resistant and two susceptible to *Striga* genotypes selected from the field screening were used to study the gene effects controlling resistance to *Striga* in pearl millet. Six basic generations each were developed for the two sets of crosses and evaluated for resistance to *Striga* in the field. Results showed significant variation for resistance to *Striga* among genotypes and genotype by environments interactions. Additive and non-additive of gene actions were responsible for three indices of *Striga* resistance assessed; area under *Striga* number progress curve, *Striga* damage rating and the number of tillers and these varied from cross to cross and from environment to environment. In addition, a genome-wide association study (GWAS) aimed at identifying genomic loci and SNP markers associated with *Striga* resistance was performed using 188 pearl millet lines and 4802 SNP markers. Genotyping was achieved using the Diversity Array Technology (DArT) high-throughput genotyping-by-sequencing (GBS). Results revealed the presence of two sub-groups and a third mixed group were identified among the lines tested. Sixteen significant SNP markers associated with area under *Striga* number progress curve (ASNPC) were identified on five chromosomes; 2, 3, 4, 5 and 7 across all the three locations. The QTLs on chromosome 5, 2 and 3 accounted for 11 to 13% phenotypic variation. Six significant SNPs were detected for tiller number and two of these SNPs located on chromosome 1 and 5 were consistent across two locations. New SNPs were identified for *Striga* resistance and number of tillers (NTL). These need to be validated and fine-mapped for their use in marker-assisted selection for *Striga* resistance in pearl millet. The study further investigated genetic variation in production of strigolactone and tillering ability in ten pearl millet lines selected from the mapping population used for GWAS. Two major strigolactones; orobanchol and 5-deoxystrigol were identified and characterized. Five pearl millet lines; 16_R, 153_R, 96_R, 172_S and 140_S produced the highest level of orobanchol. All the test lines produced significantly lower 5-deoxystrigol than IBL (susceptible control) except 158_R. The susceptible lines 172_S and140_S induced highest *Striga* germination. The lines 153_R and 196_R that produced high orobanchol had the highest number of tillers. There is a positive correlation between orobanchol production and tillers production for 153_R and 96_R and a negative correlation between orobanchol and 5-deoxystrigol. A weak positive correlation was observed between *Striga* germination and orobanchol. These results demonstrated that genetic variability in the number of tillers is related to genetic variation in Strigolactone production and could be used as a morphological marker to select lines with low susceptibility to *Striga* infestation.

This study provides useful information to plant breeders for improving pearl millet for resistance to *Striga*. With the recent availability of pearl millet reference genome, candidate genes can be identified and validated before finally used in plant breeding strategy.

DEDICATION

To my beloved husband (Mallam Ibrahim A. Koguna), our children, my mother (Hajia Attahire Jiddah) and all who contributed to the success of this work.

ACKNOWLEDGMENTS

I extend my gratitude to the West Africa Agricultural Productivity Programme for funding my training and Lake Chad Research Institute Maiduguri for nominating me for the sponsorship. I am grateful for the German Academic Exchange Service (DAAD) for funding parts of the training.

I am thankful to my supervisory team; Professors P. Tongoona, Dr. Angarawai I. Ignatius, Professor Kwadwo Ofori and Dr. John S. Y. Eleblu for their support, advice, and guidance during the planning, execution, and reviewing the content of this dissertation. I am thankful to Dr. Beatrice Elohor Ifie and Dr. Abdallah Dao for guidance and reviewing of this thesis. My appreciation also goes to ICRISAT-Kano for supporting me throughout the period of this training especially Dr. Hakeem Ajiegbe and his team.

I could not have done part of this thesis without funding from Islamic development Bank fund (IDB) Saudi Arabia and guidance from Professor Salim Albabili and supervision from Boubacar Kountche, thank you.

I would like to thank Dr. Damaris Odenyi for her assistance in conducting the polymorphic survey at BecA-ILRI. My special appreciation goes to Professor Clay Snella for his assistance during my genotyping. I am indebted to Prof. Eric Danquah, the Director of West Africa Centre for Crop Improvement (WACCI) for supporting me with funds to conduct parts of this work and to all WACCI team for their support. The support from the Federal Ministry of Agriculture and Rural Development Nigeria in paying part of the tuition fees and facilitation by Dr. Sheu Salau of World Bank is acknowledged. I am thankful to Dr. Zakari Turaki for his guidance during this training. My appreciation goes to Centre for Dryland Agriculture for giving me the opportunity to conduct my bench work in their tissue culture lab with assistance from Dr. Kabir M. Umar and Abdulmutallib.

I am grateful to my colleagues: Kachalla Mala, Abakar Mohammed, Late Abubakar Askira, Dr. Maina, Dr. Yahaya Yakubu, Jidda Umar, Simon mutah, Abubakar Umar, Jessy Yakubu, and other staff at Lake

Chad Research Institute for their friendship and technical support. I extend my gratitude to my colleagues at WACCI for their encouragement and friendship. I am indebted to my husband: Ibrahim A. Koguna and kids: Fatima, Attahire, Muhammad, Nabila, Abdallah, Ummukulsum and baby Suga.

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LIST OF ABBREVIATIONS

LCRI: Lake Chad Research Institute

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

SNP: Single Nucleotide Polymorphism

GBS: Genotyping -by-Sequencing

GWAS: Genome-Wide Association Study

GAPIT: Genome Association and Prediction Integrated Tool

QTL: Quantitative Trait Loci

ANSPC: Area under Striga Number Progress Curve

NTL: Number of Tiller

SDR: Striga Damage Rating

PMiGAP: Pearl Millet Inbred Germplasm Association Panel

SSA: Sub Saharan Africa

IGSS: Integrated Genotyping Support Service

DArT: Diversity Arrays Technology

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Brown) ranks as the 4th most important tropical cereal crop in the world after rice, maize and sorghum (FAOSTAT, 2015). It is a major staple crop over the entire Sudano-Sahelian zone of West and Central Africa (WCA). It is especially important in marginal agricultural production environments in Sub-Sahara Africa (SSA) where it provides food, feed and fodder for millions of people and livestock (Camara *et al*., 2005; Angarawai *et al*., 2008; Nambiar *et al*., 2011). India and Africa are the most important producers with more than 85% of the world's total production (FAOSTAT, 2015).

It is an important crop in Nigeria, Niger, Mali and Burkina Faso where it is either ranked first, second or third most important cereal crop (FAOSTAT, 2016). In Nigeria, the crop ranks third after maize and sorghum (Okeke-Agulu & Onogwu, 2014) as a staple cereal in Nigeria and mainly cultivated as a rainfed crop in the Sahel and Northern Sudan regions where average annual rainfall ranges from 650 to 1200 mm. Three types of pearl millet are cultivated in Nigeria namely *gero, maiwa* and *dauro*. The most widely cultivated type in the medium to high rainfall areas (1000-1200 mm annual rainfall) in Nigeria is the *Maiwa*, which is photoperiod-sensitive. It is usually planted between June and July, and most times in mixed/intercropped with sorghum. The flowering occurs towards the end of the rains, around September or October irrespective of the planting date and survives on residual soil moisture and cold harmattan weather (Angarawai *et al*., 2000).

Pearl millet is the least researched crop in terms of improvement despite its importance in food security in Nigeria (Dugje *et al*., 2006; Dawud *et al*., 2017). Although this crop is indigenous and exceptionally adapted to the region, its yields are generally less than 1 t/ha (FAOSTAT, 2016), below yields of other major cereal crops. Factors responsible for this low yield include their low harvest index, abiotic and biotic constraints such as diseases, insect pests and *Striga* infestation (Fadelmola *et al*., 2014; Dawud *et* *al*., 2017). *Striga hermonthica* is a major biotic constraint to pearl millet particularly in Northern Nigeria (Dugje *et al*., 2006; Dawud *et al*., 2017). The area of lands with *Striga* problem is increasing, and it is gradually becoming a growing pandemic and a major challenge to subsistence farmers with small land holdings (Ejeta, 2007; Pennisi, 2010). *Striga* can cause yield loss of 20 to 80 % and up to 100% when susceptible varieties are grown (Parker, 2012). Atera *et al*. (2012) reported that farmers are often forced to abandon their land due to high *Striga* infestation.

Striga is observed in most pearl millet growing areas of northern Nigeria. Though there is no recent data available on economic losses due to *Striga* in Nigeria, several participatory survey reports from Nigeria have listed it as one of the main constraints to pearl millet production (Emechebe *et al*., 2004; Dugje *et al*., 2006; Dawud *et al*., 2017). Several approaches including the use of field, pot and *in vitro* screening techniques have been developed by the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) to assess the effect of *Striga*. Emphasis has been on selection for resistant genotypes of sorghum, rice and maize (Haussmann *et al*., 2004; Amusan *et al*., 2008; Jonne *et al*., 2015), but little efforts have been directed towards identifying resistant in pearl millet.

Recent advances in molecular breeding approaches are revolutionizing research on identifying *Striga* resistance in crop plants. Use of molecular tools has led to the identification of several sources of resistance to *Striga* and individual genes conferring host plant resistance in crops like sorghum (Haussmann *et al*., 2004). In pearl millet, efforts have been made to incorporate the use of molecular markers including simple sequence repeat (Mariac *et al*., 2006; Yadav & Rai, 2013; Rajaram *et al*., 2013), diversity array technology derived markers (Supriya *et al*., 2011) and SNPs (Kountche, 2013; Moumouni *et al*., 2015) to understand associations between markers and important agronomic traits. These earlier works have resulted in the construction of genetic linkage maps. The challenge towards efficient molecular breeding for *Striga* resistance in pearl millet is the identification of significant associations between markers and quantitative trait loci underlying resistance to the weed. Association mapping was recently used in pearl millet to identify phenology, grain and stover-yield related traits (Sehgal *et al*., 2015). Kountche *et al*. (2013) identified resistance to *Striga* using a diversified germplasm gene pool. Despite the success story of the molecular marker in pearl millet breeding, this option has not yet been explored in Nigeria. The identification of sources of resistance to *Striga* and nature of genes governing such resistance is needed to enhance pearl millet production. It could be used to speed up the breeding time and to breed for reliable *Striga* resistance in new cultivars.

To identify such sources of resistance in pearl millet, information about the variability of pearl millet genotypes in response to *Striga* infestation must be obtained. Also, the potential genomic regions associated with pearl millet resistance to *Striga* that are efficient under Nigerian environments must be identified to help address this constraint. There is also the need to identify and prioritize pearl millet production constraints and farmers' preferred traits, to guide and assist in reorienting pearl millet breeding programme to meet farmers' specific needs. The general aim of this research is to access *Striga* resistance sources and knowledge that will contribute towards the improvement of pearl millet for resistance to *Striga* in northern Nigeria. The Specific objectives were to:

a) identify farmers' pearl millet preferred traits, production constraints, and perceptions of *Striga hermonthica* on production;
b) identify superior genotypes for *Striga* resistance and select resistant parents for subsequent studies;
c) identify genes that govern *Striga* resistance in pearl millet;
d) map genomic regions associated with *Striga* resistance, and
e) characterize pearl millet genotypes for their Strigolactone and tiller production.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pearl millet genetic properties

Pearl millet is a monocot C_4 grass, that is diploid (2n= 2x = 14) with about 2,500 million base genome size (Varshney *et al*., 2017). It is a highly outcrossing species, with more than 80 % outcrossing reported (Debieu *et al*., 2017). Domestication of pearl millet starts from the wild species *Pennisetum glaucum* subsp. *monodii* around northeast Mali to Lake Chad (Manning *et al*., 2011). Recently, Burgarella *et al*. (2018) used genomic approach to investigate domestication and origin of pearl millet; they reported the origin to be western Sahara by providing evidence of gene flow from wild to cultivated types. The origin of pearl millet has been debated for a long time. Chemisquy *et al*. (2010) conducted phylogenetic studies using different approaches and proposed the transfer of *Pennisetum* into *Cenchrus* genus. They proposed "*Cenchrus americanus* (L.) Morrone" as the new nomenclature for "*Pennisetum glaucum* (L.) R. Br.". The protogynous nature of pearl millet (Fig. 2.1) and its ability to produce many seeds make the crop suitable for improvement through intra and interspecific hybridization. The size of Pearl millet chromosomes is reported to be larger than most species in *Paniceae* and its peculiar nucleolar makes it suitable to study chromosome pairing between species (Manning *et al*., 2011).

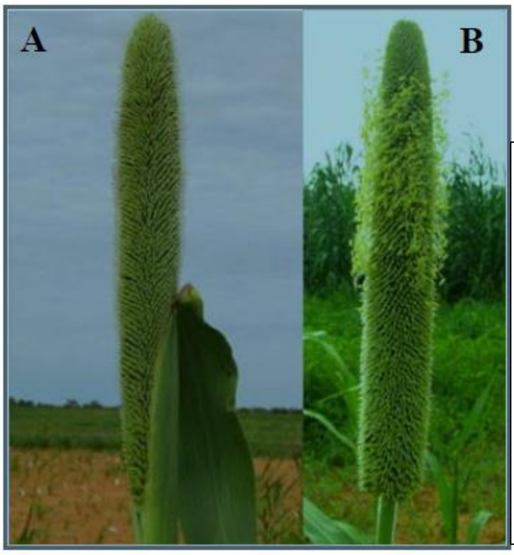


Figure 2. 1. Protogynous nature of pearl millet flowers.

The crop is hermoprodite with a markes protogyny.

(A) appearance of stigma (gynoecium) earlier, (B) appearance of the anthers (androecium) 2-3 days after.

Source: Kountche, 2013.

Pearl millet is a hardy crop that withstands harsh environmental conditions such as sandy or rocky soils. It performs better than other cereals on saline, acidic and marginal soils where other cereals cannot produce seed (Jukanti *et al*., 2016). Therefore, pearl millet serves as a crop of food security particularly in tropic and sub-tropic regions in Africa and it is becoming a crop for the future with the rapid population increase and climate change.

The crop is usually cultivated under subsistence agriculture in Africa and Asia as human food and feed for livestock. The grains are used for food and the stalk used as fodder, fencing and firewood (Rajaram *et al*., 2013). It is rich in protein, vitamins, and minerals and consumed in different ways on a daily basis in Northern Nigeria (Dawud *et al*., 2017). The grains are ground to fine flour to make the paste for pancakes, snacks, and beverages (Angarawai *et al*., 2008). It is used as a diet for diabetic patients because of its low lysine content. It is also an important diet that prevents ailments such as cancer, cardiovascular and neuron related illness (Jukanti *et al*., 2016). In North Africa, West Asia, and Brazil, the crop is used as forage, cattle and poultry feed because of its nutritional content and digestibility (Devos *et al*., 2006).

2.2 Importance of pearl millet

Pearl millet is one of the topmost important cereals in the world and serves as season forage and grain crop in the USA, Latin America, Africa, Asia and Oceania (Gulia *et al*., 2007). West and Central African regions are the world's leading producers of pearl millet grains with annual production on over 16.8 million ha and an average yield of 880 kg ha^{-1}. The largest producers in West Africa are Niger, Mali, Nigeria, Burkina Faso, and Senegal (FAOSTAT, 2016).

Pearl millet ranks third after sorghum and maize in terms of area harvested (5.2m ha) and total production (4 mt) (Okeke-Agulu & Onogwu, 2014) in northern Nigeria. It is mainly produced under rain-fed conditions in the north where rainfall is low and erratic. The grains are mainly used for making thick porridge while the stover constitutes a source of feed for cattle and material for building in northern parts of Nigeria. The grain is highly nutritious; rich in vitamins, iron, zinc (Kanatti *et al.*, 2014) and proteins (8-19%). It is also rich in dietary fiber and calories. One hundred gram of grain can provide up to 378 calories and is used for human consumption in different ways (Malik, 2015). The forage is highly digestible in the vegetative stage and it does not contain hydrocyanic acid compared to other cereal crops (Khairwal *et al*., 2007).

The crop is mainly grown by smallholder farmers under poor soil conditions in areas with low and erratic rainfall in Sudano-Sahelian areas. Farmers have been growing their landraces for centuries, the reason being it has their desired traits, however, due to high outcrossing nature of pearl millet, their trait of interest may be mixed with various unwanted traits, such as susceptibility to *Striga*. Therefore, the importance of farmers' participation and contribution in developing a new millet variety to enhance adoption cannot be overemphasized.

2.3 Farmers' participation in pearl millet improvement

Farmers' participation in plant breeding helps plant breeders to identify farmers' production constraints and preferred traits (Dawud *et al*., 2017; Egbadzor *et al.*, 2015). The technique is cost-effective and also transfers suitable varieties to farmers (Kanfany *et al*., 2016; Malaa *et al.*, 2016). Participatory plant breeding approaches in pearl millet have been conducted in some major growing areas of the crop. For example, the high adoption of early maturing pearl millet cultivar Okashana 1, also responsive to soil moisture and inorganic fertilizer, ensued from a participatory plant breeding approach in Namibia (Uno, 2005).

Farmers participated in early selection as per their preferences, while the Government of Namibia took pro-actively the rapid multiplication and dissemination of high-quality seed, thus reducing costs and speeding adoption of Okashana 1. Interestingly, a comparison conducted between improved varieties versus landraces in Namibia revealed that farmers were able to preserve the characteristics of the improved varieties after growing them along with their landraces because they grew them separately in the field (Uno, 2005). This research reveals that rapid adoption of a new pearl millet cultivar such as Okashana 1 in Namibia did not make the farmers abandon their old landraces because farmers are still cultivating the landrace considering both the climatic requirements and their lifestyle.

A participatory pearl millet varietal selection conducted in West Africa identified five varieties out of 10 initially proposed, in which the preferred traits included good grain yield, a large number of productive tillers, compact and long panicle and having good taste compared to their local variety (Omanya *et al.*, 2007). Furthermore, participatory varietal evaluation of advanced improved OPVs of pearl millet in Burkina Faso has led to the identification of MISARI-1 with high adoption rate. Participatory varietal selection of improved OPVs of pearl millet in Nigeria has led to the identification of SOSAT-C88 that was released and widely adopted. All these achievements were as a result of farmers participating in the variety development process and farmers preferred traits and production constraints are taken into consideration.

2.4 Production constraints

The productivity of pearl millet is constrained by a series of biotic and abiotic stresses. In Asia and Africa, it is cultivated in very harsh environmental conditions characterized by erratic rainfall, low soil fertility, pests, inappropriate cultural practices, diseases and *Striga hermonthica* (Sehgal *et al.*, 2012).

2.4.1 Abiotic constraints

2.4.1.1 Poor soil fertility

Poor soil fertility and occasional drought are major abiotic factors affecting increased productivity of the crop, especially in West Africa (Makuvaro *et al.*, 2017; Dawud *et al.*, 2017). It is mainly produced on marginal soil with low available water (Passot, 2016) and farmers, in general, do not apply the recommended amount of fertilizer. Moreover, this low soil fertility issue is amplified by the continuous pearl millet growing associated with demographic pressure on the arable lands. Low soil fertility in some cases creates symptoms like drought stress. This is because the crop will look yellowish and stunted. This long-term pearl millet cultivation without soil improvement strategies contributes to the deficiency of the major mineral elements (NPK) which play an important role in pearl millet development.

2.4.1.2. Drought

Drought is an important abiotic factor that considerably affects pearl millet production (Passot, 2016; Yadav *et al.*, 2017). Badolo (2015) and Drabo *et al*. (2017) reported drought as the main production constraint to pearl millet in Burkina Faso. Drought considerably affects the photosynthetic activity of pearl millet (Radhouane, 2013) and can wipe out the whole plant which consequently reduces grain yield (Karim & Rahman, 2015). Research on drought tolerance is complicated because of the uncertain nature of the frequency, duration, and intensity in relation to pearl millet growth stage. However, some studies on drought resistance mechanism have reported genotypic variation in the accumulation of abscisic acid (Wang & Huang, 2003; Saradadevi *et al.*, 2017) and osmotic adjustment in response to water stress (Henson *et al.*, 1982). Recent studies focus more on markers related to drought tolerance.

2.4.2 Biotic Constraints

2.4.2.1 Diseases

Various diseases affect pearl millet production. These include downy mildew (*Sclerospora graminicola)*, blast (*Pyricularia grisea*), *smut (Moesziomyces penicillariae*), rust (*Puccinia substriata)* and ergot (*Claviceps fusiformis).* Downy mildew is the most destructive of pearl millet diseases reported so far in most growing regions (Kumar & Vk, 2011). Annual yield loss of 20 – 40% due to downy mildew are reported worldwide, while yield losses in Africa ranged from 10 – 60% (Nene & Singh, 1976). In Mali, disease incidence generally varied from 9.8 to 77.1% and up to 85% when a susceptible variety was used. In Niger, it varied from 7.7 to 78% but up to 89% when a susceptible variety was used. In Nigeria, it was 14.5 to as high as 99.2% (Thakur *et al*., 2004). A similar trend of downy mildew incidence in these four countries was also reported by Jeger *et al.* (1998). Resistant varieties have been reported to effectively control the disease (Hess *et al*., 2008), also seed treatment with metalaxyl fungicide plus furathiocarb insecticide (Thakur *et al*., 2011), and removal of affected plants (Kountche *et al*., 2016).

Insects such as millet head miner and stem borers are the most prevalent pests of the crop, while plant-parasitic nematodes attack the crop in most areas of production (Nayak, 2010).

2.4.2.3 Parasitic weeds

The most important parasitic weed in West Africa pearl millet fields is *Striga hermonthica*. The parasite causes damage to its host with nutrient deficiency especially phosphorus deficiency (Kountche *et al*., 2016). Consequently, pearl millet faces double punishment; nutrient deficiency coupled with *Striga* parasitism. *Striga* problem affects more than 50 m ha of land under cereal production in sub-Saharan Africa. Yield losses resulting from *Striga* infestation could reach 20 % to 60 % and can lead to complete failure of the field under severe infestation when highly susceptible varieties are grown (Parker & Riches, 1993). As a result, it causes a yield loss of over US$10 billion annually (Westwood *et al*., 2012; Pennisi, 2015). *S. hermonthica* was recently listed as one of the most devastating biological constraints to food production (Pennisi, 2010). Thus, it is a serious threat to smallholder farmers (Pennisi, 2010). Control measures such as quarantine, controlled movement of farm implements, herbicides and injection of ethylene gas have effectively been used in the United States to control *S. asiatica* (Berner *et al*., 1995; Van Mourik, 2007). These control methods require technical expertise and are beyond the reach of African farmers.

Studies have been conducted to come up with efficient control measure against *Striga* menaces such as improving soil fertility and deployment of resistant crops have been reported in other crops (Haussmann *et al.*, 2000b; Rector, 2008; Tesso & Ejeta, 2011) but not in pearl millet. As a result, hand-pulling remains the only method practiced by farmers. The host-parasite relationship makes it difficult for single *Striga* control strategy to be effective. Considering this, the integrated *Striga* control management has been suggested for a long term effect (Joel, 2000). Although some of these control methods are helpful, they did not completely control *Striga*. Pearl millet production could be improved through effective conventional and modern breeding approaches using locally adapted and well-characterized germplasm.

2.5 Breeding for resistance to *Striga* in pearl millet

Genetic improvement of pearl millet for *Striga* resistance has been attempted using both conventional and molecular breeding tools (Wilson *et al.*, 2004).

2.5.1 Conventional breeding approach for resistance to *Striga*

The conventional breeding method used in improving resistance to *Striga* in pearl millet lines includes bulk methods, mass selection, and family-based recurrent selection. The bulk method was used when more than two parents are used for a cross. Mass selection is a breeding system in which seeds from individuals are bulked and used to grow the next generation. The mass selection has been effective in improving pearl millet (Rattunde *et al.*, 1989; Singh & Talukdar, 1998). Enhancing open-pollinated populations of pearl millet for resistance to *Striga* mainly depends on changing allele frequencies so that favorable alleles are increased, but at the same time, on maintaining a high degree of heterozygosity. Recurrent selection based on selfed (S_1 or S_2) family, half-sib (HS) family and full-sib (FS) family selection is used (Weltzien. & King, 1995; Kountche *et al.*, 2013). The progenies are screened for resistance to *Striga* and the remnant seed of resistant individuals are planted and recombined to give the first cycle of recombination. The process can continue until a satisfactory resistance population is developed. The use of recurrent selection (full-sib individuals) has been successfully tested for resistance to *Striga* in pearl millet (Kountche *et al*., 2013).

2.5.2 Molecular breeding approach for resistance to *Striga* in pearl millet

The advent of molecular markers provides an efficient approach to dissect complex genetic architecture (Jeremy Roberts *et al.*, 2003; Shabir *et al.*, 2017). However, such technologies have been applied to pearl millet only to a limited extent. Quantitative genetics is gradually advancing to molecular genetics, which has led to improved methods for selecting superior genotypes such as for *Striga* resistance more efficiently (Bell & Dochtermann, 2015). Some scientists have reported significant interaction for genotype-by-environment for traits related to *Striga* resistance (Haussmann *et al*., 2001; Badu-Apraku, 2010; Kountche *et al.*, 2013). Knowledge of genetic markers associated with *Striga* resistance is expected to greatly help in developing resistant/tolerant varieties. Use of DNA-based markers for the identification of QTL regions underlying resistance phenotypes is increasingly receiving attention (Haussmann *et al*., 2001; Badu-Apraku *et al.*, 2012) attention. The use of linkage mapping greatly assisted in detecting association between QTLs and stress tolerance traits in pearl millet, however, this technique has its limitation in terms of narrow genetic diversity present in the two parents that lead to low resolution (Xu *et al.*, 2017).

Association mapping is increasingly receiving attention for allele mining using the history of ancestral recombination events for the association between phenotypic trait and marker. Its advantage over QTLs mapping is that it is easier, takes less time to complete, has a higher capacity for allele detection and higher resolution power when diverse germplasm is used. Genome-wide association mapping has been used in rice, maize, sorghum, barley, and wheat to identify QTLs associated with stress resistance (Senthilvel *et al.*, 2008; Sehgal *et al.*, 2012; Rajaram *et al.*, 2013). This approach is also suitable for orphan crops that do not have reference genomes. An association study was recently used by Kountche (2013) in pearl millet for resistance to *Striga*. Of recent, Sehgal *et al*. (2015) used 346 lines of “pearl millet inbred germplasm association panel (PMiGAP)" collected from different regions of Africa and Asia for association mapping of drought tolerance (DT) traits. The use of this panel provided new insights for QTL mapping and mining of favorable alleles for important traits. Until recently, there have been few reports on the identification of alleles associated with *Striga* resistance using association mapping approach in pearl millet. (Shivhare & Lata, 2016; Kountche *et al.*, 2016). The sequencing and availability of a reference genome for pearl millet will now facilitate exploration and marker-trait associations of important agronomic traits of the crop such as yield and stress resistance. Recently, the use of *Strigolactone* (germination stimulant) to induce sprouting of *S. hermonthica* seeds has been extensively studied as a possible target for control measures.

2.5.3 Mechanism of host recognition: through the role of Strigolactone

Striga life cycle to its host (Fig. 2.2) depends on different mechanisms for coordination. Germination of *Striga* seed starts only after precondition period of moist and suitable temperatures, followed by perception and stimulation of chemicals released by the host root, termed 'germination stimulants', (Xie *et al.*, 2010). The radicle grows very close to the root of the host plant and gets itself attached to the root within 2-3 days before the seedling resource gets exhausted. When contact with host root is established, the radicle develops a *haustorium* which sticks and penetrates the host epidermal and cortical tissues of the host root and forms xylem to xylem connection thereby drastically drawing water and nutrients from the host (Ejeta, 2007). The tubercle grows underground for about three weeks depending on the genotype and grows aboveground, flowers and sheds tiny thousands of seeds at about 8 weeks. Finally dries up and dies at about 92 days. As a consequence, the host becomes stunted, with yellowing of leaves and reduced photosynthesis (Remerij & Van Ast, 1999).

