FIELD AND MOLECULAR SCREENING FOR *STRIGA* RESISTANCE IN SELECTED FINGER MILLET (*Eleusine coracana,* L. Gaertn) GERMPLASM IN WESTERN KENYA

By

SIRENGO PETER NYONGESA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BOTANY (PLANT GENETICS) IN THE DEPARTMENT OF BIOLOGICAL SCIENCES, UNIVERSITY OF ELDORET, (KENYA)

NOVEMBER, 2017

DECLARATION

This thesis is my original work and has not been submitted for any academic award in any institution; and shall not be produced in part or full, or in any format without prior written permission from the author and/or University of Eldoret.

Peter Nyongesa Sirengo Signature:_____ Date: _____
SC/PGB/047/2011

This thesis has been submitted with our approval as supervisors.

Prof Otto George Dangasuk Signature:_____ Date:_____
University of Eldoret

Dr. Lexa Gomezgan Matasyoh Signature:_____ Date:_____
University of Eldoret

Dr. Chrispus Oduori Adeti Signature:_____ Date:_____
KALRO-FCRI Kisii

Dr. Damaris Achieng Odeny Signature:_____ Date:_____
ICRISAT-Nairobi

DEDICATION

This work is dedicated to my wife, Phanice Mukhwana Nyongesa; my sons, Briston Boston Nyongesa and Samuel Wekesa; and my daughters, Brenda Nekesa and Careen Nafula for their patience while I pursued this study.

ABSTRACT

Finger millet (*Eleusine coracana*) is an importance food crop in Africa and Asia. Its grain is richer in protein, fat and minerals than other major cereals. The parasitic weed *Striga hermonthica* (Del.) Benth seriously limits finger millet production. The damage of *Striga* to cereal crop is more severe under drought and low soil fertility. The main objectives of this study were to: (i) assess the effect of *Striga* infestation on finger millet based on agro-morphological traits, (ii) determine genetic basis of resistance of finger millet to *S. hermonthica* using genome-wide selection with single nucleotide polymorphism (SNP) markers through Genotyping by sequencing (GBS) and (iii) determine genetic diversity among the selected finger millet genotypes against *S hermonthica*. One hundred finger millet genotypes were evaluated for resistance against *S hermonthica* (Del) Benth under field conditions at Alupe and Kibos sites in Western Kenya. The genotypes were planted in control and experimental plots inoculated with *Striga* and plant growth monitored to maturity. All accessions were genotyped–by-sequencing (GBS) and data analyzed using the non-reference based Universal Network Enabled Analysis Kit (UNEAK) pipeline. Genome wide association studies (GWAS) were done to establish the association of detected SNPs with *Striga* resistance based on field results. Statistical analysis of phenotypic data using Statistical Analysis System (SAS) PROC ANOVA revealed highly significant differences among genotypes for morphological traits at $P<0.05$. Six genotypes showed high resistance to *Striga* with a mean *Striga* count of 0 while the most susceptible genotype had *Striga* count mean of 69.17 at maturity. In molecular analysis 117542 SNPs from raw GBS data used in GWAS revealed that markers TP 85424 and TP 88244 were associated with *Striga* resistance in the 95 genotypes. Principal Component Analysis revealed that the first and third component axes accounted for 2.5% and 8% of total variance respectively and the genotypes were distributed according to their reaction to *Striga* weed. Genetic diversity analysis grouped the 95 accessions into three major clusters containing 32 (A), 56 (B), and 7 (C) genotypes each. All finger millet genotypes that showed resistance to *Striga* in the field were from cluster B while the most susceptible genotypes were from cluster A. Results revealed genetic variation for *Striga* resistance in cultivated finger millet genotypes and hence the possibility of marker – assisted breeding for the trait. It is suggested that more studies including more genotypes and wild relatives be carried out to understand further the resistance to *Striga* in *Eleusine* genera.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ACRONYMS AND ABBREVIATIONS

ABC-QTL	Advanced Backcross Quantitative Trait Loci
AEZ	Agro-ecological zone
BLAST	Basic local alignment search tool
BWA	Burrows wheeler alignment
CGIAR	Consultant group international agricultural research
CL3 AEZ	Coastal lowlands
CROPS	Complexity reduction of polymorphic sequence
DNA	Deoxyribonucleic acid
DNA (RAD)	Restriction site Associated DNA
EST	Expressed sequence tags
FAO	Food and Agricultural Organization
FAOSTAT	Food and Agricultural Organization Statistics
FIT	Inbreeding coefficient
GBS	Genotyping by sequencing
GWAS	Genome wide association studies
HTP	High throughput
ICRISAT	International Centre for Research in semi-arid and tropics
ISC	Integrated *Striga* control
ISM	Integrated *Striga* management
IUPAC	Internation Union of Physical and applied Chemistry
KALRO	Kenya Agricultural Livestock and Research Organizations
LH2	Lower highlands
LM4	Lower midlands
MAF	Minor allele frequency
MAS	Molecular assisted markers
NGS	Next generation sequencing
QTL	Quantitative trait loci
RRL	Reduced Representation Library
SADC	Southern African Development Community

SAS	Statistical Analysis System
SNPs	Single nucleotide polymorphisms
SOAP	Short nucleotide alignment program
SOLID	Support Oligonucleotides Ligation Detection
TBT	Tag by Taxa
TOPM	Tags On Physical Map
UNEAK	Universal Network Enabled Analysis Kit

ACKNOWLEDGEMENT

First and foremost, my gratitude goes to my supervisors Prof. Otto George Dangasuk and Dr. Gomezgani Lexa Matasyoh for their critical guidance in the course of this study. In a special context, I appreciate opportunity accorded to me by Dr. Chrispus Oduori Adeti to research at Alupe Kenya Agricultural and Livestock Research organization (KALRO) and Kibos Kenya Sugar Research Fund (KESREF) during field screening activity and for his guidance and financial support during this study.

I am also highly indebted to Dr. Damaris Achieng Odeny for her guidance and support and for the opportunity accorded to me to undertake my research at International Crops Research Institute for Semi-Arid Tropics (ICRISAT). Special thanks go to the staff of Cornell University laboratory for the acceptance to run GBS work for me in guidance of Dr. Rajneesh Paliwal of International Crops Research Institute for Semi-Arid Tropics (ICRISAT) Nairobi.

Special thanks also go to Gilda Aringo of Kenya Agricultural and Livestock Research organization (KALRO) Alupe, Dennis Odhiambo of Kenya Sugar Research Fund (KESREF) Kibos, Annis Saiyiorri and Vincent Njunge of International Crops Research Institute for Semi-Arid Tropics (ICRISAT) for their technical advice on physiological and molecular work. My kind regard goes to Aggrey Amakundu Omutsani and Olivia Mberesia of Kenya Agricultural and Livestock Research organization (KALRO), Kakamega Mary Anyinyo of KALRO Alupe for their technical assistance during the preliminary stages of this study.

I am indeed so grateful to finger millet Alliance for Green Revolution in Africa (AGRA) project through Dr. Chrispus Oduori Adeti for funding my research in Alupe and Kibos. I I acknowledge the patience and moral support of my wife Phanice and children while I pursued this study. Finally, I thank God who has been great and guided me this far.

CHAPTER ONE
INTRODUCTION

1.1 Background information

Finger millet (*Eleusine coracan*a, L. Gaertn) is one of the staple foods for many predominantly peasant communities in the semi-arid tropics of Africa (ICRISAT/FAO, 1996; Obilana and Manyasa, 2002; Oduori, 2005). It is consumed in the form of several products such as fermented and non-fermented porridge, pancake-like flat breads, fermented alcoholic and non-alcoholic beverages (Murty and Kuman, 1995; ICRISAT/FAO, 1996). The grains may also be malted and a flour of malted grain used as food for infants and the elderly (NRC, 1996).

It is the second most important millet grown in Eastern, Central and Southern Africa (House, 1995; Van Wyk and Gericke, 2000; Obilana, 2002). Due to its nutritive components, finger millet ranks fourth among other millets of the world (Obilana and Manyasa, 2002). It is the third most important cereal food crop after maize and sorghum in the North Rift Valley province of Kenya (MoALD, 1994).

Finger millet is productive in a wide range of environmental conditions and is able to tolerate annual precipitation of 290 to 422 mm, annual temperature of 11.1^{o}C to 27.4^{o}C and pH of 5.0 to 8.2 (Duke, 1979; ICRISAT/FAO, 1996; Holt, 2000), spanning from the Himalayas in Nepal, India, and throughout the middle-elevation areas of Eastern and Southern Africa. In Kenya, the main production areas are located west of the Rift valley (Oduori, 1993).

The two sulphur containing amino acids, (i.e. methionine and cysteine) are lacking in the diets of millions of the poor who live on starch foods other than millet and cassava (Oryokot, 2001). It is mitigates against protein malnutrition, particularly kwashiorkor. Finger millet is also rich in calcium, iron, phosphorus and manganese (Holt, 2000).

It is grown on over 4 million ha worldwide and is a primary food for millions in dry lands of East and Central Africa, and Southern India (Anon et al., 2004). Finger millet can grow on any soil type as long as the rainfall is higher than 800 mm per annum (Van Wyk and Gericke, 2000) and has the ability to utilize rock phosphate better than other cereals

(Flack et al., 1987).

Finger millet commands a high market price compared with other cereals in East Africa (Holt, 2000; Taken et al., 2002; Obilana et al., 2002). However, it is labour intensive especially during weeding because of its wild relative *Eleusine indica*, which is usually confused with *Eleusine coracana*, due to close similarity limiting its commercial production (Rohrbach, 1991). According to Agrawal, (1993); Musonga et al., (1993); Mitaru, (1993) low finger millet production is due to processing (de-hullers), labour during cultivation, poor technology, with research priority given to maize than finger millet and non-adoption of new technologies such as row planting. Oduori (2001) reported that farmers planting improved varieties and adopting improved management practices could imrove yields of finger millet in Keya.

As explained by CGIAR, (2001), lack of improved varieties, pests and diseases, limited uses, competition from other crops with better economic returns and lack of commercial food products are major limiting factors in finger millet cultivation. Among the poor technologies is the problem of farmers growing land races with low yield genetic potential (Oduori, 1993).

The major biological constraint to increased and a serious threat to sorghum and millet production in small holder (SH) sector in sub-Saharan Africa and India as explained by DeVeries and Toenniessen, (2001), (Rispail et al. (2007), and Teka, (2014) is attack by *Striga* or witch weeds. The genus *Striga* consists of obligate hemiparasitic root parasite, some of which are serious agricultural pests (Parker, 2009). *S. hermonthica* (Del.) Benth and *S. assiatica* (L.) Kuntze are particularly harmful to sorghum, maize and millet, but is also increasingly being found in sugar cane and rice fields (Stroud, 1993; Rodenburg et al., 2006; Aly, 2007; Ejeta, 2007; Scholes and Press, 2008; Atera and Itoh, 2011). Crop yield losses may be up to 100% when a susceptible cultivar is grown under high level of infestation (Obilana and Rammaiah, 1992; Haussmann et al., 2000). Parasitic weeds are problematic in Agricultural Production Systems (APS) in the world today as they compete with crops for nutrients, water and by habouring disease causing organisms (Parker and Riches, 1993; Press and Graves, 1995). The parasitic weeds penetrates the roots of the host plants depleting them of essential nutrients for growth resulting to

stagnation and finally low yields (Watson et al., 1998; Mohamed et al., 2006; Parker, 2009).

In Kenya, *Striga* infects about 210,000 ha causing an annual crop loss of US $40.8 million (Gethi et al., 2005; Vanauwe et al., 2008). These losses largely depend on the level of infection crop variety, soil fertility and rainfall patterns (Melker et al., 2007). The greatest impact of the parasite is on the infertile soils and the most affected are the subsistence farmers (Kabambe et al., 2008). The control of *Striga hermonthica* in cereals has proven elusive. Economically feasible and effective technologies are still to be developed (Debrah, 1994) for the cash strapped subsistence farmers in most *Striga* – stricken areas.

The analysis of genetic variation therefore becomes an essential part of plant genetics and crop improvement programs. According to Rafalski (2002) DNA polymorphisms can directly be related to phenotypic differences which could be genetically linked to its causative factor, or indicate relationships between individuals in populations. Allelic variations within a genome of the same species can be classified into three major groups that include differences in the number of tandem repeats at the particular locus such as microsatellites, or simple sequence repeats (SSRs) (Weber and May, 1989), segmental insertions/deletions (InDels) (Ophir and Graur, 1997), and SNPs (Wang et al., 1998). In order to detect and track these variations in the individuals of a progeny at DNA level, researchers have been developing and using genetic tools called molecular markers (Botstein et al.,1980).

Genetic diversity has several ‘indicators’, which are measured using various tools such as classical or Mendelian genetic analysis, that can be employed to evaluate variation in single known gene (qualitative traits), such as resistance to diseases (Smale and Mc Bride, 1996). Classical plant breeding uses the deliberate interbreeding of closely related individuals to produce new cultivars with desirable traits. As it needs a long period and several generations to select and evaluate useful genotypes, classical breeding could be limited to address global food security and meet the increasing requirements of food demands (Tester and Langridge, 2010).

Molecular plant breeding is the applications of molecular biology or biotechnology to improve or develop new cultivars, which includes two major approaches, marker-assisted selection (MAS) and genetic transformation (Moose and Mumm, 2008). MAS is a process whereby molecular markers are used for the indirect selection on traits of interest in crops and being a critical and effective method, has widely been applied in plant breeding to enhance crop yield, quality, and tolerance to biotic or abiotic stresses.

The DNA markers have been used to evaluate genetic diversity in different crop species (Cooke, 1995). Various molecular markers are being used for fingerprinting such as Restriction Fragment Length Polymorphism (RFLP) (Dubrail and Charcosset, 1998), Random Amplified Polymorphic DNA (RAPD) (Williams et al., 1990), microsatellites (Smith et al., 2000) and Amplified Fragment Length Polymorphism (AFLP) (Agarwal et al., 1999). Some of these techniques are robust and reliable (e.g., RALP and AFLP), quick (e.g., RAPD) while others are quick and reliable (e.g., microsatellites or Simple Sequence Repeats (SSR). The main limitation in the use of RFLP and AFLP markers is hybridization, radioactivity, time consuming, requires large amount of DNA and limited by the number of available probes (Bernatsky and Tanksley, 1986; Kochert, 1994; Vos et al., 1995). PCR-based molecular markers such as microsatellites and RAPD have been widely used in many plant species including finger millet for identification, phylogenetic analysis, population studies and genetic linkage mapping (Hilu, 1995; Salimath et al., 1995).

The SSR markers offer many advantages such as higher frequency of polymorphism, rapidity, technical simplicity use of fluorescence, requirement for only a few nanograms of DNA, compatible for high throughput genotyping and feasibility of automation (Semang et al., 2006). Therefore, SSRs has been used to analyze the genetic relatedness in several crop species (Varshney et al., 2001) but the cost of detection has remained high and also need prior sequence information (Powell et al., 1996).

Several low cost, high throughput methods that combine next generation sequencing with reduced-representation have been developed (Van et al., 2007; Morishije et al., 2013). Although complexity reduction of polymorphic sequence (CroPs) and restriction site

association DNA sequencing (RAD) technologies are powerful tools to detect SNPs, they can hardly be called high throughput (HTP), because on an average only ~1000 SNPs in genome pass stringent quality control (Mammadov et al., 2010). While the numbers are enough to generate genetic linkage maps of reasonable saturation and carry out preliminary QTL mapping, they are not adequate to implement genome-wide association studies (GWAS). Discovery of a large number of SNPs using GBS was demonstrated in maize (Narechania et al., 2009) and sorghum (Nelson et al., 2011) which not only increases the sequencing throughput by several orders of magnitude but also has high multiplexing capabilities (Elshire et al., 2011).

GBS has been developed as a low-cost approach for reduced representation sequencing (Elshire et al., 2011; Poland et al., 2012) and demonstrated as a simple and robust method for genome-wide profiling of complex populations (Chen et al., 2013; Lu et al., 2013). GBS uses restriction enzymes such as *Mst*I, *Pst*I or *Apek*I to reproducibly capture a targeted portion of the genome enabling high levels of multiplexing while obtaining sufficient sequencing coverage and has been successfully applied for a range of studies including genetic mapping (Elshire et al., 2011; Poland et al., 2012), assaying genetic diversity, population structure, and genomic selection (Baird et al. 2008; Lu et al., 2013). To eliminate a large portion of repetitive sequences, a type II restriction endonuclease, *ApekI*, is applied to digest DNA prior to sequencing to generate reduced representation libraries (genome complexity reduction component), which are further subjected to sequencing (Elshire et al., 2011). Thus targeted portion of the genome flanking restriction site is ligated to DNA-barcoded adaptor that enable multiplexed sequencing of many individuals on a single sequencing run.

To date the use of GBS approaches has largely focused on sequencing with the Illumina GAII and Hiseq platform which generates tens to hundred thousands of the genotyped SNP markers, ready for genetic analysis (Poland and Rife, 2012). The key components of this system are: reduced sample handling; few PCR and purification steps; no DNA size fractionation and barcoding; simultaneous marker discovery. Opportunities to apply markers to breeding or conservation biology that were often limited by the availability of appropriate bioinformatics tools have been addressed through implementation of a GBS

analysis pipeline in the Java program TASSEL (Bradbury et al., 2007) (version 4) which is specifically tailored to the GBS protocols of Elshire et al., (2011) or Poland et al., (2013) and Morris et al., (2013). Furthermore, the Tassel-GBS pipeline is not limited to specific restriction enzymes but works on nearly any restriction enzyme and barcoding approach, provided that sequence reads commence with the barcode immediately followed by the remnant of the restriction enzyme cut site (Mascher et al., 2013).

1.2 Statement of the problem

The major biological constraint to increased sorghum and millet production in small holder (SH) sector in Africa is attack by *Striga* or witch weeds (DeVeries and Toenniessen, 2001). The presence of *Striga* and its interaction with host plant can lead to high yield loss of between 10-70%, especially under heavy infestation depending on crop cultivar (Lagoke et al., 1991). Research on *Striga* control has been carried out for a long time and a wide range of technologies developed that have not been widely adopted due to mismatch between technologies and the farmers' socio-economic conditions (Atera et al., 2011). Strategies for *Striga* control require expensive resource investment in the form of labour, chemicals and equipment which most of the SH farmers cannot afford (Chivinge, Mashingaidze and Mujuru 1995; Kasembe, 1999). Also the low adoption of the control practices are as a result of limited knowledge of the problem, its biology, the labour or resource to make the needed investment, an uncertainity of potential control and return to investment, and an unwillingnessto make the long

The control of the weed has also been difficult because of its high fecundity and it's biology that allows the seed to remain viable underground for more than 10 years allowing it to persist and increase in magnitude (Van Ast & Bastiaans, 2006; Hearne, 2009). Also complete control of *Striga* on cereals has been a challenge to scientists for a long time and therefore the need to search for farmer satisfying strategies. For a long time crop improvement through conventional breeding has been going on at slow pace especially for traits controlled by quantitative gene action like *Striga* resistance. This is because of the fact that finger millet mainly is self-fertile with some amount of cross pollination (1%) mediated by wind (Jansen and Ong, 1996). The major challenge therefore is to develop methods or varieties that will help small scale farmers control

Striga effectively within a sustainable and profitable farming system (Doggett, 1988). According to Scholes and Press, (2008), the use of resistant crop cultivars is considered to be one of the most effective strategies. However, their effective deployment has been limited due to lack of understanding of genetic and phenotypic basis of adaptation of *Striga* population to their hosts. PCR-based molecular markers such as microsatellites and RAPD have been widely used in many plant species including finger millet for identification, phylogenetic analysis, population studies and genetic linkage mapping (Hilu, 1995; Salimath et al., 1995). However, the cost of detection has remained high in SSR and also need prior sequence information (Powell et al., 1996).

Therefore knowledge of the extent and distribution of genetic variation within finger millet could be an important tool for efficient collection, conservation and development of improved crops against *Striga* together with other environmental stress. Also because food security is at the heart of sustainable development in the region there is need to apply research interventions and solutions that will increase crop productivity to counteract the effects of food insecurity and climate changes. Since finger millet does not have reference genome determination of polymorphism was done using genotyping by sequencing (GBS) through TASSEL (Universal Network Enabled Analysis Kit) UNEAK pipeline. The method has the potential to simultaneously discover and score segregating markers in populations of interest.

1.3 Justification

Finger millet is usually tolerant to low rainfall, and therefore is more suitable for cultivation in arid and semi-arid areas just like sorghum compared to other grain crops (Rukuni et al., 2006). With the current climatic trends, drought resistant crops such as finger millet will be relied on to feed the worlds expanding populations (Bisht and Mukai, 2002). The crop is also known to have insignificant pest problems in comparison to other cereals as reported by Shakya et al., (1991). The control methods have been tried out with no conclusive and consistent results for the subsistence farmer due to the difficulty to deplete huge amount of seeds that have accumulated and continue to accumulate in the seed bank over years (Tenywa et al., 1999). Unfortunately, *Striga* poses a major setback to finger millet production.

According to Sorrells et al. (2003) and Proba et al. (2009), the use of modern crop improvement tools such as genomics to transfer genes from model species to the species of interest, and genetic mapping in order to identify genes controlling traits of interest can provide a more timely and robust response to crop production threats. It also provides added opportunities to develop crop varieties with multiple stress resistance. Use of crop cultivars that are resistant to *Striga* will provide most effective strategies to till with food insecurity in Kenya and neighbouring states. Therefore an approach incorporating most resistance mechanisms and screening approaches would be the way forward to the overall management of *Striga*. In Kenya, there are no finger millet varieties that have been developed to withstand *Striga* attack. Therefore, identification and adoption of *Striga* resistant genotypes could be a feasible cost-effective solution to finger millet production in soils infested by *Striga.*

This study undertook to screen Kenyan and International finger millet accessions for *Striga* resistance, investigate the genetic basis for resistance and then determine the overall genetic diversity among the finger millet germplasm using genotyping-by-sequencing protocol.

1.4 Objectives

1.4.1 General objective

To determine variations in finger millet genotypes in response to *Striga* infestation under field conditions and relate to genetic diversity through molecular characterization.

1.4.2 Specific objectives

I. To determine the effect of *Striga* infestation on finger millet agro-morphological performance
II. To determine the genetic basis of finger millet resistance to *Striga hermonthica* in the selected germplasm through Genotyping by sequencing.
III. To determine genetic diversity among finger millet genotypes showing resistance and susceptibility to *Striga* using general linear model GLM and mixed linear model MLM.

1.5 Research hypothesis

The study was based on the following alternative hypotheses:-

I. *Striga* infestation on many finger millet would have adverse effect on agro-morphological traits performance.

II. Using GBS, it is possible to identify genetic sites involved in *Striga* control in finger millet.

III. There is significant genetic diversity among finger millet germplasm that can be useful in breeding for *Striga* resistance.

CHAPTER TWO
LITERATURE REVIEW

2.1 Origin and distribution of finger millet

Finger millet is indigenous to eastern Africa, where the oldest domesticated form of the crop was found in a pre-historic site Axum hills of Ethiopia and Uganda dating back 5000 years (National Research Council, 1996; Consultative Group on International Agricultural Research, 2001; Bennetzen et al., 2003). According to Bennetzen et al., 2003, tremendous diversity in the crop exists in this region. De Wit et al. (1984) recognized five races that is to say *Eleusine, Elongata, Plana, Compcta and Vulgaris*, of which *Eleusine* is the most widely cultivated. According to ICRISAT (2008), finger millet is the most important minor millet in the tropics and grown in more than 25 countries where Africa and Asia, accounts for 12% of the global millet area. It is a potential and nutritious crop for the increasing world population, particularly in arid and semi-arid regions where it is usually ranked third in cereal production, after sorghum and pearl millet (Bisht and Mukar, 2002).

The crop was introduced to India at a very early date, probably over 3000 years ago (FAO, 1995). The annual worldwide production of finger millet is about 4.5 million tons, equally divided between India and Africa (M.S Swaminathan Research Foundation India 2003), grown on approximately 3.8 million hectares (Anon et al., 2004). In Eastern Africa, finger millet is grown in Uganda, Kenya, Tanzania, Rwanda and Burundi in Eastern region of Democratic Republic of Congo and also in Ethiopia, Sudan and Somalia (Obilana et al., 2002).

Kenya and Uganda are among the leading producers of finger millet in Africa and worldwide. In Uganda about 600,000 ha is devoted to finger millet, while in Kenya it is about 65,000 ha (Taken et al., 2002; FAOSTAT, 2008). In Kenya it is grown in Western, Rift Valley, parts of eastern and Nyanza Provinces (M'Ragwa, 1986; Pinto, 1982). It is also grown in West Africa, India, and other Asian countries including Sri Lanka and China (Fakruchin et al., 2004) of which major producers are, India, Nepal and China (ICRISAT, 2008). Table 2.1 shows millet acreage and production in Africa relative to

other regions of the world. Acrearage is high however, production is very in Africa relative to Asia. Finger millet also an important cereal in the Southern African Development Community (SADC) countries of Tanzania, Zambia, Malawi and Zimbabwe and is back up "famine food" as far south as Mozambique (Gomez, 1993; National Research Council, 1996).

Table 2.1: Millet acreage and production in Africa relative to other regions of the world, 1992-1994

Region/Country	Area (million ha)	Production (million tons)
Whole of AFRICA (28 countries)	18.50	11.36
East & Central Africa (8 countries)	3.36	2.01
Southern Africa (10 countries)	1.20	0.75
West Africa (10 countries)	13.94	8.60
ASIA	16.99	15.17
India	13.95	10.70
China (mostly foxtail millet)	1.90	3.67
USA (mostly proso millet)	0.15	0.18
Argentina (mostly proso millet)	0.04	0.06
World (all cultivated millet species)	38.10	28.38

Source: ICRISAT/FAO 1996

Poor research attention has been paid to improvement of finger millet, particularly in Africa as is evident from the scarcity of literature on the crop. The poor research attention on the crop include lack of international research and political support in sub-Saharan Africa and Asia. Because of little research effort on the crop, the yield of finger millet on farmers' fields in Kenya is low ranging from 500 to700kg ha^{-1} (Mitaruet al., 1993 and Taken et al., 2002). Slightly higher yields ranging between 680 and 1000kg ha^{-1}have been reported in Uganda and India under rainfed conditions (Tenywa et al., 1999and FAOSTAT, 2008). The higher yield in Uganda partly explains the higher production in

Uganda than Kenya (Table 2.2)

Table 2.2: Kenya and Uganda finger millet eight years annual production in tons.

Year	2000	2001	2002	2003	2004	2005	2006	2007
Kenya	44600	44600	72200	63000	50500	53100	68700	50000
Uganda	534000	584000	590000	640000	659000	672000	687000	732000

(Data Source: FAOSTAT (2008)

2.1.1 Ecology of finger millet

Finger millet is an important staple crop in many parts of Africa (AGPC, 2008), where it can grow on any soil type competing with maize for the best agricultural land in regions between 900 and 1200mm of annual rainfall (de Wet, 1995a and Van Wyk and Gericke, 2000) and is able to produce some yield during times of drought. It has ability higher to utilize rock phosphate better compared to other cereals (Flack et al., 1987). The crop is productive in a wide range of environmental conditions being able to tolerate annual temperature of 11.1 to 27.4^{o}C and pH of 5.0 to 8.2 (Holt, 2000 and ICRISAT/FAO, 1996) spanning from the Himalayas in Nepal, India, and throughout the middle-elevation areas of Eastern and Southern Africa (Holt, 2000). Millets are C_4 plants which have competitive advantage over C_3 plants under conditions of drought, high temperature, nitrogen or carbon (IV) oxide limitation (Roder, 2006; Osborne and Freckleton, 2009). C_4 plants utilize their specific leaf anatomy, known as Kranz anatomy, to fix Carbon (IV) oxide around rubisco thus reducing photorespiration (Holt, 2000; Osborn and Beerling, 2006).

2.1.2 Production in Kenya

Finger millet yields are variable, compared to other cereals, but are generally good (National Research Council, 1996). Its yields on farmers' fields are generally low, just about 15-16% of their theoretical maximum in Kenya (Takan et al., 2002). The yield has been declining since 1978 with a greater variation in hectarage than production (Mburu, 1989). According to Mitaru et al., 1993 the grain yields has been ranging between 500-750 kg ha^{-1}. In North Rift Valley region of Kenya yields range from 0.5-0.9 ton ha^{-1} (MoALD Report, 1994). Under irrigated conditions in field trials, yields of up to 5-6 tons ha^{-1} of variety P224 was obtained (National Research Council 1996). However, yield

performance trials have shown that finger millet variety Gulu-E had a yield potential of 1.9 tons ha^{-1} compared to the local variety which yielded 0.3 tons ha^{-1}at Kodich under agro-ecological zone (AEZ) Lower midlands (LM_4) in West Pokot District which is a semi-arid zone. Finger millet variety P224 had a yield potential of 4.8 tons ha^{-1} at Alupe Lower highlands (LH_2 AEZ) and 5.8 tons ha^{-1} at Mtwapa, Coastal lowlands (CL_3 AEZ), according to KARI, (1992). Zimbabwe produces between 45,000 and 90,000 tons from hectarage of 90,000 to 130,000 yr^{-1} with yields of between 350 and 750 kg ha^{-1} using variety I.E 4491, I.E 4497, I.E 5306 and I.E 6337, (Rohrbach and Mazvimavi, 1993).

2.1.3 Morphology of Finger millet

The numerous races under cultivation are primarily divided into purple and green types. The spikes are divided into straight or open, curved or closed and branched. The length of ear-heads is 5-10cm. The seed is globose about 2mm in diameter and having a range of colours from deep brown to shade of orange-red to almost white or black. The plant height range from 0.45m to 1.3m tall and from poor tillering to profuse tillering. The leaf blades are shiny green, strongly keeled and difficult to break. They are 22 to 50 cm long and 0.6 to 1.0 cm wide. The plant has an exceptionally strong root system that is difficult to pull out of ground (Van Wyk and Van Oudtshoorn, 1999).

2.1.4 Utilization

As food, the grain has good taste and is a dietary source of two sulphur containing amino acids methionine (~5%) and cysteine, an amino acid lacking in diets of many poor people's carbohydrates staples and therefore can mitigate against protein malnutrition particularly kwashiorkor (Orykot, 2001). Finger millet is also rich in calcium, iron, phosphorus, copper and manganese than maize and its sprouted seeds are nutritious and easily digested, hence recommended for expectants, lactating mothers, infants and elderly in tropics as well as providing a sustaining diet for people doing hard work, management of measles and anemia (NRC, 1996; Holt, 2000). According to NRC, (1995), the grain's protein content (7.7%) is comparable to that of rice (7.9%), but the main protein fraction (eleusinin) has high biological value, with good amount of tryptophan, cysteine, methionine, and total aromatic amino acids, which are crucial to human health and growth and are deficient in most cereals.

Regular consumption of finger millet is known to reduce the risk of diabetes due to lowering of plasma glucose level in comparison to rice and wheat and gastro-intestinal tract disorders which could be attributed to polyphenols and high dietary fiber content or presence of anti-nutritional factors in the whole finger millet flour that reduces starch digestibility and absorption (Kumari and Sumanthi, 2002). Amruthmahal et al., (2003) finding that finger millet has the highest total rapidly digestible starch (RDS), compared to rice, wheat, and sorghum grain added to explanation on why it is used for diabetes management. The high nutritive value, gives finger millet some medicinal value, making it important cereal for community-based health care programs and children feeding schemes in rural institutions in developing countries.

According to Haore et al. (2007), it is also used in traditional medicine as an internal remedy for leprosy or liver disease. Finger millet in Africa is used to make traditional beer because its amylase enzymes rapidly convert starch to sugar, that is subsequently converted to alcohol, hence it is only second to barley, the world premier beer grain (Van Wyk and Gericke, 2000). The straw makes good fodder and contains up to 61% total digestible nutrients-better than pearl millet, wheat, or sorghum (Duke, 1979). It is sold for cash and in cultural value, for example in special ceremonies like weddings and paying of bride price (Oduori 1993; NRC, 1996). Table 2 presents nutrient composition of sorghum, finger millet and other cereals in which case finger millet has highest amount of crude fibre and calcium.

Table 2.3: Nutrient composition of sorghum, millets and other cereals

Cereal	Protein (g)	Fat (g)	Crude fibre (g)	Carbohydrate (g)	Energy kcal	Calcium (mg)	Iron (mg)
Rice (brown)	7.9	2.7	1.0	76.0	363	33	1.8
Wheat	11.6	2.0	2.0	71.0	348	30	3.5
Maize	9.2	4.6	2.8	73.0	358	26	2.7
Sorghum	10.4	3.1	2.0	70.7	329	25	5.4
Finger millet	**7.7**	**1.5**	**3.6**	**72.6**	**336**	**35**	**3.9**

Source: FAO (1995)

2.1.5 Importance of small grains to household food security

According to Taylor (2003); Alumira and Rusike (2005) and FAO (2008), sorghum and millet are vitally important cereals for the maintenance of food security in Africa due to

their high levels of adaptation to African conditions as much as the two are under researched compared to other cereals. In view of that, Taylor (2003) advocated for proper research in sorghum, pearl millet and finger millet that could play an important role in offering better long-term food security than maize because they are indigenous African cereals hence are well adapted to African semi-arid and sub-tropical agro-ecological conditions. The same considerations were mentioned earlier by Rohrbach (1991) that sorghum and millet represent potential staple food for many of the poorest farm households in semi-arid areas. FAO (2006) suggested that although Zimbabwe's Natural Regions (NR) IV and V are considered inappropriate for dry land cropping, however drought tolerant crops such as sorghum, pearl millet (*mhunga*) and finger millet (*rapoko*) are suitable crops that can be grown by smallholder farmers in these regions. More so in the event of severe drought, maize can be destroyed yet drought tolerant small grain cereals such as sorghum and millet can yield some food for subsistence (Van Wyk and Gericke 2000; Rukuni et al., 2006).

2.2 Origin, occurrence and distribution of *Striga*

Striga hermonthica (Del.) Benth originated in Nuba mountain of Sudan and in parts of Ethiopia which are also known to be the origin of sorghum and pearl millet that are readily infected by the weed (Ejeta, 2007; Atera and Itoh, 2011). *S. hermonthica* is widespread in sub-Saharan Africa, and found throughout West Africa to Ethiopia, Uganda and Kenya in East Africa (Mohamed et al., 2001). It is most common on heavy black cotton soils particularly in the densely populated regions of Nyanza and Western Province Kenya, eastern and northern Uganda (Ebiyau et al., 2000; MacOpiyo et al., 2010). The weed was also confirmed by Hassan and Ransom (1998), to be on the increase in maize in the moist transitional zone in Kenya with a total affected area approximating to 400,000 ha. According to Oswald (2005), *Striga* has been in existence in farmers' fields in Western Kenya since 1936. Ayensu et al. (1984), reported serious crop losses due to *Striga* in the following regions of the world, Gambia, Senegal, Mauritania, Togo, Ghana, Tanzania, Botswana, Zwaziland, Mozambique and more locally elsewhere in Africa, Asia, Australia and the USA.

