GENETIC DIVERSITY OF *Striga hermonthica* (DEL.) BENTH. WEEDS FROM NIGERIA AND KENYA, AND THE GENETIC RESPONSES OF SELECTED HOST MAIZE LINES

BY

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A THESIS IN THE DEPARTMENT OF BIOCHEMISTRY, SUBMITTED TO
THE FACULTY OF BASIC MEDICAL SCIENCES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

December 2019

ABSTRACT

Striga hermonthica (SH) is a parasitic weed that attacks and significantly reduces the yield of maize in Africa. The genetic interactions responsible for resistance or susceptibility of hosts to the parasite and the genetic differentiation that exists between and within SH populations are not fully known. This study investigated the genetic diversity of SH populations in the largest maize producers in Sub-Saharan Africa (Kenya and Nigeria) and; the genetic responses of a susceptible (5057) and a resistant (ZD05) maize genotype to SH infestation.

The SH plants were collected from farms across western Kenya (KSH) and northern Nigeria (NSH) in October 2012 and authenticated at the Department of Botany, University of Ibadan (UIH-22774). The plants (n=1029) were then genotyped with 1576 single nucleotide polymorphism markers and indices of genetic diversity [effective alleles (N_e), Shannon's information index (I), expected (H_e) and observed heterozygosity (H_o)] were determined. Population structure and fixation index (F_{st}), were assessed to identify genetic differentiation between and within KSH and NSH populations. Two maize varieties (5057 and ZD05) were divided into four groups of nine plants each and planted in rhizotrons (root observation chambers). Seven days after planting, three groups of each maize genotype were infested with pre-germinated SH and the fourth was used as uninfested control. Root tissue was taken at 3, 9 and 22 days post infestation (DPI) and total ribonucleic acid (RNA) was extracted using standard method. The root transcriptome was sequenced using next-generation sequencing. Gene expression levels of secondary metabolism, defence, and antiapoptotic genes were determined by profiling the messenger RNA levels and comparing the $\log_2$ fold-change (LFC) between the infested and uninfested maize plants and between the genotypes. Data were analysed using two-way ANOVA at $\alpha_{0.05}$.

The two populations of SH displayed high levels of genetic diversity. KSH showed higher levels (Ne=1.41±0.01, I=0.38±0.01, Ho=0.28, He=0.25±0.0) than NSH

(N_e=1.41±0.01, I=0.332±0.01, H_o=0.21, H_e=0.20±0.00). Significant genetic differentiation (F_{st}=0.15) was observed between the two populations and between three subpopulations detected within the NSH population (F_{st} =0.053). At 3DPI, secondary metabolism and defence genes, *benzoxazineless 1* (LFC=2.5) and *chalcone synthase 2* (LFC=3.2), were upregulated in ZD05, while in 5057, antiapoptotic genes, *bax inhibitor1* (LFC=1.4) and *bcl-2 binding anthanogene-1* (LFC=1.7) were upregulated. At 9DPI, secondary metabolism and defence genes, *chalcone synthase* (LFC=-1.7) and *cellulose synthase* (LFC=-1.7), were downregulated in 5057, while secondary metabolism and defence genes, *chalcone isomerase* (LFC=2.3), *cellulose synthase* (LFC=1.5), *chitinase* (LFC=1.6) and *phenylalanine ammonia-lyase1* (LFC=1.8) were upregulated in ZD05. At 22 DPI, secondary metabolism and defence genes, *chalcone synthase* (LFC=-2.9) and *phenylalanine ammonia-lyase1* (LFC=-2.9), were down regulated in 5057, while in ZD05, secondary metabolism and defence genes, *bx13* (LFC=1.8), *chalcone synthase* (LFC=1.8), *phenylalanine ammonia-lyase* (LFC=2.6) and antiapoptotic gene, *bax inhibitor1* (LFC=1.8) were upregulated.

Striga hermonthica populations in Kenya and Nigeria are genetically distinct and ecotypes exist within Nigeria. Genes involved in secondary metabolism and defence were upregulated in the resistant maize genotype, but down regulated in the susceptible genotype. The resistant line mobilized a more comprehensive response to the parasite than the susceptible line.

Keywords: *Striga hermonthica*, Genetic diversity, Maize genotypes

Word count: 496

CERTIFICATION

I certify that this work was carried out by Unachukwu, Nnanna Nnamdi in the Department of Biochemistry, University of Ibadan, Nigeria.

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DEDICATION

This work is dedicated to the Almighty God,

and to my family and teachers.

ACKNOWLEDGEMENTS

Firstly, I would like to express my profound gratitude to my supervisor, Professor E. O. Farombi, for the opportunity to carry out my studies under his tutelage. I appreciate his support throughout my postgraduate studies and acknowledge his kind guidance and direction all through this programme. I am also particularly indebted to my supervisor at the International Institute of Tropical Agriculture (IITA), Dr Melaku Gedil, for the stimulating discussions, hard questions, insightful comments, and financial support. This work would not have been possible without the financial support of the Bill and Melinda Gates foundation.

I also want to appreciate my teachers; Professor O. O. Olorunsogo, Professor O. A. Odunola, Professor O. A. Adaramoye, Dr M. A. Gbadegesin, Dr C.O.O. Olaiya, Dr S. O. Nwozo, Dr O. A. Adesanoye, Dr O. Adeyemo-Salami, Dr S. E. Owumi and Dr I. A. Adedara. I am especially grateful to Dr O. Olanlokun, who practically saw my finishing this program as a personal challenge. I am grateful to Dr Abolaji, Dr I. Awogbindin, Mrs Nwokocha, and Mr Ige for their kind help and all the staff and students of the Department of Biochemistry, University of Ibadan. I am especially grateful to my fellow students in the Molecular Drug Metabolism and Toxicology Unit.

My heartfelt gratitude goes to all staff of Integrated Striga Management in Africa (ISMA) Project, Maize breeding unit (IITA) and the Bioscience Center, IITA Ibadan: Dr I. Rabbi, Dr M. Oluoch, Dr A. Menkir, Dr Abuelgazim Elzein, Dr A. Partene, Dr L. Stavalone, Dr A. Gisel, Victoria Iwu, Toyin Abdulsalam, Suzzy Shitta, Ebere Stanley, Kamorudeen Oladeji, Hammed Ganiyu, Yemi Fasanmade Damilola Dada. My colleagues: Yinka Ilesanmi, Queen Obi, Deborah Babalola, Yusuf Muyideen, Ruth Uwugiaren, Kemi Stanley, Adetutu Udofia, Olohimai Akalumhe, Busayo Odunaya, Wale Oladejo, Joy Egbejale, Seun Opeloye, and Alimat Adewale.

Finally, I am grateful to my family for all the sacrifices they have had to make. My wife; Olutoyosi and my little ones Chimamanda and Somtochukwu. To my parents; Mr and Mrs Unachukwu and my siblings; Ada, Ije, Emeka, Uche and Onyeka. Thank you.

Nnanna Nnamdi Unachukwu (2019)

TABLE OF CONTENTS

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

ACRONYMS AND ABREVIATIONS

12OPDA	(9S,13S) 12 Oxophytodienoic Acid
AAO	Aldehyde Oxidase,
AOC	Allene Oxidase Cyclase
AOS	Allene Oxidase Synthase
ARF	Auxin Response Factor 5
BA2H	Benzoic Acid 2-Hydroxylase
Bcl2	B-Cell Lymphoma 2
bHLH	Basic-Helix–Loop-Helix
CBP60	Calmodulin-Binding Protein
CM	Chorismate Mutase
COI	Coronatine Insensitive 1
DIBOA	2, 4-Dihydroxy-2H-1, 4- benzoxazin -3(4H)-one
DIMBOA	2, 4-Dihydroxy-7-Methoxy-2H-1, 4-benzoxazin-3(4H)-one
DMBQ	2, 4-Dimethoxy-P-Benzoquinone
DNA	Deoxribonucleic Acid
EOT	Epoxyoctadecatrienoic Acid
FDR	False Discovery Rates
GBS	Genotyping By Sequencing
H_2O_2	Hydrogen Peroxide
HSP	Heat Shock Protein
IC	Isochorismate
ICS	Isochorismate Synthase
IIA	Indole Acetic Acid
IPL	Isochorismate Pyruvate Lyase
JA	Jasmonic Acid
JAIle	Isojasmonoyll Isoleucine
JAR1	Jasmonate Resistant 1

JAZ	Jasmonate ZIM Domain
Kda	Kilodalton
LOX	Lipoxygenase
LRR	Leucine-Rich Repeat
NBS	Nucleotide Binding Site
NPR1	Non-Expressor Of Pathogenesis-related protein
OPR	Oxophytodienoic Acid Reductase
PCR	Polymerase Chain Reaction
PR	pathogenesis-related
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SA	Salicylic Acid
SAR	Systemic Acquired Resistance
SCF	Skp1-Cul-F-Box Protein
SNP	Single Nucleotide Polymorphism
tar2	Tryptophan Aminotransferase related 2
Tf	Transcription Factor

CHAPTER 1

INTRODUCTION

1.1. Background of the study

Maize (*Zea mays L.*) is one of the most important cereal crops in the world in terms of quantity produced (McCann, 2005). Maize is cultivated in every ecological zone in sub-Saharan Africa. The moist savannahs, with an annual rainfall of 1270-1590mm and relatively high amount of sunlight, are the most suitable regions for maize production. Maize production in Africa is estimated at 64 million tonnes of grains from 26.9 million hectares (FAO, 2017). In 2010, Kenya produced over 3.3 million tonnes of maize grains from over 2.3 million hectares, while Nigeria produced over 10 million tonnes from 6.7 million hectares of land (FAO, 2017). Maize grains are rich in carbohydrates, essential minerals, vitamins A, C and E, and they contain 9 per cent protein. They are good energy sources; rich in calories and dietary fibre (US Department of Agriculture, 2019). It is used as food for humans and animals, raw materials for several industries and fuel. Despite its potentials, the production is severely constricted by both abiotic and biotic factors. *Striga* spp. is thought to be the greatest biotic constraint to Africa's cereal production (Sauerborn, 1991).

The genus *Striga* contains obligate root hemiparasites which are considered to be responsible for the most extensive agricultural losses in the semi-arid tropics of Africa. *Striga* (family; Orobanchaceae) contains about 41 parasitic species of flowering plants (Raynal-Roques, 1994). They are commonly referred to as 'Witchweeds' and are found occurring naturally in Africa, Asia, America and Australia. Plants of the genus *Striga* mostly parasitize important graminaceous crops. However, one species, *Striga gesnerioides,* is an important parasite of broadleaf crops such as cowpea, tobacco and sweet potato (Musselman, 1980). On the world scale, of all the *Striga* species, *Striga hermonthica* (DEL.) BENTH. is considered to be the most economically important (Kountche 2013). No other individual weed species in Africa results in as much crop loss as this parasite (Sauerborn, 1991). A single *Striga hermonthica* plant on a host plant can lead to approximately five per cent yield loss (Parker and Riches, 1993) and where infestation is high, it can cause total crop failure (Ejeta, 2007). *Striga hermonthica* plants are highly fecund, 10000 to 200000 are small, light seeds per plant. These seeds have long viability periods and are easily dispersed by wind, water, animals and agricultural practices.

A common challenge in controlling this parasite is the inherent diversity in *Striga hemonthica* populations and its adaptation to broad geographic areas (Hearne, 2009). Effective development and deployment of technologies geared at controlling *S. hermonthica* is undermined by this variability within the parasite populations, rendering these controls method ineffective across different areas. This may also be due to the specificity of parasite populations to the different host plants (Hearne, 2009) eliciting differential reactions of host genotypes to the parasite. As an obligate out-crossing species, *S. hermonthica* has enormous within population diversity which enables the parasite to evolve and adapt to changing environmental conditions. Its physiology, including the production of large number of seeds with long viability, contributes to its high genetic diversity (Kuiper *et al*., 1996). The constant changes in cropping patterns and farming systems across regions in sub-Saharan Africa impose varying selection pressures, which can also promote evolution of new ecotypes of *S. hermonthica*. A detailed knowledge of the distribution of *S. hermonthica,* is therefore necessary to facilitate the selection of representative testing sites for host resistance screening and evaluation of other control options. Host plant genotypes with durable resistance can therefore be developed along with other control options across the prevalent broad range of ecotypes of the parasite.

Crop varieties that are resistant to *Striga hermonthica* are central to any effective *S. hermonthica* control strategy for resource-poor small holder farmers (Kim, 1994). The use these cultivars though considered to be one of the most cost-effective strategies for control of *Striga*, has their effective deployment limited by a lack of understanding of the underlying genetic and phenotypic basis of the adaptation of *Striga* populations to new host resistance phenotypes (Scholes and Press, 2008) and environments. Kim, (1994) reported the lack of genes conferring resistance to *Striga* among most African maize landraces, even though some of these landraces showed differing amounts of tolerance to the parasite. Over the last decade, research into *Striga hermonthica* resistance in wild relatives of cultivated maize including *Zea diploperennis* and *Tripsacum dactyloidehave* have led to *Striga* resistance in *Zea diploperennis* derived maize lines (Amusan *et al.,* 2008, Yallou *et al.,* 2009). A good example of resistance in maize is found in ZD05 inbred developed in the International Institute of Tropical Agriculture (IITA). This inbred, with pedigree Z.Diplo.BC2-19-4-1-#-1-13-1-B-B, was chosen due to its ability to resist *S. hermonthica* in field trials. It had a lower number of attached *S. hermonthica* plants, the parasite development was delayed and the number of attached parasites that died were higher when compared to a susceptible inbred (5057) (Menkir 2006). Menkir (2006) showed that the resistant response manifested in this maize line can be transferred successfully to other genotypes. However, the underlying mechanisms (molecular, biochemical and physiological) of this resistance are yet unknown and uncharacterized. Knowledge of the mechanisms controlling and driving resistance to *Striga hermonthica* in these lines will provide a rational and scientific basis for their exploitation. Resistance that is sustainable could then be attained in maize by stacking genes for the various resistant mechanisms in varieties intended for environments endemic for *Striga.*

1.2 Justification of the study

Scientific and physical evidence exist and is apparent that *Striga hermonthica* is highly detrimental to cereal crop production in Africa. *Striga hermonthica* infestation leads to untold damage to cereal crops, including maize, sorghum and rice, and heavy losses to the farmers, thus exacerbating existing poverty and food insecurity in Africa. These losses are especially pronounced in maize. A number of control options are constantly being developed against this parasite; they however do not effectively control the parasite. It is, therefore, important that improved and locally adaptable control methods are developed against this devastating parasite (Kim, 1994). These control methods can only be developed and effectively deployed if the biology (genetics), biochemistry, physiology and distribution of the parasite is thoroughly understood (Hearne, 2009). It is also important that the relationships between the parasite and its host are well understood, characterized and documented. Understanding these interactions will bring to the fore the various mechanisms by which these maize plants respond (both susceptible and resistant responses) to *Striga hermonthica* infestation. These mechanisms can then be included when designing programs to develop crops that are resistant to the parasite. This is because a knowledge of the molecular basis underlying resistance phenotypes is essential for crop improvement and the development of novel control strategies (Yodder and Scholes, 2010). This is because, it will allow for host resistance phenotypes to be combined and deployed in an optimal manner, enabling the development of resistant maize lines that will be used as part of a cocktail of control strategies against *Striga* (Ciotola *et al.*, 2010). Nigeria is the largest producer of maize in sub-Saharan Africa (FAO 2017). *Striga hermonthica* infestation is found in the savannah ecologies of the Northern part of the country. Kenya is the largest producer of maize in the Eastern Africa (FAO 2017) and *Striga hermonthica* infestation is occurs only in the western part of the country. These region within these countries were therefore chosen for this study.

1.3 Objectives of the study

In view of the aforementioned, this study was carried out to investigate the diversity and inherent structure in populations of *Striga hermonthica* weeds in Nigeria and Kenya. In this study, the physiological, biochemical and molecular interactions between *Striga hermonthica* and infested maize plants, was also investigated. This study was also carried out to elucidate the molecular responses of maize lines (both resistant and susceptible) to infestation by the parasite.

The specific objectives of this study are to:

1. Evaluate the genetic diversity of *Striga hermonthica* in northern Nigeria and western Kenya.

2. To investigate the presence or absence of population structure within and between the *Striga hermonthica* populations from Nigeria and Kenya and to identify, where present, specific loci undergoing selection

3. Investigate the mechanisms through which maize genotypes resist/tolerate *Striga hermonthica* infestation.

4. Assess the genetic response of a resistant and a susceptible maize genotype to *Striga hermonthica* infestation.

5. Catalogue the difference in the molecular responses of infested and uninfested plants of both lines at different time points.

6. Identify potential genes involved in the resistant and susceptible responses to the parasite.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to parasitism

Parasitism is a form of symbiosis in which one organism (the parasite) benefits at the expense of another (the host). Both organisms are usually of different species and their association is often to the detriment of the host (Larry, 2014). Infestation by parasites does not immediately lead to the death of their hosts and they will often live on, in or with the host for long periods. The hosts are often larger than the parasite.

Among the vascular plants, parasitism is found in the eudicotyledonous angiosperms only. The only environments where parasitic plants have not adapted to is the aquatic environment, and this may be because competition for water is a major driver of the evolution of plants adapted to dry land. This force does not exist in the aquatic environment (Heide-jørgensen, 2013). There are approximately 4000 species of angiosperms that form parasitic symbiosis with other plants (Nickrent *et al.,* 1998). These parasitic plants depend on their hosts for the acquisition of solutes to different extents. The parasitic mode of nutrition is diverse, both taxonomically and geographically. The evolution of parasitism is believed to have occurred independently 11 times in angiosperms, parasitic members being found in approximately 22 families and 270 genera (Nickrent *et al.,* 1998). Parasitic angiosperms can be found across a large number of biomes ranging from the arctic tundra (e.g. *Pedicularis dasyantha*), to temperate grasslands (e.g. *Orobanche minor*), to tropical rain forests (e.g. *Rafflesia* spp.) (*Ziang et al.,* 2018). Although all parasitic relationships are detrimental to the host organism (Smith and Douglas, 1987), the extent of damage done to the hosts varies dramatically. The impact of a specific parasite on a host is dependent on several factors, which include, the degree of dependency of the parasite on its host, the parasite’s size, its metabolic activities, it’s developmental stage, the susceptibility of the host, coupled with a wide range environmental factors (Graves, 1995).

Parasites can be classified based on the whether or not they possess chlorophyll in their tissues and are thus able or unable to photosynthesize - (a) hemiparasites that can photosynthesize and (b) holoparasites that cannot. Holoparasites such as *Orobanche* are entirely heterotrophic in their nutrition, while hemiparasites (e.g. *Striga* and *Bartsia*) are capable of autotrophic carbon acquisition. Hemiparasitic angiosperms can be classified further into facultative and obligate parasites. Facultative parasites such as the root hemiparasite, *Rhinanthus,* will grow and successfully reproduce in the absence of any hosts. Obligate parasites (all holoparasites and some hemiparasites such as *Striga),* on the other hand, are incapable of completing one or more stages of their life cycle without a host (Musselman, 1980). The type and degree of parasitism varies widely among parasitic angiosperms. However, one unifying feature, the haustorium is common to all parasite-host associations. This specialised parasite-derived organ, the haustorium, facilitates the attachment to and penetration of the host plant. Upon penetration, it acts as a conduit between parasite's vascular tissue and that of the host. The position of the haustorial connections on the host plant has allowed the classification of parasitic angiosperms into either root (e.g. *Striga* spp.) or shoot or aerial (e.g. Cuscuta spp.) parasites. However some species like, *Exocarpos cupressiformis,* that occur both on stems and roots exist (Coleman, 1934) and in the Orobanchaceae some species are known to infest both roots and rhizomes (Weber, 1993); therefore caution should be exercised when using the term 'root and stem parasites'. (Heide-Jørgensen, 2013).

Parasitic angiosperms are also classified on the basis of their importance as agricultural weeds. Of the families containing parasitic plants, eight contain species that are considered to be economically important weeds: Scrophulariaceae, Orobanchaceae, Santalaceae, Balanophoraceae, Convolvulaceae, Lauraceae, Viscaceae and Loranthaceae (Parker and Riches, 1993). These weeds act to reduce the productivity and/or quality of agricultural, silvicultural or forest crops (Riches and Parker, 1995). The most important genera of all parasitic weed species, in terms of economic loss to agriculture are *Striga*, *Alectra*, *Orobanche* and *Cuscuta* (Eplee and Norris, 1995), which are found in the Orobanchaceae (*Striga*, *Alectra* and *Orobanche*), and Convolvulaceae.

2.2. Taxonomy, distribution and biology of *Striga hermonthica* (DEL.) BENTH.

Striga hermonthica (Del.) Benth. (1836) is commonly known as purple witchweed. It is an obligate root hemiparasite. According to Parker and Riches (1993), *S. hermonthica* is recognized as the parasitic weed causing the highest amount of economic losses globally.

2.2.1 Taxonomic Tree

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Lamiales
Family: Orobanchaceae
Genus: *Striga*
Species: *Striga hermonthica*

2.2.2. Description

Striga hermonthica is a herbaceous annual plant between 30 and 100 cm high, with branches in larger plants. Stems and leaves are green and covered in fine hairs (trichomes). The leaves are narrowly lanceolate or elliptic, up to 1 cm wide, 2-8 cm long and are arranged on the lower half of the stem mostly in an opposite configuration, this arrangement however becomes irregular on the upper half. It has sessile flowers that are arranged in an inflorescence with a terminal spike of flowers (which may bear up to 100 flowers), and with axillary spikes that branch from the upper leaf axils. The flowers are subtended by 1-2 cm long and up to 3 mm wide bracts. They have with a fringe of ciliate hairs and a tubular Calyx that is up to 1 cm long possessing 5 ribs and 5 teeth 2-3 mm long. The flowers are asymmetrically campanulate, the tube 1-2 cm long, bent approximately halfway up in West African, Sudanese and Ethiopian populations but usually well above halfway in East African populations (Parker and Riches, 1993). Four corolla lobes are present, one is bi-lobed almost erect, the others spread out horizontally. They can be up to 2 cm across and are pink with some white markings in the throat. Both the stigma and stamens are hidden in the tube. Its seeds, numbering up to several hundreds, are approximately 0.3 mm by 0.2 mm in size and are housed in 1 cm long capsules. The root system is weak and is almost incapable of absorbing materials from the soil; however, on contact with roots of the host, the lower nodes of the plant develop branches, which can develop into secondary haustoria (CABI, 2019).

2.2.3. Distribution and habitat of *Striga hermonthica*

Striga hermonthica is distributed throughout the semi-arid tropics of Africa. It can be found from the semi-arid areas of Ethiopia to the moist savannah of West Africa, traversing the continent east to west, and down to Namibia in the south (Riches and Parker, 1995). *Striga hermonthica* is commonly associated with soils with low fertility, especially soils with low nitrogen content (Pieterse, 1991). It can thrive on light, sandy soils and heavy clay soils with low amounts of moisture and is not usually found on well-watered soils. It however tolerates abundant moisture for short periods. These factors make *S. hermonthica* extremely well adapted to the African savannah and thus its association with cereal cropping. There is a steady increase in land area parasitized by *S. hermonthica* as well as its level of infestation (Emechebe, 2004; Ejeta, 2007), and the reasons adduced for this include; movement of cereal seeds contaminated with *S. hermonthica* seeds, movement of animals, dispersal by surface water and wind, a poor understanding of the parasite and lack of effective control options (Berner *et al.*, 1994). It is possible that climate change may influence both the invasive potential and the geographic distribution of *S. hermonthica*, this is because there might be expansions of habitats that can support the growth of the parasite (Mohamed *et al.*, 2006).

2.2.4. Biology and physiology of *Striga hermonthica*

Striga hermonthica has the capacity to photosynthesize, even though it is an obligate hemiparasite. It has a complex life cycle that comprises of a series of discrete steps. A summary of its life cycle is shown in figure 2.1.

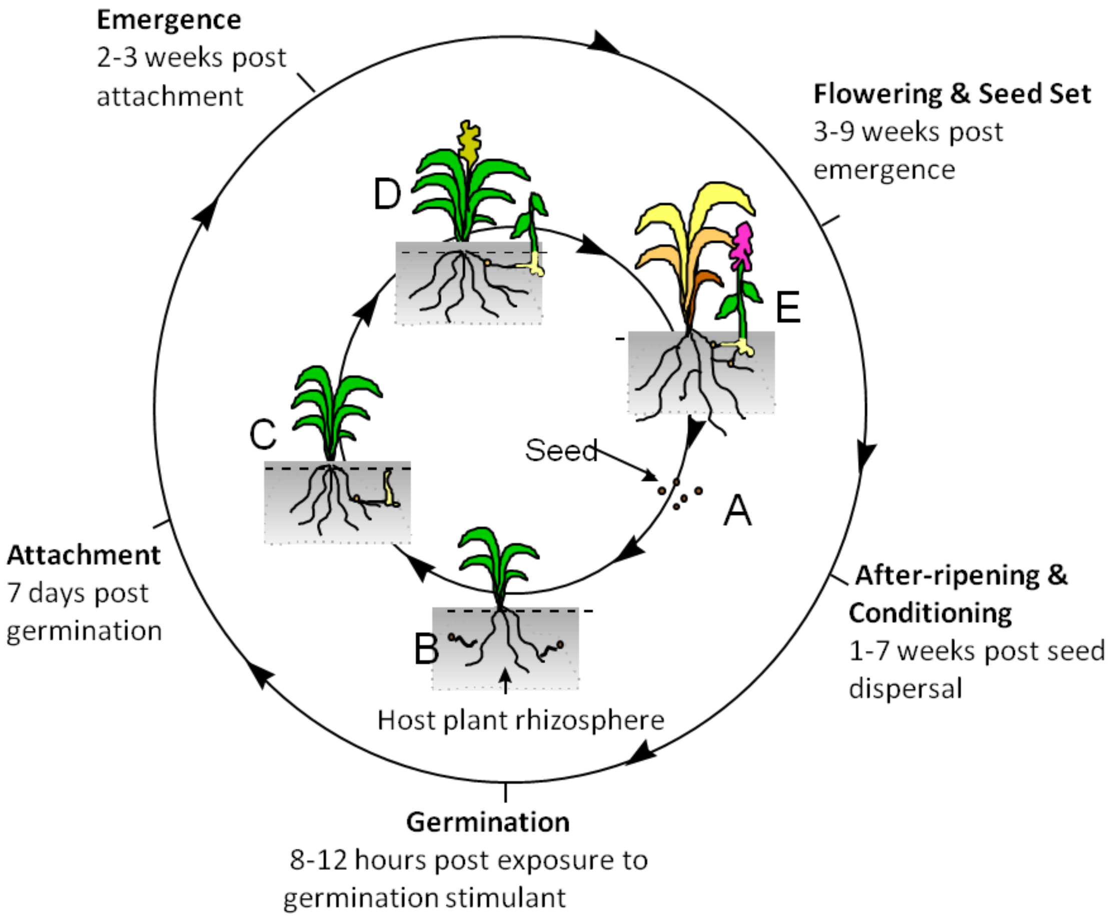

Figure 2.1. The Life Cycle of *Striga hermonthica* on a Susceptible Host.

Stages indicated:

A = after-ripening and conditioning of seed, B = seed germination, C = haustorial initiation, elongation and subsequent attachment to the host, then underground growth, D = emergence of plants from the soil, E = flowering, insect pollination, seed set and dispersal.

2.2.4.1. Pre-emergence growth.

Striga hermonthica passes through a pre-emergence stage, a seedling stage, a vegetative growth stage, a flowering stage, and finally a fruiting stage. Its tiny seeds require about 6 months of dormancy and then a conditioning period (a 7 to 14-day period, where the seeds are exposed to moisture and high temperatures) after which they germinate if germination stimulants from the roots of a suitable host are present (Parker and Riches, 1993). Conditioned *S. hermonthica* seeds can revert to the dormant state in the absence of the germination stimulant (Mohamed *et al.*, 1998). These germination stimulants are called strigolactones.

Strigolactones and strigolactone biosynthesis

Strigolactones are plant signalling compounds (hormones). They perform two major functions in plants. Firstly, they control the development of the plant as endogenous hormones and secondly, they facilitate symbiotic associations between plants and certain soil microbes as part of exudates from the roots of these plants. They however also stimulate germination of the seeds of some parasitic plants (including *S. hermonthica*) when these parasitic plant seeds are in close proximity to the roots of a strigolactones exuding plant. This parasite seed germinating ability led to the detection and recognition of strigolactones (Smith M, 2014). Characterised strigolactones, like strigol and orobranchol, are tetracyclic sesquiterpene with double lactone rings (Figure 2.2). Differences in the chemical modifications to the core strigolactone structure and differences in their stereochemical (three dimensional) conformations characterize the various individual strigolactones (Smith M., 2014). Examples of some strigolactones are shown in figure 2.2.

Carlactone is a precursor in the synthesis of strigolactones. In the plastid, all-trans β-carotene is converted to carlactone by the enzymes D27 (an isomerase) and CCD7 and CCD8 (both carotenoid cleavage dioxygenases). It is then transported into the cytoplasm where oxidization, more rings closed and functional groups are added to the rings by CYP711 family (MAX1) enzymes (Zhang *et al.*, 2014) (Figure 2.3).

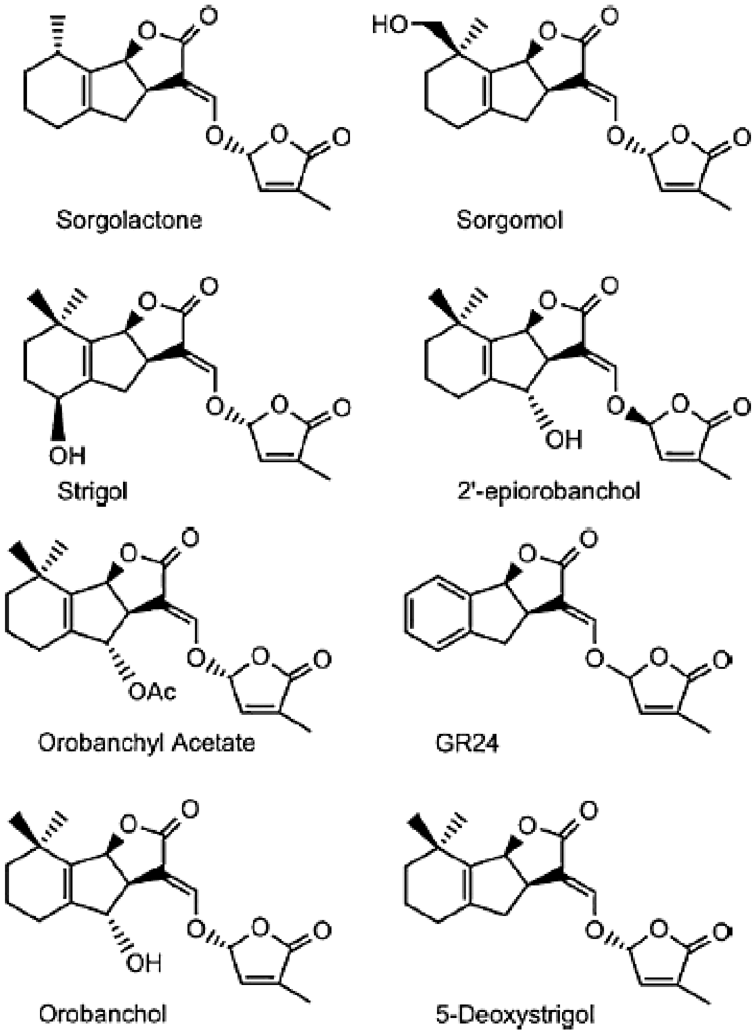
Sorgolactone
Sorgomol
Strigol
2'-epiorobanchol
Orobanchyl Acetate
GR24
Orobanchol
5-Deoxystrigol

Figure 2.2. Chemical structure of some strigolactones and GR24

(GR24 is a synthetic strigolactone with IUPAC name (±)-(3aR*,8bS*,E)-3-(((R*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one).

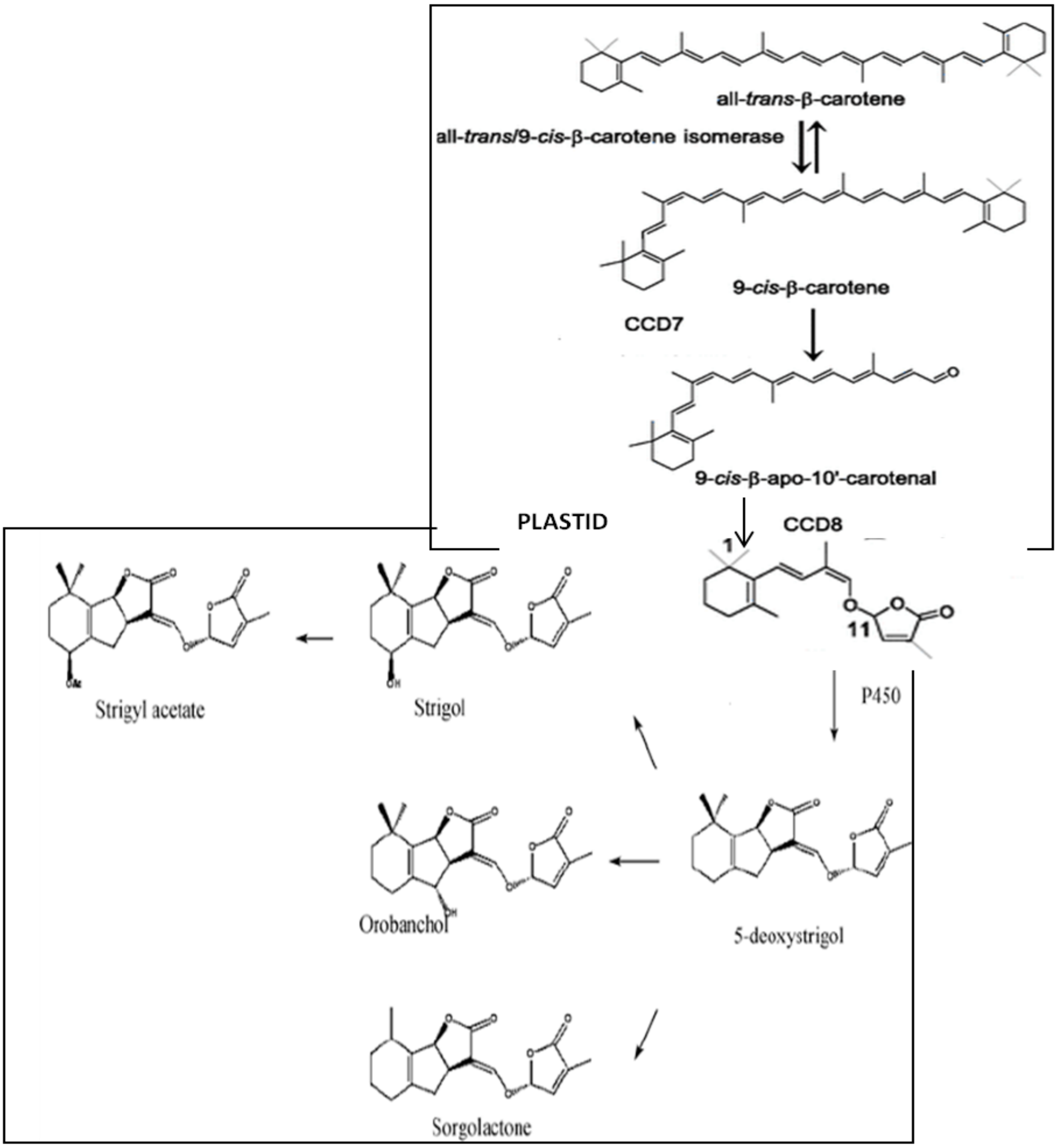

Figure 2.3. Strigolactone biosynthesis

As endogenous hormones, strigolactones, under normal conditions reduce lateral branching in both stems and root, but enhances plant height, increases secondary growth, senescence, and root hairs. Under suboptimal conditions, like low soil phosphates, strigolactone production is enhanced and it promotes root growth while limiting stem growth. Strigolactones also induces the branching of hyphae in arbuscular mycorrhizal fungi. This might be partly due to the need to encourage mycorrhizal symbiosis; mycorrhizal fungi help plants to obtain phosphates from the soil (Jansa *et al.*, 2011). However, its third function is decidedly negative, as they induce the seeds of some parasitic plants including *Striga* to germinate. A brief exposure of pre-conditioned seeds to Strigolactones can initiate germination within 8 to 12 hours (Ejeta *et al.*, 199), this seed germination is also temperature-dependent and optimal germination is achieved at 33 ^{o}C (Musselman, 1980).