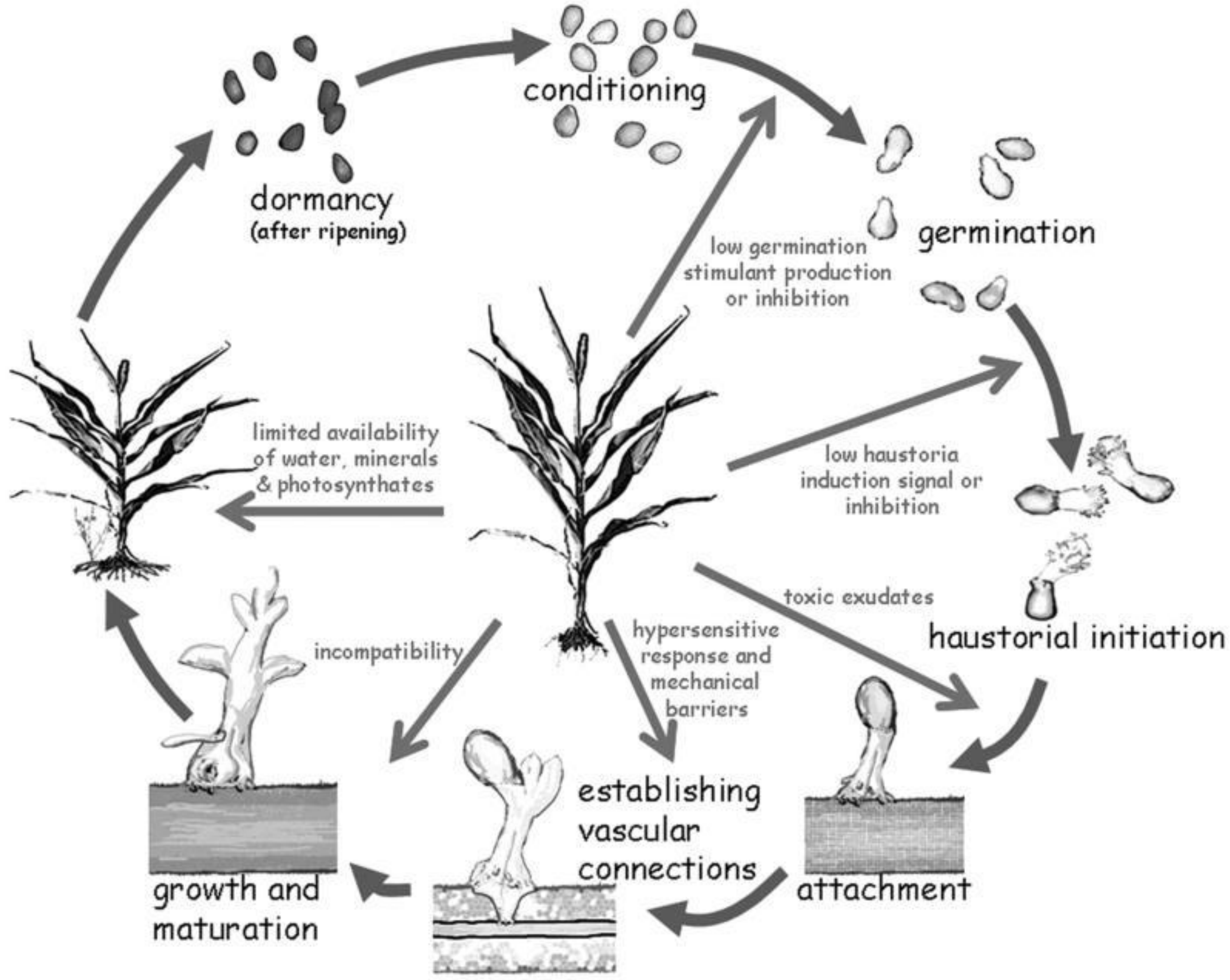


Figure 2. 2. *Striga* life cycle showing host-parasite interaction. (Adopted from Ejeta, 2007).

Three forms of germination stimulants have been identified and these include *strigolactones* (SLs), sesquiterpene lactones and dihydroquinones (Bouwmeester *et al.*, 2003), with SLs being the most active in stimulating the parasitic germination (≤10 pM) of *Striga* (Kim *et al.*, 2010). Strigolactones were first identified in cotton as a trap crop (Cook *et al.*, 1966; Cook *et al.*, 1972) and later found in roots of some cereals like maize, sorghum, and proso millet (Siame *et al.*, 1993). There are two germination stimulants that are structurally related to strigol, these are alectrol found in cowpea (Mtiller *et al.*, 1992) and sorgolactone from sorghum (Hauck *et al.*, 1992). Striga-related compounds were first named ‘strigolactones’ by Butler (1995), as these compounds are capable of inducing *Striga* germination and subsequently parasitism. In addition, it acts as rhizosphere signaling molecule for the association between arbuscular mycorrhizal fungi and inhibits tillering (Akiyama *et al*., 2005; Besserer *et al*., 2006; Jamil *et al*., 2018). For decades, highly pleiotropic plant hormones such as auxin mask the effect of specific hormones as in the case of SLs, as such they were thought as the only plant hormones responsible for tillering or branching. Until recently studies confirmed the existence of a novel long-distance signal class of hormone the SLs (Gomez-Roldan *et al*., 2008). Thus, Strigolactone acts as both an internal and rhizosphere signaling molecule. Jamil *et al.* (2012) reported a positive correlation between low tillering capacity and SLs production in rice. Also, Gomez-Roldan *et al*. (2008) found an inhibitory effect of SLs for branching in *Arabidopsis* and pea. Host crops such as pearl millet can be characterized as resistant or susceptible based on quantity and type of Strigolactone they have and their relationship with tiller production.

Research on SL variation in pearl millet genotypes can assist in identifying *Striga* resistant and susceptible genotypes to reduce yield losses in *Striga* prone areas. Differential response of pearl millet to *S. hermonthica* in terms of SL production has been reported (Jamil, 2013). Several research programmes have tried identifying resistance to *S. hermonthica* and had attempted transfer of resistance genes to adapted popular varieties (Wilson *et al*., 2004; Kountche, 2013). The best resistance mechanism demonstrated so far is the low germination stimulant in the host (Nasreldin, 2018). Genotypes possessing this attribute may be resistant because only few *Striga* seeds in proximity with the host root will be able to germinate. Several studies have been conducted to quantify and qualify SLs in different crops such as sorghum, Maize (Jamil, 2013; Charnikhova *et al*., 2017), rice (Jamil *et al*., 2012) and millet (Van Mourik, 2007). Differences have been observed in regard to the quantity and type of SL produced within the same species of family *Gramineae*. Thus, this can be exploited in identifying resistant and susceptible genotypes.

2.6 Genetic control of resistance to *Striga* in pearl millet

Understanding the genetics of a specific trait is important for effective improvement of pearl millet for tolerance to stress factors (Azizi *et al*., 2006). Studies on the type of gene action governing inheritance of traits has been a topic of debate for centuries because gene effect varies depending on the genotypes used and the test environments. Knowledge of these components will greatly increase our understanding of the nature of gene action that controls resistance, hence facilitates the development of resistant varieties.

Generation means analysis is a simple tool that use means and variances of the basic generations to estimate the gene effects. It estimates the gene effects controlling quantitative traits which have so far been applied on many crops and for many traits. What makes generation mean analysis more important is its ability to tease out epistatic effects in addition to additive and dominance effects (K. Mather & Jinks, 1982). Studies have shown that the epistatic effect can account for a significant proportion of the genetic variance of a polygenic character (Vengadessan, 2008). Therefore, knowledge of both additive and nonadditive components of the gene effect is important in the design of a breeding programme. Research on the importance of these genetic components governing the inheritance of quantitative traits has led to the design of many breeding methods that rely on the nature of gene action such as recurrent selection for general combining ability (additive effects), recurrent selection for specific combining ability (dominance effect) and the reciprocal recurrent selection (additive and dominance effects).

Striga resistance/tolerance in maize behaved qualitatively and recessive in nature (Mbogo *et al.*, 2015) which is important for its stability (Rispail *et al.*, 2007). Lane *et al.* (1997) reported that the nature of genes controlling *Striga* resistance is additive with few genes involved. Some authors also reported the quantitative nature of inheritance for resistance to *Striga* (Haussmann *et al.*, 2004; Ejeta, 2007). Similarly, Kim (1994), reported on the genetics of *Striga* emergence in a diallel study to be additive and non-additive effects while additive and dominant effects were reported for *Striga* resistance by Wang *et* *al*. (2014) and Akaogu *et al*. (2019) in maize. Grain yield in pearl millet and its components have been attributed to non-additive gene effects while G x E for productivity and *Striga* resistance traits varied in its nature and magnitude in different sets of family and populations according to Bhuri (2015) and Lal Solanki *et al*. (2017). The G x E variances for genetic variance were reported to be greater than the environmental variances for resistance to *Striga* in pearl millet, suggesting such genotypes should be evaluated in the different environments and recombined to form experimental varieties for the specific environment (Kountche *et al*., 2013). Similarly, Badu-Apraku (2010) reported interaction between genotype and environment to be significant for resistance to Striga in maize among three environments. While Haussmann *et al*. (2001) found the interaction between genotype and environment to be significant for resistance to *Striga* indices as well as grain yield in sorghum. Therefore, the aforementioned trials conducted by several researchers support the need to breed for specific sites knowing the genetics of the genotypes being environment –specific especially if the target environments differ considerably as they may consist of different Striga ecotypes. Knowledge of G x E effect in pearl millet in relation to gene effects that control resistance to *Striga* traits will go a long way to assist in designing effective breeding programme for developing resistance to *Striga* varieties.

CHAPTER THREE

3.0 Identification of Pearl Millet Production Constraints and Farmers' Preferred Traits in Jigawa State, Nigeria

3.1 Introduction

Pearl millet serves as a staple food for over 40 million people in northern Nigeria. The crop is relatively more tolerant to stress conditions such as drought, poor soil fertility, and increasing temperature when compared to major cereals grown in the Sudano-Sahelian region (Jukanti *et al*., 2016).

Despite the release of improved varieties of pearl millet by different research institutions over the past few decades, the use of improved varieties by smallholder farmers has been reported to be just about 24% (DIIVA- Nigeria, 2010). However, a recent study indicated a three-fold increase in productivity of the crop in northern parts of Nigeria. This was attributed to the adoption of improved varieties and agronomic practices (Okeke-Agulu & Onogwu, 2014). Use of improved technologies is generally low in Africa due to non-involvement of farmers in planning and designing of these technologies. It is, therefore, important to take into account the preferences and perception of farmers in planning breeding programmes in order to increase the uptake of improved varieties.

Participatory approaches have been used to document farmers knowledge and practices in rural farming communities, such method allows an analysis of challenges facing local communities for informed decision making and planning (Chambers, 1994; Mukherjee, 1995). Anthony & Persley (2017) have given several reasons why the use of improved technologies are low in most of the rural areas. These include the non-incorporation of farmers' perception and knowledge in designing the technologies.

The use of participatory research tools involving farmers and scientists is becoming popular in identifying production constraints and farmer's needs. Therefore, it is important to understand farmers' preferences before undertaking and investing in breeding programmes. Few studies have reported the use of participatory tools for pearl millet research in Nigeria (Dugje *et al.*, 2006; Okeke-Agulu &

Onogwu, 2014). For instance, Dugje *et al*. (2006) reported that farmers in Yobe State of Nigeria understood *Striga* problem and poor soil fertility as a major production constraint. However, there no documented reports of farmers preferred traits, constraints to productivity, infestation and control strategies of *Striga* in pearl millet fields in Jigawa State of Nigeria, an important pearl millet producing area. This study, therefore, was conducted to achieve the following objectives, to:

i) assess preferred traits among pearl millet farmers,

ii) understand the production constraints of pearl millet farmers and

iii) asses the level of farmers' knowledge of *Striga hermonthica* infestation.

3.2 Materials and Methods

3.2.1 Study area

The current study was carried out across 6 communities from 3 local government areas (LGAs) in Jagawa State, comprising two each from a Local Government Area (LGA) representing the Sudan savanna zone (Table 3.1). The study was conducted at Kudai and Madobi (Dutse LGA), Karfawa and Shuwarin (Kiyawa LGA) and Kafingana and Kantoga (Birninkudu LGA) (Figure 3.1). The LGAs were chosen because of the substantial production of pearl millet in these areas.

Table 3.1. Geographical information of Dutse, Kiyawa and Birninkudu local governments of Jigawa

Parameter	**Local Governments**		
	Dutse	**Kiyawa**	**Birninkudu**
Latitude	11º46’39’’N	11º47’05’’N	12º59’53’’N
Longitude	9º20’03’’ E	09º36’30’’E	8º54’35’’ E
Altitude (m.a.s.l)	444	508	459
Fadama land ha	23154	10691	16000
Annual rainfall (mm)	743	7341	8202
Total population	290322	1999391	3133732

Data source: (“Yr – Weather forecast for Kiyawa, Jigawa (Nigeria),” 2016), (Katanga *et al*., 2016)[1], (Akiode *et al*., 2014)[2]

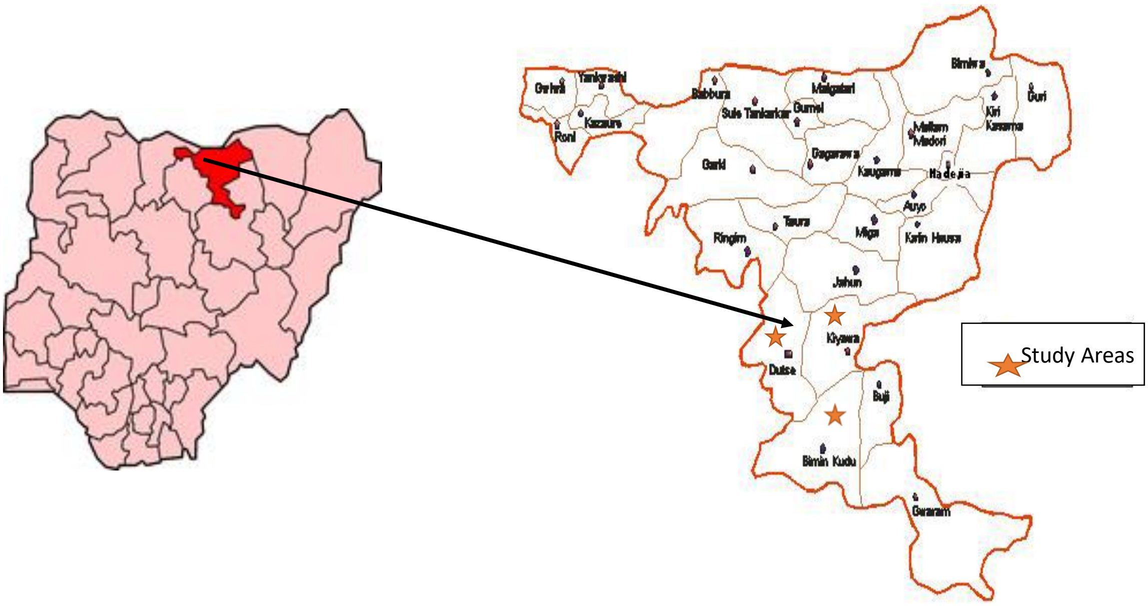


Figure 3.1. Map of Nigeria showing Jigawa State and Local Government Areas of the Study

3.2.2 Sampling procedure and data collection

The PRA was conducted in three local governments of Jigawa (Northern Nigeria) state from 22nd - 24th March 2016 when farmers are less busy with farming activities. The implementation team included a facilitator, a technician, and in each local government, an agricultural extension officer. At each of the communities visited, a local agricultural extension officer helped introduce the purpose of the visit and facilitated discussion with the group using local dialect.

A three-stage sampling approach was used to select villages for the study. In the first stage, 3 LGAs were selected based on the high volume of production of pearl millet using information obtained from local extension personnel. The second stage was a random selection of two communities from each of the 3 LGAs and the last stage involved selection of 23-24 farmers growing pearl millet for each of the communities selected in the second-stage above, making a total of 143 farmers across all communities of the study (Table 3.2). This comprised 90 farmers for the survey administered using semi-structured questionnaires and 53 farmers from all the LGAs in the focus group discussions (FGD). FGD was conducted in each LGA with eight to nine participants. This FGD exercise allowed identification of popular varieties, farmers preferred traits, local constraints to productivity, and knowledge of Striga and its control practices. The FGD sessions helped to validate the information from individual interviews using a semi-structured questionnaire. Participants in the FGD were allowed to express their opinions on the content of the discussion sessions. The discussion began with general issues and later tailored to the specific content of the objectives of the study. Probing questions were intermittently asked to get detailed explanation and clarification on certain responses.

Table 3.2. Surveyed Communities and number of farmers that participated

Villages	**LGA**	**Regions**	**No. of farmers (survey)**	**No. of farmers (FGD)**		**Dates**
				Males	**Females**	
Madobi	Dutse	South	15	4	5	22/03/2016
Kudai	Dutse	South	15	6	3	22/03/2016
Karfawa	Kiyawa	South-East	15	5	4	23/03/2016
Shuwarin	Kiyawa	South-East	15	6	3	23/03/2016
Kafingana	Birninkudu	South	15	6	2	24/03/2016
Kantoga	Birninkudu	South	15	4	5	24/03/2016

LGA = local Government Area

3.2.3 Data Analysis

The 90-questionnaire administered were retrieved, and data collected were coded and analyzed using SPSS software (Version 20.0). Responses of farmers were summarized using descriptive (SPSS, 2011).

3.3 Results

3.3.1 Respondents socio-economic attributes

The results on farmers' socio-economic characteristics revealed that about 22% were aged 31-40 and 27% were between 41-50 years of age, indicating that close to half of the respondents belonged to the active age group. There were more men (71%) than women (29%) involved in pearl millet farming activities. A substantial number (67%) of the respondents had no formal education, 20% primary education and 8% secondary education. The number of respondents with post-secondary education were very few (6%). In addition, many of the respondents were married (93%) with only 6% that were single while about 1% were divorced. Most of the respondents (56%) have been farming for more than 21-40 years while 20 and 25% of the respondents have been farming for 1-20 and 41-60 years respectively (Table 3.3).

Table 3.3. Distribution of respondents based on socioeconomic characteristics

Variable	No. of Respondents	Percentage
Age-group		
11-20	9	10
21-30	5	5.6
31-40	23	25.6
41-50	20	22.2
51-60	24	26.6
Above 60	9	10
Total	90	100
Gender		
Males	64	7
Females	26	29
Total	90	100
Education		
No formal education	60	66.7
Primary	18	20
Secondary	7	7.8
Tertiary	5	5.5
Total	90	100
Marital status		
Married	84	93
Single	5	6
Divorced	1	1
Total	90	100
Years of farming experience		
1-20	18	20
21-40	50	56
41-60	22	24
Above 60	0	0
Total	90	100

3.4 Main Staple Crops Cultivated in the Study Areas

Staple crops popular across the study areas are presented in Table 3.4. *Maiwa* type of millet was ranked as third in Madobi and second most important in Kudai, and Karfawa and Shuwarin both ranked it as the fourth most important, Kaifngana and Kantoga had the least ranking 8th and 7th respectively. Farmers reported that *maiwa* millet does not require heavy rain during flowering as it washes away the pollen. It usually flowers after the rains but the short panicle/duration type often sheds pollen during rainfall and pollen gets washed away and when this coincides with high temperature and humidity it results to ergot disease and drastically leads to a reduction in yield and grain quality (colour and taste). They also reported that when affected grains are consumed it causes drowsiness, laziness, stomach pain and in severe cases death. Farmers in Birninkudu reported that soil in Birninkudu is relatively fertile and in recent years is experiencing higher rainfall (820 mm annual rainfall), compared to the two other local governments. For this reason, they did not grow it much compared to some years back when rain was less.

On the other hand, farmers from Dutse and Kiyawa reported that their soils were poor and hence experienced severe *Striga* problems. *Maiwa* does not require much rainfall during heading. Once it heads, with little rain it can continue to survive. Farmers in Madobi reported that they adopted *maiwa* millet cultivation mainly because of its ease of work; with only one weeding and one rainfall shower you can get appreciable yield. They predicted that with climate change, *maiwa* cultivation is the only option; even the old people among the farmers can grow it. In all the local governments, *maiwa* millet is grown in the main season (June to July) irrespective of the fertility or rainfall of the site. Farmers in most of the study local government areas reported that *maiwa* millet is an important crop and is their main food crop. Farmers also stressed that *maiwa* millet is a crop that even the poor and older people can grow because it requires much less labour, fertilizer, and water.

Table 3.4. Pairwise ranking of major crops on the basis of importance

Major Crops	**Dutse**		**Birnin Kudu**		**Kiyawa**	
	Madobi	**Kudai**	**Kafingana**	**Kantoga**	**Karfawa**	**Shuwarin**
Gero millet	1	2	2	4	2	1
Maiwa millet	3	2	8	7	4	4
Sorghum	2	1	1	1	3	5
Rice	-	4	3	6	-	-
Maize	-	6	5	-	-	-
Groundnut	5	1	7	5	5	2
Cowpea	4	3	4	3	1	3
Sesame	-	5	6	2	6	-
Bambaranut	-	7	-	-	-	8
Sorrel	-	-	-	-	7	6

Pairwise ranking: high ranking indicates crop is less preferred, - denotes crop not mentioned

Pearl millet was mostly intercropped with groundnut. However, some farmers grew it in the pure stand if they had enough land to grow other legumes such as groundnut separately. Farmers grew pearl millet mainly for food and little to sell when they need cash. The two most important crops grown across most of the districts were sorghum and *gero* millet.

3.5 Farmers' Preferences for pearl millet Improvement

Farmers preferred traits in *maiwa* type of millet ranked by the respondents are presented in Table 3.5. Their importance varies across sites. Pearl millet resistant varieties to *Striga* was the most desired feature farmers would like to have in a variety except at Kafingana where it was ranked 4th most preferred trait. Tolerance to shattering came second in the list of farmers preferred pearl millet traits among farmers at Kudai, Kafingana, and Shuwarin while resistance to downy mildew was the most important trait farmers want to see in improved varieties at Kafingana. Interestingly, pearl millet farmers chose late maturity over early maturity. Their reasons were early/medium maturity varieties would usually start flowering when rains are still heavy and result in a great amount of pollen been washed away and serious infestation of ergot disease.

Table 3. 5. Farmers' trait preferences for major improvement ranked according to the importance

Variety characteristic	**Dutse**		**Birninkudu**		**Kiyawa**	
	Madobi	**Kudai**	**Kafingana**	**Kantoga**	**Karfawa**	**Shuwarin**
Maturity (Early/medium)	11	11	11	13	13	10
Yield (high)	10	8	8	8	10	8
Plant height (medium)	12	10	11	11	14	13
Panicle length (medium)	13	12	10	12	12	12
Panicle size	11	8	9	10	11	10
Grain size (bold)	8	9	9	9	9	8
Grain color (grey)	8	7	7	7	7	7
Thresh ability	9	10	7	9	9	11
Grain hardness	7	6	6	5	7	7
Taste	6	7	5	5	7	4
Storability	6	5	5	4	8	5
Panicle compactness	5	6	6	6	7	6
Panicle bristle	14	13	11	14	6	*
Downy mildew Resistance	5	4	1	3	5	2
Striga Resistance	1	1	4	1	1	1
Tolerance to lodging	3	3	3	2	2	3
Tolerance to shattering	4	2	2	3	4	2
Tillering ability	2	3	11	2	4	8
Quality local beverage	4	6	2	5	3	4

*denotes characteristic not reported

3.6 Relative importance of grain

The ranking of the utilization of *maiwa* millet grain differed among the different villages (Table 3.6). Farmers in Madobi ranked *tuwo* (thick porridge) as the most important, Kudai and Shuwarin farmers ranked *kunu* (thin porridge) as the most important use of the grain whereas Karfawa farmers prefer it made to *tsaki* (coucous). These farmers emphasized that *maiwa* millet has a very good aroma which is better than that of *gero* millet. However, they preferred cooking *tuwo* (thick porridge) with *gero* than *maiwa*, because *tuwo* made of *gero* is more palatable and cooks better. Farmers from Kantoga mostly prefer to use the grain for *dafuwarsaba* (boiled grain) while Kafingana farmers process it into a paste and fry it for breakfast as *waina*. Pearl millet grains were less frequently used for *gumba* (pounded millet grains) across the districts.

Table 3. 6. The relative importance of grain (pair-wise ranking)

Grain use	**Dutse**		**Birnin Kudu**		**Kiyawa**	
	Madobi	**Kudai**	**Kafingana**	**Kantoga**	**Karfawa**	**Shuwarin**
Tuwo (thick porridge)	1	2	5	3	5	3
Kunu (thin Porridge	2	1	3	2	2	1
Dambu (another form of couscous)	5	6	6	3	7	6
Tsaki (couscous)	7	-	4	3	1	2
Fura (thin porridge)	4	5	2	4	4	4
Dafuwarsaba (boiled grains)	3	3	-	1	3	5
Waina (fried millet paste)	6	4	1	5	5	-
Gumba (pounded millet grains)	8	7	-	6	8	7

- denotes importance not reported

3.7 Relative importance of *maiwa* millet stalks

Maiwa stalk is very important such that farmers did not adopt a *maiwa* variety that has poor stalks. All farmer groups revealed that *maiwa* is preferred not only for its grain but also its stalk. The stalk is used for many purposes (Table 3.7). Farmers from all the districts agreed that it was mostly used as fodder because of its stay-green attributes. They also used it for fencing as *maiwa* stalk is usually harder and has more tillers than *gero* millet, except Kafingana and Kudai who ranked it as 3rd and 4th most important use respectively. As firewood is expensive, farmers from Kudai and Kafingana revealed that they also use their *maiwa* stalk as firewood for cooking. Ash from the firewood is taken to the farm as a source of plant nutrients. Few farmers from Madobi compost it and use it in their farms as a source of the nutrient.

Table 3. 7. Relative importance of stalk (pair-wise ranking)

Stalk use	**Dutse**		**BirninKudu**		**Kiyawa**	
	Madobi	**Kudai**	**Kafingana**	**Kantoga**	**Karfawa**	**Shuwarin**
Fodder	1	1	1	1	1	1
Fencing	2	4	3	2	2	2
Firewood	3	2	2	3	3	3
Compost	4	-	-	-	-	-
Source of plant nutrients	5	3	4	4	4	4

- denotes importance not reported

3.8 Constraints to *maiwa* millet production identified across study areas

Constraints identified by farmers are presented (Table 3.8). These varied across communities and regions. Limited access to land was the most popular constraint all farmers alluded at Madobi and Kafingana. Poor access to seeds of improved varieties and extension services were the most important constraint for farmers at Kudai and Karfawa respectively. Farmers at Kudai and Shuwarin identified limited capital as their topmost production constraints. However, poor soil fertility was ranked very high across all study areas, followed by Striga problem and high incidence of downy mildew. Other constraints such as high cost of labour and heavy rainfall were site-specific. Famers that complained of *Striga* and downy mildew showed their frustration of these problems.

Table 3. 8. Farmers identified pearl millet production constraints across three LGA in Jigawa State of Nigeria

Production constraints	**Dutse**		**Birnin Kudu**		**Kiyawa**	
	Madobi	**Kudai**	**Kafingana**	**Kantoga**	**Karfawa**	**Shuwarin**
Low soil fertility	2	2	2	2	3	3
Striga	3	3	5	3	4	2
Downy mildew	5	5	4	5	6	7
High labor cost	5	4	5	6	7	-
Land shortage	1	-	1	7	-	5
Theft	6	-	6	1	-	8
Lack of improved seeds	-	1	2	2	-	-
Lack of capital	-	1	-	-	2	1
Access to extension agents	-	5	-	4	1	4
High rainfall	-	-	2	5	8	6

- denotes constraint not reported, LGA = Local Government Areas

3.9 Farmers management strategies for major production constraints

Farmers have adopted series of management strategies to major constraints in the study areas (Table 3.9) against *Striga* infestation, poor soil fertility, downy mildew, bird damage, smut, high labour cost and land shortage but none for lack of improved seed, lack of capital, lack of extension agents and too much rainfall. For *Striga* infestation, majority of the respondents used hand-pulling (94%) with few farmers reported the addition of urea fertilizer (3%) or land fallowing (2%). As for fertility, most farmers reported the use of ash (52%) while others reported the use of ash and inorganic fertilizer (43%), with few farmers using inorganic fertilizer and crop rotation. For downy mildew, majority of the respondents rogued infected plants (81%), whereas for bird damage farmers reported scarring (58%) and planting when birds seemed to be in their breeding seasons somewhere else (1%). To mitigate ergot disease, a few farmers (3%) adopted the spraying of insecticides while the majority of them did not take any action (97%). Also, to mitigate high labour cost, most farmers hired casual labour (63%) or used family labour (28%) with few of the respondents reducing acreage (8%) or plant on previously used fields (1%). Similarly, for the land shortage, most farmers practiced land hire (71%) and intercropping (20%).