Striga adapts very quickly to different hosts and environment attaining up to 50%

germination under moisture regimes described as permanent wilting point for its host, illustrating the serious consequences the parasite can have in arid regions (Dawoud and Sauerborn (1994). It can tolerate wide ranges of day/night temperatures $25^{o}/15^{o}C$-$40^{o}/30^{o}C$, making it a successful parasite throughout its range (Patterson et al., 1982). The ability of *S. hermonthica* to withstand a wide range of climatic conditions (Welsh and Mohamed, 2011) and parasitize different hosts (Ali et al., 2009) qualifies it to be considered among the most widely distributed known witch weeds with real invasive potential threatening cereal production worldwide (Mohamed et al., 2006). It has spread in Africa south to Angola, and north to Delta zones in Egypt. Striga has also extend its range outside the continent to Yemen and Saudi Arabia (Mohamed et al. 2001). Generally, *Striga* spp. grows in areas with annual rainfall varying from 25-150 cm per year with decrease in severity of infestation in areas of high rainfall (Mohamed et al., 1998). It is also favoured by conditions such as continuous cultivation of cereal crops, overused, depleted and infertile soils and soil moisture stress conditions (Khanet et al., 2007).

2.2.1 The *Striga* seed

Striga seeds are minute, with the average seed size being 0.2 mm wide and 0.3 mm long. A single *Striga* plant can produce up to 10,000- 500,000 seeds in one season (Ariga et al., 1997; Koich et al., 2010). The seeds are dispersed by wind, water, cattle, man and farm machinery like tractors (Euserink, 1995). The seeds can stay in the soil for 15 – 20 years and can remain viable longest in soils that are usually dry where just a fraction seeds germinate in any season in the presence of a host (Berner et al., 1995, 1997; Ariga et al., 1997).

2.2.2 Life cycle of *Striga spp.*

Most *Striga* and *Orobanche* species show a large genetic diversity and complexity due to co-evolution with host (Botanga et al., 2006; Roman et al., 2000a). For germination to occur *Striga* seed requires a period of pre-treatment, conditioning in moist warm environment for 2 to 16 days before they have the potential to germinate (Longan and Stewart, 1991; Koua et al., 2011a). Following this period, seeds germinate in response to molecules calleö strigolactones, öihydrosorogoleone, sesqluterpene, kinetin, coumarin,

jasmonate, ethylene and fungal metabolites (hydroquinones) which are released by host plant roots into the rhizosphere (Shen et al., 2006; Yoneyama et al., 2010; Cardoso et al., 2011). The root tips of the parasite develops radial swelling and haustorial hairs that function as attachment anchors and penetration pegs (Keyes et al., 2001). Successful parasitic establishment creates a strong sink of nutrients to the detriment of the host, leading to drastic growth and yield reductions (Keyes et al., 2001; Joel et al., 2007). After a connection has been established between host and parasite, it exhibits a holoparasitic subterranean stage of development at which time damage is inflicted. The *Striga* shoot then emerges from the soil, develops chlorophyllous shoots (hemiparasitic stage) and produces flowers and sets seeds 6 weeks later (Bagonneaud-Berthorne et al., 1995).

2.2.3 How *Striga* damages cereal host

The early symptoms of *Striga* damage on the cereal hosts include stunted growth, bleaching / yellowing and wilting which are evident before emergence of the parasite (Berner et al., 1995). Under severe infestation, failure of panicle formation may occur resulting to total crop loss (Agrios, 1997). *Striga* reduces crop yields in two ways: Firstly, by direct parasitism in which *Striga* derives water, mineral nutrients and photosynthetic assimilates from crop root system thereby retarding its growth and development (Press and Stewart, 1987). According to Patrick et al. (2004) and Berner et al. (1997), *Striga* inflicts most of the damage to its host while still under ground. It grows parasitically under the ground for a period of 6-8 weeks prior to emergence (Babiker, 2000). Secondly, by pathological effect in which *Striga* is known to produce toxins affecting plant growth and development (Stewart and Press, 1990). The extent of yield loss is related to the incident and severity of attack, the host's susceptibility to *Striga*, environmental factors (edaphic and climatic) and the management level at which the crop is produced. For example, Maize losses of up to 81% have been recorded in western Kenya (Ransom et al., 1990). According to MacOpiyo et al. (2010), the average losses due to *Striga* are 1.15, 1.10 and 0.99 tons per hectare for maize, sorghum and millet respectively. However, the damage can reach as high as 2.8 tons ha^{-1} in maize and sorghum in some locations with high *Striga* densities (Anderson and Halvarsson, 2011). Plate 1 is a photograph showing two plots of finger millet infested with *Striga* and the level of damage. The first plot carried a genotype that was tolerant as the damage was

minimal while the second plot comprised of genotype that was susceptible to *Striga* where by the crop had stunded growth. Arable lands are often abandoned because of the prohibitive parasite populations (Hess and Lenne, 1999). Land abandonment impact adversely on household and national food security as well as income generation (Kasembe, 1999).

A

B

Plate 1: Severely *Striga* infested plots of Finger millet at Kibos 2012 LR.

(Source: Author, 2012).

2.3 Field screening and evaluation of materials for *Striga* resistance

According to Haussmann et al. (2000) and Omanya *et al.,* (2004) field screening is still the most reliable technique to produce stable resistance to *Striga*, though, it is complex, expensive, hampered by high soils micro-variability, heterogeneity of natural infestations, and concomitant large environmental effects on *Striga* emergence. The fact that resistance to *Striga* can be greatly affected by environmental factors such as drought, soil type and fertility levels does not make screening for *Striga* any easier (Ejeta, 2007 and Amusan *et al*., 2008). An improved field testing methodology should include one or several of the following practices: i) field inoculation with *Striga* seeds, appropriate experimental design that allow high replication for example lattice designs for nursery screening followed by randomized complete block design (RCBD) on fewer genotypes, ii) specific plot layout by use of appropriate susceptible and resistant checks, evaluation in adjacent infested and un-infested plots and the use of selection indices derived from emerged *Striga* counts, *Striga* vigor, and grain yield or a host plant damage score. Multi-

location screening to obtain materials with stable performance is recommended due to the extreme variability of the parasite and significant genotype x environment interaction effects (Oswald, 2005).

In addition to multi-locational testing, many breeding strategies have been put forward by several workers (Berner *et al.,* 1995; Haussmann, 2000). Among them is characterization of crop germplasm and identification of sources of resistance and their improvement for agronomic performance.

2.3.1 *Striga* management methods and their limitations

Complete control of *Striga* on cereals has been a challenge to scientists for a long time and therefore the search for farmer satisfying strategies continue. Management of *Striga* is difficult because majority of its life cycle takes place underground and therefore when not detected before emergence will be too late to reduce crop loss (Johnson, 2005). Some *Striga* control strategies were developed and tested on farm in western Kenya including intercropping, crop rotation, catch-cropping, hand weeding, inorganic fertilizer and manure application, resistant varieties and improved fallow management (Oswald, 2005). Many researchers however, suggested that integrated *Striga* control or management (ISC or ISM) was the best strategy for short and long term *Striga* control which needed involving concerted effort of all stakeholders (Aliyu et al., 2004; Van Mourik, 2007). According to Ejeta and Gressel, (2007) strategies for management of *Striga* revolve around the options of control, containment, or eradication, with the latter being almost impossible. Based on the effect on *Striga* population, Haussmann et al. (2000) grouped *Striga* control measures into three categories: i) reduction of the soil seed bank ii) limitation of *Striga* seed production and iii) reduction/prevention of *Striga* seed dissemination to un-infested fields. Most often these control measures have had limited success leading to the conclusion that effective and affordable control measures for *Striga* being scarce as reported by Kuiper et al., (1998).

Hand weeding/hand pulling is the most widely practiced control method for *Striga* in Kenya, but due to high labour costs, it is recommended to begin 2-3 weeks after the weed begins to flower to prevent seeding (Parker and Riches, 1993; Frost, 1994). The method usually need to be continued for 3-4 years and is most economical on the least infested

fields (Ransom, 1996).

The use of trap and catch crops (e.g. cotton, cow pea, jute, soya bean, pigeon pea, chickpea, kenaf, ground nut sunflower, lablab) that induce germination of the *Striga* but are not themselves parasitized is currently one of the best methods to control agricultural root parasites (Khan et al., 2010). However, available studies indicate that trap crops need to be cultivated for at least 3 consecutive years in order to reduce parasite seed (Esilaba and Ransom (1997). However, this approach was found not to be better than continuous cultivation of maize in reducing *Striga* numbers in Kenya (Ransom and Odhiambo, 1996).

The use of nitrogen to suppress *Striga* has been demonstrated in the East and Central Africa highlands (Esilaba et al., 2000; Gacheru and Rao, 2000). Mumera and Bello (1993) found that although *Striga* infestation declined with increasing N availability, the impact was partially dependent on the severity of infestation.

Studies carried out in Kenya indicate that intercropping with cowpeas between the rows of maize significantly reduced *Striga* numbers when compared to within the maize rows (Odhiambo and Ransom, 1993). Previous on-farm trials showed that intercropping of maize and beans in the same hole in *Striga* infested farmers' fields increased maize yields by 78.6% in western Kenya (Odhiambo and Aringa, 2004). The intercrop legumes also increase soil fertility and provide shade that gives *Striga* a disadvantage (Khan et al., 2006). The disadvantage with intercropping is that it is more time consuming compared to monocropping (Khan et al., 2009). Similarly a push and pull strategy for integrated pest management showed that fodder legumes (*Desmodium uncinatum* and *D. intortum*) intercropped with maize to repel stem borers reduced *Striga* infestation in western Kenya due to allelopathic mechanisms of *Desmodium* spp. (Khan et al., 2002), that involved a germination stimulant for *S. hermonthica* and also an inhibitor for haustorial development (Vanlauwe et al., 2008).

Chemicals are grouped either as germination stimulants or herbicides, e.g. ethylene, ethephon, strigol and strigol analogues induced germination of *Striga* seeds in the absence of a suitable host thereby reducing seed reserve in the soil (Esilaba and Ransom, 1997). Among the chemicals investigated for efficacy in controlling *Striga* is Dicamba

which can provide early season control but has not proven to be consistently cost-effective (Odhiambo and Ransom, 1993). Recent on-farm trials in Kenya and Tanzania indicated that seed dressing with imazapyr and Pyrithiobac offered good *Striga* control and increased maize yields (Kanampiu et al., 2004). According to Parker and Riches (1993), effective preventive measures require to be taken through seed quarantine, *Striga* free equipment and burning material which may contain viable seeds. In general, the priority in all field projects requires provision of information where farmers can make optimum decisions on farming system.

2.3.2 Breeding for *Striga* resistance

Parasitic weed resistance in host plants is expressed either before or after host-parasite vascular bridge formation (Rispail *et al.,* 2007). Several *Striga* resistance mechanisms in sorghum have been proposed where some were tagged as potential. For example, slow *Striga* germination stimulant production by host plant, mechanical barriers to parasitization, host production of germ tube inhibitors, host production of defense chemicals (Antibiosis), post parasite attachment incompatibility, insensitivity of host to *Striga* toxin, and avoidance by development of few roots in the top soil (Berner *et al*., 1995; Haussmann and Hess, 2001). Of these resistance mechanisms the production of low *Striga* germination seed stimulant strigolactone was the most understood and is detected by differential crop varieties root exudates to stimulate *Striga* seeds germination on agar/water gel assay (Umeliara et al., 2008). Low Striga germination stimulant activity is controlled by one single gene recessively inherited gene, *lgs* (Satish et al., 2012). It was observed that a single nuclear recessive gene controls this mechanism in sorghum variety SRN 39 (Vogler *et al*., 1996). Mechanical barriers (e.g. lignification of cell walls) mechanism involves localized necrosis of host tissue that hinders parasite penetration of host tissue (Ejeta, 2007). Inhibition of germ tube exo-enzymes by root exudates that inhibit the host root penetration enzymes of the parasite retarding the germ tube (Mohamed *et al*., 2001). The existence of such mechanism in finger millet needs to be verified with progression in breeding for *Striga* resistance in this crop. Resistant varieties are defined as those that show less attach, and with few attached and / or emerged *Striga* plants (Parker and Riches, 1993). The converse to this is susceptibility. Tolerant varieties on the other hand are parasitized to the same extent as a standard variety but suffer less

damage and the converse to tolerance is sensitivity.

Haussmann *et al.,* (2000) outlined three categories of *Striga* screening methods. Laboratory screening individual for resistance mechanisms where two approaches exist: agar-gel assay (Hess *et a*l., 1992). According to Haussmann *et al.,* (2000) and Omanya *et al.,* (2004) this is a useful, fast, indirect selection method for screening for long stimulant character. However, correlation analysis showed that this resistance mechanism was ineffective in some environments, pointing to the necessity of field evaluation. Paper-roll assay method (Ejeta, 2000) allows observations of early stages of *Striga* infection and is effective for identifying early post infection resistance mechanisms, i.e. hypersensitivity reaction or incompatibility though it needs modification for large-scale application.

2.4 SNP Genotyping

SNP genotyping is the downstream application of SNP discovery to identify genetic variations. The advantages of SNPs over microsatellites and mitochondrial DNA resides in the fact that SNPs represent single base sequence nucleotide substitutions and as such they are less affected by homoplay because their origin can be explained by mutation models (Vignal et al., 2002). SNPs have been employed to quantify genetic variation, for individual identification, to determine parentage relatedness and population structure (Morin et al., 2004). SNPs have also been used to study the evolution of genes such as WAG-2 in wheat (Wei et al., 2011), Algorithms such as neighbor-joinig and maximum likelihood implemented in PHYLIP (Retief, 2000). The number of SNPs and individuals to screen are of primary importance in choosing on SNP genotyping assay, though cost of the assay and/or equipment and the level of accuracy are also important considerations. Illumina Golden gate is a commonly used genotyping assay because of its flexibility in interrogating 96 to 3,072 SNP loci simultaneously (http://www.illumina.com/).

2.4.1 Genotyping-by-Sequencing (GBS)

There have been a number of approaches developed that use complexity reduction strategies to lower the cost and simplify the discovery of SNP markers using NGS, RNA-Seq, complexity reduction of polymorphic sequences (CRoPS) (Mammadov et al., 2010), restriction-site-associated DNA sequencing (RAD-Seq) (Pfender et al., 2011), and GBS

(Davey et al., 2011). Of these methodologies GBS holds the greatest promise because it has the ability to perform SNP discovery and genotyping simultaneously besides it having a simplified library production procedure that is more amenable to use on large numbers of individuals/lines (Elshire et al., 2011). The technique can be applied to species with or without a reference genome (Chutimanitsakun et al., 2011). A two-enzyme (*PstI/Msp*I) GBS protocol, which provides a greater degree of complexity reduction and uniform library for sequencing than the original protocol using *Ape*KI, has now been developed and applied to both wheat and barley (Poland et al., 2012). Two different GBS strategies have been developed with the Ion PGM system (Poland et al., 2012a). (A) Restriction enzyme digestion, in which no specific SNPs have been identified and ideal for discovering new markers for MAS programs. The complexity of the genome under this approach is reduced by digesting the DNA with one or two selected restriction enzymes prior to the ligation of the adapters. (B) Multiplex enrichment PCR, in which a set of SNPs has been defined for a section of the genome. This approach uses PCR primers designed to amplify the areas of interest.

Barcodes are included in one of the adapter sequences, and their locations, just upstream of the RE cut-site in genomic DNA, eliminate the need for a second Illumina sequencing ("indexing") read. The barcoding strategy is similar to RAD but modulation of barcode nucleotide composition and length results in fewer sequence phasing errors (Baird et al., 2008). Compared to the RAD method, GBS is substantially less complicated; amenable to setting up an automated work flow using liquid handling work stations, generation of restriction fragments with appropriate adapters is more straightforward, single-well digestion of genomic DNA with a restriction enzyme and adapter ligation results in reduced sample handling, fewer DNA purification steps, and fragments are not size selected (Elshire et al., 2011; Poland et al., 2012). Startup costs for GBS are minimal, as it involves only (1) testing that one of your candidate restriction enzymes (or enzyme pairs) produces a suitable GBS library, and (2) optimization of the ratio of sample DNA to the PCR adapters (Elshire et al., 2011). Costs can be further reduced via shallow genome sampling coupled with imputation of missing internal SNPs in haplotype blocks.

Unlike other high density genotyping technologies which have mainly been applied to general interest "reference" genomes, the low cost of GBS makes it a powerful approach on discovering and genotyping SNPs in a variety of crop species and populations. GBS is suitable for population

studies, germplasm characterization, plant genetics, and breeding in diverse crops and it has widely been applied in many large crop genomes to saturate the mapping and breeding populations with 10–100s of 1000s of SNP markers (Poland et al., 2012; Lu et al., 2013).

Construction of GBS libraries is based on reducing genome complexity with restriction enzymes (REs) (Elshire et al., 2011). This approach is simple, quick, extremely specific, highly reproducible, and may reach important regions of the genome that are inaccessible to sequence capture approaches. Choosing appropriate Res avoids repetitive regions of genomes, and lower copy regions are targeted with two to three fold higher efficiency which tremendously simplifies computationally challenging alignment problems in species with high levels of genetic diversity (Gore et al., 2007). The GBS procedure is demonstrated with maize and barley recombinant inbred populations where roughly 200,000 and 25,000 sequence tags were mapped, respectively (Elshire et al., 2011). To date, the use of GBS approach has largely focused on sequencing with the Illumina GAII and Hiseq platform (Poland and Rife, 2012).

Startup costs for GBS are minimal, as it involves only (1) testing that one of your candidate restriction enzymes (or enzyme pairs) produces a suitable GBS library, and (2) optimization of the ratio of sample DNA to the PCR adapters (Elshire et al., 2011). The Production Pipeline determines the taxon of origin of each good, barcoded sequence read in each input FASTQ file and then checks if the read matches one of the useful tags in the production-ready TOPM. In this manner, allelic depths for each useful SNP in the TOPM are recorded for each taxon, allowing quantitative SNP calling to be performed, again either by our own binomial likelihood ratio method or, optionally, according to the method of Hohenlohe et al., (2010). Genotype files are produced in HapMap format as well as in or in the custom HDF5 format (which also records allelic depth). The ability to convert from this custom HDF5 format into VCF format also retains allelic depth and plans are underway to have them added to the TASSEL GUI in the near future (Danecek et al., 2011).

2.4.2 DNA sample Preparation

High quality genomic DNA is crucial to the success of these protocols, given that varying efficiency of digestion, ligation and amplification can have significant effects on the final marker set. Most importantly the quantity of DNA from different samples should be

evenly balanced before pooling to avoid losing markers from some individuals owing to lack of coverage. The choice of method may also be influenced by the amount of genomic DNA starting material required for example RRL 25µg pooled (Close et al., 2009); CroPs 300ng per sample (Schadt et al., 2010) and GBS 100ng per sample (Xu et al., 2012). Low sequence diversity is a problem with methods in which the restriction enzyme overhang appears at the same position in every read. Although using many barcodes an innovation of GBS that can be applied to any method usually avoids this problem, together with use of variable length barcodes (between 4 and 8 nucleotides long).

2.4.3 Pooling individuals.

Many studies use one barcode for a pool of several individuals which is useful to avoid a whole genome amplification step when amount of DNA per individual is small (Libaut et al., 2010; Emerson et al., 2010). There is also an analytical theory to suggest that such pooling improves SNP discovery and leads to better estimates of population allele frequencies (Robertson et al., 2007; Futschik and Schlotterer, 2010). In the absence of a high-quality reference genome sequence, pooling also precludes filtering on the basis of observed heterozygosity (Lu et al., 2010).

2.4.4 Illumina sequencing

With this sequence approach fragments of DNA are hybridized to a solid substrate called a flow cell. Through bridge amplification process, the bound DNA template fragments are amplified in an isothermal reaction where copies of the template are created in close proximity to the original. A clusters of DNA fragments are formed on the flow cell creating a "lawn" of bound single strand DNA molecules which are sequenced by flooding the flow cell with new class of cleavage fluorescent nucleotides and reagents necessary for DNA polymerization (Turcatti et al., 2008). A complementary strand of each template is synthesized one base at a time using fluorescently labeled nucleotides. The fluorescent molecule is excited by a laser and emits light, the colour of which is different for each of the four different bases (Appendices XXV and XXVI). The fluorescent label is then cleared off and a new round of polymerization occurs. Unlike 454 sequencing, all four bases are present for polymerization step and only a single molecule is incorporated per cycle.

The flagship Hiseq 2500 sequencing instrument from Illumina can generate up to 600 GB per run with read length of 100nt and 0.1% error rate. The Illumina technique can generate sequence from opposite ends of DNA fragment so called paired-end (PE) reads. The choice of a sequencing strategy takes into account the research goals, ability to store and analyze data, the ongoing changes in performance parameters, and the cost of NGS/TGS platforms. Some key considerations are cost per raw base, cost per consensus base, raw and consensus accuracy of bases, read length, cost per read, and availability of PE or single end reads (Glenn, 2011). The pre- and post-processing protocols such as library construction and pipeline development and implementation for data analysis are also important (McPherson, 2000; Kothiyal et al., 2009). In order to efficiently store and retrieve data from a matrix of this size (3.2 TB of uncompressed, raw data), the HDF5 storage format is used (http://www.hdfgroup.org) as well as implementation of a rapid and efficient run length compression algorithm to further decrease the storage size. At low depth and with high genetic diversity (numerous sequence tags per locus), the TBT is a sparse data matrix consisting mostly of zeros; where run length compression algorithm takes advantage of this.

2.4.5 Software for Sequence Analysis

Both commercial and noncommercial sequence analysis software are available for Windows, Macintosh, and Linux operating systems. Commercial software such as CLC-Bio (http://www.clcbio.com/) and SeqMan NGen (http://www.dnastar.com/t-sub-products-genomics-seqman-ngen.aspx) provide a friendly user interface, and are compatible with different operating systems. They require minimal computing knowledge and being capable of performing multiple downstream analyses. However, they are fairly expensive, with narrow customizability, and requiring locally high computing power. Linux-based programs have been recommended because they are often free, not specific to any sequencing platform, and less computing power hungry and, as a consequence, tend to perform faster (Wang et al., 2009). Flexibility in the parameter's choice for read assembly is another major advantage. However, most biologists are unfamiliar with Linux operating systems, its structure and command lines, thereby imposing a steep learning curve for adoption. Linux-based software such as Bowtie (Langmead et al., 2009), BWA (Li and Durbin 2009), and SOAP2/3 (Li et al., 2009 have been used widely

for the analysis of NGS data. Currently, SAM format (Li et al., 2009) output alignment files produced by the free software programs Bowtie2 (Langmead and Salzberg, 2012 or BWA (Li et al., 2009) can be read by the Tassel-GBS pipeline and converted into a "Tags On Physical Map" (TOPM) file that can be used for SNP calling. The TOPM contains all of the tags present in the master Tag Count-file and genomic positions for the subset of tags that align to a unique best position in the genome.

2.4.6 SNP Discovery and initial filtering

In theory, a SNP is identified when a nucleotide from an accession read different from the reference genome at the same nucleotide position while in the absence of reference genome, this is achieved by comparing reads at different genotypes using de novo assembly strategy (You et al., 2011). The most common application of NGS is SNP discovery, whose downstream usefulness in linkage map construction, genetic diversity analyses, association mapping, and marker-assisted selection has been demonstrated in several species beans (Cortes' et al., 2011); wheat (Allen et al., 2011; Trebbi et al., 2011); eggplant (Barch et al., 2011); Arabidopsis (Zhang and Borevitz, 2009), barley (Close et al., 2009); sorghum (Nelson et al., 2011). SNP discovery is performed for each set of tags that align to the exact same starting genomic position and strand, where the starting genomic position of a tag is defined by the cut site remnant at the beginning of the tag. Such tags, originating from the same restriction enzyme cut site and with the same orientation (but not necessarily of the same length), collectively comprise a "Tag Locus" (Appendix XXVII). To call SNPs and ensure that indels are handled consistently, a *de novo* multiple sequence alignment of all the tags in each Tag Locus is performed using the BioJava 3.0 API (Prlic et al., 2012), which implements the CLUSTAL W algorithm (Thompson et al., 1994). For each SNP in the resulting "Tag Locus Alignment", the allele represented by each tag is determined and the TBT file is consulted to tally the observed depths of each allele in each taxon. The genotype of the SNP in each taxon is then determined either by a binomial likelihood ratio method of quantitative SNP calling or, optionally, following the method of Hohenlohe et al., (2010). Putative SNPs from GBS may be of low quality for multiple reasons. The sequencing error rate for a SNP may be high because of its distance from the read start and/or its immediate sequence context (McElroy et al., 2010; Allhoff et al., 2013). Alternatively, paralogous sequence tags from

different loci may be mistakenly aligned to a single Tag Locus, resulting in spurious SNPs. To detect and filter out error-prone SNPs, the tassel-GBS pipeline relies on population-genetic parameters such as the minor allele frequency (MAF) and, in particular, the inbreeding coefficient (or "index of panmixia"), F_{IT}. Filtering based upon minimum MAF can remove spurious SNPs arising solely from sequencing error.

2.5 SNP Validation

Prior to any SNP applications, the discovered SNPs must be validated to identify the true SNPs to get an idea of the percentage of potentially false SNPs resulting from SNP discovery exercise which is accomplished using a variety of material such as a bi-parental segregating population or a diverse panel of genotypes. Usually a small subset of the SNPs is used for validation through assays such as the Illumina Golden gate (Fan et al., 2006), K Biosciences Competitive Allele Specific-PCR SNP genotyping system (KASPar) (http://www.lgcgenomics.com/) or the High Resolution Melting (HRM) curve analysis. SNP validation rates can be improved using RRL for SNP discovery and choosing SNPs within the non-repetitive sequences including predicted single copy genes and single copy repeat functions show to have high validation rates (You et al., 2011). Validation serves as an iterative and informative process to modify and optimize the SNP filtering criteria to improve SNP calling. For example, a subset of 144 SNPs from a total of 2,113,120 SNPs were validated using the Goldengate assay on 160 accessions in apple (Chagne` et al., 2012).

2.5.1 Copy number Variation

Randon et al. (2006) defined Copy number Variation (CNV) as a DNA segment of one kilo base (kb) or larger that is present at a variable copy number in comparison with a reference genome. CNVs correspond to relatively large regions of the genome that have been deleted (fewer than the normal number) or duplicated (more than the normal number) on certain chromosomes. CNVs have effects on phenotypes by altering transcription levels of genes and may have major impacts on protein sequence, structure and function. CNVs can be detected and analyzed by various methodologies at the genome-wide and locus-specific levels.

2.5.2 Genome wide association studies (GWAS)

GWAS also called whole genome association study, an examination of a genome-wide set of genetic variants in different individuals to see if any variant is associated with a trait. It provides a better resolution and considers numerous alleles which also provide faster marker-trait association than biparental population (Flint et al., 2003). GWAS requires 10000 – 100000 markers applied to a collection of genotypes representing broad genetic basis and relies on the nonrandom association between markers and traits (Gupta et al., 2005). It typically identifies common variants with small effect sizes as reported by Bush and Moore, (2012). In practice, various empirical and statistical criteria are used to call SNPs, such as a minimum and maximum number of reads considering the read depth, the quality score and the consensus base ratio for examples (You and Huo, 2011). Thresholds for these criteria are adjusted based on the read length and the genome coverage achieved by the NGS data. In assemblies generated allowing single nucleotide variants (CNV) and insertions/deletions (indels) a list of SNP and indel coordinates is generated, and the read mapping results is visualized using graphical user interface programs such as Tablet (Milne et al., 2009), SNP-VISTA (Shah et al., 2005) or Savant (Fiume et al., 2010). It typically focuses on association between single-nucleotide polymorphisms and traits like major human diseases, but can equally be applied to any other organism. In plants GWAS was first reported in Arabidopsis for flowering time and pathogen resistance genes (Aranzana et al., 2005). It was performed in rice using ~3.6 million SNPs identified genome regions associated with 14 agronomic traits (Huang et al., 2010) and on barley that has no reference genome (Pasam et al., 2012). Genome-wide analysis of CNVs has been enhanced by a comparative genome analysis using bioinformatics tools with long-range sequences (She et al., 2006). Once a CNV of interest is identified at the genome level, it needs to be analyzed more precisely at the locus level, and ultimately, the genotype and haplotype must be determined to elucidate its relationship with a particular genetic alteration (Seo et al., 2007). Locus specific CNVs are identified in conjunction with genome wide screening (Iafrate et al., 2004; Sharp et al., 2005; Wong et al., 2007) and independently through gene family studies (Ghanem et al., 1988; Trask et al., 1998) or functional analysis of genes associated with a certain phenotype (Johanson Moller et al., 1996).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Field Assay

3.1.1 Study Site

The experiments were conducted in two separate locations of the Kenya Agricultural Livestock Research Organisations (KALRO); Alupe (Busia, Kenya) and Kibos (Kisumu). Alupe lies at an altitude of 1189 m above sea level, latitude of 0^{o} $29^{'}$ N and longitude of 34^{o} $08^{'}$ E. The soil is Ferralo-orthic Acrisol with pH of 5.0 (FURP, 1987). Kibos lies at an altitude of 1135 m above sea level latitude 0^{o} S and longitude $34^{o}49^{'}$ E. The soil is black cotton with clay loam with pH of 6.55. The two sites are located in regions that are severely infested by *Striga* which poses a serious threat to cereals crops.

3.1.2 Accessions Selection and Land Preparation

Seeds of one hundred finger millet genotypes (Appendix 1) of unknown genetic background and *Striga* resistance including local and international varieties were sourced from gene bank at Kenya Agricultural and Livestock Research Organisation (KALRO) Kakamega and Alupe, for this research. *Striga* seeds that were collected from the experimental localities were used for artificial inoculation of finger millet germplasm. The experimental field was ploughed, harrowed and ridged two weeks to planting and Diammonium Phosphate (D.A.P) fertilizer applied at planting time at the rate of 170 Kg ha^{-1} in every plot after demarcation.

3.1.3 *Striga* innoculation

The field screening for *Striga* resistance was done in long and short rain seasons. The seeds of finger millet wereplanted in long rain season on 10^{th} June, 2012 at Alupe and on 20^{th} June, 2012 at Kibos. After harvesting, the collected seeds of finger millet were planted at KALRO Alupe on 19^{th} September 2012 and at Kibos on 23^{rd} for the second rain season during short rain season.

3.1.4 Experimental design for Field Screening

The experimental design was a 10 x 10 triple lattice (Appendices I and II). A plot was made of three rows of 2 m length spaced 30 cm apart between rows and later thinned to intra-row spacing of 15 cm. Plots were spaced 50 cm apart reps separated by 1 m paths

(Plate 3.2 appendix 3). Planting was in shallow furrows where Diammonium phosphate (DAP) basal fertilizer was applied followed by seed by drill before being loosely covered. For the inoculated plots, a Striga seed/sand mixture was applied by drill before fertilizer and seed application. Because *Striga* seeds are tiny (200 to 400µm), 10 grams of it was mixed with ½ kg of sterilized sand to serve as the carrier before being drilled into furrows of per plot for the purpose of providing adequate volume for rapid and uniform *Striga* infestation (Doggett, 1970). Three weeks after germination of finger millet, the rows were thinned to an intra-row spacing of 15 cm (Plate 3.3 appendix III). Weeding was done three times throughout the crop season. However, the removal of weeds from finger millet plots inoculated with *Striga* was by hand pulling with effect from second weeding. Duduthrin pesticide was applied at two weeks interval to prevent crop attack by shoot fly and the stalk borer. C.A.N fertilizer (27:0:0) was used to top dress the crop three weeks after thinning.

3.1.5 Field data collection

The data collected were for: the seedling vigor, *Striga* count at vegetative stage per plot of respective genotypes, days to 50% flowering, *Striga* count at 50% crop flowering and at maturity, plant height, ear exertion, ear shape, lodging percentage, ear length and ear width on the main stalk, number of fingers on the main stalk, stand count, and grain yield. Seedling vigor was taken at three week after emergence on a scale of 1 to 3 where 1 = highly vigorous, 2 = vigorous and 3 = less vigorous. Ear shape was also rated on a scale of 1 = open, 2 = curved and 3 = fist. *Striga* count at vegetative stage was done up to but before the crop began to flower. The days to 50% flowering was done on the day when half of the plants in each plot had flowered and finally *Striga* counting was done when the crop had reached physiological maturity. Lodging percentage was determined by the number of lodged plants in a plot expressed as a percentage of plant stand. Ear length was take as distance from receptacle to the tip of head while ear width was taken as distance across and near the tip of mature head. Plant height was the measured length in cm from the base of the plant at soil level to tip of the main stalk head at physiological maturity. This was done on five representative plants in each plot and average recorded. The ear exertion was taken as the distance between ligule of the flag leaf and the base of the head. The number of fingers was obtained from the average of total number of fingers of five

plants plants per plot. Plant stand was a count of the number of plants per plot at physiological maturity. Yield per plot was the weight of clean grain resulting from threshed and winnowed plot harvest. Yield in kg ha^{-1} was extrapolated from yield per plot.The data collected were for: the seedling vigor, *Striga* count at vegetative stage, days to 50% flowering, *Striga* count at 50% crop flowering and at maturity, plant height, ear exertion, ear shape, lodging percentage, ear length and ear width on the main stalk, number of fingers on the main stalk, stand count, and grain yield. Seedling vigor was taken at three week after emergence on a scale of 1 to 3 where 1 = highly vigorous, 2 = vigorous and 3 = less vigorous. Ear shape was also rated on a scale of 1 = open, 2 = curved and 3 = fist. *Striga* count at vegetative stage was done up to but before the crop began to flower. The days to 50% flowering was done on the day when half of the plants in each plot had flowered and finally *Striga* counting was done when the crop had reached physiological maturity. Lodging percentage was the number of lodged plants in a plot expressed as a percentage of plant stand. Ear length was take as distance from receptacle to the tip of head while ear width was taken as distance across and near the tip of mature head. Plant height was the length in cm from the base of the plant at soil level to tip of the main stalk head at physiological maturity. This was done on five representative plants in each plot and average recorded. The ear exertion was taken as the distance between ligule of the flag leaf and the base of the head. The number of fingers was obtained by dividing the total number of fingers from five plants by five plants measured. Plant stand was a count of the number of plants per plot at physiological maturity. Yield per plot was the weight of clean grain resulting from threshed and winnowed plot harvest. Yield in kg ha^{-1} was extrapolated from yield per plot using the following formula:

$$Y = \left[10000X\left(\frac{X}{1000}\right)\right]/A$$

Where Y = yield in $kgha^{-1}$

X = plot yield in g

A = plot area = no. of rows x row spacing x row length (3x0.3mx2m)

3.1.6 Field Data Statistical Analysis

Data on morphological traits and *Striga* effect were subjected to analysis of variance (ANOVA) procedure using Statistical Analysis System (SAS) software version 2003. Means were separated using Fischer's least significant (LSD) test at $P \leq 0.05$).

3.2 Molecular Assay

3.2.1 DNA Extraction

Reagents and apparatus for purification of Plant Genomic DNA were:

Lysis Buffer PA1, RNase A, Elution Buffer PG, Binding Buffer PB

Wash Buffer PAW, Wash Buffer PAW2, Tissue lyser II, Steel beads

Collecting tubes 2ml self-lock, Micro-centrifuge tubes (1.5ml and 2ml), Centrifuge, ISOLATE II Filter (violet), ISOLATE II Plant DNA Spin Column, Incubator, Eppendorf pipette and Eppendorf tubes, Vortex machine, Medical hand gloves, Tips

The procedure followed is outlined in the ISOLATE II Plant DNA Kit (Bioline) and is as follows:

150mg of fresh weight of plant material of each sample was homogenized in 2ml collecting tube containing two steel beads.

400µl of Lysis Buffer PA1 was added before fitting on the Lyser II machine for cell disruption for 3mins. This was followed by addition of 10µl of RNase A to the lysate mixed thoroughly and incubated at 65^0Cfor 10 min.