The carbohydrate and lipid reserves of *Striga* seeds are low in comparison with other seeds (Parker and Riches, 1993), and much of the lipid reserve is utilised in haustorial initiation and formation. This low nutrient reserves lead to a loss in the capacity of germinated seedlings to form competent haustoria within three to five days.

Haustorial induction and development

After germination, the haustorium develops. It is a specialised parasite-derived organ that facilitates both the attaching of the parasite to the host and the penetrating of its tissues. Upon penetration, it acts as a conduit between host and parasite vascular tissue. In response to stimuli provided by the roots of the host plant, the haustorium develops from the tips of *Striga* radicals. Some flavonoids, quinines and phenolics have been implicated in the induction of haustorial development. Of these, 2, 4-dimethoxy-p-benzoquinone (DMBQ) has been isolated directly from the roots of host plants. DMBQ is produced when lignin is oxidatively degraded and decarboxylation of phenolic acids. According to Keyes *et al*. (2007) from studies on *Striga asiatica,* reactive oxygen species (H_2O_2) regardless of its origin (from the plant or the parasite), interacts with host-derived phenols and peroxidases to release the benzoquinones which then serve as a cue for the initiation of haustorial formation. *Striga* is thought to, in a process termed semagenesis, elicit its host to produce the signal required for its development (Yoder and Scholes, 2010). *Striga* radicles generate Hydrogen peroxide (H_2O_2) which provides the appropriate substrate for peroxidases derived from host plant to catalyse the breakdown of the hosts' cell wall components. These cell wall components are used as substrates to generate benzoquinones that will induce the formation of haustoria (Keyes *et al.,* 2007). In this model, xenognosins are extracted from the root surface of the hosts; this ensures that the parasite is in close proximity with its host before it commences haustorial development (Keyes *et al.,* 2007). Kim *et al*, (1998) however indicated that constitutive production of an activated oxygen species mediates host recognition and thus haustorial formation. This allows the parasite to exploit abundant host enzymes to produce the diffusible recognition signals. (The synthesis of haustioria inducing factors is shown in figure 2.4) These signals induce the development of the haustoria. Sticky hairs are found all over the developing haustorium enabling it to anchor to the root of a suitable host. At the parasite-host interface, the haustorial cells undergo division and elongation and slips between the epidermis and cortex of the host's root. There is no disruption in the cell wall of the host at the site of invasion, suggesting that the penetration occurs between host cells rather than through them. This is again observed as the endodermal cells are neither damaged nor crushed during penetration of host endodermis by *Striga hermonthica.*

The penetrating haustorium advances by moving between the primary and secondary wall of the endodermis (Neumann 1999). Haustorial cells push the host cell walls aside by the dissolving the middle lamella and the mechanical pressure by the advancing parasitic cells results in a change in the host cell shape (Joel and Losner-Goshen 1994). It is thought that either parasite or host-encoded cell wall degrading enzymes are involved in the invasion, these enzymes have however not been directly identified (Yodder, 2010). Dorr, 1997 using scanning electron microscopy showed that during invasion, *Striga asciatica* (a close relative of *S. hermonthica*) cells use a specialised structure, called the osculum, to perforate the vascular system of the host and form xylem-to-xylem connections (Figure 2.5).

The anchoring and penetration steps are essential if *Striga* (an obligate parasite) is to survive, as water and host-derived nutrients can now be transferred to the parasite (Press and Graves, 1995). The parasite depends totally on the host until it emerges from the soil, this is because its seeds are very small and thus do not contain sufficient stored nutrients.

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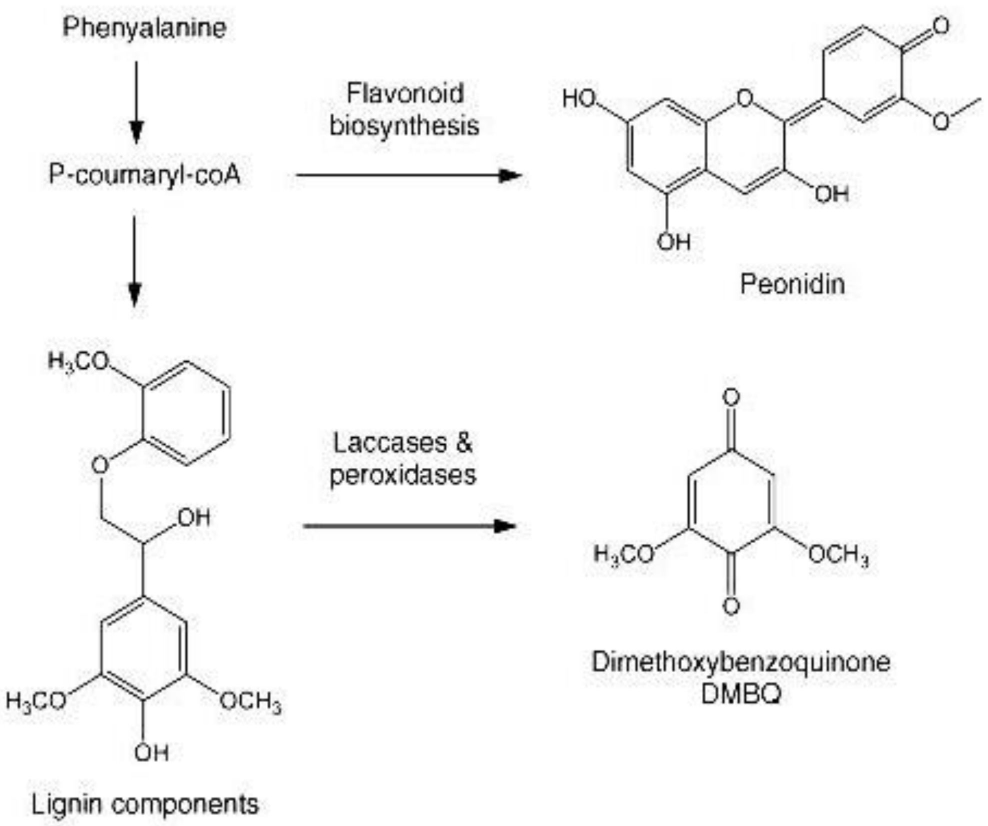

Figure 2.4. Synthesis of haustoria inducing factors.

Peonidin and dimethoxy benzoquinone are both haustoria inducing factors. Peroxidases and laccases act on lignin components to produced DMBQ. (Adapted and modified from Yoder and Scholes, 2010).

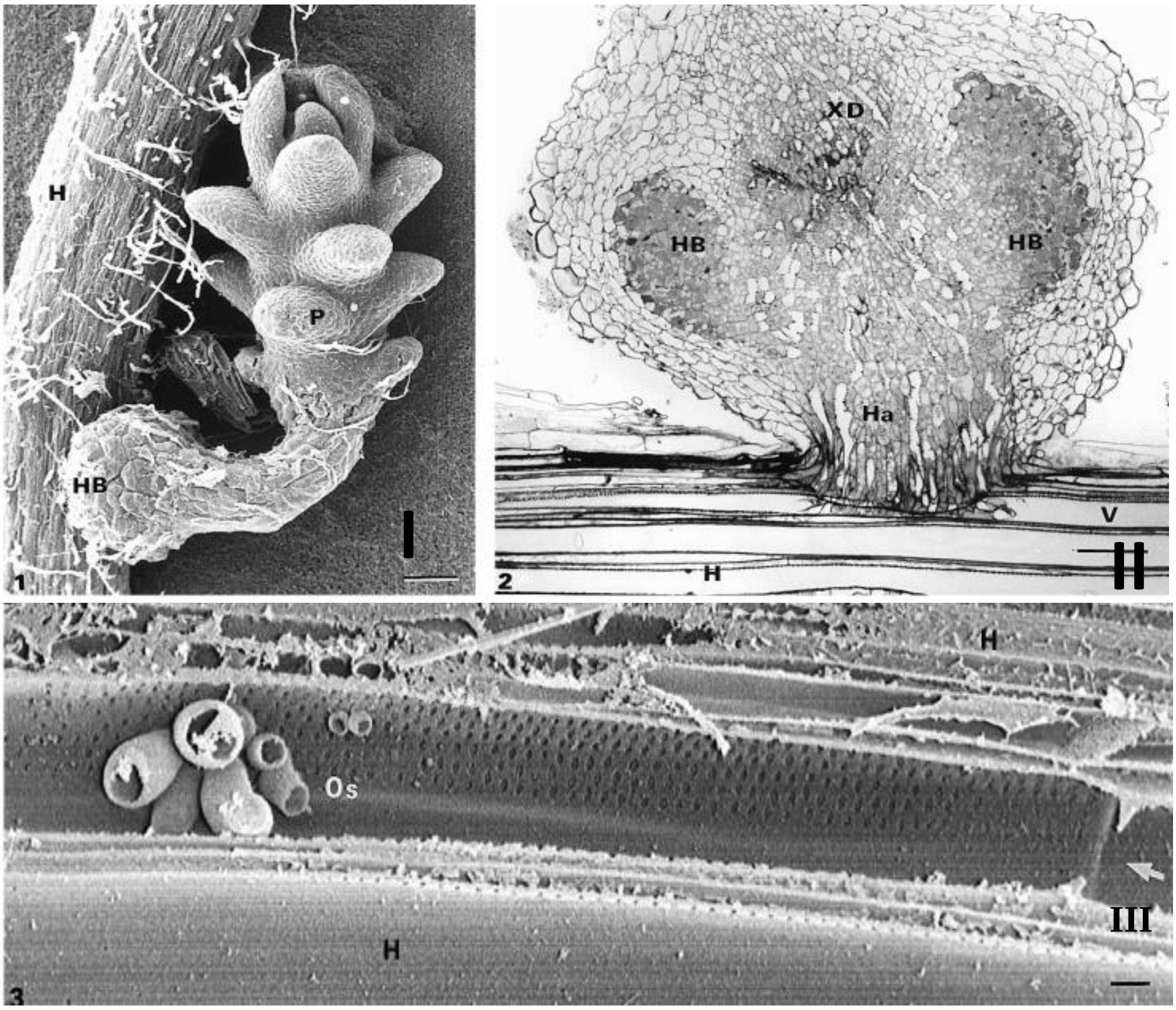

Figure 2.5. The development of a *Striga* plant attached to the root of a host.

I). An attached *Striga* plant (P) growing on the root of a host plant (H). The hyaline body is marked HB.

II) The longitudinal section of the hyaline body (HB) of the haustorium (Ha) of the parasite and the root of its host (H). V the host vessel showing spreading parasitic cells

III) A longitudinal section of a haustorium (Ha) that is penetrating the root of a host (H). As seen the pitted vessel of the host is penetrated by a number of oscula (Os) (The tips of the Oscula show openings). (Adapted and modified from Dorr, 1997)

One unknown key issue in parasitic plant research is how the parasite plant manages to traverse the barriers provided by a host during a compatible invasive process without activating defensive mechanisms (Pe´rez-de-Luque, 2013). Mayer (2006) theorized that either there are biochemical or physiological similarities between the host and the parasite because they are both higher plants or that the parasitic plant can somehow subvert the activation of host defence responses.

2.2.4.2. Post emergence growth.

The shoots of *Striga* tubercles grow in a negatively geotropic manner. The culmination of this growth for a proportion of attached parasites is emergence from the soil. Upon emergence from the soil, *Striga* shoots become chlorophyllous and are capable of autotrophic nutrition. Despite this, its relatively low photosynthetic ability (between 0.5 and 8.0μmol $m^{-2}s^{-1}$) means its dependence on the host will not cease (Press *et al.*, 1986). The parasite maintains a higher transpiration rate than its hosts (Shah *et al.*, 1987) due to its stomata being almost permanently open. This leads to a rapid transfer of materials from the host, especially if the humidity in the environment is low (CABI, 2019). Osmosis is also involved in the movement of solutes from the host to the parasite.

The continued movement of host solutes into the developing *Striga* tubercle is achieved by the maintenance of more negative osmotic potentials in the parasite. Parasitic plants employ a number of substances to supplement/maintain a negative osmotic pressure on their hosts; these include amino acids, organic acids, polyols, other carbohydrates and xylem-mobile cations (Richter and Popp, 1992; Ehleringer and Marshall, 1995; Tennakoon *et al.*, 1997).

The main carbohydrate osmoticum present in *Striga* is the polyol mannitol, which accounts for an excess of 75% of the parasite’s soluble carbohydrates (Press *et al.*, 1986). Some roots may develop at the base of the parasites’s stem and form secondary haustoria if the roots come into contact with the roots of other hosts (not necessarily from the primary host). These secondary haustoria have similar structures and are believed to function in a similar manner to primary haustoria (Parker and Riches, 1993). Temperatures between 30-35°C favour growth, as do soils with low humidity. Emerged plants flower within five to six weeks after initial attachment to the host

(Parker and Riches, 1993). *S. hermonthica*, an obligate out-crosser, depends on different insects to pollinate it (Musselman *et al.*, 1983). Viable seed is produced within two weeks and is shed within four weeks of flower opening (Parker and Riches, 1993).

2.3. Responses of hosts to *Striga* infestation

Striga hermonthica parasitizes Gramineae (Poaceae): maize, millet, sorghum, sugar cane and rice, causing a deleterious impact on the growth and performance of all its cereal hosts. Its deleterious effects on the host plant is attributable to a combination of factors including loss of water, nitrogen, minerals and carbohydrate by the host, a distortion of the efficiency of photosynthesis in the host (Press and Graves, 1991) and a disruption in the host's root/shoot allometry. Typically, infected plants have lower total biomass accumulation, lower grain yield and are noticeably shorter (due to lower internode elongation) than uninfected counterparts (Taylor *et* al., 1996; Frost *et al.*, 1997). The impact of this parasitic plant has also been suggested to be due to the action of a *Striga*-derived toxin (Musselman 1980). The severity of the impact of *S. hermonthica* on its hosts is moderated by host and parasite genotype, host nutrition, growth stage of the host at the time of infection and infection density (Cechin and Press 1993a, 1993b, 1994; Graves *et al.*, 1989; Gurney *et al.*, 1999). Upon infection, the host plants display symptoms of stunting, show chlorosis and even die (Dorr, 1997). Upon heavy infestation on maize in Kenya, losses estimated at 80% are common (Bebawi *et al*, 1984). About 30–40% of the total maize crop growing area of Togo, Mali and Nigeria is infested (De Groote *et al*, 2008). Certain plants in the host range of *Striga* show varying amounts of resistance, tolerance or susceptibility to attacks by the *S. hermonthica*, and the mechanism of this observed host response to the parasite differs from one host plant to another (Timko and Scholes, 2013)

2.3. Taxonomy, distribution and production constraints of Maize

Maize (*Zea mays*) is widely grown across a range of agroecological environments throughout the world. It evolved in Mexico, Central America about 6000 years ago, but arrived in Africa through various introductions about 500 years ago (McCann, 2005). It is presently a leading food crop in Africa. Approximately 1000 million tons of maize is produced worldwide, with Africa producing 7.6%. The largest maize producer in West Africa is Nigeria with approximately 10.8 million tons while Kenya produces just over 3.5 million tonnes, making it the largest maize producer in East Africa (FAO, 2017).

2.3.1. Taxonomic Tree

Kingdom: Plantae
(Unranked): Angiosperms
(Unranked): Monocots
(Unranked): Commelinids
Order: Poales
Family: Poaceae
Subfamily: Panicoideae
Tribe: Andropogoneae
Genus: Zea
Species: Z. mays
Subspecies: Z. mays subsp. Mays

2.3.2. Distribution and Importance

Maize is grown at latitudes varying from the equator to slightly above 50^o North and South and from sea level to over 3000m above sea level, under heavy rainfall and in semi-arid conditions, in cool and very hot climates and with growing cycles ranging from three to six months. The wide distribution of maize production is an indication of its excellent capacity to adapt to many environments. Its production in West Africa has greatly expanded in recent years, from the traditional maize growing areas in the forest zone to the high potential areas of the savannas. Among the grains, maize has the highest annual production globally (FAO, 2017).

Over 50 species of maize exist, and these species have varying grain shapes, textures, colours, and sizes. Although maize is a grain crop, it is consumed as a vegetable in Africa and many parts of the world. Its grains contain large quantities of carbohydrates, vitamins, essential minerals and protein (Okoruwa and Kling, 1996), with high dietary fibre and calorie content, and are thus a good energy source. Maize is majorly used as livestock feed and industrial raw material in industrialized countries.

Households with low-income in Eastern and Southern Africa expend 30−50% of their earning on maize, where it is used as food for these families and their livestock, fuel and raw materials for industries. It is a major calorie and income source in the savannahs and mid-altitudes of West and Central Africa. It can be processed into various end uses at the homestead level and on industrial scale as brewer grits. (Abdulrahaman and Kolawole, 2006). Different countries prepare and process maize into various products depending on usage. Ground maize and maize flour is made into a variety of meals in different regions of Africa. Maize can be boiled, grilled or roasted on its cob and enjoyed as a light meal in many parts of Africa. Popcorn is also popularly consumed all over Africa and the world. Abdulrahaman and Kolawole, 2006).

2.3.3. Disease incidence and constraints

Despite its potential, the production of maize has been greatly constrained by both abiotic and biotic factors. Drought, variable sunshine and low soil nitrogen significantly affect maize production. In addition, the humid tropical climates can cause stored maize grains to decay. Biotic factors common in sub-Saharan Africa include maize diseases (like downy mildew, leaf blight, rust, stalk and ear rots, leaf spot, and maize streak virus), insect pests including various species of stem borers, ear borers, grain moths, beetles, weevils, and the parasitic weed *Striga* (*Striga hermonthica* and *S. asiatica*). These factors result in between 30 and 90 per cent loss of the crop during cultivation, after harvesting and during storage. The parasitic weed *Striga hermonthica* is the most destructive weed species in Africa resulting in extensive losses of all its cereal hosts (Sauerborn, 1991). *Striga* infestation causes yield losses that range from 15 to 75 per cent (Oswald and Ransom, 2001). This yield loss is exacerbated by the fact that the parasite thrives under low fertility and low moisture conditions and in locations with very poor farming systems within Africa. In these regions farmer’s resources are scarce and they have little or no means with which to control the parasite, this further impoverishes the already relatively poor, smallholder farmers.

2.3. Resistance, tolerance and susceptibility to *Striga* in maize

In general terms of research into witchweeds, tolerance is defined as the potential of a host plant to function optimally in the presence of attached parasites, while resistance relates to the potential of a host to prevent the parasite from forming attachments to its roots (Kim and Adetimirin, 1997). Resistance by hosts can take different forms. These forms may involve both generic and specialized defence mechanisms mobilized simultaneously or independently, to disrupt important points during the parasite's growth cycle (Timko and Scholes, 2013). It is important to mention that, host species that are completely resistant to parasite are yet to be found, and it has been observed that resistance to a particular parasite is more commonplace in wild relatives of the host as opposed to the cultivated (domesticated) germplasm (Hearne, 2009). Some host plant resistance mechanisms have been put forward (Ejeta *et al.,* 1993) and they are broadly divided into pre-attachment and post-attachment resistance (Scholes and Press 2008; Rodenburg *et al.,* 2010). Pre-attachment resistance is seen in a lack of or a decrease in the generation of germination stimulants, inhibition of germination, haustorium formation inhibition or reduction, partial inhibition of haustorium development and formation of mechanical barriers to infection like thickened cell walls in the roots of the host (Timko and Scholes 2013). With these mechanisms, a host can prevent the parasite from attaching. Post attachment resistance occurs following the successful connection of the parasite to the vascular system of the host. According to Timko and Scholes (2013) post-attachment resistance can take a number of forms, which include:

1) Abiosis, which involves the root cells of the host producing and releasing cytotoxic compounds (like phytoalexins), when attacked by the parasite.

2) Preventing the ingress and growth of parasite by forming of physical barriers (for instance cell wall lignification and suberisation).

3) A hypersensitive response (HR) that involves programmed cell death (PCD) at the point of parasite attachment to effectively halt penetration and prevent parasite development as such retarding the establishment of a functional vascular continuity with the host.

2.4. Gene expression in host defence responses and signalling pathways involved in host defence responses.

A number of genes, gene families and transcription factors have been found to be involved in a host's response to parasites in general and to members of the Orobanchaceae in particular. Similar groups of genes are recorded to have been upregulated during resistance responses to Orobanche and *Striga*, and the Salicylic and Jasmonic acid signalling pathways are increasingly becoming implicated in the control of resistance to parasitic plants by hosts (Timko and Scholes, 2013).

2.4.1. Salicylic acid signalling pathway.

The phenolic compound, salicylic acid (SA, 2-hydroxy benzoic acid), comprises of an aromatic ring to which a hydroxyl group or its functional derivative is attached. As reported by Dempsey *et al*, (2011) salicylic acid is an established plant signal molecule (hormone); it is known to regulate some aspects of plant growth and development, as well as disease resistance (Vlot *et al*, 2009). Humans use SA and its acetylated derivate (aspirin) as important pharmacological agents. Salicylic acid is commonly used to treat acne, warts and psoriasis, while aspirin is widely used in treating fever, inflammation, and pain. Aspirin also reduces the risk of stroke, heart attack (AFHS 2018) and some cancers (Patrignani and Patrono, 2016)

There is evidence for two distinct SA biosynthetic pathways in plants (Figure 2.6). One is the isochorismate (IC) pathway and the other phenylalanine ammonia-lyase (PAL) pathway. Although both biosynthetic pathways have not been fully elucidated, both of them start with the end product obtained from the shikimate pathway, which is chorismate. (Dempsey *et al*, 2011)

2.4.1.1. Biosynthesis of salicylic acid

Plants and some bacteria are hypothesised to synthesise SA through the same pathway, and this led to the identification of the isochorismate (IC) pathway. A number of genes that encode isochorismate synthase (ICS), the enzyme that catalyses the conversion of chorismate to isochorismate, have been recognised in plant species (Dempsey and Klessig 2017). In the phenylalanine pathway, the enzyme, phenylalanine ammonia-lyase catalyses the conversion of phenylalanine (Phe) to *trans*-cinnamic acid (*t*-CA) and ammonia (NH_3). After which *t*-CA is non-oxidatively converted to Benzoic acid (BA). An inducible benzoic acid-2-hydroxylase (BA2H) is proposed to convert BA to Salicylic acid.

2.4.1.2. Salicylic acid signalling in plant innate immunity and defence

Plants have an innate immune system which protects them from invasion by pathogenic organisms. Salicylic acid (SA) induces resistance responses to diseases in plants (Dempsey *et al.*, 2011, Yang *et al.*, 2013). This system proactively protects plants against fungal, bacterial and viral pathogens. These pathogens have a signature called ‘pathogen-associated molecular pattern (PAMP)’ and this pattern can be perceived by plant pattern recognition receptors (PRRs). A PRR consists of a “receptor” domain to transduce the PAMP signal (Vidhyasekaran, 2015).

According to Vidhyasekaran (2015), this signal is delivered by Salicylic acid (SA) and it initiates defence gene transcription. The PAMP signalling system triggers SA biosynthesis by generating a specific calcium ion ($Ca^{2+)}$ signature in the cytosol, which is received and decoded by Calmodulins. Calmodulin-binding protein 60 G (CBP60g) is a protein that is involved in activating SA biosynthesis. Activation of the isochorismate synthase in SA biosynthesis pathway is triggered by the calcium signature signals transduced to CBP60g.

Reactive oxygen species (ROS) also acts upstream of SA accumulation. Intracellular accumulation of Benzoic acid (BA) is caused by the presence of Hydrogen peroxide (H_2O_2). Benzioc acid (BA) is converted to SA by benzoic acid 2-hydroxylase (BA2H). This enzyme is inducible, and it is synthesized de novo in response to increased BA level. Therefore, it leads to an increase in the cellular concentration of SA. Another ROS Nitric oxide (NO) induces phenylalanine ammonia lyase (PAL), a key enzyme in of salicylic acid biosynthesis, thereby activating the biosynthesis. Salicylic acid responsive defence-related genes are activated by increased expression of transcription factors in response to SA signalling.

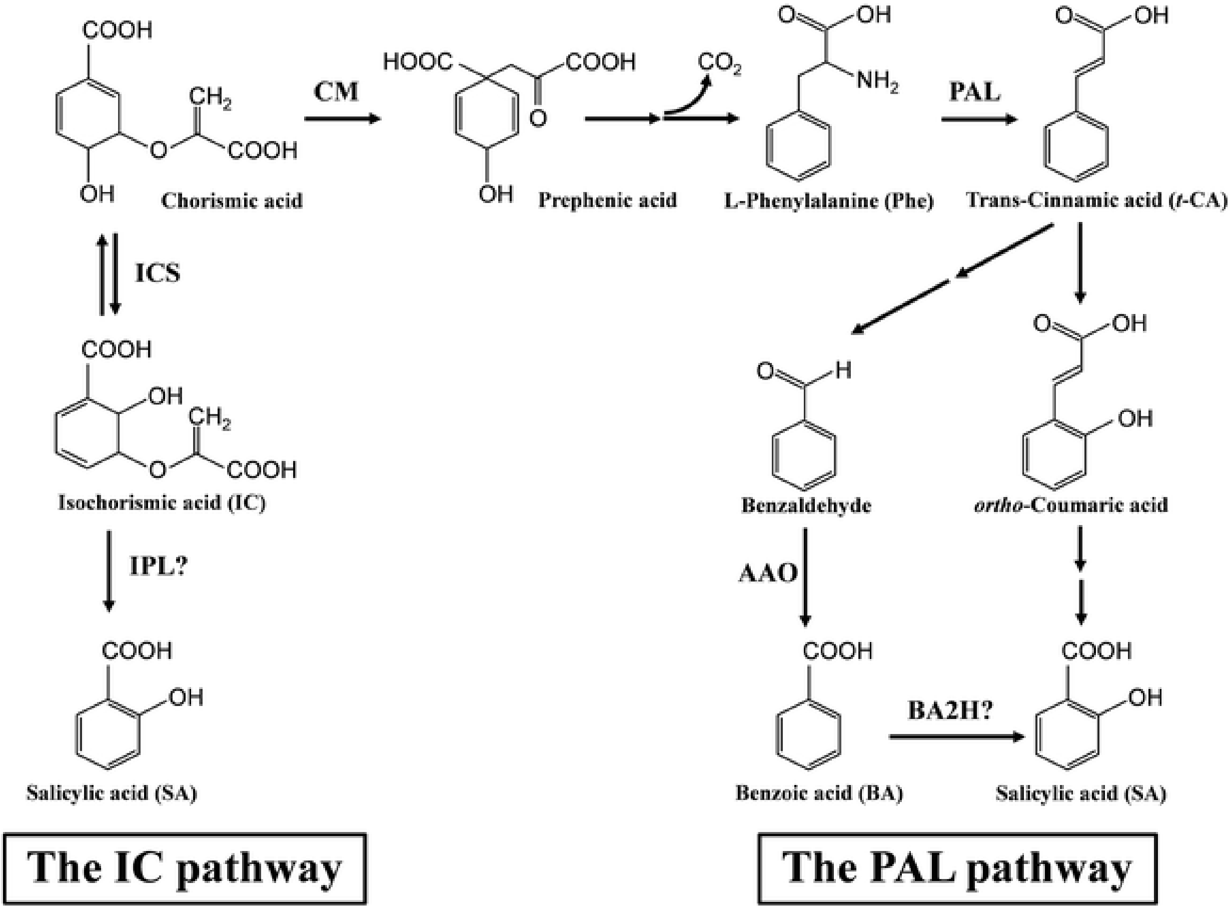

Figure 2.6. Biosynthesis of Salicylic acid

Plants are thought to produce Salicylic acid through two biosynthetic pathways.

The abbreviated enzymes are as follows: *ICS* isochorismate synthase, *IPL* isochorismate pyruvate lyase, *CM* chorismate mutase, and *PAL* phenylalanine ammonia-lyase, *AAO* aldehyde oxidase, *BA2H* benzoic acid 2-hydroxylase. A *Question mark* indicates an enzyme that has not been definitively identified (Dempsey and Klessig 2017).

NPR1 (non-expressor of pathogenesis-related protein) is a master regulator of the SA-mediated induction of defence genes that directly binds SA. In the absence of pathogen challenge, an NPR_1 protein forms an oligomer in the cytoplasm upon induction it becomes localized in the nucleus. The proteasome continuously clears NPR_1 from the nucleus, thus its activity as a co-activator is repressed and defence responses initiated only when necessary. SA accumulation is induced by Pathogen/PAMP exposure and the induced SA causes targeted degradation, in a SA concentration-dependent manner, of two NPR_1 paralogues (NPR_3 and NPR_4). NPR_3 and NPR_4 have been identified as adaptor proteins of the CUL_3E_3ligase. After a plant is infected SA concentration increases and it binds to NPR_4 causing it to release NPR_1. Transcription of defence genes is then activated by the free NPR_1. NPR_1 is a cofactor of TGA transcription factors, and it enhances the binding of TGA transcription factors to the promoter of *PR1* gene to activate transcription of *PR1* gene (Vidhyasekaran, 2015).

The SA signalling system, apart from activating local resistance, is also involved in activating systemic acquired resistance (SAR) observed in distal (systemic) tissues. SAR is a heightened state of defence against a broad spectrum of pathogens. It is salicylic acid-dependent and activated throughout a plant following a localized infection. SAR is associated with priming of defence, which results in a faster and stronger induction of defence mechanisms after pathogen attack (Conrath 2011; Po-Wen *et al.*, 2013). It involves extensive reprogramming of transcription. Changes in the expression of about 2,000 genes is mediated by SA. Such a broad effect on gene transcription may be associated with extensive chromatin remodelling. The offspring of plants that have been primed show a faster and higher accumulation of defence-related genes transcripts in the salicylic acid signalling pathway and enhanced disease resistance upon inoculation with virulent pathogens, indicating that the priming can be epigenetically inherited from plants that were exposed to a disease (Pastor *et al.*, 2013).

The epigenetic basis of this phenomenon is indicated by the fact that when one generation of the plant was skipped from the stressor, the SAR was still found to be sustained (Luna *et al.*, 2012). DNA methylation is also thought to play an important role in transgenerational SAR. This is because hypomethylated genes also transmit transgenerational SAR. These genes then direct the priming of SA-dependent defences in the following generations. (Vidhyasekaran, 2015)

2.4.2. Jasmonic acid signaling pathway

Jasmonic acid (JA) and its derivatives, Jasmonates (JAs) are lipid-derived signalling molecules. They are involved in the regulation of many developmental processes and in regulating adaptive responses to stresses in plants. JAs belong to of oxygenated fatty acid collectively known as oxylipins. Oxylipins are derived either from enzymatic or autoxidation of free or membrane esterified fatty acids (Borrego and Kolomiets, 2013). Oxylipins are termed eicosanoids in mammals. The major subgroup of enzymatically derived mammalian oxylipins are leukotrienes, prostaglandins, prostacyclins, thromboxanes, lipoxins, eoxins, hydroxyeicosatetraenoic acids and epoxyeicosatrienoic acids (Buczynski *et al.,* 2009). In mammals, they regulate a number of physiological processes, including vasoconstriction, vasodilation, response to pain and generation of fever. In addition to receptor ligand signalling, oxylipins also have direct antimicrobial activity (Prost *et al.,* 2005) and can alter the redox status of the cell (Park *et al.,* 2013).

2.4.2.1. Overview of maize Jasmonic Acid biosynthetic pathway.

In the maize genotype B73, the synthesis of jasmonate (Figure 2.7) begins with 13 lipoxygenase (13 LOXs) catalyzing the production of 13 (S) hydro peroxy octadeca 9, 11, 15 trienoic acid (13 HPOT, (9Z, 11E, 13S, 15Z) 13, from α linolenic acid (C 18:3). The 13LOX product 13HPOT is catalysed into an epoxide, 12, 13 (S) epoxyoctadecatrienoic acid (12, 13, EOT), by Allene Oxide Synthase (13 AOS). Allene Oxide Cyclase (AOC) then catalyses the cyclisation of 12, 13 (S) EOT yielding a member of the jasmonate family of oxylipins (9S,13S) 12 oxophytodienoic acid (12OPDA). It is predicted that 12,13(S) EOT undergoes self-cyclisation in a stereospecific manner and the enzyme AOC just holds it in the appropriate configuration (Borrego and Kolomiets, 2013, Hofmann *et al.,* 2006). The above steps of JA biosynthesis takes place in the plastid. Inside the peroxisome, 12OPDA is reduced via Type II 12 oxophytodienoic acid reductases (OPRs). Type II OPRs reduce the cyclopentenone cis (+) 12OPDA into the cyclopentanone, OPC8: 0 (8[3 oxo 2 cis [(Z) 2pentenylcyclopentyl] octanoic acid).

member of the jasmonate family of oxylipins (9S,13S) 12 oxophytodienoic acid acid (C 18:3).