Table 3. 9. Management Strategies for major production constraints across three LGA in Jigawa State of Nigeria

Constraints	**Mechanisms**	**Percentage of respondents**
Declining soil fertility	- Ash	52
	- Inorganic fertilizer	3.5
	- Ash and inorganic fertilizer	43.5
	- Crop rotation	1
Striga coping mechanism	- Hand pulling;	94.5
	- Crop rotation	0
	- Intercropping	0
	- Urea application	3.5
	- Fallowing	2
Downy mildew	- Hand pulling	81
	- No action is taken	19
Birds damage	- Scaring birds	58
	- Planting to escape peak population	1
	- Not a problem	41
Ergot	- Spray insecticides	3
	- No action is taken	97
High labor cost	- Hire casual labor	63
	- Reduction in acreage	8
	- Family labour	28
	- Use of previously used field	1
Land shortage	- Intercropping	20
	- Land hire	71
	- Addition of more fertilizer	1
	- Not a problem	8

3.10 Farmers' knowledge about *Striga*

The extent of the *Striga* constraint and control strategies for managing the menace is presented in Table 3.10. *Striga* infestation was reported by 79% of respondents as serious menace in their fields for over two decades now and this has constrained productivity of their major staples (pearl millet, sorghum, maize, and rice) with varying degrees of susceptibility to *S. hermonthica*. Most of the farmers reported that *Striga* damage is more severe when *maiwa* millet is heading (42%) or at flowering stage (39%) with 18% of the respondents reporting *Striga* damage at pearl millet seedling stage as the most severe. Farmers revealed cultivation of two types of *maiwa* millet; the late maturing type with long panicles and early maturity type that have short panicle (Fig. 3.2).

Figure 3. 2. The long and short panicle types of *maiwa* millet as identified by the farmers.

Among the two types of *maiwa* millet reported by farmers, the majority (77%) indicated that the short panicle types were more resistant to *Striga hermonthica* than the long panicle type while 12% of the respondents revealed that both types of *maiwa* millet were resistant/tolerant to *Striga*. All the respondents, however, agreed that *maiwa* millet is more tolerant to *Striga* than *gero* millet or sorghum. Most (79%) of the farmers used hand-pulling to control *Striga* in their fields. The farmers also revealed that they have no access to improved *maiwa* millet seeds so the majority of them used their own saved seeds (80%) with no seed treatment applied prior to planting (79%). All the respondents (100%) desired access to improved resistant varieties of the crop.

Table 3. 10. Understanding farmers' knowledge of *Striga hermonthica* and control strategies

Question asked	**Answer**	**Percent**
For how long is *Striga* a problem in your field?	< 20 years	21
	>20 years	79
At what growth stage is *Striga* a problem?	- Seedling	18
	- Heading	42
	- Flowering	39
	- Maturity	1
Among the varieties you grow which is most resistant/ tolerant?	- Long panicle	2
	- Short panicle	77
	- Both	12
	- None is resistant	9
How do you control *Striga*?	- Hand pulling/hoe	79
	weeding	4
	- Use of urea	7
	- Weeding and urea app.	
What is your source of seed?	- Other farmers	9
	- Own saved seed	80
	- Market	4
	- Agro-dealers	7
Do you treat your seed before sowing?	- Yes	21
	- No	79
Would you like to have *Striga* resistance cultivar?	- Yes	100
	- No	0

3.4 Discussion

3.4.1 Pearl Millet Farmers’ Preference Traits

The overwhelming majority of the respondents desired to have new pearl millet varieties with resistance to *Striga*, tolerance to shattering, downy mildew, lodging tolerance, good quality local beverage, panicle compactness, storability, taste, grain hardness, grain colour (grey), grain size (big), thresh-ability (ease), high yield, panicle size, medium maturity, moderate height, panicle length and panicle bristle and preferred attributes of their landraces.

Dugje *et al*. (2006) have reported similar findings that farmers rated *Striga* as the most important constraint in Yobe State of Nigeria and were willing to adopt resistant varieties. Preference to medium to late varieties expressed in this study as they are associated with delayed onset of flowering that helped in reducing amount of pollen loss due to rainfalls and reduced incidence of ergot disease has been reported by earlier workers (John, 2002; Thakur *et al.*, 2011; Miedaner & Geiger, 2015; Lubadde *et al.*, 2016). Absence of early maturing varieties that are believed to be resistant to ergot disease has influenced farmers’ preference for late maturing cultivars that mature when the incidence of ergot is minimal.

Preference for medium and tall plants (1 ± 0.2 m) was due to ease of harvesting as noted by Owere *et al.* (2014) in finger millet, and reduced lodging which occurs in taller plants. In addition, Owere *et al.*, (2014) reported that farmers prefer tall varieties because very short varieties are more susceptible to attacks by rodents, damage by rain splash, ground walking birds and grain damage by termites. They also reported that grains on shorter varieties were harder for birds to feed on. Market and consumer acceptance determined the choice for grey coloured and bold grains, and these grain types are also most suitable for good local beverage (*Kunun zaqi*) and taste. This finding corroborates with the findings of (Achot *et al.*, 2014)These indicate that the principal use of pearl millet in the study areas is for food (thick porridge, thin porridge and fried as cake) and making local beverage (*Kunun zaqi*). In addition, grey coloured grains were less preferred by birds compared to whitish coloured grains. Grain colour, therefore, is associated with its utilization of the crop as well as market value. These were also pointed out by Biradar & Tomer (1999). All the farmers, however, concurred that there was a need for improvement in the current cultivars.

Other traits desired by farmers included: tolerance to shattering, tolerance to lodging, downy mildew resistance, good quality brew, high tillering ability, panicle compactness, storability, taste, and grain hardness.

3.4.2 Farmers identified constraints to pearl millet production across three local government areas of Jigawa State Nigeria

Poor soil fertility was identified across all villages visited as the leading constraints to production of pearl millet. Soils were very poor and farmers harvest little to nothing without applying fertilizers. This is one of the reasons why many opt for the cultivation of *maiwa* types since they give relatively good yield even in soils with sub-optimal fertility. This finding is consistent with earlier reports by Ismaila *et al.* (2010), Ibrahim *et al.* (2015), and Lubadde *et al.* (2016), in which farmers had indicated poor soil fertility as a major constraint to pearl millet production. However, Manyong (2009) identified had identified *Striga* infestation and poor access to inputs as their major production constraints.

Striga hermonthica infestation and downy mildew disease were the major biotic stresses reported across all study areas. This study confirmed the increasing trend of *Striga* infestation incidence and severity in pearl millet growing areas. This persistent pattern has been noted by several surveys over the years by farmers' responses and susceptibility of cultivars which were initially resistant due to the emergence of new races/biotypes of the parasitic weeds and use of infested seeds. There was a high incidence of *Striga* at Shuwarin, Madobi, Kudai, and Kantoga compared to Karfawa and Kafingana. Other important constraints identified were based on farmer groups from the different villages. This finding is consistent with Lubadde *et al.* (2016) that farmers from different areas have different production constraints.

3.4.3 Farmers' perceptions of *Striga hermonthica*

Surveyed farmers recognized *Striga* as a major problem in pearl millet production. *Striga* was named differently by different ethnic groups. These names were associated with the visible effects of the weeds on crop plants. Some of the local names are "*Kuduji"* in Madobi, "*wuta wuta"* and "*maakasha"* meaning witchcraft, fire and killer respectively. This demonstrated that farmers were aware of the devastating effects of this pest. They went further to attribute the emergence of the pest with poor soil fertility and were able to highlight symptoms of infested millet plants. All the two main *maiwa* varieties together with the other cereals were susceptible to *S. hermonthica.* Farmers from all the study areas reported that *Striga* incidence has been on the rise for the past few years, this trend has been observed by Emechebe *et al.* (2004). Most respondents reported higher severity and damage by *Striga* at heading and flowering period while other respondents reported higher severity at the seedling stage. Short panicle *maiwa* types were assumed by farmers to be more tolerant to *Striga* infestation over the long panicle types, they also alluded that some previously *Striga* tolerant short panicle types are now showing susceptibility, indicating a possible breakdown of tolerance in those cultivars.

Farmers across the study areas observed that *Striga* damage to pearl millet was of two kinds; underground and aboveground damage and strongly suggested underground damage was more harmful than the aboveground. These assertions have been confirmed by empirical results from Emechebe *et al.* (2004). Several conflicting reasons were advanced to be responsible for increased incidence and severity of *Striga* infestation by farmers, with farmers at Madobi suggesting that inadequate resource to purchase desired inputs such as fertilizers and high cost of labor as responsible for the increased incidence of *Striga*. This point of view has been supported by Atera *et al.* (2012) who studied incidence of *Striga* in maize, while farmers at Kafingana and Kudai areas believed increased incidence of *Striga* is due to poor soil fertility resulting from continuous cropping without adequate fertilizer application, recurrent problems of erosion and dispersal of *Striga* seeds by grazing animals as main reasons from increasing trend of *Striga* menace. These findings indicate that pearl millet growers were well informed of the devastating effects of *Striga* on their fields and desirous of sustainable solutions that will help address the menace of *Striga* fields.

3.4.4 Farmers management strategies for *Striga* infested fields

Farmers have adopted different methods towards addressing a variety of problems resulting from *Striga* infestation of their fields. These include use of manual hoe-weeding and or hand pulling emerged *Striga* plants, application of fertilizers, rotation of crops, application manure and land fallowing. Use of hoe weeding and hand pulling were the most popular among local farmers. Farmers reported that these old control methods (such as hand hoeing or hand pulling, application of manure, land fallowing and cereal rotation other than legumes) were obtained from parents and elders. Similarly, a small proportion of farmers reported the use of more efficient methods to control *Striga* infestation. These include use of urea fertilizer, spraying of herbicide, rotating pearl millet with legumes like cowpea. Most of the farmers that reported these latter methods were often younger ones and those with good contact with extension personnel.

Preference was given to control measures that resulted in increased yield, reduced *Striga* emergence, increased vigour of the crop, improved soil fertility while methods that lead to increased need of labour, add to cost of production, increased chance of crop damage and lower quality and quantity of grain yield were generally not favored by most of the growers across the study areas. Use of control measures by farmers was determined by several factors, such as household needs, available resources, potential advantages and disadvantages of the method, thereby leading to the use of different strategies by different communities. For instance, use of hand pulling and intercrop of pearl millet with legumes like cowpea/groundnut was more popular at Madobi and Kafingana areas while these combinations were not being used in several other places surveyed, while at Kudai and Kantoga and Karfawa and Shuwarin apart from hand pulling which is widely adopted, application of urea fertilizer was used by some farmers. In severely infested fields, farmers with limited resources that cannot support the use of appropriate control strategies abandon their fields. Use of urea fertilizer was the least preferred by most farmers due to the high cost of the fertilizers and occasional scarcity of the fertilizers, especially in rural markets. Farmers lamented poor access to fertilizers despite substantial investments made by the government in subsiding farmer inputs like fertilizers and improved seeds. Interventions targeting reduction of *Striga* menace among farming communities will lead to significant positive impacts on the well-being of these farmers due to high yield losses associated with *Striga* problems.

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 pearl millet for biotic stress is a priority objective. *Striga* is particularly a serious challenge to millet production in Jigawa state, Nigeria. Development of new pearl millet varieties should include criteria such as resistance to *Striga* and downy mildew, tolerance to shattering and lodging, good beverage quality, high tillering ability, panicle compactness, storability, taste, grain hardness, and boldness and grey grain colour. *Striga* infestation was a major problem for main staple crops that provide food and nutrition security to communities across study areas. Menace by *Striga* is a serious constraint to farming communities visited and this is likely to become worse unless appropriate control measures are put in place. Farmers have adopted local control measures techniques for controlling *Striga*, including hand pulling or hoe weeding, urea application, intercropping and combination of urea application and hand pulling. Farmers' perceptions on the rising incidence and severity of *Striga hermonthica* shows that deployment of affordable and sustainable control measures will be adopted by small-holder farmers in these areas. Therefore, there is a need for quick intervention by research and extension to salvage the conditions of these farmers to ensure improved food and nutrition security of these communities.

CHAPTER FOUR

4.0 Phenotyping Pearl millet germplasm for resistance to *Striga*

4.1 Introduction

Striga [(*Striga hermonthica* (Del.) Benth)] is one of the major biotic factors constraining pearl millet production in several tropical and subtropical regions of sub-Saharan Africa including Nigeria. It can cause yield loss ranging from 20 to 80% in Africa and up to 100% in worst situations, leading to the consequential significant negative impact on food security in these regions (Rodenburg *et al*, 2005; Badu-Apraku & Akinwale, 2011; Teka, 2014).

Most of the available methods for *Striga* control are either expensive or too complicated for low income, low literacy, and subsistence smallholder African farmers. Resistant crop cultivars have long been proposed as a means of reducing losses due to *Striga* under the low-cost input subsistence farming in Africa (Ejeta *et al*., 1993). However, development of resistant cultivars has been hindered by the paucity of resistance genes in the crop germplasm, specificity of resistance, existence of *Striga* morphotypes, ecological variant races of the parasite and lack of fast and reliable screening techniques (Yagoub *et al*., 2014). Field screening with *Striga* inoculum has been effective in assessing the response of genotypes to *Striga hermonthica*. However, field screening with artificial *Striga* infestation is not always feasible because of parasite dissemination risks (Haussmann *et al*., 2001); and it may not always be reliable because weed pressure and distribution cannot be controlled by the researcher.

Pearl millet and *S. hermonthica* are highly out-crossing species, which results in each plant in a pearl millet population having a different genotype and therefore carrying potentially different alleles for *Striga* resistance or susceptibility. Similarly, the high variability of *Striga* strains/biotypes (Reda *et al*., 2010) carries potentially different alleles for virulence, which call for a greater effort to continuously search for potential sources of resistance from diverse gene pools. Pearl millet is a genetically diverse crop, and this has offered a great opportunity for searching for sources of resistance and tolerance. The identification of sources of *S. hermonthica* resistance and their incorporation into breeding schemes would be a useful approach to combat the damage caused by the parasite in pearl millet. The objective of the present research was to screen for superior genotypes for *Striga* resistance among the germplasm available in Nigeria and identify resistant parents for subsequent studies.

4.2 Materials and Methods

4.2.1 Location

The experiment was carried out under field conditions at Birninkudu Local Government Area of Jigawa State, Nigeria in 2015. Birninkudu is located at 12°59’53’’N, 8°54’35’’ E and 47 km south of Duste the capital city of the state. The climate is semi-arid, characterized by a long dry season and a short wet-season with a mean annual temperature of about 25°C; relative humidity ranges from 80% in August to 23% between January and March. The total annual rainfall ranges from 600-1000 mm. The site was chosen because it is one of the hot spots of *S. hermonthica* infestation in north-east Nigeria (Personal communication Angarawai Ignatius, ICRISAT).

4.2.2 Pearl millet germplasm

Pearl millet germplasm used comprised of 240 S_2 genotypes that included 156 from the International Crop Research Institute for Semi-Arid Tropics (ICRISAT), 82 landraces collected by the Lake Chad Research Institute (LCRI) across Northern Nigeria (Figure 4.1) and two local pearl millet accessions obtained from the location where evaluation was carried were included as checks (appendix 2).

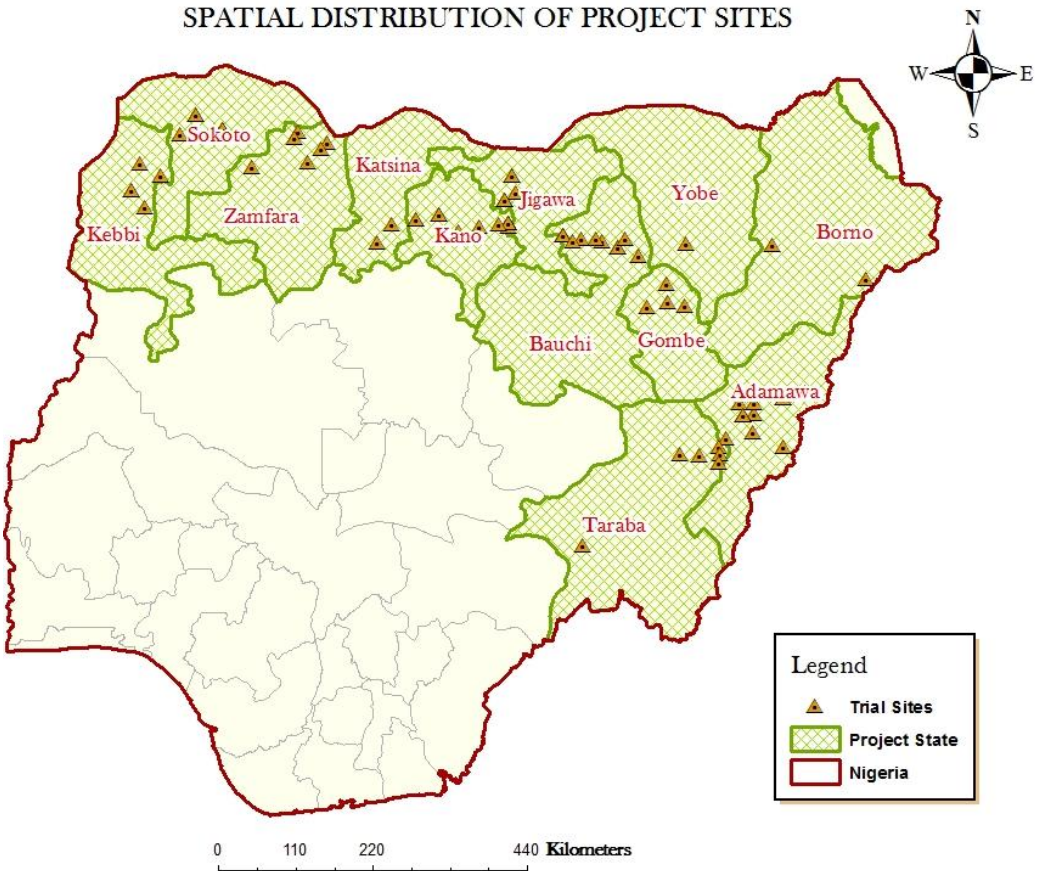


Figure 4. 1. Map of Nigeria showing locations of pearl millet landrace collections used in this study.

4.2.3 Cultural practices and field evaluations

The trial was conducted under natural infestation in a field that was severely infested with *S. hermonthica* and infestation augmented artificially under rainfed condition of 2015 at Birninkudu (12°59’53’’N, 8°54’35’’ E). The augmentation was done according to the method of *Striga* infestation developed by IITA’s Maize programme. *Striga* seeds were collected on sorghum field from the previous planting season and mixed with fine sand in the ratio of 1:99 by weight. A scoop was used to infest each hole with approximately 5,000 germinable *Striga* seeds. The experimental set up was 20 × 12 Alpha lattice design with two replications. Each plot comprised of two rows of 5 m long each with between row spacing of 0.75 m, and 0.50 m within the rows. Three to five seeds were planted per hill and were thinned to one per hill two weeks after planting, resulting in a total of 20 plants per plot. Data were recorded on five randomly selected plants and the average taken. Compound fertilizer (N15: P15: K15) fertilizer was applied at a rate of 50 kg ha^{-1} two weeks after sowing pearl millet. One hoe-weeding was done before *Striga* emergence, thereafter handpicking was done each time there was weed emergence other than *Striga*.

4.2.4 Data collection

Data were collected on the following quantitative characters:

- A number of panicles **(NP),** determined by counting all productive panicles (panicles with seeds) per plot at maturity.
- Panicle weight (**PW**), determined by harvesting all productive panicles per plot, sun-drying for 10 days and the weight determined in kg ha^{-1}using a beam balance
- Grain yield (**GY**), was estimated by weighing the grain yield per plot using a measuring scale after drying the panicles in the sun to about 10% moisture content, threshing, winnowing, and cleaning. The grain yield per plot was converted to kg ha^{-1} using the formula (10000 x yield per plot) / (plot size x 1000).
- Number of emerged *Striga* plants (Sc60, Sc90, and Sc140), *Striga* plants were counted after the appearance of the first *Striga* plant in the field that was 60 days after sowing (DAS), followed by 90 days and at 140 on plot basis (Fig. 4.2).

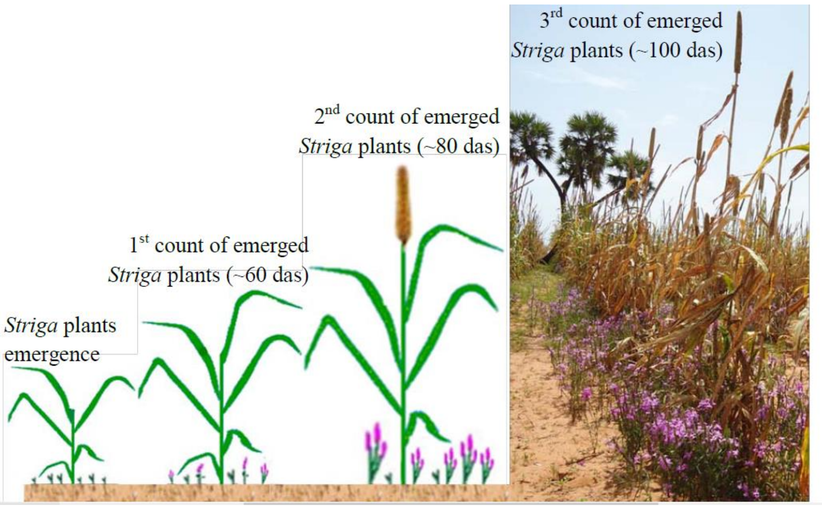


Figure 4. 2. Successive counts of emerged *Striga* plant throughout the season. The figure illustrates the continued counts of emerged *Striga* plants in the plot (Source: Kountche, 2013)

- *Striga* vigour (Sv60, Sv90, and Sv140): Each *Striga* count was accompanied by visual estimation of the vigour of emerged *Striga* plants, based on the average height of the emerged *Striga* plants and the extent of *Striga* branching as described in Figure 4.3 and table 4.2 (Haussmann *et al*., 2000a).

Figure 4. 3. Illustration of *Striga* vigour classes. Source: Kountche, 2013

Table 4. 1. *Striga* vigour classes

Score	***Striga* height (cm)**	**Number of *Striga* branches**
1	≤5	No branch
2	6 – 20	No branch
3	6 – 20	≥ 1
4	21 – 30	≤ 5
5	21 – 30	> 5
6	31 – 40	≤ 10
7	31 – 40	> 10
8	> 40	≤ 10
9	> 40	> 10
0	No emerged *Striga* plant	

Source: Kountche, 2013

- *Striga* severity (**SEV**): This was calculated by multiplying the *Striga* count and the average *Striga* vigour. *Striga* severity was assessed in each plot following each *Striga* count (Haussmann *et al*., 2000a).

In host-plant reaction to *Striga* studies, low values of *Striga* traits (*Striga* emergence, vigour, and severity) indicate resistance to *Striga*, and high values report susceptibility to *Striga*.

Genotypes were classified as resistant or susceptible using a scale described by Singh & Emechebe (1997) as follows:

1. Resistant = no *Striga* emergence on plot and no *Striga* symptom observed on the plant.
2. Moderately resistant = few *Striga* emergence (2-3) per plot but no *Striga* symptoms observed.
3. Tolerant = *Striga* emergences on host plant but no significant yield reduction
4. Susceptible = >5 *Striga* plant emerged per plot and plants show severe *Striga* symptoms associated with stunted growth and yield reduction.

4.2.5 Statistical model and data analysis

Prio to the statistical analysis, data on *Striga* count, vigour and severity were square root transformed. The linear model of observations in alpha lattice design used is as follow:

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

Where y_{ijk} is the value of the observed trait for ith treatment received in the kth block within jth replicate (superblock),

τ_i is the fixed effect of the ith treatment (i = 1, 2… t);

ρ_j is the effect of the jth replicate (superblock) (j = 1, 2… r);

β_{jk} is the effect of the kth incomplete block within the jth replicate (k = 1, 2…s)

e_{ijk} is an experimental error associated with the observation of the ith treatment in the kth incomplete block within the jth complete replicate.

The data obtained from the study were subjected to analysis of variance (ANOVA) using PROC GLM in SAS 9.3. Pearson's correlation coefficient was used to test associations among the measured traits using SAS 9.3. Principal component analysis (PCA) and cluster analysis using the same software was then performed.

4.3 Results

4.3.1 Performance of pearl millet genotypes under *Striga* infestation

The analysis of variance for *Striga* resistant traits and yield components revealed a highly significant difference ($p<0.05$) among the pearl millet accessions (Table 4.2).

Table 4. 2. Mean square from the analysis of variance *Striga* parameters, yield and yield components of pearl

Source	**Sc60**	**Sc 90**	**Sc 140**	**Av_Se**	**Sv 90**	**Sv140**	**SS90**	**SS1 40**	**Np**	**Pw**	**Gy**
Block (Rep)	0.01	0.49*	0.67	0.25*	0.83	1.09	4.78*	14.89	84.97	1108593	13683.03
REP	0.01	1.01	1.01	0.49	0.21	0.1	4.22	18.49	0.93	294884.7	19452.5
Entry	0.1**	1.30**	2.72**	0.90**	1.85**	4.74**	12.49**	42.91**	1055.65**	1861186**	389625.06**
CV	436.3	105.4	92.0	91.6	109.2	86.1	118.4	116.0	13.2	65.5	14.7
Mean	0.0	0.5	0.7	0.4	0.7	1.2	1.4	2.8	59.3	1579.3	858.9

Sc60, Sc90, Sc140 denote Striga count at 60, 90 and 140 days after planting respectively;Av_se=average Striga emergence; Sv90, Sv140 denote Striga vigour at 90 and 140 days after planting; Ss90, Ss140 denote Striga severity at 90 and 140 days after planting; Np, Pw,Gy denote number of panicles, panicle weight, and grain yield respectively

There were significant ($p<0.05$) differences among genotypes for *Striga* count, *Striga* vigour, *Striga* severity, number of panicles, panicle weight and grain yield (Table 4.3). Fifteen genotypes mostly from Nigerian collections had no emerged *Striga* shoots and were described as resistant in this study. Ten genotypes described as tolerant in this study had 1 to 4 *Striga* shoots/plot, but no significant yield loss observed. They were made up of collections from ICRISAT with only one from Nigeria. Two checks (Farmers' local1 and Farmers' local 2) had no emerged *Striga* shoots. *Striga* vigour ranged from 1-5 and severity ranged from 1-16 (Table 4.4). The number of panicles formed by the pearl millet varieties ranged from 37- 131 for the resistant genotypes with an average of 72.1 ha^{-1}. Panicle weight (Pw) varied from 1.780 kg ha^{-1} to 3.612 kg ha^{-1} with a mean of 2.542.5 kg ha^{-1}. Grain yield ranged from 1530.47 kg ha^{-1} to 2549.33 kg ha^{-1} with an average of 1831.55 kg ha^{-1} (Table 4.4).

Fifteen genotypes (IP12049, IP13222, IP20679, IP20593, IP20962, IP20710, IP20446, IP20580, MPMG11032, IP20753, IP20604, IP12101, IP20750, IP20467 and IP13238) considered as susceptible, had emerged *Striga* shoots (1 to 4 *Striga* shoots) with relatively significant yield loss, *Striga* vigour and severity ranging from 2-6 and 3-20 respectively (Table 4.5).