Isolate II Filter (violet) was placed into new 2ml collection tube and loaded lysate onto the columns of respective samples, followed by centrifugation for 2min at 11,000rpm. Clear flow- through was collected and discarded the Isolate II Filter. Where not all liquid passed through the filter, centrifugation was repeated. Where a pellet was visible in the flow-through, the clear supernatant was transferred without disturbing the pellet to a 1.5ml micro-centrifuge tube.

450µl of Binding Buffer PB was added and mixed thoroughly by pipetting up and down 5 times or by vortexing.

ISOLATE II Plant DNA spin column (green) was placed into a new collection column Tube (2ml) and sample loaded (max. 700µl). This was followed by centrifugation for 1 min at 11,000rpm and the-flow through discarded. For higher volumes the loading and centrifugation steps was repeated.

Added 400µl of Wash Buffer PAW1 to the ISOLATE II Plant DNA spin column and centrifuge for 1 min at 11,000rpm and discarded flow-through.

Added 700µl of Wash Buffer PAW2 to the ISOLATE II Plant Spin Column, centrifuge for 1 min at 11,000 rpm and discarded the flow-through.

Added another 200µl of Wash Buffer PAW2 to the ISOLATE II Plant Spin Column, centrifuged for 2 min at 11,000rpm in order to remove the wash buffer and to dry the silica membrane completely.

Placed ISOLATE II Plant Spin Column into new 1.5 ml micro-centrifuge tube. Pipetted 50µl of Elution Buffer PG (65ºC) onto the membrane.

Incubated the ISOLATE II Plant DNA Spin Column for 5 min at 65ºC. Centrifuged for 1 min at 11,000rpm to the elute DNA.

Repeated this step with another 50µl Elution Buffer PG (65ºC) and eluted into the same tube.

3.2.2: Agarose gel preparation and Electrophoresis

Gel casting frame was prepared and the desired number of combs placed depending on DNA samples made.

0.8 g of Agarose powder was dissolved in 100 ml of 1 X TBE (0.1M Tris base, 0.1M boric acid and 0.02M EDTA; pH 8.0) buffer in a conical flask microwave on high for 2 minutes. The mixture was heated in a microwave for 3 minutes for agarose to dissolve. The gel was left to cool for five minutes on the bench at 25ºC before adding 5 µl gel red (Biotium, USA) which is less mutagenic, then poured in a horizontal gel tray fitted with appropriate gel combs. The gel was left to set for 40 minutes then combs removed carefully and the tray immersed in an electrophoresis tank that contained 1 x TBE buffer. 2 µl of extracted of DNA for each genotype was mixed with 1 µl of 3x loading dye that

contains bromophenol blue, xylene cyanol FF, a high density glycerol reagent and deionized water. Lambda (ƛ) DNA IEcoRI + Hind 111 500µg/ml, 100µg Promega MADISON, WI U.S.A. was loaded alongside the DNA samples in order to check the integrity of the DNA. The DNA was then subjected to electrophoresis at 80v for 45 minutes. The DNA was visualized under UV light using a UV documentation system (Bio-IT™, Ultra-Viole Products, Cambridge, (UK).

3.2.3 Quantification of DNA

Quantification of DNA was done using Quibit® 2.0 Fluorometer (Invitrogen by Life technologies corporation, USA) as outlined below:

Requirements

Standards #1 and #2

Qubit™ Reagent (Broad range)

Qubit™ Buffer

Quibit® 2.0 Fluorometer

1x n µl Qubit™ Reagent (Broad range)was mixed with 199 x n µl Qubit™ Buffer to make Qubit™ Working solution.(Note that n was=number of standards plus number of samples).

The standards #1 and #2 were prepared by mixing 190 µl of working solution with 10 µl of respective standards from kit making final volumes of 200µl each.

The user samples were also prepared by mixing 180-199µl of working solution with 1-20µl of genomic DNA samples making final volumes of each sample at 200µl.

After all samples were prepared, all assay tubes were vortex for 2-3 seconds and incubated at room temperature for 2 min.

The tubes were then read in Qubit® 2.0 Fluorometer on broad range scale. The respective sample DNA quantity was recorded as shown in appendix XXII column eight of the table.

3.2.4 Library Preparation

The quantified DNA of the 95 genotypes was packed into the 96-plex/wells together with one blank to act as a control (Appendix XX). The DNA samples that were sent for GBS had quantity ranging from 30 to 100ng/µl (Appendix XXII). The DNA was then submitted to Institute of Genomic diversity (Cornell University, Ithaca, New York, USA) for genotyping by sequencing. Library preparation and sequencing followed the protocol described in Elshire et al., (2011a, b). Restriction enzyme *ApeK*I was used for genomic digestion because of its methylation sensitivity and uniform distribution of cut sites across finger millet genome. The barcoded samples were then pooled in 96-plex and sequenced in 1 lane of Illumina Hiseq 2500 (Illumina, San Diego, CA, USA) (Plate 2 Appendix XXIII). Six microliter of DNA was taken from eight of the 95 samples at random loaded in each well and run for 2 hours at 80 volts. The eight samples were digested using restriction enzyme HIND-III to check for quality and quantity in library preparation with well layout as shown in Appendix XXIV.

3.2.5 Molecular Data Analysis by SNP Calling

Genotyping by sequencing was performed on 95 genotypes, which comprised of a set of 77 land races from Gene bank Kenya and 18 land races from different regions of the world. Because finger millet does not have reference genome, association between phenotypic and traits, GBS data was determined by running on UNEAK (Universal network enabled analysis Kit) production pipeline as explained in Lu et al., (2013). Full description of UNEAK protocol was obtained by logging to (http://www.maizegenetics.net/gbs-bioinformatics). Quality filtering was performed primarily using built in function in VCF tools (Daneceke et al., 2011). All bioinformatics and Subsequent analysis were performed on High Computing Machine Workstation with 60 GB of RAM running Ubuntu.

3.6 SNP Calling to confirm markers showing association with Striga

This was performed using general linear model (GLM) and mixed linear model (MLM). GLM performs association analysis using a least squares fixed effects where TASSEL utilizes a fixed effect linear model to test for association between segregating sites and

phenotypes. It accounts for population structure using covariates that indicate degree of membership in underlying population. A MLM is one which conducts analysis using both fixed and random effects giving it the ability to incorporate information about relationship among individuals. Raw SNPs were filtered to include only sites with 80% coverage across sample and minor allele frequencies ≥ 0.05, and only samples with ≥ 25% coverage across the remaining sites. After assigning reads, Single-nucleotide polymorphisms (SNPs) were called using the TASSEL GBS pipeline (Glaubitz et al., 2014). UNEAK commands were run as TASSEL plugins via the commands in the following format (Linux or Mac operating system).

3.6.1 Population Structure Analysis

Population structure was determined using the program fast STRUCTURE (Raj et al., 2014) an updated version of the program STRUCTURE (Pritchard et al., 2000) designed to handle large SNP data set rapidly.

CHAPTER FOUR

RESULTS

4.1 Field Data Analysis.

The field data was based on the following variables whose means were calculated for significance ($p \leq 0.05$) as shown in Table 4.1

Table 4.1: Overall statistical summary of means for *Striga* inoculated and un-inoculated finger millet genotypes.

Variable	*Striga* inoculated mean	*Striga* un-inoculated
Seedling vigour	2.13 **	1.97
Striga count at vegetative	5.27 **	0
Striga count at 50% flowering	13.80**	0
Days to 50% flowering	88.80**	93.47
Plant height	53.64**	68.72
Ear shape	2.30 ^{ns}	2.28
Lodging %	12.74 ^{ns}	11.76
Ear exertion	11.08**	12.97
Stand count	23.76 ^{ns}	24.43
Ear length	5.67**	7.32
Ear width	2.47**	5.99
Number of fingers	5.22 ^{ns}	5.37
Striga count at maturity	25.75**	0
Yield in kg ha^{-1}	609.94**	1074.4

Key: ns = not significant ** = Statistical significant at $P \leq 0.05$

Table 4.2 Analysis of variance for the thirteen agro-morphological traits of one hundred genotypes inoculated with *Striga*

		Mean squares						
Source	Df	Seedling vigour	Striga count at vegetativ	Striga count at 50% F	Striga ct at maturity	D50%F	Plant ht (cm)	Ear shp
Rep	2	6.05**	282.9**	1158.2**	22559.5**	301.91^{ns}	5188.96**	4.19**
Stg	1	3.14**	4745.5**	33307**	148754.6**	6078.31**	41735.2**	0.10^{ns}
Rep*Stg	2	2.37**	282.9**	1381.6**	22559.5*	130.19^{ns}	1659.76**	0.37^{ns}
entNO	99	1.05**	63.95^{ns}	231.8^{ns}	1732.32^{ns}	307.71**	287.84**	2.74**
Stg*entNo	99	0.50^{ns}	65.83^{ns}	131.1^{ns}	1732.32^{ns}	97.70ns	145.14**	0.65^{ns}
LSD(0.05)		0.10	8.64	16.39	34.55	11.23	11.71	0.1
CV%		38.06	304.23	220.2	312.22	1.26	17.39	34.15

Cont'd		**Mean squares**						
Source	Df	Ear lt (cm)	Ear wd (cm)	Lodging %	Ear Ex (cm)	Stand count	Number of Fingers	Yield Kgha^{-1}
Rep	2	47.7**	11.59^{ns}	4320.9**	57.26**	1367.21**	12.61**	12806883**
Stg	1	496**	2166.6**	9.53^{ns}	713.33**	15.03^{ns}	1.06^{ns}	33959189**
Rep*Stg	2	20.2**	20.1**	1582.4**	61.14**	378.29**	13.12**	6800424**
entNO	99	10.2**	3.97^{ns}	514.06**	21.44**	325.97**	2.00^{ns}	1015596**
Stg*entNo	99	0.90^{ns}	3.19^{ns}	263.07^{ns}	4.84^{ns}	75.37^{ns}	1.96^{ns}	265332.2^{ns}
Mean inoc		5.67	2.47	11.76	11.07	23.76	5.22	609.94
LSD(0.05)		1.10	2.57	16.52	2.26	12.40	1.15	79.91
CV%		122.9			17.1	46.89	24.94	77.27

Key: ** = Statistical significant ($p = \leq 0.05$), ns = not significant, df = degree of freedom, ct = count, D50%F = days to 50% flowering, ht = height, shp = shape, lt = length, wd = width, Ex = exertion, stg = Striga, entNo = entry number. Of these eleven parameters showed high mean significance difference, and 4 did not show mean significant difference at $p \leq 0.05$. The 100 genotypes of finger millet infected with *Striga* had significantly higher reaction syndrome compared with their respective *Striga* free control.

Table 4.1 shows the following:

4.1.1. Seedling vigour

There was significant difference between the plots of finger millet that were inoculated and those that were not inoculated with *Striga* amongst the replicas at $p \leq 0.05$, where by inoculated seedling were more vigorous (Tables 4.1 and 4.2). The genotypes that were highly vigorous included I.E 4491, I.E 6165, KACIMI 15, GBK 029661, KACIMI 11, I.E 2957, I.E 4795, PR 202, GBK 000463, VL 149 and GBK 043081. The vigor score of

the inoculated plots were significantly higher than the non-inoculated *Striga* controls at early vegetative stage (Appendix 4).

4.1.2 *Striga* count

Striga count was done at vegetative stage, 50% of crop flowering and crop maturity and showed the following results:

(a) *Striga* count at vegetative stage

The mean *Striga* count for inoculated plots was 5.27 while in the un-inoculated plots was 0 giving a high significant difference (Table 4.1). The mean *Striga* count ranged from 0 to 13.4 plants in respective genotypes (Appendix VIII). The following genotypes showed immunity to *Striga* at vegetative stage, I.E 4497, I.E 4795, VL 149, GBK 000516, I.E 2217, GBK 027199, KACIMI 24, GBK 026992, GBK 008339, GBK 029724, KACIMI 36 and KACIMI 7 (Appendix VIII). The only genotype that was recorded to have the highest mean significant difference at this stage was GBK 000409 and had mean *Striga* count of 13.4 (Appendix 8).

(b) *Striga* count at 50% flowering

The mean *Striga* count at this stage in the inoculated plots was 13.80 giving a high significance difference at $p \leq 0.05$. (Table 4.1 and 4.2). Nine genotypes were immune to *Striga,* having mean *Striga* count of 0 while the genotype that had the highest mean *Striga* count at this stage was I.E 4816 (Appendix VIII). This same genotype was the only one that showed high mean significance difference among the one hundred genotypes that were screened on the field for *Striga* susceptibility. The genotypes that showed immunity to *Striga* included; I.E 4497, I.E 6165, I.E 2957, I.E 2440, I.E 4795, PR 202, I.E 2217, GBK 043081 and GBK000463 (Appendix VIII).

(c) *Striga* count at crop maturity

The mean *Striga* count among the inoculated genotypes was 25.75 (Table 3, Appendices VII and VIII). The mean *Striga* count obtained as per respective genotypes ranged from 0 to 69.2 plants (Appendix VIII). The genotypes that had highest mean *Striga* at maturity were the checks which included GBK000900, GBK027300, GBK011113, GBK029744, GBK029715, GBK008292, GBK000369 and GBK000549. The mean of 69.2 was obtained for genotype I.E 5306 (Appendix VIII). The genotypes that were immune /or had lowest mean *Striga* count at 50% flowering displayed the same property at crop

maturity.

4.1.3 Days to 50% flowering

The first genotype flowered in 53 days and was GBK036821. It was also a high yielding type (Appendix 8). None among the highly resistant and highly susceptible genotypes were in the early maturing bracket. The mean for days to 50% flowering in the *Striga* free plots of finger millet was 93.5 days while the *Striga* inoculated plots was 88.8 days. Thus the finger millet in the plots inoculated with *Striga* matured earlier unlike the *Striga* free plots. The days to 50% flowering ranged from 53 to 101 (Appendix VIII). Thus there was high significance difference between the *Striga* inoculated plots of finger millet and the ones that were *Striga* free at $p < 0.05$.

4.1.4 Plant height

The mean height for *Striga* inoculated plants was 53.64 cm while the *Striga* free plants was 68.72 cm (Table 4.1 and Appendix XI). Thus the finger millet in plots inoculated with *Striga* had the shortest height compared to *Striga* free plots showing high mean significant difference between replications in the inoculated and *Striga* free plots. Among the high yielding variety was GBK 036821 which is also resistant to *Striga* and had no effect on its growth. Other high yielders that were affected by *Striga* included GBK029722, GBK029793, KACIMI 72, KACIMI 22, KACIMI 20, GBK 000802, KACIMI 77 and KACIMI 42. KACIMI 17 had low variation in their mean height and their yield were drastically affected. Among the medium yielders that had high *Striga* count at maturity included GBK 027300. The low yielders with high *Striga* count at maturity were; GBK 000900, GBK 011082, GBK 029744 and GBK 011113.

4.1.5. Ear shape

The mean of ear shape for *Striga* free plots was 2.30 while the *Striga* inoculated plots was 2.28 (Table 4.1 and appendix XII). There was therefore no significant difference between the *Striga* inoculated plots of finger millet and the *Striga* free plots.

4.1.6 Ear length

The mean for *Striga* free plots was 7.32 cm while the *Striga* inoculated plots had mean of 5.67 cm (Table 4.1 and appendix XIII). The results therefore showed high significant difference at $p \leq 0.05$ between the *Striga* inoculated plots and the *Striga* free plots. The mean ear length was in the range of 4 cm to 9.05 cm (Appendix VIII). Among the top

resistant genotypes the one that had highest ear length was KACIMI 47 that gave 8.4 cm. Among the least resistant genotypes with long ear was GBK 029715 which was 8.12 cm. Of the top resistant genotypes with least ear length were I.E 2440 and GBK 029661. None among the least resistant genotypes had lowest ear length.

4.1.7. Ear width

The *Striga* free plots had a mean width of 5.99 while the *Striga* inoculated had a mean width of 2.47 (Table 4.1 and appendix XIV). There was mean significant difference in ear width between *Striga* inoculated plots and *Striga* free plots. The mean ear width ranged from 1.5 to 6.82 cm (Appendix VIII). There were two genotypes resistant to *Striga* but had low ear width and included; I.E 4491 and GBK 029661. Among the resistant genotypes to *Striga* that had highest width was KACIMI 47. The genotype that was highly susceptible to *Striga* but among those with highest ear width was GBK 008292.

4.1.8. Lodging percentage

There was no significant difference between the *Striga* inoculated plots and the *Striga* free plots as shown in Table 4.1 and Appendix XV. The *Striga* weed therefore has no effect on lodging of the respective finger millet genotypes. The genotype with lowest lodging percentage in the resistant category was I.E 2217. The mean lodging percentage ranged from 0 to 43 (Appendix VIII). None of the resistant genotypes was in the highly lodged panel of finger millets. Among the highly susceptible to *Striga* and highly lodged was GBK 000369 (Appendix VIII).

4.1.9. Ear exertion

There was significant difference on ear exertion between the *Striga* inoculated plots of finger millet and the *Striga* free plots. The ear exertion mean for *Striga* free plots was 13cm while the *Striga* inoculated plots was 11.1cm (Table 4.1 and 4.2). The mean for ear exertion ranged from 7.5 cm to 16 cm among respective genotypes (Appendix VIII). In the resistant panel of finger millet to *Striga* KACIMI 36 had the highest ear exertion value of 14.1cm (Appendix VIII). Genotype I.E 4497 was extremely susceptible to *Striga* while the rest were moderately resistant.

4.1.10. Stand count.

The mean stand count for *Striga* free plots was 24.4 while the *Striga* inoculated plots was

23.8 (Table 4.1 and appendix XVII) implying lack of significant difference between the two categories as far as treatment was concerned. The highest mean stand count was exhibited by KACIMI 17 that had a mean of 31.58 plants. Only one genotype I.E 4115 among the resistant to *Striga* had high plant stand (Appendix VIII).

4.1.11. Number of fingers

The mean number of fingers for *Striga* free plots was 5.37cm while that for *Striga* inoculated plots was 5.22cm (Table 4.1 and 4.2), hence no significant difference between the *Striga* inoculated plots and *Striga* free plots. The mean number of fingers among finger millet genotypes ranged from 3.7 to 7 (Appendix VIII). Among the resistant genotypes with high number of fingers was I.E 4115 while in the highly susceptible group to *Striga* was GBK 008292. The ones with least number of fingers in the resistant category were GBK 029661, I.E 2440 and KACIMI 7. None among the highly susceptible genotypes had lowest number of fingers (Appendix VIII).

4.1.12. Grain yield

The mean grain yield ranged from 35.5kgha^{-1} to 1573 kg ha^{-1} (Appendix 8). The mean grain yield for *Striga* free plots was 1074.4kgha^{-1} and the *Striga* inoculated mean grain was 609.94kgha^{-1} (Table 4.1 and Appendix 8). There was significant difference between *Striga* inoculated plots and non-inoculated plots (Table 4.2). Among highest yielders was KACIMI 47 that is also resistant to *Striga*. None among the highly susceptible genotypes was a high yielder. Two of the resistant genotypes had very low yield and included GBK 029661 and I.E 2440. Among the low yielders in the susceptible panel was GBK 000900.

4.2.1 Selection for *Striga* resistance

This was based on the population of *Striga* that emerged from each plot per respective genotype monitored from early stage of growth to physiologal maturity of the crop. Table 4.3 below indicates the genotypes that had none to the lowest number of *Striga* population out of the one hundred genotypes that were screened for resistance.

Table 4.3: Finger millet genotypes selected for high resistance to *Striga.*

Serial Number	Variety	Entry Number
1	I.E 2217	24
2	I.E 4491	5

3	KACIMI 47	26
4	I.E 6537	4
5	KACIMI 30	20
7	I.E 4115	7

4.2.2 Selection for low resistance

This was similarly based on the population of *Striga* that emerged in the respective plots per genotype. Table 4.4 below shows the panel of genotypes that had highest *Striga* number out of the one hundred genotypes.

Table 4.4: Genotypes selected for low resistance to *Striga.*

Serial Number	Variety	Entry Number
1	I.E 5306	11
2	GBK000900	97
3	GBK027300	22
4	GBK029744	80
5	GBK029715	67
6	GBK011113	78
7	GBK008292	62
8	GBK011126	79
9	GBK000369	87
10	GBK000549	98

4.3 Molecular Results

4.3.1 Genetic diversity of finger millet for *Striga* resistance using molecular markers

A total of 17 GB Fastq.gz (~ 60 GB fastq.txt) raw sequence data was obtained from Cornell University laboratories Ithaca New York, USA from which 117,542 SNPs single-end 64-bp reads were obtained from raw GBS dataset that was used for genome wide

association studies (GWAS) analysis for *Striga* resistance (Figure 1, Appendices XXV, XXVI and XXVII).

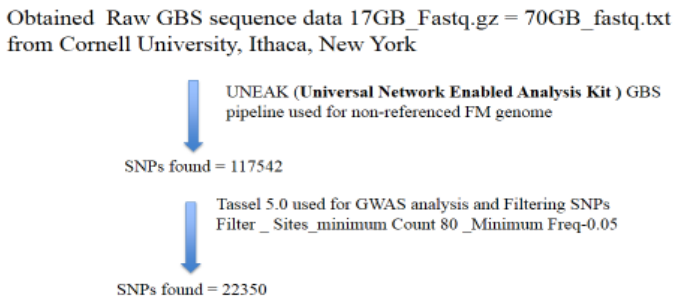


Figure 1: Summary of the GBS result of the HapMap file

The HapMap file was obtained from Cornell University laboratory Ithaca, New York.

After filtering, the complete dataset resulted in the selection of 22,350 sites across the 95 genotypes. Table 4.5 below shows the HapMap genotype files generated using single letters to represent phase unknown, and diploid genotypes. Heterozygotes and monozygotes were represented by IUPAC nucleotide codes namely: A = A/A; C = C/C; G = G/G; T = T/T; M =A/C; R = A/G; W = A/T; S = C/G; Y = C/T; K = G/T and N = missing data

Table 4.5: The HapMap files of the 63 entries of finger millet among the 95 genotypes.

Taxa	PC 1	PC 2	PC 3	Haplotype
FM_GP10	-4.39775	3.154309	1.938215	T;Y;Y;Y;Y;Y;W;K;N;T...
FM_GP11	-7.89314	2.061789	8.150765	K;C;Y;Y;Y;C;W;K;A;K...
FM_GP12	-9.31874	12.32787	-13.4802	K;Y;N;Y;T;N;N;T;A;K...
FM_GP13	18.25793	-25.5499	2.586355	N;Y;N;Y;T;C;N;K;G;N...
FM_GP14	30.1367	8.46259	-1.64855	T;Y;C;Y;Y;C;W;G;G;G...
FM_GP15	-11.6071	11.96489	-1.7462	N;Y;N;C;T;C;N;T;A;T...
FM_GP17	15.62622	-19.098	-6.81512	T;T;N;N;C;T;W;K;R;G...

FM_GP1	-11.6365	-7.15388	-11.3562	N;C;Y;Y;Y;Y;A;K;G;T...
FM_GP21	19.27961	-32.9381	-1.75989	T;Y;N;T;N;T;W;G;N;K...
FM_GP22	-5.84832	5.383294	-3.80779	N;T;T;Y;N;C;T;G;G;T...
FM_GP23	-19.3515	5.340974	24.06419	T;C;T;C;Y;T;N;T;A;T...
FM_GP24	-3.65163	13.91614	-10.425	T;T;C;C;C;Y;T;K;G;G...
FM_GP25	35.06066	6.135511	4.811163	K;C;Y;Y;N;Y;W;K;A;T...
FM_GP26	-17.0025	2.881768	22.7412	G;N;T;Y;C;C;N;K;N;K...
FM_GP27	-9.55533	-3.28729	-10.7591	T;Y;C;N;C;Y;W;G;N;T...
FM_GP28	-12.2951	6.558644	-9.20099	T;Y;Y;C;C;Y;N;N;G;G...
FM_GP29	-2.92913	-11.2873	15.11956	G;N;Y;Y;Y;Y;N;T;G;N...
FM_GP30	-11.8522	8.224259	7.368297	N;Y;Y;T;T;Y;W;T;R;K...
FM_GP32	-8.47744	16.25641	-9.54788	T;T;T;C;C;Y;T;K;N;K...
FM_GP33	-15.9839	-10.1681	18.80095	T;T;T;C;T;N;N;K;A;K...
FM_GP34	24.0056	8.287489	-0.93963	N;Y;Y;Y;C;T;T;K;A;N...
FM_GP35	-19.5758	0.389689	27.38488	T;T;Y;Y;Y;Y;T;K;R;K...
FM_GP3	-11.9976	-7.70413	-15.447	T;T;T;Y;N;Y;W;N;G;T...
FM_GP44	-16.1051	6.891485	20.51329	T;Y;Y;Y;N;Y;A;K;A;G...
FM_GP45	31.42922	7.031455	12.56948	N;N;T;C;T;Y;N;K;R;G...
FM_GP46	-19.2779	-1.73684	22.09652	K;Y;C;C;C;C;A;K;G;T...
FM_GP48	-19.5068	0.895896	22.71134	T;Y;Y;Y;Y;Y;T;K;N;K...
FM_GP50	0.116288	13.75498	-1.75568	N;T;C;Y;N;Y;W;T;G;G...
FM_GP52	35.8667	9.840527	8.208302	T;Y;C;T;C;Y;N;K;G;K...
FM_GP53	23.23557	2.873849	-0.9229	T;T;T;Y;N;Y;W;K;A;N...
FM_GP55	32.59412	10.25578	1.891272	T;Y;Y;N;C;Y;T;K;N;K...
FM_GP57	-11.0992	4.061519	-2.68313	T;Y;T;Y;T;C;W;N;N;T...
FM_GP58	1.516685	11.70126	9.542977	K;C;C;Y;T;Y;T;K;G;K...
FM_GP60	-6.86787	-29.6789	-13.6574	T;N;C;N;N;Y;A;T;A;K...
FM_GP61	-12.1924	13.83638	2.26358	N;Y;T;Y;N;Y;N;K;N;N...
FM_GP63	31.70574	9.07898	-1.30607	K;T;C;T;N;Y;W;K;G;T...
FM_GP65	-8.73097	13.32335	-13.0094	K;T;Y;Y;T;Y;N;G;N;K...
FM_GP67	13.18953	-1.28891	5.890087	G;N;T;Y;N;C;W;G;N;N...
FM_GP70	-14.1475	-9.39725	-14.3427	N;C;N;Y;N;Y;A;G;G;T...
FM_GP71	-8.37954	8.231428	-6.91852	T;C;Y;T;C;C;W;N;G;T...
FM_GP72	20.40855	-12.8009	2.376856	T;N;C;N;Y;N;N;T;A;T...

FM_GP73	-12.64	-1.51211	-10.9068	N;T;N;C;Y;T;A;N;R;T...
FM_GP75	19.37384	-31.0883	-0.33938	T;N;Y;Y;Y;Y;A;K;R;N...
FM_GP76	-6.82491	10.08528	-9.3035	K;T;T;Y;N;Y;N;G;R;K...
FM_GP77	-13.1285	1.297694	-5.69489	N;C;T;N;N;T;T;T;N;G...
FM_GP78	-6.10135	16.34464	-11.9782	N;T;N;Y;C;Y;W;K;A;N...
FM_GP7	-17.471	0.232483	24.82886	K;Y;Y;Y;C;T;W;K;R;K...
FM_GP80	36.01646	7.371938	5.699596	T;T;Y;C;T;Y;W;T;G;T...
FM_GP81	-8.1337	15.55246	-18.6926	T;Y;Y;N;N;N;W;G;A;G...
FM_GP82	-7.59208	-29.1877	-13.3588	T;Y;C;C;C;C;W;T;N;G...
FM_GP83	-14.4484	-11.5402	-0.00703	N;C;Y;C;Y;Y;T;T;A;K...
FM_GP84	0.515264	-22.405	-8.82829	T;T;C;Y;C;Y;N;K;R;G...
FM_GP86	-6.3745	-15.5748	-4.46295	N;Y;C;Y;Y;Y;N;K;N;K...
FM_GP87	-7.89306	-13.7149	0.072567	N;Y;Y;Y;Y;Y;T;K;R;N...
FM_GP89	-8.3707	-2.19941	-11.364	G;C;C;Y;N;Y;A;G;G;T...
FM_GP90	-11.9694	-4.74803	-15.0476	T;Y;C;Y;T;Y;W;G;A;N...
FM_GP91	12.0451	3.818151	-3.36709	T;T;T;Y;Y;T;A;K;N;T...
FM_GP93	-7.55681	17.01001	-22.1882	N;T;T;C;N;Y;W;G;A;G...
FM_GP94	-9.10023	17.03901	-8.41556	T;Y;N;T;N;C;N;G;N;N...
FM_GP95	-11.8788	-12.5563	11.8429	N;T;C;N;N;T;A;G;A;N...
FM_GP96	-3.35357	-6.60551	4.423523	K;C;Y;C;T;Y;N;T;G;T...
FM_GP98	34.46874	9.743627	7.220517	T;Y;N;C;N;T;W;K;N;T...
FM_GP99	17.66963	1.604101	0.36699	T;N;N;T;C;Y;N;K;A;T...

Key: FM = Finger millet; GP = Genotypes; PC1 = Principal component 1; PC2 = Principal component 2; PC3 = Principal component 3.

4.3.2 Phylogenetic analysis

Genetic diversity analysis was done on the same 95 finger millet genotypes. The dendrogram was generated through neighbor-joining method of TASSEL software. The genotypes were grouped into three major clusters (A, B and C) based on reaction to *Striga* (Figure 2). Cluster A comprised of 32 genotypes of which were 27 Kenyan genotypes and 5 were exotic genotypes from India 1, Uganda (2), Malawi (1), and 1 from Zambia (1). Cluster B comprised of 56 genotypes of finger millet. Cluster B was further divided into two sub clusters: B1 and B2. Of the thirty four accessions grouped into sub-

cluster B1, 28 were from eastern Africa (Kenya 27 and Uganda 1), two from southern Africa (Zimbabwe), one from western Africa (Nigeria), two from Asia (India and Nepal) and one from Europe (Germany). Cluster B2 had 22 genotypes of which 21 were from eastern Africa (Kenyan 20 and Uganda 1) and India (1). Cluster C had seven genotypes in total, out of which 4 genotypes were from southern Africa (Zimbabwe) and 3 from Kenya.

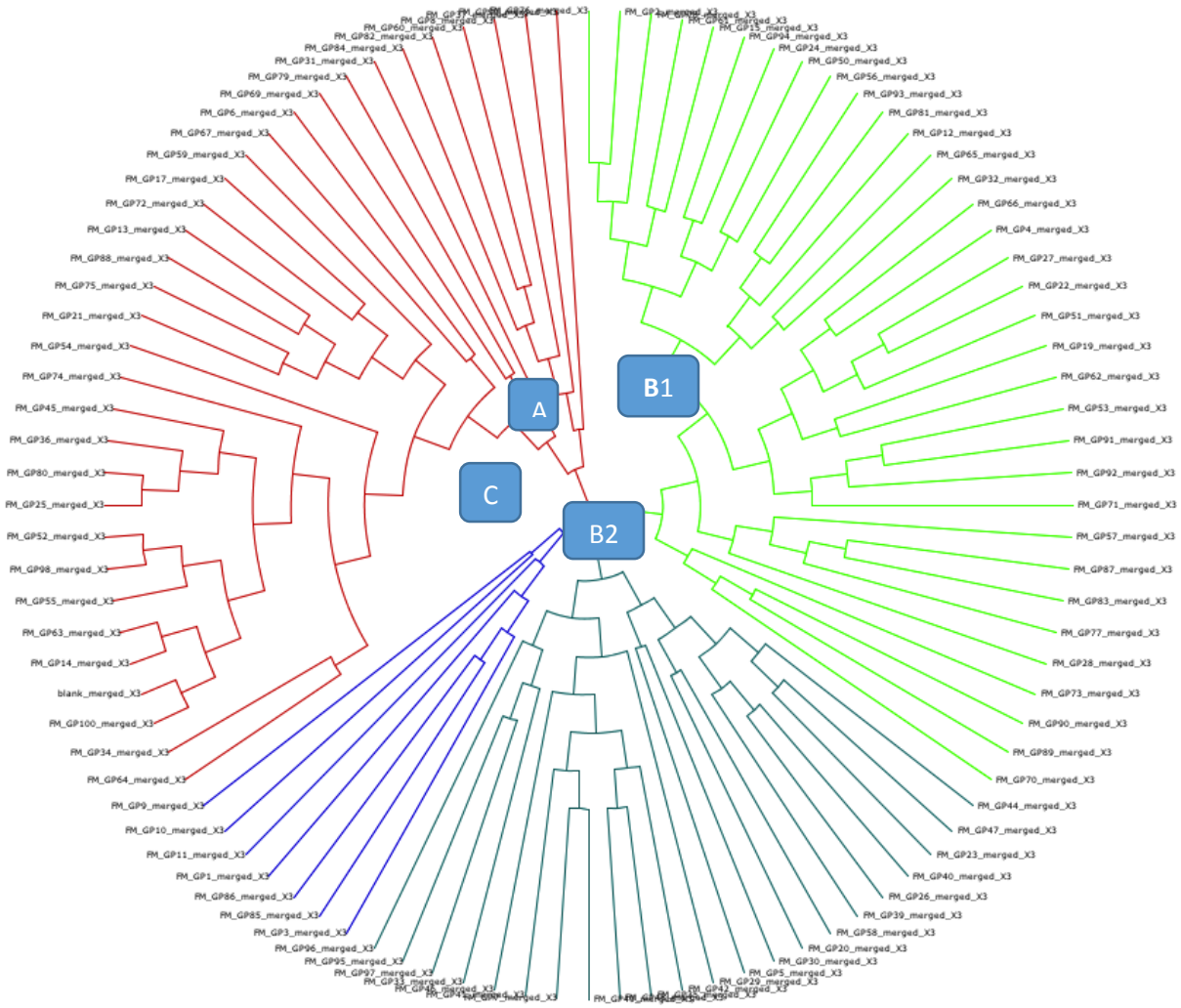


Figure 2: Phylogenetic analysis of 95 finger millet genotypes

The genotypes were generated through neighbor-joining method of TASSEL software in response to *Striga* in two environments in Kenya. Three major clusters are shown with a

further sub-cluster within the biggest pool (blue and Green). The same genotypes were further used to perform genome-wide association studies (GWAS) for *Striga* resistance. The genotypes are represented by entry numbers.

4.3.3 Cluster analysis for the 95 inbred lines

From Table 4.6, the genotypes that showed susceptibility to *Striga* were mostly fromcluster A and included; GBK000549, GBK000462, GBK029715 and GBK029744. At least two of the susceptible genotypes were also found in cluster B1 i.e. GBK027300, GBK011113, GBK040568 and one of them I.E 5306 was found in cluster C. Cluster B was further split into two sub-clusters that had fifty six genotypes in total out of the 95. All the genotypes that showed high resistance to *Striga* belonged to cluster B. Thus Genotypes I.E 2217, 1.E 6537 were from sub-cluster B1 while genotypes I.E 4115, I.E 4491, KACIMMI 24, KACIMMI 30, KACIMMI 47, were from sub-cluster B2 (Table 4.6). Similarly the genotypes that were tolerant belonged to cluster B and included; KACIMI 16, KACIMMI 36, KACIMMI 49, KACIMMI 73, BUSIBWABO-1, OMUGA-G, GBK029793, GBK000516. They were high yielders despite supporting high population of *Striga* at maturity (Appendix VIII). The clustering pattern revealed highly diverse nature of composite collection based on racial and regional diversity.