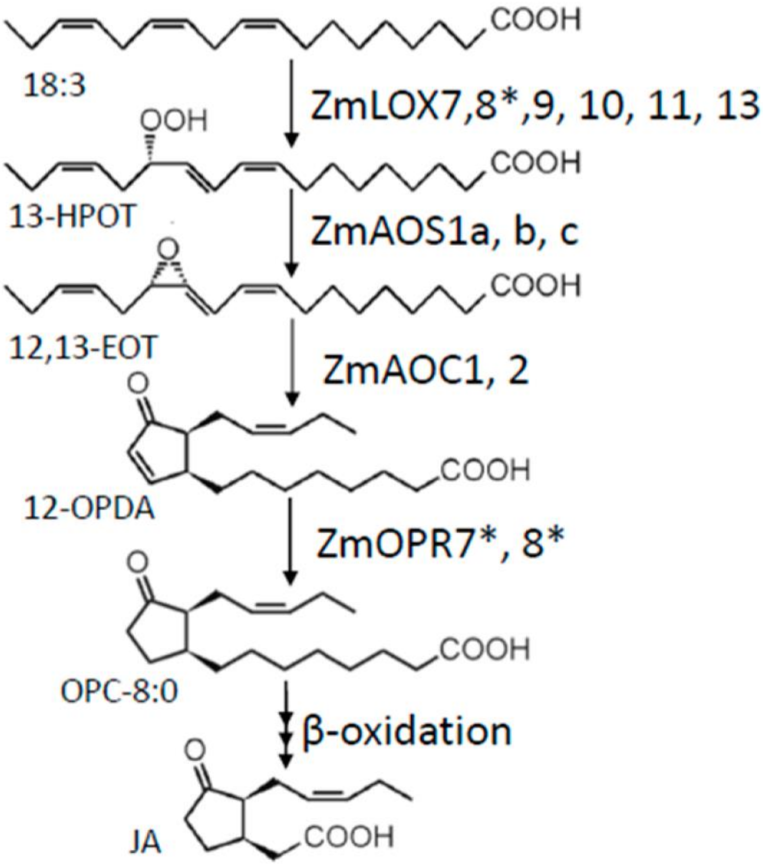

Figure 2.7. Overview of maize Jasmonic Acid biosynthetic pathway.

Genes marked with * have been functionally characterised and other genes are predicted from phylogenic analysis. The abbreviations are as follows

18:3: α linolenic acid
ZMLOX: Zea mays lipoxygenase
ZMAOS: Zea maysAllene Oxide Synthase
ZMAOC: Zea mays allene Oxide Cyclase
OPR: 12 oxophytodienoic acid reductases (s).
13 HPOT: 13 (S) hydro peroxy octadeca 9, 11, 15 trienoic acid (, (9Z, 11E, 13S, 15Z)
12, 13, EOT: 12, 13 (S) epoxyoctadecatrienoic acid
12OPDA: (9S,13S) 12 oxophytodienoic acid
OPC8: 0 (8[3 oxo 2 cis [(Z) 2pentenylcyclopentyl] octanoic acid).

This is followed by three rounds of betaoxidation that are performed by ACX (acyl coenzyme A (CoA) oxidases), MFP (multifunctional proteins), and KAT (3ketoacylCoA thiolases) 6. Which then give rise to the product Jasmonic acid (JA). JAR1 (JASMONATE RESISTANT 1) enzyme then conjugates JA with Isoleucine (Staswick *et al.,* 2002) to give (+) 7 isojasmonoyllisoleucine (JAIle). The only JA receptor currently identified in plants, the SCF (COI1) receptor and isojasmonoyllisoleucine (JAIle), was found to be its most effective ligand (Fonseca *et al.,* 2009).

2.4.2.2.. Jasmonic acid signalling in plant innate defence

In the presence of an abiotic or a biotic stimulus, coronatine insensitive 1 (COI1) is bound by jasmonic acid- isoleucine conjugate (JA-Ile). Coronatine insensitive 1 (COI1) is a component of the Skp1-Cul-F-box protein (SCF) E3 ligase complex. This binding causes the degradation of jasmonate ZIM domain (JAZ) proteins mediated by the proteasome. Jasmonate ZIM domain (JAZ) proteins repress MYC Transcription factors (TF) in the absence of stimulation. The transcription of JA signalling components, such as the basic-helix–loop-helix (bHLH) master transcription factor MYC2 and its close homologs MYC3 and MYC4 are thus liberated from repression (Sheard *et al.,* 2010; Xie *et al.,* 1998). They then bind to G-box or G-box-like sequences found in the promoters of Jasmonic acid-responsive genes (Dombrecht *et al.,* 2007; Ferna´ndez-Calvo *et al.,* 2011) and interact with the MED25 subunit of the plant mediator complex (Kidd *et al.,* 2011). Thus acting as a bridge between DNA-bound TFs and the RNA polymerase II transcription apparatus required for transcription (Chen *et al.,* 2012).

2.4.3. Genes involved in host defence responses.

A study in rice using susceptible and resistant rice lines by Swarbrick *et al.,* (2008) showed the upregulation of a number of genes in the resistant line but not the susceptible lines. These genes include genes encoding hypersensitive response proteins, glucanases (PR-2), endochitinases (PR-3) and thaumatin-like proteins (PR-5). Also, upregulated were a number of genes that code for enzymes involved in defence-related secondary metabolism, these include chalcone synthase, phenylalanine ammonia lyase (PAL) and naringenin 3-dioxygenases (involved in the biosynthetic pathway of diterpene phytoalexin). Several genes encoding cytochrome P450 monoxygenases (P450s), genes encoding proteins of the pleiotropic drug resistance (PDR) subfamily of ABC transporters, a number of genes encoding resistance-like proteins or homologs of resistance genes and some transcription factors (members of the WRKY family) were upregulated. Another study by Hiraoka *et al* (2008), using incompatible and compatible hosts, showed a similar pattern of expression. *Lotus japonica* infected with *Striga* (incompatible host) showed upregulation of genes involved in the following processes; phytoalexin synthesis, pathogenesis related proteins, defence response, cell-wall fortification and detoxification of reactive oxygen species. From the above studies, the following genes and gene families have been implicated in resistance to *Striga*: -

1) Disease resistance genes (R genes)

Disease resistance genes also known as R genes that code for plant nucleotide-binding-site-leucine-rich-repeat (NBS-LRR) proteins. These proteins have a nucleotide binding site (NBS) domain and a leucine-rich repeat (LRR) domain, while their amino- and carboxy-terminal domains are variable. These proteins enable the detection of pathogens, like bacteria, viruses, fungi, nematodes, insects and oomycetes (McHale *et al.*, 2006). Pathogen effectors are recognised by them, triggering defence responses from plants. Effectors for parasitic plant are yet to be identified (Yoder and Scholes 2010). A CC-NBS-LRR R protein (named RSG3-301), predicted from a full-length gene sequence, has been implicated in the resistance cowpea cultivars to SG3 (a race of *S. gesnerioides*).

2) Pathogenesis-related proteins

Pathogenesis related (PR) proteins are proteins encoded by the host plant but induced by various pathogens or related situations like the presence of chemicals that can initiate stress or that mimic the effects of pathogens (Bol *et al.,* 1990). So far, there are 14 recognised families of PRs (Van Loon and Van Strien, 1999). (Table 2.1). These proteins were originally implicated in defending against pathogens like viruses, bacteria, and fungi, but many of them have been found to be upregulated in the response of resistant genotypes to parasites. For example, a cowpea accession (IT97K-499-35) that shows resistance to a specific race of S. gesnerioides (SG3). Upon infestation it showed highly elevated levels of PR-5 transcript compared to a susceptible interaction, a non-host interaction or to uninfected plants. Also, in the resistance response of sunflower to O. Cumana, the expression levels of many induced PR genes were elevated (Letousey, (2007). This is similar to what was observed by Hiraoka *et al.*, (2008) in sorghum to *S. hermonthica*. Swarbrick *et al.*, (2008) showed that in response to *S. hermonthica*, rice upregulated transcripts encoding PR-2 (glucanases), PR-3 (endochitinases) and PR-5 (thaumatin-like proteins).

3) Transcription factors

Transcription factors involved in plant defence include the WRKY family proteins. WRKY family proteins are involved in the regulation of plant defence response pathways (Eulgem and Somssich, 2007). WRKY proteins possess at least one conserved DNA-binding region, known as WRKY domain in its N-terminal and a zinc-finger-like structure at its C terminal. The WRKY domain consists of about 60 amino acids that contain N-terminal heptapeptide WRKYGQK (Eulgem *et al.*, 2000). It generally binds to the DNA element termed the 'W' box (C/TTGACT/C) (Eulgem *et al.*, 2000, Turck *et al.*, 2004, van Verk *et al.*, 2008). This 'W' box occurs in the promoters of genes under the control of WRKY proteins. A number of defence-related genes, including PR genes, contain a 'W' box in their promoter regions (Eulgem *et al.*, 2000). Members of the WRKY family of transcription factors were upregulated in the resistant interaction between Nipponbare (a *Striga* resistant cultivar of rice) and *S. hermonthica* (Swarbrick et al. 2008).

4) Genes involved in secondary metabolic processes

A number of other genes involved in secondary metabolic process were found to be upregulated in the resistant rice cultivar (nipponbare) when infested with *Striga hermonthica* (Swarbrick *et al.*, 2008). These include several genes encoding cytochrome P450 monoxygenases (P450s), a number of genes that code ABC transporters, Naringenin,2-oxoglutarate 7 3-dioxygenase, Chalcone synthase DII, Phenylalanine ammonia-lyase, Isoflavone reductase homolog IRL etc.

Table 2.1. Pathogenesis related proteins

Family	Member Properties	Gene symbols
PR-1	Acidic unknown protein	ϒpr1
PR-2	Basic b-1,3-glucanase (I), acidic/basic b-1,3-glucanase (II, III)	ϒpr2 [Gns2(`Glb')]
PR-3	Basic chitinase (I), Acidic chitinase (II)	ϒpr3 Chia
PR-4	Type I, II chitinase	ϒpr4 Chid
PR-5	Acidic/basic thaumatin-like (TL), osmotin proteins	ϒpr.5
PR-6	Basic protease inhibitor	ϒpr6 Pis (`Pin')
PR-7	Endoprotease	ϒpr7
PR-8	Acidic chitinase (III), acidic/basic chitinase (IV)	ϒpr8 Chib
PR-9	Lignin-forming peroxidase	ϒpr9 Prx
PR-10	Ribonuclease-like proteins	ϒpr10
PR-11	Basic Chitinase (V)	ϒpr11 Chic
PR-12	Plant defensin	ϒpr12
PR-13	Thionin	ϒpr13 Thi
PR-14	Lipid-transfer protein	ϒpr14 Ltp

Adapted and modified from Van Loon and Van Strien, 1999

Taken together, present molecular studies on *Striga* - host interactions indicate that the host plants respond by eliciting biotic stress-related defence pathways. These pathways sometimes have similar mechanisms to those activated by other pests and pathogens and pathways induced by abiotic stresses like drought (Timko and Scholes, 2013). The factors that determine the virulence of a parasite or that make a parasite choose a specific host are not well understood (Timko and Scholes, 2013). Understanding the above, as well as the differences and similarities in responses by the susceptible and resistant hosts to parasitism, will enable the crafting of methods to successfully introduce durable resistance into hosts.

2.5. Control measures

Many control measures have been suggested and/or developed for *S. hermonthica* (Hearne, 2009, Teka, 2014). Control measures currently being applied include crop rotation, intercropping, herbicide treatment (Musselman, 1987; Eplee *et al.,* 1991), use of high nitrogen fertilizer (Kim and Adetimirin, 1997; Kim *et al.,* 1997), uprooting by hand (Akobundu, 1991), planting of catch and trap crops (Egley *et al.*, 1990), herbicide seed dressing (De Groote *et al.,* 2008) and growing only of resistant/tolerant varieties (Parker, 1991; Kling *et al.*, 2000). However, it is observed that these methods, when used individually are not completely effective in controlling *S. hermonthica* infestations (Bozkurt 2014). It is therefore necessary to combine these different methods, which have been adapted to the local farming systems, for use against the parasite. All the control options mentioned above have both technical and practical limitations such as: relatively high cost of deployment (herbicide treatment, high nitrogen fertilizer), lack of suitable break crop or intercrop and limited utility if only adopted by isolation (Hearne, 2008). Suggested potential control methods include host resistance from within and outside (wild) crop species, biological control (fusarium, desmodium, Arbscular mychorrhiza etc), and genetic transformation of either *S. hermonthica* or its maize host (Hearne, 2008). However, these potential methods also suffer limitations like the methods presently deployed against *Striga*. These limitations include non-availability of resistant maize seeds, lack of means of deployment of the technology (Hearne 2008) and limited public acceptance

The most practical *S. hermonthica* control approach for resource-poor small holder farmers is host crop varieties that are tolerant and/or resistant to *Striga* (Kim, 1994).

To be effective this approach has to be a component for any integrated *Striga* control strategy (Haussmann *et al.,* 2001).

2.5.1. Host plant resistance

Resistance by host plants is thought to be the most practical means of controlling parasitic weeds. Using approaches that include biochemistry, plant genetics, plant breeding, and molecular biology, noteworthy advancements have been made in the design of methods for evaluating hosts for resistance to *S. hermonthica,* and this has led to the identification of novel sources of host resistance (Teka, 2014). Kim *et al*., (1999) reported the scarcity of *Striga* resistance gene among African maize landraces, tolerant plants were however identified. Lines developed from a temperate germplasm background initially provided sources of resistant genes to maize inbred lines (IITA, 1983), after which some other sources were identified from tropical germplasms.

The first set of inbred lines sources of *Striga* resistance show different levels of partial resistance and were thus not satisfactory. This led to further search for resistance in wild relatives of cultivated maize including *Zea diploperennis* and *Tripsacum dactyloides, w*hich show higher levels of resistance to *Striga hermonthica*. Reports of *Striga* resistance in maize have emerged over the last decade with lines derived from *Zea diploperennis* proven to have potential for *Striga* control include Zd282, Zd290, Zd467, Zd472 and Zd551 (Yallou *et al.,* 2009). Menkir (2006) showed that the resistant response manifested in this maize line can be transferred successfully to other genotypes. The underlying resistance mechanisms of this maize line are yet uncharacterized. Knowledge of the mechanism of resistance to *S. hermonthica* in ZD05 will provide a rational and scientific basis for their exploitation and sustainable resistance could be attained in maize. Evidence by Rich and Ejeta (2008) has however shown that mechanisms of resistance in maize can occur due to reduced stimulation of *Striga* seed germination, reduced haustorial induction, evasion of the parasite through root architecture, escape through early maturity, resistance to attachment, and incompatibility or a combination of these mechanisms.

If source germplasm that have varying mechanisms of resistance to *Striga hermonthica* are identified, the genes that code for these different mechanisms can be pyramided into maize lines intended for cultivation in environments that are endemic to *S.*

hermonthica (Menkir, 2006). This is to obtain more lines with durable and stable polygenic resistance to the parasite. *Striga hermonthica* however, has the ability to overcome host resistance making breeding host plants against the parasite a difficult and complicated venture. This ability has been attributed to high levels of genetic variation within and between parasite populations (Koyama, 2000). This trait also thwarts other measures used to control the parasite, leading to control that is observed to vary in effectiveness seasonally and across geographic locations (Hearne, 2009).

2.6. Genetic diversity in *Striga hermonthica*

Genetic diversity is defined as the variability in the genetic makeup among individuals within a single species. It is the genetic differences between populations of a single species and among individuals within a population. It also refers to variation in the nucleotides, genes, chromosomes, or whole genomes of organisms. At its most elementary level, it is represented by differences in the sequences of nucleotides. As an obligate out-crossing species, *S. hermonthica* is expected to have very large within and between population genetic variations. This variation can help the parasite to evolve and adapt to changing environmental conditions (Koyama, 2000). The constant changes in cropping patterns and farming systems across regions in sub-Saharan Africa impose varying selection pressures, which can also promote the evolution of new ecotypes of *S. hermonthica*. Using isozymes and random amplification of polymorphic DNA (RAPD), East African *S. hermonthica* populations were observed to be genetically distinct from those from West Africa (Koyama, 2000). Amplified fragment length polymorphism (AFLP) markers could not identify any inherent population differentiation in *S. hermonthica* genotypes from Kenya (Gerthi *et al.*, 2005). It was however stated that it is possible the genes involved with pathogenicity and virulence may not be in the genomic regions that were sampled (Gerthi *et al.*, 2005). A study using micro-satellite markers showed Malian *S. hermonthica* populations to have considerable genetic diversity. There was, however, little differentiation among the populations and no host specificity was apparent (Estep *et al.*, 2011). Ethiopian *S. hermonthica* populations were shown to possess very high genetic diversity; geography and distance played the largest roles in the observed genetic diversity with host specificity again not being too significant (Welsh & Mohamed, 2011). A synthesis of different diversity studies conducted in various locations across Africa with different markers types so far agree that genetic distance increases as geographic distance increase but disagree on the presence or absence of witchweed populations that are specific to a particular host. Recent studies by Estep *et al.,* (2011) and Welsh & Mohamed, (2011), however point to the presence of races. Estep *et al.*, (2011) found different races of the parasite and suggested the need for a more extensive study involving large numbers of population collected from different geographic areas and varying host crops using reproducible, neutral and co-dominant markers. Single Nucleotide Polymorphisms (SNPs) are co-dominant markers. They have become the most widely used markers for genotyping because they are numerous in the genome (Dechamps *et al.,* 2012)

2.7. Single nucleotide polymorphism (SNP), next-generation sequencing technology and genotyping by sequencing (GBS).

Variations in the sequence of a plant's DNA accounts for many of the differences observed between individual plants or varieties. These observed differences range from plant development, tolerance to stress, and yield to nutritional quality. Most of the natural genetic variation in organisms is represented by SNPs or small insertions or deletions (Kruglyak, 1997). In the context of population genetics and diversity, SNPs are single base pair positions at a particular locus in genomic DNA where different sequence alternatives (alleles) exist, with the rarer allele, in this case, being defined as greater than one per cent in the population (Brookes 1999).

Single Nucleotide Polymorphism (SNP) is now the most widely used genotyping marker (Deschamps *et al.,* 2012). They are flexible, fast, cost-effective and are amenable to automation. This is observed in their increasing use in the Next Generation Sequencing (NGS) Technology platforms. SNPs are now the focus of large-scale genotyping projects in humans, model organisms and crop plants. Next generation sequencing (NGS) platforms are high-throughput sequencing technologies that have the demonstrated capacity to sequence DNA at unprecedented speed, quantity and quality thereby enabling excellent scientific achievements and novel biological applications. The development of these high-throughput sequencing technologies is driven by the high demand for low cost sequence technologies (Jiangfeng *et al.,* 2014). NGS relies on massively parallel sequencing and imaging techniques to yield several hundreds of millions to several hundreds of billions of

DNA bases per run (Shendure and Ji, 2008). Several NGS platforms, such as Roche 454 FLX Titanium, Illumina MiSeq and HiSeq2500, Ion Torrent PGM are commercially available. Relative to Sanger sequencing data and to each other, these platforms generate different base read lengths, different error rates, and different error profiles. NGS technologies have increased the speed and throughput capacities of DNA sequencing and this has led to dramatically reduced overall sequencing costs.

New applications like the sequencing of ancient DNA samples have become possible in the wake of NGS technology (Jiangfeng *et al.*, 2014). They have recently been used for resequencing and to sequence whole genomes. According to Elshire *et al.,* (2011) the genomes of several organisms have been sequenced and vast amounts of single nucleotide polymorphisms discovered and used for investigating genetic diversity, map construction and for performing genome-wide association studies.

2.7.1. Genotyping-By-Sequencing

Advanced genome sequencing technology such as Genotyping-by-sequencing (GBS) has aided in the discovery and genotyping of millions of genome-wide DNA polymorphisms like SNPs (Elshire *et al.,* 2011). It is a cost-effective, simple and highly multiplexed sequencing method for constructing reduced representation library that can be used in the study of genetic analyses and to address biological research questions in many organisms (Baird *et al.,* 2008, Elshire *et al.,* 2011). By using restriction enzymes (REs) that are sensitive to methylation, repetitive regions of genomes can be avoided and regions with lower copy numbers targeted with two to three-fold higher efficiency (Jiangfeng *et al.*, 2014). Genotyping-by-sequencing (GBS) makes use of high throughput, short-read sequencing to provide low cost genotyping with high information content. GBS tremendously facilitates genetic studies that were previously very expensive to carry out. Genotyping-by-sequencing has grown to become be a great instrument for genetic mapping (Baird *et al.,* 2008; Elshire *et al.*, 2011), breeding applications, and diversity studies (Fu, 2012; Lu *et al.*, 2012).

2.8. Transcriptomics

Gene expression by hosts on infection by parasites can be interrogated by studying an organism’s transcriptome. The transcriptome is the whole set of transcripts and their quantity in a cell, tissue, organ or a whole organism at a particular time, in a specific developmental stage or under special physiological conditions (Wang *et al.,* 2009). The analysis of the transcriptome is useful for the interpretation of functional elements of the genome, to understand development and disease or to determine the transcriptional structure of genes, like transcription start sites, 5' and 3' ends, posttranslational modifications and splicing processes. Transcriptomics is the study of the transcriptome, which is all the RNA transcripts that are produced by the genome, under a specific circumstance or in a specific cell. It is done using high-throughput methods like RNA-seq and microarray. The main purpose of such a study is to catalogue all species of transcripts, such as mRNAs, small interfering RNAs or non-coding RNAs. It involves determining the transcriptional structure of genes, in terms of their start sites, 5‘and 3′ ends, splicing patterns and other post-transcriptional modifications and quantifying the changing expression levels of each transcript during development and under different conditions (Wang *et al.,* 2009). The presence of and access to a maize reference genome assembly has made transcriptome analysis possible (Schnable *et al.,* 2009).

Several methods exist for exploring and quantifying the transcriptome, they are broadly divided into hybridisation-based or sequence-based methods (Wang *et al.,* 2009). The approaches involving hybridisation-based include various forms of micro-arrays while the sequence-based methods include ribonucleic acid sequencing (Also known as RNA-Seq). Ribonucleic acid sequencing also called whole transcriptome shotgun sequencing (WTSS), is a highly sensitive and accurate tool for measuring expression across the transcriptome. RNA-seq uses next-generation sequencing (NGS) platforms and offers advantages that include lack of the background noise commonly observed in microarrays as a result of cross-hybridisation (Okoniewski and Miller, 2006, Royce *et al.,* 2007). The advantages of RNA-Seq also include an improved alternative splice variants detection ability (Mortazavi *et al.,* 2008; Sultan *et al.,* 2008), a large potent range (greater than 9000 fold) across which gene expression can be estimated, and the capacity to identify and quantify the expression of paralogs that are quite alike (Mortazavi *et al.,* 2008; Wang *et al.,* 2009; Grabherr *et al.,* 2011).

In plants and animals, ribonucleic acid sequencing is used to study the expression of genes at different developmental stages and under different conditions. It has been used in the study of gene expression in *Arabidopsis thaliana*, mouse, human cells (Mortazavi *et al.,* 2008; Lister *et al.,* 2008; Cloonan *et al.,* 2008; Marioni, 2008; Morin *et al.,* 2008) and rice (Swabrick *et al.,* 2008). It has been used in maize to; study root development (*Scott et al.,* 2016), understand how the roots of a maize variety responds to deficiency in Nitrogen (He *et al.,* 2016) and explore its tolerance to the presence of Nicosulfuron (Liu *et al.,* 2014) e.t.c.

Many studies have been done to investigate the interactions between diverse parasites and host plants. A study on the interaction between *Cuscuta spp* and a susceptible and resistant host plant shows that tomato (*Solanum lycopersicum*), through a hypersensitive-type response, resists infection by *Cuscuta reflexa*. In its response, the cells of the tomato at the infection site secrete soluble phenylpropanoids and show an increased accumulation and activity of peroxidases. Peroxidases are important for linking phenylpropanoids with other components of the cell wall such as proteins, pectins, or cellulose fibres. This cell wall that has been modified is hypothesized to block the site of infection, containing the parasite's hautorium, thus effectively halting *Cuscuta Reflexa* movement through the tissues of the tomato plant (Kaiser *et al.,* 2015). Swabrick *et al*., (2008) in a different study involving two different rice genotypes, one susceptible and the other resistant to infestation by *Striga hermonthica* revealed differences in the molecular responses of both genotypes. The resistant genotype upregulated many genes involved in plant defence like pathogenesis-related proteins, ATP-binding cassette transporters, genes involved in the metabolism of phenylpropanoid and WRKY transcription factors. While the susceptible plant showed a global downregulation of these genes. It particularly downregulated genes involved with plant growth regulation, genes involved in signalling and metabolism, and genes involved in biogenesis of cellular components and cell division (Swarbrick *et al.,* 2008).

CHAPTER 3

MATERIALS AND METHODS

3.1. Chemicals and reagents

Restriction enzyme (EcoR1), HindIII size standard, Quant-iT™ PicoGreen™ dsDNA Reagent and Qubit™ dsDNA HS Assay Kit were obtained from Thermo Fisher Scientific Inc. USA. Restricton enzyme ApeKI and T4 DNA ligase were procured from New England Biolabs, USA. Lambda DNA was obtained from Promega, USA. The synthetic strigolactone, GR-24 ((±)-(3aR*,8bS*,E)-3-(((R*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one) was obtained from Dr Sarah Hearne (CIMMYT, Mexico. RNAstable was obtained from Biomatrica Inc. California USA. ZYMO research RNA clean and concentrator-5 kit was obtained from ZYMO research corp, USA. ScriptSeq Complete (plant leaf or root) library preparation kit, ScriptSeq index kit, StarScript Reverse Transcriptase, Failsafe DNA polymerase was procured from Illumina Inc. USA. D1000 ScreenTape & Reagents were procured from Integrated sciences Pty limited, Australia. Qiagen buffer PB (Binding buffer) and QIAgen MinElute Kit were procured from Qiagen Hilden, Germany. RNAse kits, DNase kits, Diethyl pyrocarbonate, Boric acid, TRIZMA base, hydrochloric acid, Ethylenediaminetetraacetic acid (EDTA), sodium chloride, Cetyl triammonium bromide (CTAB), chloroform, Isoamyl alcohol, Potassium sulphate, Calcium chloride 2 hydrate, Magnesium sulphate 7 hydrate, Agarose gel tablets, Disodium hydrogen orthophosphate 7 hydrate, Ammonium nitrate, Ethylenediaminetetra-acetic acid ferric monosodium salt, Manganese II sulphate 1 hydrate, Zinc sulphate-7-hydrate, Copper II sulphate 5 hydrate, Boric acid, Disodium molybdate 2 hydrate, Cobalt sulphate 7 hydrate, Mercaptoethanol and ethanol were procured from Sigma-Aldrich, Germany. The chemicals used in this study were of molecular biology grade.

3.2. Procedures and preparation of reagents.

3.2.1 Sample collection

3.2.1.1. Maize

Seeds of two inbred maize lines (5057 (Tlalt. 7844 x TZSR)) and ZD05 (Z. Diplo-BC_4-19-41-#-3-1-b-1-b*6) were obtained from Dr Abebe Menkir, a maize breeder in the International Institute of Tropical Agriculture (IITA). The 5057 is a maize genotype that is susceptible to *Striga,* while ZD05 is resistant to *Striga* infestation (Menkir, 2006, Menkir *et al.*, 2016). The seeds were stored in the cold room of the Bioscience Center, International Institute of Tropical Agriculture and used as required.

3.2.1.2. *Striga hermonthica*

Striga hermonthica leaves were sampled from farms located in regions, of two African countries, with prevalent *Striga hermonthica* infestation (Western Kenya and Northern Nigeria), as shown Table 3.1. The sampling was done between October and December in 2012 by the student with the help of staff of IITA-kano, in Nigeria and IITA-Kenya in Kenya. Leaves and seeds were collected from randomly selected evenly dispersed natural *Striga hermonthica* populations (not from inoculated farms). In each collection site, leaf tissue was randomly collected from *Striga hermonthica* plants growing on hosts at least 1 meter apart. Leaves from at least fifteen *S. hermonthica* plants per host species per farm were collected in separate bags. All the leaves from a *S. hermonthica* plant were harvested and immediately kept in individual bags that contain silica gel. The silica gel desiccated the samples and prevent degradation of their deoxyribobnucleic acid (DNA). Also ripened seeds from twenty plants, from each of the hosts were collected from the same farms where the leaves were collected. Ripened seeds were obtained from plants that have completed flowering cycle (that is, those with flowers only on the uppermost parts of the plant or those without any flowers) and contain healthy, intact, and unshattered capsules. The seeds were collected by cutting off the floral head(s), placing and allowing it to dry in paper bags, then beating the bags and progressively sieving the contents through a 250 micron and a 150-micron sieve. The seeds obtained were collected and stored in labelled plastic bags. A total of two hundred and fifty-four *S. hermonthica* plant samples were collected from nine different districts in western Kenya. The Nigerian samples amounted to seven hundred and seventy–five *S. hermonthica* samples from thirty-seven (37) locations across fourteen (14) states. The *Striga* plants were collected from four host crops Maize, sorghum, millet and rice (Maize, sorghum and millet in Nigeria and maize, sorghum and rice in Kenya). More locations were sampled in Nigeria than in Kenya because Northern Nigeria in many times larger than western Kenya. The *Striga hermonthica* plants were authenticated at the Department of Botany, University of Ibadan, voucher number UIH-22774.

3.2.2. Surface sterilization of seeds

Procedure for surface sterilization was modified from Girton (1936).

3.2.2.1. Maize seeds

Maize seeds were placed into 500ml conical flask, 250ml of distilled water was poured into the flask, 10ml of 1% sodium hypochlorite and 2 drops of tween-20 were added into the mixture. The mixture was stirred intermittently for 10 minutes and then rinsed thoroughly several times with double-distilled water.

3.2.2.2. *Striga hermonthica* seeds

The *Striga hermonthica* seeds were mixed, surface-sterilized with a 1 % sodium hypochlorite solution containing 3 drops of tween-20 (to break the surface tension). The mixture was stirred intermittently for 5 minutes in a sterile 500ml conical flask. After which, the surface-sterilized seeds were washed 3 to 4 times with double distilled water.

3.2.3. Pre-conditioning of *Striga hermonthica* seeds

The surface sterilized *S. hermonthica* seeds were put into a Petri dish lined with 90mm Whatman glass microfiber filter paper and sterile distilled water was sprinkled on the filter paper to get it moist. The Petri dish was covered with aluminium foil and kept in the incubator at 28 ^{0}C for 20 days for preconditioning.

Table 3.1. *Striga hermonthica* sample collection sites

S/N	Country	State/district	Town/collection location	Longitude	Latitude	Host crop
1	Kenya	Butere	Sabatia	0.09994	34.7391	Maize
2	Kenya	Homabay	Homabay/Rodi road	-0.5879	34.4791	Maize/sorghum
3	Kenya	Matungu	Nambale-Mumias road	0.39098	34.4767	Maize
4	Kenya	Migori	Migori	-1.0487	34.3053	Maize
5	Kenya	North Teso	Bumula	0.3711	34.2451	Maize
6	Kenya	Nyando	Ahero	-0.0107	34.0563	Maize/sorghum
7	Kenya	Sabatia	Butere	0.09994	34.7391	Maize
8	Kenya	Siaya	Ugenya	-0.2071	34.2681	Maize/sorghum
9	Kenya	South Teso	Adungosi	0.51836	34.1401	Rice/Maize
10	Kenya	Webuye	Matete	0.344	34.4755	Maize
11	Nigeria	Abuja	Zuba-Kaduna expressway	9.24478	7.21419	Sorghum
12	Nigeria	Abuja	Zuba-Gwagwalada road	9.04744	7.19246	Sorghum
13	Nigeria	Abuja	Zuba-Garki road	9.13341	7.34418	Sorghum
14	Nigeria	Abuja	7km from airport junction	9.02921	7.17461	Sorghum
15	Nigeria	Abuja	9km from airport junction	9.0676	7.20557	Sorghum
16	Nigeria	Abuja	Gwarko	12.8576	5.1689	Maize
17	Nigeria	Abuja	Zuma rock	9.13198	7.22855	Sorghum/Maize
18	Nigeria	Adamawa	Sabongarimbalhwana	10.5337	13.1629	Sorghum
19	Nigeria	Adamawa	Sabongarimbalhwana	10.5371	13.1466	Maize
20	Nigeria	Bauchi	Bishi	10.2531	10.1064	Maize
21	Nigeria	Bauchi	Bishi	10.2531	10.1064	Sorghum
22	Nigeria	Bauchi	Gar Alkaleri	11.9569	7.68472	Maize
23	Nigeria	Bauchi	Gar Alkaleri	11.9569	7.68472	Millet
24	Nigeria	Bauchi	Gwaltukura Dass	9.25306	9.50136	Maize
25	Nigeria	Bauchi	Gwaltukura Dass	9.25306	9.50136	Sorghum
26	Nigeria	Bauchi	Gwaltukura Dass	10.5371	13.1466	Sorghum
27	Nigeria	Bauchi	KafinMadaki, Ganjuwa	10.7315	9.77375	Sorghum
28	Nigeria	Bauchi	Rigingain	10.0526	9.09825	Sorghum
29	Nigeria	Benue	Yandev	7.3667	9.05	Sorghum

S/N	Country	State/district	Town/collection location	Longitude	Latitude	Host crop
30	Nigeria	Borno	Askira Uba	10.8207	13.1868	Sorghum
32	Nigeria	Borno	Askira Uba	10.8252	13.1936	Maize
33	Nigeria	Borno	Grium Hawul	10.97	12.0168	Maize
34	Nigeria	Borno	Grium Hawul	10.97	12.0168	Sorghum
35	Nigeria	Gombe	Konkeje akko LGA	10.5054	11.5733	Sorghum
36	Nigeria	Gombe	Konkeje akko LGA	10.5081	11.5772	Maize
37	Nigeria	Jigawa	Babura	12.7602	9.01108	Maize
38	Nigeria	Jigawa	Babura	12.7602	9.01108	Millet
39	Nigeria	Jigawa	Babura	12.7602	9.01108	Sorghum
40	Nigeria	Jigawa	Yarda birninwa	12.8031	10.2545	millet
41	Nigeria	Kaduna	Kasuwan Maganin	10.4204	7.71731	Sorghum
42	Nigeria	Kaduna	Zaria	11.1667	7.63333	Sorghum
43	Nigeria	Kaduna	Zaria	11.1856	7.63328	Maize
44	Nigeria	Kaduna	Zaria/Zikri	11.1858	7.63328	Sorghum
45	Nigeria	Kaduna	Zaria/Zikri	11.1863	7.59942	Maize
46	Nigeria	Kano	Danbata doguwa doruwa	12.2861	8.64878	Millet
47	Nigeria	Kano	Danbata doguwa doruwa	12.2861	8.64878	Sorghum
48	Nigeria	Kano	Minjibir	12.1783	8.65917	Millet
49	Nigeria	Kano	Minjibir wase	12.1783	8.65917	sorghum
50	Nigeria	Kano	Tudun wada	11.2483	8.37111	Maize
51	Nigeria	Kano	Tudun wada	11.2483	8.37111	Sorghum
52	Nigeria	Katsina	Dayi-Malunfashi	11.9569	7.68472	Maize
53	Nigeria	Katsina	Dayi-Malunfashi	11.9569	7.68472	Millet
54	Nigeria	Katsina	Dayi-Malunfashi	11.9569	7.68472	sorghum
55	Nigeria	Kebbi	Wali Argungu	12.7102	4.86694	sorghum
56	Nigeria	Kebbi	Wali Argungu	12.7193	4.86389	Millet
57	Nigeria	Kogi	Yogbo leke	7.9974	8.4467	Sorghum
58	Nigeria	Niger	2km E on Bida road	9.32542	5.06875	Sorghum
59	Nigeria	Niger	Kwakwara	9.36226	5.02541	Sorghum
60	Nigeria	Niger	Mokwa	9.32393	5.14815	Sorghum
61	Nigeria	Niger	Mokwa	9.35186	5.02839	Maize

S/N	Country	State/district	Town/collection location	Longitude	Latitude	Host crop
62	Nigeria	Niger	Tatabu-mokwa road.	9.24616	4.96107	Millet
63	Nigeria	Sokoto	bisallam-dangeshuni	12.8216	5.38164	Millet
64	Nigeria	Sokoto	bisallam-dangeshuni	12.8216	5.38164	Sorghum
65	Nigeria	Zamfara	Mahuta-bungudu	12.2385	6.62578	Millet
66	Nigeria	Zamfara	Mahuta-bungudu	12.2385	6.62578	sorghum

3.2.4. Deoxyribonucleic Acid (DNA) extraction

The procedure used to extract DNA from the *Striga hermonthica* leaf tissue was modified from Doyle and Doyle (1990).