The number of panicles formed ranged from 23- 61 for the susceptible genotypes with an average of 35.37 ha^{-1}. Panicle weight (Pw) varied from 501 kg ha^{-1} to 1.087 kg ha^{-1} with a mean of 7.483 kg ha^{-1}. Grain yield ranged from 99.55 kg ha^{-1} to 310 kg ha^{-1} with an average of 352.46 kg ha^{-1} (Table 4.5)

Table 4. 3. *Striga* parameters, yield and yield components (number of panicles and panicle weight) of pearl millet

Genotypes	**Sc60**	**Sc90**	**Sc140**	**Av_Se**	**Sv90**	**Sv140**	**Ss90**	**Ss140**	**Np**	**Pw**	**Gy**
Local checks											
Farmers'local1	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	53	2267	1754.67
Farmers'local2	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	56	2130	1076.00
Fifteen most resistant											
MPMG11074	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	84	2898	2549.33
IP 20990	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0 (0.7)	0(0.7)	59	2916	2528.34
MPMG11089	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0 (0.7)	0(0.7)	109	2755	2066.60
IP20717	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0 (0.7)	0(0.7)	56	2844	2009.33
MPMG11079	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0 (0.7)	0(0.7)	56	2389	1946.29
MPMG11051	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0 (0.7)	2(0.7)	51	3612	1856.00
MPMG11104	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	84	2619	1700.00
MPMG11015	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	72	2498	1652.67
IP 20511	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	71	2550	1646.65
MPMG11080	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	91	2432	1631.24
IP 20414	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	37	2234	1641.33
IP 12136	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	99	2515	1581.00
IP 20940	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	77	2159	1580.00
MPMG11108	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	111	1937	1548.00
MPMG11112	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	0(0.7)	131	1780	1530.47
Mean	0	0	0	0	0	0	0	0	79.2	2542.53	1831.15
Range	0	0	0	0	0	0	0	0	94	1832	1018.86
LSD	**0.18**	**1.08**	**1.35**	**0.77**	**1.50**	**2.12**	**3.17**	**6.46**	**15.43**	**765.2**	**249.57**

LSD: Least Significant Difference at p = 0.05; Values in bracket are transformed; Values outside the bracket are untransformed; Sc60, Sc90, Sc140 denote Striga count at 60, 90 and 140 days after planting respectively;Av_se=average Striga emergence; Sv90, Sv140 denote Striga vigour at 90 and 140 days after planting; Ss90, Ss140 denote Striga severity at 90 and 140 days after planting; Np, Pw,Gy denote the number of panicles, panicle weight, and grain yield respectively

Table 4. 4. *Striga* parameters, yield and yield components (number of panicles and panicle weight) of pearl

Genotypes	Sc60	Sc90	Sc140	Av_Se	Sv90	Sv140	Ss90	Ss140	Np	Pw	Gy
Ten most tolerant											
IP 13225	0(0.7)	3(1.7)	3(1.9)	2(1.5)	3(1.9)	5(2.2)	8(2.8)	9(3.9)	36	2965	2698.67
IP 20513	0(0.7)	1(1.1)	2(1.4)	1(1.3)	2(1.3)	4(2.1)	3(1.6)	13(2.6)	76	2819	2448.67
IP 20695	2(1.6)	4(2.1)	4(2.1)	3(2.0)	4(2.1)	4(2.1)	16(4.1)	16(4.1)	77	3165	2217.78
MPMG11094	0(0.7)	1(1.0)	1(1.0)	0(0.9)	1(1.1)	2(1.3)	1(1.1)	2(1.3)	63	2752	1828.67
IP 17436	0(0.7)	1(1.0)	2(1.4)	0(1.1)	2(1.3)	3(1.7)	2(1.3)	5(2.1)	79	2703	1776.00
IP 13240	0(0.7)	2(1.3)	3(2.0)	1(1.2)	2(1.3)	4(1.7)	5(1.9)	16(3.3)	97	2450	1648.00
IP 13226	0(0.7)	1(1.1)	2(1.6)	1(1.2)	2(1.3)	5(2.2)	3(1.6)	9(3.1)	71	2376	1634.00
IP 12135	0(0.7)	0(0.7)	1(1.0)	0(0.8)	0(0.7)	1(1.1)	0(0.7)	1(1.1)	101	1933	1477.66
IP 12068	0(0.7)	1(1.0)	1(1.1)	1(1.0)	1(1.1)	1(1.1)	1(1.1)	2(1.4)	55	1918	1394.00
IP 20729	0(0.7)	2(1.4)	2(1.4)	1(1.2)	1(1.2)	3(1.7)	2(1.4)	4(2.1)	84	2851	1368.67
Mean	0.2	1.6	2.1	1	1.8	3.2	4.1	7.7	73.9	2593.2	1849.2
Range	0-2	1-4	1-3	1-3	1-4	1-4	1-16	1-15	36-101	1918-2965	1368-2698
Fifteen most susceptible											
IP 12049	0(0.7)	2(2.0)	3(1.7)	2(1.4)	3(1.7)	4(2.1)	5(2.2)	11(3.2)	23	740	409.55
IP 13222	0(0.7)	1(1.1)	1(1.1)	0(0.7)	2(1.3)	3(1.5)	3(1.6)	18(4.2)	53	949	392.00
IP 20679	0(0.7)	3(1.7)	4(2.1)	2(1.6)	3(1.9)	4(2.1)	8(2.8)	16(4.1)	35	733	378.23
IP 20593	1(1.0)	2(1.6)	2(1.6)	2(1.4)	3(1.9)	5(2.2)	6(2.5)	9(3.1)	35	1087	375.35
IP 20962	0(0.7)	2(1.4)	2(1.4)	1(1.2)	2(1.5)	4(2.0)	3(1.7)	5(2.2)	24	578	377.33
IP 20710	0(0.7)	2(1.6)	2(1.6)	1(1.4)	3(1.9)	4(2.1)	6(2.5)	8(2.9)	41	675	358.67
IP 20446	0(0.7)	2(1.6)	4(2.0)	2(1.5)	4(2.0)	5(2.3)	7(2.7)	18(4.2)	61	678	344.06
IP 20580	0(0.7)	2(1.6)	2(1.6)	1(1.4)	3(1.7)	4(2.1)	5(2.3)	8(2.9)	29	725	356.89
MPMG11032	0(0.7)	2(1.6)	3(1.9)	2(1.5)	3(1.9)	3(1.9)	6(2.5)	9(3.1)	25	501	351.82
IP 20753	0(0.7)	1(1.1)	2(1.6)	1(1.2)	2(1.3)	3(1.9)	3(1.6)	6(2.5)	51	944	329.33
IP 20604	0(0.7)	1(1.0)	3(1.7)	1(1.3)	2(1.4)	5(2.2)	4(1.8)	12(3.3)	29	626	340.17
IP 12101	1(1.0)	2(1.4)	2(1.4)	1(1.3)	2(1.4)	3(1.9)	3(1.7)	3(2.1)	31	803	337.33
IP 20750	0(0.7)	1(1.1)	2(1.3)	1(1.1)	2(1.3)	2(1.4)	3(1.6)	6(2.1)	32	573	311.84
IP 20467	0(0.7)	4(2.1)	5(2.2)	3(1.3)	3(1.7)	3(1.9)	11(3.2)	18(3.9)	31	785	314.34
IP 13238	2(1.4)	5(2.3)	11(3.3)	6(2.3)	4(2.1)	6(2.4)	20(4.5)	18(6.0)	36	837	310.00
Mean	**0.27**	**2.13**	**3.2**	**1.73**	**2.73**	**3.87**	**6.2**	**11**	**37.73**	748.93	352.46
Range	**1-2**	**1-4**	**1-11**	**1-6**	**2-4**	**2-4**	**1-11**	**3-15**	**38**	586	99.55
LSD	**0.18**	**1.08**	**1.35**	**0.77**	**1.50**	**2.12**	**3.17**	**6.46**	**15.43**	**765.2**	**249.57**

Least Significant Difference at p = 0.05; Values in bracket are transformed; Values outside the bracket are untransformed; Sc60, Sc90, Sc140 denote Striga count at 60, 90 and 140 days after planting respectively;Av_se=average Striga emergence; Sv90, Sv140 denote Striga vigour at 90 and 140 days after planting; Ss90, Ss140 denote Striga severity at 90 and 140 days after planting; Np, Pw,Gy denote number of panicles, panicle weight and grain yield respectively

4.3.2 Assessing the relationship between *Striga* parameters and yield parameters across genotypes

There were high negative correlations between all the *Striga* parameters measured and the number of pearl millet panicles and panicle weight, except *Striga* count at 60 DAS (r = 0.00) (Fig. 4.4). Similarly, there were high negative correlations between all the *Striga* parameters with pearl millet grain yield except for *Striga* count at 60 DAS (r = 0.10).

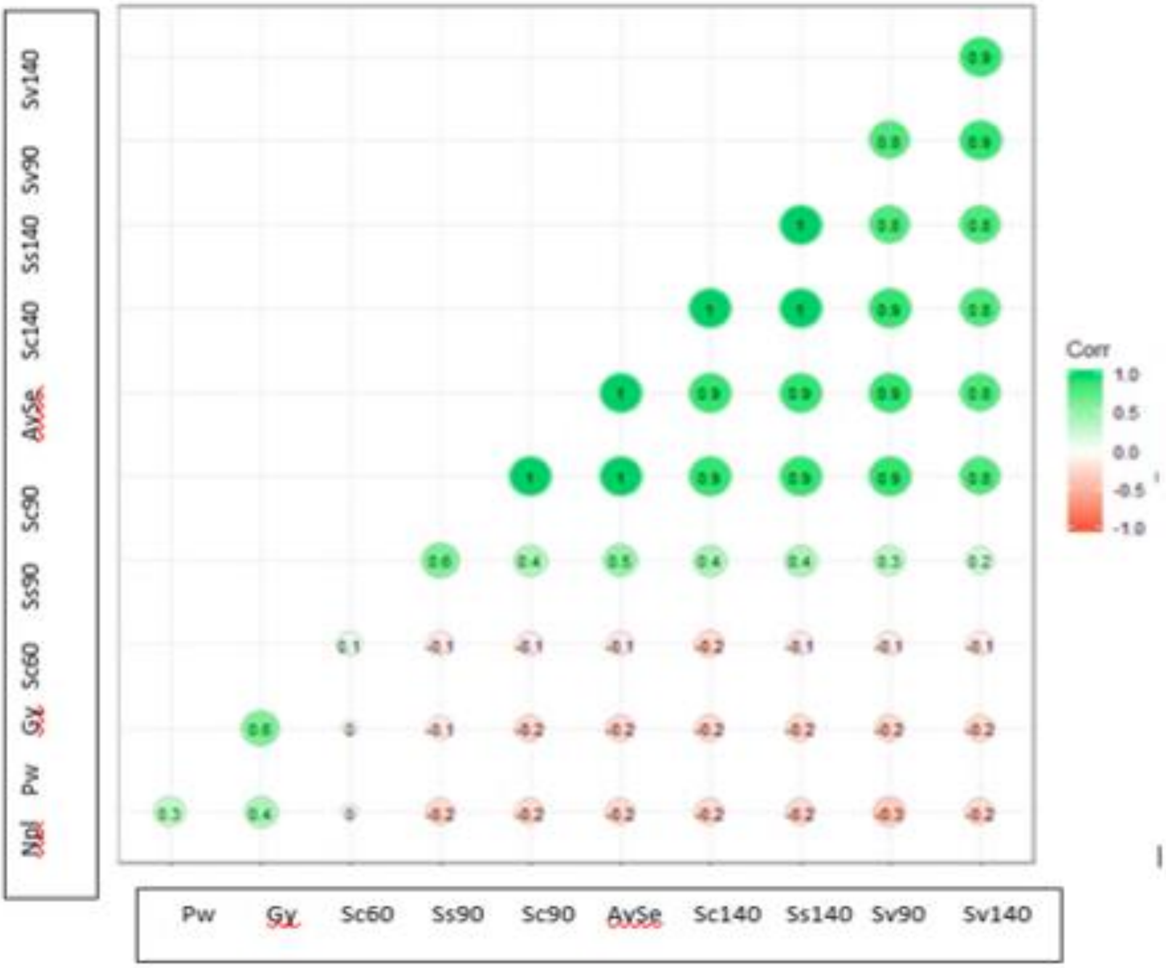


Figure 4. 4. Pattern of relationship between *Striga* resistance parameters and yield parameters. Sc60, Ss90, Sc90, AvSe, Sc140, Ss140, Sv90, SvV140 denote Striga count at 60 days after planting, Striga severity at 90 days after planting, Striga count at 90 days after planting, average Striga count, Striga count at 140 days after planting, Striga severity at 140 days after planting Striga vigour at 90 days after planting and Striga vigour at 140 days after planting respectively; Pw,, Npl, and Gy denote panicle weight, number of panicles, and grain yield respectively

4.3.3 Relative importance of *Striga* and yield parameters

The first two principal components accounted for 80.00% of the total variance and have eigenvalues greater than 1(Table 4.5). The first PC accounted for 61.67 % whereas the second accounted for 18.33 %.

The first PC (61.7) was associated with *Striga* count at 90 days after sowing (Sc90), *Striga* count at 140 days after sowing (Sc140), average *Striga* emergence (Av._Se), *Striga* vigour at 90days after sowing (Sv90), *Striga* vigour at 140days after sowing (Sv140), *Striga* severity at 90days after sowing (Ss90) and *Striga* severity at 140 days after sowing (Ss140D). The second PC was associated with traits such as number of panicles formed (Np), panicle weight (Pw) and grain yield (Gy).

Table 4. 5. Eigenvectors and values from the two principal component axes used

Traits	**Prin1**	**Prin2**
Sc60	0.181	0.211
Sc90	**0.373**	0.050
Sc140	**0.372**	0.044
A_Se	**0.379**	0.062
Sv90	**0.357**	0.015
Sv140	**0.341**	0.004
Ss90	**0.369**	0.095
Ss140	**0.368**	0.063
Np	-.117	**0.435**
Pw	-.104	**0.613**
Gy	-.077	**0.607**
Eigenvalue	6.784	2.016
Proportion	0.617	0.183
Cumulative	0.617	0.800

Sc60, Sc90, Sc140, and A_Se denotes *Striga* count at 60 days, 90 days, 140 days and average *Striga* emergence respectively. Sv90= *Striga* vigor at 90 days, Sv140= *Striga* vigor at 140 days, Ss90= *Striga* severity at 90 days, Ss= *Striga* severity at 140 days, Np= number of tillers, Pw= panicle weight, Gy= grain yield

4.3.4 Relatedness of genotypes based on principal *Striga* and yield parameters

The agglomerative hierarchical clustering dendrogram illustrated the relationship among the 240 accessions (Figure 4.4) based on the traits that contributed most to the first two (2) principal components (Table 4.6). Three main clusters were identified. A (Pink cluster) had 78 accessions, cluster B (blue cluster) had 108 accessions while cluster C (yellow cluster) had 54 accessions.

Cluster A included pearl millet genotypes which are low to medium yielding and tolerant to *Striga* infestation. Cluster B included low yielding genotypes which are susceptible to *Striga* infestation while cluster C included medium to high yielding pearl millet genotypes which are resistant to *Striga* infestation.

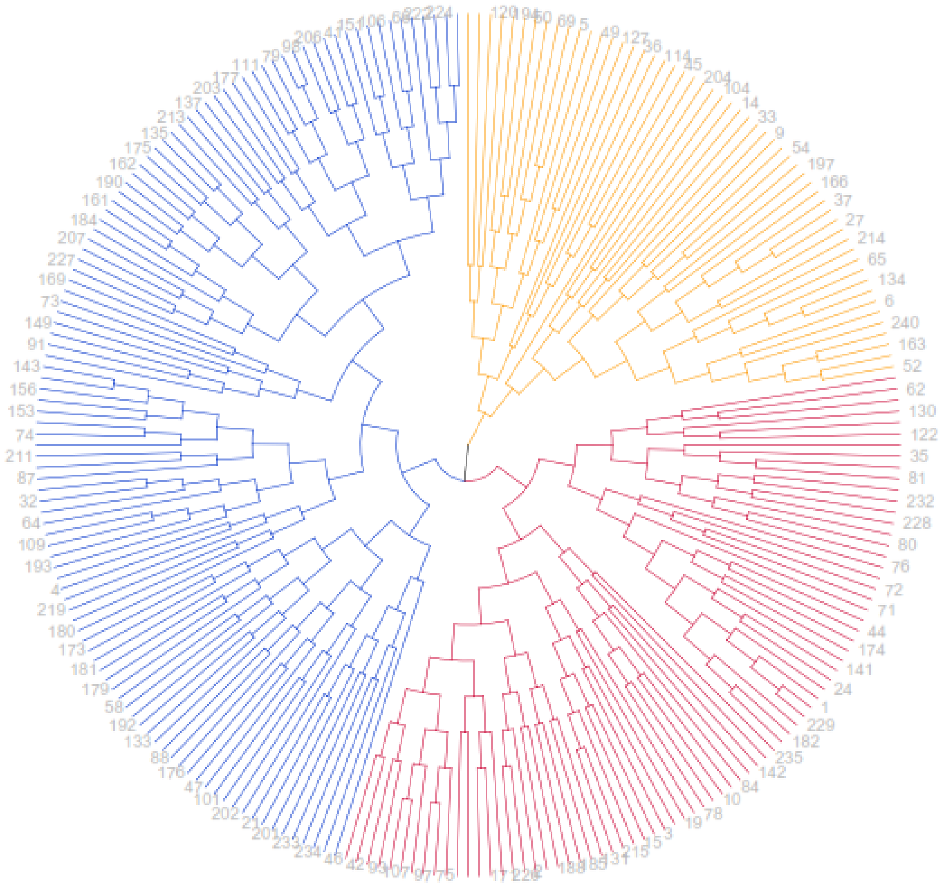


Figure 4. 5. Agglomerative Hierarchical Clustering Dendrogram constructed based on yield and *Striga* resistance parameters of pearl millet. The colors Pink, Blue, and Yellow are referred to as A, B, and C respectively. The numbers 1,2,3…240 refer to the first, second, third…last entry used in Table 4.1 in the text.

4.4 Discussion

A broad range of variation in *Striga* resistance traits was exhibited in this study which could be attributed to climatic conditions or the genetic background of the varieties. Only 15 genotypes including the two farmers' local (Farmers' local1 and Farmers' local 2) were identified without *Striga* emergence and with good agronomic performance in this study. This indicates that these genotypes based on phenotypic data were resistant to *Striga* infestation. These lines can be potential sources for developing a breeding programme for *Striga* resistance and yield improvement in pearl millet. Similar results were reported by Aladele & Mustapha (2003), Wilson *et al.* (2004) and Kountche (2013) who reported an absence or a low number of emerged *Striga* suggests resistance.

On the other hand, ten (IP13225, IP20513, IP20695, MPMG11094, IP17436, IP13240, IP13226, IP12135, IP12068, IP20729) genotypes supported moderate to high number of *Striga* with relatively high for grain yield, indicating that they are tolerant to *Striga*. Tolerant varieties are important in situations where resistant varieties are lacking. Kim (1994) and Kim *et al.* (2002) recommended the use of tolerant varieties of maize in the absence of resistant varieties because they produce a higher yield than the susceptible varieties under *Striga* infestation. In this study, the effect of *Striga* attack was more pronounced on fifteen genotypes. These genotypes supported a more or equal number of *Striga* as in the tolerant genotypes but with a significantly lower yield. These suggest susceptibility. The average yield of landrace pearl millet varieties in Nigeria ranged between 700-800 kg ha^{-1} (Aminu-Kano *et al.*, 1998). The yield of the fifteen genotypes reported as susceptible in this study ranged between 300-400 kg ha^{-1} which is far less than the expected yield, suggesting they are susceptible.

Furthermore, the yield performance and the *Striga* resistance traits were examined. There were significant negative correlations between all the *Striga* parameters measured and the number of productive panicles formed, except for *Striga* count at 60 DAS which was negative but not significant. This shows that panicle formation in pearl millet was significantly reduced by *Striga* infestation but at Sc60 there was no much *Striga* to cause a significant effect. This is in line with Kroschel (2001) and Olupot (2011) who reported that panicle formation is negatively affected by *Striga* infestation in sorghum. Furthermore, there were significant negative correlations between all the *Striga* parameters measured and panicle weight. However, *Striga* count at 60 DAS was not significantly correlated to panicle weight which may be as a result of low *Striga* emergence and vigour at this first count. This revealed that *Striga* attack as expected would reduce panicle weight. Ezeaku *et al*. (2015) found *Striga* infestation parameters to adversely affect panicle weight in pearl millet. Similarly, significant negative correlations were detected between most of the *Striga* infestation parameters and grain yield except for *Striga* count at 60 DAS, *Striga* severity at 90 DAS and at 140 DAS which were negative but not significant. These may be as a result of few *Striga* plants at the first count and the pearl millet plants showed some level of tolerance. The result further indicates that the more the *Striga* emergence and vigour the more yield is reduced. Mesfin & Temam (2016), Ezeaku *et al*. (2015), Aladele & Mustapha (2003) and Dugje & Adam (2012) found similar results in pearl millet.

Information from PCA gives an overview of the interrelationship in the whole set of data that explains the largest proportion of the variance which could be exploited by plant breeders to execute a breeding programme aimed at improving pearl millet for *Striga* resistance. The results in this study reveal that out of eleven parameters used, ten (Sc90, Sc140, Av._Se, Sv9, Sv140, Ss90. Ss140D Np, Pw and Gy), contributed to the variability observed among the genotypes. This indicates that these parameters can be used for selecting superior genotypes to be incorporated into the pearl millet breeding programme aiming at *Striga* resistance and yield improvement. In the present study, the PCA analysis divided the total variance into 10

PCs out of which the first two PCs contributed a substantial amount of diversity among the genotypes under study. This is consistent with the results obtained by Dugje and Adam (2012). The authors found that *Striga* emergence, *Striga* vigour, *Striga* severity, number of tillers, number of panicles, and grain yield among others contributed to the differences observed among the genotypes. They concluded that the yield-related parameters should be considered while selecting parents for hybridization programme in yield improvement of pearl millet.

Cluster analysis grouped the genotypes into three main clusters A (pink), B (blue) and C (yellow). The cluster A had 78 accessions, B had 108 accessions while C had 54 accessions. Cluster A included low to medium yielding *Striga* tolerant varieties with 70% of the genotypes having grain yield more than 800 kg ha^{-1}. This cluster could be further divided into two sub-clusters according to the yield levels. The sub-cluster A_1 included 19 genotypes with grain yield above 900 kg ha^{-1}. These can be used as donor parents in breeding for tolerance to *Striga* infestation although it is associated to increase in *Striga* seed bank in the soil. Cluster B included low yielding and susceptible to *Striga* varieties with 90% of the genotypes having grain yield less than 800 kg ha^{-1}. This cluster can further be divided into two sub-clusters; B_1 consisted of susceptible genotypes with grain yield less than 500kg/ha while B_2 consisted of susceptible genotypes with a relatively higher number of panicles that ranged between 23 to 100, panicle weight 670 to 1768 kg ha^{-1}and grain yield 329 to 900 kg ha^{-1}. Genotypes that were grouped in this cluster indicate that yield parameter contributed greatly in discriminating the genotypes. This was revealed by its high contribution to the first two principal components. Cluster C included medium to high yielding, *Striga* resistant varieties with 83.3% of the genotypes having grain yield greater than 1000 kg ha^{-1}. This grouping implies that genotypic variability existed between the accessions. Therefore, yield improvement and *Striga* resistance can be achieved with this germplasm.

4.5 Conclusion

Potential sources of resistance based on phenotypic data were found in this screening which included MPMG11074, IP20990, MPMG11089, IP20717, MPMG11079, MPMG11051, MPMG11104, MPMG11015, IP20511, MPMG11080, IP20414, IP12136, IP20940, MPMG11108, and MPMG11112. The clustering of the genotypes was predominantly based on their response to *Striga* and grain yield. Based on their performance with respect to yield and the response to *Striga* the genotypes screened were grouped into low to medium yielding and tolerant, low yielding and susceptible, and medium to high yielding and resistant.

CHAPTER FIVE

5.0 Generation Mean Analysis for *Striga* Resistance and Yield Contributing Traits in Pearl Millet [*Pennisetum Glaucum* (L.) R.Br.]

5.1 Introduction

Pearl millet is the staple food and fodder crop of millions of poor rural families in Africa and Asia (Shivhare & Lata, 2016; Senthilvel *et al*., 2008). Among other constraints, pearl millet production is affected by the parasitic weed *Striga hermonthica* (Del.) Benth. (Kountche *et al.*, 2013; Dawud *et al*., 2017). *Striga hermonthica* is an obligate parasitic plant that causes serious biotic constraint to cereal production thereby affecting the livelihood of over 100 million people in Africa (Sauerborn, 1991). In extreme cases, a severe infestation of *Striga* can result in complete loss of the crop and the abandonment of otherwise productive fields (Ejeta, 2007; Amusan *et al.*, 2008). Over the past several decades, many attempts have been made to identify and breed crop cultivars resistant to *Striga* infestation (Wilson *et al*., 2004; Kountche, 2013). However, adapted and resistant cereal cultivars capable of meeting the needs of the small-scale African farmers remain elusive. However, breeding for resistance to *Striga* in pearl millet, with the scarcity of donor source and known mechanism of resistance, has been challenging. Badu-Apraku *et al.* (2008) reported that favorable genes are expected to make an equal contribution to the improvement of a trait if their mode of inheritance is additive in nature. Better knowledge of the mode of gene action controlling the inheritance of resistance would enhance the identification and development of resistant varieties (Akanvou & Doku, 1998). Gethi and Smith (2004) reported additive gene effect to be more important for resistant to Striga in maize. However, Mbogo *et al.* (2015) and Badu-Apraku *et al.* (2008) reported preponderance of dominance-gene effect responsible for resistance to *Striga* in maize. However, for the pearl millet varieties adapted to and resistant/tolerant to *Striga* in Nigeria, little is known about the gene action involved in the resistance/tolerance to the noxious weed. Information about gene effects including additive, dominance gene effects (a and d) and the three types of non-allelic gene interactions, that is, additive x additive (aa), additive x dominance (ad) and dominance x dominance (dd) are very important in designing an effective gene deployment strategy in a resistance improvement programme. Generation mean analysis is a simple and useful technique for estimating these genetic effects as well as epistasis. The nature of epistasis is also useful in deciding breeding procedures to be adopted for the improvement of quantitative characters. The objective of this study was to identify gene effects influencing resistance/tolerance to *Striga hermonthica* in two crosses of pearl millet

5.2 Materials and methods

5.2.1 Location

The experiments were conducted in fields that were severely infested with *S. hermonthica* under rainfed conditions in 2017 at three environments in northern Nigeria; Birninkudu Prison farms (11°48’N, 9°47’E, 435m) (E_1) Jigawa state, Makoda (12°.33’N, 8°.53’E, 253m) (E_2) Kano state and Bui (12°43’42N, 4° 9’0E, 223m) (E_3) Kebbi state Nigeria. The climate in the locations is semi-arid, characterized by a long dry season and a short wet season with mean annual temperature and rainfall of about 25°C and 800mm, 34°C and 775mm, and 28°C and 807mm respectively.

5.2.2 Pearl millet population development

Two *Striga* resistant (Nyoko-2, IP20511) and two *Striga* susceptible (IP20446, IP13238) pearl millet genotypes selected from previous screening by ICRISAT in 2014 were crossed to produce F_1 generations. The F_1 generations were selfed and backcrossed to the susceptible (P_1) and resistant (P_2) parents to produce F_2, BC_1P_1, and BC_1P_2 generations, respectively. Experimental units comprised populations of the six generations (Table 5.1).

Table 5. 1. Two sets of 6 basic generations of pearl millet used in this study

Generations	**Cross 1 (C_1)**	**Cross 2 (C_2)**
P_1 (susceptible)	IP20446	IP13238
P_2 (resistant)	Nyoko-2	IP20511
F_1	IP20446 × Nyoko-2	IP13238 × IP20511
F_2	IP20446 × Nyoko-2	IP13238 × IP20511
BC_1P_1	(IP20446 × Nyoko-2) × IP20446	(IP13238 × IP20511) × IP13238
BC_1P_2	(IP20446 × Nyoko-2) × Nyoko-2	(IP13238 × IP20511) × IP20511

5.2.3 Experimental design and Cultural practices

Two sets of six generations; P_1, P_2, F_1, F_2, BC_1P_1, and BC_1P_2 were evaluated under *Striga* sick plots. Experimental plots were ploughed and harrowed and made into ridges. Seeds were hand-sown 3-5 seeds/ hill and were thinned to one per hill two weeks after planting. Entries were grown in a randomized complete block design in three replications. The experimental units were two-row plots for the P_1, P_2, and F_1 generations, a four-row plot for the BC_1P_1 and BC_1P_2 generations, and six-row plots for the F_2 progeny generation. The length of the rows including one-meter alleys was 6.5 meters with 0.75 meters between rows. Two rows were planted for P_1, P_2 and F_1, four rows for BC_1P_1 and BC_1P_2, and six rows for F_2, were used to sample adequately genotypic variability within generations. A compound fertilizer Nitrogen Phosphorus and Potassium (15:15:15) was applied at a rate of 50 kg per hectare two weeks after sowing. A hoe-weeding was carried out prior to *Striga* emergence and thereafter weeds were hand pulled.