Table 4.6: Membership cluster for the 95 inbred lines from phylogeny tree.

Cluster A (Red)	Cluster B1 (green)	Cluster B2 (blue)	ClusterC (Dark blue)
KACIMMI 77	GBK000568	KACIMI 17	I.E 4497
P4C3	I.E 6165	KACIMI 49	GBK039217
GBK000520	GBK011113	I.E4816	GBK043268
GBK000451	GBK008292	KACIMI 73	I.E 4491
GBK008299	I.E 5873	KACIMI 47	I.E 5306
GBK029805	GBK000784	BUSIBWABO-1	KACIMI 11
GBK000549	I.E 2217	KACIMI 36	I.E 5870

GBK008339	GBK000516	KACIMI 30
KACIMI 7	GBK029821	OMUGA G
GBK029744	U15 X P283	OMUGA P
U-15	GBK029798	OKHALE-1
KACIMI 22	I.E 2957	KACIMI 6
GBK000462	GBK029199	SERERE-1
GBK029793	P 224 CV	KACIMI 72
GBK000463	GBK029678	KACIMI 42
GBK000493	I.E 6537	I.E 4115
UFM 138	VL149	KACIMI 20
PR 202	GBK027300	KACIMI 24
GBK000409	GBK000692	P 283
I.E 2606	I.E 6337	GBK000900
GBK000802	GBK008292	GBK000831
GBK029715	GBK029701	GBK026992
KACIMI 15	GBK008348	
GBK029724	GBK033446	
GBK011126	GBK040568	
P 224	GBK029847	
GBK033416	GBK000369	
GBK029820	GBK033414	
GBK000828	GBK011082	

GBK029661	GBK043081		
NANJALA-BROWN	GBK000449		
	GBK000909		
GBK029807	GBK000482		
	GBK003821		
32	34	22	7

4.3.4 SNP markers showing association with *Striga* resistance

Some markers that were detected using mixed linear model (MLM) analysis were similarly detected in general linear model (GLM) analysis (Table 4.7). This confirmed the reliability of GLM in genome wide association studies (GWAS). The markers identified were TP85424 and TP88244 (Table 4.7).

Table 4.7: Presentation of SNP markers showing significant association with *Striga* resistance using GLM and MLM.

GLM 60% filter 0.05						
Trait	Marker	Locus_pos	marker_F	marker_p	perm_p	markerR2
AlupSfree	TP11346	11346	11.77614	8.71E-05	0.966	0.2876
AlupSfree	TP16436	16436	13.46379	1.87E-05	0.54	0.28372
AlupSfree	TP25285	25285	11.43916	9.62E-05	0.973	0.28983
AlupSfree	TP53302	53302	12.4427	5.71E-05	0.885	0.34173
AlupSfree	TP68225	68225	15.04937	6.97E-06	0.271	0.30923
Alupinoc	TP68225	68225	14.36346	1.08E-05	0.343	0.30987
Alupinoc	TP86696	86696	18.17384	7.98E-05	0.93	0.21652
kibosSfre	TP7986	7986	12.58671	6.76E-05	0.864	0.33936
kibosSfre	TP53302	53302	22.36557	2.44E-07	0.006	0.40889
KibosIno	TP14093	14093	14.49574	1.25E-05	0.384	0.33984

KibosIno	**TP85424**	**85424**	**14.12507**	**1.26E-05**	**0.388**	**0.31724**
KibosIno	**TP88244**	**88244**	**11.76539**	**5.74E-05**	**0.871**	**0.27191**
			MLM 60% filter 0.05			
kibosIno	TP70567	70567	8.0447	9.04E-04	0.26291	1.19121
KibosIno	TP78789	78789	8.03945	9.93E-04	0.27239	1.19121
KibosIno	**TP85424**	**85425**	**9.72326**	**2.59E-04**	**0.31777**	**1.19121**
KibosIno	**TP88244**	**88244**	**8.51908**	**6.09E-04**	**0.27301**	**1.19121**

Alupsffree = Alupe *Striga* free, Alupinoc = Alupe inoculated with *Striga*, Kibosfree = Kibos *Striga* free, KibosInoc = Kibos inoculated with *Striga*

4.3.5 Population structure of the 95 inbred lines

Based on analysis of the first three Principal component analysis (PCA) of the fourteen components there was a cumulative proportion of 8% (Fig.3, appendix XXIX). The results also provide evidence for genetic variation for response to *Striga* in finger millet which is the first study reported so far. Although only 95 accessions were used, there is likelihood that there are more novel sources for resistance to *Striga* within cultivated and wild germplasm.

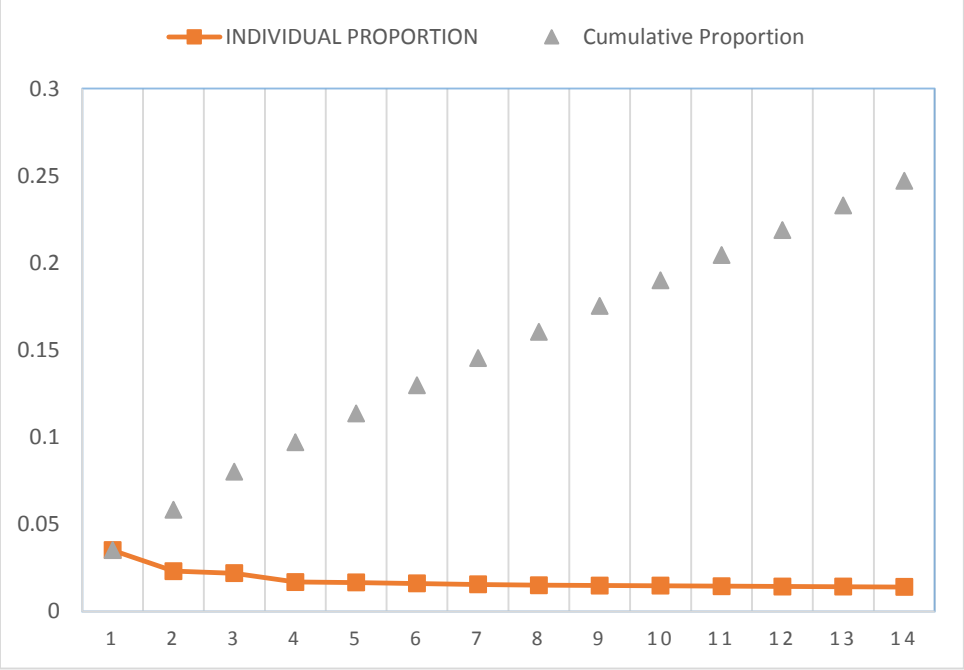


Figure 3: PCA presentation graphically on individual and cumulative proportion.

4.3.6 Multidimensional scaling: A confirmation of population structure

The purpose of multidimensional scaling (MDS) in this case was to provide a visual representation of the pattern of proximities (i.e. similarities or distances) among a set of objects or the meaning of the MDS is to visualize the level of similarity of individual cases of the dataset. It was performed on the data set to validate the population structure. It is among the many multivariate techniques that aim to reveal the structure of data set by plotting points in one or two dimensions. The 95 genotypes were not clearly classified into three broad groups as there was overlapping of sub-populations (Figure 4). The clusters were collinear with the population structure. It is extremely similar to principal component analysis (PCA), with the main difference being that for MDS the raw SNP scores are first converted into matrix of distances between all samples (Figure 4 and appendices XXVII and XXVIII). The conversion is necessary because PCA does not function on datasets where some elements are missing and the stochastic nature of GBS ensures that essentially every data set will have at least some missing data and frequently quite a bit (Wallance et al., 2015). The MDS plot provides a bit of some separation of the accessions into two sub-populations that are overlapping, a confirmation of population structure and the clustering pattern that was observed in phylogenetic analysis. The over-lapping is in conformity where by the resistant and tolerant genotypes were put in the cluster B.

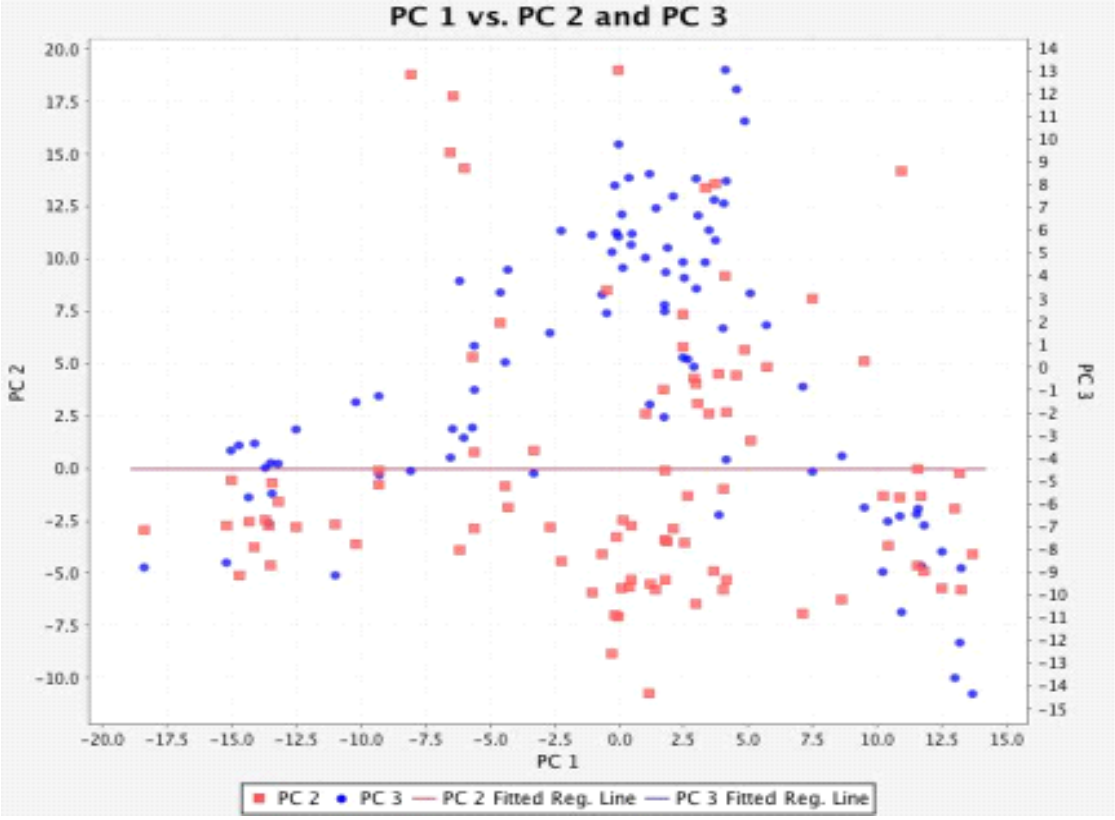


Figure 4: Multiple dimensional scaling for the entire collection with Colours depicting corresponding subpopulations.

4.3.7 Genome wide association studies

GWAS also called association mapping studies focuses on polymorphism in candidate genes that are suspected to have roles in controlling phenotypic variations for one specific trait of interest (Thornsberry et al., 2001). Using the few genotypes from the HapMap shows that diversity within inbred lines of finger millet was as a result of copy number variation (CNV) in response to reaction to *Striga* (Fig. 5). These variations have involved deletion, insertions and duplication as can be observed in the consensus sequence among the eight genotypes below**:**

1. KACIMMI 73

   CAGCAAAACGCCAAGCACAGA**TGGG**CAACTGCTCGGG**C**AGAAAAAA
   AAAAAAAAAAAAAAAAAA

KACIMMI 73

CAGCAAAACGCCAAGCACGGA**TGGG**CAACTGCTCGGG**C**AGAAAAAA AAAAAAAAAAAAAAAA AA

2. KACIMMI 49

CAGCAAGCTACGGGAGAAAACCAACCTCGCCAC**TGGGG**CCGAAGCA GAAAAAAAAAAAAAAA AA

KACIMMI 49

CAGCAGGCTACGGGAGAAAACCAACCTCGCCAC**TGGGG**CCGAAGCA GAAAAAAAAAAAAAAA AA

3. GBK000516

CAGCAAACACGAGGTCTGATCGCTCCCTCTCACTTT**TGG**CTCCACTGC TGAAAAAAAAAAAAAA

GBK000516

CAGCGAACACGAGGTCTGATCGCTCCCTCTCACTTT**TGG**CTCCACTGC TGAAAAAAAAAAAAAA

4. KACIMMI 36

CAGCAAGGCAGTTTTTCCATCCCGAGAAACCTCAAGCTTCCAACAGA**T GTG**TCAGCTGAAAAAA

KACIMMI 36

CAGCAAGGCAGTTTTTCCATCCCGAGAAACCTCAAGCTTCCAACGGA**T GTG**TCAGCTGAAAAAA

5. KACIMMI 24

CAGCAAAGGGGGGAAGCAGAAGGCGTTCCCCGAC**GGG**CGGTGGCTG AAAAAAAAAAAAAAAAA

KACIMMI 24

CAGCAAAGGGGGGAAGCGGAAGGCGTTCCCCGAC**GGG**CGGTGGCTG AAAAAAAAAAAAAAAAA

6. BUSIBWABO-1

CAGCACCGTCGAGTCG**TGG**AGCGATGACGGCGGGAGCAGAAAAAAA
AAAAAAAAAAAAAAAA AA
BUSIBWABO-1

CAGCACCGTCGAGTCG**TGG**AGCGATGACGGCGGGGGCAGAAAAAAA
AAAAAAAAAAAAAAAAAA

7. KACIMMI 16
   CAGCAAGCCTCGGCAGAGCGGAGAGGGA**TGG**C**GG**CAA**GG**CAGAAAA
   AAAAAAAAAAAAAAAAAA
   KACIMMI 16

   CAGCAAGCCTC**GG**CAGAGC**GG**AGAGGGG**TGG**CGGCAAGGCAGAAAA
   AAAAAAAAAAAAAAAAAA

8. KACIMMI 65
   CAGCAAGCTACAGCAGGAGAGATGAGCTG**TGGG**CGCACTGCAGAAA
   AAAAAAAAAAAAAAAAAA
   KACIMMI 65
   CAGCAAGCTACAGCAGGAGAGATGAGCTG**TGGG**CGCCCTGCAGAAA
   AAAAAAAAAAAAAAAA

Figure 5: Eight paired end reads trimmed to 64 base paired arrangement of SNPs.

The eight were among the genotypes that showed moderate resistance to Striga from the 95 genotypes.

CHAPTER FIVE
DISCUSSION

5.1 Effects of *Striga* infestation on finger millet morphological traits.

The F-values for most of the quantitative traits (i.e. seedling vigour, *Striga* count at vegetative stage, *Striga* count at 50% flowering, plant height, ear exertion, ear length, ear width, *Striga* count at crop maturity and crop yield) were statistically significant except for ear shape, lodging percentage, stand count and number of fingers in the two environments. This was an indication that the composition of finger millet germplasm used in screening for *Striga* resistance had sufficient genetic variation for the traits. Some genotypes were observed to have low variation among the treatment and the control despite carrying high *Striga* population with respect to the agro-morphological traits, hence seemed to to tolerant to *Striga*. The experiment was carried out in localities that are hot spot to Striga prevalence hence results obtained on the field are very significant. Similar results on variance in response to *Striga* by genotypes has been reported by Ramasamy et al. (1996), Sivagurunathan, (2005) and Ya Zhini, (2006) for traits such as plant height, days to 50% flowering, ear head length and width, peduncle length, panicle exertion and grain yield.

5.2.1 Seedling vigour

Seedling vigour is an important characteristic in many cereals for its yield and biomass determining property and breeding programs (Botwright et al., 2002; Richards and Lukacs, 2002; Rebetzke et al., 2004). The mean for seedling vigour was higher in the genotypes that were infested with *Striga* compared to *Striga* free plots. Thus genotypes that had high seedling vigor had least Striga count or none at vegetative stage, days to 50% flowering to crop maturity. According to Ransom and Odhiambo, (1995) early maturing maize has the ability to escape the phytotoxic effects of *Striga* through vigorous early growth before *Striga* cause serious damage to the plant. Seedling vigour had high significant negative relationship with *Striga* count at both days to 50% flowering and maturity, traits that also had negative relation with yield. The same genotypes had low lodging percentage apart from the accession PR 202 that was highly lodged. These similarity suggest that a genotype with high seedling vigor is likely to be resistant to

Striga but would probably lodge, which agreed with Roozrokh et al. (2002) findings on chicken pea. Similarly the NRC (1996) also listed robust growth, early vigour, resistance to *Striga* and blast disease as important traits in finger millet breeding. Overally, effect of *Striga* on plant vigor influences primary productivity, increasing ground mortality and lowering seed production capacity particularly among the susceptible genotypes.

5.2.2 *Striga* count

In this study, particicular genotypes that had no *Striga* or low *Striga* number recorded throughout physiological development, responded positively to evaluated agro-morphological traits. It was also evident that accessions which responded negatively to *Striga* infestation were poor grain yielders. For instance six poor yielding genotypes did not support *Striga* suggesting they could be carrying *Striga* resistance genes but deficient in yield conferring genes. Such trait could be introduced to productive genotypes that are highly susceptible *Striga* through gene transfer to improve yields. The results showed high mean *Striga* count among accessions at physiological maturity compared to that at vegetative and 50% flowering. The early attachment of *Striga* seedlings to roots is a function of *Striga* seed density, and host plant characteristic such as root architecture (Van Delfit, 1997; Gurney et al., 1999; Kim and Ademitirin, 2001; van Ast and Bastian, 2006). Early attachments result in severe damage to the host under controlled conditions (Cechin and Press, 1993b; Gurney et al., 1999) or in the field (Weber et al., 1995: Abayo et al., 1996). This is in agreement with the findings in this study where by genotypes that had high *Striga* count at maturity of crop had low mean yield. *Striga* count at flowering and at maturity were highly positively correlated and the two were negatively correlated to yield. This is in agreement with Haussmann et al. (2000) who reported of *Striga* being deleterious parasitic weed on cereals. The positive relationship between *Striga* counts and agro-morphological performance was expected as a *Striga* susceptible genotype would likely show similar behaviour at all stages of plant development.

5.2.3 Days to 50% flowering

The high significant difference between *Striga* counts and days to 50% flowering, plant height and crop yield all point to the fact that *Striga* has deleterious effect on finger millet (Haussmann et al., 2000). This shows a high indication that infected plants struggle to

reach maturity earlier in order to survive environmental stress (Shah et al., 1987). This is in tandem with report by Ransom and Odhiambo, (1995) where studies done on maize varieties in Kenya found that early maturing maize landraces were more tolerant to *Striga* than late maturing land races through a mechanism termed 'the escape mechanism'. According to Ransom and Odhiambo, (1995), early maturing maize has the ability to escape the phytotoxic effects of *Striga* through vigorous early growth before *Striga* cause serious damage to the plant. The parasitic weeds keep their stomata permanently opened because much water is withdrawn from the host inducing drought symptoms (Stewart and Press, 1990). Early maturity is one attribute to avoid *Striga* infestation as was demonstrated in resistant genotypes of finger millet. The nutrient uptake by host plant (finger millet) was reduced by the *Striga* and could be a factor to affect the flowering and reduced millet production because there is general effect on primary productivity/or growth and development. Therefore *Striga* causes adverse effects on growth and development of agro-morphological traits in host plant which justifies the first hypothesis that *Striga* infestation has effect on finger millet agronomic performance.

5.2.4 Plant height

The high significant differnce between *Striga* counts with respect to plant heights, ear lengths, ear widths, ear exertions and grain yields as shown in Table 4.1 were expected as *Striga* infestation has the effect of competing the host plant for nutrients parasitically through haustourial development as a bridge with host. This is in agreement with report by Hausmann et al. (2000) that *Striga* retards plant growth, reducing plant height and consequently yielding. Gebremedhin et al. (2000), made similar observation that during sorghum flowering and grain filling periods there is significant reduction in stem height due to *Striga* infestation. The greater the relative reduction, the greater the *Striga* susceptibility. As explained by Musselman (1987) and Parker (1999), two species of *Striga, Striga asiatica* (L) Kuntze and *Striga hermonthica* (Del) Benth caused economic losses to important cereal crops such as sorghum, millet, maize, and rice in Africa of which *S. hermonthica* has a marked influence in growth and allometry of its host plant. Similar explanation was also reported by Press et al. (1999), that plants infected by *Striga* show low levels of indole-3-acetic acid. Odongo and Abayo, (1999) reported that maize varieties susceptible to *Striga* have reduced growth leading to short plants. Frost et al.,

(1997) reported that the attachment of *Striga* on the root system affected and reduced the plant height of host plant by taking substantial amount of nutrients from host plant. Similarly Gurney et al. (1999) and Swabrick et al. (2009), reported that the parasite produces phytotoxic substances that affects crop's growth, with even low levels of infection resulting in dehydration and loss of vigor, stunting, and biomass and grain yield reduction. Gebremedhin et al. (2000), made similar observation that during sorghum flowering and grain filling periods there is significant reduction in stem height due to *Striga* infestation. The greater the relative reduction, the greater the *Striga* susceptibility.

5.2.5 Ear shape

The results obtained showed no mean significance difference for the *Striga* inoculated plots and *Striga* free ones owing to the fact that it is a qualitative trait.

5.2.6 Ear length

The varieties that had huge mean difference in length of ears also gave large difference in mean yields particularly highly susceptible genotypes of finger millet. Similarly, Bondale et al. (2002) found grain yield per plant to be significantly influenced by finger length and finger width among finger millet genotypes from diverse regions of India. Studies done by Van Ast and Bastiaans (2006), showed that sorghum responds to *Striga* parasitism through changes in dry matter allocation, in particular sorghum infested with *Striga* has a reduced panicle and stem fraction while leaf and root fraction is increased. Thus *Striga* weed had serious effect on growth and development of ear length.

5.2.7 Ear width

In this study there was high significant difference between ear width and *Striga* counts. Thus smaller size of the ear results to smaller panicles that limit proper formation and development of finger millet seeds. These results are clearly in agreement with Press et al. (1999) who outlined that *Striga* can impose effects on the hosts even in its early and underground stage of development, which might be attributed to the production of phytoxins by parasite affecting growth and physiology of the hosts. He also attributed this as due to low levels of indole-3-acetic acid. Frost et al. (1997) reported that the attachment of Striga on the host root system affected and reduced the plant weight of host plant by taking substantial amount of nutrients from host.

5.2.8 Lodging percentage.

There was no significant difference between lodging and *Striga* infestation an indication that *Striga* does not have adverse effect on center of gravity of plant because some genotypes that had high lodging percentage among the infested plots similarly had high grain yield. This was in agreement with report by Duke, (1978) that lodging could be due to heavy heads associated with high yield in finger millet leading toppling plants. However, this did not agree with results given by Sallah and Afribeh (1998) and Kim (1994) who reported that grain yield was negatively affected by stalk lodging caused by *Striga* infestation among the maize varieties. The positive relationship between lodging and yield is in contrast to findings in wheat and barley, where lodging causes up to 40% yield losses (Kelbert et al., 2004). The effect of lodging could be compensated through gene transfer between highly resistant genotypes to *Striga* with those that are susceptible in order to promote their stem stability.

5.2.9 Ear exertion

The mean ear exertion showed highly significant difference between inoculated genotypes and non – inoculated genotypes an indication that *Striga* has an adverse effect on its growth. This is in agreement with findings by Odongo and Abayo (1999), who reported that maize varieties susceptible to Striga have reduced growth leading to short plants. Study by Drewhan and El Hiweris (1979) showed that xylem sap from infected plants contained lower quantities of cytokinin and gibberellins and higher quantities of ABA than sap from uninfected plants, suggesting that perturbation in the balance of growth regulators may contribute to the changes in the host architecture. Greater concentration of ABA in the xylem sap reported here would be expected to reduce leaf expansion (Zang and Davies, 1990). ABA has also been shown to enhance the root: shoot ratio and reduce stem growth (Trewavas and Jones 1991)

5.2.10 Stand count

This is a parameter determining the mean of plant populations per genotype. There was no significant interaction between the *Striga* count and stand count. As explained by Bacaltchuk and Ulrich (1983), plant stand establishment is an important characteristic in wheat and being highly correlated to plant height. Plant stand has positive relationship

with *Striga* count particularly in susceptible genotypes. Thus the more the finger millet plants, the higher the incidence of *Striga* infestation reflecting increase in pest severity with increased host density (Mundt, 2002). Plant stand has positive relationship with yield (Steppuhn, 1997 and Holen et al., 2001).

5.2.11 Number of fingers

There was no significant difference between number of fingers and *Striga* count in this study an indication that its number and formation in finger millet is solely influenced by genes rather than by environment. As supported by Ademitrin et al. (2000), pre-flowering stress due to *Striga* parasitism was higher than post flowering stress and resulted in higher reduction for ears per plant (44%) than reduction for other yield components (12-29%).

5.2.12 Crop yield

The mean yield for *Striga* inoculated genotypes was 609.9 $kgha^{-1}$ while the mean for *Striga* free genotypes was 1074.4 $kgha^{-1}$. The reduction in yield due to *Striga* infestation was approximately 43%. This is in tandem with report by M'Boob, (1986) that yield losses of maize due to *Striga* infestation in Nigeria was estimated at 70%, while losses in Africa was about 40% representing an annual losses of about US $7 billion. The infestation of crop by *Striga* results in chlorosis, wilting, stunting and death, with losses ranging from slight to 100% (Agrios, 1997). It is also in agreement with Shawemimo (2006), who reported that *Striga* infestation in sorghum reduced plant height, panicle weight, 1000 grain weight and grain yield by 13.7, 35.9, 52.9, 64.5 and 52.6% respectively. The parasitic weeds penetrate the roots of the host plants depleting them of essential nutrients for growth resulting to stagnation and finally low yields (Watson et al., 1998). Successful parasitic establishment creates a strong sink of nutrients to the detriment of host, leading to drastic growth and yield reductions (Keyes et al., 2001; Joel et al., 2007). Rodenburg et al., (2008), outlined that several photosynthetic parameters are reduced in sorghum plants infected with *S. hermonthica* including electron transport rate through the photosystem II and photochemical quenching. Report by Frost et al. (1997), had shown that the negative effect on photosynthesis relates with reduced conductance, which is possibly the consequence of elevated abscisic acid (ABA) levels of sorghum

plants infected by *S. hermonthica*. Not only ABA, but also other plant hormones such as cytokinin and gibberellin levels are altered in sorghum plants infected with *Striga* relative to control plants (Taylor et al., 1996). The results of this study agree with those of Ramaiah (1991) on sorghum, and millet that *Striga* infestation causes substantial reduction in yield components, increases yield loss and eventually economic loss of crops to the farmers(s).

5.3 Variation in finger millet genotypes for *Striga* resistance

Genetic diversity is a basic requirement for crop improvement programmes. The genetic variation within and between species is generated by mutation, sexual reproduction and natural selection. Efficient use of conserved bio-diversity requires information about the degree and distribution of genetic diversity. The variation in the genetic make up and its interaction with environment indicates the observable pattern of diversity. Determination of diversity using molecular markers provides opportunity to select appropriate parents for crop improvement with higher precision. The importance of increased use of genetic resources in enhancing genetic potential of crop alleviates biotic and abiotic stresses thereby broadening genetic base of crop (Banks, 1976)

5.4 GBS Analysis and phenotypic association with *Striga* tolerant traits.

Genetic and phenotypic correlation between traits are quite important because they indicate the association in response to other characters that could occur during selection. The results obtained using SNP markers through GBS analysis showed high significance and some association between some genetic loci/ or sites. As reported by Stuber et al. (1966), some characters of economic importance like yield are complex in inheritance and may involve several related genes. Genetic correlation for traits measured showed that grain yield, ear length, ear width, ear exertion were affected by *Striga* more so by susceptible genotypes. The clustering of the 95 genotypes with respect to reaction to *Striga* is an indication that resistance is genetically controlled and occurring in particular gene loci. According Bush and Moore (2012), genome wide association studies typically identifies common variants with small effect sizes. Similarly the variants that were tolerant to *Striga* belonged to the same cluster B an indication that susceptibility to the weed occurs when the gene is in homozygous recessive state. Similar results were

reported by Vogler et al. (1996), who observed that a single nuclear recessive gene controls this mechanism in sorghum variety SRN 39.

5.5 Population structure and Phylogenetic analysis

Population structure analysis with fastSTRUCTURE (Raj et al., 2004) separated the finger millet genotypes into three primary clusters. Phylogenetic analysis closely corresponds with the structure analysis, whereby the inferred clusters matched major branch points in the phylogeny.

The results also provided evidence in genetic variation for response to *Striga* in finger millet which is the first study to be reported so far. This results also justifies the second hypothesis which stated that there could be genetic diversity among the finger millet genotypes in response to *Striga*. It revealed three groups depending on the germplasm with the resistant genotypes being separated from the susceptible ones. This separation was due to differences in the reaction of the 95 types of germplasm to *Striga* infestation. This findings is consistent with results of Menkir et al., (2012b) who showed that *Striga* – resistant hybrids were separated from *Striga* tolerant hybrids but contrary to the results of Badu-Apraku and Lum (2007) who reported that the clustering of inbred lines was independent of the genetic background of genotypes. Although only 95 accessions were used, there is likelihood that more novel sources for resistance to *Striga* could be available within cultivated and wild germplasm.

All the eight genotypes that were selected for moderate resistance were from same cluster B implying the high reliability of the results obtained in field screening and verification by molecular work. Therefore the molecular markers that were obtained through GLM and MLM with respect to resistance to *Striga* confirm the same. The resistance has come about due to copy number variation through insertion and deletion

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The 100 genotypes of finger millet assessed using agronomic traits had various levels of response to *Striga* infestation: (i) The resistant genotypes immune to *Striga* infestation were least affected; (ii) Moderately resistant genotypes with a limited level of *Striga* infestation were least affected. Genotypes susceptible to *Striga* menace were highly affected in terms of productivity and morphological traits

2. The genetic molecular markers analysis for tolerance to *Striga* revealed the following: From the GBS analysis, it was observed that finger millet genotypes inoculated with *Striga* at Kibos had the markers TP 85424 and TP 88244 present in both GLM and MLM. This indicated that the two markers were stringent, hence confirming the reliability of GBS in genome wide association studies.

3. The genetic diversity analysis, divided the genotypes into three sub-populations (A, B and C) and all appeared to have an admixture of alleles. Thus Cluster A consisted majorly of susceptible genotypes which included; GBK000549, GBK000542, GBK029715 and GBK029744 agreeing with results from agronomic traits. All genotypes that showed high resistance to *Striga* were in cluster B and they included I.E 2217, I.E 6537, I.E 4115, KACIMMI 24, KACIMI 30 and KACIMMI 47.

   Similarly the seven tolerant genotypes equally belonged to cluster B2 and include KACMMI 49, GBK000516, KACIMMI 65, KACIMMI 36, KACIMMI 16, KACIMI 73 and BUSIBWABO-1. At least two of the susceptible genotypes were also found in cluster B1 (i.e. GBK027300, GBK011113, GBK040568 and one of them I.E 5306 was found in cluster C). Cluster C also comprised of susceptible genotypes and include I.E 4497, GBK039217, GBK043268, I.E 4491, KACIMMI 11 and I.E 5870.

6.2 Recommendations

1. The molecular markers identified should be validated across a large germplasm set so as to confirm validity/or strength of the two approaches and shared with breeders to enhance efficient selection for resistance to *Striga*.
2. Further GBS work be done on the selected lines for resistance to *Striga* and compare gene interaction with genotypes that are susceptible to *Striga* but possess traits that will promote high overall performance of crop.
3. Probably the most important point is simply establishing working parameters for genotyping by sequencing (GBS) in the species that open doors to many other analysis that rely on extensive genotyping.
4. Population structure and phylogenetic analysis be prioritized in checking for diversity among the large panel of finger millet in order to improve on genetic base for resistant variants

REFERENCES

Abayo, G. O., Ransom, J. K., Gressel, J., and Odhiambo, G. D. (1996). *Striga hermonthica control with acetolactate synthase inhibiting herbicides applied to maize seed with target-site resistance.* In: Moreno M.T, Cubero J.I, Berner D, Joel, D, Musselman L.J. and Parker C. (eds). *Advances in parasitic plant research.* Sixth International Parasitic Weed Symposium, Cordoba, Spain, pp. 761-768.

Adetimirin, V. O., Aken'Ova., M. E., and Kim., S. K. (2000). Effects of *Striga hermonthica* on yield components in maize. *J. of Agric. Sci*. 135: 185-191.

Agrawal, B. L., Siame, J. A., and Uprichard, G. T. (1993). *Status of finger millet* (*Eleusine coracana* Gaertn.) in Zambia. 1993. In: Riley, K.W., Gupta S.C., Seetharam, A. and Mushonga, J. N. (ed.). *Advances in small millets*. pp. 21-28. New Delhi: Oxford and IBH

Agrawal, R. K, Brar, D. S., Nandi, S., Huang, N., and Khush, G. S. (1999). Phylogenetic relationships among Oryza species revealed by AFLP markers. *Theor. Appl. Genet*, 98: 1320–1328.

Agrios, G. N. (2005). *Plant Pathology*. 5th ed. London: Elsevier Academic Press.

Agrios, G. N. (1997). *Plant Pathology*. 4th ed. Academic Press, San Diego, CA 635

Ali, R. A., El-Hussein, A., Mohamed, K., Babiker, A. (2009). Specificity and genetic relatedness among Striga hermonthica strains in Sudan. *Life Sci Int J* 3:1159-1166.

Aliyu, L., Lagoke, S. T. O., Carky, R. J., Kling, J., Omotayo, O., Shebayan, J. Y. (2004). Technical and economic evaluation of some Striga control packages in maize in the Nigeria Guinea Savanna. *Crop protection* 23, 65-69.

Allen, A. M., Barker, G. L., Berry, S. T et al. (2011). "Transcript-specific, single-nucleotide polymorphism discovery and linkage analysis in hexaploid bread wheat (Triticum aestivum L.)," *Plant Biotechnology Journal*, 9(9), 1086– 1099.

Allhoff, M., Schönhuth, A., Martin, M., Costa, I. G., Rahmann, S., et al. (2013). Discovering motifs that induce sequencing errors. BMC Bioinformatics 14 Suppl 5: SI. Alumira J. and Rusike J, 2005. The Green Revolution in Zimbabwe. *Journal of Agricultural and Development Economics*, 2(1), 50-66.

Amruthmahal, A., Urooj, A., and Puttaraj, S. (2003). In vitro starch digestibility and nutritionally important starch fractions in cereals and their mixtures. *Starch/Starke* 55, 94-99.

Amusan, I. O., Rich, P. J., Menkir, A., Housley, T., and Ejeta, G. (2008*).* Resistance to Striga hermonthica in maize inbred line derived from Zea diploperennis. *New phytologist* 178, 157-168

Anon. (2004). *Finger Millet.* Encyclopedia Home Page.[online] Available: (http://www.encyclopedia4u.com/f/finger-millet.html (verified 07 Oct. 2008).

Aranzana, M. J., Kim, S., Zhao, et al. (2005). "Genome-wide association mapping in Arabidopsis identifies previously known flowering time and pathogen resistance genes*," PLoS Genetics*, 1(5), p. e60.

Ariga, E. S., Ransom, J. K., Odhiambo, G. D., Abayo, G., and Ndungu, D. K. (1997). Potential of using cotton and other trap crops for Striga hermonthica management in cereals in Kenya. In: Adipala E, Tusiime G and Okori P (eds.), *Proceedings of the 16th Biennial Weed Science Society Conference for Eastern Africa. Kampala*: WSSEA. Pp 247-254

Atera E. A., Itoh, K., & Onyango, J. C. (2011). Evaluation of ecologies and severity of Striga weed on rice in sub-Saharan Africa. *Agr.and Biol. J. of N. America* 2: 752-760.