Principle

The extraction of DNA from plant cells is based on the fact that cells can be lysed and their intracellular contents exposed into an environment conducive for nucleic acids. The other constituents of the cell are then selectively removed by both physical and chemical methods until only nucleic acids are left. They are then cleaned and an appropriate enzyme (RNase) is used to degrade the RNA present in the sample, thus leaving only DNA.

Procedure for DNA extraction

The reagents used for DNA extraction are Cetyl triammonium bromide (CTAB) extraction buffer [CTAB, 1.0 M TRIS-HCl, 0.5M Ethylenediaminetetraacetic acid (EDTA), 5 M Sodium Chloride (NaCl), Mecarptoethanol], 70 % Ethanol, Iso-propanol and RNase. Leaf tissue (about 0.1g) was ground into fine powder by shaking for 4 minutes at a speed of 500 strokes/min using GenoGrinder-2000. 800 ul of freshly prepared modified CTAB extraction buffer (200 mM Tris, pH 7.5; 50 mM EDTA, pH 8.0; 2 M NaCl; 2 % CTAB; 1 % beta-mercaptoethanol) was added to the ground powder in a 1.5 ml extraction tube and incubated at 60^o C in a water bath for 30 minutes with continuous gentle rocking. The tubes were then removed from the water bath, gently tapped and centrifuged at 3500 rpm for 10 mins. About 500 ul of the aqueous phase was transferred into new tubes, and 600 ul of chloroform isoamyl alcohol (24:1) added. The solution was gently mixed and centrifuged at 3500 rpm for 10 min. The upper aqueous layer was transferred to fresh strip tubes and the process repeated. About 400ul of the upper aqueous layer was transferred into fresh strip tubes and 600 ul of 100 % ice-cold isopropanol (2-propanol) was added. The tubes gently inverted about 50 times and then put in the freezer (-20 °C) for 60 minutes. They were then centrifuged at 3500 rpm for 20 min to form a pellet at the bottom of the tube and the supernatant discarded. The pellet was washed with 400ul of 70 % ethanol, centrifuged for 15 min and the ethanol decanted. This process was repeated, and the pellet was air-dried and reconstituted with double distilled water (Doyle and Doyle, 1990).

3.3.4.1. DNA quantity and quality Assessment

The quality and quantity of the DNA was assessed by two methods

(i) Agarose gel electrophoresis
(ii) Spectrophotometry

Gel electrophoresis

Principle

Biomolecules can be separated by applying an electric field to move the charged molecules through an agarose matrix. The biomolecules are separated by size in the agarose gel matrix. The size of the pores within the agarose gel control the rate at which a molecule moves from one end of the gel to another. The negatively charged phosphodiester groups form the backbone of the DNA molecule and thus the molecule will migrate towards the positively charged electrode when an electric current flows through the gel. Thus, the larger weight molecules, will migrate only a very short distance from the wells.

Procedure

The quality of DNA was checked in an 0.8% agarose gel, prepared by boiling 0.8 g of agarose in 100 ml of 1X TBE. The gel was then cooled to about 60 ^{o}C and 5ul ethidium bromide added. The mixture was poured on a gel tray to solidify. A mixture of 3ul of the DNA solution and 3 ul of bromophenol blue loading dye was prepared and loaded onto the gel and the gel ran at 80 volts for 60 minutes.

Nanodrop Spectrophotometer

Principle

A nanodrop spectrophotometer works by measuring the absorbance of double-stranded DNA at a wavelength of 260 and calculates the corresponding concentration of the

DNA solution based on the Beer-Lambert equation (as modified for the spectrophotometer): $c = A/ (E \times b)$

where

c = concentration in ng/µL,

A = absorbance in AU,

e = wavelength-dependent extinction coefficient in ng-cm/µL.

b = path length in cm (The generally accepted extinction coefficients for nucleic acids are; Double-stranded DNA: 50, Single-stranded DNA: 33 and RNA: 40)

The ratio of the OD at 260nm to the OD at 280nm is used to determine the purity of the DNA solution. A value of between 1.8 and 2.0 is considered as pure DNA.

Procedure

DNA Concentration was also measured by pipetting 2 µL of DNA solution on the pedestal of the Nanodrop spectrophotometer and reading the corresponding concentration off a computer screen. The ratio of the absorbance at 260 nm/280 nm was also noted to confirm the purity of DNA.

3.2.5 Genotyping by sequencing

Genotyping-by-sequencing was performed as described in Elshire *et al.,* (2011)

Principle

Genotyping by sequencing (GBS) involves sequencing by synthesis. The order of nucleotides in a DNA strand is deduced as the complimentary copy of the strand that is synthesized. Restriction enzymes are used to reduce the complexity of the organism’s genome. The DNA is digested, and polymerase chain reaction is performed to increase the number of restriction fragments. These fragments then form a library that is sequenced and about 100 base pairs (bp) single-end reads are obtained. The reads are aligned to the organism reference genome and a suitable pipeline is used to identify single nucleotide polymorphisms (SNPs).

Reagents

PicoGreen, Dimethyl sulphoxide, Lambda DNA solution (diluted serially from 0 to 200 ng/ul), Low-salt Tris-EDTA buffer, ApeKI and NEB buffer (100 mM NaCl, 50 mM Tris-HCl, 10 mM MgCl2, 100 µg/ml BSA) (pH 7.9), T4 DNA ligase and Adapters, Qiagen buffer PB (Binding buffer), Qiagen MinElute and Protocol Phusion® High-Fidelity Polymerase chain reaction Master Mix (Phusion DNA Polymerase, 5 mM $MgCl_2$ and 200 µM of each dNTP)

Procedure

The concentrations of the DNA samples extracted were adjusted to working solutions of at least 10 ng/µl. 95 DNA samples were then pipetted into a 96-well optical plate and one randomly placed well left blank. 5 µl (500 ng) each of 10 randomly selected samples per plate were digested using *EcoR1* and run on 1% standard agarose along with λ *HindIII* size standard after which the DNA samples were lyophilized and sent for genotyping using the 'Genotyping by sequencing' (GBS) method.

Fluorimetric quantification of DNA

In preparation for GBS, the DNA was quantified by fluorimetry.

Fluorimetry principle

This assay is based on the fact that the free dye does not fluoresce, however upon binding to double-stranded DNA, it exhibits a greater 1000-fold fluorescence enhancement and it is highly selective for dsDNA over ssDNA and RNA. The absorbance is directly proportional to the concentration of DNA in the solution.

Procedure for fluorimetry

Preparation of DNA Standard Curve

Lambda DNA was diluted serially from 0 to 200ng/ul with low-salt Tris EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8.0). Working PicoGreen quantification reagent (100 µl) was mixed with 100µl of the DNA and incubated for 5 minutes at room temperature. After incubation, 200µl of each dilution was pipetted into a microplate in three replicates and the concentration read using the Biotek plate reader (Table 3.2) at

Table 3.2. Optical reading for DNA concentration standard curve

DNA concentration(ng/µL)	Reading 1	Reading 2	Reading 3	Average
0	16165	16458	16463	16362
4	19042	20050	19416	19502.67
8	23401	21628	22762	22597
12	27440	25379	24933	25917.33
16	37172	30514	30514	32733.33
20	35991	37826	37826	37214.33
24	45218	40957	43132	43102.33
28	45946	42845	45372	44721
32	44931	49704	54007	49547.33
36	47322	52368	52368	50686
40	57725	57725	57289	57579.67
44	60400	59720	68785	62968.33
48	66838	62746	66838	65474
52	72382	76261	72382	73675
60	81660	81666	81678	81668
70	92778	92773	92775	92775.33
80	103889	103887	103889	103888.3
100	127111	129113	129154	128459.3
120	144430	145431	146432	145431
140	170538	170567	170556	170553.7
160	192794	192785	192776	192785
180	214003	213002	212005	213003.3
200	237223	237222	237221	237222

an emission wavelength of 485nm/20nm and an excitation of 530nm/25nm. The average absorbance was plotted against DNA concentration to give the standard curve. The same process was done to the test DNA sample and their concentrations read off the standard curve.

DNA digestion

The DNA samples in solution were digested separately with a selective restriction enzyme known as *Ape*K1. *Ape*K1 is a type II restriction endonuclease that recognises a degenerate 5 bp sequence (GCWGC, where W can be A or T), creates a 3 bp 5' overhang, and is partially methylation-sensitive. The digest cocktail consisting of 2µl of Neb buffer, 1µL of ApeKI and 5µL nuclease free water, was prepared and added to 12µl of 100ng/µL of DNA. The cocktail was then kept on ice and the solution was pipetted continuously to mix evenly, and the plate sealed and placed in a thermocycler with a heated lid and allowed to incubate for 2 hours at 75 ℃, followed by cooling at 4 ℃.

Ligation of adapters to DNA

Genotyping by sequencing (GBS) involves the use of common and barcoded adapters. These were then ligated to the digested DNA fragments. The adapters (1.5 ng/µL) was briefly centrifuged and 3 µl was added to each digest product. The ligation cocktail (10x T4 ligase buffer, T4 DNA ligase and nuclease free water) was prepared at room temperature and added to the DNA-Adapter mix. The samples were properly mixed and incubated for 1 hour in a thermocylcer with a heated lid at 22 ℃, 30 minutes at 65˚C, and then cooled at 4 ℃. Equal volumes of ligated DNA were then pooled in a reagent reservoir containing 5X the total volume Qiagen buffer PB, such that the total amount pooled is close to 2000 ng. The DNA was thoroughly mixed again by pipetting and then cleaned and concentrated using MinElute.

Size selection

The pooled ligated DNA was visualized using two per cent low melting point agarose. Bands within the range 400-800 bp was cut, placed in a 2 ml tube and eluted from the gel with a Qiagen MinElute. For 400mg of gel, one MinElute was used.

Enrichment of ligated DNA via Polymerase Chain reaction (PCR).

The ligated DNA fragments were pooled and amplified with primers that are complementary to the adapter sequences. The PCR reaction mix was prepared by adding the PCR cocktail (2 µL of Primer 1, 2 µL Primer 2, 25 µL of Phusion HF master mix (NEB) and 7µL nuclease free water) to 14 µL of digested DNA. The samples were properly mixed and run in a thermocylcer with a heated lid at 100 °C. The PCR program used was as follows: An initial denaturation step at 98 °C for 30 seconds, then 10 cycles of denaturation 98°C for 10 seconds, annealing at 65°C for 30 seconds, and elongation at 72°C for 45 seconds, and then a final elongation step at 72 °C. The PCR product was stored for 5 minutes at 4 °C. The polymerase chain reaction products were run on 2% low melting agarose gel and visualized.

Sequencing and SNP calling

The library was purified and quantified using Agilent BioAnalyzer 2100. The fragment sizes and the presence of adapter dimers were evaluated. The DNA library was then processed and sequenced on the Illumina HiSeq 2500. The SNPs were called from the sequencing results using the UNEAK (Universal Network Enabled Analysis Kit) GBS pipeline (Lu *et al.,* 2013), which is part of the TASSEL 3.0 bioinformatics analysis package (Bradbury *et al.*, 2007) (Version: 3.0.166 Date: April 17, 2014). This method was used because it does not require the organism’s reference genome. Single nucleotide polymorphisms are discovered within pairs of matched sequence tags and filtered through network analysis as described by Huang *et al.,* (2014) and Lu *et al.,* *(*2013). Also, another genotyping by sequencing discovery pipeline, using *Striga* transcriptome, available on Tassel (Version: 3.0.166 Date: April 17, 2014) was used. In this instance, sequence reads were mapped to *Striga* transcriptome.

3.2.6. Preparation of 40% Long-Ashton solution.

The Long Ashton solution was prepared as amended by Cechin and Press (1994), to provide 1mM NH_4NO_3 in order to ensure that *S. hermonthica* germination and attachment was not impeded by excessive nitrogen.

Procedure

Stock solutions of the macronutrients and micronutrients were prepared to the concentrations listed below, (table 3.6), Working solutions were then prepared by mixing appropriate amounts of the stock solutions with water in a 2000 L drum. The resulting solution was used to nourish the plants throughout the experiments.

3.3.7 Preparation of 1mg/L GR24 solution

GR24 [(±)-(3aR*,8bS*,E)-3-(((R*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy) methylene)-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one)] is a synthetic strigolactone that is used to mimic the effects of strigolactones.

Principle

Gr24 is nonpolar substance and will thus dissolve in only non-polar solvents. It must however be administered in water. Acetone has a polar C=O bond, which can interact with the dipoles of water to form hydrogen bonds it is thus soluble in water.

Procedure.

1µg of GR24 was dissolved in 1 ml of acetone. The mixture is swirled slowly until the GR24 is completely dissolved. The solution was then made up to 1 L with double distilled water, stored at -20 °C and used as required.

Table 3.3. Chemical composition of 40% Long Ashton Solution (Hewitt, 1966), amended to provide 1mM NH_4NO_3.

Compound	Formula	Stock solution gdm^{-3}	Final conc. of nutrient solution fed rhizotrons
Macronutrients			
Potassium sulphate	K_2SO_4	21.75	0.8 mM
Calcium chloride 2 hydrate	$CaCl_2.2H_2O$	73.47	1.6 mM
Magnesium sulphate 7 hydrate	$MgSO_4.7H_2O$	46.0	0.6 mM
Disodium hydrogen orthophosphate 7 hydrate	$Na_2HPO_4.7H_2O$	22.28	0.53 mM
Ammonium nitrate	NH_4NO_3	50.25	1.0 mM
Micronutrients			
Ethylenediaminetetra-acetic acid ferric monosodium salt	$C_{10}H_{12}N_2O_8FeNa$	8.23	0.04 mM
Manganese II sulphate 1 hydrate	$MnSO_4.1H_2O$	1.69	4.0 nM
Zinc sulphate 7 hydrate	$ZnSO_4.7H_2O$	0.29	0.4 nM
Copper II sulphate 5 hydrate	$CuSO_4.5H_2O$	0.25	0.4 nM
Boric acid	HBO_3	3.1	0.02 mM
Disodium molybdate 2 hydrate	$Na_2MoO_4.2H_2O$	0.12	0.2 nM
Cobalt sulphate 7 hydrate	$CoSO_4.7H_2O$	0.053	0.08 nM
Sodium chloride	NaCl	5.85	0.04 mM

3.2.8 Assembly of rhizotron

A Rhizotron is a transparent bioassay growth chamber that allows for real-time monitoring of plant roots without destroying the plant. These were used to investigate the attachment and growth of *Striga hermonthica* throughout the study.

Materials

a) Black and clear Sheets of perspex glass, (30 cm x 40 cm x 2 mm)
b) Glass rods (40 cm x 1 cm x 1 cm)
c) Sheet of black cotton fabric (30cm x 40 cm)
d) A sheet of black polyethylene plastic material
e) Felt
f) Fold back clips
g) Silver sand

Procedure

Washing and sterilizing of river sand

River sand obtained from the Lagos bar beach was washed by running water through a pipe continuously into a drum containing the sand. The running water removes all organic matter from the sand. The sand was sterilized by pouring boiling water into the drum with the washed river sand until the water level is just above the level of the sand. This was left to stand for about one hour, after which the water was drained and the sand allowed to dry.

Construction of rhizotron

The Rhizotron was constructed by gluing 1 cm thick glass rods to both sides of a black Perspex glass sheet and a felt strip to the bottom of the glass sheet. Washed, sterilized and dry river sand was poured unto the black Perspex glass and a sheet of black cotton fabric placed on the surface of the river sand, a clear sheet Perspex glass was then placed upon the fabric. The entire setup was wrapped with a black polyethylene plastic material to shield the root zone from light and five fold-back clips were used to firmly hold the rhizotron set up together (figure 3.2).

Introduction of plant material into the rhizotrons

One surface-sterilized maize seed was placed per rhizotron, on the black cotton fabric, at a depth of about 2 cm. The maize lines, in the rhizotrons, were infested with *Striga hermonthica* seeds, by using a brush to carefully spread 2 g of *Striga hermonthica* seeds all over the rhizotron surface and especially on the exposed roots of the maize plants. The rhizotrons were fed two times (morning and evening) daily, with nutrient solution (40% Long Ashton solution containing 1molm^{-1} ammonium nitrate), to provide a total volume of 250 ml of nutrient solution per rhizotron per day.

3.2.9. Collection of maize root exudates

The germination ability of exudates of the roots of the maize plants was tested in two experiments. In the first experiment, to determine the number of germinated *Striga hermonthica* seeds, seeds from the maize lines (5057 and ZD05) were sown in pots filled with washed sharp sand. The plants were maintained in 2 stands per pot with 5 replicates per variety in a tunnel screen house. Root exudates were collected from each pot 14 days after planting by passing water through the sand until 45 ml was collected per pot. Sterile Petri dishes were lined with a glass microfibre filter, and approximately 100 *Striga* seeds were transferred to the surface of each filter paper, followed by the corresponding root exudate until the filter was richly moistened. The dishes were wrapped with aluminium foil, incubated at 37°C in an incubator and examined for germination after 72 hours. The experiment was repeated, the samples were incubated for eleven days, and only seeds with elongated haustoria were counted.

The second experiment was done to determine the ability of the maize line to stimulate radicle elongation. In this experiment, twenty surface-sterilized seeds from the ZD05 and 5057 maize lines were left to sprout in a Petri-dish that was lined with moistened filter paper. Five germinated seeds of both genotypes were randomly selected, fixed in a felt plug and suspended in a test tube that was filled with 45 ml of nutrient solution (40% Long Ashton solution containing 1 mol m^{-3} ammonium nitrate). The whole tube was wrapped with aluminium foil and kept in the screen house. The maize plants grew for a 30-day period. Then, the nutrient solution was replaced with 30 ml of distilled water. After 48 hours, the maize plants were gently removed from the solution, and the solution was transferred into Falcon tubes to the 30 ml mark.

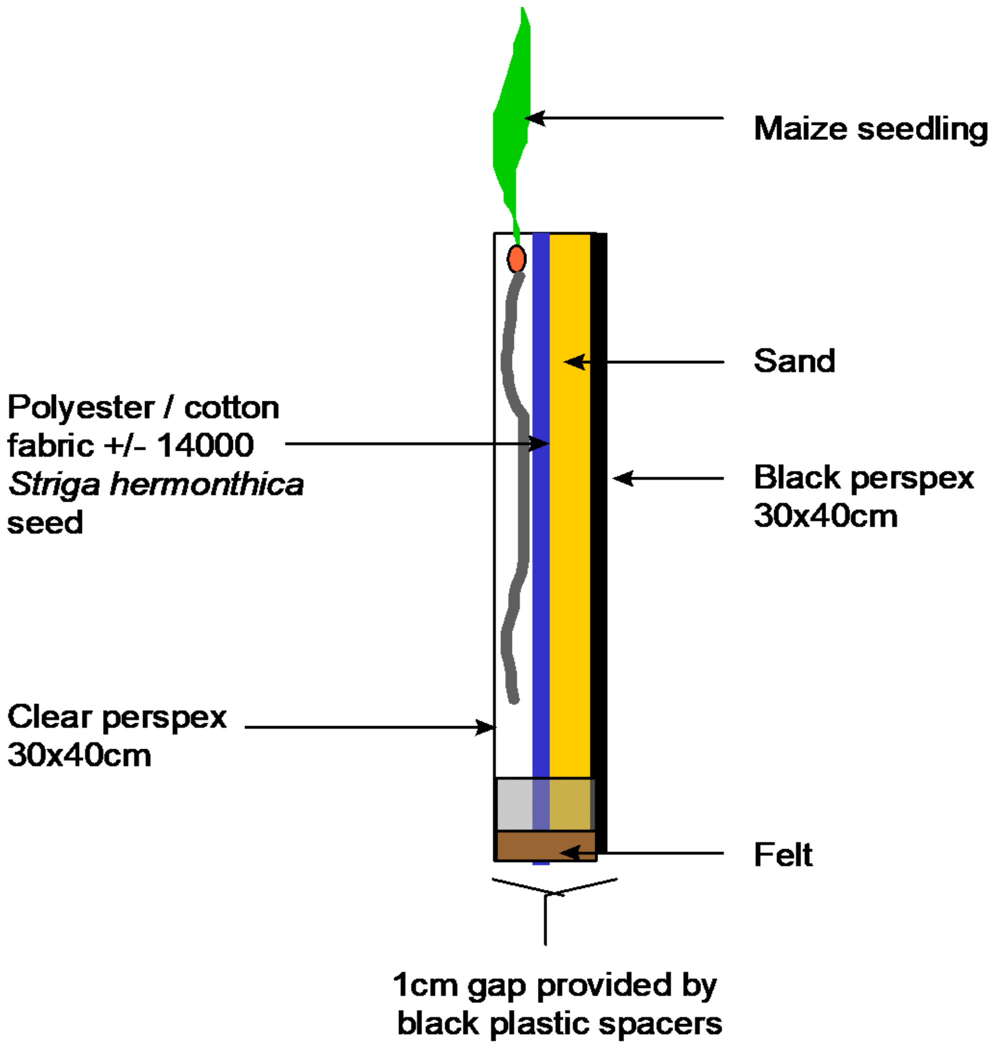

Figure 3.1. Illustration of a rhizotron

The dry weight of the roots of each plant were taken and recorded. The extracted maize root exudates were pipetted into Petri dishes with 100 preconditioned *Striga hermonthica* seeds. The Petri dishes were wrapped in aluminium foil and kept in a controlled environment (incubator) for 96 hours at 28°C and number of *Striga hermonthica* seeds that had germinated were counted under a microscope.

In both cases, the *S. hermonthica* seeds were incubated in synthetic germination stimulant GR-24 (1 ppm) and distilled water as positive and negative controls, respectively.

3.2.10 Measurement of root indices

Maize seeds were planted in large pots (25l) in a screen house. Five pots from each genotype were infested with *Striga hermonthica,* and five were left uninfested. At 28 days after planting, plant roots were carefully washed to remove all the soil, and the lengths of all the major roots (crown, seminal and primary roots) on each plant were taken using a measuring tape. The total major root length was determined by summing the lengths of the individual roots (a major root was defined as any root that originates from the base of the plant, as opposed to a lateral root that originates from another root).

3.2.11 Sample collection for maize transcriptomics study

Maize plants were grown in rhizotrons and infested with *S. hermonthica* seeds. At the three, nine and twenty- two days post infestation, root tissue was collected from each plant by removing the maize plant from the rhizotron, briefly cleaning the roots to remove extra seeds, and cutting the roots of the plants into smaller bits into aluminium foil on ice. Three plants were simultaneously harvested, bulked and dropped into liquid nitrogen. The samples were collected in three replicates with each replicate consisting of three bulked plants. This was repeated at each collection time point.

3.2.12. RNA Extraction.

Principle

The extraction of RNA from plant cells is based on the fact that cells can be lysed and their intracellular contents exposed into an environment conducive for nucleic acids. After which all the other constituents of the cell are then selectively removed by both physical and chemical methods until only nucleic acids are left. They are then cleaned and an appropriate enzyme (DNase) is used to degrade the DNA present in the sample.

Procedure

An oven-baked mortar was used to grind 0.20 g of plant root in liquid nitrogen. The finely ground material was transferred into an Eppendorf tube and 1.5 ml of warm (65 °C) CTAB buffer was added. The samples were heated at 65 °C for 15 minutes with vertexing at 5-minute intervals. The tubes were then centrifuged at 3500rpm for 5 minutes and 10ml of the supernatant transferred into another tube. 1ml of chloroform–isoamyl alcohol (24:1) was added to the transferred supernatant, the mixture vortexed and spun at 3500rpm for 15 minutes. 750 ul of the supernatant was again transferred into another tube and 450ul of ice-cold 2-propanol added and the solution mixed by inversion. The tubes were spun for 20 minutes at 7000g. The supernatant was discarded, and the pellet washed with 70 % ethanol. The pellet was air-dried, and the extracted nucleic acid reconstituted in 100 µl RNase and DNase-free water.

DNA digestion

DNA was removed from the extracted RNA samples. Each RNA sample was treated by pipetting 5 µl of DNase1 and 5 µl digestion buffer to 10 µg RNA made up to 40 µl with nuclease free water. The mixture was then incubated at room temperature for 15 minutes.

RNA Purification

The purification was done using a commercially available kit (ZYMOresearch RNA clean and concentrator -5 kit)

Procedure

To each sample, two volumes of RNA binding buffer was added and the mixture vortexed. An equal volume of 100% ethanol was then added to the mixture. The mixture was transferred into a Zymo-Spin IC column in a collection tube and centrifuged for 30 seconds at 7000rpm. The flow-through was discarded and 400 µl RNA prep buffer was added to the column and centrifuged for 30 seconds at 7000rpm. The flow-through was again discarded and 700 µl RNA prep wash buffer was added to the column and centrifuged for 30 seconds at 7000 rpm. This was repeated with 400 µl and centrifuged for 2 minutes. The spin column was placed in an RNase free tube and 15 µl of DNase/RNase free water added directly to the column matrix and the column centrifuged at 7000rpm for 30 seconds. The purified RNA was packed in Eppendorf tubes and sent for sequencing.

3.2.13. RNA-Seq (RNA Sequencing)

The Illumina Miseq sequencing platform was used for sequencing.

The RNA-Seq procedure can be broadly divided into:

1) Library preparation

2) Sequencing and preliminary bioimformatics

3.2.13.1. Library preparation

cDNA libraries for Illumina Miseq platform are done by preparing the RNA pool and then the cDNA library

Materials

- Agilent Tapestation and D1000 HS kit
- Thermal cycler
- Pipettes and filter tips (separate sets for pre and post PCR stages)
- Magnetic separation stand
- Microfuge
- Qubit double-stranded DNA fluorimeter and reagents
- Ampure XP magnetic beads
- RNAclean XP magnetic cleanup beads

- ScriptSeq Complete (plant leaf or root) library preparation kit (Illumina)
- ScriptSeq index kit (Illumina)
- Failsafe DNA polymerase (illumina)
- PCR plates, Eppendorf tubes
- 1ug DNA free RNA from plant samples
- EACC spike in control (LifeTech)

3.2.13.1.1. RNA pool preparation

The ScriptSeq Complete Kit (Plant Leaf) from Illumina INC was used. It involves removing ribosomal RNA (rRNA) from the extracted RNA samples and cleaning up the resultant RNA.

1) Ribosome depletion of the RNA (with ribozero reagents)

a) Preparation of magnetic beads

The Magnetic Core Kit components were allowed to equilibrate to room temperature for 30 minutes and the magnetic Beads tube (5.4 ml or 225 uL/sample) was mixed by gentle vortexing and placed in a magnetic stand until complete separation. The supernatant was discarded, and 5.4 ml of RNase-Free Water was added and the solution vortexed at medium speed. The process was repeated until the solution appeared clear. The pellet was resuspended in 1500 µl (60u L/sample) of Magnetic bead Resuspension solution and 65uL of the washed magnetic beads was aliquoted into new 1.5 uL RNase free tube. 1 uL of RiboGuard RNase inhibitor was added and the mixture was vortexed.

b) Ribosome and rRNA depletion

Five micrograms of DNA-free RNA (at least 500 ng) was dissolved in RNase-Free water and the resulting solution treated as shown in table 3.4 and incubated for 10 minutes at 68˚C, then 5 minutes at room temperature. The treated RNA was added to 1.5ml microcentrifuge tube containing the washed Magnetic Beads (prepared in ‘a’ above) and immediately mixed and vortexed at medium setting for 10 seconds. After which it was incubated at room temperature for 5 minutes, vortexed again and incubated for 5 minutes at 50 °C. The microcentrifuge tubes were then immediately

Table 3.4. Composition of RNA treatment solution

Reagent	Amount
1-5µg of DNA-free RNA	20 µL
rRNA Removal Solution	8 µL
Reaction Buffer	4 µL
RNase free water	11 µL

placed on a magnetic stand until the solution appeared clear. The supernatant was transferred to a labelled RNase-free tube placed on ice. The rRNA-depleted sample was then purified as follows, RNA clean XP solution was brought to room temperature and vortexed and 60 ul of the vortexed RNAclean XP solution was added to 1.5 ml microfuge tube containing 85-90 uL of rRNA depleted RNA and mixed by pipetting. The mixture was allowed to stand for 15 min and then placed on a magnetic stand. The supernatant was removed, and the beads washed twice with 200 ul fresh 80% ethanol and air-dried for 15 min. 11 ul of water was added and the tubes placed on a magnetic stand for 2 minutes. The supernatant was collected and mixed by pipetting.

3.2.13.1.2. cDNA library preparation

a) RNA fragmentation and cDNA (complementary DNA) Synthesis

For each sample to be fragmented, a fragmentation mix was prepared by pipetting the 9µl rRNA depleted RNA, 1 µl RNA fragmentation solution and 2 µl of cDNA synthesis primer. The solution was mixed and incubated at 85°C for 2 minutes in a thermocycler and then placed on ice. After which mixture was added to the cDNA synthesis cocktail described in table 3.5, and the resulting mixture incubated at 25 °C for 5 min followed by 42 °C for 20 min. The reaction mixture was then cooled to 37 °C and 1.0µl of Finishing Solution was added to each reaction. The mixture was incubated again at 37 °C for 10 min and then at 95 °C for 3 min, cooled to 25 °C and then brought to room temperature.

b) Addition of terminal-Tags to cDNA

From each sample, a tagging cocktail was prepared by pipetting 7.5 µL Terminal Tagging Premix and 0.5 µL DNA Polymerase into a 20 µL tube. The resulting solution was mixed thoroughly by pipetting and then 8.0 µl of the Master Mix was added to each reaction and the solution mixed by pipetting. The reaction mixture was incubated at 25 °C for 15 minutes, then at 95 °C for 3 minutes and then cooled to 4 °C.

Table 3.5. cDNA synthesis cocktail

Reagent	Amount
cDNA Synthesis PreMix	3.0 µl
100 mM DTT	0.5 µl
StarScript Reverse Transcriptase	0.5 µl

c) Purification of the tagged samples

The tagged samples were purified as follows:

45ul Ampure XP solution was added, mixed and placed on a magnetic stand for 15 minutes. The supernatant was pipetted out and washed twice with 20 ul of 80% ethanol. The beads were air-dried for 15 minutes and 24 ul water was added. The tubes containing the beads were allowed to stand for 2 min and then placed on a magnetic stand after which 22.5ul of the supernatant was transferred to a fresh tube

d) Enrichment of cDNA library via PCR

The PCR Master mix was prepared as shown in table 3.6, and the PCR ran with the following conditions: an initial denaturation step at 95°C for 1 minute, 15 cycles of: denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, elongation at 68°C for 3 minutes and then final elongation at 68 °C for 7 minutes and the samples stored at 12 °C. The libraries were quantified by Qubit and visualized on Agilent Tapestation. Concentrations above 5 ug/ml, with a visible tapestation peak between 200-800bp, were selected.

e) Purification of pooled library sample

Equal quantities of libraries, based on the qubit values, were pooled and purified as follows;

AmpureXP solution was brought to room temperature for 30 minutes and vortexed vigorously. 120 uL AmpureXP solutions was added to 200 uL of the pooled library, mixed and left to stand for 15 minutes. The tubes were then placed on a magnetic stand and the supernatant removed. The beads were washed twice with 200 uL freshly prepared 80% ethanol; air-dried for 15 minutes and 50ul of water is added. The mixture was allowed to stand for 2 min before placing it on a magnetic stand. 45uL of the supernatant was transferred to a fresh tube and the library pool quantified by Qubit and visualized on Agilent Tapestation.

Table 3.6. Enrichment PCR cocktail

Reagent	Amount
di-tagged cDNA	22.5 μL
FailSafe PCR PreMix E	25 μL
Forward PCR Primer	1 μL
Index PCR Primer	1 μL
FailSafe PCR Enzyme	0.5 μL

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3.2.13.2 Sequencing and preliminary bioinformatics

3.2.13.2.1. cDNA Sequencing

The cDNA library was then sequenced on the Illumina MiSeq sequencing system (Illumina inc). The MiSeq System uses Illumina sequencing by synthesis technology (SBS). This SBS sequencing chemistry uses a proprietary reversible terminator-based method that detects single bases as they are incorporated into massively parallel DNA strands. Fluorescent terminator dyes are imaged as each dNTP is added and then cleaved to allow incorporation of the next base. With all 4 reversible, terminator-bound dNTPs present during each cycle, natural competition minimizes incorporation bias. Base calls are made directly from signal intensity measurements during each cycle

(Miseq datasheet). The single nucleotide polymorphism (SNP) marker data received was further filtered using the TASSEL software (Bradbury, 2007) to retain only polymorphic SNPs with less than 100 missing values and a minimum and maximum allele frequency of 0.05 and 0.95 respectively

3.3 Data analysis and statistics

3.3.1. Data analysis for the determination of genetic diversity

Basic diversity measurements for each population, including the total number of alleles (Na), Number of effective alleles (Ne), Shannon’s information index (Shannon, 1948), number of private alleles, expected (gene diversity) and observed heterozygosity, were calculated using Power Marker (Liu and Muse, 2005) and GenAlex version 6.41 (Peakall and Smouse, 2006) software. Nei’s genetic distance between the populations collected from Nigeria and Kenya as well as between pairs of populations within Nigeria and Kenya was calculated using Power marker and GenAlex version 6.41 software. For individual-level analysis, pair-wise genetic distance (identity-by-state, IBS) matrix was determined using PLINK (Purcell *et al.,* 2007). A Neighbour-joining tree of all collected *Striga hermonthica* samples was constructed using MEGA vs 6. (Tamura *et al.,* 2013).