5.2.4 Data collection

In each experiment, 20 plants from homogenous generations (P_1, P_2, and F_1), 40 plants from backcross generations [(BC_1P_1) and (BC_1P_2)] and 60 plants from F_2 generations were assessed in each replicate on the following parameters:

- **NTL**: number of tillers/plant
- **ASNPC**: Area under the *Striga* number progress curve (ASNPC) is a measure of the total *Striga* emergence throughout the season which was calculated using the successive *Striga* counts, as outlined by Haussmann *et al*. (2000a).

$$\boldsymbol{ASNPC} = \sum_{i=0}^{n-1} \left[\frac{Y_i + Y_{(i+1)}}{2}\right] (t_{i+1} - t_i)$$

- **SDR**: *Striga* damage syndrome was scored per plant using the scale of 1-5; where 1 = no damage, indicating normal plant growth and high level of resistance/tolerance, and 5 = complete collapse or death of the pearl millet plant, that is, highly susceptible.

Striga damage rating was used as an index of tolerance while ASNPC was the index of resistance. Each of the plants sampled for assessment was tagged.

5.2.5 Data Analysis

Analysis of variance for all parameter studied was done using the general linear model (GLM) procedure in SAS 9.4 software (*SAS/STAT*, 2014) to determine significant differences among environments, genotypes, and their interactions. Generation means were calculated for each of the 6 generations on an individual plant basis using SASQuant macro in SAS (Gusmini, *et al.*, 2007). Components of genotypic variance (Phenotypic P, environmental E, Genotypic G, and additive A) were estimated from generation variance as follows:

$$\sigma^2 P = \sigma^2 F_2 \ , \quad \sigma^2 E = \frac{\sigma^2 P_1 + \sigma^2 P_2 + (2 \times \sigma^2 F_1)}{4}, \quad \sigma^2 G = \sigma^2 P - \sigma^2 E, \quad \sigma^2 A = (2 \times \sigma^2 F_2) - (\sigma^2 BC_1 P_1 - \sigma^2 BC_1 P_2)$$

Generation means and variances, computed from each of the individual plant selected for each generation, were combined to estimate the gene effects (Mather & Jinks, 1977). Additive, dominance, and epistatic effects were partitioned according to Hayman's GMA procedure (Hayman, 1958; Gamble, 1962). Main genetic components consisted of additive variance [a] and dominance variance [d]. Interaction components (epistasis) were described as additive x additive [aa], additive x dominance [ad], and dominance x dominance [dd] where [m] = F_2 mean; additive gene effects [a] = $BC_1P_1 – BC_1P_2$; dominance gene effects [d] = $-0.5P_1 - 0.5P_2 + F_1 - 4F_2 + 2BC_1P_1 + 2BC_1P_2$; additive x additive gene effects [aa] = $-4F_2 + 2BC_1P_1 + 2BC_1P_2$; additive × dominance gene effects [ad] = $-0.5P_1 + 0.5P_2 + BC_1P_1 – BC_1P_2$ and dominance x dominance gene effects [dd] = $P_1 + P_2 + 2F_1 + 4F_2 - 4BC_1P_1 - 4BC_1P_2$.

The model for a generation mean, Y, is: $Y = m + \alpha a + \beta d + \alpha 2aa + 2\alpha\beta ad + \beta 2dd$

Where, Y, the observed generation means; m, F_2 means; a, additive gene effects; d, dominance gene effects; aa, additive x additive epistatic gene effects; ad, additive x dominance epistatic gene effects and dd, dominance x dominance epistatic gene effects. α and β represent the coefficients for the genetic effects for the particular generation being estimated (Hayman, 1958; Mather & Jinks, 1982). The data were analyzed over three data collection times for each trait.

5.3 Results

5.3.1 Variation for *Striga* resistance and yield parameters in pearl millet lines

Analysis of variance revealed significant differences ($P < 0.01$) for all characters in the six generations and three environments in Cross1. A similar trend was observed in Cross 2 for all the traits (Table 5.2). The population × environment interaction for both crosses was significant ($P < 0.01$) except for NTL in Cross 2.

Table 5. 2. Analysis of variance of traits for two sets of six basic generations ((P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2) measured in three environments

SOV	**DF**	**Mean square**		
		ASNPC130	**SDR**	**NTL**
Cross 1				
Environment(E)	2	612348**	201.4**	2352.7**
Population	5	348031**	967.5**	732.1**
Population × E	10	35073.9**	79.1**	1133.9**
Replication	2	70830.6**	283.2**	2786**
Error	1780	8575.97	4.09428	44.0931
Cross 2				
Environment (E)	2	671024**	847.8**	1405.51**
Population	5	373249**	789.8**	410.9**
Population × E	10	289028**	45.4**	201
Replication	2	63166.4**	102.6**	575.5**
Error	1780	10647.34	5.59012	112.2397

ASNPC130 = area under *Striga* number progress curve, SDR = *Striga* damage rating, NTL = number of tillers/plant, SOV = Sources of variation, DF = degrees of freedom, E x P and Rep denote; environment by population interaction and replication respectively. * Significant at 5% levels of probability, ** Significant at 1% levels of probability.

5.3.2 Variation for *Striga* resistance and number of tillers in pearl millet lines

- **Cross 1 (IP20446 × NYOKO)**

There were significant differences (P 0.05) for the traits measured for all the generations across the environments (Table 5.3). Backcross to parent 2 (E_1) had the least ASNPC while the susceptible parent P_1 (E_3) had the highest. ASNPC ranged from 27 – 152 across the environments. Similarly, BC_1P_2 (E_1) was the least damaged generation (0.6) while P_1 (E_1) was the highest SDR (6.7). The generation with the highest tiller number was F_2 (22) in E_1 while P_1 (E_2) had the least (10.5).

- **Cross 2 (IP13238 × IP20511)**

The resistant parent P_2 (E_1) had the least ASNPC while F_2 (E_2) had the highest. The ASNPC across environments ranged from 32.7 to 160. As for SDR, P_2 and BC_1P_2 (E_1) had the least damage (1) while BC_1P_1 (E_2) had the highest damage (6.2). Number of tillers was highest for BC_1P_2 (15.6) in E_1 and least in BC_1P_1(7.5) in E_3.

Table 5. 3. Means of traits for Cross 1 of six generations evaluated under *Striga* infestations in three environments

Generation	**Env_1**			**Env_2**			**Env_3**		
	ASNPC 130	**SDR**	**NTL**	**ASNPC 130**	**SDR**	**NTL**	**ASNPC 130**	**SDR**	**NTL**
P_1	120	6.7	10.4	106	4.2	12.6	152	6	13.9
P_2	38.6	0.7	17	37.7	1.4	11.9	78.4	2.3	13.4
F_1	49.9	1	13.9	67	2	11.1	123	3	13.8
F_2	57.4	0.9	22.8	53.8	2.6	11	148	3.4	12.7
BC_1P_1	139	6	11.5	114	4.5	12.6	171	5.8	12.5
BC_1P_2	27.8	0.6	20.9	48.9	2	11.8	127	2.5	13
LSD	15	0.47	2.41	14.26	0.65	0.81	40.03	1.89	0.71

ASNPC130 = area under *Striga* number progress curve, SDR = *Striga* damage rating, NTL = number of tillers/plant, E_1 E_2, and E_3 denote; environment 1, environment 2 and environment 3 respectively.

Table 5. 4. Means of traits for Cross 2 of six generations evaluated under *Striga* infestations in three environments

Generations	**Env_1**			**Env_2**			**Env_3**		
	ASNPC	**SDR**	**NTL**	**ASNPC**	**SDR**	**NTL**	**ASNPC**	**SDR**	**NTL**
P_1	84.5	4.5	10	108	5.6	11.5	155	5.6	10.6
P_2	32.7	1	14	34	1.7	11.9	83.5	2.2	14
F_1	53.1	1.5	12.7	50.5	3.2	12.6	153	4.2	10.3
F_2	54.8	2.2	13.1	160	5.2	11.9	131	4.4	8.4
BC_1P_1	102	3.8	14.3	131	6.2	11	153	5.9	7.5
BC_1P_2	41.1	1	15.6	80.1	4.4	11.7	116	3.4	10.6
LSD	22.38	1.18	0.59	36.75	0.76	5.05	29.39	1.35	0.71

ASNPC = area under *Striga* number progress curve, SDR = *Striga* damage rating, NTL = number of tillers/plant, E_1 E_2, and E_3 denote; environment 1, environment 2 and environment 3 respectively.

5.3.2 Genetic estimates of components of variation for all studied characters

- **Cross 1 (IP20446 × NYOKO)**

Phenotypic and environmental variances for ASNPC were highest in E_3 (19412, 13761) (Table 5.5). Additive and genetic variances for ASNPC were negative in E_2. E_3 had higher genetic (5651) and dominance variance (10063) for ASNPC. The additive variance was positive in all environments for NTL. As for SDR, E_3 had larger phenotypic, environmental and additive variances, followed by E_2. E_2 had higher genetic (2.04) and dominant (1.25) variances. Genetic and dominant variances were zero to negative respectively in E_1. Similarly, NTL in E_1 had the largest components of variance except for dominance variance that expressed negative values for all the environments.

- **Cross 2 (IP13238 × IP20511)**

The estimate of environmental variance was highest in E_3 and least in E_1. E_2 and E_3 had a higher phenotypic and environmental variance for ASNPC respectively (Table 5.6). Phenotypic and environmental variance for SDR was highest in E_3 and least in E_1. Environment 3 had the largest phenotypic and environmental variance for NTL with E_2 having the least. The positive genetic variance was observed for all the traits ASNPC (E_3). The additive variance was positive for ASNPC and SDR in all the three environments. Dominance variances were negative for ASNPC and SDR in all the environments.

Table 5.5. Genetic components of variation for all studied characters of Cross 1 in three environments

Traits	**Phenotypic**			**Environmental**			**Genetic**			**Additive**			**Dominance**		
	E_1	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**
ASNPC	2571.9	15609	14555	2303.5	2387	32720	268.4	13222	-18165	955.5	22962	7826	-687.1	-9740	-25991
SDR	6.25	8.45	10.64	3.47	6.47	9.62	2.78	1.98	1.02	7.66	3.49	5.24	-4.88	-1.5	-4.23
NTL	12.71	7.81	25.47	8.23	6.54	19.67	4.48	1.27	5.8	-21.89	-7.76	-10.71	26.37	9.03	16.52

ASNPC=; area under *Striga* number progress curve, SDR = *Striga* damage rating, NTL= number of tillers/plant, E_1, $E_{2,}$ and E_3 denote environment 1, environment 2 and environment 3respectively

Table 5.6. Genetic components of variation for all studied characters of Cross 2 in three environments

Traits	**Phenotypic**			**Environmental**			**Genetic**			**Additive**			**Dominance**		
	E_1	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**
ASNPC	3308.2	2225	19412	2821.5	2257	13761	486.8	-32.01	5651	1975	-1530	-4412	-1488	1498	10063
SDR	0.89	6.36	7.45	1.04	4.32	7.42	-0.15	2.04	0.02	-0.93	0.79	4.15	0.78	1.25	-4.13
NTL	234.31	9.25	101.9	18.2	7.68	11.87	216.1	1.57	90	392.3	4.14	164.2	-176.2	-2.57	-74.17

ASNPC =; area under *Striga* number progress curve, SDR = *Striga* damage rating, NTL= number of tillers/plant, E_1, E_2 and E_3 denote environment 1, environment 2 and environment 3respectively.

5.3.3 Estimates of gene effects using generation means for the studied characters

- **Cross 1 (IP20446 X Nyoko-2)**

Estimates of the six parameters, i.e. additive (a), dominance (d), additive by additive (aa), additive by dominance (ad), dominance by dominance (dd) and F_2 means (m) are presented in Table 5.6. The result indicated that the mean effects (m) were highly significant for the variables in all the environments. Most of the gene effects were significant ($P < 0.05$) with few exceptions. Among the main effects, (a) is larger than (d) for ASNPC and SDR except for NTL while among the interaction effects (aa) is larger than (ad) and (dd) for ASNPC130 and SDR. (dd) is larger than (aa) and (ad) for NTL. The (d) and (dd) are in the opposite direction for SDR and NTL except for ASNPC (E_3) hence the nature of epistasis is duplicate.

The (a) effects were positive and significant for ASNPC (E_1, E_2) and SDR (E_1, E_2, E_3). Negative significant values for these parameters were detected for NTL (E_1).

With regards to (d) effects, positive and significant effects were observed for SDR (E_1), and ASNPC (E_2) while negative significant effects were observed for NTL (E_1). With respect to (aa) effects, positive significant effects were detected for ASNPC and SDR (E_1 and E_2) and NTL (E_2), however, negative values were recorded for NTL (E_1).

Additive x dominance gene effects (ad) were found to be positive and significant for ASNPC (E_1 and E_2) and SDR (E_1 and E_3). Number of tillers had negative and highly significant gene effects in E_1. Concerning the (dd) effects, negative significant effects were found for D50%F E_2, ASNPC (E_1 and E_2) and SDR (E_1).

- **Cross 2 (IP20446 X Nyoko-2)**

The mean effects (m) were highly significant for the variables in all the environments. The gene effects were not significant ($P < 0.05$), for some variables depending on the environment (Table 5.6). Among the main effects, (d) is larger than (a) except for ASNPC and SDR while among the interaction effects (aa) is larger than (ad) and (dd) for D50%F, ASNPC, and SDR. The (d) and (dd) are in the opposite direction for ASNPC and same direction for SDR (E_1) and NTL (E_1 and E_3) hence the nature of epistasis is more of duplicate.

Additive effects (a) were positive and significant for ASNPC (E_1, E_2) and SDR (E_1, E_2, and E_3). Negative significant values for these parameters was observed for NTL (E_3). With regards to dd effects, a negative significant effect was observed for ASNPC (E_2) and NTL (E_1).

Additive x additive gene effects (aa) was positive and significant for ASNPC130 and NTL (E_1), however, a negative significant effect was detected for ASNPC (E_2).

Concerning (ad) effects, positive significant effects were observed for ASNPC and SDR both in Environment 1. The dominance x dominance (dd) effects were found negative and significant for SDR (E_2) and NTL (E_1).

Table 5. 5. Gene effects and their standard errors for traits associated with resistance and tolerance to *Striga* (Cross 1)

Traits	**m**			**a**			**d**		
	E_1	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**
ASNPC	57.41±4.29***	53.8±3.52***	148±10.38***	111.05±8.52***	64.81±9.92***	43.75±25.33	74.12±47.88	105.33±46.01*	10.61±121.97
SDR	0.9±0.07***	2.57±0.19**	3.39±0.2***	5.38±0.2***	2.44±0.44***	3.33±0.42***	6.96±0.95***	1.93±2.17	1.68±2.35
NTL	22.82±1.14***	10.95±0.23***	12.73±0.75**	-9.37±0.98***	0.82±0.49	-0.5±0.81	-26.27±7.63***	3.83±2.59	-0.03±5.52

ASNPC =; area under *Striga* number progress curve, SDR = *Striga* damage rating, NTL= number of tillers/plant, E_1, E_2 and E_3 denote environment 1, environment 2 and environment 3respectively.

Table 5. 6. Gene effects and their standard errors for traits associated with resistance and tolerance to *Striga* (Cross 2)

Traits	**m**			**a**			**d**		
	E_1	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**
ASNPC	54.81±3.78***	160.3±9.31***	131.36±8.99***	61.26±8.15***	50.65±11.73***	37.02±18.81	62.16±43.67	-240±73.3***	45.34±116.94
SDR	2.22±0.17***	5.17±0.22***	4.42±0.24***	2.78±0.26***	1.78±0.46***	2.53±0.52***	-0.6±1.74	0.07±2.43	1.34±2.8
NTL	13.14±0.27***	11.86±0.21***	8.42±0.35***	-1.36±0.86	-0.68±0.62	-3.15±1.01**	-7.86±3.51*	-1.12±2.72	0.58±4.66

ASNPC =; area under *Striga* number progress curve, SDR = *Striga* damage rating, NTL= number of tillers/plant, E_1, E_2 and E_3 denote environment 1, environment 2 and environment 3respectively

Table 5. 7. Hayman's epistatic gene effects by environment (Cross 1)

Traits	**aa**			**ad**			**dd**			
	E_1	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**	**Epistasis**
ASNPC	103.69±34.2**	110±33.89**	3.11±92.21	70.25±15.63***	30.89±15*	6.88±40.19	-178.4±78.61*	-157.9±77.95*	-122.1±202.41	DDD
SDR	9.72±0.69***	2.72±1.64***	2.87±1.66	2.35±0.34***	1.04±0.71	1.52±0.73*	-13.72±1.61***	-6.13±3.6	-5.14±3.88	DDD
NTL	-26.49±.53***	5±1.88**	-0.13±4.63	-6.08±1.57***	0.49±0.81	-0.72±1.25	16.86±10.69	-7.12±2.28	4.13±8.03	DDD

ASNPC =; area under *Striga* number progress curve, SDR = *Striga* damage rating, NTL= number of tillers/plant, E_1, E_2 and E_3 denote environment 1, environment 2 and environment 3respectively, DDD= dominant type of epistasis, DDC= complementary type of epistasis

Table 5. 8. Hayman's epistatic gene effects by environment (Cross 2)

Traits	**aa**			**ad**			**dd**			
	E_1	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**	**E_1**	**E_2**	**E_3**	**Epistasis**
ASNPC	67.66±31.42*	-219.6±60.71**	11.44±73.59	35.37±13.32**	13.82±17.66	1.13±31.8	-131.3±72.21	40.69±109.34	-2.92±197.91	DDD
SDR	0.68±1.27	0.52±1.79	1.01±2.01	1.06±0.54*	-0.17±0.71	0.83±0.86	-1.76±2.73	-7.87±4*	-3.64±4.63	CDD
NTL	7.17±2.79**	-2.07±2.07	2.6±3.53	0.67±1.29	-0.52±1.01	-1.43±1.53	-17.56±5.95**	5.39±4.61	6.17±7.81	CDC

Where ASNPC =; area under *Striga* number progress curve, SDR = *Striga* damage rating, NTL= number of tillers/plant, E_1, E_2 and E_3 denote environment 1, environment 2 and environment 3respectively, DDD= dominant type of epistasis, DDC= complementary type of epistasis

5.4 Discussion

5.4.1 Variation for *Striga* resistance and yield parameters in pearl millet lines

Significant difference among genetic materials is a prerequisite for any breeding programme to make progress in selection. The results of this study indicate the presence of significant differences among the pearl millet generations, indicating the existence of genetic variation and the possibility of selection for *Striga* resistance. Significant differences among environments indicated that test environments were different from each other. In fact, the site Birninkudu is located in the Sahel whereas Makoda and Bui sites are in Sudan savanna. Genetic variation was found in pearl millet for pearl millet quantitative traits by Kumar (2016) and for *Striga* emergence and SDR in Maize by Mbogo *et al*. (2015).

5.4.2 Variation for *Striga* resistance and yield parameters in pearl millet lines

The mean ASNPC and SDR for the *Striga* resistant parent and or BC_1P_2 were generally lower than the susceptible parent P_2. This result shows that plant resistance to *Striga* was manifested and expressed by a lower number of emerged *Striga* plants and less damage sustained by the host. It also suggests that screening progenitors were effective in differentiating the resistant from susceptible genotypes. These results are consistent with the observations reported in maize (Mbogo *et al*., 2015) in which the susceptible parent had highest *Striga* emergence and highest *Striga* damage rating while the resistant parent showed the reverse. When backcrossed to resistant parent, population distribution skewed towards the resistant parent P_2, but when backcrossing was performed to P_1 population distribution skewed to susceptible parent P_1. In general, backcrossing to resistant parent increases the frequency of alleles for resistance hence skews towards resistance direction. Backcrossing to susceptible parent increases alleles for susceptibility. Backcross to parent 2 population in C_1 (E_1) resulted in more resistant plants than the resistant parents. The F_1 had the shortest plants in C_1 (E_1) whereas BC_1P_2 had the shortest plants in C_2 (E_1) while having tallest plants in both crosses in E_2. This indicates that alleles responsible for the increase and decrease in plant height are present in these genotypes. The mean NTL having a higher count in one cross and not the other or one environment, not another indicates that genotypes express this trait differently and in response to the environment. Both backcrosses exceeded their respective recurrent parents in most instances which indicate the prevalence of allelic and non-allelic interactions for genetic control of important traits in pearl millet. Singh *et al*. (2017) also reported similar results in pearl millet for yield components in which both the crosses performed better than their parents in most cases.

5.4.3 Genetic estimates of components of variation for all studied characters of the two crosses

The present study revealed that both additive and non-additive components of variances played an important role in the expression of studied traits. The manifestation of the components of variance was generally inconsistent for both crosses in all the environments.

In Cross 1 additive variance for all the traits though expressed in different environments played a major role in the expression of these traits. A similar trend was observed for cross 2 for the additive variance. Mrema *et al.* (2017) found similar results in which additive variance played a major role in the expression of *Striga* resistance trait in sorghum.

In addition, non-additive genetic variance was higher in the expression of ASNPC in Cross 1 and for the same trait for SDR (E_3) NTL (E_3) in Cross 2, These results suggested both additive and non-additive variance components played some role in determining the total genetic variability in C_1 and C_2 across the three environments, indicating predominance of both environmental and genotypic components of variances. The above findings are in line with Jeeterwal *et al.* (2018) and Singh *et al.* (2017) who found that both additive and non-additive variance played important role in the expression of several traits including number of tillers in millet.

5.4.4 Estimates of gene effects for the studied characters

The same sign for dominance (d) and dominance × dominance (dd) type of interaction was recorded for most of the traits in some environments. It indicates that these traits depicted the duplicate type of epistatic effect (Dvojković *et al*., 2010). On the other hand, in cross 2, SDR and NTL displayed duplicate type of nonallelic gene interaction. This scenario for the two traits except for ASNPC further confirms the prevalence of dominance effects (Arya *et al.*, 2013). Presence of duplicate epistasis indicates that variability in segregating generations may be reduced which hinder the selection process; hence it is difficult to utilize them in the breeding programme. On the other hand, the complementary type for some traits suggested the possibility of a considerable amount of heterosis for these traits in the particular crosses (Punia *et al.*, 2011).

The mean (m) for all the studied attributes of the two crosses across environments is significant. indicating the effect of common genes and common environment. The sign associated with the estimates of additive effects (a) and dominance effects (d) indicates the parent which concentrates the highest number of genes or positive alleles for increasing the traits, therefore, additive gene effect (a) was significant for ASNPC in C_1 and C_2 ($E_1$and E_2), SDR in C_1 and C_2 (E_1, E_2, and E_3), suggested preponderance of additive genetic effect in the inheritance of *Striga* resistance. This is in agreement with Gethi & Smith (2004), who reported the importance of additive gene effect to be more important than non-additive gene effects in the expression of *Striga* resistance traits measured in maize. Also, Mrema *et al.* (2017) reported additive gene effect to control resistance to *Striga* in sorghum. The potential of improving the performance of these characters by simple selection could be effective. On the contrary, Mbogo *et al*. (2015), reported the dominance gene effect to be more important for *Striga* emergence and *Striga* damage rating. Presence of dominance indicates that selection should be delayed until heterozygosity is reduced in the population. Khattak *et al.* (2004) and Punia *et al*. (2011) reported that traits which are highly controlled by dominance than additive genes can be improved through either pedigree, bulk or single seed descent to reduce heterozygosity until dominance effect is reduced in the population. The significant but negative values of (d), (aa), (ad) and (dd) for some traits in some environments showed that negative alleles were also dispersed in the parents involved in the cross. The negative sign of dominance in a cross for any trait indicates that dominance effects were contributed by the parents having alleles responsible for low values for the trait, for example, NTL in Cross 1 (E_1). Thus, selection for these traits should be delayed until when desirable segregants become available

The significant (aa) effects in the inheritance of ASNPC and SDR in (E_1 and E_2) and NTL in (E_2) in cross 1 and ASNPC and NTL in Cross 2 suggest that early selection for these traits might not be effective for *Striga* breeding.

Additive x dominance type of gene actions were found to be positive and significant for ASNPC (E_1 and E_2) and SDR (E_1 and E_3) in Cross 1, while in Cross 2 for ASNPC and SDR both in environment 1). Positive significant (ad) in the inheritance of these traits revealed that both additive and dominance effects are involved in the genetics of the traits. The negative and significant effects for NTL in Cross 1 (E_1) suggest an interaction between increasing and decreasing alleles, thus providing evidence of dispersion of genes in the parents (Mather & Jinks, 1982). Yi-Hong *et al*. (2014) and Mbogo *et al*. (2015) reported the importance of additive x dominance gene interaction to govern *Striga* resistance parameters under conditions of *Striga* infestation. It is, therefore, suggested that selection should be delayed till later generations and interaction should be fixed by selection under selfing conditions. Godasara *et al*. (2010) also found a different magnitude of gene effects governing different traits in different crosses of pearl millet.

The nature of gene effects from this study indicated that gene action varied from cross to cross across the environments. Hence, each cross must be handled separately for a specific character in each environment.

5.5 Conclusion

In conclusion, variability exists among the populations, environments and population x environment. The three environments were contrasting enough for this study. Additive gene effect was found to be more important for *Striga* resistance traits in both crosses but varied across environments. This can be exploited by fixing these traits through single seed descent by selfing method of plant breeding, while non-additive gene effects may be exploited by using the cyclic method of breeding involving selection and hybridization of desirable segregants from concerned crosses.

CHAPTER SIX

6.0. Genome-Wide Association Studies for Resistance to *Striga* in Pearl Millet

6.1 Introduction

To overcome the pearl millet *Striga* constraint, many efforts have been made by the national programmes including collaboration with international breeding programmes in order to identify new sources of resistance and develop pearl millet varieties resistant to the parasitic weed (Wilson *et al*., 2004; Kountche, 2013). However, this was done mainly through conventional breeding techniques with little integration of molecular techniques. Resistance to *Striga* in pearl millet has been reported to be controlled quantitatively (Wilson *et al*., 2004). As such, molecular markers that are linked to the genomic regions conferring host plant resistance to *Striga* can bring about rapid progress in the development of resistant varieties that will be subsequently incorporated to farmer-preferred varieties through marker-assisted selection (Mutengwa *et al*., 2005). Efforts have been made to develop molecular markers associated with *Striga* resistance in other cereals such as sorghum, maize and rice with the progress of identifying quantitative trait loci (QTLs) (Grenier *et al*., 2007; Kapran *et al*., 2007; Rispail *et al*., 2007). However, less effort has been made in the identification of QTLs in pearl millet. Kountche (2013) identified several QTLs for resistance to *Striga* on seven linkage groups in a bi-parental population in pearl millet.

Molecular markers are completely heritable, as a result using markers to select for a moderate to the high heritable quantitative trait, as the case of *Striga* resistance, will be more effective and less expensive compared to only morphology-based phenotyping. The use of molecular markers reduces time thereby combining the steps of multiple evaluations and sometimes reaching maturity in the field before selection to only one field trial where genotyping of seedlings is equal to the evaluation of resistance and crossing. The resolution of identified QTLs through linkage mapping is limited due to the relatively few recombinants generated from the two parents used to develop a mapping population. This resolution can be improved using Genome-Wide Association Study (GWAS) approach. The main advantage of this approach is that it exploits phenotypic and genetic variations present across a natural population with a high resolution and then draws inferences based on past recombination events. In addition, the technique is cost effective and does not require the development of a mapping population (McCarthy *et al*., 2008). Thus, it complements linkage mapping. Moreover, GWAS are powerful tools to identify the genetic variation that underlie many phenotypes in plants. Recently, GWAS has been applied in many cereals such as maize (Hindu *et al*., 2018), rice (Patishtan *et al*., 2018), sorghum (Spindel *et al*., 2018), and foxtail millet (Jaiswal *et al*., 2018) to identify markers associated with a range of traits of interest. However, in pearl millet, there are few reports of the use of GWAS to map traits of interest such as resistance to *Striga*.