Attere, A. F. (1993). *Distribution and collection of Finger Millet (Eeusine) in East and Southern Africa*. In: Riley, K. W., Gupta, S.C., Seetharam, A. and Mushonga, J.N. (ed.). *Advances in small millets*. pp 375-380.New Dehli: Oxford and IBH.

Ayensu, E. S., Dogget, H., Keynes R. D., Marton-Lefevre, T., Musselman L. J., Parker, C., and Pickering, A. (1984). *Striga biology and control*. ICU/IDRC, France

Babiker, A. G. T. (2000). *Striga research in the Sudan*: *Towards an intergrated control strategy*, Agricultural Research Corporation, Wad Medani, Sudan, 22-24 May 2000.

Bacaltchuk, B., and Ullrich, S. E. (1983). Stand establishment traits of barley genotypes of different plant heights. *Crop Science* 23, 64-68.

Badu-Apraku, B., and Lum, A. F. (2007). Agronomic performance of Striga resistant early- maturing maize varieties and inbred lines in the savannas of West and Central Africa. *Crop Sci*. 47, 737– 750. doi:10.2135/cropsci2006.04.0245. L.

Bagonneaud-Berthome, V., Arnaud, M. C., Fer, A. (1995). A new Experimental approach to the chemical susceptible maizenhybrids. *Crop science* 37, 711-716

Baird, N. A., Etter, P. D., Atwood et al. (2008). Rapid SNP discovery and genetic mapping using RAD markers, *PloS ONE*, 3(10), Article ID e3376.

Banks, D. J. (1976). Peanuts: Germplasm resources. *Crop Sci.* 16, 499-502.

Barchi, S., Lanteri, E., Portis et al. (2011). "Identification of SNP and SSR markers in eggplant using RAD tag sequencing," *BMC Genomics*, 12, article 304.

Bashir, A., Klammer, A. A., Robins, W. P et al. (2012). "A hybrid approach for the automated finishing of bacterial genomes," *Nature Biotechnology*, 30(7), 701–707.

Batley, J., Barker, G., O'Sullivan, H., Edward, K. J., and Edwards, D. (2003). Mining for single nucleotide polymorphisms and insertions/deletions in maize expressed sequence taq data. *Plant Physiology*, 132(1), 84-91.

Bennetzen, J. L., Dida, M. M., Manyera, N. W. M., Devos, K. M. (2003). *Characterization of genetic diversity in finger millet* (*Eleusine coracana*). [Online] Available: [2003Jul. 10]

Bernado, R. (2008). Molecular markers and selection for complex traits in plants: learning from the last20 years. *Crop Science*, 48(5), 1649-1664

Bernardo, R. (2002). *Breeding for quantitative traits in plants*. Stemma Press, Woodbury, MN., USA.

Bernatsky, R., and Tanksley, S. D. (1986).Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. *Genetics* 112, 887–898.

Berner, D. K., Kling, J. G., Singh, B. B. (1995). *Striga* research and control, a perspective from Africa. *Plant Disease* 79, 652-660.

Berner, D. K., Winslow, M. D., Awad, A. E., Cardwel, K. F., Mohan, Raj, D. R., and Kim, S. K. (1997). *Striga research methods*: A manual. International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria.

Bisht, M. S., and Mukai, Y. (2002). Genome organization and polyploid evolution in the genus Eleusine (Poaceae). *Plant Systematics and Evolution* 233, 243–258.

Bondale, K. V., Bhave, S. G., Pethe, U. B. (2002). Genetic variability, correlation and path analysis in finger millet (*Eleusine coracana* Gaertn.). *Journal of soils and crops:* 12: 187-191)

Bondale, K.V. (1993). *Present status of small millets production in India*. p. 117–121. In Riley K.W., S.C. Gupta, A. Seetharam and J.N. Mushonga (ed.) *Advances in Small Millets.* Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi.

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

Botstein, D., White, R. L., Skolnick, and Davis, R. W. (1980). Construction of a genetic linkage map using restriction fragment length polymorphisms. *American Journal of Human Genetics,* 32(3), 314-331.

Botwright, T. L., Condon, A. G., Rebetzke, G. J and Richards, R. A. (2002). Field evaluation of early vigour for genetic improvement of grain yield in wheat. *Australian Journal of Agricultural Research* 53:1137-1145.

Bradbury, P. J., Tian, F., Brown, P. J. et al. (2011), "Genome-wide association study of leaf architecture in the maize nested association mapping population," *Nature Genetics*, 43(2), 159–162.

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y et al. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* 23: 2633-2635. doi:10.1093/bioinformatics/btm308.

Buckler, E. S., Holland, J. B., Bradbury P. J. et al. (2009). "The genetic architecture of maize flowering time," *Science*, 325(5941), 714–718.

Bush, W. S., and Moore, J. H. (2012). Lewitter, Fran; Kann, Maricel, eds. "Chapter 11: genome-wide association studies". *PLoS Comput Biol*. **8**(12): e1002822. doi:10.1371/journal.pcbi.1002822. PMC 3531285 . PMID 23300413

Butler, L. G. (1995). *Chemical communication between parasitic weed Striga and its crop host*. A new dimension in alleloche Agricultural Experiment Station Purdue University: West Lafayette, IN 47907, pp.156-166.

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

Cechin, I., and Press, M. C. (1983b). The influence of nitrogen on growth and photosynthesis. *Plant, Cell and Environment* 16, 237-247.

Chagné, D., Crowhurst, R. N., Troggio, M. et al. (2012). "Genome-wide SNP detection, validation, and development of an 8K SNP array for apple*," PLoS ONE*, 7(2), ID e31745.

Chen, Q., Ma, Y., Yang, Y., Chen, Z., Liao, R., et al. (2013) Genotyping by genome reducing and sequencing for outbred animals. *PloS one* 8: e67500.

Chivinge, O. A., Mashingaidze, A. B., and Mujuru, T. (1995). Response of short season maize cultivars to Striga infestation. *African Crop Science Journal*, 3(4): 505-510.

Chutimanitsakun, Y., Nipper, R. W., Cuesta-Marcos, A., et al. (2011). Construction and application for QTL analysis of a Restriction Site Association DNA (RAD) linkage map in barley, *BMC Genomics* 12(4).

Close, T. J., Bhat, P., RLonardi, S., et al. (2009). "Development and implementation of high-throughput SNP genotyping in barley," *BMC Genomics,* 10(582).

Consultative Group on International Agricultural Research. (2001). CGIAR Research: *Areas of Research-Millet*. [Online] Available:

Cooke, R. J. (1995). Variety identification of crop plants. In: Skerrit J.H. and Appels R. (eds). New Diagnostics in Crop Science. *Biotechnology in Agriculture* No. 13. CAB International, Wallingford, UK, pp. 33–63.

Cortés, A. J., Chavarro, M. C., and Blair, M. W. (2011). "SNP marker diversity in common bean (Phaseolus vulgaris L.)," *Theoretical and Applied Genetics*, 123(5), 827–845.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., et al. (2011). The variant call format and VCF tools. *Bioinformatics* 27: 2156-2158.

Davey, J. W., Hohenlohe, P. A., Etter, P. D, Boone, J. Q., Catchen, J. M., et al. (2011). Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nat Rev Genet* 12: 499-510.doi:10.1038/nrg3012.

Davey, J. W., Hohenlohe, P. A., Etter, P. D., Boone, J. Q., Catchen, J. M, Blaxater M. L. (2012). "Genome-wide genetic marker discovery and genotyping using next-generation sequencing", *Nature Reviews Genetics*, 12(7), 499-510.

Dawoud, D., and Sauerborn, J. (1994). Impact of drought stress and temperature on *Striga hermonthica* and *Alectra vogelii* at early growth stages. *Exp. Agric* 30:249-257

Debrah, S. K. (1994). Socio-economic constraints to the adoption of weed control techniques: The case of Striga control in West African semi-arid tropics. *Ins. I. Pest Mgt* 40, 153-158.

DeVries, J., and Toenniessen, G. (2001). *Securing the Harvest Biotechnology, Breeding and Seed Systems for African crops* CABI Publishing, New York (2001)

De Wet, J. M. J., Prasada Rao, K. E., Brink, D. E., and Mengesha, M. H. (1984). Systematics and evolution of *Eulisine coracana*. *Am. J. Bot*. 71: 550.

De Wet, J. M. J. (1995). *Finger millet, Eleusine coracana.* In: Smartt J, Simmonds NW (eds). *Evolution of Crop Plants*. Longman, Singapore, pp137-140

Dida, M. M., Srinivasachary, Sujatha Ramakrishna, Bennetzen J. L., Gale M. D and Devos K. M (2007). The genetic map of finger millet. *Eleusine coracana, Theo. Appl. genet,* 114:321-332.

Dida, M. M., Wanyera, N., Harrison Dunn, M. L. N., Bennetzen, J. L., Devos K. M. (2008). *Population structure and diversity in finger millet* (*Eleusine coracana*) *germplasm*. Tropical Plant Biol1: 31–141. doi: 10.1007/s12042-008-9012-3

Doggett, H. (1988). *Witchweed (Striga*) In: G. Wingley (ed.) Sorghum. Second (ed). Longman Scientific and Technical, London. pp. 368-404.

Drewhan, D. S. H., and El Hiweris, S. O. (1979). *Changes in growth regulating substances, 80 References in Sorghum vulgare infected by Striga hermonthica*. In: Musselman, L.J., Worsham, A.D. and Eplee, R.E. (eds). Proceedings, 2nd International Symposium on Parasitic Weeds, Raleigh, 1979. North Carolina State University, Raleigh, pp. 144-155.

Dubrail, P., and Charcosset, A. (1998). Genetic diversity within and among maize populations: a comparison between isozyme and nuclear RFLP loci. *Theor. Appl. Genet,* 96: 577–587.

Duke, J. A. (1979). Ecosystematic data on economic plants. *Quarterly Journal of crude Drug Research.* 17: 91-110

Duke, J. A. (1978). *The quest for tolerant germplasm*. ASA Special Symposium 32. *Crop tolerance to suboptimal land conditions*. No. 32, America society of Agronomy, Madison, USA. pp 1-61

Ebiyau, J., Eselle, J. P., and Oryokot, J. (2000). *Striga research activities in sorghum at Serere Agricultural and Animal Production Research Institute (SAARI), Uganda*. In: *Breeding for Striga Resistance in cereals* (Haussmann, B.I.G. Hess D.E., Koyama, M.L., Grivet, L, Rattude, H.F.W.and Geiger, H.H. eds.). Proceedings of a workshop held at IITA, Ibadan, Nigeria. 18-20 August 1999. IITA, Ibadan, Nigeria. Margraf Verlag, Wiekersheim, Germany. pp. 307-311.

Ejeta, G. (2000). *Molecular mapping of Striga resistance genes in sorghum*. P.173. In B.I.G. Haussmann, Hess D.E, Koyama M.L, Grivet L, Rattunde H.F.W, Geiger H.H (ed.) *Breeding for Striga Resistance in Cereals*. Proceedings of a Workshop, IITA, Ibadan, Nigeria, 18-20 August 1999. Margraf, Weikersheim, Germany.

Ejeta, G. (2007). Breeding for *Striga* resistance in sorghum: exploitation of an intricate host-parasite biology. *Crop Science* 47 (S3):S216-S227.

Ejeta, G., and Gressel, J. (2007). *Integrating new technologies for Striga control-towards ending the witch-hunt*. World Scientific Publishing Co. Pte, Ltd., Singapore

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S, Mitchell, S. E. (2011a). A robust, simple genotyping-by- sequencing (GBS) approach for high diversity species. *PloS ONE* 6(5):e19379.doi:1371/journal.pone.0019379.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler E. S, Mitchell, S. E. (2011b). *Powerpoint presentation for rapidly genotyping highly diverse species.*

Emberton, J., May, J., Yuan, Y., SanMiguel, P., and Bennetzen, J. L. (2005). Gene enrichment in maize with hypomethylated partial restriction (HMPR) libraries. *Genome Research*, 15(10), 1441-1446.

Esilaba, A. O and Ransom, J. K. (1997). *Striga* in the Eastern and Central African Countries: A literature Review. *Technical Report Series No.1.African Highlands Initiative, ICRAF, Nairobi*.39pp.

Fahey, J. W. (1998). Underexploited African Grain Crops: A nutritional Resource. *Nutr. Rev*. 1998, (56), 282-285[CrossRef] [PubMed]

Fakrudin, B., Kulkan R. S., Shashidhar, H. E., Hittalmani, S. (2004). Genetic diversity assessment of finger millet, Eleusine coracana (Gaerth) germplasm through RAPD analysis. *Biodiversity International Newsletter* 138:50-54.

Fan, J. B., Chee, M. S., and Gunderson, K. L. (2006). "Highly parallel genomic assays," *Nature Reviews Genetics*, 7(8), 632–644.

FAO. (1995). Sorghum and millets in human nutrition. *FAO Food and Nutrition series, (*27). FAO. Rome, Italy.

FAO. (2006). *World agriculture*: *towards 2030/2050*. Prospects for food, nutrition, agriculture and major commodity groups, Food and Agriculture Organization of the United Nations, Global Perspective Studies Unit, Rome

FAOSTAT. (2008). *2000-2007 finger millet production in Kenya and Uganda*. Food and Agricultural Organization annual cereals production statistics. Available:

http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor (verified 30 Oct. 2008).

Fiume, M., Williams, V., Brook, A., and Brudno, M. (2010). "Savant: genome browser for high-throughput sequencing data," *Bioinformatics*, 26(16), 1938–1944.

Flint-Garcia, S. A., Thornsberry, J. M., and Edward, S. B. (2003). "Structure of linkage disequilibrium in plants," *Annual Review of Plant Biology*, 54, 357–374.

Frost, H. M. (1994). *Striga Research and Survey in Kenya*. National Agricultural Research Project, KARI/ODA Crop Protection Project. Final Report. 61pp.

Frost, D. I., Guerney, A. L., Press, M. C & Scholes, J. D. (1997). *Striga hermonthica* reduces photosynthesis in sorghum: The importance of stomatal limitations and a potential role for ABA? *Plant Cell Environ*. 20: 483-492

Gacheru, E., and Rao, M. R. (2001). Managing *Striga* infestation on maize using organic and inorganic nutrient sources in western Kenya. *International Journal of Pest Management* 47, 233-239.

Garvi, M. R., Saitoh, K., and Gharrett, A. J. (2010). "Application of single nucleotide polymorphisms to non-model species: a technical review," *Molecular Ecology Resources*, 10(6), 915–934.

Gebremedhin, W., Goudriaan, J., and Naber, H. (2000). Morphological, phonological and water use dynamics of sorghum varieties (*Sorghum bicolor*) under *Striga hermonthica* infestation. *Crop Prot*., 19(1):61-68

Gethi J G, Smith M E, Mitchell S E, Kresovich S, 2005. Genetic diversity of *Striga hermonthica* and S. asiatica populations in Kenya. *WeedRes* 45:64–73.

Gethi, J. G., Smith, M. E. (2004). Genetic responses of single crosses of maize to *Striga hermonthica* (Del.) Benth. And *Striga asiatica* (L.) Kuntze. *Crop Sci*. 44: 2068-77.

Ghanem, N., Uring-Lambert, B., Abbal, M., Hauptmann, G., Lefranc, M. P., Lefranc, G. (1988). Polymorphism of MHC class III genes: definition of restriction fragment linkage groups and evidence for frequent deletions and duplications. *Hum Genet* 79:209-218.

Glaubitz, J., Casstevens, T., and Lu, F. (2014). TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline. *PLoS One* 9(2): e90346.

Glenn, T. C. (2011). "Field guide to next-generation DNA sequencers," *Molecular Ecology Resources*, 11(5), 759–769.

Ghosh, S., Malhotra, P., Lalitha, P. V., Guha-Mukherjee, and Chauhan, V. S. (2002). Novel genetic mapping tools in plants: SNPs and LD-based approaches. *Plant Science*, 162(3), 329-333.

Gomez, M. I. (1993). *Preliminary studies on Grain Quality, Evaluation for Finger Millet as a Food and Beaverage use in the Southern African Region*. In: K.W., Gupta S.C., Seetharam, A. and Mushonga, J.N. (ed.). *Advances in Small millets*, pp. 289-296. New Dehli: Oxford and IBH.

Gore, M., Bradbury, P., Hogers, R., Kirst, M., Verstege, E., van Oeveren, J., et al. (2007).Evaluation of target preparation methods for single-feature polymorphism detection in large complex plant genomes. *Crop Sci.* (47), S135–S148. doi: 10.2135/cropsci2007.02.0085tpg

Gupta, P. K., Varshney R. K., Sharma P.C., and Ramesh. (1999). Molecular markers and their applications in wheat breeding. *Plant breeding*, 118(5), 369-390.

Gupta, P. K., Rustgi, S., and Kulwal, P. L. (2005). "Linkage disequilibrium and association studies in higher plants: present status and future prospects," *Plant Molecular Biology*, 57(4), 461–485.

Gupta. P., K, Rustgi, S., and Mir, R. R. (2008). Array-based high-throughput DNA markers for crop improvement. *Heredity* (*Edinb.*) (101), 5–18. doi: 10.1038/hdy.2008.35

Gurney, A. L., Press, M. C., and Scholes, J. D. (1999). Infection time and density influence the response of sorghum to the parasitic angiosperm Striga hermonthica. *New phytol.* 143: 573-580.

Haore, D. B., Skerman, P. J., and Riveros, F. (2007). *Eleusine coracana (L.) Gaertn. Gramineae.* FAO Grassland Species Profiles.

Hassan, R. M., and Ransom, J. K. (1998). *Determinants of the incidence of severity of Striga infestation of maize in Kenya*. In: *Maize technology development and transfer:* A GIS application for research in planning in Kenya (Hassan, R.M. and Ransom, J.K.eds.). CABI, Wallingford, UK. pp. 163-174.

Haussmann, B. I. G., Hess, D. E., Welz, H. G., and Geiger, H. H. (2000). Improved methodologies for breeding *Striga* resistant Sorghums. *Field Crops Research* 66: 195-21

Haussmann, B. I. G., and Hess, D. E. (2001). *Striga control: mechanisms and strategies forpromoting sustainable sorghum production in Africa with special emphasis on host plant resistance.* p. 101-117. In I. Akintayo and J. Segoe (ed.).*Towards sustainable sorghum production, utilization, and commercialization in West and Central Africa:* proceedings of a Technical Workshop of the West and Central Africa Sorghum Research network, 19-22 April 1999, Lome, Togo.

Hearne, S. J. (2009). Control – the *Striga* conundrum. *Pest Manag*. *Sci* 65: 603-614.

Hedrick, P. W. (1999). "Perspective: highly variable loci and their interpretation in evolution and conservation," *Evolution*, 53(2), 313–318.

Hess, D. E., Ejeta, G. (1992). Inheritance of resistance to *Striga* in sorghum genotype SRN 39. *Plant Breed* 109:233-41

Hess, D. E., and Lenne, J. M. (1999). *Host-Plant Resistance to Striga in Sorghum and Millet.* ICRISAT, Bamako, Mali.

Hohenlohe, P. A., Bassham, S., Etter, P. D., Stiffler, N., Johnson, E. A, et al. (2010). Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. *PLoS Genet* 6: e1000862 Available: http://www.pubmedcentral.nih.gov/article render.fcgi?artid=2829049&tool=pcentrez&rendertype=abstract

Holen, D. L., Brucker, P. L., Martin, J. M., Carson, G. R., Wichman, D. M., and Berg, J. E. (2001). Response of winter wheat to stimulated stand reduction. *Agronomy Journal* 93:364-370.

Holt, J. (2000). *Investigation into the biology, epidemiology and management of finger millet blast in low-input farming systems in E. Africa*. [Online] Available: http//www.wisard.org/wizard/shared/asp/projectsummary.asp?

[2003, Jul. 08]

Huang, X., Wei, X., Sang et al. (2010). "Genome-wide-association studies of 14 agronomic traits in rice landraces," *Nature Genetics,* 42(11), 961–967.

ICRISAT/FAO (1996). *The world Sorghum and millet economies: facts, trends and outlook*. ICRISAT, Patancheru, India and FAO, Rome. 68pp

ICRISAT. (2008). Archival Report. . http://intranet/ddg/admin%20Pages2009/Documents/Archival_2008.pdf

Iafrate, A. J., Feuk, L., Rivera, M. N., Listewnik, M. L., Donahooe, P. K., et al. (2004). Detection of large-scale variation in human genome. *Nat Genet* 36:949-951.

Jansen, P. C. M., and Ong, H.C. (1996). *Eleusine coracana* (L) *Gaertne*r cv. *Group Finger Millet*. In: Grubben, G. J. H and Partohardjono, S. (Editors). *Plant Resources of South-East Asia* No. 10 Cereals. Bakhuys Publishers, Leiden, Netherlands.pp. 90-95

Jander, G., Norris, S. R., Rounsley, S. D., Bush, D. F., Levin, I. M., and R. L. (2002). Last, "Arabidopsis map-based cloning in the post-genome era," *Plant Physiology*, 129(2), 440–450.

Joel, D. M., Hershenhorn, J., Eizeberg, H., Aly, R., Ejeta, G., Rich, J. P., Ransom, J. K., Sauerborn, J., Rubiales, D. (2007). Biology and management of weedy root parasites. *In Horticultural Reviews*, (33), 267-349.

Johanson, Moller, M., Chaudhary, R., Hellmèn, E., Höyheim, B., Chodhary, B., Andersson, L. (1996). Pigs with dominant white coat color phenotype carry a duplication of the KIT gene encoding the mast/stem cell growth factor receptor. *Mamm Genome* 7:822-830

Johnson, A. (2005). New South Wales. *Witch weed*. http;//www.wyong.nsw.gov.au/environment/Weeds_category_one_Witchweed.pdf.

Kabambe, V., Katunga, L., Kapewa, T., and Ngwira, A. R (2008). Screening legumes for integrated management of witchweeds (*Alectra vogelii* and *Striga asiatica*) in Malawi. *Afr. J of Agric. Res*. 3: 708-715.

Kanampiu, F., Mbogo, P., and Massawe, C. (2004). *Multi-locational testing of herbicide-resistant maize to control Striga.* In: Integrated approaches to higher

productivity in the new millennium (Friesen, D.K. and Palmer, A.F.E.eds). Proceedings of the 7th Eastern and Southern Africa Regional Maize Conference. 5-11 February 2002. Nairobi, Kenya. CIMMYT (International Maize and Wheat Improvement Centre) and KARI (Kenya Agricultural Research Institute). pp 169-172.

Kasembe, E. (1999). *The effect of different cowpea cultivars and time of ridging on witchweed (Striga asiatica L. Kuntze) management in the smallholder farming sector of Zimbabwe*.MPhil Thesis, Crop Science Department, University of Zimbabwe.

Keyes, W. J., O'Malley, R. C., Kim, D., Lynn, D. G. (2000). Signaling organogenesis in parasitic angiosperms: Xenognosin generation, perception, and response. *Journal of Plant Growth Regulation* 19:217-231.

Keyes, W. J., Palmer, A. G., Erbil, W. K., Taylor, J. V., Apkarian, R. P., Weeks, E. R., Lynn, D. G. (2007). Semagenesis and the parasitic angiosperm Striga asiatica. *Plant Journal*, 51:707-716.

Keyes, W. J., Apkarian, R. P., et al. (2001). Dancing together: social controls in parasitic plant development. *Plant Physiology*, 127(4), 1508-1512.

Khan, Z. R., Midega, C.A.O., Hassanali, A., Pickett, J. A., and Wadhams, L.J. (2007). Assessment of different legumes for the control of Striga hermonthica in maize and sorghum. *Crop Sci*. 47: 730-736.

Khan, Z. R., Midega, C. A. O., Wanyama, J. M., Amudavi, D. M., Hassanali, A., Pittchar, J., and Pickett, J. A. (2009). Integration of edible beans (Phaseolus vulgaris L.) into the push–pull technology developed for stemborer and Striga control in maize-based cropping systems. *Crop Prot*. 28: 997-1006.

Khan, Z. R, Pickett, J. A., Wadhams, L. J., Hassanali, A., and Midega, C. A. O. (2006). Combined control of Striga hermonthica and stemborers by maize- Desmodium spp. intercrops. *Crop Prot*. 25: 989-995.

Khan, Z. R., Hassanali, A., Overholt, W. A., Khamis, T. M., Hooper, A. M., Pickett, J. A., Wadhams, L., and Woodcock, C. M. (2002). Control of witchweed *Striga hermonthica* by intercropping with *Desmodium* spp. and mechanisms defined as allelopathic. *Journal of Chemical Ecology*, 28, 1871-1885.

Kim, S. K., Akintunde, A.Y., and Walker, P. (1999). Response of maize inbreds during development of *Striga hermonthica* infestation. *Maydica* 44:318-333.

Kim, S. K., and Adetimirin, V. O. (2001). Conditioning effects of *Striga hermonthica* seed on field performance of maize. Crop Protect. 20: 159-161.

Kim, S. K. (1994). Genetics of maize tolerance of *Striga hermonthica*. *Crop science* 34: 960-967

Kim, S. K., and Winslow. (1991). *Progress in breeding for Striga tolerance resistance at IITA.* pp. 493-499. In: K. Ransome, L.J Musselman, A.D Worsham, G. Parker (eds.). Proc. 5th Int. Symposium on Parasitic Weeds, 24-30 June 1991. Nairobi, Kenya, CIMMYT, Mexico.

Kochert, G. (1994*). RFLP technology*. In: Philips R.L. and Vasil I.K. (eds), *DNA-Based Markers in Plants*. Kluwer Academic Publishers, Dordrecht, pp. 8–38.

Konishi, S., Izawa, T., Lin, S. Y., et al. (2006). "An SNP caused loss of seed shattering during rice domestication," Science, vol. 312, no. 5778, pp. 1392–1396.

Kothiyal, P., Cox, S., Ebert, J., Aronow, Greinwald, J. H., and Rehm, H. L. (2009). "An overview of custom array sequencing", *Current Protocols in Human Genetics* 61(7) 1-17.

Kuiper, E., Groot, A., Noordover, E.C.M., Pieterse, A.H., and Verkleij, J. A. C. (1998). Tropical grasses vary in their resistance to *Striga aspera, Striga hermonthica,* and their hybrids. *Canadian Journal of Botany* 76:2131-2144.

Kumari, P. L., and Sumathi, S. (2002). Effect of consumption of finger millet on hyperglycemia in non-insulin dependent diabetes mellitus (NIDDM) subjects. *Plant Foods for Human Nutrition* 57:205-213.

Lagoke, S. T., Parkinson, V., and Agiunbiade, R. M. (1991). Parasitic weeds and control methods in Africa. In: S.K. Kim (ed). pp. 3-15. *Combating Striga in Africa: Proc. International Workshop organized by IITA,* ICRISAT, and IDRC, 22-24 August, 1988. IITA, Ibadan, Nigeria.

Langmead, B., Trapnell, C., Pop, M., and Salzberg, L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology*, 109(3), R25.

Langmead, B., Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9: 357–359

Li, H., and Durbin. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, 25(14), 1754-1776.

Li, R., Yu, C., Li, Y et al. (2009). "SOAP2: an improved ultrafast tool for short read alignment". Bioinformatics, 25(15), 1966-1967.

Li, R., Li, Y., Fang, X., et al. (2009). "SNP detection for massively parallel whole-genome resequencing," *Genome Research*, 19(6), 1124–1132.

Libault, M., Farmer, A., Joshi, T., et al. (2010). "An integrated transcriptome atlas of the crop model Glycine max, and its use in comparative analyses in plants," *Plant Journal*, 63(1), 86–99. .

Lu, T., Lu, G., Fan et al. (2010). "Function annotation of the rice transcriptome at single-nucleotide resolution by RNA-seq," *Genome Research*, 20(9), 1238–1249.

Lu, F., Lipka, A. E., Elshire, R. J., Glaubitz, J. C., Cherney, J., et al. (2013). Switch grass genomic diversity, ploidy and evolution: novel insights from a network-based SNP discovery protocol. *PloS Genet* 9: e1003215.

MacOpiyo, L., Vitale, J., and Sanders, J. (2010). *AN ex-ante assessment of a Striga control programme in East Africa.* Kilimo Trust, pp6-25.

Mammadov, J. A., Chen, W., Ren, R., et al. (2010). Development of highly polymorphic SNP markers from the complexity reduced portion of maize (Zea mays L.)

genome for use in marker-assisted breeding. *Theoritical and Applied Genetics*, 121(3), 577-588.

Manyong, V. M., Nindi S. J., Alene A. D., Odhiambo G. D., Omanya G., Mignouna H. D., and Bokanga, M. (2008). *Farmers' perception of Imazapyr-Resistant (IR) maize technology on the control of Striga in Western Kenya*. Nairobi, Kenya: African Agricultural Technology Foundation, pp3-50.

Mascher, M., Wu, S., Amand, P. S., Stein, N., Poland, J. (2009). Application of Genotyping-by-sequencing on semiconductor sequencing platform: A Comparison of Genetic and Reference-Based Marker Ordering in Barley. *PLos One* 8: e76925.

M'Boob, S. S. (1986). A regional programme for West and Central Africa. In: Proceedings FAO/OAU all African Government Consultation on *Striga* control, Maroua, Cameroon, 20-24. October 1986. FAO, Rome, 190-194.

Mburu, C. (1989). *Cropping systems, production technology and utilization of small millets with special reference to finger millet in Kenya* Pages 305-307. In small millets in global agriculture. Seetharam, A., Riley, K.W., and Harinayana, G., ed: New Dehli: oxford and IBH.

McElroy, K. E., Luciani, F., Thomas, T. (2012). GemSIM: general error Model based simulator of next-generation data. *BMC genomics* 13: 74.

McLendon, R., Friendman, A., Bigner, et al. (2008). "Comprehensive genomic characterization defines human glioblatoma genes and core pathways", *Nature*, 455(7216), 1061-1068.

McCarthy, M. I., Abecasis, G. R., Cardon et al. (2008). "Genome-wide association studies for complex traits: consensus, uncertainty and challenges," *Nature Reviews Genetics*, 9(5), 356–369.

McPherson, J. D. (2009). Next-generation gap. Nature Methods, 6(11), S2-S5.

Menkir, A., Makumbi, D., and Franco, J. (2012b). Assessment of reaction patterns of hybrids to *Striga hermonthica* (Del.) Benth. Under artificial infestation in

Kenya and Nigeria. *Crop Sci.* (52)2528–2537. doi:10.2135/cropsci2012.05.0307

Metzker, M. L. (2010). Sequencing technologies – the next generation. *Nat Rev Genet* (11)31-46. doi: 10.1038/nrg2626.

Mgonja, M. A. (2005). Finger millet: research revival in East Africa. *SATrends Issue* 59 October 2005 (ICRISAT monthly newsletter).

Milne, I., Bayer, M., Cardle, L. et al. (2009). "Tablet-next generation sequence assembly visualization," *Bioinformatics*, 26(3), 401–402.

M.S. Swaminathan Research Foundation India, n.d. Kolli Hills. (2003). *Diversity of millets.* [Online] Available: http://www.mssrf.org/fris9809/kolli-millets.html.

Mitaru, B. N., Karugia, J. T., and Munene, C. (1993). *Finger millet production and utilization in Kenya*. In: Riley, K.W., Gupta, S.C., Seetharam, A. and Mushonga, J.N. (ed.). *Advances in small millets*. pp. 247-254. New Dehli: Oxford and IBH.

MoA. (1989-2004). Ministry of Agriculture Annual Reports 1989–2004. Ministry of Agrisculture Headquarters, Kilimo House, Nairobi Kenya.

Mohamed, A. H., Ejeta, G., Butler, L. G., and Housley, T. L. (1998). Moisture content and dormancy in *Striga asiatica* seeds. *Weed Res*. (38), 257-265.

Mohamed, A. H., Rich, P., Housley, T. L., and Ejeta, G. (2001). In-vitro techniques to screen for mechanisms of Striga resistance in Sorghum.p. 96-100. *In* A. Fer et al. (ed.) Proc. 7th Int. parasitic Weed Symposium, Nantes, France. 5-8 June 2001. Faculté des Sciences, Univ. de Nantes, Nantes, France.

Mohamed, K. I., Papesx, M., Williams, R., Benz, B. W., Peterson, A. T. (2006). Global invasive potential of ten parasitic witch weeds and related *Orobanchaceae*. *Ambio* (35), 1–10.

Moose, S. P., and Mumm, R. H. (2008). Molecular plant breeding as the foundation for 21 century crop improvement. *Plant Physiol.* 147, 969–977. doi: 10.1104/pp.108.118232

Morin, P. A., Luikart, G., and Wayne. R. K. (2004). "SNPs in ecology, evolution and conservation," *Trends in Ecology and Evolution*, 19(4), 208–216,

Morishige, D. T., Klein, P. E., Hilley, J. L., Sahraeian, S. M., Sharma, A., et al. (2013). Digital genotyping of sorghum- a diverse plant species with a large repeat-rich genome. *BMC Genomics* 14: 448.

Morris, G. P., Ramu, P., Deshpande, S. P., Hash, C. T., Shah T., et al. (2009). *Population genomic and genome-wide association studies of agroclimatic traits in sorghum*. Proc Natl Acad Sci U.S.A 110: 453-458.

Mumera, L., and Bello. (1993). *Striga infestation in maize and sorghum relative to cultivar, herbicidal activity and nitrate*. *In*: Proceedings, 9th East African Weed Science Society Conference, Nairobi, 1983, pp. 758-763.

Mundt, C. C. (2002). Use of multiline cultivars and cultivar mixtures for disease management. *Annual Review of Phytopathology* 40:381-410. NRC

Murty, D. S., Bello, S. A., Aladele, S. E. (1995). Screening sorghum for resistance to *Striga* under artificial field inoculation. *International Sorghum and Millets Newsletter.* 36: 84-86.

Mushonga, J. N., Muza, F. R., and Dhliwayo, H. H. (1993). *Development, current and Future Research Strategies on Finger Millet in Zimbabwe*. In: Riley, K.W., Gupta Setharam, A. and Mushonga, J.N. (ed.). *Advances in small millets*. pp. 11-19. New Dehli: Oxford and IBH.

Musselman, L. J. (1987). *Parasitic weeds in Agriculture*. Striga CRC Press, Boca Raton, pp 317

Musselman, L. J., Bharathalakshmi, S. B., Safa, D. A., Knepper, K. I., Mohamed, C. L., White. (1991). *Recent research on the biology of Striga asiatica, S. gesnerioides* and *S. hermonthica*. Pages 31–41 in SK Kim, ed. *Combating Striga in Africa*. Proceedings, International Workshops organized by International Institute of Tropical Agriculture (IITA),International Crops Research Institute for the Semi-

Arid Tropics(ICRISAT), and International Development Research Centre (IDRC), August 22–24, 1988. IITA, Ibadan, Nigeria.

Musselman, L. J., and Press, M. C. (1995). *Introduction to parasitic plants*. In: Press M.C., Graves J. D. (ed.). *Parasitic Plants*. Chapman & Hall, London, UK, 1–13.

Narechania, A., Gore, M. A., Buckler, E. S., et al. (2009). Large –scale discovery of gene enriched SNPs. *The plant Genome*, 2(2), 121-133.

National Research Council. (1996). *Lost crops of Africa*; Volume I Grains. National Academy Press, Washington, DC

Nelson, J. C., Wang, S., Wu, Y., et al. (2011). Single nucleotide polymorphism discovery by high-throughput sequencing in sorghum. *BMC Genomics*, 12, article 352.