3.3.2. Data analysis for the determination of population structure

The population structure inherent in the samples was determined using ADMIXTURE and R. Analysis of molecular variation (AMOVA) was used to determine the fixation index (F_{ST}).

Procedure

Population Structure

A Ward's minimum variance hierarchical cluster dendrogram was built from the IBS matrix using the Analyses of Phylogenetics and Evolution (ape) package (Paradis *et al.,* 2004) implemented in R (R core team, 2015). Thereafter two approaches were used to determine the population structure of the samples

i) A model-based maximum likelihood estimation of individual ancestries using ADMIXTURE (Alexander *et al.,* 2009). This assumes that the loci are in linkage equilibrium and the ancestral populations are in Hardy-Weinberg equilibrium (Frichot *et al.,* 2014). In the ADMIXTURE analysis, we varied the number of subpopulations (K) from 1- 10 and determined the best value of the K after considering the 10-fold cross-validations. The K value with the lowest cross-validation error (Alexander *et al.,* 2011) was then selected and the goodness of fit of the results with the clustering pattern of the hierarchical tree examined.

ii) Discriminant Analysis of Principal Components (DAPC) (Jombart *et al.*, 2010) that is free from the above assumptions.

Discriminant Analysis of Principal Components (DAPC) was done using 'adegenet' (Jombart 2008). DAPC involves first inferring optimal clusters number of PCA-transformed SNP data by varying the possible number of clusters from 2 to 40, using k-means analysis. After which assessing the best supported model by Bayesian Information Criterion is selected. The results obtained from the hierarchical tree, ADMIXTURE and DAPC analysis were then compared.

Fixation index (F_{ST})

Fixation index (F_{ST}) is a measure of differentiation due to genetic structure. It is used to determine if distinct subpopulations exist with a larger population. The values of the fixation index F_{ST} range from zero to one. A value of zero indicates no population structuring, while one indicates that the two populations share no alleles. Wright (1978) suggested the following qualitative guidelines when interpreting F_{ST}. A range 0.0 to 0.05 may be considered as indicating little genetic differentiation, 0.05 to 0.15 indicates moderate genetic differentiation, and 0.15 to 0.25 indicates great genetic differentiation, while values of F_{ST} above 0.25 indicate very great genetic differentiation. The fixation index (F_{ST}) and standardized F_{ST} (F'_{ST}) were assessed using Analysis of Molecular Variance (AMOVA) implemented in GenAlex 6.41. Correlations between pairwise F_{ST} values and geographical distance matrices were calculated using Mantel test (Mantel, 1967), after 1000 random iterations, as implemented on GenAlex software version 6.41.

3.3.3. Data analysis for identification of potential loci under selection within she *Striga hermonthica* populations.

To determine the presence markers under selection within the populations we observed, hierarchical Bayesian method as described in Beaumont and Balding (1995) and implemented in BayeScan 2.1 software (Foll and Gaggiotti, 2008) was used.

Principle

BayeScan works based on the assumption that allele frequencies within populations follow a multinomial-Dirichlet distribution (Balding and Nichols 1995, Rannala and Hartigan, 1996). The F_{ST} parameters are also assumed to be a function of population-specific components shared among all loci (β) and of locus-specific components shared among all populations (*α)*. For a given locus, departure from neutrality is assumed when the locus-specific component is required to explain the observed pattern of diversity. BayeScan directly infers the posterior probability of each locus to be under the effect of selection by defining and comparing two alternative models: one model includes the locus-specific component, while the other excludes it (Fischer *et al.,* 2011). The ratio of the model posterior probabilities is used to calculate the posterior odds (PO), which measures how much more likely the model with selection is, compared to the model without selection (Balding and Nichols, 1995, Fischer *et al*

.2014). When using the same prior for both models the posterior odds are reduced to the Bayes Factor. Jeffreys (1961) proposed a logarithmic scale for model choice defined as: barely worth mentioning if $\log_{10}PO < 0.5$, substantial if $\log_{10}PO > 0.5$, strong $\log_{10}PO > 1.0$, very strong if $\log_{10}PO > 1.5$) and decisive evidence for accepting a model ($\log_{10}PO > 2.0$). The estimated alpha coefficient indicates the strength and direction of selection. A positive value of alpha suggests diversifying selection, whereas negative values suggest balancing or purifying selection. (Foll and Gaggiotti, 2008). When a given posterior odds threshold (chosen according to Jeffrey's scale of evidence), defines a set of outlier markers, the corresponding expected proportion of false positives (FDR) among outlier markers can be calculated. This process was reversed in Bayescan, by first choosing a target FDR, and then looking selecting the highest posterior odds threshold achieving this FDR. A q-value, which is the FDR analogue of the p-value was therefore defined. The q value of given locus is the minimum FDR at which this locus may become significant. .

Procedure

The SNP data obtained loaded into the software BAYESCAN and the estimation of model parameters was automatically tuned on the basis of short pilot runs (10 pilot runs, length 5000, and a burn-in of 50000). The sample size was set to 5000 and the thinning interval to 10 resulting in a total chain length of 100000 iterations. False Discovery Rate (FDR) was used to control for multiple testing. To identify loci under selection, the posterior distribution of α_i was used. If the value obtained is positive it suggests that the locus is undergoing directional selection, while if it is negative it implies that stabilizing selection is occurring and homogenizing the allele frequencies in the populations. The tested loci were then ordered by their estimated posterior probabilities. An R function (as provided in BAYESCAN) was used to identify and plot outlier loci.

3.3.4. Data analysis for effects of *S. hermonthica* infestation on a susceptible and a resistant maize genotype and determination of physiological and biochemical mechanisms of resistance to *Striga hermonthica* by maize

Statistical analyses were performed on Excel and MINITAB software using one-way analysis of variance (ANOVA) and a post hoc Tukey test. P-value less than 0.05 was considered significant.

Analysis of covariance (ANCOVA) implemented in R software, was used estimate the relationship between the rates of development of *Striga hermonthica* plants attached to the roots of the susceptible and resistant genotype.

3.3.5. Bioinformatics/data analysis for the transcriptomics experiment.

3.3.5.1. Quality Control

After sequencing, the quality of the obtained sequence data was evaluated with FASTQC software. After which the paired-end reads obtained per sample were trimmed to an average read length of 137 nucleotides using TRIMMOMATIC software.

3.3.5.2. Read Mapping

These reads were mapped against the maize genome using STAR software resulting between 33 to 53 per cent of uniquely mapped reads. The mapped reads were then loaded into CUFFLINKS software for assembly. The assembled transcripts were processed by CUFFMERGE software to produce the final transcriptome assembly.

3.3.5.3. Differential Gene Expression Analysis

The expression catalogue obtained for the three time points were analysed with CUFFDIFF and CUMMERBOND software to obtain the differential expression results as well as test for statistical significance of differentially expressed gene /transcripts. For each time point and each maize genotype, the transcripts from the infested plants and thee uninfested plants were compared and the upregulated and downregulated genes for each genotype in every time point was identified. A transcript was taken as up or downregulated at each time point if in comparison with the uninfested tissue it shows a $\log_2$ fold-change greater than 1.5 (for genes that were upregulated) and less than -1.5 (for genes that were downregulated) and a p-value below 0.01. The transcripts IDs were compared against three repositories;

1) Ensembl Plants BioMart (http://plants.ensembl.org/biomart/martview/00cafbbb 1dc f6fc44bcee79033497445)

2) Gramene (ftp://ftp.gramene.org/pub/gramene/CURRENT_RELEASE/gff3/zea mays/gene_function/B73v4.gene_function.txt),

BioMart is a repository to share biological data, sequences, convert identifiers and support data enrichment analysis, while Gramene is a curated, open-source, integrated data resource for comparative functional genomics in crops and model plant species.

3) The Kyoto Encyclopaedia of Genes and Genomes (KEGG)

KEGG is a database resource for understanding high-level functions and utilities of the biological system from molecular-level information.

The first repositories were used to identify the corresponding genes, while the third was used to identify pathways involved in the resistant or susceptible response.

3.4. Experiments

3.4.1 Experiment 1: Studies on genetic diversity of *Striga hermonthica* populations in Nigeria and Kenya.

3.4.1.1. Introduction

Genetic diversity is the total number of genetic characteristics in the genetic makeup of a species. It refers to the variation of individual genes (polymorphism). A higher amount of variation increases the chances that certain members of the population individuals will possess a suitable variant allele that will enhance their survival. These individuals will in turn reproduce and transfer this variant to their progeny thus propagating the variant and the organism which will in turn reproduce and continue the population into subsequent generations. It therefore provides a means through which organisms can adapt to changing surroundings (National Biological Information

Infrastructure). The dynamics within the gene-pool of a species is closely associated with several life-history traits. For example, highly fecund, out-crossing species should have higher proportions of polymorphic loci, more alleles per polymorphic locus and more genetic diversity (Hamrick and Nason 1996; Dubois *et al.,* 2003; Nybom, 2004). As an obligate out-crossing species that produces a very high number of seeds (high fecundity) that can remain viable for very long periods of time, *S. hermonthica* will have high amounts of genetic variations within its population. This can increase the parasite's ability to change evolve and adjust to various circumstances (Koyama, 2000). The constant changes in cropping patterns and farming systems across regions in sub-Saharan Africa impose varying selection pressures, which can also promote the evolution of new ecotypes of *S. hermonthica.*

This high level of genetic variation would enable *S. hermonthica* to defeat host plant resistance (Koyama, 2000) and other control methods. This will lead to differences in the amount of control achieved in diverse locations at different times (Hearne, 2009). Estep *et al.,* (2011) and Bozkurt *et al.,* (2013) reported high amounts of diversity within *S. hermonthica* plants from countries in West and East Africa. However, these samples did not represent the entire regions where *S. hermonthica* may be found within these countries or all the *S. hermonthica* host crops. This study was therefore designed to investigate the extent of diversity of *S. hermonthica* populations across regions infested by the parasite in Northern Nigeria and Western Kenya. The objectives of this experiment are to:

a) Characterise the extent of diversity of *S. hermonthica* populations collected in Nigeria

b) Characterise the extent of diversity of *S. hermonthica* populations collected in Western Kenya.

3.4.1.2 Procedure

Striga hermonthica leaves were sampled as described in Materials and Methods under section 3.2.1.2. DNA was extracted from the leaf samples, quantified and digested, as described in Materials and Methods under sections 3.2.4. The DNA was then lyophilised and genotyped using the genotyping by sequencing (GBS), as described in

Materials and Methods under sections 3.2.5 to generate Single Nucleotide Polymorphism (SNP) markers.

3.4.1.3. Experimental protocol

The Single Nucleotide Polymorphism (SNP) markers derived from GBS were used to genotype the 1029 *Striga hermonthica* samples and the SNP data were analysed for measures of genetic diversity for the groups as follows;

I. Individual samples.
II. By their country of origin.
III. By their host plants.

Genetic diversity measurements was done between the population collected in Nigeria and the population collected in Kenya, as well as between pairs of populations within Nigeria and Kenya were calculated.

3.4.2. Experiment 2 (A): Determination of genetic differentiation and population structure in Kenya and Nigerian *Striga hermonthica* populations.

3.4.2.1 Introduction

Population structure is the presence of a systematic difference in the frequencies of allele between subpopulations within a population, this is most likely because these subpopulations have differing ancestries. The main reason why population stratification exit is non-random mating between groups, this may be because these groups are separated physically, and consequently, different alleles are being fixed by these different groups. If populations are subdivided, they can evolve apart, somewhat independently; this allows the populations to diversify. Genetic differentiation is defined as an accumulation of differences in allelic frequencies between completely or partially isolated populations due to evolutionary forces such as selection or genetic drift. Different environmental conditions subject plant populations to different selection pressures and these plants become increasingly genetically heterogeneous (Linhart and Grant, 1996). This may be differences in habitat like elevation, exposure, and the availability of moisture. These conditions often create ecological barriers that prevent gene flow thus leading to interpopulation genetic separation (Abbasi *et al.*, 2016).

Studies carried out in Africa with different genetic markers show that there is a relationship between the genetic distance and the geographic distance between parasite populations. They however do not agree that *S. hermonthica* populations specifically infest only a given host within Africa (Koyama, 2000; Gethi *et al.*, 2005; Estep *et al.*, 2011; Welsh & Mohamed, 2011; Bozkurt *et al.*, 2015). There is therefore a need for studies that involve *S. hermonthica* sampled across different locations and hosts. The objectives of this experiment are to

1) To investigate the population structure and genetic differentiation inherent in *S. hermonthica* populations collected from Kenya and Nigeria.

2) To investigate and determine the presence or absence of potential loci under selection

3.4.2.2 Procedure Experimental design

Single Nucleotide Polymorphism (SNP) markers derived from GBS were used to genotype the 1029 individuals and and the samples were analysed in groups as follows.

I. The Nigerian population

II. The Kenyan population

III. All the samples collected

a) Population Structure

Two approaches were used to investigate the population structure within the samples;

i) A model-based maximum likelihood estimation of ancestries using ADMIXTURE (Alexander *et al.*, 2009).

ii) Discriminant Analysis of Principal Components (DAPC) (Jombart *et al.*, 2010)

The results obtained from the hierarchical tree, ADMIXTURE and DAPC analysis were then compared.

b) Genetic Differentiation (Fixation Index (F_{ST}))

Fixation index (F_{ST}) is a measure of differentiation due to genetic structure. It is used to determine if distinct subpopulations exist within a larger population. The values of the fixation index (F_{ST}) ranges from zero to one. This analysis was used to determine the amount of differentiation between the Nigerian and Kenya samples with the host plants and geographical locations as factors. Mantel test (Mantel, 1967) was used to determine the correlation between geographical distance and pairwise F_{ST}.

Experimental protocol

Only populations with detectable structuring were used for this experiment. They include.

(i) The three subpopulations observed within the Nigerian *S. hermonthica* population.

(ii) The Kenyan *S. hermonthica* population as defined by host plant

(iii) The entire *Striga* population collected

3.4.3. Experiment 2 (B). Identification of potential loci under selection within the *Striga hermonthica* populations.

3.4.3.1 Introduction

In the genomic era, selection refers to any non-random, differential propagation of an allele as a consequence of its phenotypic effect. (Vitti *et al.,* 2013). The detection of molecular signatures of selection is one of the major concerns of modern population genetics, as it provides insight into the mechanisms leading to population divergence and differentiation (Fariello *et al.,* 2013). It has become crucial in biomedical sciences, where it can help to identify genes related to disease resistance (Albrechtsen *et al*. 2010; Fumagalli *et al*. 2010; Cagliani *et al*. 2011), adaptation to climate (Lao *et al*. 2007; Rees and Harding 2012), or altitude (Simonson *et al*. 2010). Selection may act in a number of ways: it may be directional selection, in which an allele is favoured and so propagated (positive selection) or disfavoured (negative selection, also called purifying selection). It may also be balancing selection, where multiple alleles are maintained at an appreciable frequency within the gene pool (Vitti *et al.,* 2013). These multiple alleles may give rise to opposing phenotypic effects, for example, large and small body sizes may be maintained within the population to the exclusion of intermediate sizes. A trend that is often further described as diversifying or disruptive selection (Vitti *et al.,* 2013). However, when intermediate phenotypic values are favoured, whether by balancing selection of co-dominant alleles or by positive selection of alleles that underlie intermediate phenotypes, the trend is called stabilizing selection. An example is where the presence of the heterozygote is selected for, as seen in the selection of 'AS' in the Hemoglobin-B gene (HBB) due to its ability to resist malaria (Allison AC. 1954. Pasvol *et al*. 1978, Kwiatkowski. 2005). Other classical examples of balancing selection are; the major histocompatibility complex (MHC) system in mammals (Hughes and Nei 1988; Takahata and Nei 1990), the disease-response genes (R-genes) in plants (Stahl *et al*. 1999), and the self-incompatibility system in plants (Wright 1939).

This study seeks to establish the presence of signatures of selection by identifying potential loci (outlier loci) undergoing selection within populations that show significant genetic differentiation to determine some of the molecular forces driving genetic differentiation.

The BayeScan analysis was used to determine potential markers under selection in populations that showed significant amounts of differentiation.

The populations detected by population structure analysis;

A. The Nigerian and the Kenyan *Striga hermonthica* population collected

B. The three Nigerian populations observed by population structure analysis.

C. The *Striga hermonthica* population with Sorghum, rice and maize respectively as host plants in Kenya.

D. One Kenyan and three Nigerian subpopulations.

3.4.4. Experiment 3: Interactions between *S. hermonthica* and a susceptible and a resistant maize genotype.

3.4.4.1 Introduction

The growth and development of maize are impacted negatively by *S. hermonthica* and this has been attributed to a combination of factors. Firstly, resource withdrawal; this is the removal of water, carbon and nitrogen by the attached parasite. Secondly, *Striga*-induced changes on host allometry resulting in elevated respiratory demand and a higher incidence of self-shading of lower leaf areas. Thirdly, a lowering in the rate of canopy carbon fixation in infected hosts (Press, 1995). It has also been hypothesised that the impact of *S. hermonthica* may be due to the action of a *Striga*-derived toxin (Musselman 1980), but till date, there is little evidence supporting this hypothesis. As a consequence of these, typically *S. hermonthica*-infected plants have lower total biomass accumulation, lower grain yield and are noticeably shorter (due to lower internode elongation) than uninfected counterparts (Cechin and Press, 1993a, 1993b, 1994; Frost *et al.*, 1997; Graves *et al.*, 1989; Gurney *et al.*, 1995; Taylor *et al.*, 1996). In addition to lower biomass accumulation, the pattern of biomass partitioning in infected hosts is often disrupted such that biomass is preferentially allocated to the roots of the host rather than to the shoot. This results in an elevation of the root to shoot ratio in *S. hermonthica*-infected plants. The severity of the impact of *S. hermonthica* on its hosts is moderated by host and parasite genotype, host nutrition, infection time and infection density (Cechin and Press 1993a, 1993b; Graves *et al.*, 1989). The objective of these experiments is to elucidate the biochemical and physiological interactions between susceptible and resistant maize lines and *S. hermonthica*.

This was divided into experiments 3A and 3B.

A) Determination of the ability of maize plants to resist the attachment of *S. hermonthica* plants and the effect of *S. hermonthica* plant attachment on host plant height

B) Determination of the effect of *S. hermonthica* infestation on the root/shoot ratio by weight

3.4.4.2. Experiment 3A. Determination of the ability of maize plants to resist the attachment of *S. hermonthica* plants and the effect of *S. hermonthica* plant attachment on host plant height

Procedure

Surface sterilised maize seeds from two maize lines 5057 and ZD05 were germinated for five days in a moist filter paper-lined Petri dish. Seedlings from both maize lines were randomly divided into three groups (groups A, B and C), comprising five individuals each, and transferred to individual rhizotrons by placing them on the black cotton fabric at depth of about 5cm. The rhizotrons were nourished with nutrient solution (40% Long Ashton solution containing 1molm^{-1} ammonium nitrate) daily. The first set (group A) was infested with untreated *S. hermonthica* seeds, while the second set (group B) was infested with untreated *S. hermonthica* seeds treated with GR24, a synthetic germination stimulant, for 48 hours and the third set (group C) was not infested and used as control. At 20 days old, the plants were infested by using a brush to carefully spread 2 g of *Striga hermonthica* seeds all over the rhizotron surface and especially on the exposed roots of the maize plants. The number of attached *Striga hermonthica* plants on the roots was observed and counted at three to four-day intervals from the first day attached plants were observed. Similar to what was described by Amusan *et al.,* (2008), the growing *Striga hermonthica* plants were categorised into five developmental stages, viz: L1= plants with 2-4 partially horizontal opened leaves, L2= plants with 6-8 partially horizontal opened leaves, L3 = plants with 10-12 partially horizontal opened leaves, L4 = more than twelve partially horizontal opened leaves, Lx = Discoloured, dying or dead *S. hermonthica* plants.

The plant height (distance from the base of the stem to the base of the tassel) was measured. The number of *S. hermonthica* plants in each of these developmental stages were also identified and counted along with the total number of attached plants until the susceptible maize plants started dying and these was taken as an index of the ability to resist infestation by the parasite.

Experimental protocol

Seeds from both maize genotypes were divided into 3 groups (each group consisted of five replicates) grown on rhizotrons and treated as follows;

Group 1- Uninfested ZD05 plants used as Control

Group 2- ZD05 plants infested with 2 g of preconditioned *Striga hermonthica* seeds

Group 3- ZD05 plants infested with 2 g of preconditioned and pre-germinated *Striga hermonthica* seeds

Group 4- Uninfested 5057 plant used as Control

Group 5- 5057 plants infested with 2g of preconditioned *Striga hermonthica* seeds

Group 6- 5057 plants infested with 2g of preconditioned and pre-germinated *Striga hermonthica* seeds.

3.4.4.3. Experiment 3B: Determination of root-shoot ratio by weight of *Striga hermonthica* infested maize.

Procedure

Maize seedlings from varieties 5057 and ZD05 were transferred to individual rhizotrons by placing them on the black cotton fabric at depth of about 5cm and the rhizotrons were nourished with nutrient solution (40% Long Ashton solution containing 1molm^{-1} ammonium nitrate) daily. At 30 days old the plants (five susceptible, five resistant) were infested by using a brush to carefully spread 2g of *Striga hermonthica* seeds all over the rhizotron surface, especially on the exposed roots of the maize plants growing. Both maize lines were divided into two groups (n = 5). The first set was infested with untreated seeds, while for the second set was not infested and used as control. At the end of the experiment (53 days post infestation), the maize plants were carefully removed from the rhizotrons, separated into roots and shoots and then dried in an oven (at 45 °C) till they lost all moisture (this was determined by weighing daily until the plant tissue showed constant weight). The roots and the shoots were then weighed and the root to shoot ratio determined.

Experimental protocol

Seeds from both maize genotypes were divided into groups (each group consisted of five replicates) grown on rhizotrons and treated as follows.

Group 1- Uninfested ZD05 plants used as control.

Group 2- ZD05 plants infested with 2 g of preconditioned *Striga hermonthica* seeds.

Group 3- Uninfested 5057 plant used as control.

Group 4- 5057 plants infested with 2g of preconditioned *Striga hermonthica* seeds.

3.4.5 Experiments 4: Determination of physiological and biochemical mechanisms of resistance to *Striga hermonthica* by maize

3.4.5.1 Introduction

Striga hermonthica seeds have very specific requirements; they have to undergo conditioning (warm stratification that enables *Striga* seeds to become responsive to germination stimulants) before they can germinate and attach to their hosts. This includes stimulation by chemical compounds exuded by host to germinate and signals from other chemical compounds from the host plants for subsequent haustorial development (Joel *et al.,* 2007). The ability of a host to successfully prevent the *S. hermonthica* seed from obtaining one or more of these requirements or attaining any of its growth stages can confer resistance to *Striga hermonthica* infestation on that host.

Resistance to parasitic weeds can be expressed before or after host-parasite vascular bridge formation (Rispail *et al.*, 2007). A number of mechanisms have been identified by different studies on a number of host crops. Cherif-Ari *et al.* (1990) showed that low root length density might be one mechanism adopted by certain sorghum varieties to avoid *Striga* parasitism. This is because the lower the density of the roots the less the likelihood of contact between *S. hermonthica* seeds and the roots of the host. According to Mohamed *et al.,* (2010), low production of *Striga* seed germination stimulants, production of germination inhibitors, low production of the signal required for haustoria initiation and a hypersensitive response (characterised by a distinct necrotic area on the host root at the attachment site) are all mechanisms through which sorghum plants discourage parasitic establishment. Studies by Amusan *et al.,* (2008) revealed the accumulation or deposition of an unidentified substance at the haustoria–host interface in a resistant maize genotype. These investigations have elucidated a number of mechanisms and potential mechanism of tolerance and resistance to infestation by *S. hermonthica* by some host crops.

According to Joel *et al*., (2007) testing of crop resistance to *Striga* in the field provides little information concerning the mechanisms of resistance in the host plant. It is therefore important to characterise these mechanisms of defence against the parasite and to identify developmental stages of the parasite that are vulnerable to such defence (Hood *et al.,* 1998). This present study investigates the specific physiological and biochemical mechanisms deployed by ZD05, a resistant maize genotype, in a bid to resist or tolerate infestation by the parasite.

This comprised of experiments A, B and C.

A) Determination of germination stimulant ability of maize root exudates from susceptible and resistant maize lines.

B) Determination of total root length of major roots on ZD05 and 5057

C) Determination of growth rate of attached *S. hermonthica* plants growing on maize genotypes with contrasting characteristics

3.4.5.2. Experiment 4A. Determination of germination stimulant ability of maize root exudates from susceptible and resistant maize lines

Procedure

Maize root exudates were obtained from five plants per variety through two methods.

1. From plant grown in test tubes with no soil

2. From potted plants with river sand.

The extracted maize root exudates obtained by both collection methods, as described in the Methods section 3.2.9, were pipetted (150 µl) into petri dishes with preconditioned (20 days) *Striga hermonthica* seeds. Five replicates of each maize variety were established. The Petri-dishes were wrapped in aluminium foil and kept in an (incubator). *S. hermonthica* seeds suspended in the exudates obtained from plants grown in test tubes were incubated for 96 hours at 28°C and the number of germinated *S. hermonthica* seeds counted under a microscope. The number of seeds germinated per unit maize root weight was determined by dividing the number of *S. hermonthica* seeds germinated by the dry weight of the maize roots form which the exudates were collected. While seeds suspended in root exudates collected from potted plants were incubated for 48 hours at 28°C and the number of germinated *S. hermonthica* seeds counted under a microscope and then the samples were incubated for nine more days, and only germinated seeds with elongated haustoria were counted. *Striga hermonthica* seeds were also exposed to 150 µl of synthetic germination stimulant GR-24 (mg/l) and distilled water as positive and negative controls.

Experimental protocol

The exudates obtained through both methods were individually tested for their ability to stimulate germination. The preconditioned *Striga hermonthica* seeds were divided into 4 groups (each group consisted of five replicates) and treated as follows.

Group 1- Suspended in distilled water (negative control).

Group 2- Suspended in the root exudates of ZD05

Group 3- Suspended in the root exudates of 5057

Group 4- Suspended in 1ppm GR24 (positive control).

3.4.5.3. Experiment 4B: Determination of total root length of major roots on the resistant (ZD05) and susceptible (5057) genotypes.

Procedure

Ten surface sterilised maize seeds per line (two maize lines were used for the experiment, 5057 and ZD05) were planted in 25 L large pots (one seed per pot) filled with a (1:1) topsoil–river sand mixture, in a screen house. The pots were watered as required. At 28 days after planting all the soil was carefully washed off the plant roots and the number of major roots on each plant was counted. A major root was defined as any root that originated from the base of the plant as opposed to a lateral root that originates from another root). Also, the length of each major root was taken using measuring tape and the total length of the major roots of each plant determined by summing the lengths of the individual major roots.

3.4.5.4. Experiment 4C: Determination of growth rate of attached *S. hermonthica* plants on a susceptible and a resistant maize genotypes.

Procedure

Ten germinated sterilised maize seeds per line (two maize lines were used for the experiment, 5057 and ZD05) were transferred to individual rhizotrons by placing them on the black cotton fabric at depth of about 5 cm and the rhizotrons were nourished with nutrient solution (40% Long Ashton solution containing 1molm^{-1} ammonium nitrate) daily. When the plants were 30 days old, they were infested by using a brush to carefully spread 2 g of *Striga hermonthica* seeds all over the rhizotron surface especially on the exposed roots of the maize plants growing in the rhizotrons. *Striga hermonthica* plants (one on a major root and one on a lateral root selected) were selected and tagged per maize plant. At 3-4 days interval the number of leaves on the two selected *Striga hermonthica* plants on each maize plant were counted. ANCOVA was used to compare the rates of development of *Striga hermonthica* plants attached to the roots (both lateral and major roots) of both genotypes.

Experimental protocol

Group 1- ZD05 plants infested with 2g of *Striga hermonthica* seeds

Group 2- 5057 plants infested with 2g of *Striga hermonthica* seeds

3.4.6. Experiment 5: Gene expression profiling of maize genotypes

Introduction

A few studies have been done to investigate the molecular responses of host plants to parasitic plants using a variety of technologies. These technologies deduce and quantify the transcriptome. The transcriptome is the complete set of transcripts in a cell, and their quantity, for a specific developmental stage or physiological condition.

Understanding the transcriptome is essential for interpreting the functional elements of the genome and revealing the molecular constituents of cells and tissues. Understanding the transcriptome is also essential for understanding development and disease (Wang *et al.,* 2009). These technologies are broadly divided into hybridisation or sequence-based approaches. Hybridisation-based approaches typically involve incubating fluorescently labelled cDNA with custom-made microarrays or commercial high-density oligo microarrays (Wang *et al.,* 2009). In contrast to microarray, sequence-based approaches directly determine the cDNA sequence. RNA-Seq (RNA sequencing) employs novel high-throughput DNA sequencing methods and deep-sequencing technologies.

Microarray technology was used to investigate the changes in gene expression in the roots of cowpea (*Vigna unguiculata L.*) during susceptible and resistant interactions with *Striga gesnerioides*. Huang *et al.,* (2012). was observed that genes and pathways involved in signal transduction, programmed cell death and apoptosis, and defence response to biotic and abiotic stress were differentially expressed in the early resistance response; at the later time point, enrichment was primarily for defence-related gene expression, and genes encoding components of lignification and secondary wall formation. In susceptible interactions, multiple defence pathways were repressed, including those involved in lignin biosynthesis and secondary cell wall modifications, while cellular transport processes for nitrogen and sulphur were increased. Swarbrick *et al.,* (2008), while investigating rice cultivars undergoing a susceptible or resistant interaction with *Striga hermonthica*, also found differences between the resistant and susceptible interactions. The resistance reaction was characterised by upregulation of defence genes, including pathogenesis-related proteins, pleiotropic drug resistance ABC transporters, genes involved in phenylpropanoid metabolism and WRKY transcription factors. While the susceptible interaction was characterised by large-scale downregulation of gene expression, particularly within the functional categories - plant growth regulator signalling and metabolism, biogenesis of cellular components and cell division. It was also observed that there was a similarity between these changes in gene expression and those associated with resistance to microbial pathogens.

The present study was carried out using RNA-Seq to interrogate the molecular responses of two maize lines to infestation by *Striga hermonthica*. The objectives of this study are;

a) To elucidate the biochemical and molecular interactions between susceptible and resistant maize lines and *S. hermonthica*.

b) To elucidate the biochemical mechanisms through which resistant maize lines resist or tolerate infestation by *S. hermonthica*.

Procedure

Seeds of ZD05 and 5057 maize genotypes, 3 replicates each (n = 3 for each replicate), were surface sterilised and germinated by placing them in a petri dish lined with filter paper and sprinkling water on the paper to keep it wet for 48 hours. The germinated seeds were transferred into separate rhizotrons, assembled as described in the Methods section (3.3.8) above. Seven days later, preconditioned *S. hermonthica* seeds were placed on the roots of the maize plants. Some plants were uninfested and served as control. At three, nine- and twenty-two-days post infestation, root samples from infested and uninifested maize plants were harvested by removing the maize plant from the rhizotron, briefly cleaning the roots to remove ungerminated *S. hermonthica* seeds. The roots of three plants were cut into aluminium foil placed on ice, which was then wrapped and dipped into liquid nitrogen. The samples were collected in three replicates with each replicate consisting of three bulked plants. This was done at each collection time point. Total RNA was extracted from the root samples as described in the Methods sections 3.2.12 and converted to complementary DNA, which was then sequenced (Methods section 3.3.13). The sequencing results were taken through bioinformatics analyses (Methods section 3.3.5). Data were analysed using two-way ANOVA, and the Benjamini-Hochberg multiple testing correction was applied to the data at $\alpha_{0.01}$ and the differentially expressed transcripts were identified as described in the Methods sections 3.2.11, 3.2.12 and 3.3.5 respectively.

Experimental protocol

Three sets of plants representing each harvest time point were used

Set 1 (The roots of the plants were harvested 3 days post infestation)

Group 1- Uninfested ZD05 used as Control
Group 2- Uninfested 5057 plants used as Control
Group 3- ZD05 plants infested with 2g of preconditioned *Striga hermonthica* seeds.
Group 4 - 5057 plants infested with 2g of preconditioned *Striga hermonthica* seeds.

Set 2 (The roots of the plants were harvested at 9 days post infestation)

Group 1- Uninfested ZD05 plants used as Control
Group 2- Uninfested 5057 plants used as Control
Group 3 - ZD05 plants infested with 2g of preconditioned *Striga hermonthica* seeds.
Group 4- 5057 plants infested with 2g of preconditioned *Striga hermonthica* seeds.

Set 3 (The roots of the plants were harvested at 22 days post infestation)

Group 1- Uninfested ZD05 plants used as Control
Group 2- Uninfested 5057 plants used as Control
Group 3- ZD05 plants infested with 2g of preconditioned *Striga hermonthica* seeds.
Group 4- 5057 plants infested with 2g of preconditioned *Striga hermonthica* seeds.

At the end of each time point, the roots of the plants were harvested, and total RNA extracted from them.

CHAPTER 4

RESULTS

4.1. Genetic diversity of *Striga hermonthica* populations in Nigeria and Kenya

The Kenyan *Striga hermonthica* population had a higher effective number of alleles than, higher observed and expected heterozygosity, and unbiased expected heterozygosity (1.405 ± 0.009, 0.282, 0.246 ± 0.004 and 0.246 ± 0.004) respectively for Kenya) than the Nigerian samples (1.320 ± 0.008, 0.209, 0.204 ± 0.004 and 0.204 ± 0.004 respectively for Nigeria). It also had a higher Shannon’s information index (0.380 ± 0.006 for Kenya and 0.332 ± 0.005 for Nigeria) (Table 4.1). In both the Nigerian and Kenyan *Striga hermonthica* populations, the observed heterozygosity was higher than the expected heterozygosity. Conversely, the Nigerian samples had a higher number of different alleles (that is, Allele richness, Na) (1.999 ± 0.001) than the Kenyan population (1.964 ± 0.005). It also had more unique alleles that are unique it compared to the Kenyan population (61 for the Nigerian population and 11 for the Kenyan population).