GWAS was recently conducted in pearl millet to identify regions associated with resistance to *Striga* (Kountche, 2013), to characterize and study the population structure in Senegalese pearl millet landrace (Hu *et al*., 2015b) and to mine favorable alleles for grain iron and zinc (Prasad *et al*., 2017). The recently published pearl millet genome by Varshney *et al*. (2017) will make it possible to use GWAS to precisely identify single nucleotide polymorphisms (SNPs) associated with traits of interest. The microarray-based marker technology, Diversity Array Technology (DArT) is useful for constructing high-density maps and mapping QTL and its efficiency and low-cost advantage over other technologies makes it a technology of choice. It also has the advantage of reducing genome complexity together with high-throughput next-generation sequencing (NGS) technologies (Qiu *et al*., 2012). DArTseq markers based on genotyping-by-sequence (GBS) technology have been successfully applied for linkage mapping, QTL identification in bi-parental mapping population, GWAS, genetic diversity, and marker-assisted and genomic selection based on the GBS technology (Sánchez-Sevilla *et al*., 2015).

The objective of this study was to identify SNP markers associated with resistance to *Striga* and tiller phenotype in pearl millet lines for breeding for varieties resistant to *Striga*.

6.2 Material and Methods

6.2.1 Plant Materials

A total of 200 entries from the initial collections comprising of 82 landraces of diverse genetic backgrounds collected in the north-east and north-western parts of Nigeria and 156 lines from ICRISAT gene bank were used (Table 6.1 supplementary). Ten checks were added to the 200 making a total of 210 genotypes.

6.2.2 Phenotypic Data

Resistance to *Striga* assessment for the 210 association panel was conducted during the rainy season of 2017 between June to November at three locations Birninkudu, Makoda, and Bui in Nigeria. These sites have been identified as hotspots for *Striga*. At each site, the experimental set up was 42 × 5 alpha lattice design with three replications. Each plot comprised of two rows of 5 m long each with between row spacing of 0.75 m, and 0.50 m within the rows and an empty row in between each plot to reduce shading effects. The area under *Striga* number progress curve (ASNPC) and number of tillers (NTL) were used as phenotypic data for this study. Out of the 210 genotypes 188 were considered for the analysis.

6.2.3 DNA extraction and genotyping

Genomic DNA was extracted from fresh leaves sampled on 2-week old plants as described in Mariac *et al.* (2006) into 96 sample collection well kit from LGC. The two kits were sent to Integrated Genotyping Support Service (IGSS) facility at BeCA-ILRI Nairobi for DNA extraction using an in-house protocol available on (https://ordering.igss-africa.org/files/DArT_DNA_isolation.pdf). To ensure good quality DNA, a quality check was assessed based on 0.8 % agarose gel and 50ng/ul to 100ng/ul and the amount ranging between 30ul to 50ul. The sample was shipped to Diversity Arrays Technology (DArT) genotyping service providers at Australia (https://www.diversityarrays.com/) Genotyping was carried out using Genotyping-By- Sequencing (GBS) technology as described by Elshire *et al.* (2011) using DArTseq GBS (1.0) high-density SNP. Generated sequences were aligned to the pearl millet reference genome (Varshney *et al.*, 2017b).

6.2.4 Data filtering process and DArTseq SNP calling

To ensure high-quality SNP markers, the DArtTseq SNPs that can cause false positives were removed. SNPs with more than 20 % missing data were rouged (SNPs with less than 80 % call rate were also removed). Those with 50 % heterozygosity were pruned along with SNPs with < 5% minor allele frequencies (MAF) using TASSEL 5.1 (Bradbury *et al.*, 2007). Finally, 4802 DArtTseq SNPs out of the initial 22,548 called SNPs and 188 pearl millet lines were considered after filtering and data control process for subsequent analyses.

6.2.5 Analysis of Data

6.2.5.1 Phenotypic data

The phenotypic data were analyzed using the linear mixed effects (lme4) model with lines as fixed factors and replications, blocks and block within replications as random factors to estimate the best linear unbiased prediction (BLUP) means of the pearl millet lines using the lme4 package. Variance components were further estimated assuming intercept as fixed with lines, replications, and block within replications as random (Bates *et al*., 2018). Broad-sense heritability was estimated as the proportion of the total variance explained by the genetic variance.

6.2.5.2 Population structure analysis and linkage disequilibrium

Prior to conducting trait marker association, population structure was investigated using the 4802-good quality DArTseq derived SNP markers as described in 6.2.4. The population structure, referred to as "Q" matrix (Pritchard *et al.*, 2000) was assessed using a discriminant principal component analysis (DAPC) method implemented in the R package "adegenet" (Jombart & Bateman, 2008). The SNP markers were scored as 0, 1, 2 and "-" which represents major, minor, heterozygous and missing alleles respectively. The compressed mixed linear model (CMLM) was used which takes effects of individuals as random and then use it to determine the extent of relatedness between individuals through the Kinship (K) matrix as a variance-covariance matrix between individuals (Zhang *et al.*, 2010) and assign individuals to a group based on relatedness.

Linkage disequilibrium was performed using GAPIT package implemented in R statistical software with the selected 4802 DArTseq-derived SNP markers. This was estimated as squared allele frequency correlation and each pair of loci was declared significant at the threshold of $P \geq 3$.

6.2.5.3 Genome-wide association mapping

The phenotypic data for ASNPC and NTL and the cleaned SNPs were used to conduct GWAS using the compressed mixed linear model (cMLM) (Zhang *et al.*, 2010) with GAPIT (Lipka *et al.*, 2012) package in R. This model used compression approach to group the individual markers for analysis. This grouping of individuals based on marker relationship and the population structure improve the statistical power of the MLM model to detect the true association between traits and markers. The equation used for the mixed linear model was as follows:

$$Y = X\beta + Zu + e$$

Where Y = vector of phenotype measured, β = unknown vector with fixed effects (including SNP markers, population structure (Q) and the intercept); u = an unknown vector of random additive genetic effects such as effects of multiple QTL for individuals; X and Z are the known design matrices; and e = the unobserved vector of residuals. The u and e vectors are assumed to be normally distributed with a mean of zero and a variance (Zhang *et al.*, 2010).

To identify significantly associated SNPs, the Bonferroni correction test for false positives at 5% was found to be too stringent instead a less stringent approach based on the bottom 0.1 percentile distribution of the p distribution (–log (*p* values) > 3 that was proposed and used by Pasam *et al*. (2012) in spring barley was adopted. Quantile-quantile (QQ) plots were further used to examine the MLM fitness for ASNPC and NTL.

6.3. Results

6.3.1. Phenotypic variation

The summary statistics generated by the linear mixed effects analysis and broad-sense heritability (h^2) are presented in Table 6.1. The range revealed variation among the pearl millet lines used in this study. Both ASNPC and NTL had moderate to high broad sense heritability in all the three environments.

Table 6. 1. Summary statistics of pearl millet lines in three *Striga* hot spots

Environments	Trait	Min	Max	Range	Mean	SD	h^2
1	ASNPC	16.03	505.03	16.0-505.0	69.18	40.96	0.8
	NTL	2.0	22	2.0-22	12	4	0.5
2	ASNPC	14.38	289.14	214.4-289.1	67.55	35.13	0.60
	NTL	5	28	5-28	10	3	0.6
3	ASNPC	30.66	5283.74	30.7-5283.7	330.37	776.90	0.90
	NTL	2.0	19	2-19	11	3	0.7

1= Birninkudu, 2= Makoda, 3= Bui, ASNPC= area under *Striga* number progress curve, NTL=number of tillers per plant, Min= minimum, Max= Maximum, SD= standard deviation, h^2 = heritability

6.3.2. Marker distribution on the linkage group, linkage disequilibrium, and population structure

The number of SNPs resulting from the cleaning pipeline and imputation was composed of 4802 covering all the seven Linkage Groups (LG) of the pearl millet genome. The average number of SNPs per LG was 686. The LG3 had the highest number of SNPs (817) while the LG7 consisted of the lowest number (560).

Based on the genotypic data, rapid linkage disequilibrium (LD) decay was observed. Indeed, the R^2 estimate of LD ranged between 0 and 0.85 and declined below 0.2 at about 3kb (Fig. 6.1).

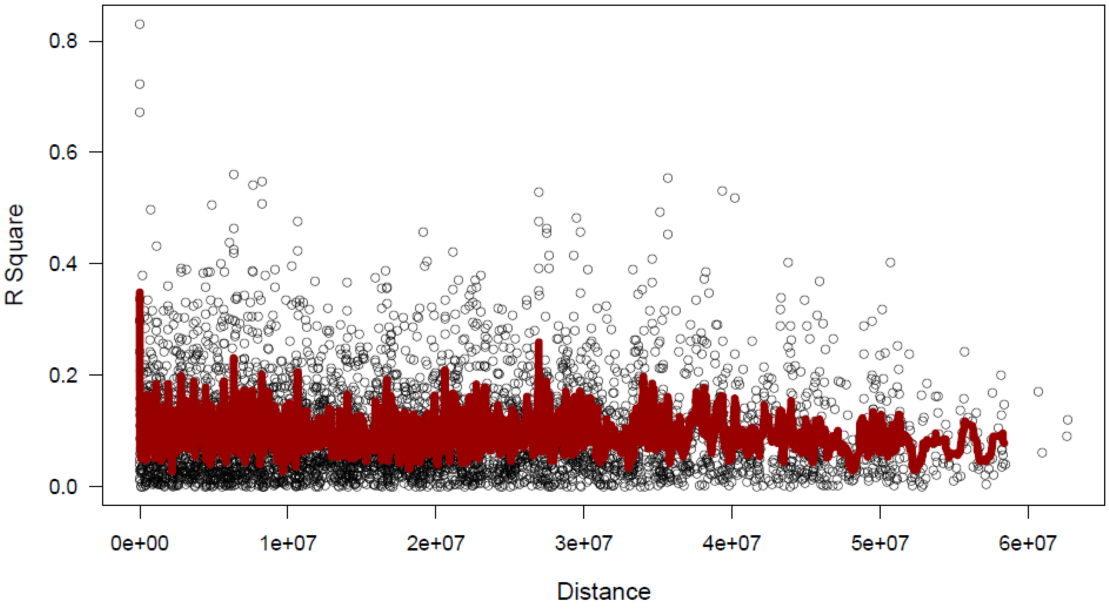


Figure 6. 1. Linkage disequilibrium (LD) decay plot of the pearl millet lines.

Analysis of population structure of the 188 pearl millet lines using the STRUCTURE software, for K ranging from 1 to 10 and by inferring on Delta K identified the most suitable K value that was used for the determination of the genetic cluster K = 2 (Fig. 6.2).

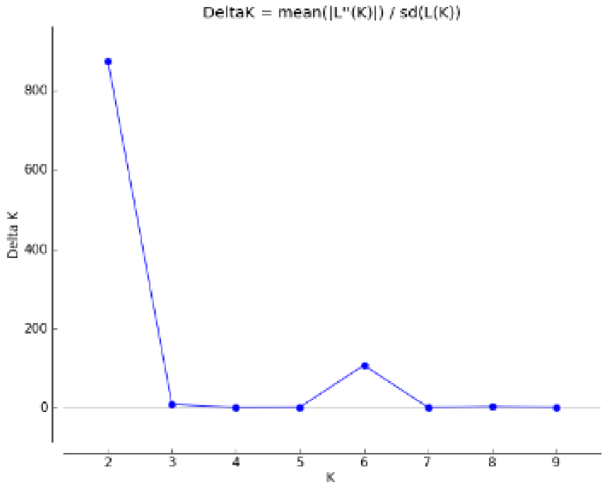
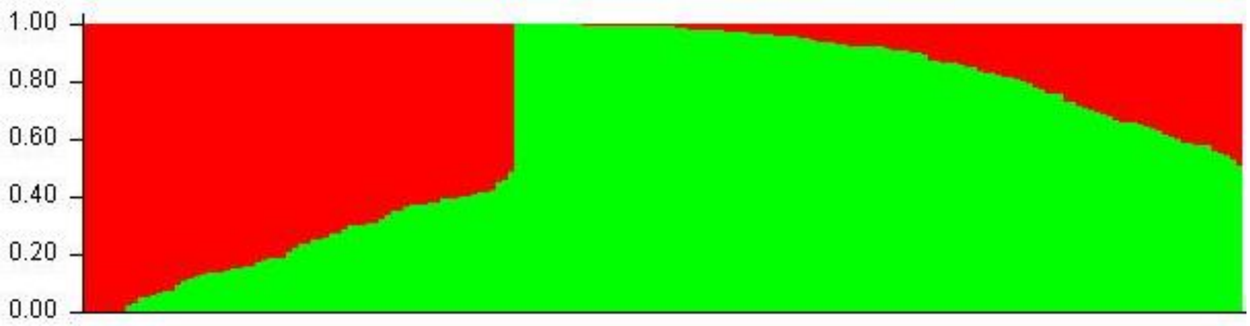
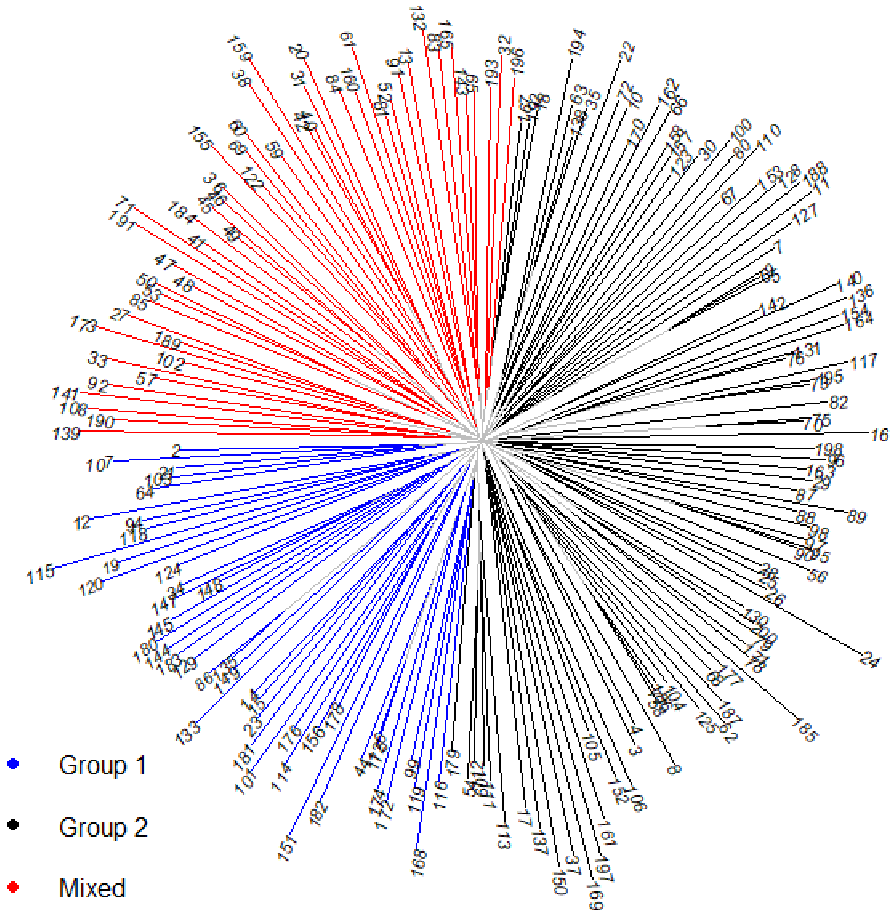


Figure 6. 2. The magnitude of Δ K as a function of K for 188 pearl millet lines using 4802 DArT seq SNP markers.

The number of subpopulations identified using STRUCTURE was visualized using the DAPC method. The method revealed a genetic separation among the lines dividing them into two subpopulations (Fig. 6.3). cluster 1 (Q1) encompassed 42 genotypes while Cluster 2 (Q2) had 91 genotypes. Fifty-five genotypes were mixtures of the two clusters (which forms another cluster). The neighbor-Joining (NJ) tree that was constructed further revealed two main groups and a mixed group similar to the groupings of population structure analysis. Group 1 consist of 27 collections from Nigeria and 15 collections from ICRISAT lines, group 2 had 41 and 50 lines from Nigeria and ICRISAT, respectively.

(a)





(b)

Figure 6. 3. Estimated population structure (a) and neighbor-joining tree (b) of 188 pearl millet lines.

It appeared that these groupings are based on resistance to *Striga* and tiller phenotype rather than their origin (Fig. 6.4). Indeed, Q2 was positively correlated to resistance to *Striga* (r = 0.07) and tiller phenotype (r = 0.06) and Q1 negatively correlated with both the two traits (r = -0.09 and r = -0.06). The cluster Q1 and Q2 were highly negatively correlated (r = -1.00).

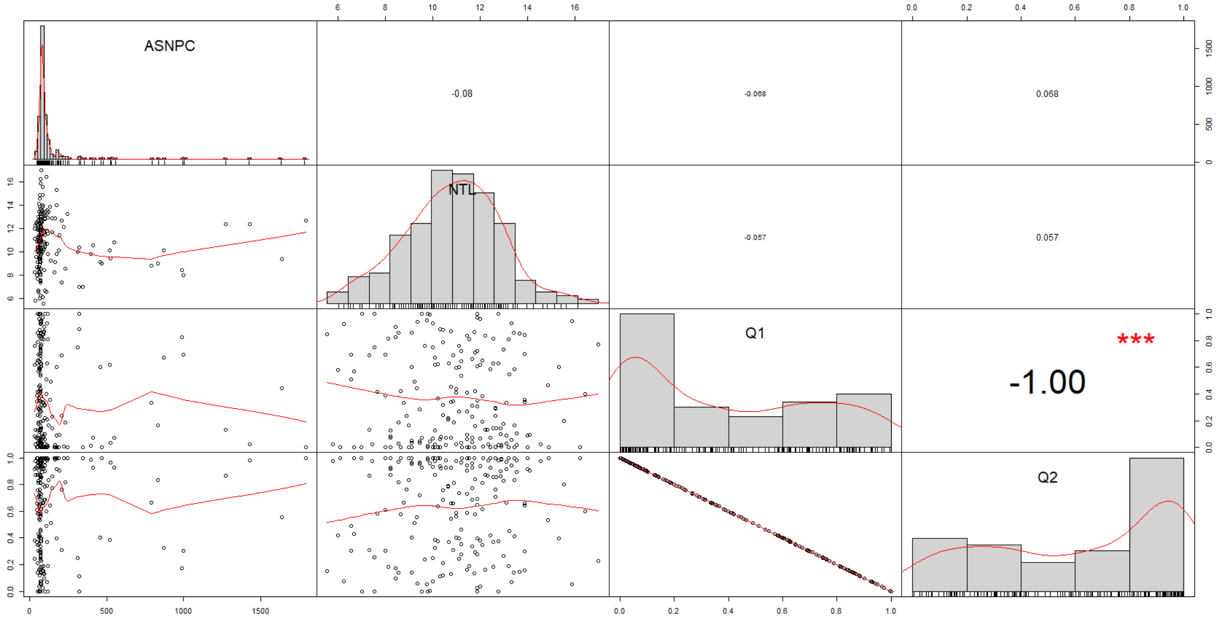
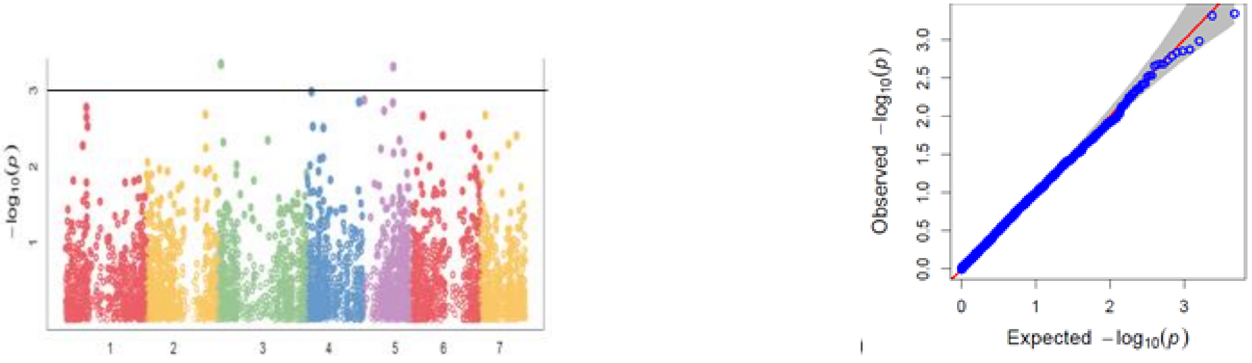


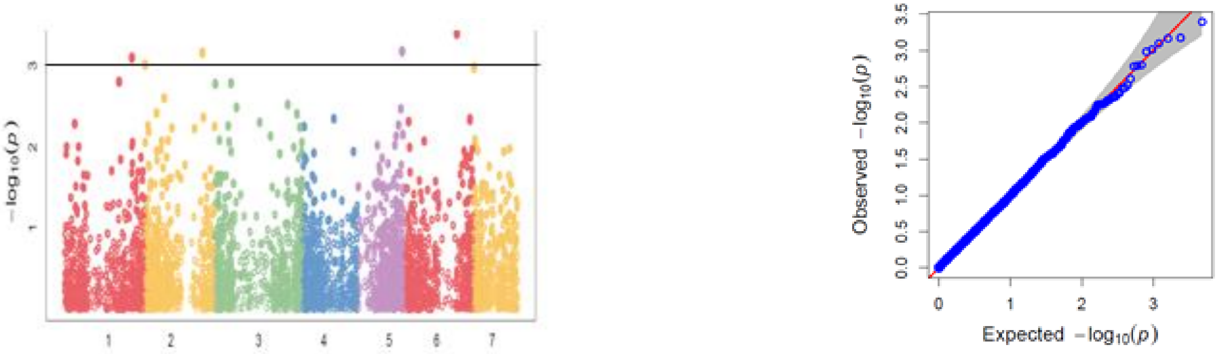
Figure 6. 4. Relationship between observed variables and membership probability.

6.3.3 Association mapping

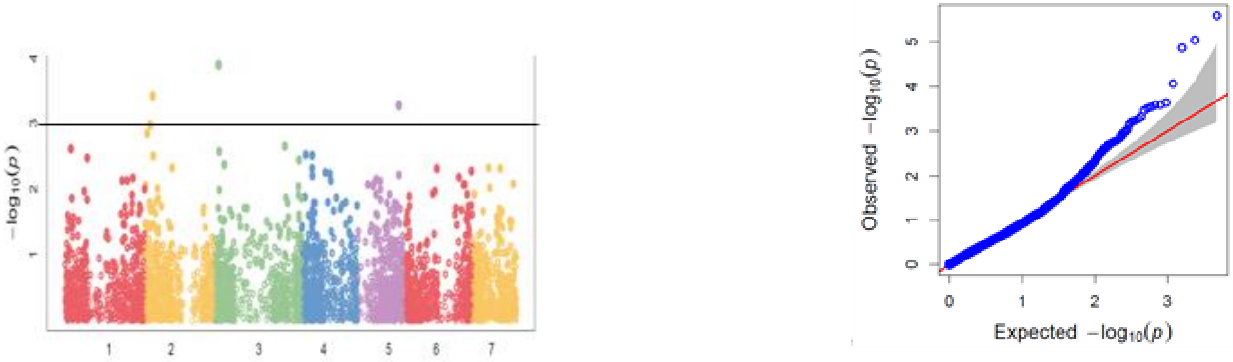
Manhattan plots were used for the graphical presentation of the significant association of SNP markers to *Striga* resistance and number of tillers (Fig 6.5A and Fig 6.5 B). Quantile-quantile (Q-Q) plot almost had a perfect fit between the observed and the expected *p*-values, except for ASNPC at Makoda and Bui (Fig. 6.5 A and Fig 6.5 B). No significant deviation between the observed –$\log_{10}$P and expected –$\log_{10}$P values for ASNPC and NTL were observed. However, a slight deviation was observed for ASNPC at Makoda and Bui.



(a) ASNPC

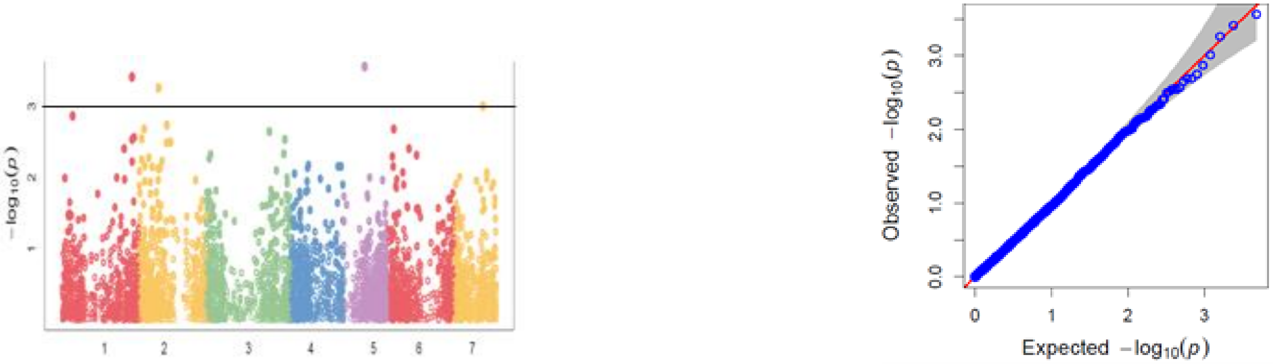


(b) NTL

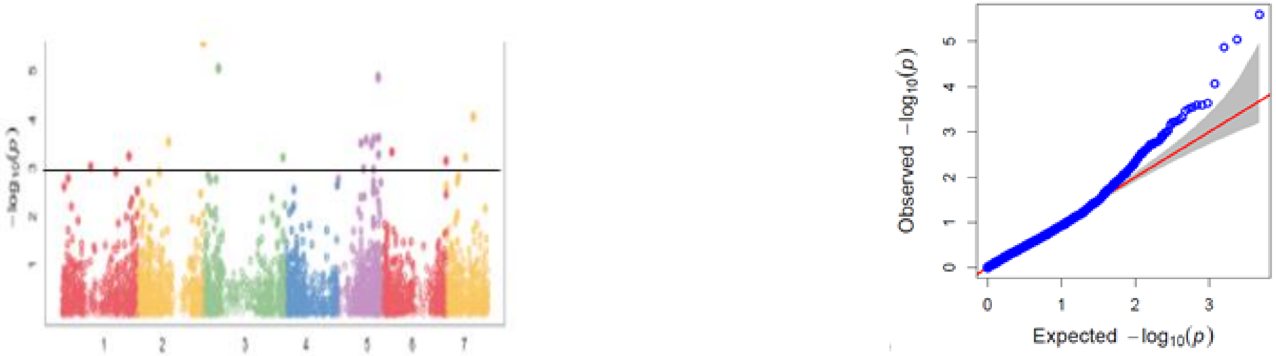


(c) ASNPC

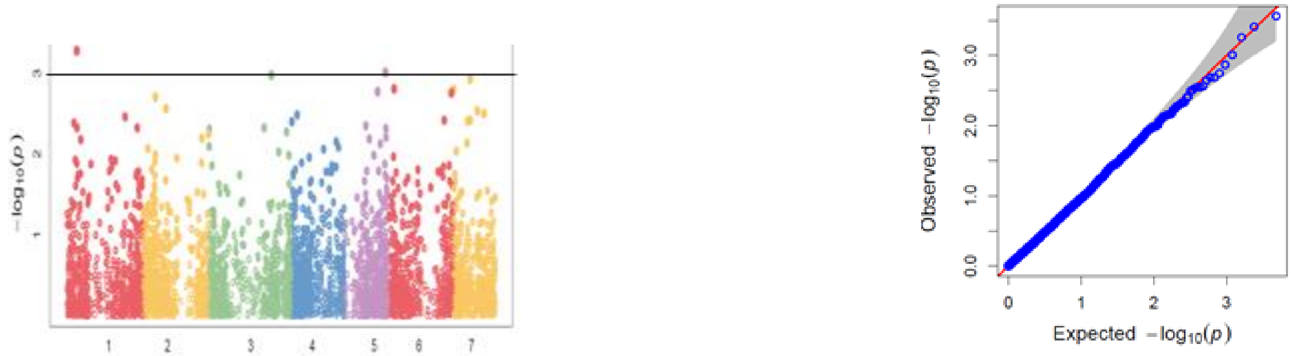
Fig 6.5 A: Manhattan and Quantile-quantile plot for ASNPC (a) at Birninkudu, NTL (b) at Birninkudu(a) and ASNPC (c) at Makoda



(d) NTL



(e) ASNPC



(f) NTL

Fig 6.5 B: Manhattan and Quantile-quantile plot for NTL (d) at Makoda, ASNPC € at Bui and NTL (f) at Bui

A total of 3 significant (P ≤ 0.001) associations were detected for *Striga* resistance at LG 3, LG 5 and LG 4 (Fig. 6.5A) with alleles for increased resistance to *Striga* (Table 6.2). Each of these SNPs as explained by the significant marker accounted for 7% of the variations in the trait. Two markers; S1_100178482 and S5_100176827 located at LG1 and LG5 and at the same chromosome positions with the same allelic effect showed significant association with NTL at Birninkudu and Bui locations (Table 6.2) and the proportion of variation explained by these SNPs ranged from -16 to 54 % at both locations.