Obilana, A. T., Manyasa, E. O., Kibuka, J. G., and Ajanga, S. (2002). *Finger millet blast (fmb) samples collection in Kenya*: Passport data, analyses of disease incidence and report of activities. ICRISAT, Nairobi, Kenya.

Odhiambo, G. D., and Ariga, E. S. (2004). *Effect of intercropping maize beans on Striga incidence and grain yield. In*: *Integrated Approaches to Higher Productivity in the new millennium* (Friesen, D. K. and Palmer, A. F. E. (eds.). Proceedings of the 7th Eastern and Southern Africa Regional Maize Conference.5-11 February 2002, Nairobi, Kenya. CIMMYT (International Maize and Wheat Improvement Centre) and KARI. pp 183-186.

Odhiambo, G. D., and Ransom, J. K. (1994). *Preliminary evaluation of long-term effects of trap cropping and maize management on Striga*. *In*: *Biology, and management of Orobanche* (Pieterse A.H, Verkleij J.A.C and ter bora S.J. eds). Proceedings of the 3rd International workshop on *Orobanche* and related *Striga* research. Royal Tropical Institute, Amsterdam. The Netherlands. pp 505-512.

Odongo, M. O., and Abayo, G. (1999). *Performance of maize varieties under Striga and Striga free environment in western Kenya*. Proceedings of the 6th Biennial

Kenya Agricultural Research Institute (KARI) Scientific Conference. Agronam Services Ltd (ed). November 9-13 1998, Nairobi, Kenya. Kenya Agricultural Research Institute, Nairobi, Kenya. pp 390-394.

Oduori, C. O. A. (1993). *Small Millets Production and Research in Kenya. In*: Riley, K.W, Gupta, S.C, Seetharam, A. and Mushonga, J.N. (ed.). *Advances in small millets.* pp. 67-73. New Dheli: Oxford and IBH.

Oduori, C. O. A., and Kanyenji, B. (2005). *Finger Millet in Kenya: Importance, Advances in R&D, Challenges and Opportunities for Improved Production and Profitability*. In Mgonja MA, Lenné JM, Manyasa E and Sreenivasaprasad S. (eds.). 2007. *Finger Millet Blast Management in East Africa*. Creating opportunities for improving production and utilization of finger millet. Proceedings of the First International Finger Millet Stakeholder Workshophttp://ubeds.build.squiz.co.uk/__data/assets/pdf_file/0005/127607/Finger-Millet-Blast-Ref-Manual.pdf#page=116

Oduori, C. O. A. (2000). *Finger Millets*. Kenya Agricultural Research Institute. [Online] Available [2004, Apr. 10]

Ogborn, J. E. A. (1987). *Striga controls under present farming conditions*. In L.J. Musselman (ed.) *Parasitic weeds in agriculture*. CRC Press Inc., Boca Raton, Florida. 1:145-158.

Okou, D. T., Steinberg, K. M., Middle, C., Cutler, D. J., Albert, T. J., and Zwick, M. E. (2007). microarray-based genomic selection for high-throughput resequencing. *Nature Methods*, 4(11), 907-909.

Omanya, G. O., Haussmann, B. I. G., Hess, D. E., Reddy B. V. S., Mukuru, S. Z., Welz H. G.,and Geiger, H. H. (2001). *Variation for Indirect and Direct Measures of resistance of sorghum (Sorghum bicolor* (L.) Moench]. Dissertation, University Hohenheim, Stuttgart, Germany.

Omanya, G. O., Haussmann, B. I. G., Hess, D. E., Reddy, B. V. S., Kayentao, M., Weiz, H. G., and Geiger, H. H. (2004). Utility of indirect and direct selection traits for

improving *Striga* resistance in two sorghum recombinant inbred populations. *Field crops Research* 89:237-252.

Ophir, R., and Graur, D. (1997). Patterns and rates of indel evolution in processed pseudogenes from humans and murids. *Gene*, 205(1-2), 191-202.

Oryogot, J. (1994). *Striga*: Strategies for its control-a review. *African Crop Science Conference Proceedings* 1, pp 224-226

Osborne, C. P., and Freckleton, R. P. (2009). Ecological selection pressure for C4 photosynthesis in the grasses. *Proc. R. Soc. B.* 276: 1753-1760

Osborne, C. P., and Beerling, D. J. (2006). *Nature's green revolution: the remarkable evolutionary rise of C4 plants*. Philosophical Transaction of the Royal Society of London, Series B, 361, 173-194

Oswald A, 2005. Striga control-technologies and their dissemination. *Crop protection* 24:333-342.

Pasam, R. K., Sharma, R., Malosetti, M., et al. (2012). "Genome-wide association studies for agronomical traits in a worldwide spring barley collection," *BMC Plant Biology*, 12, article 16.

Parker, C., Riches, C. R. (1993). *Parasitic Weeds of the World: Biology and control.* CAB Int., Walliford. U.K.

Parker, C. (2009). Observations on the current status of Orobanche and Striga problems worldwide. *Pest Manag. Sci*. 65: 453-459

Patterson, D. T., Musser, R. L., Flint, E. P., Eplee, R. E. (1982). Temperature responses and potential for spread of witch weed (*S. lutea/S. asiatica*) in the United States. *Weed Sci* 30:87–93.

Patrick, J. R., Grenier, C., and Ejeta, G. (2004). *Striga* resistance in the wild relatives of sorghum. *Crop Science* 44: 2221-2229.

Pieterse, A. H., and Persche, C. J. (1983). The witchweeds (Striga spp). A review. Abstracts of Tropical Agriculture 9: 9-37

Pfender, W. F., Saha, M. C., Johnson, E. A., Slabaugh, M. B. (2011). Mapping with RAD (restriction-site associated DNA) markers to rapidly identify QTL for stem rust resistance in *Lolium perenne*. *Theor Appl Genet*, 122(8):1467-1480.

Poland, J. A., Brown, P. J., Sorrells, M. E., Jannick, J. L. (2012). Development of high-density genetic maps of barley and wheat using a novel two enzyme genotyping-by-sequencing approach. *Plos ONE*, 7(2), Article ID e32253.

Poland, J. A., Brown, P. J., Sorrells, M. E., and Jannink, J. L. (2012a). Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. *PLoS ONE* 7:e32253.doi: 10.1371/journal.pone.0032253

Powell W, Machray G.C, and Proven J, 1996. Polymorphism revealed by simple sequence repeats. *Trends in Plant Science*, 1(7), 215-222.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, T., Tingey, S., and Rafalski, J. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed*.3: 225-238.

Press, M. C., Scholes, J. D., and Watling, J. R. (1999). *Parasitic plants: Physiological and ecological interaction with their hosts.* In: Press M.C, Scholes, J. D. and Barker, M.D (eds). *Physiological plant ecology*. Blackwell Science, Oxford pp. 175-197.

Press, M. C., and Graves, J. D. (1995). Parasitic plants. Chapman and Hall, London. Press, M.C. and Stewart, G.R. 1987. Growth and photosynthesis in Sorghum bicolor infected with Striga hermonthica. Ann. Bot. 60, 657-662.

Press, M.C., and Stewart G.R, 1987. Growth and photosynthesis in sorghum bicolor infected with Striga asiatica. *Annals of Botany* 60: 657-662.

Prichard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.

Prliç, A., Yates, A., Bliven, S. E., Rose, P. W., Jacobsen, J., et al. (2012). BioJava: an open source framework for bioinformatics in 2012. *Bioinformatics* 28: 2693-2695.

Rafalski, A. (2002). "Applications of single nucleotide polymorphisms in crop genetics," *Current Opinion in Plant Biology*, 5(2), 94–100.

Raj, A., Stephens, M., and Prichard, J. K. (2014). FastSTRUCTURE: Variational inferecnceof population structure in large SNP datasets. *Genetics* 197:573-589. Doi:10.1534/genetics.114.164350

Ramaiah, K. V. (1987). *Breeding cereal grains for resistance to Witch-weed*. In: LJ Musselman (ed.). *Parasitic weeds in Agriculture* vol. 1. CRC press, Boca Raton, FL. pp. 227-242.

Ramasamy, P., Subbaraman, N., Venkatachalam, R., and Soundraapandian. (1996). Evaluation of enviroments in finger millet genotypes, Inti. Small Small Millets *Newslett.* 37:78-79

Ranson, J. K. (1996). *Integrated management of Striga spp. in the agriculture of sub-Saharan Africa. In*: Proceedings of the second international Weed Control Congress. Copenhagen. Pp. 623-628.

Ranson, J. K., and Odhiambo, G. D. (1995). Effect corn (*Zea mays*) genotypes which vary in maturity length on *Striga hermothica* parasitism. *Weed Technology* 9:63-67

Ransom, J. K., Wawire, N. W. O., and Thomas, Compton, M. A. (1990). *Yield losses due to Striga. In*: National *Striga* Weed Workshop, Kisumu, Kenya, 18-21 June 1990. 8p.

Ransom, J. K., Odhainabo, G. D., Eplee, R. E., Diallo, A. O. (1996). *Estimates from field studies of the phytotoxicity effects of Striga spp. on maize*. In: Moreno MT, Parker C, editors. *Advances in parasitic plant research*. Proceedings of the 6th International Symposium on the Parasitic Weed. Cordoba, Spain, 16–18 April 1996. p. 327– 333.

Rebetzke, G. J., Botwright, T. L., and Moore, C. S., Richards, R. A., Condon, A. G. (2004). Genotypic variation in specific leaf area for genetic improvement of early vigour in wheat. *Field Crops Research* 88:179–189.

Retief, J. D. (2000). "Phylogenetic analysis using PHYLIP," *Methods in Molecular Biology*, 132: 243–258.

Richards, R. A., and Lukacs, Z. (2002). Seedling vigour in wheat - sources of variation for genetic and agronomic improvement. *Australian Journal of Agricultural Research* 53:41-50.

Riches, C. R., and Parker, C. (1995). *Parasitic plants as weeds*. In: M.C. Press and J.D. Graves (eds): *Parasitic plants*. Chapman and Hall, London. 226-255.

Riley, K.W., Gupta, S. C., Setharam, A., and Mushonga, J. N. (1993). (ed.). *Advances in small Millets*: Proc. Second International Small Millets Workshop, April 1991. Bulawayo, Zimbabwe.

Rispail, N., Dita, M. A., González Verdejo, C., Pérez-de-Luque, A., Castillejo, M-a., Prats E., Román, B., Jorrín, J., Rubiales, D. (2007). Plant resistance to parasitic plants: molecular approaches to an old foe. *New Phytol*; 173: 703-12.

Robertson, G., Hirst, M., Bainbridge, M., et al. (2007). "Genome-wide profiles of STAT1 DNA association using chromatin immunoprecipitation and massively parallel sequencing," *Nature Methods*, 4(8), pp. 651–657.

Romay, M. C., Millard, M. J., Glaubitz, J. C., Peiffer, J. A., Swarts, K. L., Casstevens, T. M.,et al. (2013).Comprehensive genotyping of the USA national maize inbred seed bank. *Genome Biol.* 14, R55. doi: 10.1186/gb-2013-14-6-r55

Rodenburg, J., Bastiaans, L., Schpendonk, A. H. C. M., van der Putten, P. E. L., van Ast A., Dingemanse, N. J., et al. (2008). *CO-assimilation and chlorophyll fluorescence as indirect selection criteria for host tolerance against Striga*. *Euphytica*, 160, 75-87

Rodenburg, J., Bastiaans L., Kropff M. J. (2006). *Characterization of the host tolerance to Striga hermonthica*. Euphytica. 147:353-365.

Rohrbach, D. D., and Mazvimavi, K. (1993). *Opportunities to Exploit a Premium Market Niche for Finger Millet in SADCC Region*. In: Riley, K.W., Gupta, S.C.,

Seetharam, A. and Mushonga, J. N. (ed.). Advances in small finger millets. pp. 309-324. New Dehli: Oxford and IBH.

Rohrbach, D. (1991). *The impact of new sorghum and millet technologies in the evolving grain market of Southern Africa*, Pages 51-60. Proceedings of the International Sorghum and Millet Conference, 8-12 July 1991. Corpus Christi, Texas, USA.

Rooney, L. W., McDonough, C. M. (1986). *Food quality consumer acceptance of Pearl millet. In*: "Proceeding of International pearl millet workshop 7-8 April 1986", (J.R Witcombe and S.R Beckerman ed.), ICRISAT Patancheru, India (1987).

Roozrokh, M., Golozani, K. G, and Javanshir, A. (2002). Relationship between seed vigour and field performance in chickpea (Cicer arietinum L.). *Seed and Plant* 18:156-169.

SAS Institute. 2003. SAS/STAT user's guide. Release 9.1. ed. SAS Institute Inc., Cary, NC, USA.

Rounsley, S., Marri, P. R., Yu, Y., et al. (2009). "De novo next generation sequencing of plant genomes", *Rice*, 2(1), 35-43.

Rukuni, M., Tawonezvi, P., Eicher, C., Munyuki-Hungwe, M., and Matondi, P. (2006). Zimbabwe's Agricultural Revolution Revisted, University of Zimbabwe Publications. Harare, Zimbabwe.

Rumble, S. M., Lacroute, P., Dalca, A. V., Fiume, M., Sidow, A., and Brudno, M. (2009). "SHRiMP: accurate mapping of short color-space reads". PloS Computational Biology, 5(5), Article ID e1000386.

Sahu, B. B., Sumit, R., Srivastava, S. K., and Bhattacharya, M. K. (2012). Sequence based polymorphic (SBP) marker technology for targeted genomic regions: its application in generating a molecular map of the Arabidopsis thaliana genome. *BMC Genomics* 13:20, doi: 10.1186/1471-2164-13-20.

Sand, Paul, Robert Eplee, and Randy Westbrooks. (1990). *Witchweed Research and Control in the United States.* Champaign, IL: Weed Science Society of America.

SAS Institute, 2003. SAS/STAT user's guide. Release 9.1. (ed.). SAS Institute Inc., Cary, NC, USA.

Satish, K., Gutema, Z., Grenier, C., Rich, P. J., and Ejeta, G. (2012). Molecular tagging and validation of microsatellite markers linked to the low germination stimulant gene (lgs) for *Striga resistance* in sorghum [*Sorghum bicolor* (L.) Moech]. *Theor. Appl. Genet*. 124, 989-1003.

Sauerborn, J. (1991). *The economic importance of the phytoparasites Orobanche and Striga.* P.137- 143. In J.K. Ranison et al. (ed.) Proc. 5th Int. Symp. Parasitic weeds, Nairobi, Kenya. 24-30 June 1991. CIMMYT, Nairobi, Kenya.

Schadt, E. E., Turner, S., and Kasarskis, A. ((2010). "A window into third-generation sequencing," *Human Molecular Genetics*, 19(2), R227–R240, 2010.

Scholes, J. D., Press, M. C. (2008). *Striga* infestation of cereal crops-an unsolved problem in resource limited agriculture. *Current Opinion in Plant Biology* 11: 180-186.

Seo, B. Y., Park, E. W., Ahn, S. J., Lee, S. H., Kim, J. H., et ai. (2007). *An accurate method for quantifying and analyzing copy number variation in porcine KIT by an oligonucleotide ligation assay*. BMC Genet 8:81.

Semagn K, Bjørnstad A and Ndjiondjop M. N, 2006. Principles, requirements and prospects of genetic mapping in plants, *African Journal of Biotechnology* 5(25): 2569-2587

Shah, N., Teplitsky, M. V., Minovitsky, S., et al. (2005). "SNP-VISTA: an interactive SNP visualization tool," *BMC Bioinformatics*, 6(1), article 292.

Shah, N., Smirnoff, N., Stewart, G. R. (1987). Photosynthesis and stomatal characteristics of Striga hermonthica in relation to the parasitic habit. *Physiol Plantarum* 69:699–703.

Sharp, A. J., Locke, D. P., McGrath, S. D., Cheng, Z., Bailey, J. A., et al. (2005). Segmental duplications and copy number variation in the human genome. *Am J Hum Genet* 77:78-88

She, X., Liu, G., Ventura, M., Zhao, S., Misceo, D., et al. (2006). A preliminary comparative analysis of primate segmental duplications show elevated substitution rates and great-ape expansion of intra-chromosomal duplications. *Genome Res* 16:576-583

Shendure, J. A., Porreca, G. J., and Church, G. M. (2008). "*Overview of DNA sequencing strategies,*" Current Protocols in Molecular Biology, chapter 7, no. 81, pp. 7.1.1–7.1.11.

Sivagurunathan, M. (2005). *Genetic and molecular studies on quantitative and qualitative traits in finger millet*. (Eleusine coracana (L.) Gaertn.) M.Sc.(Ag.), Thesis submitted to T.N.A.U., Coimbatore, India.

Smale, M., and McBride, T. (1996). '*Understanding Global trends in the use of wheat diversity and international flows of wheat genetic resources'*, Part 1 of CIMMYT 1995/96 World Wheat Facts and Trends: Understanding Global Trends in the Use of Wheat Diversity and International flows of Wheat Genetic Resources, Centro international de Mejoramiento de Maiz Y Trigo (CIMMYT), Mexico.

Smith, J. S. C., Kresovich, S., Hopkins, M. S., Mitchell, S. E., Dean W.L., Woodman, R.E., Lee, M., and Porter, K. (2000). Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. *Crop Sci*., 40: 226–232.

Sorrells, M. E., Gustafson, J. P., Somers, D., Chao, S., Benscher, D., et al. (2011). Reconstruction of the Synthetic W7984×Opata M85 wheat reference population. *Genome* 54: 875–882.

Steppuhn, H. (1997). *Increasing plant density in spring wheat to ameliorate the effects of salinity on grain yield.* Transactions of the American Society of Agricultural Engineers (ASAE) 40: 1599-1606.

Stewart, G. R, and Press, M. C. (1990). The physiology and biochemistry of parasitic angiosperms. *Annu Rev Plant Mol Biol* 41:127–151.

Stroud, A. (1993). *Control of weeds*. In dry farming in Africa Macmillan Press pp 172-187. Hong Kong: Technical Centre for Agriculture and Rural Co-operation.

Stuber, C. W., Moll, R. H., and Hanson, W. D. (1966). *Crop. Sci*. 6, 455-458

Swabrick, J. T., Timmins, S. N., and Bullen, K. M. (1999). The biology of Australian weeds. Ligustrum lucidum Aiton and Ligustum sinense Lour. *Plant Protection Quarterly,* 14: 122-130

Tadesse, M., and Kebede, Y. (1993). *Finger Millet Importance and Improvement in Ethiopia*. In: Riley, K.W., Gupta, S.C., Seetharam, A. and Mushonga, J.N. (ed.). *Advances in small millets*. pp. 51-59. New Dehli: Oxford and IBH.

Taken, J. P., Muthumeenakshi, S., Sreenivasaprassad, S., Akello, B., Bandyopadhyay, R., Coll, R., Brown, A. E., & Talbot, N. J, 2002. *Characterization of finger millet blast pathogen populations in East Africa and strategies for disease management*. [online] Available: [2003, Jul.08]

Taylor, A., Martin, J., and Seel, W. E. (1996). Physiology of the parasitic association between maize and witchweed (S. hermonthica): is ABA involved? *Journal of Experimental Botany* (47), 1057-65. http://dx.doi.org/10.1093/jxb/47.8.1057

Taylor, J. R. (2003). *Overview importance of sorghum in Africa* [Online]. Available from: http://www.sciencedirect.com/science?_[Access 31 July 2008].

Tenywa, J. S., Nyende, P., Kidoido, M., Kasenge, V., Oryokot, J., and Mbowa, S. (1999). Prospects and constraints of finger millet production in eastern Uganda. *African Crop Science Journal* 7:569-583.

Tester, M., and Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. *Science* 327, 818–822. doi: 10.1126/science.1183700

Thompson, J. D., Higgins, D. G., Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position- specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673-4680.

Thornsberry, J. M., et al. (2001). Dwarf8 polymorphisms associate with variation in flowering time. *Nat. Genet*; 28: 286-289

Teka, H. B. (2014). Advance research on Striga control: A review. *Africa Journal of Plant Science,* 8(11), 492-506.

Tian, F., Bradbury, P. J., Brown, P. J., et al. (2011). "Genome-wide association study of leaf architecture in the maize nested association mapping population," *Nature Genetics,* 43(2), 159–162.

Trask, B. J., Friedman, C., Martin-Gallardo, A., Rowen, L., Akinbami, C., et al. (1998). Member of the olfactory receptor gene family are containedin large blocks of DNA duplicated polymorphically near the ends of human chromosomes. *Human Mol Genet* 7:13-26.

Trebbi, D., Maccaferri, M., de Heer, et al. (2011). "High-throughput SNP discovery and genotyping in durum wheat (Triticum durum Desf.)," *Theoretical and Applied Genetics,* 123(4), 555–569.

Trewavas, A. J., and Jones, H. G. (1991). *An assessment of the role of ABA in plant development.* In *Abscicic Acid: Physiology and Biochemistry* (eds W. J. Davies and H. G Jones), pp. 169-188. BIOS Scientific, Oxford.

Turcatti, G., Romieu, A., Fedurco, M., and Tairi, A. P. (2008). "A new class of cleavable fluorescent nucleotides: synthesis and optimization as reversible terminators for DNA sequencing by synthesis," *Nucleic Acids Research,* 36(4) article e25.

Umeliara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda- Kamiya, N., et al. (2008). Inhibition of branching by new terpenoid plant hormones. *Nature*, 455, 195-200.

Upadhyaya, H. D., Gowda, C., Pundir, R., Reddy, V., and Sube, S. (2006). Development of core subset of finger millet germplasm using geographical origin and data on 14 quantitative traits. *Genetic Resources and Crop Evolution* 53:679-685.

Van Ast, A., and Bastiaans, L. (2006). The role of infection time in the differential response of sorghum cultivars to *Striga hermonthica* infection. *Weed Res.* 46: 264–274.

Van Delft, G. J. (1997). *Root architecture in relation to avoidance of Striga hermonthica infection*. PhD thesis, University of York, UK.

Van Deynze, A., Stoffel, K., Buell, C. R., Kozik, A., Liu, J., van der Knaap, E., Francis D. (2007). Diversity in conserved genes in tomato. *BMC Genomics*, 8:465.

Vanlauwe, B., Kanampiu, F., Odhiambo, G. D., De Groote, H., Wadhams, H. J., and Khan, Z. R. (2008). Integrated management of *Striga hermonthica*, stemborers, and declining soil fertility in Western Kenya. *Field Crops Res*. 107: 102-115.

Van Mourik, T. (2007). *Striga hermonthica seed bank dynamics: process quantification and modeling.* PhD thesis, Wageningen University, The Netherlands. ISBN: 978-90-8504- 692-9.With summaries in English, French and Dutch. http://library.wur.nl/wda/dissertations/dis4240.pdf

Van Orsouw, N. J., Hogers, R. C., Jansen, A., et al. (2007). Complexity reduction of polymorphic sequences (CroPS): a novel approach for large-scale genomes. *PloS ONE*, 2(11).

Van Wyk, B. E., and Gericke, N. (2000). *People's plants: a guide to useful plants of southern Africa.* Arcadia, Pretoria, South Africa: Briza Publications.

Varshney, R. K., Kumar, A., Balyan, H. S., Roy, J. K., Prasad M, et al. (2001). *Characterization of microsatellites and development of chromosome specific STMS markers in bread wheat*. Plant Mol Biol Rep 18:5–16. doi: 10.1007/bf02824006

Vignal, A., Milan, D., SanCristobal, M., and Eggen, A. (2002). "A review on SNP and other types of molecular markers and their use in animal genetics," Genetics Selection Evolution, vol. 34, no. 3, pp. 275–305.

Vogler, R. K., Ejeta, G., Buttler, L. G. (1996). Inheritance of low production of *Striga* germination stimulant in sorghum. *Crop Science*, 36:1185-1191.

Vos, P., Hogers, R., Bleeker, et al. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, 23(21), 4407-4414.

Wallace, J. G., Upadhyaya, H. D., Vetriventhan, M., et al. (2015). *The genetic makeup of global barnyard millet germplasm collection*. Plant Genome 8. Doi:10.3835/plantgenome2014.10.0067

Wang, S., Meyer, E., Mckay, J. K, Matz, M. V. (2012b). *RAD: a simple and flexible method for genome-wide genotyping.* Nat Methods 9: 808-810. Available: http//www.nlm.nih.gov/pubmed/22609625.

Wang, W., Wei, Z., Lam, T. W., Wang, J. (2011). Next generation sequencing has lower sequence coverage and poorer SNP detection capability in the regulatory regions. *Scientific Reports,* vol. 1, article 55.

Wang, D. G., Fan, J. B., Siao, et al. (1998). Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science,* vol. 280, no. 5366, pp.1077-1082.

Watson, A. K., Ciotola, M, and Peden, D. (1998). *Controlling of the noxious striga weed.* International Development Research Centre, Ottawa, Canada. Weed Res. 35: 303-309.

Waugh, R., Jannink, J. L., Muehlbauer, G. J, and Ramsay, L. (2009). "The emergence of whole genome association scans in barley," *Current Opinion in Plant Biology*, 12(2), 218–222.

Weber, J. L., and May, P. E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics*, 44(3), 388-396.

Weber, G., Elemo, K., Lagoke, S. T. O., Awad, A., and Oikeh, S. (1995). Population dynamics and determinants of *Striga hermonthica* on maize and sorghum in savanna farming systems. *Crop Prot*. 14: 283-290.

Wei, Z., Wang, W., Hu, P., Lyon, G. J., and Hakonarson, H. (2011). "SNVer: a statistical tool for variant calling in analysis of pooled or individual next-generation sequencing data," *Nucleic acids research*, 39(19), article e132.

Welsh, J., and McClelland. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research*, 18(24), 7213-7218.

Williams, J. G. K., Kubelik, A. K., Livak, K. J., Rafalski, J. A., and Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, 18(22), 6531-6535.

World Bank. (2004). 'Millet production growing in Africa, Available at www.worldbank. org/htm/cigar/newsletter r.

Wong, K. K., Deleeuw, Dosanjh, N. S., Kimm, L. R., Cheng, Z., et al. (2007). A comprehensive analysis of copy-number variations in human genome. *Am J Hum Genet* 80: 91-104.

Xu, X., Liu, X., Ge, S., et al. (2012). "Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes," *Nature Biotechnology*, 30(1), 105–111.

Ya Zhini. (2006). Studies on genetic variability, association, diversity and drought tolerance in finger millet (*Eleusine coracana* (L.) Gaertn.) genotypes MSc. (Ag.), Thesis Coimbatore, India: T.N.A.U.

You, F. M., Huo, N., Deal, K. R. et al. (2011). "Annotation-based genome-wide SNP discovery in the large and complex Aegilops tauschii genome using next-generation sequencing without a reference genome sequence," *BMC Genomics*, vol. 12, article 59.

Yuan, Y., SanMigue, P. J., and Bennetzen. (2003). High-Cot sequence analysis of the maize genome. The Plant Journal, vol. 34, no. 2, pp. 249-255.

Zhang, X., and Borevitz, J. O. (2009). "Global analysis of allele-specific expression in Arabidopsis thaliana," *Genetics*, 182(4), 943–954.

Zhang, J., and Davies, W. J. (1990a). Does ABA in the xylem control the rate of leaf growth in soil-dried maize and sunflower plant? *Journal of Experimental Botany* 41: 1125-32

Zhang, J., and Davies, W. J. (1990b). *Change in the concentration of ABA in xylem sap as a function of changingsoil waterstatus can account for changes in leaf conductance and growth*. Plant, Cell and Environment 13: 277-85

APPENDICES

APPENDIX I: The 100 Finger millet variants (test entries) used in the experiment

ENT. NO	GENOTYPE	ENT. NO	GENOTYPE	ENT. NO	GENOTYPE	ENT. NO	GENOTYPE	ENTRY NO	GENOTYPE
1	I.E 4491	21	GBK000463	41	KACIMMI 20	61	GBK008278	81	GBK029798
2	I.E 6165	22	GBK027300	42	KACIMMI 6	62	GBK008292	82	GBK029820
3	I.E 4497	23	I.E 4816	43	KACIMMI 65	62	GBK008299	83	GBK033414
4	I.E 6537	24	I.E 2217	44	KACIMMI 17	64	KACIMMI 77	84	GBK033416
5	OMUGA-P	25	KACIMMI 7	45	KACIMMI 22	65	GBK029199	85	GBK039217
6	KACIMMI 15	26	KACIMMI 47	46	KACIMMI 24	66	GBK029678	86	GBK043268
7	I.E 4115	27	VL 149	47	KACIMMI 49	67	GBK029715	87	GBK000369
8	GBK029661	28	GBK043081	48	KACIMMI 72	68	GBK029722	88	UFM 138
9	I.E 5870	29	OKHALE-1	49	KACIMMI 42	69	GBK029724	89	GBK000482
10	KACIMMI 11	30	OMUGA-G	50	GBK000516	70	GBK03821	90	GBK000909
11	I.E 5306	31	P 224	51	GBK000692	71	GBK040568	91	GBK008348
12	I.E 2957	32	P224 CV	52	GBK008339	72	GBK000409	92	GBK033446
13	PR 202	33	P 283	53	GBK029701	73	GBK000449	93	U15XP283
14	GBK000451	34	P4C3	54	GBK029793	74	GBK000462	94	GBK000784
15	I.E 5873	35	SERERE-1	55	GBK029805	75	GBK000493	95	GBK000831
16	I.E 4795	36	U-15	56	GBK029821	76	GBK000568	96	GBK026992
17	I.E 2606	37	N-BROWN	57	GBK029847	77	GBK0011082	97	GBK000900
18	I.E 2440	38	GULU-E	58	KACIMMI 36	78	GBK011113	98	GBK000549
19	I.E 6337	39	BUSIBW-1	59	GBK000802	79	GBK011126	99	GBK029807
20	KACIMMI 30	40	KACIMMI 73	60	GBK000828	80	GBK029744	100	GBK000520

Key: I.E =International Eleusine, CVR = Chakol Variant, U = Uganda, P = urple

N = Nanjala, GBK = Gene Bank Kenya, G = Green, KACIMI = KARI African Centre for Crop Improvement McKnight Foundation Millet

APPENDIX II: Field experimental layout

(i) Treatment combinations on each unit of the design

(ii) Design = Triple Lattice with 100 entries

(iii) Alupe 2012LR *Striga* Screening Nursery

Plots		1	2	3	4	5	6	7	8	9	10
Replicates	Blocks										
APPENDDIX II contd…											
1	1	92	62	82	52	12	22	32	72	42	2
	2	97	47	7	87	27	37	17	57	67	77
	3	10	90	80	40	50	100	30	20	60	70
	4	34	44	94	84	54	14	74	4	24	64
	5	49	79	29	19	39	9	59	69	89	99
	6	33	73	63	43	93	23	53	3	13	83
	7	76	86	26	46	16	36	6	56	96	66
	8	51	11	61	41	91	81	21	31	1	71
	9	25	15	85	35	75	45	65	95	5	55
	10	28	78	48	18	8	58	88	68	38	98
2	1	44	46	42	50	48	43	49	47	41	45
	2	70	65	68	69	66	61	63	64	62	67
	3	33	31	36	39	34	37	35	40	38	32
	4	99	92	98	100	96	94	93	95	91	97
	5	12	16	13	19	18	11	20	15	14	17
	6	30	25	24	21	29	27	26	28	23	22
	7	56	54	58	59	55	52	60	57	53	51
	8	84	86	88	82	81	87	89	85	90	83

	9	2	5	8	6	10	7	9	4	3	1
	10	72	75	77	79	78	73	76	71	80	74
3	1	4	22	77	13	59	31	100	68	86	45
	2	38	29	83	47	74	20	65	51	6	92
	3	8	17	94	62	26	40	71	53	85	49
	4	43	34	98	80	11	89	57	25	2	66
	5	14	23	69	41	87	78	60	5	96	32
	6	55	82	73	10	64	37	91	19	28	46
	7	56	97	15	1	70	42	79	88	24	33
	8	93	61	30	84	48	7	39	75	52	16
	9	54	72	36	81	9	63	95	50	27	18
	10	12	76	44	99	67	90	35	58	3	21

(i) Treatment combinations on each unit of the design

(ii) Design = Square Lattice = Triple Lattice (10 x10)

(iii) Kibos 2012 *Striga* Screening Nursery Layout

Plots		1	2	3	4	5	6	7	8	9	10
Replicates	Blocks										
1	1	33	1	70	56	97	15	79	42	88	24
	2	89	25	66	80	11	57	34	98	43	2
	3	26	17	62	85	40	49	71	53	8	94
	4	93	30	16	48	7	39	75	52	84	61
	5	18	36	72	81	50	27	9	54	63	95
	6	35	76	12	90	3	44	21	67	58	99
	7	6	38	20	83	29	47	74	92	51	65
	8	78	14	32	5	60	23	41	96	87	69

	9	31	4	86	45	59	100	22	13	77	68
	10	10	37	91	19	64	55	28	82	46	73
2	1	19	49	69	89	79	29	9	59	99	39
	2	94	44	14	54	74	4	64	84	34	24
	3	63	33	23	53	13	3	73	83	93	43
	4	96	66	56	46	16	6	26	86	76	36
	5	95	5	65	55	15	25	35	45	75	85
	6	97	67	7	77	57	37	27	17	47	87
	7	92	72	52	2	12	82	32	42	22	62
	8	50	100	10	70	60	40	20	30	80	90
	9	68	28	18	8	58	88	98	48	38	78
	10	91	51	21	61	71	31	41	1	81	11
3	1	91	94	97	95	96	100	8	93	99	92
	2	73	79	78	76	75	80	72	77	71	74
	3	26	30	27	29	28	25	23	24	21	22
	4	83	89	86	87	88	82	84	85	90	81
	5	5	3	7	10	8	1	9	6	4	2
	6	39	32	38	37	36	31	35	33	40	34
	7	47	46	49	42	50	44	48	43	45	41
	8	13	12	11	19	14	20	18	16	17	15
	9	67	65	63	62	66	70	69	61	64	68
	10	53	55	51	56	60	54	59	52	58	57

NOTE: The field layout was triple lattice or square lattice.

APPENDIX III: Plates

Plate 3.1

Plate 3.2

Plate 3.3

Plate 3.4

Plate 3.5

Plate 3.6

Plate 3.7

Plate 3.8

Plate 3.9

Plate 3.10

Plate 3.11

Plate 3.12

(Source, Author, 2012)

Key:

Plate 3.1: Section of land that had been prepared for planting finger millet in 2012 LR season at Alupe

Plate 3.2: Plot of finger millet before thinning process at Alupe LR 2012

Plate 3.3: Plot of finger millet after thinning process

Plate 3.4: Plots of finger millet being top dressed at Alupe.

Plate 3.5: Technical staff assigned to guard and scare birds on maturing plots of finger millet before harvesting.

Plate 3.6 Members scoring several data on each plot of finger millet at crop maturity LR 2012.

Plate 3.7: Heads of finger millet 4 weeks to harvesting

Plate 3.8: Taking data on the ear shapes on respective plots of finger millet

Plate 3.9: A single head of finger millet with curved ear shape mg X2

Plate 3.10: Elutriator machine at Kibos research station.

Photo 3.11: Part two of elutriator machine

Photo 3.12: part 3 of the elutriator machine where counting of *Striga* seeds are estimated from soil samples collected from farms.