A neighbour-joining, dendrogram clustered the samples into two unequal major groups. The smaller group contained all the Kenyan samples, while the larger comprised all the Nigerian samples (Fig. 4.1). The group comprising samples from Nigeria were divided into three major sub-clusters, with each sub-clusters having some minor subgroups within, while the group with Kenyan samples had only some minor subgroups. (Fig.4.1).

Genetic diversity analysis of the three major sub-clusters observed in the Nigerian population revealed that group 3, consisting of samples collected from the north-eastern part of Nigeria had the highest amount of genetic diversity. The second most diverse group was sampled from the north western part of the country, while samples from the central region showed the least amount of genetic diversity (Table 4.2). Group 3 had a higher effective number of alleles and higher observed and expected heterozygosity as well as a higher Shannon’s information index (Ne = 1.337 ± 0.0121, Ho = 0.229, and He = 0.209 ± 0.004, µHe = 0.2099 ± 0.004)) than group one (Ne = 1.312 ± 0.012, Ho =0.2, and He = 0.195 ± 0.004 and µHe = 0.196±0.004) and two (Ne = 1.272 ± 0.0131 , Ho = 0.193, He = 0.164 ± 0.005 and µHe = 0.165 ± 0.005) (Table 4.2). In all the groups the observed heterozygosity was higher than the expected heterozygosity (Table 4.2). Group 3 also had a higher Shannon’s information index (0.33 ± 0.006) than group 1 (0.260 ± 0.0060) and group 2 (0.313 ± 0.006) (Table 4.2.).

It was however observed that the samples in group 2 had a higher number of alleles (Na) as well as a higher number of unique alleles than both groups 1 and 3 (Group 2: Na = 46 and Unique alleles = 1.959 ± 0.005, Group 1: Na = 1.885±0.008 and Unique alleles = 10 and, group 3: Na = 1.917 ± 0.007 and Unique alleles = 19 and) (Table 4.2).

Table 4.1. Allelic patterns across populations of *Striga* collected in Kenya and Nigeria.

Parameter	Kenya ± S.E.	Nigeria ± S.E.
Ne	1.405 ± 0.009	1.320 ± 0.008
Ho	0.282	0.209
He	0.246 ± 0.004	0.204 ± 0.004
μHe	0.246 ± 0.004	0.204 ± 0.004
I	0.380 ± 0.006	0.332 ± 0.005
Na	1.964 ± 0.005	1.999 ± 0.001
Private Alleles	11	61

S.E. = Standard Error, Ne = No. of Effective Alleles, Ho=observed Heterozygosity, He = Expected Heterozygosity, μHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He. I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Na = No. of Different Alleles, Private Alleles = No. of Alleles Unique to a Population.

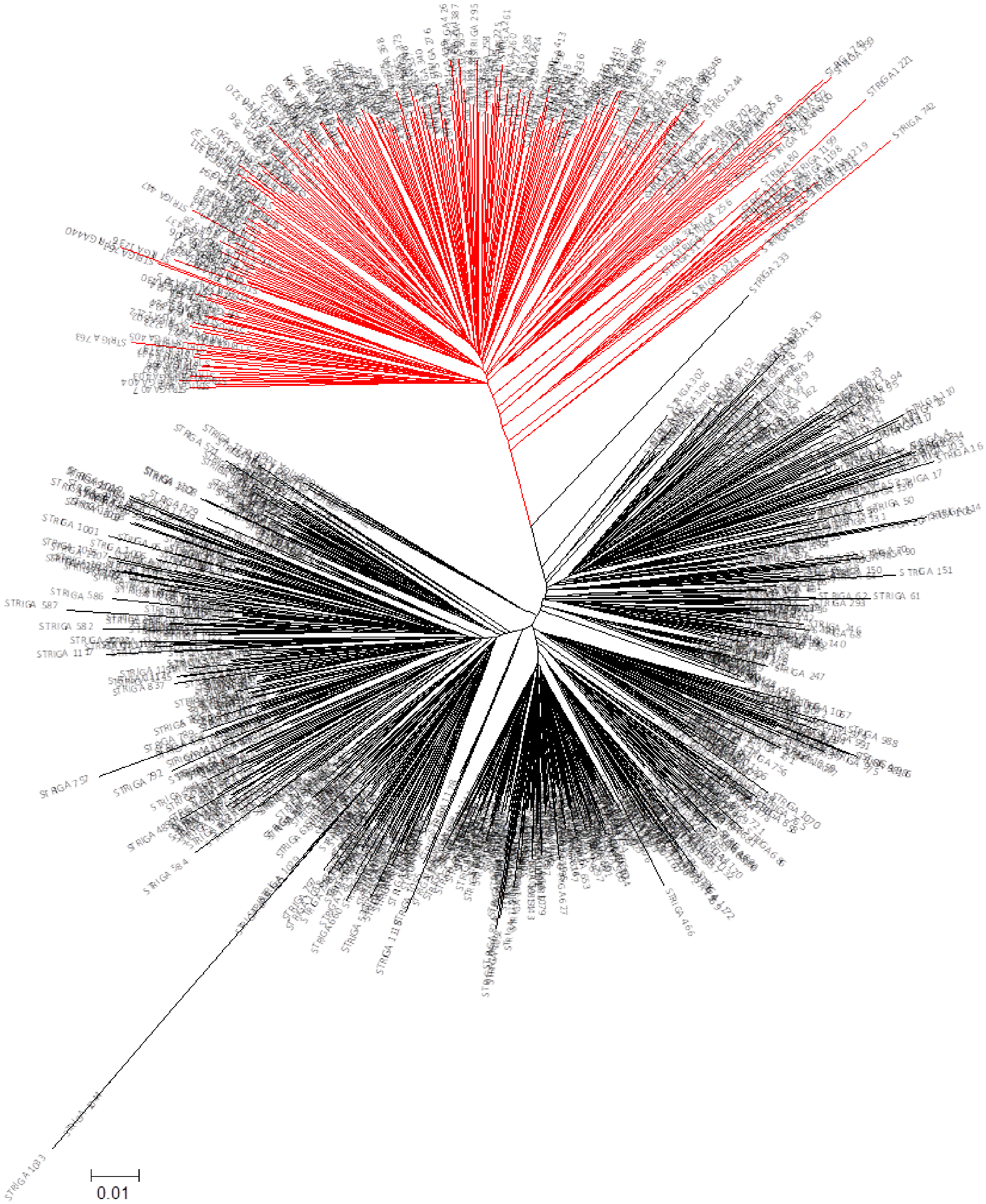


Figure 4.1. Neighbour-joining tree of all collected *Striga hermonthica* samples.

Nigerian samples in black showing the three major subgroups, and the Kenyan samples in red.

Table 4.2. Allelic patterns of populations observed in Nigeria.

Population	1 ± S.E.	2 ± S.E.	3 ± S.E.
Ne	1.272 ± 0.013	1.312 ± 0.012	1.337 ± 0.012
Ho	0.193	0.2	0.229
He	0.164 ± 0.005	0.195 ± 0.004	0.209 ± 0.004
µHe	0.165 ± 0.005	0.196 ± 0.004	0.2099 ± 0.004
I	0.260 ± 0.006	0.313 ± 0.006	0.33 ± 0.006
Na	1.885 ± 0.008	1.959 ± 0.005	1.917 ± 0.007
No. Private Alleles	10	46	19

S.E. = Standard Error, Ne = No. of Effective Alleles, Ho=observed Heterozygosity, He = Expected Heterozygosity, µHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He. I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Na = No. of Different Alleles, Private Alleles = No. of Alleles Unique to a Population.

1 = North west

2 = Central

3 = North East

4.2. Population Structure and Genetic Differentiation in Kenya and Nigerian *Striga hermonthica* Populations.

4.2.1 Population Structure

The Ward’s minimum variance hierarchical cluster dendrogram showed the entire *S. hermonthica* population to consist of one Kenyan and one Nigerian population. The Nigerian population was divided into three subpopulations (Figure 4.2).

ADMIXTURE analysis showed the samples to cluster progressively. K = 2 separated the samples by country of origin (Kenya and Nigeria). This was followed by further subdivision at k = 3 and at k = 4, the samples from Nigeria was divided into three subpopulations (Figure 4.2.).

DAPC analysis also showed the samples to be made up of four populations (Figure 4.2 and 4.3), which consist of the Kenyan samples and three Nigerian samples sub-populations.

The results obtained above were consistent and showed good correspondence (Figure 4.2.), thus the population structure within the samples had been correctly identified.

The Nigerian *S. hermonthica* samples collected from different hosts did not show structuring when clustered based on their host plants. However, Among the Kenyan samples, the samples with rice as their host clustered away from the samples that had maize and sorghum as their host (Figure 4.4).

A plot of each Kenyan *Striga hermonthica* samples at its geographical coordinates, showing its ancestral group revealed that all the Kenyan samples belong to the same ancestral group regardless of their sampling location. (Figure 4.5). However, when the Nigerian samples were plotted at its geographical coordinates, showing their ancestral groupings, it was observed that samples with the same ancestry grouped together at approximately three large locations; The North-western, Central and North-eastern parts of Nigeria These observed groups did not consist of purely genetically homogeneous groups of individuals, as some individuals from the one observed population shared ancestry with individuals from other populations. (Figure 4.6).

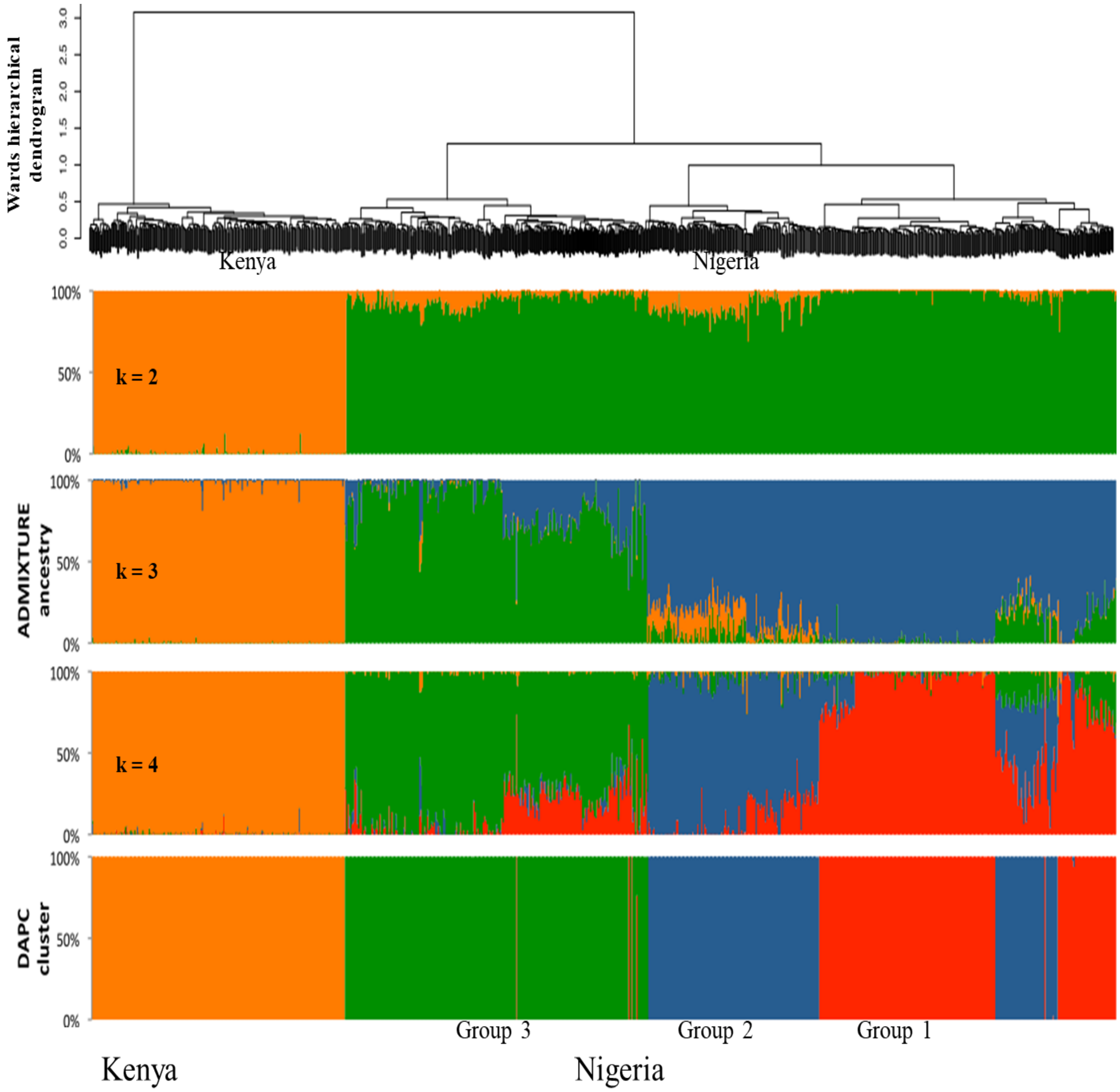


Figure 4.2. Wards hierarchical dendrogram, ADMIXTURE and DAPC plots showing population structure of the sampled *S. hermonthica* plants.

ADMIXTURE is seen to split the samples at progressively into the Kenyan and Nigerian populations at k = 2, into the Kenyan and two Nigerian subpopulations at k = 3 and, into the Kenyan and the three Nigerian subpopulation at k = 4. This coincide with the arms of the dendrogram and the groups of the DAPC plot.

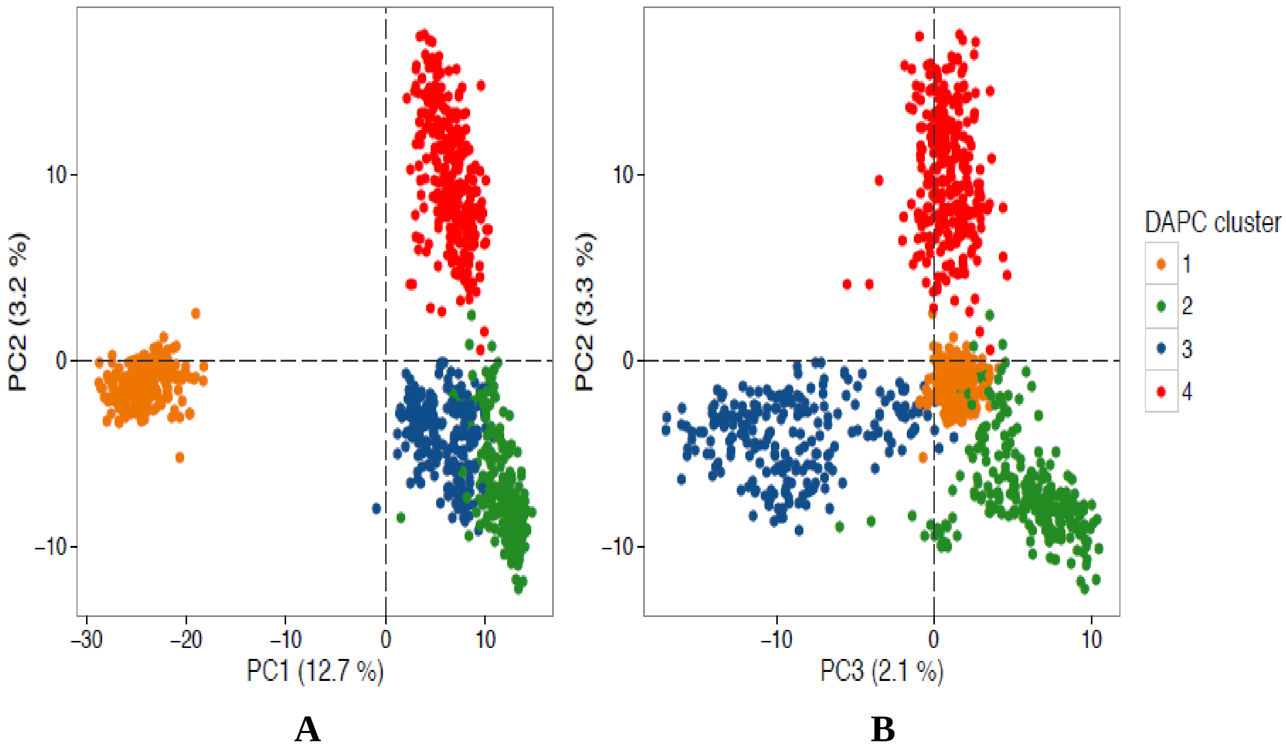


Figure 4.3. DAPC plots of the sampled *S. hermonthica* plants.

(A) Each sample is plotted along PC1 and PC2.

(B) Each sample is plotted along PC 2 and PC3

The groups show consistency with the population structure at K = 4.

Key

1 = Kenyan *Striga hermonthica* samples.

2 = Nigerian (Group 3) *Striga hermonthica* samples.

3 = Nigerian (Group 2) *Striga hermonthica* samples.

4 = Nigerian (Group 1) *Striga hermonthica* samples.

The percentages are the proportion of variance explain by each principal coordinate

PC1 explains 12.7% of the observed variance

PC2 explains 3.2 % of the observed variance

PC3 explains 2.1% of the observed variance

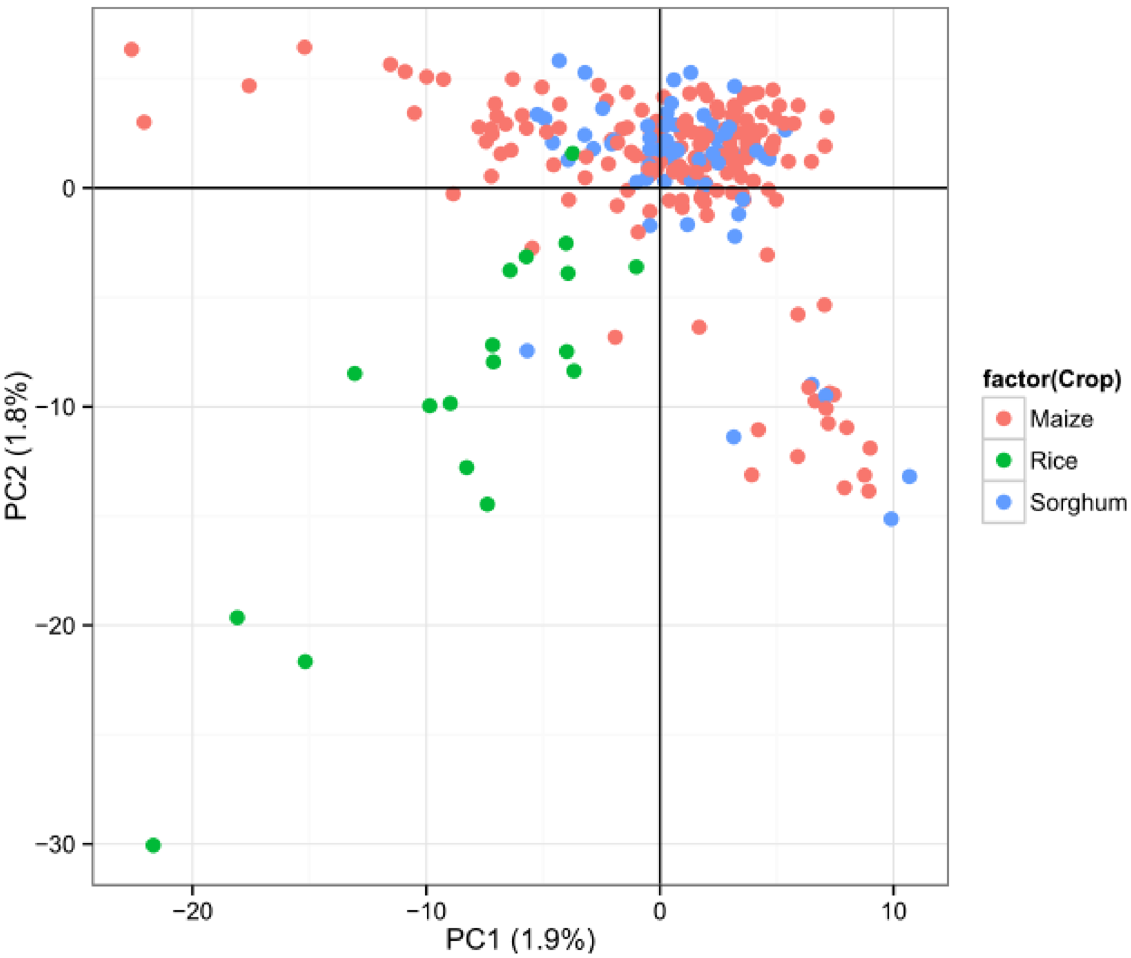


Figure 4.4. DAPC plot of Kenyan *Striga hermonthica* samples with hosts as maize, rice and sorghum.

Samples from sorghum and maize (red and blue) are seen to cluster away from those from rice (in green).

The percentages are the proportion of variance explain by each principal coordinate

PC1 explains 1.9% of the observed variance
PC2 explains 1.8 % of the observed variance

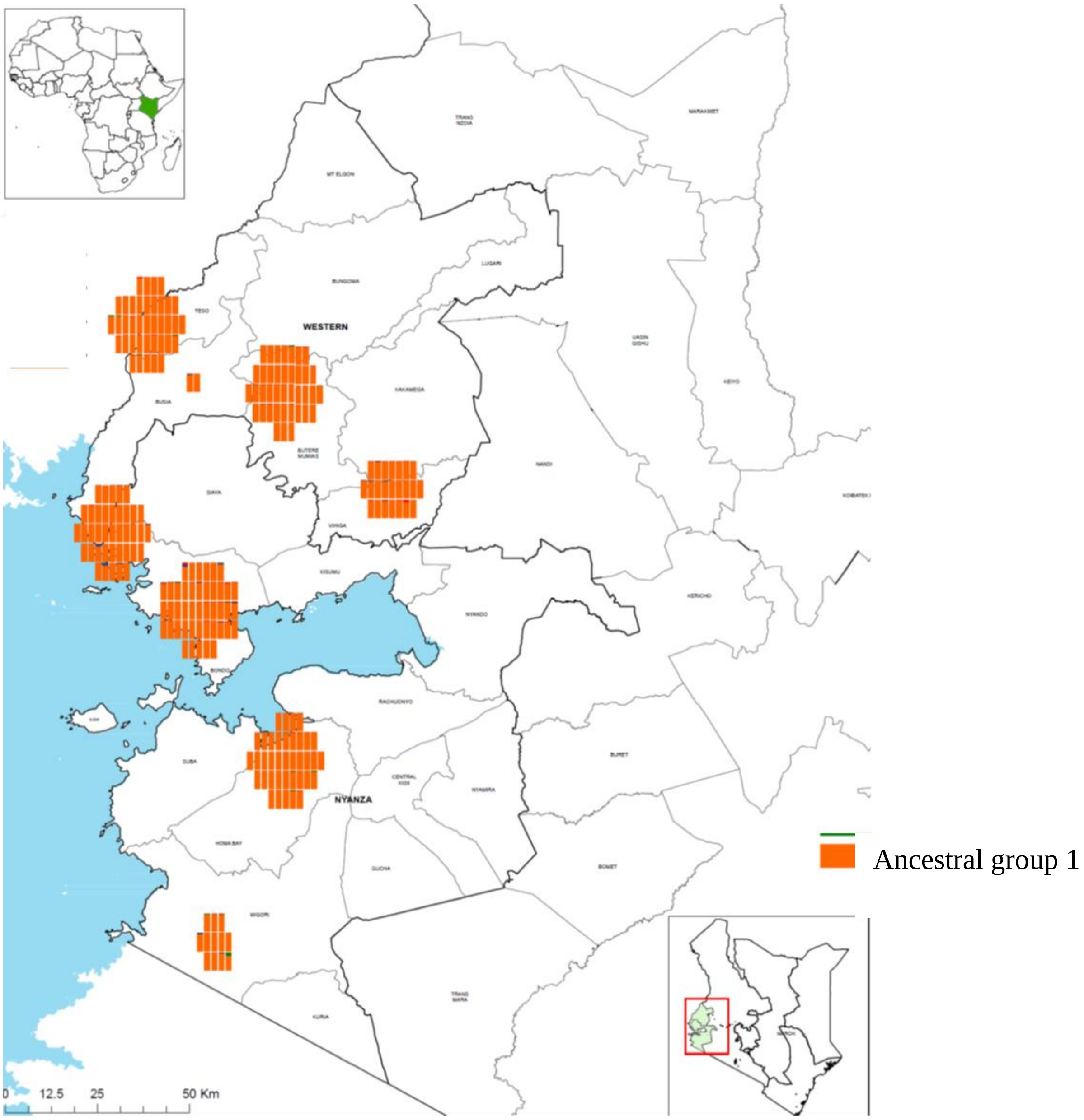


Figure 4.5. A plot of each Kenyan *Striga hermonthica* samples at its geographical coordinates showing its ancestral group

The top inset is the map of Africa showing the location of Kenya while the bottom insert is a map of Kenya showing the study location.

Each rectangle represents a plant, and the colour scheme represents the ancestral group(s) of each plant as determined by the study and shown in Fig. 4.2.1 at k = 4.

All the Kenyan samples appear to belong to a single ancestral group.

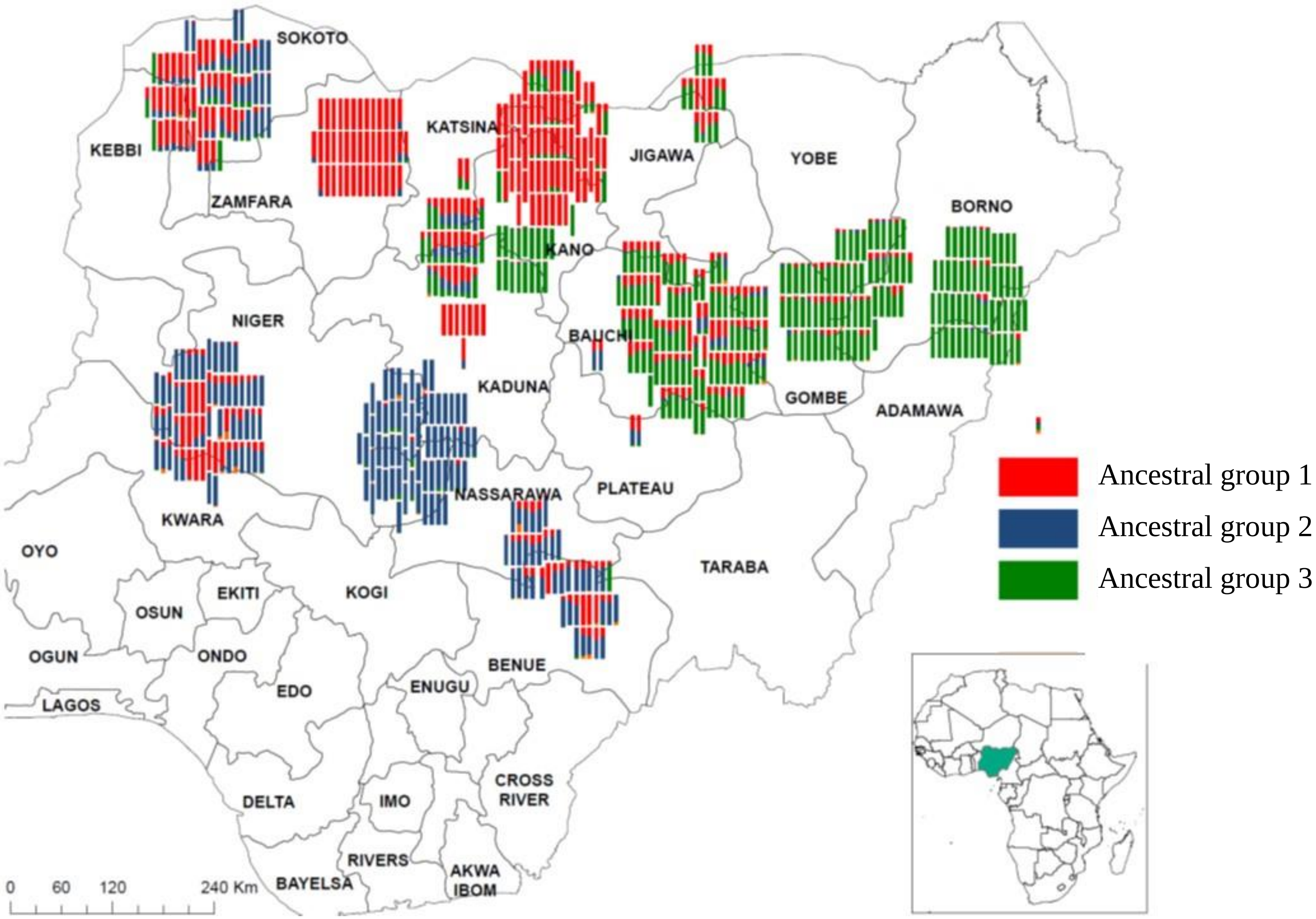


Figure 4.6. A plot of each Nigerian *Striga hermonthica* samples at its geographical coordinates, showing its ancestral group.

The inset is the map of Africa showing the location of Nigeria. Each rectangle represents a plant, and the colour scheme represents the ancestral group(s) of each plant as determined by the study and shown in Fig. 4.2.1 at K = 4.
The Nigerian samples belong to three major ancestral groups concentrated majorly in separate regions in the country.

4.2.3.1 Genetic Differentiation

Genetic Differentiation

Analysis of molecular variance (AMOVA) across all samples revealed that 15 per cent of the total variation among samples was accounted for by genetic variance between the two countries, while the remaining 18 per cent and 67 per cent of the variance was among and within all the samples respectively (Table 4.3.). A moderate to high level of genetic differentiation (as shown by the F_{ST} values) ($F_{ST} = 0.15$, $\alpha_{0.001}$) was observed between *S. hermonthica* populations from the two countries (Table 4.3).

Genetic differentiation within Kenya and Nigeria

The Kenyan plants had an F_{ST} value of 0.021 ($\alpha_{0.001}$) among samples from different locations (Table 4.4) and Mantel's test detected a strong relationship between the geographic distance between sampling locations and their pairwise F_{ST} values ($R^2 = 0.33$, $\alpha_{0.01}$, Fig 4.7). The level of statistically significant differentiation (as shown by the F_{ST} values) was observed between *Striga* plants with rice and maize as hosts (0.05, $\alpha_{0.001}$) and rice and sorghum as hosts (0.05, $\alpha_{0.001}$), whereas the F_{ST} value between samples with maize and sorghum as hosts was not statistically significant (0.002, $\alpha_{0.1}$) (Table 4.5).

Genetic Differentiation within Nigeria

The three clustering methods used to investigate the *S. hermonthica* samples collected in Nigeria showed the presence of three subpopulations (Fig. 4.2.1) based on the sampling locations. Statistically significant differentiation (as shown by the F_{ST} values) was observed between *Striga* plants in group 1 and group 2 (0.06, $\alpha_{0.001}$) and group 1 and group 3 (0.06, $\alpha_{0.001}$), and between group 2 and group 3 (0.04, $\alpha_{0.001}$) Table 4.6). Mantel's test did not detect a statistically significant relationship between the geographic distance between sampling locations and their pairwise linearized F_{ST} values ($R = 0.0604$, $\alpha_{0.2}$) (Fig 4.8). When host plants were used to group the samples, very low genetic differentiation was observed between maize and pearl millet ($F_{ST} = 0.02$), and between maize and sorghum ($F_{ST} = 0.01$), and between sorghum and Pearl millet ($F_{ST} = 0.07$) which were all significant ($\alpha_{0.01 \text{ to } 0.001}$).

Table 4.3. Summary of Analysis of Molecular variance (AMOVA) for all *S. hermonthica* samples collected in Kenya and Nigeria.

Source	% variation
Between populations*	15
Among samples	18
Within samples	67

F-Statistics	Values	P
F_{ST}	0.15	0.001
F_{IS}	0.21	0.001
F_{IT}	0.33	0.001
F'_{ST}	0.20	

* Nigerian and Kenyan population P = significance.

Table 4.4. Summary Analysis of Molecular variance (AMOVA) of *S. hermonthica* samples collected from Kenya based on their collection locations.

Source	% variation
Among Pops*	2
Among Samples	1
Within Samples	97

F Statistics	Value	P
F_{ST}	0.021	0.001
F_{IS}	0.011	0.240
F_{IT}	0.032	0.028
F'_{ST}	0.029	

*Pops= samples from different locations in Kenya

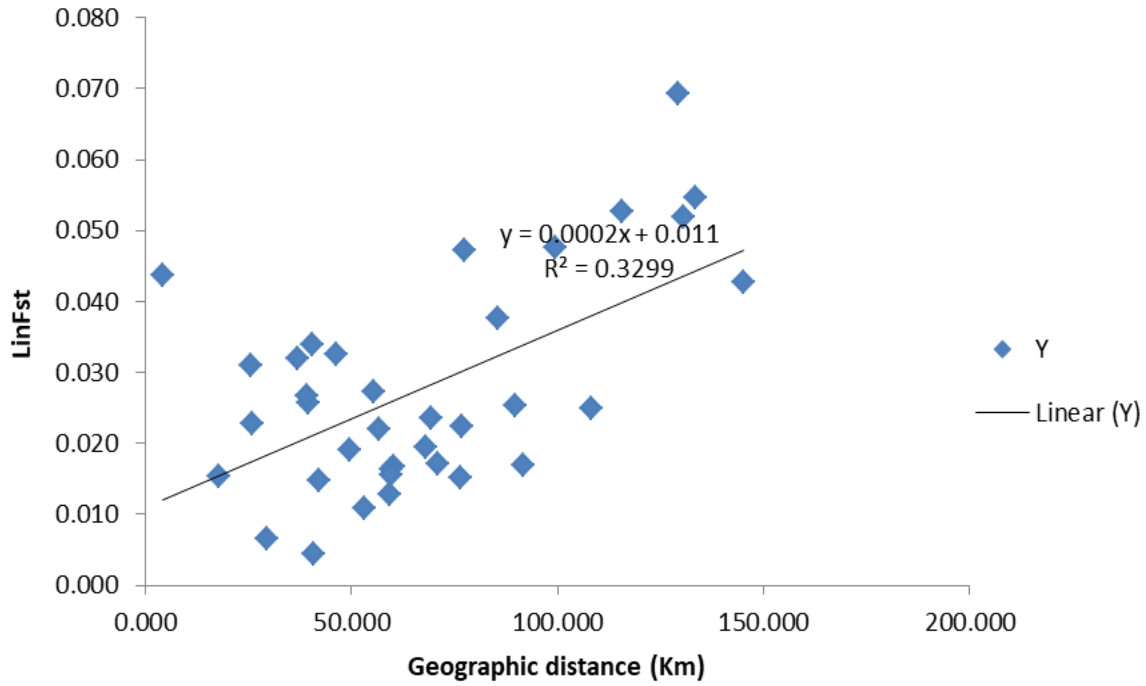


Figure 4.7. Mantel's test. A regression plot showing the relationship between linearized F_{ST} of the Kenyan *Striga* samples and the distance between their sampling sites.