Similarly, at Makoda location, the MLM analysis revealed three SNPs that were significantly associated with *Striga* resistance on LG 2, LG 3 and LG 5 with percent variation explained ranging between 1 – 2 %. Two of the three SNPs have alleles for increasing resistance to *Striga*. At the same location, four SNPs were found to be significantly associated with NTL at LG 1, LG 2, LG 5 and LG 7 out of which 3 were having alleles for the increasing number of tillers (Table 6.2).

Bui location had the highest SNPs (10) that were significantly associated with *Striga* resistance out of which 5 of the SNPs contributed to the increase in resistance to *Striga* allelic effect. These SNPs covered LG 2, LG 3, LG 5 (two SNPs at this LG) and LG 7.

Table 6. 2. SNP markers significantly associated with *Striga* resistance, and NTL from the pearl lines

Env		SNP	Chrom	Position	P.value	MAF	Major	Minor	PEV	Allelic effect
1	ASNPC	S3_100129087	3	9985743.00	0.0005	0.194	G	A	0.07	-0.87
		S5_100108433	5	102939908.00	0.0005	0.136	G	T	0.07	-0.78
		S4_100174590	4	16798456.00	0.0010	0.170	G	T	0.07	-0.08
	NTL	**S1_100178482**	**1**	**35445362.00**	0.0005	0.168	C	T	0.01	-0.16
		S5_100176827	**5**	**150517116.00**	0.0010	0.242	C	T	0.02	0.54
		S6_100158366	2	101021645	0.0018	0.168	G	A	0.01	0.83
		S6_100168693	6	23357306	0.0020	0.191	G	A	0.01	-0.86
2	ASNPC	S3_100061688	3	13105704.00	0.0001	0.096	G	A	0.01	0.76
		S2_100139058	2	27808386.00	0.0004	0.085	G	C	0.01	-10.37
		S5_100161851	5	143730667.00	0.0005	0.207	T	C	0.02	-1.44
	NTL	S5_100177024	5	76573072.00	0.0003	0.051	A	G	0.02	-0.41
		S1_100120099	1	251598407.00	0.0004	0.085	C	T	0.01	0.17
		S2_100158366	2	72058316.00	0.0006	0.160	G	A	0.01	0.48
		S7_100112619	7	104301745.00	0.0010	0.202	C	T	0.02	0.22
3	ASNPC	S2_100130569	2	242314725.00	0.0000	0.109	C	A	0.13	-65.06
		S3_100135194	3	54919009.00	0.0000	0.117	T	G	0.12	202.91
		S5_100132540	5	148977037.00	0.0000	0.109	G	T	0.11	64.33
		S7_100176346	7	99195586.00	0.0001	0.122	C	T	0.09	130.51
		S5_100169990	5	149810553.00	0.0002	0.096	C	T	0.08	-70.01
		S5_100110705	5	131747154.00	0.0003	0.104	A	G	0.08	-299.95
		S5_100135226	5	104728081.00	0.0003	0.125	T	C	0.08	-114.03
		S2_100155678	2	114456482.00	0.0003	0.074	G	T	0.08	158.78
		S5_100146453	5	85460626.00	0.0003	0.173	C	T	0.08	-23.60
		S5_100135042	5	124499939.00	0.0003	0.082	A	G	0.08	55.17
	NTL	**S1_100178482**	**1**	**35445362.00**	0.0005	0.168	C	T	0.08	-0.16
		S5_100176827	**5**	**150517116.00**	0.0010	0.242	C	T	0.07	0.54

In bold are the consistent SNP markers across Birninkudu and Bui, Env= Environment Chrom= Chromosome number, PEV= percentage of variation explained, MAF= Minor allele frequency

6.4 Discussion

Pearl millet is an important staple grain whose productivity is being constrained by several biotic and abiotic factors including *Striga*, especially in North Eastern Nigeria. This research addressed the latter part of the problem by investing extent of genetic variation in a collection of pearl millet lines from different growing areas of Nigeria and ICRISAT in order to identify genomic regions and SNPs underlying the genetic architecture of the traits. The result showed extensive variation were present in the materials evaluated. This is corroborated with an earlier report of genetic variability in pearl millet germplasm by earlier workers (Sattler *et al*., 2018). Kountche *et al*. (2013) reported genetic variation for resistance to *Striga* in pearl millet and demonstrated a significant response to recurrent selection in a *Striga*-resistant genepool. Screening pearl millet germplasm for identification of source of resistance to *Striga* is a prerequisite to identify resistant source which can be incorporated into the genepool to solve the *Striga* menace.

Furthermore, the moderate to high broad sense heritability observed for the studied trait in all the three locations suggest that most of the phenotypic variation can be attributed to genetic variation. Kountche (2013) also found high heritability estimate for ASNPC in pearl millet evaluated in two locations. Also, Sankar *et al*. (2018) found high heritability (81 %) for the number of tillers in pearl millet. Therefore, QTLs with major effects for these two traits can be to some extent identified in the population.

Population structure is an important factor to consider when conducting association studies.

The presence or absence of population structure needs to be controlled, because it may lead to type 1 error (Zhu *et al*., 2008). For example, Drabo, (unpublished thesis, 2016) conducted GWAS in pearl millet for Downy mildew (DM) in Burkina Faso using general linear model and found eight markers, however when he used the mixed linear model in the same study the number of markers decreased to four. In this study using STRUCTURE, the optimal number of K (groups) was determined to be 2 based on the ΔK method (Evanno *et al*., 2005). Pearl millet lines seem to be grouped into two subpopulations from the structure result with an admixture group. The two groups and the mixed were obvious in the neighbour joining tree and appeared that the clustering is based on resistance to *Striga* and tiller phenotype rather than their origin or where they were collected. Similar results were reported by Sattler *et al*. (2018) who found a strong genetic mixture of pearl millet from WCA. This admixture is most likely because pearl millet is a highly outcrossing species (about 85%) which can cause a vast gene flow among genotypes. Diversity studies using molecular markers conducted by an earlier worker (Lewis, 2010) also reported a predominately mix population of pearl millet genotypes which do not have distinct groups. Furthermore, Kanfany (unpublished thesis, 2017) found that pearl millet population was grouped not base on origin rather panicle length. Also, Oumar *et al*. (2008) and Bashir *et al*. (2015) reported that pearl millet from West and Central Africa structuring was based on agronomic traits rather than origin or location of collection.

For an outcrossing species such as pearl millet, GWAS require high-density markers in the population, and this marker requirement is determined by the extent of linkage disequilibrium (LD) within the population. Linkage disequilibrium is a measure of non-random association of alleles at two or more loci; the faster the LD decay, the better is the mapping resolution.

The LD decay rate within this study was average (r^2 about 0.2) across the whole genome close to 6kb. The observed rapid LD decay in the panel reflects the diversity within the germplasm. This rapid LD decay in pearl millet was also noted in Senegalese pearl millet genotypes (Hu *et al*., 2015a). Therefore, this rapid LD decay observed in this panel makes it a good population for genome-wide association study with high-density markers. Significant SNPs associated with ASNPC and NTL were identified. The genome-wide scan should facilitate in identifying regions in the genome associated with quantitative traits, such as in this study if there is enough linkage disequilibrium.

A total of 16 SNPs were significantly associated with ASNPC at a threshold of –log (p values) > 3 in the three locations respectively. Kanfany (unpublished thesis, 2017) also used the threshold of (-log(p values) > 3) in pearl millet for downy mildew resistance to declaring significant marker-trait associations. Gemenet *et al*. (2015) used a lower threshold value (-log(p values) > 2) to declare significant associations in pearl millet for phosphorus-related traits. The identified SNP markers in this study were located on LG 2, LG 3, LG 4, LG 5 and LG 7 out of the seven chromosomes. Kountche (2013) in a study revealed that even though the same mapping population was used, the expression of QTLs differs from location to location. Some QTLs may express in one location but not another while others express consistently among test environments. Such QTLs are more reliable across environments.

Furthermore, the marker-trait association revealed two SNP markers (S1_100178482, S5_100176827**)** significantly associated with NTL across These two consistent markers account for 1 to 8% of the variation in tiller number. Four SNP markers were associated with NTL in environment 2 only and account for 1 to 2% of the phenotypic trait. The consistent markers could be considered as reliably associated with tiller production. Thus, these identified markers could be considered as new markers associated with tiller number under Northern-Nigeria environment.

6.5 Conclusion

Based on the mixed linear model, 16 SNPs were identified that were significantly associated with *Striga* resistance under northern Nigeria environment. These SNPs explained 1 to 13% variation of the *Striga* resistance phenotype and could be considered as new markers identified for the first time in pearl millet associated with *Striga* resistance.

Six SNPs that were significantly associated with tiller number account for 1 to 8 % phenotypic variation of the quantitative trait. Two of the six SNPs; S1_100178482, S5_100176827 were consistent across two environments; Birninkudu and Bui. They were considered as the most reliable markers and are located on LG 1 and LG 5 with similar allelic effect and explained 1 to 8 % of the phenotypic variation. The use of the identified SNPs could fast track the identification of individuals with desirable alleles when selecting genotypes that are resistance to *Striga* in pearl millet.

CHAPTER SEVEN

7.0 Strigolactone Quantification and Tillering Phenotype in Pearl Millet Lines

7.1 Introduction

Parasitism by the root-parasitic plant, *Striga* (*Striga hermonthica* L.), is a major threat to pearl millet production in sub-Saharan Africa, often leading to complete failure of pearl millet crops. The parasite has also been reported to have a serious effect on other cereals such as maize and sorghum (Ejeta, 2007; Scholes & Press, 2008; Badu-Apraku *et al*., 2013).

Over the years, efforts have been made to develop satisfactory low-cost and efficient control strategies. However, it has been a great challenge to achieve much control due to the complexity of the parasite being outcrossing; its abundant viable seed production and its ability to parasitize the host while still underground. Control strategies have utilized cultural and chemical methods, as well as the development of *Striga* resistant cultivars.

Pearl millet crop like the other *Striga* hosts (sorghum, maize, and rice) secretes root exudates, strigolactones, into the rhizosphere usually under nutrient-deficient conditions to induce *Striga* germination and subsequent parasitism (Jamil *et al*., 2014). Strigolactone (SLs) also act as a signal for hyphal branching of arbuscular mycorrhizal fungi, leading to phosphorus release to host plant and regulation of branching or tillering (Umehara *et al*., 2008), especially under low nitrogen or Phosphorus conditions in the soil (Jamil, 2013). Jamil (2013) studied the activity of Strigolactone and correlated to tillering in rice. He found a negative correlation between tillering, Strigolactone and *Striga* infection and concluded that high tillering rice cultivars were able to resist *Striga* as a result of low Strigolactone production. This suggests that plants that secrete low strigolactones may resist *Striga* infestation than cultivars that secretes high amount of Strigolactone.

Therefore, knowledge of the level of strigolactone production, *Striga*-resistance status and tillering ability in pearl millet is essential for identification of resistant germplasm to benefit breeding efforts targeted at the development of *Striga* resistant cultivars. Furthermore, the use of tillering could provide an easy, farmer-friendly method for field identification of germplasm with efficient pre-attachment resistance for pearl millet farmers in *Striga*-prone areas.

The main objective of this research was to assess the role of the level of strigolactone secretion in pearl millet resistance to *Striga*.

The specific objectives were:

- quantify and characterize Strigolactone in pearl millet,
- determine the strength of the relationship between strigolactone level and tillering in pearl millet.

7.2. Materials and methods

7.2.1 Location of experiment

The experiment was conducted at Salim Albabili Lab, King Abdallah University of Science and Technology (KAUST), Kingdom of Saudi Arabia.

7.2.2 Pearl millet lines used in the study

Five *Striga* resistant and five *Striga* susceptible pearl millet lines were selected based on emerged *Striga* and crop performance from 200 mapping populations collected from Lake Chad Research Institute, Nigeria and International Research Institute, Nigeria and International Crops Research Institute (ICRISAT), Niamey. A *Striga* resistant (29A) and a *Striga* susceptible (IBL) pearl millet lines developed by KAUST were used as controls (Table 7.1). The susceptible control was SOSAT-C88 and the resistant control was PS202 a wild progenitor of pearl millet that were advanced to inbred lines in KAUST and were designated as IBL and 29A respectively.

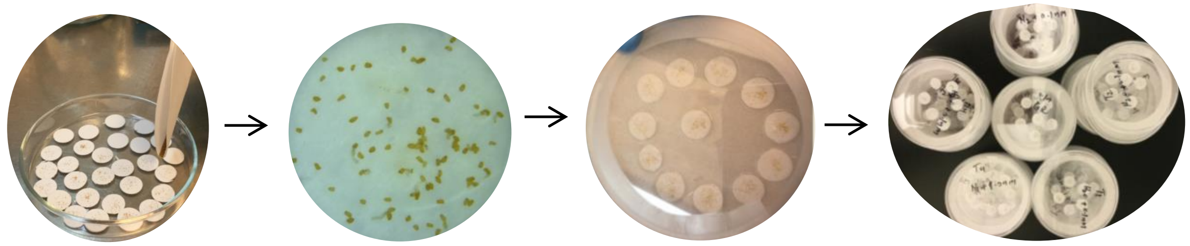
Table 7. 1. Origin and field reaction of the selected pearl millet lines used in this study

Entries	Genotypes	Origin	Field reaction
16_R	MPMG11085-2-5-1-1-5	LCRI	Resistant
64_R	MPMG11107-2-10-1-1-3	LCRI	Resistant
96_R	MPMG11104-8-1-1-2	LCRI	Resistant
153_R	1216188-1-2-1-2	ICRISAT	Resistant
158_R	1216199-3-1-1-1	ICRISAT	Resistant
87_S	MPMG11080-3-1-1-2	LCRI	Susceptible
140_S	1216130-10-2-1-6	ICRISAT	Susceptible
141_S	1216130- 10-2-1-7	ICRISAT	Susceptible
172_S	1216221-1-2-1-5	ICRISAT	Susceptible
197_S	1216273-5-1-1-2	ICRISAT	Susceptible
29A_R	PS202	KAUST	Resistant (Check)
1BL_S	SOSAT-C88	KAUST	Susceptible (Check)

7.2.3 Strigolactone quantification and characterization

Pearl millet seeds for each line were sterilized, placed on filter paper in 9mm petri dish which 3ml sterile water was added and subsequently sealed with parafilm. The seeds were pre-germinated in the dark for 23 hours then transferred to light for 24 hours. Thereafter, the pre-germinated seeds were planted in pots containing silver sand of about 750 ml. Each entry had 4 replications and 7 plants per pot. During this time, 1L of half-strength modified Hoagland's nutrient solution with 100 % Phosphorus (+P HS) was supplied to the growing plants for 3 weeks after which the plants were subjected to phosphate starvation by irrigating with phosphorus deficient nutrient (-P HS) for 7 days to induce Strigolactone (SLs) production (Mohemed *et al*., 2016). On the 7th day after phosphorus starvation, each pot was washed with 3 liters Mili-Q water followed by 3L –P (HS), which was allowed to drain freely to reduce the accumulated SLs from the rhizosphere. After 24h, 1L root exudate was collected from each pearl millet line by adding 1.5L –P HS and allowing it to drain into well-labeled bottles for SLs quantification and *Striga* bioassay. Out of the 1L root exudate, 950ml was used for the SLs quantification. The samples were passed through a C18-Fast column and SLs eluted with 4 ml acetone. Thereafter, it was quantified using Liquid chromatography-mass spectrometry (LC-MS).

For the bioassay, 50 to 100 pre-conditioned *Striga* seeds were used. The preconditioning was done by cleaning the Striga seeds of sand and debris, thereafter kept moist in the dark on 28-30 ^{0}C for 14 days. The seeds were evenly distributed on 5 glass fiber paper discs (25 mm) in each petri dish (9 cm diameter) (Fig. 7.1). A 50 ml portion of the collected 1L root exudate was used. Three dilution sets were prepared; 3x, 9x and 27x. To make a dilution, in each 300 µl of sample 300 microl of MiliQ water was pipetted to each tube first and followed by 300 µl of the samples. The mixture was vacuum centrifuged for 40 minutes to evaporate the acetone and leaving SLs + water only. Two hundred and fifty µl from the mixture was pipetted into 3x tube followed by addition of 750 µl MiliQ water (this gives 3x dilution). Each entry in a petri dish was replicated four times. Distilled water and GR24 (synthetic Strigolactone) were used as resistant and susceptible controls, respectively. The Petri dishes containing discs spread with *Striga* were sealed with parafilm and then wrapped with aluminum foil and incubated at 30 ^{0}C. After 48 h, data were recorded on germinated and non-germinated *Striga* under a microscope and percent germination calculated.



(a) (b) (c) (d)

Figure 7. 1. Conditioning *Striga* seeds and root exudate application and arrangement for bioassay. *Striga* spreading on disc fiber paper (a), about 50-100 spread *Striga* seeds on disc (b), twelve spread discs arranged in a petri dish for conditioning (c), after conditioning, five discs arranged in each petri dish and root exudates applied (d).

7.2.4 Tiller phenotype characterization

The 12 pearl millet lines that were used for SLs quantification were used for tiller characterization. Ten seeds for each millet line was sterilized and pre-germinated. Two pre-germinated seeds from each line were selected. Four pots for each line was used (8 plants/line) in a completely randomized design and thinned to 1 plant/pot two weeks after planting in a greenhouse. Two liters of Hoagland's nutrients solution that has 100% Phosphorus was supplied in 48-hour interval. The experiment was kept for 10 weeks. Data on tiller number per plant was determined by manual counting.

7.2.5 Statistical analysis

Data on *S. hermonthica* germination and tillering phenotype were analyzed statistically by ANOVA in Genstat 9.2 package release 9.2 (PC/Windows XP) (VSN International Ltd, UK). The relationship between the SLs, *S. hermonthica* infection and tillering was analyzed by multivariate analysis analysis in R statistical software.

7.3 Results

7.3.1 Strigolactone quantification and characterization

Two strigolactones; 5-deoxystrigol and orobanchol were detected in all the pearl millet lines. The amount of SLs secreted by the various genotypes differed significantly ($p < 0.05$) (Fig. 7.2). All the pearl millet lines generally secreted higher levels of orobanchol than 5-deoxystrigol except 158_R which showed the reverse. Among the resistant lines, 16_R, 153_R and 96_R produced significantly ($p < 0.05$) higher levels of orobanchol while among the susceptible lines 172_S and 140_S secreted the highest amount. Resistant control (Wild) produced the highest SLs. All the test lines show statistically same level of 5-deoxystrigol production which is slightly lower than the susceptible control (IBL). However, 158_R produced statistically same level of 5-deoxystrigol with IBL.

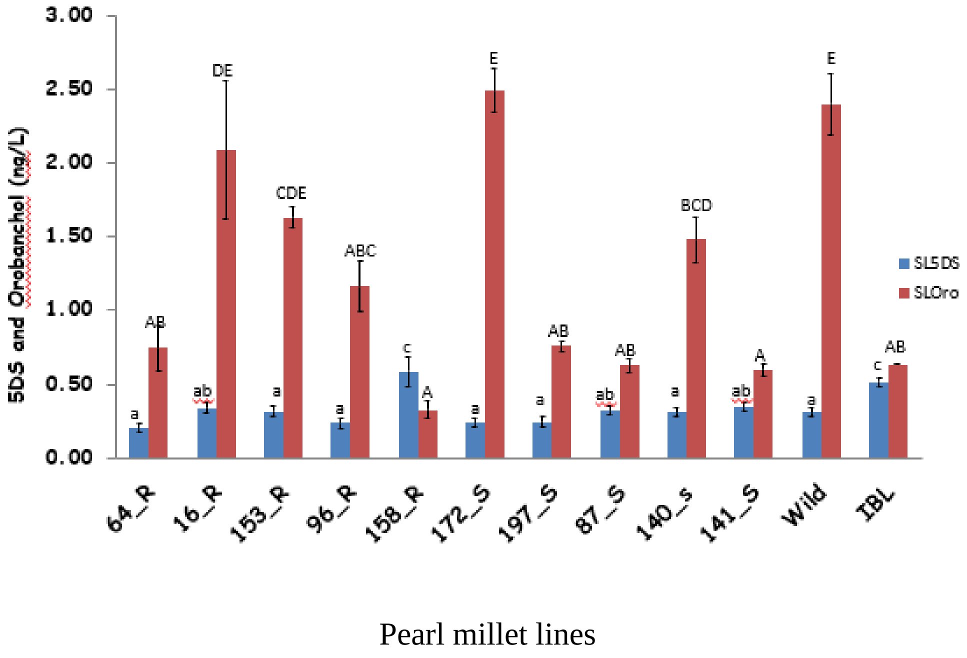


Figure 7. 2. Strigolactone concentration in the root exudates of pearl millet lines. Bars represent mean ± Standard error (n =4). Least significant differences of means at P = 0.05 were determined by ANOVA. Letters indicate the significance group after Duncan's pairwise comparisons ($P<0.05$).SL5DS and SLOro denote 5-deoxystrigol and orobanchol respectively.

7.3.1.1 *Striga* seed germination

Significant ($p < 0.05$) variation among the pearl millet lines was observed for their *Striga* germination induction (Fig. 7.3). Application of pearl millet root exudates collected from the 10 test lines resulted in high *Striga* germination by 172_S (28%) and 140_S (28%) which was not significantly different from that of IBL, the susceptible control. However, GR24 induced the highest germination of *Striga*. On the other hand, 158_R, 87_S, and 141_S induced low germination which was not significantly different from the wild control. The mock (water) did not induce *Striga* germination.

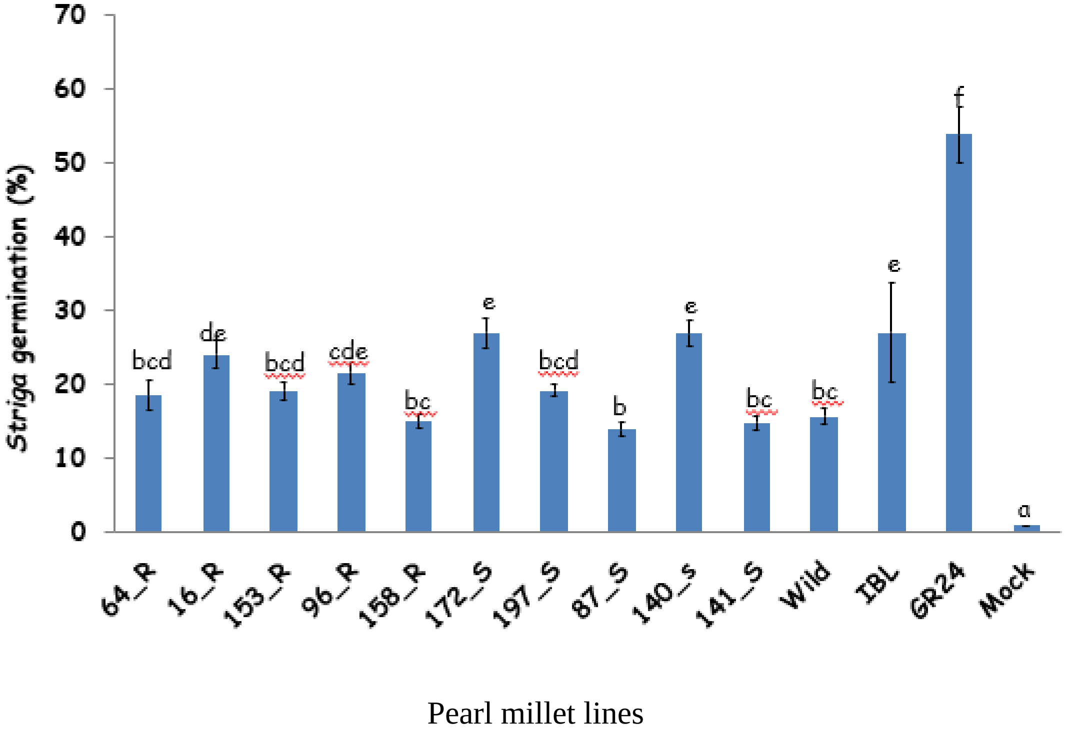


Figure 7. 3. *Striga hermonthica* germination (%) induced by pearl millet root exudates. Bars represent mean ± Standard error (n =4). Least significant differences of means at P = 0.05 were determined by ANOVA. Letters indicate the significance group after Duncan's pairwise comparisons (P<0.05).

7.3.3 Tillering phenotype

Significant ($p < 0.05$) variation among the pearl millet genotypes was observed for tiller number (Figs. 7.5). The resistant lines 153_R and 196_R had the highest tiller count among the test lines while the wild (resistant control) has the highest tillers among all. However, all the lines had a higher number of tillers compared to the susceptible control (IBL) which had only one tiller (Fig. 7.4).

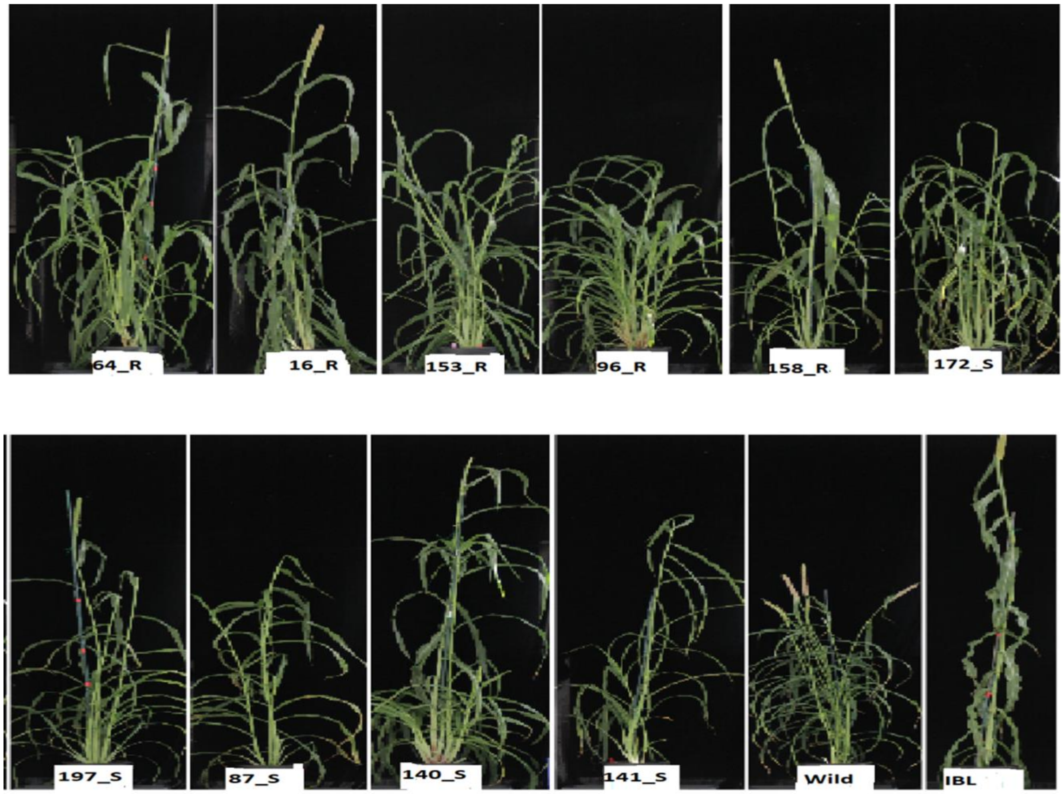


Figure 7. 4. Differences in tillering among pearl millet lines.