APPENDIX IV: ANOVA Table: Seedling Vigor

Source	DF	SS	MS	F-value	P>F	Significance.
Rep	2	12.147	6.07	9.98	<.001	**
Stg	1	3.147	3.14	5.17	0.0232	NS
Rep*Stg	2	4.754	2.37	3.91	0.0205	NS
EntNo	99	104.398	1.05	1.73	<0.001	**
Stg*entNo	93	46.819	0.50	0.83	0.8744	NS

t -Grouping Mean N Stg

A 2.12 485 Inoculated

B 1.97 477 Sfree

KEY:

Rep = Replica, Stgct = Striga count, Rep*stgct = Replica interaction by *Striga* count
Ent No =entry number, Stg*entNo. = *Striga* interaction by entry number
*= Significant ($P \leq 0.1$), **= Highly significant ($P \leq 0.05$)
NS= Not significant

APPENDIX V: ANOVA Table: Striga count at vegetative

Source	DF	SS	MS	F-value	P>F	Significance.
Rep	2	568.854	282.92	4.57	0.0197	NS
Stg	1	4745.53	4745.53	76.59	<.0001	**
Rep*Stg	2	565.84	282.92	4.57	.0107	NS
EntNo	99	6331.78	63.95	1.03	.4013	NS
Stg*entNo	99	6122.74	65.83	1.06	.3319	NS

t Grouping Mean N Striga

A 5.26 481 Inoculated
B 0.00 498 Striga free

APPENDIX VI: ANOVA Table: Striga count at 50% flowering

Source	DF	SS	MS	F-value	P>F	Significance.
Rep	2	2763.23	1158.2	5.20	.0021	**
Stg	1	33307.4	33307.4	149.45	<.0001	**
Rep*Stg	2	2763.23	1381.6	620	.0021	**
EntNo	99	22947.2	231.8	1.04	.3820	NS
Stg*entNo	93	21493.3	231.1	1.04	.3913	NS

t Grouping Mean N Stg

A 13.79 481 Inoculated
B 0.00 498 Striga free

APPENDIX VII: ANOVA Table: Striga count at maturity

Source	DF	SS	MS	F-value	P>F	Significance.
Rep	2	45119.02	22559.51	13.79	<.0001	**
Stgct	1	148754.6	148754.6	90.92	<.0001	**
Rep*Stgct	2	45119.02	22559.51	13.79	<.0001	**
EntNo	99	171500	1732.32	1.06	.3356	NS
Stg*entNo	99	171500	1732.32	1.06	.3356	NS

t Grouping Mean N *Striga*

A 25.74 554 Inoculated
B 0.00 547 *Striga*

APPENDIX VIII: Morphological traits mean with Striga inoculated for field screening

Ent.No	Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13
1	I.E 4491	3.0	0.8	1.5	3.5	84.7	43.3	5	2	2.8	8.3	2.8	5.6	113
2	I.E 6165	3.0	0.2	0.0	0.0	87	67.5	7.3	5.7	0	12.6	12.5	7	200
3	I.E 4497	2.5	0.0	0.0	0.0	82	50.0	4.0	1.0	0	7.5	1	5.5	22.2
4	I.E 6537	1.9	0.0	0.8	0.9	87.8	53.5	5.8	3	24	13.4	7.8	4.9	318.1
5	OUGA P	2.3	0.6	1.0	2	90	52.3	6.5	2.5	19.2	12.7	11.6	5.8	83.4
6	KACIMI 15	3.0	0.3	0.3	1.0	93.7	48.6	5.3	3	0	9	1.3	3.7	66.67
7	I.E 4115	2.2	0.4	2.8	6.4	84.4	49.6	5.6	2.8	9.6	11.4	29	5.3	618.9
8	GBK029661	3.0	0.0	0.5	0.5	89	55.0	4	1	5	13	1	4	66.7
9	I.E 5870	2.5	0.0	1.5	15.5	83	50.0	5.5	2.5	0	12	3	5.5	155
10	KACIMI 11	3.0	1.0	1.0	1.0	90	45	4	2	0	14	1	4	83
11	I.E 5306	2.2	2.0	12.2	69.2	91.7	58.7	5	1.7	9.7	12	25.7	5.2	213.9
12	I.E 2957	3.0	0.0	0.0	9.0	87	30.0	5	2	3.5	10.5	1	5.5	41.65
13	PR 202	3.0	0.0	0.0	12	88	60.0	8	3	30	12	1	7	63.9
14	GBK000451	1.9	6.6	15.1	38.5	74	58.3	5.7	2.7	7	12	28.2	5.4	861.1
15	I.E 5873	2.4	0.2	0.8	1.0	91.5	46.1	4.5	2.5	14.4	15.2	3.8	4.8	7.43
16	I.E 4795	3.0	0.0	0.0	0.0	90	55.0	8	3	0	14.1	1.3	6	63.9
17	I.E 2606	2.7	0.14	1.9	19.9	92.3	38.3	4.3	2	0	11.7	22	5.4	358
18	I.E 2440	2.8	0.25	0.0	0.25	98.5	39.5	4	2	15.5	12	2	4.5	59.3
19	I.E 6337	2.8	1.5	5.0	19.3	91.7	49.0	5	2.3	14.5	12.1	22.5	6.2	859
20	KACIMI 30	1.8	1.0	4.6	6.2	84.2	53.5	6	2.7	14.7	10.4	28.5	5.1	537.1
21	GBK000463	3.0	0.0	0.0	0.0	101.2	60.0	7	2	0	16	1	5	94.4
22	GBK027300	1.9	1.1	3.1	63.8	93.2	61.3	5	2.8	12.6	12.9	23.8	4.9	510.2
23	I.E 4816	1.7	6.2	18.1	31.0	83.3	55.0	6.2	2.8	12.5	11.3	23.4	5.6	1019.5
24	I.E 2217	2.7	0.0	0.7	0.83	89	43.3	4	2	1.5	11.7	2.7	4.8	36.7
25	KACIMI 7	2.1	0.3	1.2	1.7	94.3	40.4	5.5	2.3	6.4	11.7	17.1	4.7	75
26	KACIMI 47	1.6	0.5	2.3	5.1	83	64.4	6.8	3	21.3	13.4	27.9	5.5	1094.4
27	VL 149	3.0	0.0	0.0	0.0	53	53.2	8	4	0	10.1	2	5.7	113.9
28	GBK043081	3.0	0.0	0.0	0.0	85	60.0	7	3	0	11	2	5	88.9
29	OKHALE-1	2.2	7.5	14.4	31.0	87.8	67.2	8.2	2.8	18.3	13.2	30	5.7	774
30	OMUGA G	1.9	2.2	7.1	10.7	90	59.9	6.3	2.3	23.4	13.6	23.4	5.4	947.2

APPENDIX VIII contd….														
31	P 224	2.3	2.0	5.1	11.3	84.2	48.5	5.5	2.5	15.7	11.1	22.5	4.6	577.8
32	P 224 CV	2.1	4.5	13.3	27.2	84.3	57.9	5.8	2.5	23.4	12.4	22.9	5.2	520.4
33	P 283	2.0	3.8	11.1	19.1	88	56.4	5.8	2.2	11	13.2	26.7	5.1	534.3
34	P4C3	2.3	7.8	17.8	31.3	84.3	48.5	4.8	2.3	9	13.9	25	5.3	562.0
35	SERERE-1	2.3	0.9	5.3	10.7	88.8	44.6	5.5	2	25.8	11.7	21.3	4.8	414
36	U-15	2.3	2.8	4.3	15.1	90	47.7	5.8	2.5	7.9	11.8	28.7	5.5	618.5
37	N BROWN	1.6	5.3	9.9	19.2	87	48.1	6	2.3	23.5	12.5	19.1	4.2	430.6
38	GULU-E	2.2	6.3	11.8	21.3	86.8	54.6	6	2.3	12.9	12.5	26.7	5.1	630.6
39	BUSIBW-1	1.4	2.8	7.8	16.9	85.3	55.1	5	2.3	43.6	12.9	28	4.7	1200
40	KACIMI 73	1.4	1.6	4.1	10.2	84	68.0	7.3	3.2	20.8	13.8	28.7	5.8	1134.2
41	KACIMI 20	1.8	3.7	8.0	40	91.7	63.4	7.8	3.7	19.3	12.7	26.5	5.5	916.7
42	KACIMI 16	2.3	2.3	7.8	12.8	82.7	55.5	6.5	2.5	14.5	14.8	30.1	5	917.6
43	KACIMI 65	2.4	6.2	12.2	25.7	77	52.9	6	2.3	9.4	10.4	27.3	5.1	751.9
44	KACIMI 17	1.6	2.7	8.4	14.5	82.8	62.9	5.8	2.5	12.2	12.9	31.6	5.2	1165.7
45	KACIMI 22	1.8	2.4	4.2	9.2	85	61.3	6	2.5	24.7	11	27.3	5.2	994.4
46	KACIMI 24	2.1	0.1	0.8	7.58	90.3	55.8	7.2	2.5	3.7	11.2	25.4	5.3	1013
47	KACIMI 49	1.8	4.8	14.3	21	84.5	65.7	7.2	3.2	17.7	13.4	28.8	6.2	983.3
48	KACIMI 72	1.7	2.5	7.8	18.4	87.8	62	6.7	2.3	11.1	14.8	31	4.8	1040.8
49	KACIMI 42	2.2	4.3	11.4	22.3	87	54.9	7.8	2.7	8.8	13.2	28.5	5.8	926.9
50	GBK000516	2.0	0.0	1.7	6.73	84.4	57.4	6.4	2.6	5.2	13.6	26.7	5.1	1133.3
51	GBK000692	1.9	3.8	8.9	35.7	97.5	59.2	5	2.3	14.7	14.1	24.8	5.2	563
52	GBK008339	1.5	0.3	1.3	8.17	84.3	59.8	5.3	2.8	10.4	13	25.8	5.3	775
53	GBK029701	2.1	2.4	7.3	18.9	96.3	53.4	5.7	2.5	16.6	10.4	21.4	5.2	742.2
54	GBK029793	1.4	2.0	11.1	20.1	81.2	58.4	5.2	2.6	7.5	13.9	26.9	5.3	1103.3
55	GBK029805	2.0	0.5	2.9	17.2	87.7	48.9	5.2	2.3	8.7	10.2	24.8	5.3	371.3
56	GBK029821	2.2	1.1	2.8	28.8	96.3	57.5	4.8	2.3	8.3	10.5	27.3	5.5	413
57	GBK029847	2.0	0.8	2.3	19.1	97.2	58.9	7.2	3	3.7	12.2	25.7	5.3	427.8
58	KACIMI 36	2.2	0.1	1.8	5.42	83.8	52.5	6.2	2.3	10	14.2	24.1	5.2	748.2
59	GBK000802	1.8	1.4	2.4	30.6	88.7	52.1	5	2.2	6.3	12.8	26.9	5	976,9
60	GBK000828	2.6	0.7	1.8	20.6	99	50.3	5.8	2.5	9.7	11.4	20.8	5.1	315.7
61	GBK008278	2.2	3.0	9.7	29.6	92.4	53.2	6.8	2.4	14.4	12.6	26.3	5.6	589.9
62	GBK008292	1.9	1.7	6.1	43.8	92.8	52.6	6	2.7	8.3	12.5	26.8	6	719.5
63	GBK008299	1.8	3.3	4.8	25.3	89.2	42.9	5.2	2.3	12.7	10.4	25.3	5.3	452.8
64	KACIMI 77	2.3	5.6	14.2	26.8	84	48.7	5	2.2	11.2	13.4	28	6.4	803.7
65	GBK029199	2.1	0.1	2.1	21.8	96.2	55.4	5.5	2	8.8	11.3	22	5.6	467.6
66	GBK029678	2.4	2.8	7.7	16.9	89	39.2	4.3	2	12.6	9.4	23.3	5.3	462.9
67	GBK029715	1.8	2.1	8.7	45.6	98	59.3	7.3	2.7	15.8	13.5	67.4	5.8	824.1
68	GBK029722	1.8	0.4	2.3	22.7	93.7	58.5	6.7	2.3	12.8	13.7	27.5	5.3	1055
69	GBK029724	2.2	0.3	1.1	21.8	97.8	62.8	4.8	2.3	7.8	12.3	23.1	5.7	608.3
70	GBK003821	1.4	1.0	6.7	13	72.7	73.3	8	2.7	15.1	13.8	30.1	6	1670
71	GBK040568	2.7	4.8	7.2	31.2	93.5	41.3	4	2.2	9.7	10	23.7	4.9	512
72	GBK000409	1.5	13.4	14.7	40.9	71.3	47.8	4.3	3.3	11.2	8	24.4	4.5	901.4
73	GBK000449	2.2	1.3	4.0	14	81	48.9	6.3	2	18.4	12	26	5.1	572.2
74	GBK000462	1.9	0.7	3.8	49.6	91	48.4	4.3	2.5	2.5	11.7	27.7	5.3	665.7
75	GBK000493	1.9	1.8	6.6	15.1	83.2	51.4	3.8	2.2	22.8	15.2	22.5	4.8	620.4
76	GBK000568	2.2	3.8	12.0	34.3	96.7	46	5.3	2.3	12.5	10.6	21.2	4.9	143.5
77	GBK011082	2.3	2.0	4.7	38.6	98	50.7	5.4	3	2.2	10	25.1	5.7	475
78	GBK011113	1.4	0.9	4.0	45.3	98.8	55.9	5	2.3	12.8	11.4	27.6	5.2	304.7
79	GBK011126	1.9	3.7	7.9	43.8	91	53.8	5.8	2.3	8.3	11.3	27	5.2	708.3
80	GBK029744	2.0	3.5	6.5	59.5	90.8	63	6.2	2.4	16	11.9	22	4.9	706.5
81	GBK029798	1.7	2.3	8.5	20.3	92.2	47.3	5.2	2.5	15.3	10.4	24.8	5.4	408.4
82	GBK029820	1.7	0.8	5.8	33.4	91.8	67.3	6.5	3	5.9	13.7	25.7	5.6	575.9
83	GBK033414	2.2	2.2	12.1	25.2	90.6	53.9	7.4	2.8	6.8	12.7	20.6	4.8	442.6
84	GBK033416	2.7	1.3	2.8	20	83.8	55.2	5.8	2.5	13.8	11	21.8	5.8	557.4
85	GBK039217	2.4	1.5	5.3	31.7	99.7	51.4	5.5	2.7	2.2	9.1	27.1	5.8	398.2
86	GBK043268	1.9	2.1	7.8	28.8	92.4	62	5.4	2.2	18.3	13.1	26.6	5.5	464.4
87	GBK000369	2.0	8.5	16.0	42.3	84	59.2	5.3	2.5	32	11.9	22	5.1	339.8
88	UFM 138	1.9	3.3	9.3	15.4	86.2	48.6	4.8	2	6.5	12.5	26.4	5.1	638
89	GBK000482	2.3	1.8	9.4	34.9	90	52.1	5.2	2.2	14	10	25.2	5	568.5
90	GBK000909	1.8	3.3	15.3	40.3	94.8	56.9	6	2.5	10.7	13.1	27.1	5.3	463.9
91	GBK008348	2.1	8.3	15.9	29.3	93.8	44.3	4.2	1.8	18.3	12.7	24.8	5	620.4
92	GBK033446	2.2	0.7	1.8	14.8	94.4	52.2	5.5	2.7	3.9	12.1	23.8	5.4	447.2

93	U-15XP283	1.9	6.6	14.5	26.58	84.8	49.9	4.8	2.5	9.8	12.9	27.8	5	708.3
94	GBK000784	2.2	2.6	11.8	25.3	90.7	47.9	5	2.3	7.3	9.4	23.6	5.3	400.9
95	GBK000831	2.4	0.5	3.0	13.8	94.6	42.5	4.8	2.6	6.5	10.9	23.2	5.6	245.4
96	GBK026992	2.2	0.1	1.5	16.4	86.4	54.3	5	2	16.2	11.4	17.9	5	412
97	GBK000900	2.1	2.5	9.4	68.1	92.8	54.2	5.7	2.5	9.3	12.7	22.3	5.4	367.6
98	GBK000549	2.1	1.1	5.6	41.5	93.0	50.2	4.8	1.8	9.6	11.7	23.8	5.3	613
99	GBK029807	2.2	0.4	1.7	13.8	101	56.8	5.7	2.2	2.4	12.2	20.3	5.6	878.7
100	GBK000520	1.8	8.3	3.2	20.3	65	58.8	4.8	3.3	5.5	10.4	27.6	6.8	906.5

Key:

1. Seedling vigor 2. Mean *striga* count at vegetative stage 3. Mean *Striga* count at 50% flowering

4. Mean *Striga* count at crop maturity 5. Mean days to 50% crop flowering 6. Mean plant height

7. Ear length 8. Ear width 9. Lodging percentage 10. Ear exertion 11. Stand count 12. Number of fingers 13. Mean Yield in $kgHa^{-1}$

APPENDIX IX: Morphological traits mean without *Striga*

Ent.No	Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13
1	I.E 4491	3.0	0	0	0	97	58	8	3	1	7	2	4	155.6
2	I.E 6165	3.0	0	0	0	101	67.5	7.3	5.7	0	12.6	12.5	7	300
3	I.E 4497	3.0	0	0	0	114	65	6	4.0	0	5	10	7	61
4	I.E 6537	1.8	0	0	0	91.5	63	6.5	5.1	40.5	14.5	7	4.8	422
5	OUGA P	1.5	0	0	0	96	70.6	7.6	5.8	19.3	12.5	15.3	6.3	701
6	KACIMI 15	2.0	0	0	0	96.7	56	5.8	4	0	9	2	3.7	600
7	I.E 4115	2.2	0	0	0	88.8	61.3	7.1	5.3	8	11.9	26.2	5.2	1212
8	GBK029661	3.0	0	0	0	100	63	5	2	10	18	1	4	200
9	I.E 5870	2.5	0	0	0	88	56.0	6	2.5	0	12	3	5.5	750
10	KACIMI 11	3.0	0	0	0	90	54	5	2.6	0	14	4	4	650
11	I.E 5306	2.0	0	0	0	94.6	61.3	7.3	5.5	11	12.3	23.3	5.2	783
12	I.E 2957	3.0	0	0	0	107	45	6	2	7	14	1	6	27.8
13	PR 202	3.0	0	0	0	97	55.0	9	2	60	13	1	3	38
14	GBK000451	2.3	0	0	0	88.8	64	7.7	7.6	3.2	12.8	23	5.4	1018.8
15	I.E 5873	2.7	0	0	0	100	61	7.3	3.7	23.3	16	2	4.7	135
16	I.E 4795	3.0	0	0	0	98	68	6.7	6.5	0	14.7	1.5	5.5	130.5
17	I.E 2606	2.0	0	0	0	97	60.5	5.8	5.1	0	13.3	21	5.8	695
18	I.E 2440	3.0	0	0	0	106.5	67	5	1	30	13.5	1.5	5	100
19	I.E 6337	3.0	0	0	0	92.3	58	7	5.9	3.7	13.8	18.3	6.7	777.8
20	KACIMI 30	1.5	0	0	0	84.7	58.2	7.7	6.2	16.7	10.6	25.7	4.8	1000
21	GBK000463	2.1	0	0	0	107	60.0	7.8	4.8	0	16	4	5	300
22	GBK027300	1.8	0	0	0	98.3	76	7.3	5.1	17	13.8	23.2	4.8	1303.7
23	I.E 4816	1.0	0	0	0	78.8	70.3	9.2	9.0	8	12.8	30.5	6.3	1805
24	I.E 2217	2.7	0	0	0	103.3	61	7.3	5.3	2.7	14	3.3	4.3	307
25	KACIMI 7	1.8	0	0	0	89	60	7.0	5.8	4.5	14.1	22.5	5.3	1187.8
26	KACIMI 47	1.8	0	0	0	81.5	72.3	9.5	7.8	12.2	14.3	25.3	5.5	1453.7
27	VL 149	3.0	0	0	0	97.5	52.5	6.5	4	0	9.2	1	5.0	150
28	GBK043081	3.0	0	0	0	91	44	7	3	0	11	2	5	178
29	OKHALE-1	1.8	0	0	0	86.7	73.3	9.6	8.3	13.8	13.7	26.8	6	1641
30	OMUGA G	1.8	0	0	0	87	71.8	8.0	6.3	12.7	14.9	22.7	5.3	1321
31	P 224	2.0	0	0	0	89.7	62.3	7.4	6.8	20.3	11.3	25	4.3	840
32	P 224 CV	2.0	0	0	0	89.5	65.3	7.5	4.6	33.3	13.4	21.8	5.0	1011
33	P 283	1.8	0	0	0	90.2	69.3	7.7	6.1	9.7	13.6	28.6	5.3	814
34	P4C3	1.8	0	0	0	87.7	65.2	7.3	6.5	9	13.9	28.3	5.2	1627
35	SERERE-1	1.8	0	0	0	93	68.3	7.3	6.4	25.8	11.7	26	5.3	1723
36	U-15	2.0	0	0	0	87.3	59.8	7.1	6.3	7.9	11.8	29.8	5.2	1131
37	N BROWN	1.2	0	0	0	92	75	8.5	6.1	23.5	12.5	21	4.5	1064
38	GULU-E	2.3	0	0	0	87.6	63.5	7	6.3	12.9	12.5	28	4.8	1196.3
39	BUSIBW-1	1.5	0	0	0	84.2	70.2	5.7	6.8	18.8	13.7	27.2	4.3	1388
40	KACIMI 73	1.6	0	0	0	87.5	69.2	9.5	7.1	22.7	13	26	5.8	1354

APPENDIX IX CONTD……..														
41	KACIMI 20	1.6	0	0	0	90.2	76.3	9.2	7	18.7	12.4	27.6	5.6	1555
42	KACIMI 16	2.2	0	0	0	88.3	69.2	8.7	7.1	12.3	16	32	4.8	1184
43	KACIMI 65	2.5	0	0	0	91	52.2	7.3	7	5.7	10.5	25	5.4	1104
44	KACIMI 17	1.6	0	0	0	85.5	67.3	7.8	6.4	9.2	13	31.5	5.0	1503.7
45	KACIMI 22	1.7	0	0	0	89	71.2	8	7.3	30.2	11.9	29	5.0	1830
46	KACIMI 24	2.2	0	0	0	88.5	66.3	6.6	7.6	4.2	11.3	26.7	5.5	1202
47	KACIMI 49	1.6	0	0	0	83.6	68.2	8.9	6.8	17.3	13.6	28.8	6.3	1518.5
48	KACIMI 72	1.5	0	0	0	89.2	75.6	8.4	7.4	7.7	15.6	30.8	4.5	1666.7
49	KACIMI 42	1.8	0	0	0	88.3	65.8	10.3	8.0	4.8	13.3	33.2	5.8	1638
50	GBK000516	2.2	0	0	0	86.5	62.8	8	6.9	5.5	14.5	26.3	4.7	1062.9
51	GBK000692	2.0	0	0	0	98.7	76.8	6.3	5.5	11.8	15.9	22.7	5.3	664
52	GBK008339	1.5	0	0	0	93.8	65.8	6.6	5	2.7	13.4	22.2	5.2	936
53	GBK029701	2.0	0	0	0	93	74.8	8.4	5.7	20.8	11.9	24.5	5.7	1422
54	GBK029793	1.7	0	0	0	87	64	5.8	5.2	10.3	14.4	24	5.2	1033
55	GBK029805	1.8	0	0	0	96.5	66	7	5.3	10.7	11.4	26.3	5.6	889.8
56	GBK029821	2.3	0	0	0	104	71.3	6.7	5.1	0.8	10.8	26.5	5.7	736.1
57	GBK029847	2.0	0	0	0	100.2	74	9	6.7	5.7	12.5	24.3	5.3	618.5
58	KACIMI 36	2.0	0	0	0	89	65.3	8	6.7	7.8	14.8	27.3	5.3	1228.7
59	GBK000802	1.5	0	0	0	92.2	70.3	7.2	6.9	5.7	14.4	26.3	5.2	1672
60	GBK000828	2.6	0	0	0	98.8	69.7	7.9	5.2	8.2	12.5	20.2	5.7	426.8
61	GBK008278	2.0	0	0	0	102.2	68.7	8.7	6.3	11.2	13.4	27.8	5.7	1224
62	GBK008292	1.8	0	0	0	100	76	7.6	5.8	4.3	13.9	28.5	6.3	607
63	GBK008299	1.7	0	0	0	97.2	72.9	6.7	5.7	2.7	11.3	26.5	5.7	1224
64	KACIMI 77	2.0	0	0	0	89.2	67.3	7	6.4	10.3	14.7	28.8	8.2	1529
65	GBK029199	2.3	0	0	0	102.3	76.2	7.7	5.4	12	11.7	26.5	6	807
66	GBK029678	1.8	0	0	0	91.7	73.5	6.1	6.3	15	11.1	26.6	5.7	853
67	GBK029715	2.0	0	0	0	99.7	80.5	8.7	7.1	26.2	14.6	25	5.8	1478.7
68	GBK029722	1.7	0	0	0	97.3	79	7.5	5.5	18.3	15.4	28.2	5.2	1722
69	GBK029724	2.2	0	0	0	105.5	74.5	6.3	4.6	12.3	12.4	20	5.5	661
70	GBK003821	1.5	0	0	0	92.3	73.5	9.5	9.9	15.1	14.2	25.5	6.3	1444
71	GBK040568	2.4	0	0	0	92	63	5.3	5.1	7.8	11.9	28.3	5.3	1552
72	GBK000409	2.0	0	0	0	91.2	59.8	6.1	4.2	12.8	8.1	24	5.5	1056
73	GBK000449	2.0	0	0	0	92.2	71	7.4	7.5	7.2	13.4	27	5.0	867
74	GBK000462	2.0	0	0	0	95.6	61.6	6.1	5.4	10.2	11.8	28.4	5.8	916
75	GBK000493	1.7	0	0	0	89.7	75.8	4.4	4.4	4.8	16.9	25.6	4.7	1046
76	GBK000568	2.0	0	0	0	97.8	65.7	6.9	5.7	18.3	12.2	25.8	5.3	1016.6
77	GBK011082	2.3	0	0	0	102.2	72.5	6.5	5.5	6.3	10.9	22.5	5.5	756
78	GBK011113	1.8	0	0	0	100	78.2	6.3	5.7	1.5	12.5	27.8	5.5	891
79	GBK011126	2.0	0	0	0	94.2	69.2	7.1	7.6	7.3	12.3	24.6	5.2	1112.9
80	GBK029744	2.4	0	0	0	97.5	67.5	6.8	5.4	26.5	12.2	16.7	5.0	799
81	GBK029798	1.2	0	0	0	92	74.1	7.7	6.9	11	11.2	27	5.5	1535
82	GBK029820	1.5	0	0	0	99.2	73.3	7.2	5.4	7.7	14.8	22.5	5.3	1050
83	GBK033414	2.2	0	0	0	95.5	76.7	10.1	6.6	4.7	14.1	20.2	4.7	812
84	GBK033416	3.0	0	0	0	94	64.2	7.5	6.3	6.8	11.4	22.2	5.3	558
85	GBK039217	2.6	0	0	0	104.5	62.5	7.4	5.1	19.2	9.8	27	5.7	934
86	GBK043268	1.8	0	0	0	82.8	75.9	6.6	6.7	19	3.7	25.3	5.3	950.9
87	GBK000369	1.8	0	0	0	82.2	69.5	6.8	4.9	35.8	12.1	19.5	5.3	405
88	UFM 138	1.8	0	0	0	97.3	63.5	6.0	5	1.3	13.3	28.2	5.2	1075
89	GBK000482	2.4	0	0	0	98.2	64.3	6.5	4.9	17	10.5	23.2	5	556
90	GBK000909	1.7	0	0	0	98.7	82.2	7.7	4.9	10.8	14.2	27.5	5.5	1498
91	GBK008348	1.5	0	0	0	88.5	73.1	5.6	5.4	17	10.6	29.2	5	1552.2
92	GBK033446	1.8	0	0	0	101	72.7	7.1	4.4	3.6	13.5	29.8	5.5	885
93	U-15XP283	1.8	0	0	0	88.8	62.2	7.4	6.8	4.3	13.3	28.2	5.2	1181.4
94	GBK000784	2.2	0	0	0	91.8	66	5.9	5.2	1.3	10.8	23.5	5.5	1140.7
95	GBK000831	2.3	0	0	0	101.8	64.2	5.3	5.4	5.7	12.5	25.5	6.0	897
96	GBK026992	2.2	0	0	0	94.6	62.4	6.1	5	21.4	12.3	13.4	5.2	269.4
97	GBK000900	2.0	0	0	0	100.7	76.8	7.3	6.2	4.8	13.3	22.2	5.3	799
98	GBK000549	2.0	0	0	0	101.8	70.7	6.8	4.7	18	12.7	25.3	5.5	737.9

99	GBK029807	2.2	0	0	0	96.4	85.7	7.4	5.8	1.4	13.7	19.6	6.2	898.9
100	GBK000520	2.0	0	0	0	92.8	56.3	5.7	5.2	1.3	12.3	27.3	5.5	1070

Key for appendix 9:

1. Seedling vigor 2. Mean *striga* count at vegetative stage 3. Mean *Striga* count at 50% flowering

4. Mean *Striga* count at crop maturity 5. Mean days to 50% crop flowering 6. Mean plant height

7. Ear length 8. Ear width 9. Lodging percentage 10. Ear exertion 11. Stand count 12. Number of fingers 13. Mean Yield in $kgHa^{-1}$

APPENDIX X: ANOVA Table: Days to 50% flowering

Source	DF	SS	MS	F-value	P>F	Significance.
Rep	2	603.84	301.91	2.88	0.0566	NS
Stg	1	6078.31	6078.31	58.01	<.0001	**
Rep*Stg	2	260.39	130.19	1.24	0.2892	NS
EntNo	99	30463.98	307.71	2.94	<.0001	**
Stg*entNo	93	9086.33	97.70	0.93	0.6572	NS

t Grouping Mean N *Striga*

A 93.46 495 *Striga* free
B 88.79 478 Inoculated

APPENDIX XI: ANOVA Table: Plant height

Source	DF	SS	SS	F-value	P>F	Significance
Rep	2	10377.91	5188.96	45.62	<0.0001	**
Stg	1	41735.18	41735.19	366.94	<0.0001	**
Rep*Stg	2	3319.52	1659.76	14.59	<0.0001	**
EntNo	99	28496.11	287.84	2.53	<0.0001	**
Stg*entNo	93	13497.74	145.14	1.28	0.0483	*

t Grouping Mean N Stg

A 68.72 500 Sfree

B 53.64 480 Inoculated

APPENDIX X11: ANOVA Table: Ear shape

Source	DF	SS	MS	F-value	P>F	Significance.
Rep	2	8.387	4.19	6.84	0.0011	**
Stg	1	0.105	0.10	0.17	0.6793	ns
Rep*Stg	2	0.747	0.37	0.61	0.5440	ns
EntNo	99	271.66	2.74	4.48	<.0001	**
Stg*entNo	93	61.357	0.65	1.08	0.302	ns

t Grouping	Mean	N	*Striga*
A	2.30	500	*Striga* free
A	2.28	480	Inoculate

APPENDIX XIII: ANOVA Table: Ear length

Source	DF	SS	MS	F-value	P>F	Significance.
Rep	2	95.48	47.74	47.2	<.0001	**
Stgct	1	496.26	496.26	490.65	<.0001	**
Rep*Stgct	2	40.36	20.18	19.95	<.0001	**
EntNo	99	1009.91	10.20	10.09	<.0001	**
Stg*entNo	93	83.33	0.90	0.89	0.7664	ns

t Grouping	Mean	N	*Striga*
A	7.32	500	*Striga* free
B	5.67	479	Inoculated

APPENDIX XIV: ANOVA Table: Ear width

Source	DF	SS	MS	F Value	P>F	Significance.
Rep	2	23.18	11.59	2.1	0.1231	ns
Striga count	1	2166.60	2166.60	392.65	<.0001	**
Rep*Stgct	2	40.21	20.10	3.64	.0266	*
Entry No	99	393.46	3.97	0.72	.9795	ns
Stg*entryNo	93	297.03	3.19	0.58	.9994	ns

t Grouping	Mean	N	Stg
A	5.99	500	Sfree
B	2.47	479	Inoculated

APPENDIX XV: ANOVA Table: Lodging percent

Source	DF	SS	MS	F-value	P>F	Significance.
Rep	2	8641.82	4320.91	19.07	<.0001	**
Stgct	1	9.53	9.53	0.04	.8375	ns
Rep*Stgct	2	3164.78	1582.39	6.99	.0010	**
EntNo	99	50891.6	514.06	2.27	<.0001	**
Stg*entNo	99	24465.8	263.07	1.16	.01532	ns

t Grouping	Mean	N	*Striga*
A	12.74	481	Inoculated
A	11.76	500	*Striga* free

APPENDIX XVI: ANOVA Table: Ear Exertion

Source	DF	SS	MS	F Value	P>F	Significance.
Rep	2	114.52	57.26	13.51	<.0001	**
Stgct	1	713.33	713.33	168.33	<.0001	**
Rep*Stgct	2	122.29	61.14	14.43	<.0001	**
EntNo	99	2122.91	21.44	5.06	<.0001	**
Stg*entNo	93	450.16	4.84	1.14	0.1808	Ns

t Grouping	Mean	N	*Striga*
A	12.97	500	*Striga* free
B	11.07	481	Inoculated

APPENDIX XVII: ANOVA Table: Stand count

Source	DF	SS	MS	F-value	P>F	Significance.
Rep	2	2734.43	1367.21	10.7	<.0001	**
Stg ct	1	15.03	15.03	0.12	0.7316	ns
Rep*Stgct	2	756.59	378.29	2.96	.0523	*
EntNo	99	32271.6	325.97	2.55	<.0001	**
Stg*entNo	93	7009.24	75.37	0.59	0.9991	ns

t Grouping	Mean	N	*Striga*
A	24.43	500	*Striga* free
A	23.76	481	Inoculated

APPENDIX XVIII: ANOVA Table: Number of fingers

Source	DF	SS	MS	F Value	P>F	Significance.
Rep	2	25.22	12.61	7.23	.0008	**
Stga count	1	1.06	1.06	0.61	.4353	ns
Rep*Stgct	2	26.23	13.12	7.52	.0006	**
Entry No	99	198.16	2.00	1.15	.1673	ns
Stg*entNo	93	182.48	1.96	1.12	.2092	ns

t Grouping	Mean	N	*Striga*
A	5.37	500	*Striga* free
A	5.22	479	Inoculated

APPENDIX XIX: ANOVA Table: Crop Yieldkgha-1

Source	DF	SS	MS	F Value	P>F	Significance.
Rep	2	25613766.0	12806883	30.59	<.0001	**
Stgct	1	33959188.5	33959189	81.12	<.0001	**
Rep*Stgct	2	13600848.1	6800424	16.24	<.0001	**
EntNo	99	100543950.8	1015596	2.43	<.0001	**
Stg*entNo	95	25206559.8	265332.2	0.63	.9971	ns

t Grouping	Mean	N	*Striga*
A	1074.40	495	*Striga* free
B	609.94	516	Inoculated

APPENDIX XX: Finger millet *Striga* GBS PCR layout.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	**GP 1**	**GP9**	**GP19**	**GP27**	**GP35**	**GP45**	**GP53**	**GP61**	**GP70**	**GP78**	**GP86**	**GP94**
B	**GP 2**	**GP10**	**GP20**	**GP28**	**GP36**	**GP46**	**GP54**	**GP62**	**GP71**	**GP79**	**GP87**	**GP95**
C	**GP3**	**GP11**	**GP21**	**GP29**	**GP37**	**GP47**	**GP55**	**GP63**	**GP72**	**GP80**	**GP88**	**GP96**
D	**GP 4**	**GP12**	**GP22**	**GP30**	**GP39**	**GP48**	**GP56**	**GP64**	**GP73**	**GP81**	**GP89**	**GP97**
E	**GP5**	**GP13**	**GP23**	**GP31**	**GP40**	**GP49**	**GP57**	**GP66**	**GP74**	**GP82**	**GP90**	**GP98**
F	**GP6**	**GP14**	**GP24**	**GP32**	**GP41**	**GP50**	**GP58**	**GP67**	**GP75**	**GP83**	**GP91**	**GP99**

G	**GP7**	**GP15**	**GP25**	**GP33**	**GP42**	**GP51**	**GP59**	**GP65**	**GP76**	**GP84**	**GP92**	**NA**
H	**GP8**	**GP17**	**GP26**	**GP34**	**GP44**	**GP52**	**GP60**	**GP69**	**GP77**	**GP85**	**GP93**	**GP100**

APPENDIX XXI: GBS vocabulary / Terminology

GBS Vocabulary comprise of the following:

Taxa -meaning the individual sample

Key file: This is the text file containing

- Sample information
- Barcode
- Flow cell and lane number
- Sample ID

Barcode which is the unique DNA sequence associated with each taxa

Sequence file is the text file containing DNA sequences information from Illumina

- Qseq or Fastq file

Read the DNA sequence produced in sequencing.