($R^2 = 0.33$, $\alpha_{0.01}$. $r = 0.57$)

Table 4.5. Genetic differentiation (F_{ST}) of Kenyan *Striga* plant samples from maize, sorghum and rice.

Hosts	F_{ST}	P	F'_{ST}
Between Maize and Rice	0.049	0.001	0.067
Between Maize and Sorghum	0.002	0.108	0.003
Between Rice and Sorghum	0.047	0.001	0.066

F_{ST} =fixation index= (variance among populations/total variance)

F'_{ST}= Standardized F_{ST}

Table 4.6. Genetic differentiation (F_{ST}) of the subpopulations of *Striga* plants observed in Nigeria

Populations	F_{ST}	*P*	F'_{ST}
Between group 1 and group 2	0.057	0.001	0.077
Between group 1 and group 3	0.061	0.001	0.081
Between group 2 and group 3	0.043	0.001	0.061

F_{ST} =fixation index = (variance among populations/total variance),

F'_{ST} = Standardized F_{ST} = (F_{ST}/F_{ST} max), P = significance.

Group 1 = *Striga* population in the North-western region,

Group 2 = *Striga* population in the North-central region,

Group 3 = *Striga* population in the North-eastern region.

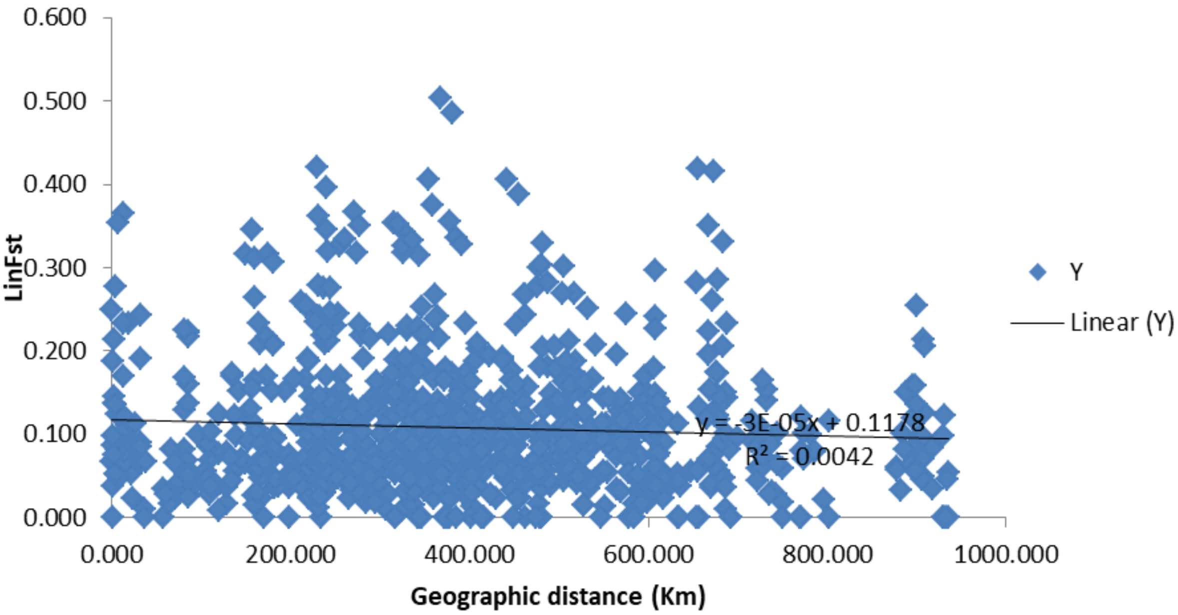


Figure 4.8. Mantel's test. A regression plot showing the relationship between linearized F_{ST} of the Nigerian *S. hermontica* samples and the distance between their sampling sites.

(R^2 = 0.004, $\alpha_{0.2}$, r = 0.064).

4.3. Identification of potential loci under selection within the *Striga hermonthica* populations.

It was observed that between the Nigerian and Kenyan populations, nine markers were potentially under selection (Table 4.7, Fig 4.9). These markers had a false discovery rate (FDR) greater than or equal to 3%, and log (PO) greater than or equal to 1.5 this corresponds to ‘strong selection’ on the Jeffery’s scale. All nine markers also showed positive α values and their F_{ST} values were high (mean $F_{ST} = 0.45$) indicating that the markers are undergoing positive selection.

Analysis of the Kenyan populations parasitizing maize, sorghum and rice, revealed three markers to be potentially under selection, FDR greater than or equal to 5%, and log (PO) greater than or equal to 0.8 corresponding to ‘substantial selection and above’ on Jeffreys’ scale. All 3 markers also showed positive α values, however their F_{ST} values were low (mean F_{ST}: 0.079) (Figure 4.10, Table 4.7).

The three subpopulations detected within Nigeria revealed 3 markers to be potentially under selection, FDR greater than or equal to 4%, and log (PO) greater than or equal to 1 corresponding to ‘strong selection and above’ on Jeffery’s scale. All of which also showed positive α values and relatively high F_{ST} (mean F_{ST}: 0.28) indicative of positive selection (Fig 4.11, Table 4.7).

When the total *S. hermonthica* population were split into the four populations observed between Nigeria and Kenya by population structure analysis, 23 markers with FDR greater than or equal to 2%, and log (PO) greater than or equal to 1.6 corresponding to ‘very strong selection and above’ on Jeffreys scale of evidence were detected as potentially under selection (Fig 4.12, Table 4.7). All the 23 markers showed positive α values and relatively high F_{ST} (mean F_{ST}: 0.39) indicative of positive selection.

Table 4.7. A Table showing observed outlier SNPs indicating positive selection.

Striga hermonthica populations	SNP markers	log10(PO)	qval	FDR%	alpha	F_{st}	Jeffery's scale
Nigerian and Kenyan populations	S1_44330351	1.05	0.03	3.12	1.15	0.43	Strong
	S1_88147395	1.3	0.02	2.49	1.19	0.44	Strong
	S1_88147383	1.38	0.02	2.16	1.21	0.44	Strong
	S1_89595816	1.43	0.02	1.85	1.22	0.44	Strong
	S1_88147307	1.6	0.02	1.5	1.26	0.45	Very strong
	S1_89964233	1.83	0.01	1.26	1.29	0.46	Very strong
	S1_16207640	1.89	0.01	1.19	1.29	0.46	Very strong
	S1_41324735	1.9	0.01	1.16	1.29	0.46	Very strong
	S1_88147333	1.96	0.01	1.08	1.28	0.46	Very strong
Kenyan *S. hermonthica* populations with maize and sorghum, and rice as host	S1_24018441	0.81	0.05	4.51	1.61	0.07	Substantial
	S1_1398074	2.92	0	0.08	1.86	0.08	Decisive
	S1_1398103	3.4	0	0.04	1.84	0.08	Decisive
Three subpopulations detected in Nigeria	S1_86280954	1.02	0.04	4.19	1.2	0.24	Strong
	S1_13796644	1.39	0.02	1.95	1.56	0.31	Very strong
	S1_5050153	1000	0	0	1.5	0.29	Decisive
Four populations detected in the entire samples (One Kenyan and Three Nigerian)	S1_59773391	0.74	0.02	2.281	0.925	0.287	Substantial
	S1_68986987	0.96	0.02	1.848	1.114	0.324	Substantial
	S1_12310584	0.99	0.02	1.573	1.02	0.304	Substantial
	S1_79552698	1.18	0.01	1.297	1.06	0.31	Strong
	S1_71839114	1.19	0.01	1.115	1.071	0.313	Strong
	S1_71839111	1.21	0.01	0.926	1.078	0.314	Strong
	S1_86664776	1.38	0.01	0.731	1.122	0.322	Strong
	S1_714301	1.4	0.01	0.593	1.113	0.32	Strong
	S1_5050153	1.61	0	0.454	1.192	0.336	Very strong
	S1_71386300	1.81	0	0.366	1.296	0.358	Very strong
	S1_22032080	1.92	0	0.311	1.201	0.337	Very strong
	S1_44330351	1.96	0	0.267	1.524	0.407	Very strong

SNP markers	log10(PO)	qval	FDR%	alpha	F_{st}	Jeffery's scale
S1_93290730	2.09	0	0.187	1.234	0.344	Very strong
S1_12318033	2.3	0	0.151	1.244	0.345	Decisive
S1_63091033	2.34	0	0.129	1.246	0.346	Decisive
S1_19341585	2.35	0	0.107	1.279	0.353	Decisive
S1_51165418	2.35	0	0.107	1.229	0.342	Decisive
S1_78236336	2.62	0	0.055	1.305	0.358	Decisive
S1_19915743	2.74	0	0.04	1.31	0.359	Decisive
S1_45843014	2.74	0	0.04	1.289	0.354	Decisive
S1_89488869	3.1	0	0.012	1.316	0.36	Decisive
S1_88147383	3.7	0	0.004	1.47	0.393	Decisive
S1_88147395	3.7	0	0.004	1.479	0.395	Decisive
S1_16207640	1000	0	0	1.669	0.437	Decisive
S1_41324735	1000	0	0	1.827	0.472	Decisive
S1_60453242	1000	0	0	1.451	0.389	Decisive
S1_88147307	1000	0	0	1.746	0.453	Decisive
S1_88147333	1000	0	0	1.752	0.455	Decisive
S1_89575380	1000	0	0	1.579	0.417	Decisive
S1_89575402	1000	0	0	1.656	0.434	Decisive

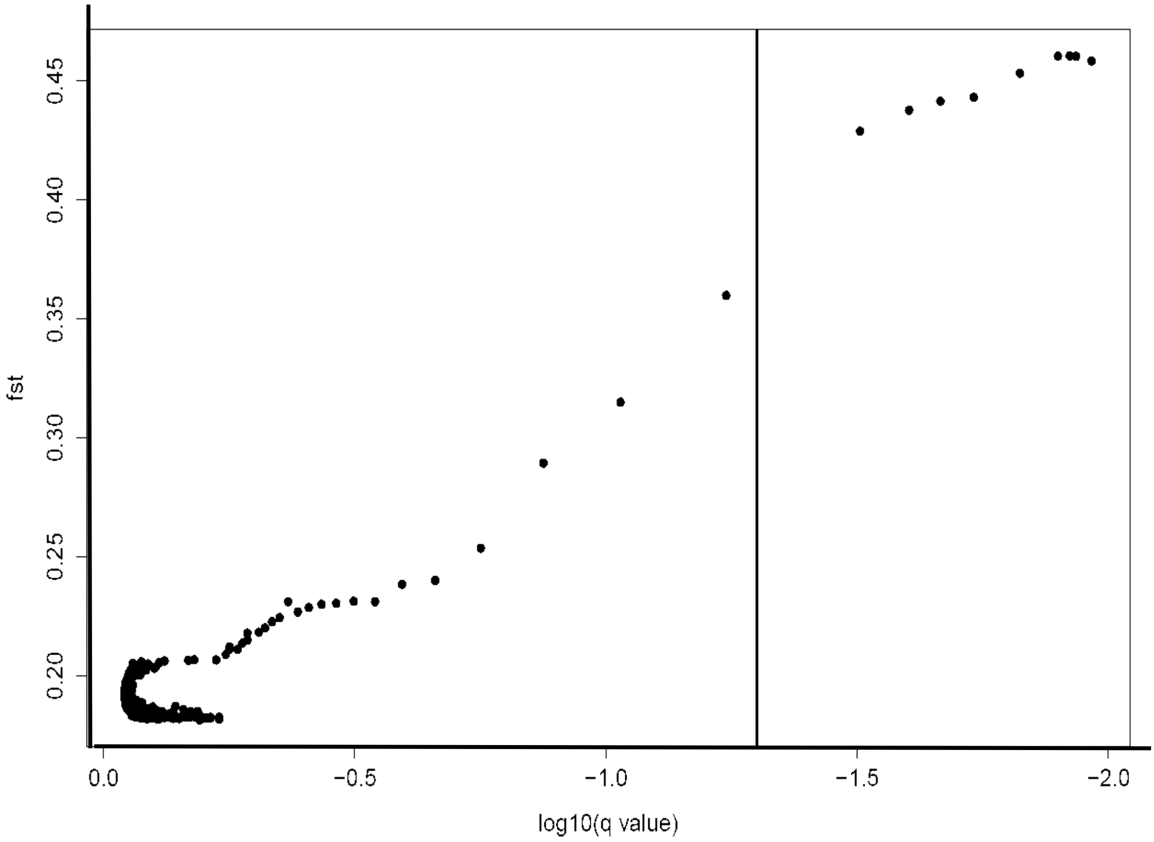


Figure 4.9. A plot of the F_{ST} of each marker against the posterior odds (PO) of that maker being under selection within the Nigerian and Kenyan populations.

The vertical line shows the critical PO used to identify outlier markers Log_{10} (q value) = -1.3. Markers on the right side of the vertical line are outliers; markers with high F_{ST} are indicative of positive selection.

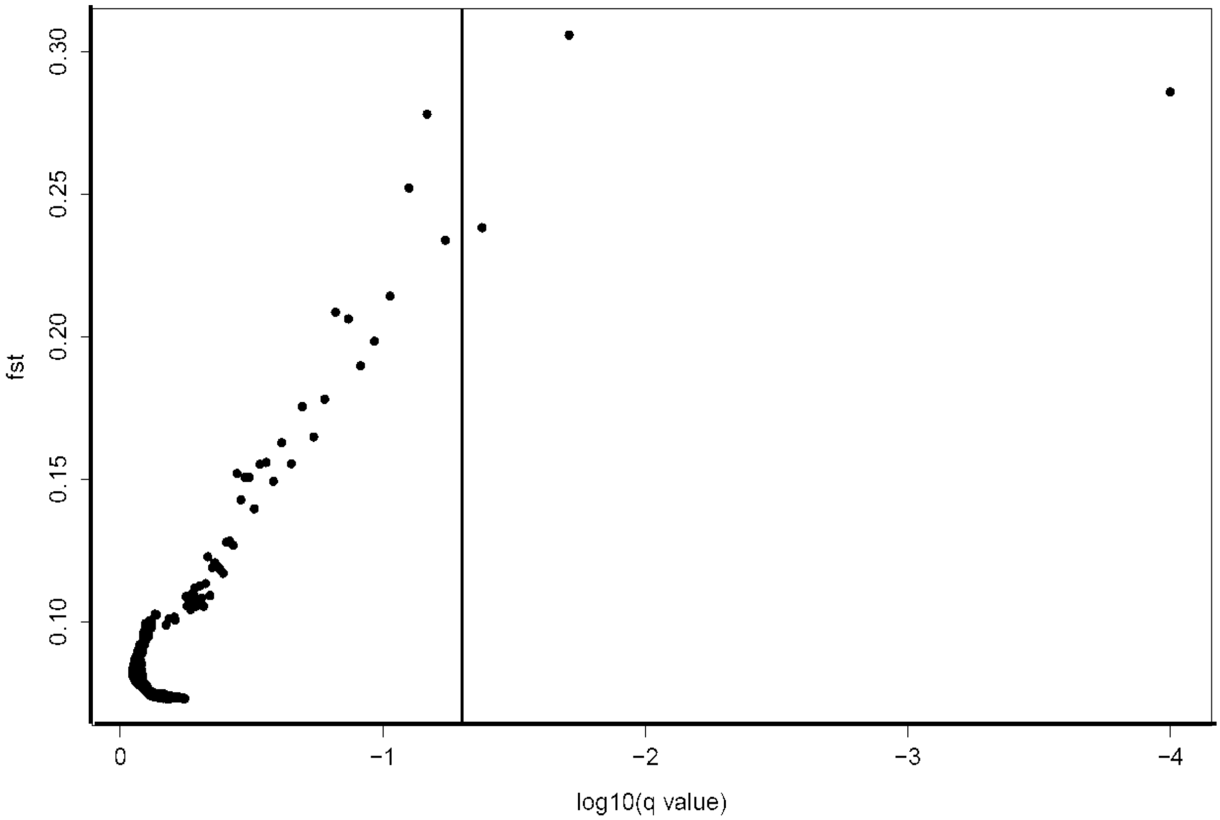


Figure 4.10. A plot of the F_{ST} of each marker against the posterior odds (PO) of that maker being under selection within the three subpopulations Nigeria.

The vertical line shows the critical PO used to identify outlier markers Log_{10} (q value) = -1.3. Markers on the right side of the vertical line are outliers; markers with high F_{ST} are indicative of positive selection.

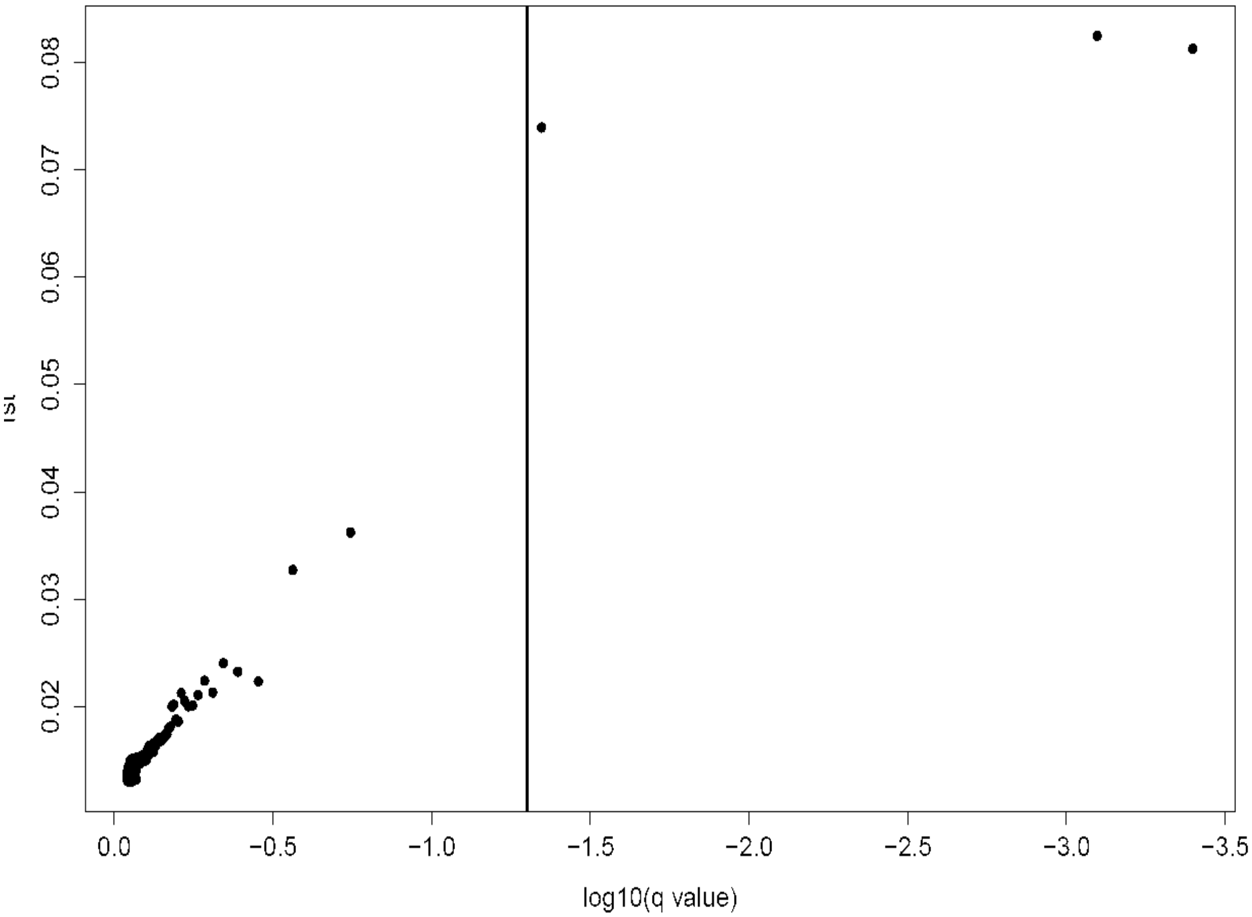


Figure 4.11. A plot of the F_{ST} of each marker against the posterior odds (PO) of that maker being under selection within the Kenyan Host plant populations.

The vertical line shows the critical PO used to identify outlier markers Log_{10} (q value) = -1.3. Markers on the right side of the vertical line are outliers; markers with high F_{ST} are indicative of positive selection.

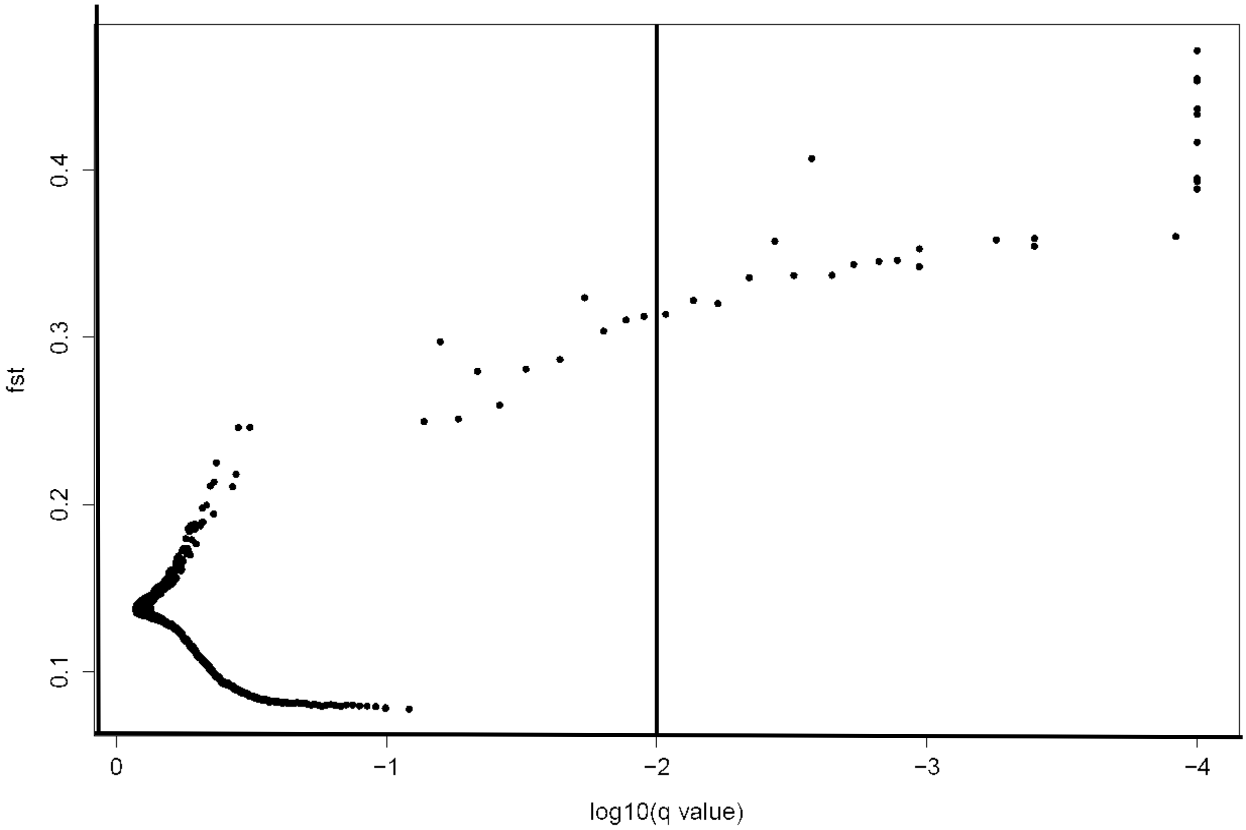


Figure 4.12 A plot of the FST of each marker against the posterior odds (PO) of that maker being under selection within the four populations observed by population structure analysis.

The vertical line shows the critical PO used to identify outlier markers Log_{10} (q value) = -2. Markers on the right side of the vertical line are outliers; markers with high F_{ST} are indicative of positive selection.

4.4. Effects of *S. hermonthica* Infestation on a Susceptible and a Resistant Maize Genotype.

4.4.1. The ability of maize plants to resist the attachment of *S. hermonthica* plants.

Differences were observed in the number of attached plants, as well as, in the growth and development of *S. hermonthica* plants on the roots of the two inbred lines, 5057 (susceptible) and ZD05 (resistant).

The susceptible plants (5057) in both groups A (maize plants infested with *S. hermonthica* seeds that were not pregerminated with GR24) and B (maize plants infested with *S. hermonthica* seeds that were pregerminated with GR24) supported a progressively higher number of attached *Striga hermonthica* plants than the resistant genotype (ZD05). In group A, statistically significant difference in the number of *Striga hermonthica* plants attached to ZD05 and 5057 was observed at 32 DPI (ZD05 = 4 ± 2, 5057 = 11.2 ± 11.6) and this difference remained statistically significant till the end of the experiment (Table 4.8). The experiment ended at 46 DPI because two susceptible maize plants (5075) died before the next observation period (49DPI). The plants in group B showed statistically significant difference in the number of *Striga hermonthica* plants attached to ZD05 and 5057 was at 46 DPI (ZD05 = 6±2.739, 5057 = 12±5.48) and this difference remained statistically significant till the end of the experiment (Table 4.9.). The experiment ended at 53 DPI because two susceptible maize plants (5075) died before the next observation period (56DPI).

Developmental stages of attached *Striga hermonthica* plants on both maize genotypes

The resistant maize (ZD05) plants had all the attached *S. hermonthica* plants at L1 (plants with 2-4 partially horizontal opened leaves). On 5057 (the susceptible maize genotype), 50% of the attached *Striga* plants were at the L1 (plants with 2-4 partially horizontal opened leaves) stage and 50% at the L2 (plants with 6-8 partially horizontal opened leaves) stage. Ten days later, at 25DPI, 44% of the *S. hermonthica* plants attached to ZD05 were at the L1 (plants with 2-4 partially horizontal opened leaves) stage, 33% (0.6/maize plant) at the L2 (plants with 6-8 partially horizontal opened leaves) stage and 22% were at the L3 (plants with 10-12 partially horizontal opened leaves) stage. At this time point 80% (3.4/maize plant), 8% and 16% of the attached *Striga* plants on 5057 were at the L1 (plants with 2-4 partially horizontal opened leaves), L2 (plants with 6-8 partially horizontal opened leaves) and L3 (plants with 10-12 partially horizontal opened leaves) stages respectively. At 34DPI ZD05 had about 7 % dying or dead *Striga* plants while 5057 had no dead or dying *Striga* plants (Figures 4.14 and 4.15.).

All the *S. hermonthica* plants attached to both genotypes of the group B plants were at L1 (plants with 2-4 partially horizontal opened leaves), at 18DPI. Ten days later at 28DPI, the *S. hermonthica* plants (0.6/Maize plant) on ZD05 remained at the L1 (plants with 2-4 partially horizontal opened leaves stage), while 80% and 20% of the *S. hermonthica* plants on 5057 were at the L1 (plants with 2-4 partially horizontal opened leaves) and L2 (plants with 6-8 partially horizontal opened leaves) stages respectively. At 39DPI, ZD05 had about 43% at the L1 (plants with 2-4 partially horizontal opened leaves) stage, 35 % at the L2 (plants with 6-8 partially horizontal opened leaves) stage, 7% at the L3 stage, no plants at the mature stage and 22 % dying or dead while 5057 had about 50 % at the L1 (plants with 2-4 partially horizontal opened leaves stage), 25 % at the L2 (plants with 6-8 partially horizontal opened leaves) stage, 7 % at the L3 (plants with 10-12 partially horizontal opened leaves) stage, 14 % mature plants and 3 % dead or dying plants (Figures 4.13 and 4.14.).

As the experiment progressed the susceptible line (5057) had more attached *Striga* at every level of development whether or not the *S. hermonthica* seeds were pre-germinated with GR24.

Table 4.8. Number of *Striga hermonthica* attached at to the roots of both genotypes from 7 DPI to 46 DPI in group A (Group infested with *S. hermonthica* that was not pregerminated).

Days post infestation (DPI)	ZD04 ± SD	5057± SD
7DPI	0 ± 0	0 ± 0
11DPI	0.2 ± 0.4	0 ± 0
14DPI	1 ± 1	0.8 ± 1.3
18DPI	1.2 ± 1.3	1.2 ± 2.2
21DPI	1.4 ± 1.1	2.4 ± 2.5
25DPI	1.8 ± 1.3	4.6 ± 4.5
28DPI	2.2 ± 1.5	7.2 ± 6.4
32DPI*	4 ± 2	11.2 ± 8.1
35DPI*	5.6 ± 2.7	15.2 ± 7.9
39DPI*	10.2 ± 4.3	19.6 ± 10.5
42DPI*	11.4 ± 5.9	25 ± 8.36
46DPI*	11.8 ± 5.9	31.8 ± 8.2

* = Significant at $\alpha_{0.05}$

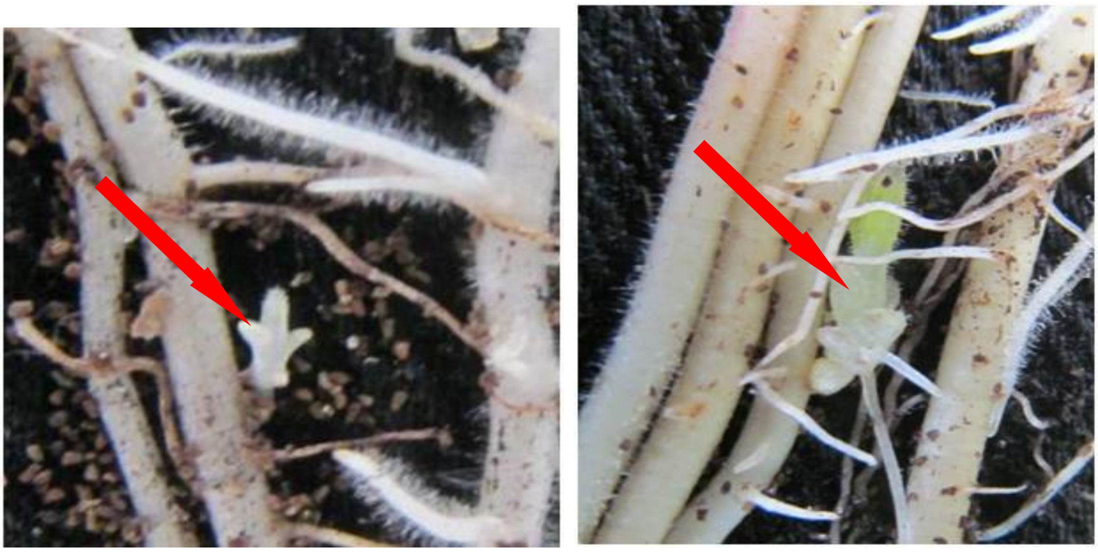
SD= Standard deviation

Table 4.9. Number of *Striga hermonthica* attached at to the roots of both genotypes from 7 DPI to 53 DPI in group B (Group infested with *S. hermonthica* that was pregerminated by adding Gr24)

Days post infestation (DPI)	ZD04 ± SD	5057 ± SD
7DPI	0 ± 0	0 ± 0
11DPI	0 ± 0	0 ± 0
14DPI	0 ± 0	0 ± 0
18DPI	0.2 ± 0.5	0.2 ± 0.5
21DPI	0.2 ± 0.5	0.4 ± 0.9
25DPI	0.2 ± 0.5	0.8 ± 1.1
28DPI	0.6 ± 0.6	1.2 ± 1.8
32DPI	1 ± 1	2.4 ± 3.3
35DPI	2 ± 1.7	3.6 ± 4.5
39DPI	2.8 ± 1.6	5.6 ± 5.7
42DPI	5 ± 2.7	9.4 ± 6.7
46DPI*	6 ± 2.7	12 ± 5.5
49DPI*	7.4 ± 3.7	15.8 ± 6.6
53DPI*	11.6 ± 4.7	21.4 ± 5.6

* = Significant at $\alpha_{0.05}$

SD= Standard deviation



LI (2-4 leaves) L2 (6-8 leaves)

L3 (10-12 leaves) L4 (Plants with 13 leaves and above)

Plate 4.1. Growth Stages of attached *Striga* plants

The arrows show the opened leaves of the parasite (*Striga hermonthica*).