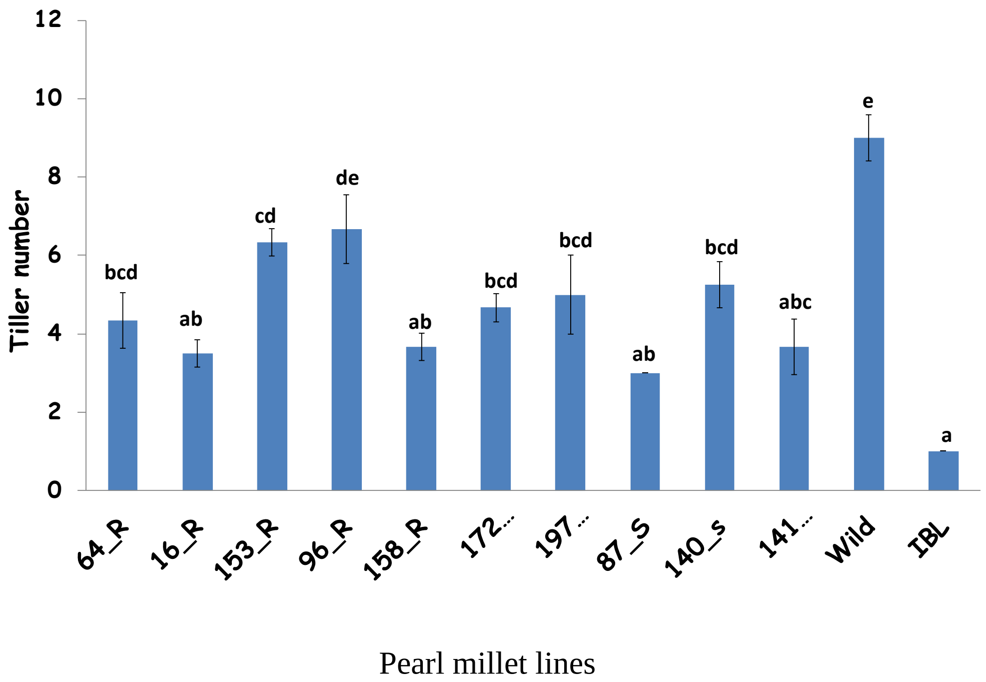


Figure 7. 5. Number of tillers in a series of pearl millet lines. Bars represent mean ± Standard error (n =4). Least significant differences of means at P = 0.05 were determined by ANOVA. Letters indicate the significance group after Duncan's pairwise comparisons ($P<0.05$).

7.3.3 Relation among SLs, *Striga* germination and tillering in pearl millet

A principal component biplot displayed the relationship between SLs (5-deoxystrigol and orobanchol), *S. hermonthica* germination and tillering (Fig. 7.6). There was a negative correlation between orobanchol and 5-deoxystrigol. In addition, the number of tillers and orobanchol were positively correlated whereas the number of tillers and 5-deoxystrigol were negatively correlated.

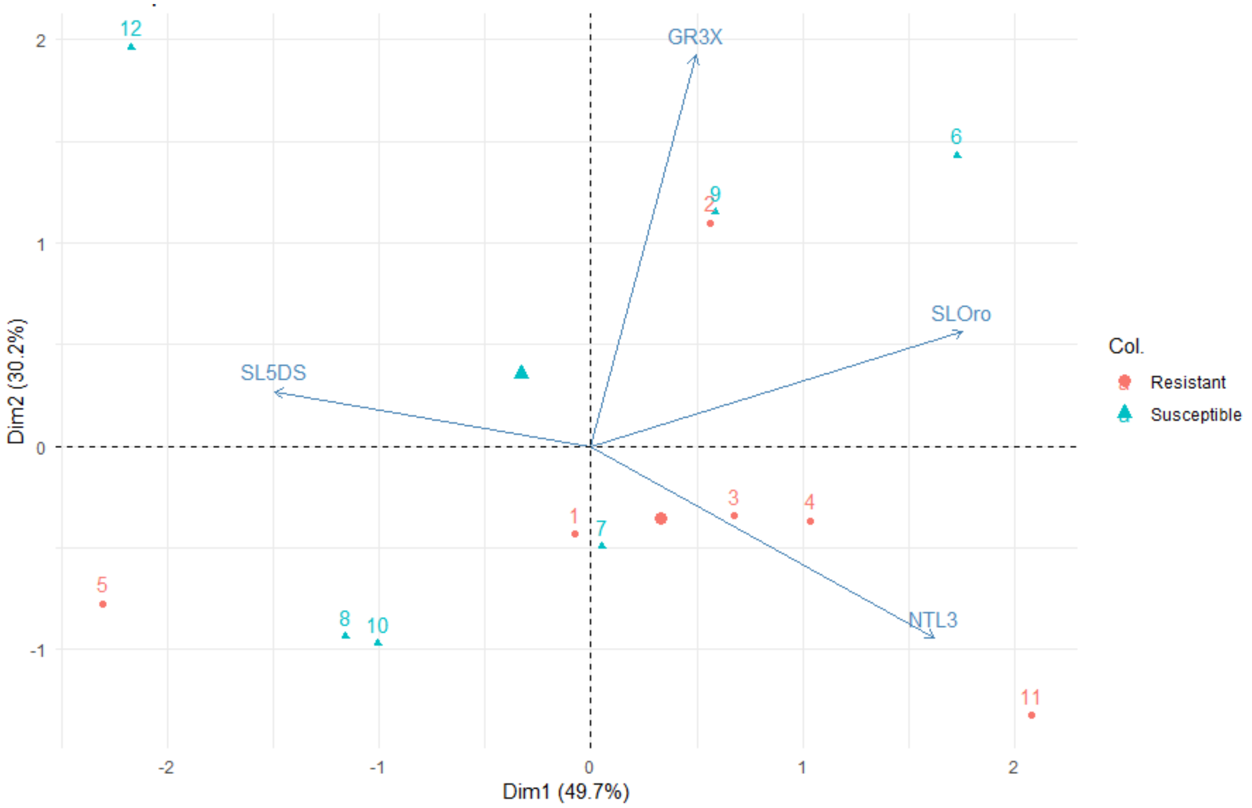


Figure 7. 6. Principal components biplot of pearl millet lines.

Number of tillers (NTL3), SLs (SLOro, S5DS) and *Striga* germination (GR3X). 1, 2, 3…12 denotes 16_R, 64_R, 96_R, 153_R, 158_R, 87_S, 140_S, 141_S, 172_S, 197_S, 29A_R, and IBL_S entries respectively.

7.4 Discussion

The results revealed that irrespective of the source of the pearl millet lines, morphological group or field reaction to *Striga*, the pearl millet lines all secrete 5-deoxystrigol and orobanchol as the major SLs. In addition, low secretion of 5-deoxystrigol was observed in most of the pearl millet lines except 158_R which resulted in high amount of 5-deoxystrigol The production of different types of SLs and in different proportions by the host crop is in line with studies conducted on SLs by Yoneyama *et al*. (2012) in Chinese milk vetch, Jamil (2013) in rice and sorghum, Jamil *et al*. (2014) in pearl millet and Nasreldin (2018) in sorghum. This suggests that 5-deoxystrigol and orobanchol are major SLs that are found in host plants and there is genotypic variation in SLs production. Awad *et al*. (2006) examined root exudates of maize, sorghum and pearl millet and found 5-deoxystrigol to be major stimulant among others produced by all the crops. However, Mohemed *et al*. (2016) confirmed high secretion of Orobanchol in sorghum including the resistant genotypes (Framida, Hakika, SRN39, Tetron, and IS9830). Furthermore, Jamil *et al*. (2012) observed high level of *ent*-2′-*epi*-5-deoxystrigol in rice whereas Xie *et al*. (2007) reported *ent*-2-*epi*-orobanchol as the major stimulant in tobacco. In addition, parasitic plants get attracted to stimulants with different levels of sensitivity (Bouwmeester *et al*., 2003), consequently, the mechanism contributes to the recognition of the host by the parasitic plant. This implies that major stimulants differ from crop to crop and even among the same species. The variation in the secretion of SLs among different pearl millet genotypes might be a guide in selecting genotypes that are less susceptible to *Striga* infestation (Jamil *et al*., 2014).

Germination bioassays from the pearl millet lines induced high germination in the susceptible lines; 172_S, 140_S as in IBL. As expected, the synthetic germination stimulant GR24 had the highest *Striga* germination. Most of the genotypes screened have high levels of orobanchol which might be associated with low *Striga* germination activity. Although the susceptible lines 87_S and 141_S induced low *Striga* germination similar to the resistant lines, it may not be due to low stimulant production. This result is in line with the findings of Yoneyama *et al*. (2010) in sorghum in which the resistant cultivar SRN39 induced similar levels of *Striga* germination as a susceptible cultivar. Mohemed *et al*. (2018) also used sorghum root exudate to screen for *Striga* germination activity and found SL orobanchol to be associated with the low germination-inducing activity. This finding suggests that genotypes that produce more of orobanchol might be a good strategy to select for lines that are good inducers of arbuscular mycorrhizal fungi at the same time have reduced *Striga*–inducing activity. It also implies that it is not always that the resistant cultivars (field resistance which is affected by environment) produce high orobanchol and less 5-deoxystrigol like most of the genotypes.

Relationship between SLs and tiller number has been reported by Umehara *et al*. (2008) in which SLs inhibit tillering/branching in rice and Arabidopsis. Studies have shown that under nutrient deficiency especially Phosphorus, plants modify their architecture (such as tillering/branching among others) by the hormone SLs to adapt to the harsh situation (Jamil *et al*., 2012; Umehara *et al*., 2008). Jamil *et al*. (2012) reported that rice genotypes that have low tiller number and high SLs are likely to induce more *Striga* germination while rice that has a high number of tillers and produces a low amount of SLs induce few *Striga* germination. In this study, the pearl millet lines that show high tiller numbers and low SLs might be indicative of genotypes that are less prone to *Striga* infestation.

The negative correlation between orobanchol and 5-deoxystrigol, and number of tillers with 5-deoxystrigol, suggest that high tillering pearl millet lines produce more of orobanchol and not 5-deoxystrigol. Entry 153_R, 69_R and wild have high number of tillers and relatively high orobanchol. The low production of 5-deoxystrigol by these genotypes could make them better resist *Striga* infestation than the high 5-deoxystrigol producers. Recently, orobanchol was reported in the xylem of tomato and Arabidopsis, suggesting it is involved in the modification of tillering/branching (Kohlen *et al*., 2011). Jamil (2013) reported that orobanchol could be among other SLs identified and involved in rice tillering if there is a positive correlation between an increase in tillering and increase in orobanchol production and negative correlation with *Striga* germination-inducing activity. This suggests that also in pearl millet, orobanchol may regulate tillering, in those genotypes that show correlation in orobanchol concentration in the root exudate as measured in this study. Surprisingly, the vector of *Striga* germination (GR3x) deviates slightly from 5-deoxystrigol and positively correlated to orobanchol even though the correlation is weak. This could be explained by other resistance mechanisms that may be present in the genotypes as was the case with rice cultivars in which Jamil *et al*. (2012) found *Striga* germination was correlated positively with the SLs. Cissoko *et al.* (2011) reported a post-attachment form of resistance in rice genotypes that behave similarly to the finding in this study. Jamil (2013) also observed a negative correlation between rice tillering and orobanchol, suggesting a different type of resistance rather than pre-attachment resistance that was expected from the type of SLs. It is hence necessary that screening for *Striga* resistance in pearl millet should not focus on the SLs production but should look beyond, such as post-attachment resistance, incompatibility, and field experiment on *Striga* hot spots.

7.5 Conclusion

Genetic variation exists among the pearl millet lines for SLs production and concentration. Also, genetic variation among the pearl millet lines for tiller production exist, 153_R and 96_R were identified as resistant lines with high tillers.

The two Strigolactones; orobanchol and 5-deoxystrigol identified and quantified exhibited significant negative correlation. Orobanchol was generally produced in higher concentration than 5-deoxystrigol in the pearl millet lines

A positive relationship existed between tiller number and orobanchol production. The pearl millet lines 153_R and 96_R had high number of tillers and relatively high orobanchol production, implying that orobanchol is involved in tiller production in pearl millet. However, a weak positive correlation was found between *Striga* germination and orobanchol indicating other sources of resistance exist apart from the pre-attachment resistance among some lines tested.

CHAPTER EIGHT

8.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

8.1 General conclusions

Interviewed farmers consider *Striga* infestation, downy mildew and high labour cost as the main production constraints. They were also aware of *Striga* menace and stressed that infestation is likely to become worse if not adequately controlled. As a result, farmers consider *Striga* resistance, resistance to downy mildew and tolerance to shattering as the most important traits to be included in pearl millet cultivars.

Phenotypic variation for resistance to *Striga* and other agronomic traits were observed among the germplasm originated from Northern Nigeria and ICRISAT. Fifteen genotypes that were identified as resistant from the screening were free of emerged *Striga* shoots and had high yield, 10 tolerant genotypes supported *Striga* shoots but with appreciable yield while 15 susceptible genotypes supported *Striga* shoots and low grain yield. The genotypes were grouped into three clusters with *Striga* parameters and yield parameters as the most discriminating factors.

To exploit the pearl millet genetic material for the knowledge on genes controlling the inheritance of *Striga* resistance, two sets of crosses developed were evaluated for resistance to *Striga*. Both additive and non-additive type of gene action are important in the control of inheritance of resistance to *Striga*, *Striga* damage rating and the number of tiller with the preponderance of additive gene effect. The nature of gene effects varied from a cross to cross and among the environments.

Sixteen single nucleotide polymorphism (SNPs) markers were identified as significantly associated with resistance to *Striga* combined in all the environments. The QTLs located on LG 5, 2 and 3 explained 11 to 13 % phenotypic variation. Six SNP markers were identified to be associated to number of tillers and two of these located on LG 1 and LG 5 were consistent across Birninkudu and Bui locations and were found on the same linkage group and same position. These identified SNPs associated with ASNPC and number of tillers particularly the consistent markers could be considered new markers.

The Strigolactones; 5-deoxystrigol and orobanchol were identified among all the pearl millet lines in varying concentrations. Pearl millet lines 16_R, 153_R, 96_R, 172_S, and 140_S secrete high amounts of orobanchol. The susceptible lines 172_S and140_S stimulates high *Striga* germination. Two lines were identified as resistant; 153_R and 196_R had high number of tillers and produced higher amounts of orobanchol. The two identified strigolactones were negatively correlated while number of tillers and orobanchol were positively correlated. *Striga* germination skewed slightly to orobanchol. The positive correlation between the Strigolactone; orobanchol and number of tillers for some genotypes suggests that tiller number may be an important morphological marker for indirect selection of pearl millet genotypes that are less affected by *Striga* infestation.

8.2 Recommendations

To sustain pearl millet production and increase the rate of adoption particularly in Jigawa state Nigeria, research in pearl millet improvement should consider *Striga* resistance, resistance to downy mildew and tolerance to shattering as identified farmers' preferred traits and constraints such as *Striga* infestation, downy mildew and high labour cost in new variety development.

The variations observed among germplasm for resistance to *Striga* can be exploited in pearl millet improvement programme.

For the cross and environment that is controlled by additive genes, there is room for exploitation by fixing these traits through recurrent selection and or single seed descent by selfing method of plant breeding, while for the non-additive gene effects, these may be exploited by using the cyclic method of breeding involving selection and hybridization of desirable segregants from concerned crosses

SNP markers associated with *Striga* resistance and number of tillers identified in this study should be verified since the pearl millet genome has been published before transferring the genes to farmer-preferred varieties.

Identification of the mechanism for strigolactone based pre-attachment resistance in this study could be used to transfer resistance to susceptible pearl millet genotypes. This resistance along with resistance mechanisms such as post attachment resistance will go a long way to improve durability for resistance in pearl millet. Genotypes that show positive correlation in the number of tillers with low *Striga* germination induction could be potential indirect morphological markers for selecting pearl millet genotypes that are less attacked by *Striga*.

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from+Af&source=bl&ots=BI1wJRW1bf&sig=MNmSRg9Ni9AI2jE5AESDdINuLJs&hl=en&sa=X&ved=2ahUKEwi6jvmrmqvdAh

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APPENDICES

Appendix 1. Participatory Rural Appraisal

➢ Focus group discussions topics

Topics	Evaluation
Major crops grown	Ranking
Production constraints	Ranking
Prefered traits	Ranking
Grain uses	Ranking
Stalk uses	Ranking

➢ Questionnaire semi structure interviews

1. Interview date:
2. Name of interviewer:
3. Starting time:
4. Ending time

A Farmers identification

5. Name:
6. Phone number:
7. Age:

   11-20 21-30 31-40 41-50 51-60

8. Gender

   1. Male 2. Female

9. Level of education

   1. No formal education 2. Primary 3. Secondary 4. Tertiary 5. Others

10. Marital status

    1. Married 2. Single 3. Divorced

11. Years of farming experience

    1. 1-20 2. 21-30 3. 31-40 4. 41-50 5. 51-60 6. Above 60

B. Coping mechanisms for major production constraints

Problem	Coping mechanism	Percentage of responses
Declining soil fertility	1. Ash	
	2. Inorganic fertilizer	
	3.Ash and inorganic	
	4.crop rotation	
Striga coping mechanism	1. Hand pulling;	
	2. crop rotation	
	3. intercropping	
	4. Urea application	
	5. Abandon field	
Downy mildew	1. Hand pulling	
	2. No action taken	
Birds damage	1. scaring birds	
	2. planting to escape peak population	
	3. Not a problem	
Smut	1.Spray insecticides	
	2. No action taken	
High labor cost	1. Hire casual labor	
	2.Reduction in acreage	
	3.Family labour	
	4.use of previously used field	
Land shortage	1. Intercropping	
	2.Land hire	
	3. Addition of more fertilizer	
	4.Not a problem	

C. Assessing the extent of *Striga* constraint and control strategy

Questions	**Response**	**Percentage**
For how long is *Striga* a problem in your field?	1. Less than 20yrs 2.More than 20yrs	
At what growth stage is *Striga* a problem?	1. Seedling 2. Heading 3.Flowering 4.Maturity	
Among the varieties you grow which is most resistant/ tolerant?	1. Long panicle 2. short panicle 3. Both 4. None is resistant	
How do you control *Striga*?	1. Hand pulling 2.use of urea 3. weeding and urea app.	
What is your source of seed?	1. Other farmers 2.Own saved seed 3. Market 4. Agro-dealers	
Do you treat your seed before sowing?	1. yes 2. No	
Would you like to have *Striga* resistance cultivar?	1. yes 2. No	

D. Level of adoption by farmers

Questions	**Response**	**Percentage**
Do you know of other improved variety apart from your local one?	1. yes 2. No	
Do you inquire about improved seeds from extension agents or agro dealers?	1. yes 2. No	
Any pearl millet production training in your locality?	1. yes 2. No	
Have you ever been trained on *Striga* control?	1. yes 2. No	
How many times do extension agents visit you?	1. Non 2. <_3 times 3.<_ 6 times	
Who advise you on the choice of chemicals and fert.	1. E/agents 2. From experience 3. Friends 4.Trained in school	

Appendix 2: List of genotypes

Nº	Name	Origin	Nº	Name	Origin	Nº	Name	Origin
1	MPMG11004	Nigeria	81	MPMG11123	Nigeria	161	1216181	ICRISAT
2	MPMG11011	Nigeria	82	MPMG11124	Nigeria	162	1216182	ICRISAT
3	MPMG11012	Nigeria	83	1216002	ICRISAT	163	1216185	ICRISAT
4	MPMG11014	Nigeria	84	1216003	ICRISAT	164	1216186	ICRISAT
5	MPMG11015	Nigeria	85	1216006	ICRISAT	165	1216188	ICRISAT
6	MPMG11016	Nigeria	86	1216011	ICRISAT	166	1216189	ICRISAT
7	MPMG11017	Nigeria	87	1216017	ICRISAT	167	1216190	ICRISAT
8	MPMG11018	Nigeria	88	1216022	ICRISAT	168	1216192	ICRISAT
9	MPMG11019	Nigeria	89	1216024	ICRISAT	169	1216193	ICRISAT
10	MPMG11020	Nigeria	90	1216026	ICRISAT	170	1216194	ICRISAT
11	MPMG11021	Nigeria	91	1216028	ICRISAT	171	1216196	ICRISAT
12	MPMG11022	Nigeria	92	1216031	ICRISAT	172	1216199	ICRISAT
13	MPMG11023	Nigeria	93	1216032	ICRISAT	173	1216202	ICRISAT
14	MPMG11024	Nigeria	94	1216033	ICRISAT	174	1216204	ICRISAT
15	MPMG11025	Nigeria	95	1216034	ICRISAT	175	1216205	ICRISAT
16	MPMG11026	Nigeria	96	1216036	ICRISAT	176	1216206	ICRISAT
17	MPMG11027	Nigeria	97	1216037	ICRISAT	177	1216207	ICRISAT
18	MPMG11028	Nigeria	98	1216038	ICRISAT	178	1216208	ICRISAT
19	MPMG11030	Nigeria	99	1216039	ICRISAT	179	1216209	ICRISAT
20	MPMG11031	Nigeria	100	1216040	ICRISAT	180	1216211	ICRISAT
21	MPMG11032	Nigeria	101	1216041	ICRISAT	181	1216212	ICRISAT
22	MPMG11033	Nigeria	102	1216042	ICRISAT	182	1216213	ICRISAT
23	MPMG11034	Nigeria	103	1216043	ICRISAT	183	1216215	ICRISAT
24	MPMG11035	Nigeria	104	1216045	ICRISAT	184	1216216	ICRISAT
25	MPMG11037	Nigeria	105	1216047	ICRISAT	185	1216218	ICRISAT
26	MPMG11038	Nigeria	106	1216048	ICRISAT	186	1216221	ICRISAT
27	MPMG11039	Nigeria	107	1216056	ICRISAT	187	1216222	ICRISAT
28	MPMG11040	Nigeria	108	1216057	ICRISAT	188	1216224	ICRISAT
29	MPMG11041	Nigeria	109	1216058	ICRISAT	189	1216225	ICRISAT
30	MPMG11042	Nigeria	110	1216059	ICRISAT	190	1216226	ICRISAT
31	MPMG11043	Nigeria	111	1216064	ICRISAT	191	1216227	ICRISAT
32	MPMG11044	Nigeria	112	1216065	ICRISAT	192	1216229	ICRISAT
33	MPMG11046	Nigeria	113	1216066	ICRISAT	193	1216230	ICRISAT
34	MPMG11047	Nigeria	114	1216067	ICRISAT	194	1216233	ICRISAT
35	MPMG11050	Nigeria	115	1216068	ICRISAT	195	1216234	ICRISAT
36	MPMG11051	Nigeria	116	1216071	ICRISAT	196	1216237	ICRISAT
37	MPMG11052	Nigeria	117	1216072	ICRISAT	197	1216238	ICRISAT
38	MPMG11053	Nigeria	118	1216073	ICRISAT	198	1216239	ICRISAT

39	MPMG11055	Nigeria	119	1216074	ICRISAT	199	1216240	ICRISAT
40	MPMG11057	Nigeria	120	1216075	ICRISAT	200	1216241	ICRISAT
41	MPMG11059	Nigeria	121	1216077	ICRISAT	201	1216244	ICRISAT
42	MPMG11060	Nigeria	122	1216078	ICRISAT	202	1216245	ICRISAT

Continued

Nº	Name	Origin	Nº	Name	Origin	Nº	Name	Origin
43	MPMG11068	Nigeria	123	1216080	ICRISAT	203	1216246	ICRISAT
44	MPMG11070	Nigeria	124	1216082	ICRISAT	204	1216247	ICRISAT
45	MPMG11074	Nigeria	125	1216083	ICRISAT	205	1216250	ICRISAT
46	MPMG11076	Nigeria	126	1216084	ICRISAT	206	1216252	ICRISAT
47	MPMG11077	Nigeria	127	1216086	ICRISAT	207	1216256	ICRISAT
48	MPMG11078	Nigeria	128	1216087	ICRISAT	208	1216259	ICRISAT
49	MPMG11079	Nigeria	129	1216088	ICRISAT	209	1216262	ICRISAT
50	MPMG11080	Nigeria	130	1216090	ICRISAT	210	1216264	ICRISAT
51	MPMG11081	Nigeria	131	1216093	ICRISAT	211	1216266	ICRISAT
52	MPMG11083	Nigeria	132	1216094	ICRISAT	212	1216267	ICRISAT
53	MPMG11084	Nigeria	133	1216095	ICRISAT	213	1216268	ICRISAT
54	MPMG11085	Nigeria	134	1216097	ICRISAT	214	1216269	ICRISAT
55	MPMG11087	Nigeria	135	1216099	ICRISAT	215	1216270	ICRISAT
56	MPMG11089	Nigeria	136	1216101	ICRISAT	216	1216272	ICRISAT
57	MPMG11090	Nigeria	137	1216106	ICRISAT	217	1216273	ICRISAT
58	MPMG11091	Nigeria	138	1216109	ICRISAT	218	1216275	ICRISAT
59	MPMG11092	Nigeria	139	1216110	ICRISAT	219	1216278	ICRISAT
60	MPMG11093	Nigeria	140	1216111	ICRISAT	220	1216280	ICRISAT
61	MPMG11094	Nigeria	141	1216112	ICRISAT	221	1216282	ICRISAT
62	MPMG11095	Nigeria	142	1216116	ICRISAT	222	1216283	ICRISAT
63	MPMG11096	Nigeria	143	1216120	ICRISAT	223	1216284	ICRISAT
64	MPMG11098	Nigeria	144	1216123	ICRISAT	224	1216286	ICRISAT
65	MPMG11099	Nigeria	145	1216126	ICRISAT	225	1216287	ICRISAT
66	MPMG11100	Nigeria	146	1216130	ICRISAT	226	1216289	ICRISAT
67	MPMG11102	Nigeria	147	1216132	ICRISAT	227	1216290	ICRISAT
68	MPMG11103	Nigeria	148	1216133	ICRISAT	228	1216291	ICRISAT
69	MPMG11104	Nigeria	149	1216138	ICRISAT	229	1216292	ICRISAT
70	MPMG11105	Nigeria	150	1216139	ICRISAT	230	1216294	ICRISAT
71	MPMG11107	Nigeria	151	1216141	ICRISAT	231	1216296	ICRISAT
72	MPMG11108	Nigeria	152	1216142	ICRISAT	232	1216304	ICRISAT
73	MPMG11109	Nigeria	153	1216144	ICRISAT	233	1216305	ICRISAT
74	MPMG11110	Nigeria	154	1216146	ICRISAT	234	1216307	ICRISAT
75	MPMG11111	Nigeria	155	1216154	ICRISAT	235	1216308	ICRISAT
76	MPMG11112	Nigeria	156	1216160	ICRISAT	236	1216309	ICRISAT

77	MPMG11113	Nigeria	157	1216166	ICRISAT	237	1216312	ICRISAT
78	MPMG11114	Nigeria	158	1216171	ICRISAT	238	1216315	ICRISAT
79	MPMG11119	Nigeria	159	1216175	ICRISAT	239	Farmer's local1	Nigeria
80	MPMG11120	Nigeria	160	1216179	ICRISAT	240	Farmer's local2	Nigeria