GBS Taq. The DNA sequence starts with cut site remnant and having additional sequence without Barcodes

Taqs by Taxa (TBT) Matrix of GBS taqs (row) with taxa (columns)

A read is a single sequence in the FASTQ output file generated by the GBS assay

A **good, barcoded read** is a sequence read with a perfect match to one of the barcodes provided in a barcode key file and with no N's in the sequence following the barcode up to the trim length. Under the current implementation, reads are trimmed to 64bp (not including the barcode).

A **tag** refers to a unique sequence (excluding the barcode) up to a specified length (currently 64bp) from one or more "good, barcoded reads". A given tag is typically observed in numerous good, barcoded reads of identical sequence (up to the trim length).

For our purposes, a **taxon** refers to a nameable entity from which one or more DNA samples can be taken.

APPENDIX XXII: Project detail that was send to Cornell University Laboratory

Project name	Source lab	Plate name	Well	Sample name	Pedigree	population	Sample DNA	Sample volume	Sample DNA prepared
FM	ICRISAT Nairobi	FM striga	A01	FM GP1	Germplasm	Inbred	47.5	40	1900
“	“	“	B01	GP2	“	“	85.5	40	3420
“	“	“	C01	GP3	“	“	95	40	3800
“	“	“	D01	GP4	“	“	95	40	3800
“	“	“	E01	GP5	“	“	76	40	3040
“	“	“	F01	GP6	“		76	40	3040
“	“	“	G01	GP7	“	“	47.5	40	1900
“	“	“	H01	GP8	“	“	57	40	2280
“	“	“	A02	GP9	“	“	85.5	40	3420
“	“	“	B02	GP10	“	“	76	40	3040
“	“	“	C02	GP11	“	“	95	40	3800
“	“	“	D02	GP12	“	“	66.5	40	2660
“	“	“	E02	GP13	“	“	66.5	40	2660
“	“	“	F02	GP14	“	“	57	40	2280
“	“	“	G02	GP15	“	“	57	40	2280
“	“	“	H02	GP17	“	“	38	40	1520
“	“	“	A03	GP19	“	“	93.8	40	3752
“	“	“	B03	GP20	“	“	38	40	1520
“	“	“	C03	GP21	“	“	38	40	1520
“	“	“	D03	GP22	“	“	38	40	1520
“	“	“	E03	GP23	“	“	38	40	1520
“	“	“	F03	GP24	“	“	38	40	1520
“	“	“	G03	GP25	“	“	38	40	1520
“	“	“	H03	GP26	“	“	38	40	1520
“	“	“	A04	GP27	“	“	38	40	1520
“	“	“	B04	GP28	“	“	47.5	40	1900

APPENDIX XXII CONTD…….

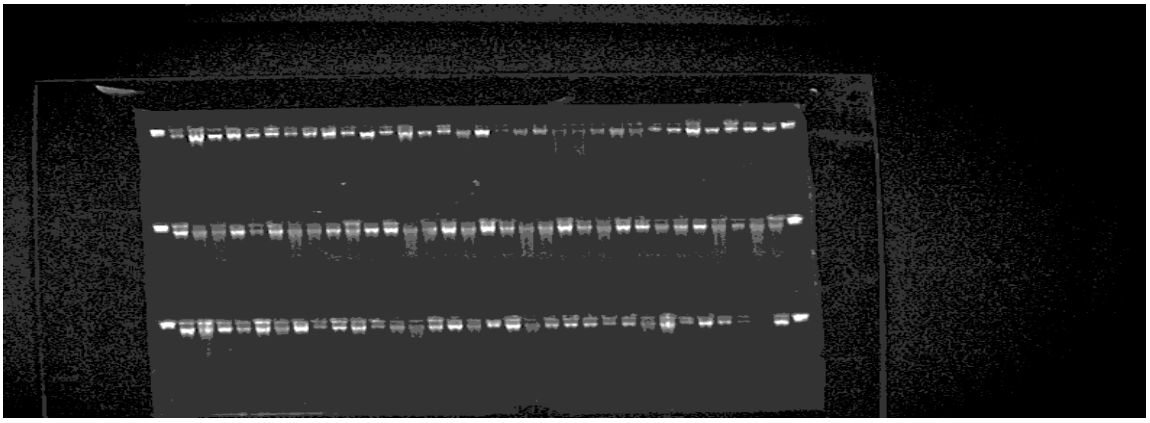
“	“	“	C04	GP29	“	“	47.5	40	1900
“	“	“	D04	GP30	“	“	66.5	40	2660
“	“	“	E04	GP31	“	“	66.5	40	2660
“	“	“	F04	GP32	“	“	66.5	40	2660
“	“	“	G04	GP33	“	“	85.5	40	3420
“	“	“	H04	GP34	“	“	76	40	3040
“	“	“	A05	GP35	“	“	57	40	2280
“	“	“	B05	GP36	“	“	38	40	1520
“	“	“	C05	GP37	“	“	38	40	1520
“	“	“	D05	GP39	“	“	57	40	2280
“	“	“	E05	GP40	“	“	38	40	1520
“	“	“	F05	GP41	“	“	57	40	2280
“	“	“	G05	GP42	“	“	38	40	1520
“	“	“	H05	GP44	“	“	38	40	1520
“	“	“	A06	GP45	“	“	57	40	2280
“	“	“	B06	GP46	“	“	47.5	40	1900
“	“	“	C06	GP47	“	“	57	40	2280
“	“	“	D06	GP48	“	“	76	40	3040
“	“	“	E06	GP49	“	“	38	40	1520
“	“	“	F06	GP50	“	“	38	40	1520
“	“	“	G06	GP51	“	“	76	40	3040
“	“	“	H06	GP52	“	“	47.5	40	1900
“	“	“	A07	GP53	“	“	85.5	40	3420
“	“	“	B07	GP54	“	“	57	40	2280
“	“	“	C07	GP55	“	“	38	40	1520
“	“	“	D07	GP56	“	“	47.5	40	1900
“	“	“	E07	GP57	“	“	66.5	40	2660
“	“	“	F07	GP58	“	“	47.5	40	1900
“	“	“	G07	GP59	“	“	38	40	1520

APPENDIX XXII CONTD…….

“	“	“	H07	GP60	“	“	76	40	3040
“	“	“	A08	GP61	“	“	57	40	2280
“	“	“	B08	GP62	“	“	47.5	40	1900
“	“	“	C08	GP63	“	“	47.5	40	1900
“	“	“	D08	GP64	“	“	57	40	2280
“	“	“	E08	GP65	“	“	38	40	1520
“	“	“	F08	GP66	“	“	38	40	1520
“	“	“	G08	GP67	“	“	47.5	40	1900
“	“	“	H08	GP69	“	“	38	40	1520
“	“	“	A09	GP70	“	“	76	40	3040
“	“	“	B09	GP71	“	“	66.5	40	2660
“	“	“	C09	GP72	“	“	66.5	40	2660
“	“	“	D09	GP73	“	“	47.5	40	1900
“	“	“	E09	GP74	“	“	66.5	40	2660
“	“	“	F09	GP75	“	“	47.5	40	1900
“	“	“	G09	GP76	“	“	76	40	3040
“	“	“	H09	GP77	“	“	47.5	40	1900
“	“	“	A010	GP78	“	“	57	40	2280
“	“	“	B010	GP79	“	“	57	40	2280
“	“	“	C010	GP80	“	“	47.5	40	1900
“	“	“	D010	GP81	“	“	57	40	2280
“	“	“	E010	GP82	“	“	38	40	1520
“	“	“	F010	GP83	“	“	57	40	2280
“	“	“	G010	GP84	“	“	66.5	40	2660
“	“	“	H010	GP85	“	“	38	40	1520
“	“	“	A011	GP86	“	“	66.5	40	2660
“	“	“	B011	GP87	“	“	97.5	40	3900
“	“	“	C011	GP88	“	“	38	40	1520
“	“	“	D011	GP89	“	“	57	40	2280

APPENDIX XXII CONTD……									
“	“	“	E011	GP90	“	“	57	40	2280
“	“	“	F011	GP91	“	“	57	40	2280
“	“	“	G011	GP92	“	“	57	40	2280
“	“	“	H011	GP93	“	“	57	40	2280
“	“	“	A012	GP94	“	“	38	40	1520
“	“	“	B012	GP95	“	“	57	40	2280
“	“	“	C012	GP96	“	“	38	40	1520
“	“	“	D012	GP97	“	“	66.5	40	2660
“	“	“	E012	GP98	“	“	57	40	2280
“	“	“	F012	GP99	“	“	38	40	1520
“	“	“	G012	Blank	NA	NA	NA	NA	NA
“	“	“	H012	GP100	“	“	66.5	40	2660

APPENDIX XXIII: 95 DNA samples of Finger millet GBS plate



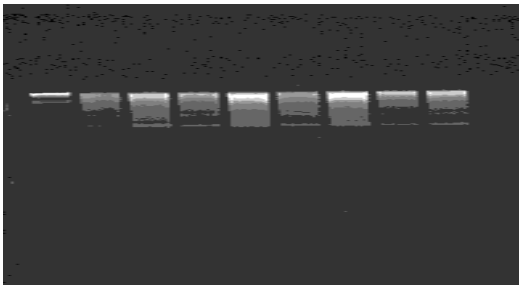
The gel picture layout of DNA samples was as follows:

LANE1: Lambda50ng/µL GP1 GP2 GP3 GP4 GP5 GP6 GP7 GP8 GP9 GP10GP11 GP12 GP13 GPP14 GP15 GP17 GP19 GP20 GP21 GP22 GP23 GP24 GP25 GP26 GP27 GP28 GP29 GP30 GP31 GP32 GP33 GP34 Lambda50ng/µL

LANE2: Lambda50ng/µL GP35 GP36 GP37 GP39 GP40 GP41 GP42 GP44 GP45 GP46 GP47 GP48 GP49 GP50 GP51 GP52 GP53 54 GP55 GP56 GP57 GP58 GP59 GP60 GP61 GP62 GP63 GP64 GP66 GP67 GP65 GP69 Lambda50ng/µL

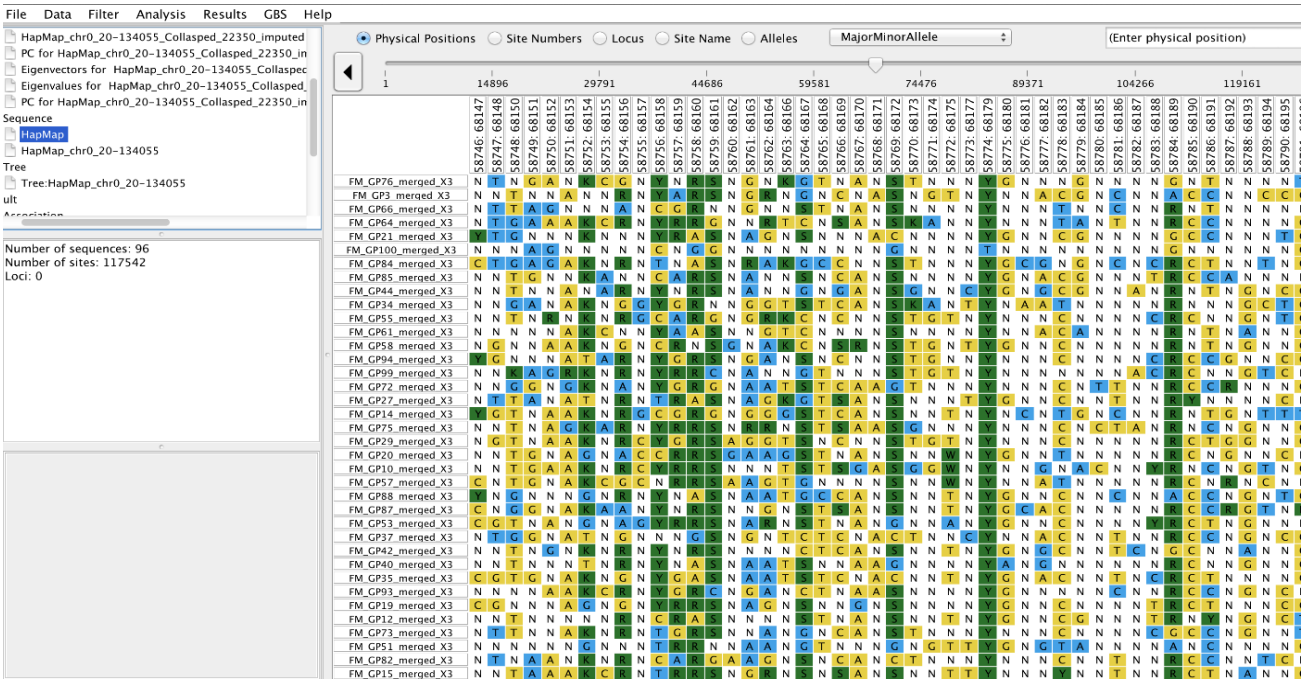
LANE3:Lambda50ng/µL GP70 GP71 GP72 GP73 GP74 GP75 GP76 GP77 GP78 GP79 GP80 GP81 GP82 GP83 GP84 GP85 GP86 GP87 GP88 GP89 GP90 GP91 GP92 GP93 GP94 GP95 GP96 GP97 GP98 GP99 Blank GP100 Lambda50ng/µL

APPENDIX XXIV: Plate of Digested DNA samples with RE Hind III

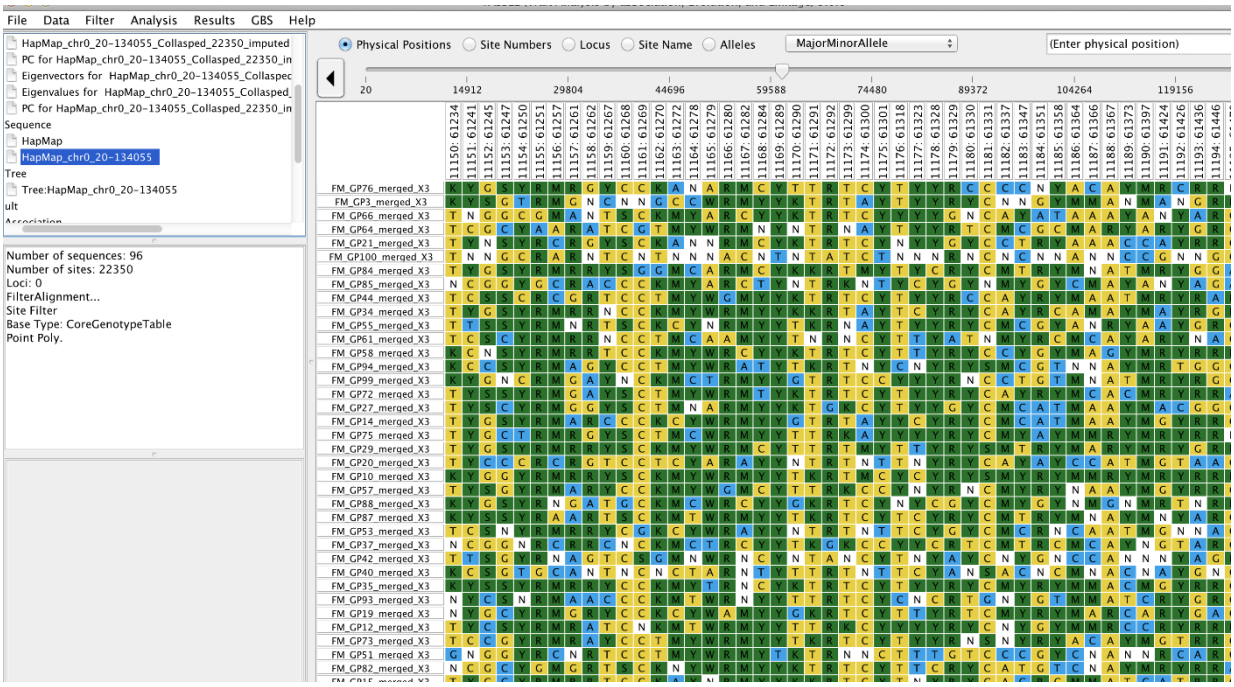


The well layout was Lambda, GP3, GP4, GP7, GP11, GP47, GP76, GP84 and GP87 respectively. Note: 6microlitre of the above selected DNA samples was loaded in each well and run 2 h at 80V

APPENDIX XXV: Hapmap file before filtering



APPENDIX XXVI: Filtered HapMap genotype file



APPENDIX XXVII: 27 paired end reads trimmed to 64bp arrangement of SNPs among the 95 genotypes

1. KACIMMI 73
   CAGCAAAACGCCAAGCACAGATGGGCAACTGCTC**GGGC**AGAAAAAA
   AAAAAAAAAAAAAAAAA

   KACIMMI 73

   CAGCAAAACGCCAAGCACGGATGGGCAACTGCTC**GGGC**AGAAAAAA
   AAAAAAAAAAAAAAAA AA

2. KACIMM 42
   CAGCAAGCCTCGATGCATCGATGAAAAATA**GGGG**CATGCCTCGATGC
   AGAAAAAAAAAAAAAA

   KACIMMI 42
   CAGCAAGCCTCGATGCATTGATGAAAAATA**GGGG**CATGCCTCGATGC
   AGAAAAAAAAAAAAAA

3. GBK000828
   CAGCAATATCAGCAGGCCGGCATGAGCCATTATGCAAATAATGCTGTG
   CCT**GGGG**AGCAGAAAA

GBK000828
CAGCAATATCAGCAGGCCGGCATTAGCCATTATGCAAATAATGCTGTG
CCT**GGGG**AGCAGAAAA

4. GBK029701
CAGCAA**GGG**AACCAAAATGCTCGTGCCACACAGCCTCCTGATCGTGG
AAGCAGAAAAAAAAAAA

GBK029701
CAGCAA**GGG**AACCAAAATGCTCGTGCCCCACAGCCTCCTGATCGTGG
AAGCAGAAAAAAAAAA A

5. GBK008278
CAGCAAGCCGCTGGTGGTCATCGTGGAAGAGCCCCAGCACGA**GG**CCT
TCATGCGCTGGCTGAAA

GBK008278
CAGCAAGCCGCTGGTGGTCATCGTGGAAGAGCCCCAGCACGA**GG**CTT
TCATGCGCTGGCTGAAA

6. GBK000692
CAGCAAGT**GGGG**CTGGTGCGGCAACACCCCCGACCACTGCGGCGCGG
GCTGAAAAAAAAAAAA

GBK000692

CAGCAAGT**GGGG**CTGGTGCGGCAACACCGCCGACCACTGCGGCGCGG
GCTGAAAAAAAAAAAA

7. KACIMMI 49
CAGCAAGCTACGGGAGAAAACCAACCTCGCCACT**GGGG**CCGAAGCA
GAAAAAAAAAAAAAAAA

KACIMMI 49

CAGCAGGCTACGGGAGAAAACCAACCTCGCCACT**GGGG**CCGAAGCA
GAAAAAAAAAAAAAAAA

8. KACIMMI 22
CAGCAAGCCGGCGGGTCGTCCGTGTGACCTCGGACGT**GGGGG**CAGAA
AAAAAAAAAAAAAAAA

KACIMMI 22
CAGCAAGCCGGC**GGG**TCGTCCGTGTGACCTCGGACGTGGTGGCAGAA
AAAAAAAAAAAAAAAA

9. GBK000802
CAGCAAGGAAGCTCTTTTGGATAGG**TT**GGGGATTTGTCTTTCG**TT**AGT
TTTTTTGGCTGAAAAA

GBK000802
CAGCGAGGAAGCTCTTTTGGATAGGTT**<u>GGGG</u>**A**TTT**GTC**TTT**CGTTAGT
TTTTTTGGCTGAAAAA

10. GBK029847
CAGCAAAAGAAGTCGGTTGGAGCTTCTTGT**<u>GGG</u>**TCACCTTCTTCGGCC
TTGTAGCAGAAAAAAA

GBK029847
CAGCAAAAGAAGTCGGTTGGAGCTTCTTGT**<u>GGG</u>**TCATCTTCTTCGGCC
TTGTAGCAGAAAAAAA

11. GULU-E
CAGCAAGGCGACGC**<u>GGGG</u>**AGAACGACGTGGGTGAGCAGAAAAAAAA
AAAAAAAAAAAAAAAAAA

GULU-E
CAGCAAGGCGACGC**<u>GGGG</u>**AGAATGACGTGGGTGAGCAGAAAAAAAA
AAAAAAAAAAAAAAAAAA

12. KACIMMI 72
CAGCAAGCCGCGGCCGCGA**<u>GGG</u>**AGCAGGCGTCGATGATGCAGATGCA
GAAAAAAAAAAAAAAA

KACIMMI 72
CAGCAAGCCGCGGCCGCGA**<u>GGG</u>**AGCAGGTGTCGATGATGCAGATGCA
GAAAAAAAAAAAAAAA

13. GBK029793
CAGCAAGCAGGCGGGCGGGGGCGGGGCCGCCGCCGGGCA**<u>GGGGGGG
GG</u>**CCGCAGAAAAAAAAA

GBK029793
CAGCAAGCAGGCGGGC**<u>GGGGG</u>**CGGGG**CC**GCCG**CC**GGGCAGGGGTGG
GG**CC**GCAGAAAAA AAAA

14. GBK029821
CAGCAAGCTCCATGCATAC**TT**CTAGACAGTTTTTGATTTC**TT**GCCCGA
ACCTGCTGAAAAAAAA

GBK029821
CAGCAAGCTCCATGCATACTTCTAGACAG**TTT**CTGA**TTT**CTTGCCCGA
ACCTGCTGAAAAAAAA

15. GBK008292
CAGCAAGATCCGAGCGCGGTAGAGGCCCCTCCAGGCGTGGCGGTGGC
CAGATCC**<u>GGG</u>**CGCTGAA

GBK008292
CAGCAAGATCCGAGCGCGGTAGAGGCCCCTCCATGCGTGGCGGTGGC
CAGATCC**GGG**CGCTAA

16. GBK008299
CAGCAAAAGCTTATTTGCTGATGTGCGTGTGCATCACCTTTTTTTTTGT
GTGTGATGAAGCAGA

GBK008299
CAGCAAAAGCTTATTTGCTGATGTGCGTGTGCATCACCTTTTTTTTTTT
GTGTGATGAAGCAGA

17. KACIMMI 77
CAGCAAAGAAATGAATGACCTCGATCCTTCCTTCCCTTTTTTTTTTTTTT
TTTTTTTTT**GGG**AA

KACIMM 77

CAGCAAAGAAATGAATGACCTCGATCCTTCCTTCCCTTTTTTTTTTTTTT
TTTTTTTTT**GG**AA

18. GBK000516
CAGCAAACACGAGGTCTGATCGCTCCCTCTCACTTT**TGG**CTCCACTGC
TGAAAAAAAAAAAAA

GBK000516
CAGCGAACACGAGGTCTGATCGCTCCCTCTCACTTT**TGG**CTCCACTGC
TGAAAAAAAAAAAAA

19. GBK029805
CAGCAAGCGCTTGTTCATGCAGGTGATCATTCTGTGCCGAGTACATCA
TTGGCAGAAAAAAAA

GBK029805
CAGCAAGCGCTTGTTCATGCA**GG**TGATCATTTTGTGCCGAGTACATCA
TT**GG**CAGAAAAAAAA

20. KACIMMI 36
CAGCAA**GG**CAGTTTTTCCATCCCGAGAAACCTCAAGCTTCCAACAGAT
GTGTCAGCTGAAAAA

KACIMM 36
CAGCAA**GG**CAGTTTTTCCATCCCGAGAAACCTCAAGCTTCCAACGGAT
GTGTCAGCTGAAAAA

21. KACIMM 17
CAGCAA**GGG**AGAGGTTGC**GG**ACGCCATCA**GG**CGCGCACA**GG**CTGAA
AAAAAAAAAAAAAAAAA

KACIMMI 17
CAGCAA**GGG**AGAGGTTGT**GG**ACGCCATCA**GG**CGCGCACAGGCTGAA
AAAAAAAAAAAAAAAAA

22. KACIMM 24
CAGCAAAGGGGG**G**AAGCAGAAGGCGTTCCCCGACGGGCGG**TGG**CTG
AAAAAAAAAAAAAAAAA

KACIMMI 24
CAGCAAAGGGGGGAAGCGGAAGGCGTTCCCCGACGGGCGG**TGG**CTG
AAAAAAAAAAAAAAAAA

23. BUSIBWABO-1
CAGCACCGTCGAGTCG**TGG**AGCGATGACGGCGGGAGCAGAAAAAAA
AAAAAAAAAAAAAAAAA

BUSIBWABO-1
CAGCACCGTCGAGTCG**TGG**AGCGATGACGGCGGGGGCAGAAAAAAA
AAAAAAAAAAAAAAAAA

24. KACIMMI 20
CAGCAACAGCGACCGCATGCCA**GGGG**TGGCAGTGGCGGCAGAAAAA
AAAAAAAAAAAAAAAAA

KACIMMI 20
CAGCGACAGCGACCGCATGCCA**GGGG**TGGCAGTGGCGGCAGAAAAA
AAAAAAAAAAAAAAAAA

25. GBK008339
CAGCAAAGA**GGG**ACGGTGT**GG**TGGC**GG**CGAT**GG**AGAATCCCTTCAC
TGGCTGAAAAAAAAAAA

GBK008339
CAGCAAA**GGGGG**ACGGTGT**GG**T**GG**CGGCGAT**GG**AGAATCCCTTCAC
T**GG**CTGAAAAAAAAAAA

26. KACIMMI 16
CAGCAAGCCTCGGCAGAGCGGAGAGGGA**TGG**C**GG**CAA**GG**CAGAAAA
AAAAAAAAAAAAAAAAA

KACIMMI 16
CAGCAAGCCTC**GG**CAGAGC**GG**AGAGGGG**TGG**CGGCAAGGCAGAAAA
AAAAAAAAAAAAAAAAA

27. KACIMMI 65
CAGCAAGCTACAGCAGGAGAGATGAGCTGT**GGG**CGCACTGCAGAAA
AAAAAAAAAAAAAAAAA

KACIMMI 65
CAGCAAGCTACAGCA**GG**AGAGATGAGCTGT**GGG**CGCCCTGCAGAAA
AAAAAAAAAAAAAAAA

APPENDIX XXVIII: PCA 22350 Matrix after SNP filtering.

<Use>	Covariate	Covariate	Covariate
<Format>	Num	Num	Num
<Trait>	PC 1	PC 2	PC 3
FM_GP76_merged_X3	-0.11251	-3.26812	5.913744
FM_GP3_merged_X3	4.547976	4.455568	12.20515
FM_GP66_merged_X3	-0.16294	-7.00073	7.985085
FM_GP64_merged_X3	-13.4755	-4.59608	-4.19039
FM_GP21_merged_X3	-6.43848	17.76162	-2.69943
FM_GP84_merged_X3	-0.47843	8.510707	2.377386
FM_GP85_merged_X3	4.113632	9.188636	13.06372
FM_GP44_merged_X3	8.625565	-6.26332	-3.8914
FM_GP34_merged_X3	-10.1853	-3.58021	-1.52615
FM_GP55_merged_X3	-13.6853	-2.43135	-4.40411
FM_GP61_merged_X3	2.991135	-6.46613	3.457041
FM_GP58_merged_X3	1.191694	-5.52338	-1.6235
FM_GP94_merged_X3	-0.27188	-8.86249	5.064355
FM_GP99_merged_X3	-5.59471	0.769492	-0.98403
FM_GP72_merged_X3	-5.67532	5.312348	-2.64425
FM_GP27_merged_X3	4.148541	2.695107	8.176304
FM_GP14_merged_X3	-12.4988	-2.78273	-2.71883
FM_GP75_merged_X3	-6.0069	14.33631	-3.08701
FM_GP29_merged_X3	3.863906	4.524906	-6.47299
FM_GP20_merged_X3	4.14508	-5.32961	-4.04642

APPENDIX XXVIII contd……..			
FM_GP10_merged_X3	1.757056	-0.11101	2.743667
FM_GP57_merged_X3	0.148332	-2.45907	4.373086
FM_GP88_merged_X3	-8.05924	18.78205	-4.54386
FM_GP87_merged_X3	2.470973	5.784499	0.42984
FM_GP53_merged_X3	-9.29567	-0.76225	-1.25891
FM_GP37_merged_X3	2.898239	4.28308	0.025924
FM_GP42_merged_X3	13.67624	-4.083	-14.3297
FM_GP40_merged_X3	13.23733	-5.80851	-8.81443
FM_GP35_merged_X3	11.70188	-1.33442	-8.72517
FM_GP93_merged_X3	-0.01602	-7.04836	9.788218
FM_GP19_merged_X3	5.080264	1.313512	3.253019
FM_GP12_merged_X3	1.431989	-5.77821	6.986494
FM_GP73_merged_X3	3.483314	2.627636	6.025033
FM_GP51_merged_X3	0.50013	-2.73269	5.864412
FM_GP82_merged_X3	3.732189	13.57792	5.575582
FM_GP15_merged_X3	1.803416	-5.32518	4.175092
FM_GP9_merged_X3	-2.24175	-4.40802	5.996946
FM_GP1_merged_X3	2.981254	4.059843	8.283764
FM_GP11_merged_X3	2.656028	-1.30411	0.360156
FM_GP67_merged_X3	-3.29947	0.840432	-4.65213
FM_GP91_merged_X3	-4.40798	-0.85462	0.229871
FM_GP65_merged_X3	1.877067	-3.49963	5.250661
FM_GP69_merged_X3	-6.16939	-3.90455	3.792223
FM_GP25_merged_X3	-13.1943	-1.58685	-4.22316
FM_GP77_merged_X3	4.056438	-0.99078	7.198134

APPENDIX XXVIII contd…….			
FM_GP13_merged_X3	-6.52185	15.08748	-3.9612
FM_GP78_merged_X3	0.112952	-5.7038	6.713561
FM_GP90_merged_X3	3.059224	3.126281	6.667044
FM_GP54_merged_X3	-13.4264	-0.67076	-5.54349
FM_GP26_merged_X3	10.40242	-3.67261	-6.759
FM_GP33_merged_X3	9.492964	5.143408	-6.1527
FM_GP8_merged_X3	-0.02019	19.03561	5.740463
FM_GP70_merged_X3	4.861542	5.655438	10.80681
FM_GP46_merged_X3	11.57328	-0.01803	-6.2014
FM_GP80_merged_X3	-14.3346	-2.56222	-5.70093
FM_GP30_merged_X3	4.020866	-5.78863	1.718999
FM_GP83_merged_X3	5.704094	4.822098	1.854039
FM_GP97_merged_X3	10.93249	14.17969	-10.7412
FM_GP28_merged_X3	2.095732	-2.87569	7.512148
FM_GP62_merged_X3	3.681598	-4.88247	7.351745
FM_GP31_merged_X3	-5.60083	-2.85239	0.948253
FM_GP79_merged_X3	-4.30209	-1.87419	4.282535
FM_GP49_merged_X3	12.99436	-1.90815	-13.6283
FM_GP39_merged_X3	12.49646	-5.72098	-8.08453
FM_GP7_merged_X3	10.20513	-1.31634	-8.97185
FM_GP63_merged_X3	-14.0984	-3.7793	-3.33418
FM_GP32_merged_X3	0.465538	-5.29227	5.384083
FM_GP48_merged_X3	10.86545	-1.38804	-6.52974
FM_GP98_merged_X3	-13.5449	-2.75236	-6.84222
FM_GP5_merged_X3	7.108842	-6.90615	-0.84282

APPENDIX XXVIII contd…….			
FM_GP81_merged_X3	0.384515	-5.66615	8.325625
FM_GP59_merged_X3	-15.0161	-0.55778	-3.64774
FM_GP47_merged_X3	11.80738	-4.90418	-6.9386
FM_GP22_merged_X3	1.768883	-3.38906	2.463204
FM_GP17_merged_X3	-4.59093	6.952748	3.285751
FM_GP52_merged_X3	-15.1962	-2.69997	-8.57167
FM_GP96_merged_X3	1.744544	3.747839	-2.18697
FM_GP2_merged_X3	1.177626	-10.7579	8.491536
FM_GP24_merged_X3	-1.04566	-5.94562	5.809732
FM_GP95_merged_X3	7.48076	8.12138	-4.57825
FM_GP89_merged_X3	1.025098	2.599789	4.81232
FM_GP92_merged_X3	-2.67394	-2.80829	1.514809
FM_GP60_merged_X3	3.33605	13.42366	4.609736
FM_GP23_merged_X3	11.51959	-4.64256	-6.44065
FM_GP45_merged_X3	-10.9783	-2.67329	-9.12849
FM_GP50_merged_X3	-0.65471	-4.06957	3.192375
FM_GP41_merged_X3	13.18793	-0.23266	-12.0848
FM_GP6_merged_X3	-9.27146	-0.10922	-4.71448
FM_GP74_merged_X3	-14.7075	-5.10769	-3.42562
FM_GP71_merged_X3	2.524609	-3.51964	3.929108
FM_GP86_merged_X3	2.475777	7.348569	4.620048
FM_GP36_merged_X3	-18.3714	-2.92426	-8.78202

APPENDIX XXIX: Cumulative principal component values

PC	Eigenvalues	Individual Proportion	Cumulative Proportion
1	**5741.6**	**0.035188**	**0.035188**
2	**3771.7**	**0.023115**	**0.058303**
3	**3578.5**	**0.021931**	**0.080234**
4	2753.3	0.016874	0.097108
5	2707.5	0.016593	0.1137
6	2624.5	0.016085	0.12979
7	2537.5	0.015551	0.14534
8	2456.3	0.015054	0.16039
9	2422.4	0.014846	0.17524
10	2410.3	0.014772	0.19001
11	2368	0.014512	0.20452
12	2337.3	0.014325	0.21884
13	2317.1	0.014201	0.23305
14	2293.2	0.014054	0.2471
15	2261.6	0.01386	0.26096
16	2236.7	0.013708	0.27467
17	2232.7	0.013684	0.28835
18	2201.2	0.013491	0.30184
19	2180.6	0.013364	0.31521
20	2172.1	0.013312	0.32852

Three terms were added to the data tree after running PCA. The first are PCs (column 1), the second Eigen values (column 2) and the last Eigen vectors. The chart function in the result model is used to graph the first three PCs, the individual Eigen value contribution and the cumulative Eigen contributions.