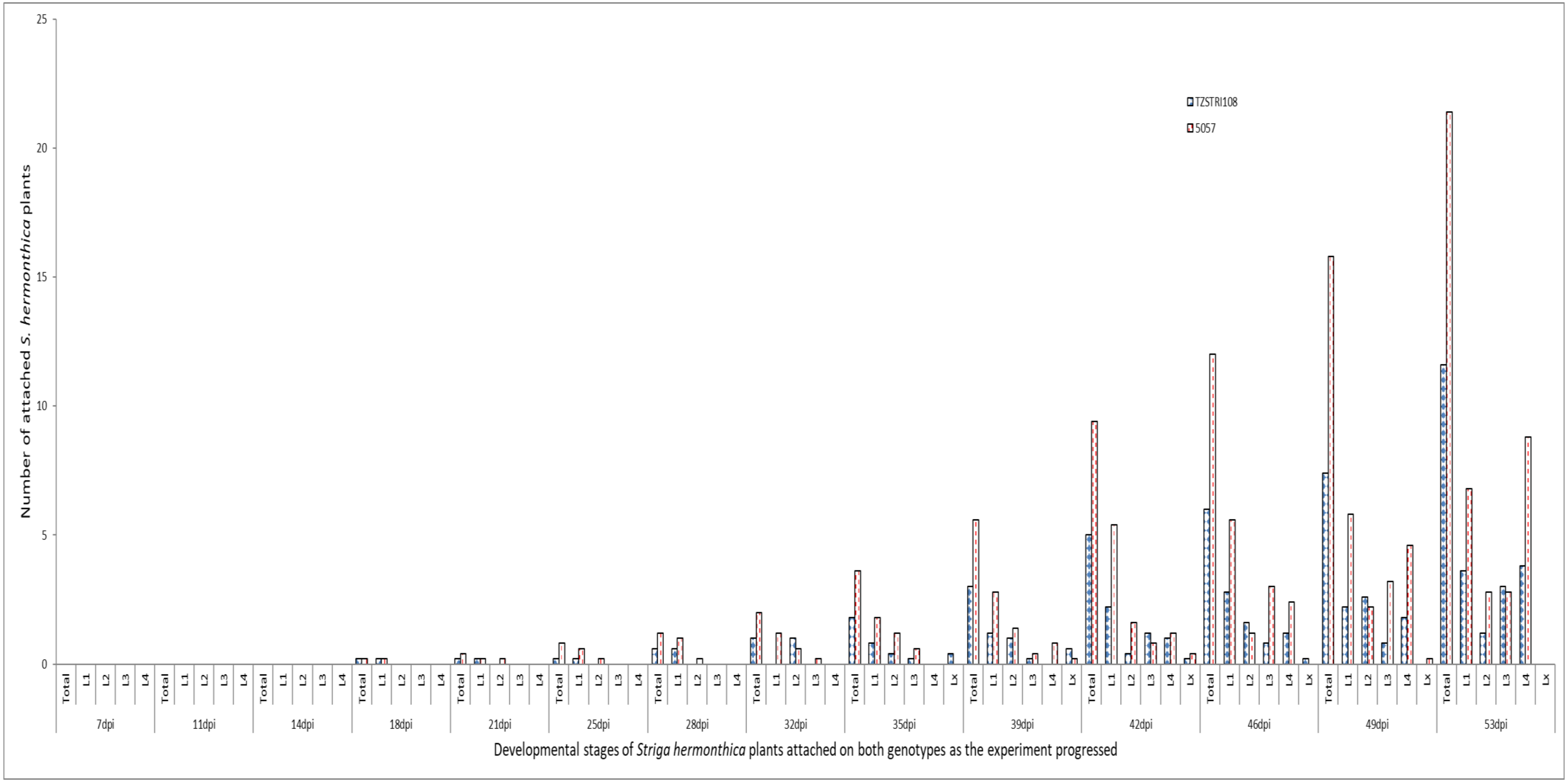


Figure 4.13. Histogram of the mean number of attached *S. hermonthica* on resistant and susceptible maize lines in group A (No GR24 treatment).

L1= 2-4 partially horizontal opened leaves, L2= 6-8 partially horizontal opened leaves, L3 =10-12 partially horizontal opened leaves, L4 = Mature *Striga* plants, Lx= Discoloured, dead or dying plants.

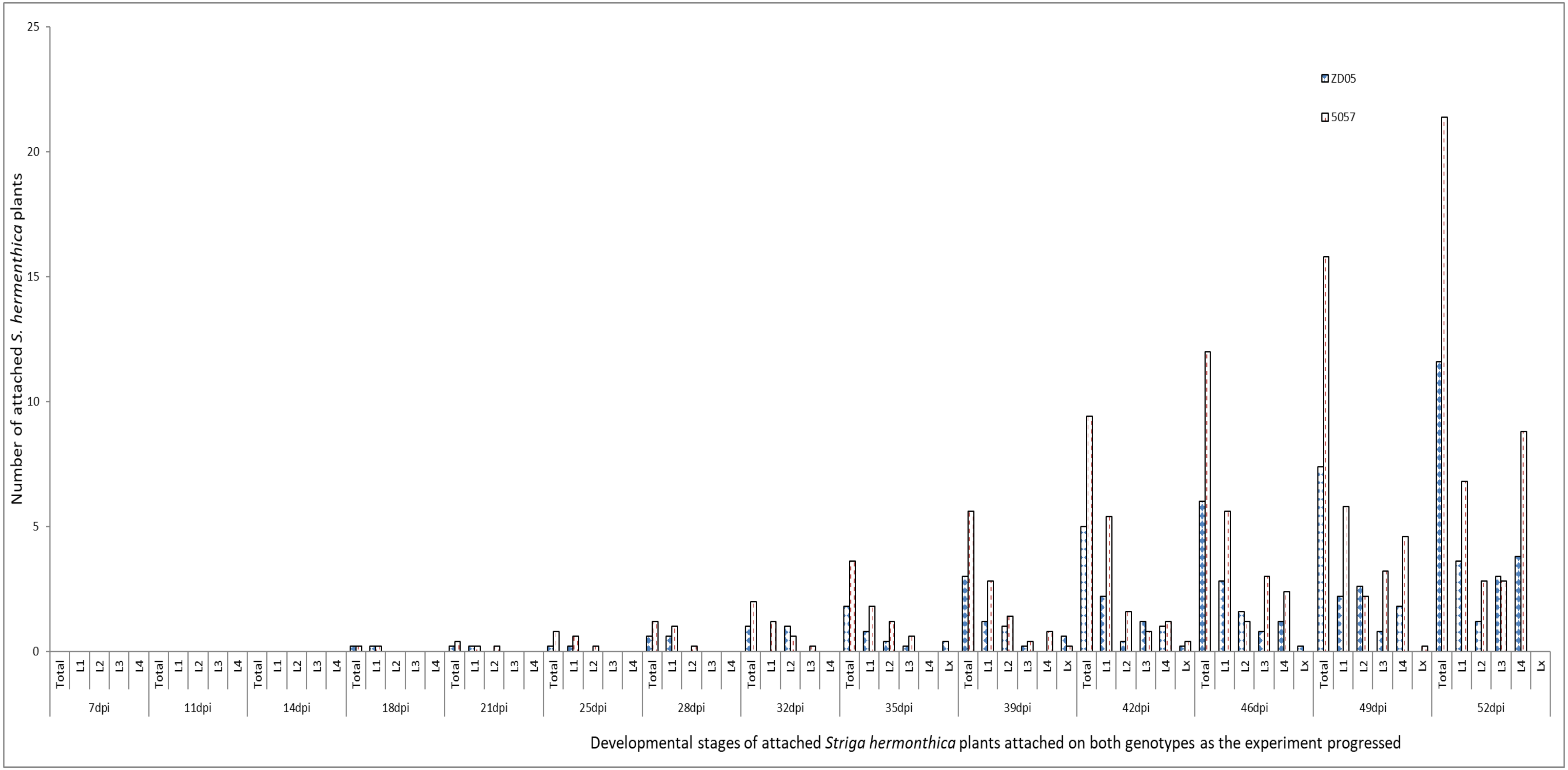


Figure 4.14. Histogram of the mean number of attached *Striga hermonthica* on resistant and susceptible maize lines in group B (GR24 treatment).

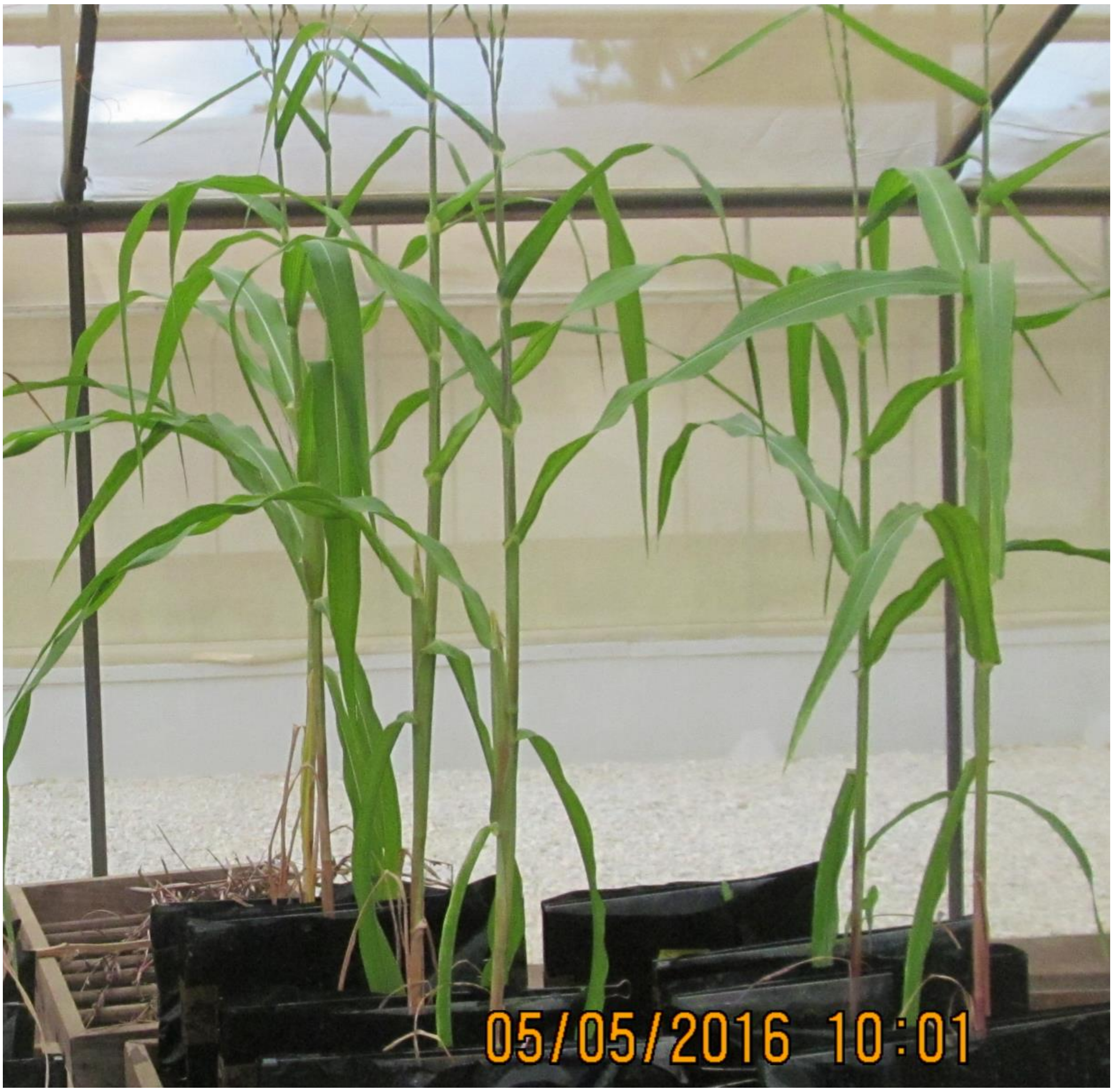
L1= 2-4 partially horizontal opened leaves, L2= 6-8 partially horizontal opened leaves, L3 =10-12 partially horizontal opened leaves, L4 = Mature *Striga* plants, Lx= Discoloured, dead or dying plants.

4.4.2. Effect of *S. hermonthica* infestation on the height of the plants.

Striga hermonthica infestation led to observable differences in the heights of ZD05 and 5057 (Plates 4.2 and 4.3). Both maize lines did not differ significantly in their heights without *S. hermonthica* infestation. (ZD04 = 81.50±4.95 cm and 5057 = 93.96±19.33 cm) (Figure 4.15).

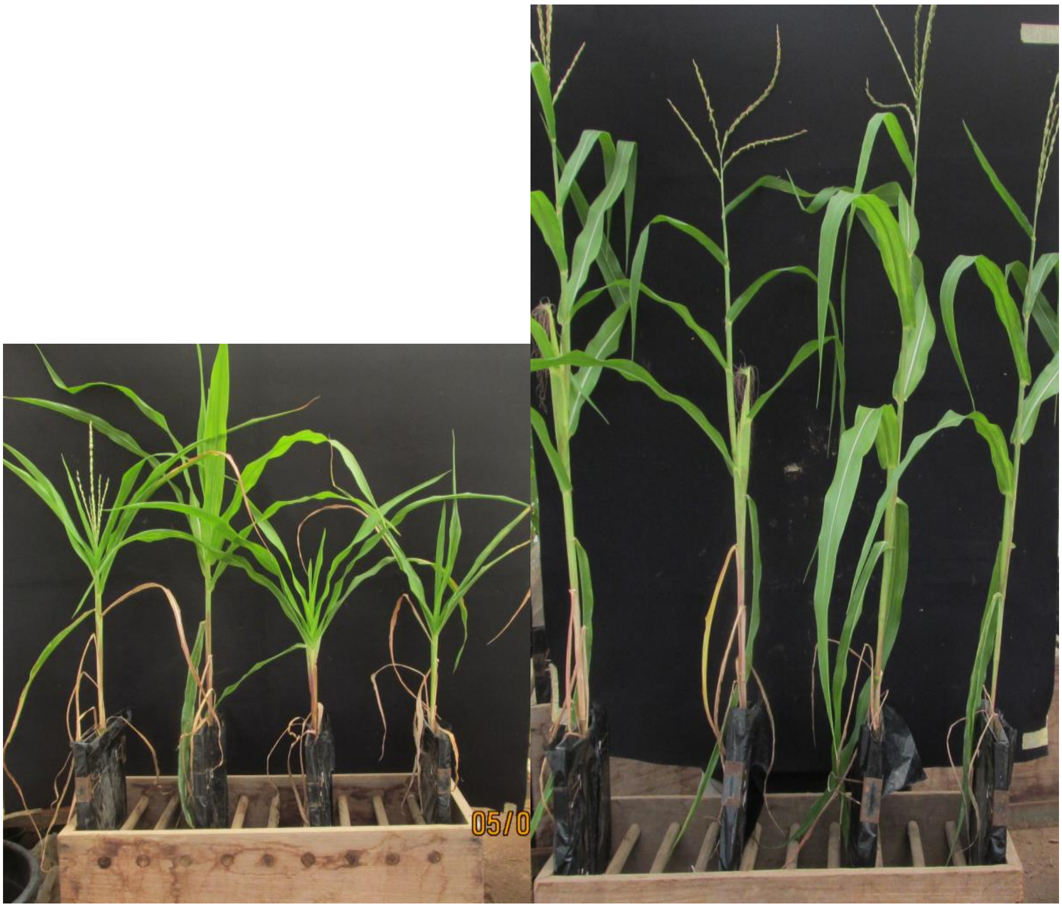
The ZD05 lines that was infested with *Striga hermonthica* seeds had significantly taller shoots at than the infested 5057, regardless of whether they were infested with were pre-germinated seeds or untreated seeds (i. e. Seeds that were not treated with GR24 to induce their germination). The ZD05 plants infested with untreated *S. hermonthica* seeds had a mean height of 69.20 ± 4.80 cm, while the 5057 plants infested with untreated *S. hermonthica* seeds had an average height of 30.60±7.00 cm. When pre-germinated *S. hermonthica* seeds were used to infest both maize lines, ZD05 plants had an average height of 73.60 ± 4.60 cm and the 5057 plants had an average height of 42.4 ± 5 cm (Figure 4.15).

There was no significant difference between the heights of the infested ZD05 line (both those infested with pre-germinated *S. hermonthica* seeds and those that were infested with *S. hermonthica* seeds that were not treated with Gr24) and the uninfested ZD05 (69.20 ± 4.80 cm, 73.60 ± 4.60 cm, and 81.50 ± 4.95 cm respectively) (Figure 4.15). The infested 5057 lines (regardless of the treatment of the seeds) were significantly shorter ($\alpha_{0.05}$) than the uninfested (5057 infested with untreated seeds = 30.60 ± 7.00 cm, 5057 infested with pregerminated seeds = 42.40 ± 5.00 cm, uninfested plants = 93.96 ± 19.33 cm) (Figure 4.15). There was however no significant difference between the heights of the 5057 plants infested with pregerminated *S. hermonthica* and those infested with untreated seeds (Figure 4.15).



ZD05 5057

Plate 4.2. Uninfested maize plants (of both genotypes) growing.



5057 ZD05

Plate 4.3. *S. hermonthica* infested maize plants (of both genotypes) with growing in rhizotron

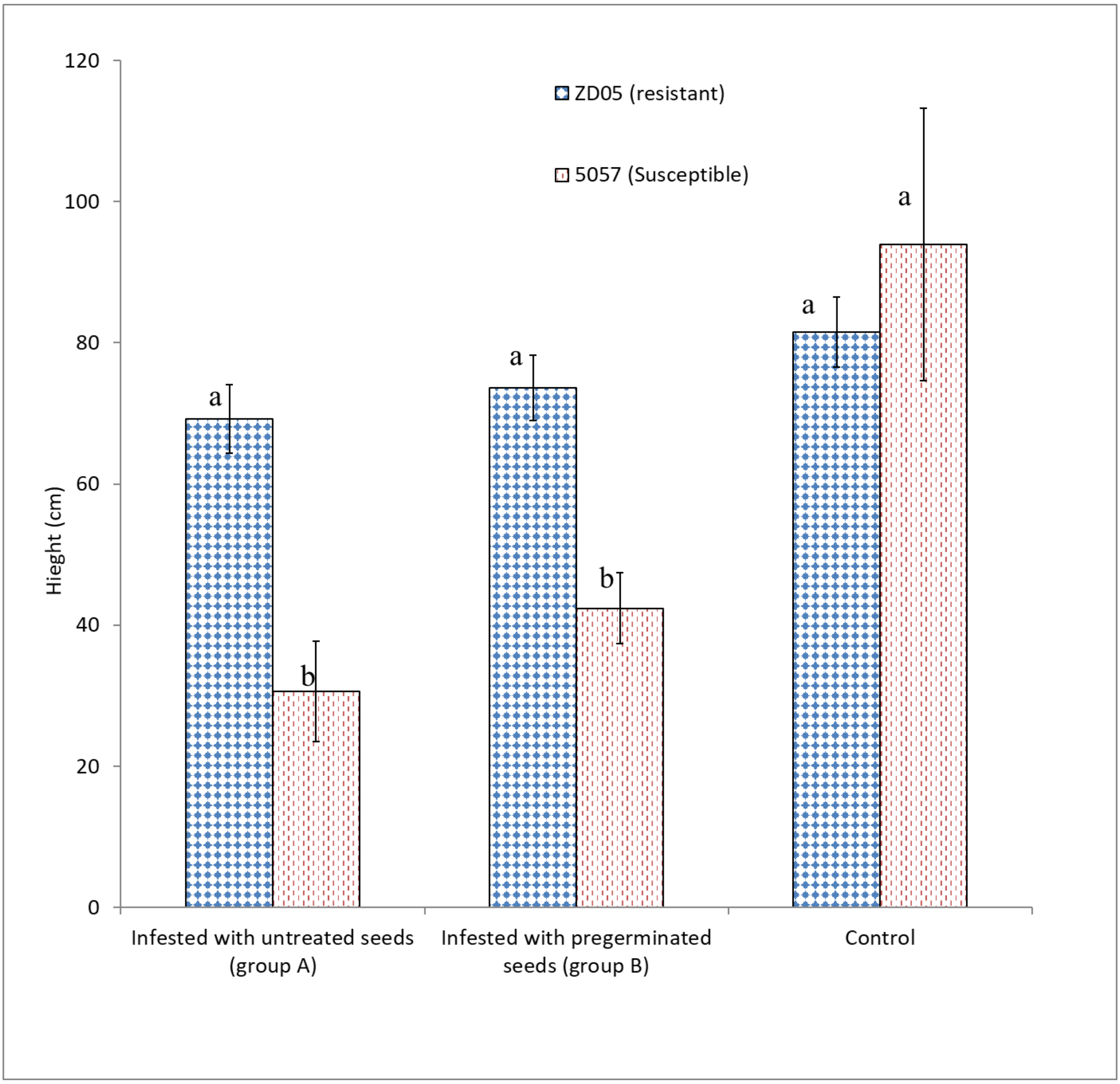


Figure 4.15. The plant height (cm) of both maize genotypes upon infestation with *Striga hermonthica* (infested and control).

The different letters (a and b) in the graph show significant differences among groups at $\alpha_{0.05}$.

The bars show Standard deviation.

4.4.3. Root-Shoot ratio by weight of *Striga hermonthica* infested maize.

The biomass partitioning in the susceptible genotype (5057) was significantly altered due to infestation by *Striga hermonthica*, giving rise to a larger root to shoot ratio when compared to the resistant genotype (ZD05).The infested ZD05 had a significantly lower root/shoot ratio by weight than the infested 5057 (ZD05 = 0.63 ± 0.2, 5057 = 1.9 ± 0.6, $\alpha_{0.00}$) (Figure 4.16).

The root/shoot ratio by weight of the infested 5057 maize plants was significantly higher than the root/shoot ratio by weight of the uninfested 5057 maize plants. The infested ZD05 plants had a higher root/shoot ratio than the uninfested ZD05 plants (uninfested ZD05 = 0.37 ± 0.19, infested ZD05 0.63 ± 0.2), but this difference was however not statistically significant. (4.16)

The difference in the root/shoot ratio between the control groups, uninfested 5057 (0.32±0.07) and uninfested ZD05 (0.37±0.19) was not statistically significant. (Figure 4.16).

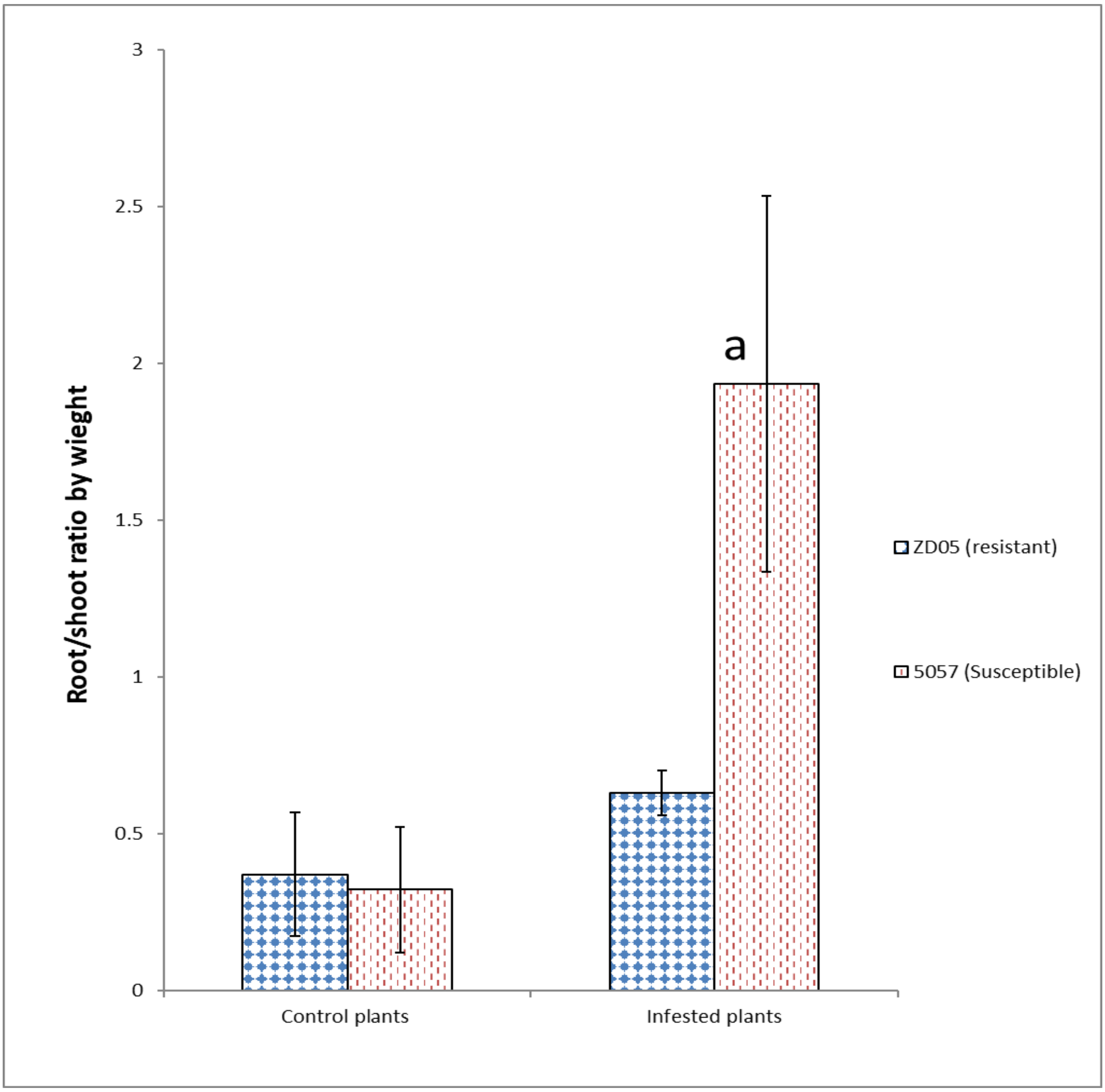


Figure 4.16. A histogram showing the root/shoot ratio by weight of *Striga* infested maize plants.

a = significant at α $_{0.05.}$

The bars show Standard deviation.

4.5. Physiological and biochemical mechanisms of resistance to *Striga hermonthica* by maize

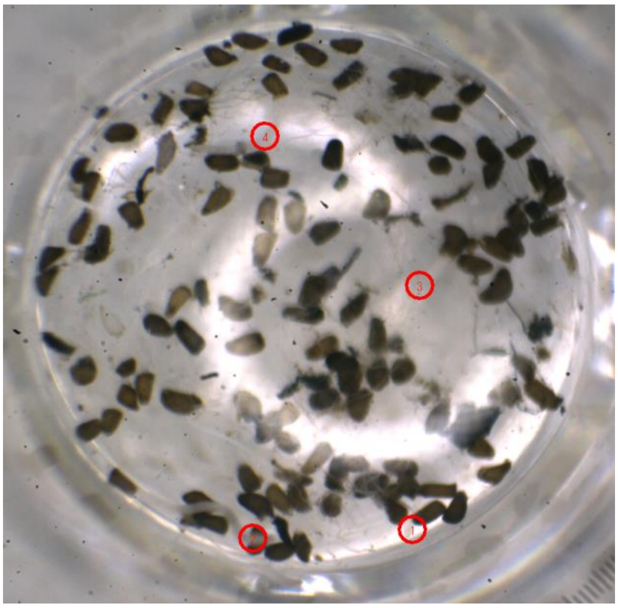
4.5.1. Germination stimulant ability of maize root exudates from susceptible and resistant maize lines.

Root exudates of maize plants stimulated the germination and radicle elongation of *S. hermonthica* to different extents (Plates 4.4. and 4.5).

The root exudates, derived from plants grown in pots, of the susceptible plant (5057) stimulated the germination of significantly more *S. hermonthica* seeds within the time of the experiment, when compared to the root exudates or the resistant genotype (ZD05) (5057 = 1.6 ± 0.11 germinated seeds in 2 days, ZD05 = 0 ± 0 germinated seeds in 2 days, $\alpha_{0.05}$) (Figure 4.17). Gr24, a synthetic germination stimulant that was used as the positive control, induced the germination of more *S. hermonthica* seeds than the exudates of both maize genotypes. *S. hermonthica* seeds that were suspended in distilled water (which was also used as the negative control) did not germinate. A larger number of *S. hermonthica* seeds that were suspended in the root exudates susceptible genotype showed elongated radicles than when compared to the resistant genotype (*S. hermonthica* seeds with elongated radicles suspended in the root exudates of 5057 = 2.8 ± 0.5, *S. hermonthica* seeds with elongated radicles suspended in the root exudates of ZD05 = 0.2 ± 0.2, $\alpha_{0.05}$) (Figure 4.18). More *S. hermonthica* seeds that were suspended in Gr24 developed elongated radicles than seeds suspended in the exudates of both maize genotypes.

Also, the differences in the number of *Striga hermonthica* germinated by the exudates collected from test tubes and the number per unit of root weight between the susceptible and resistant lines were statistically significant (α0.05). TSTR1108 = 9.4 ± 1.5, 5057 = 26.4 ± 9.96, and TSTR1108 = 31.4 ± 4.2 and 5057 = 191.3 ± 8.5 per plant and per unit root weight respectively (Figure 4.19 A). There was also a significant difference in the number of *Striga hermonthica* seeds germinated per unit root weight by both the susceptible (5057) and resistant lines (ZD05) (*S. hermonthica* seeds germinated per unit root weight by 5057 = 110.66 ± 78.1, *S. hermonthica* seeds germinated per unit root weight by ZD05= 36.4 ± 9.7, $\alpha_{0.05}$) (Figure 4.19 B).

A



B

Plate 4.4. *Striga hermonthica* seeds treated with exudates from 5057 and ZD05.

A = Seed treated with exudates from ZD05

B = Seeds treated with exudates from 5057

The red circles show germinating *Striga hermonthica* seeds

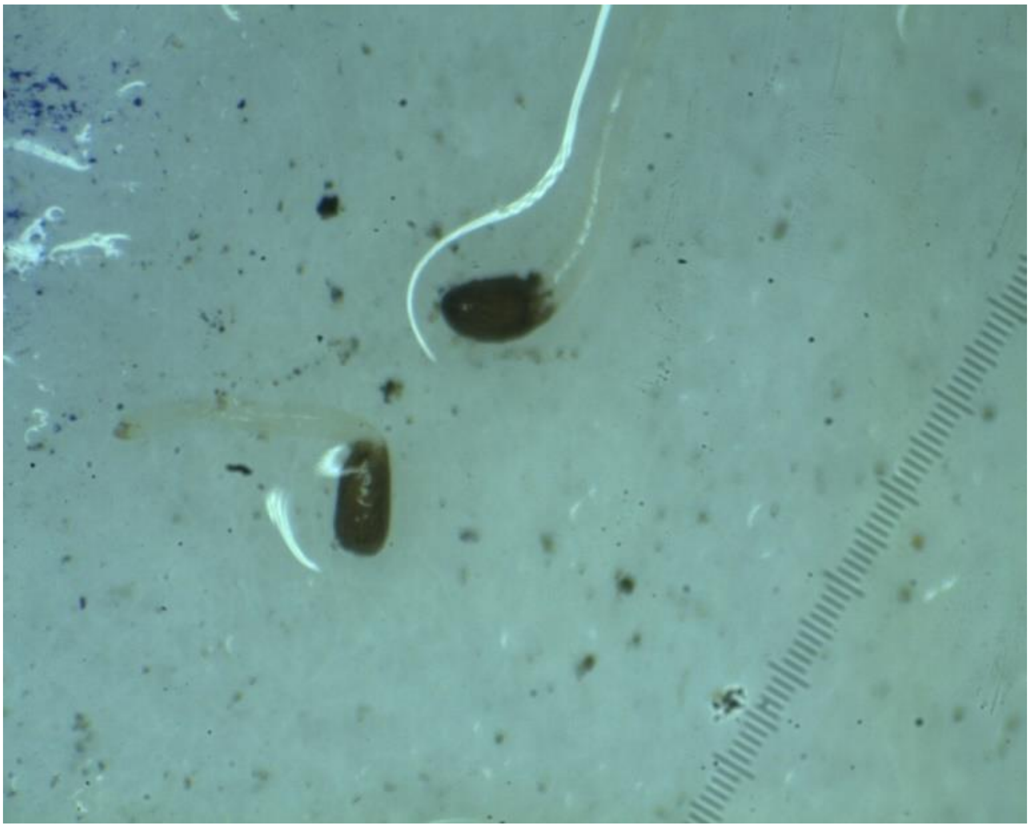


Plate 4.5. Germinated *S. hermonthica* seeds with elongated radicles

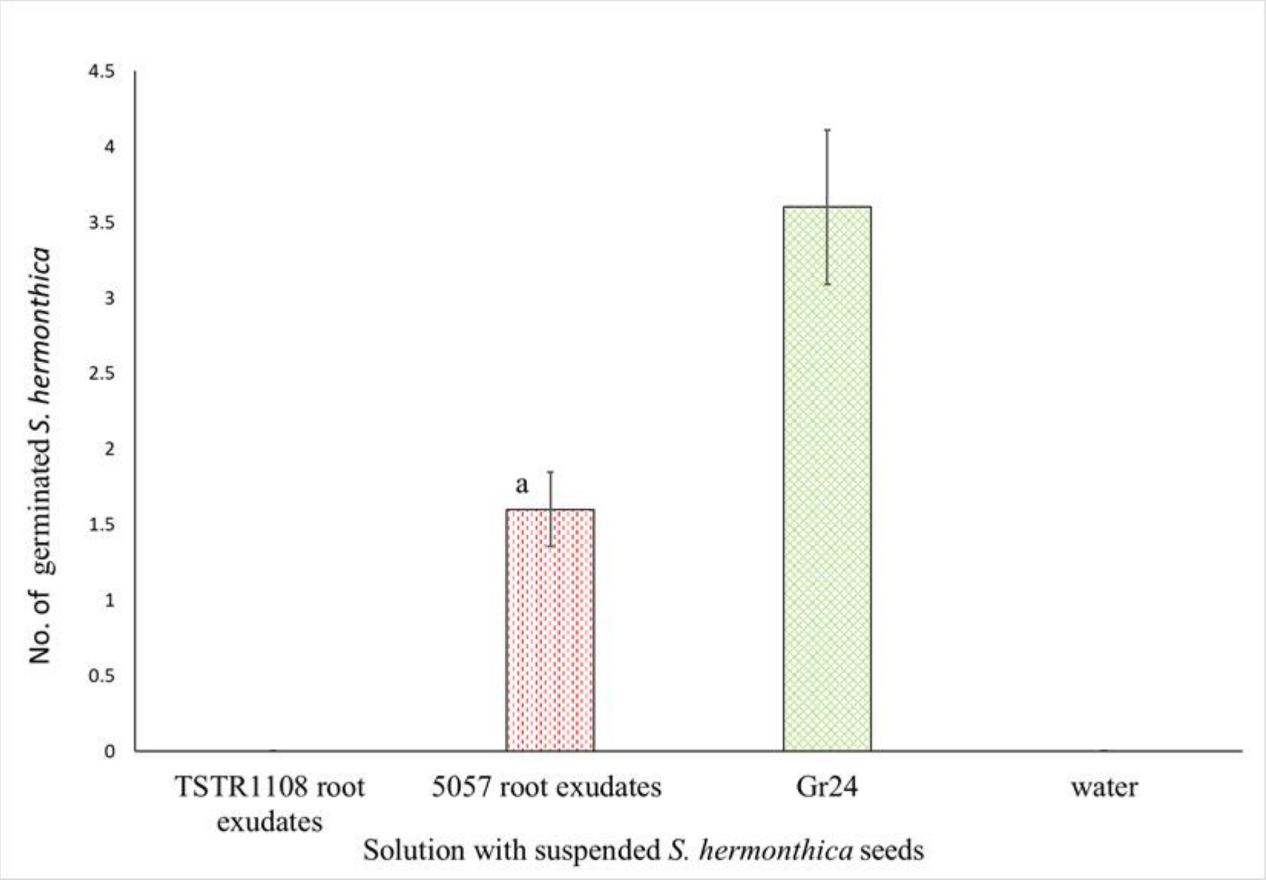


Figure 4.17. Number of *Striga hermonthica* seeds germinated by GR24 and distilled water and root exudates of the maize lines.

The bars show Standard deviation.

a = Significantly different from TSTR1108

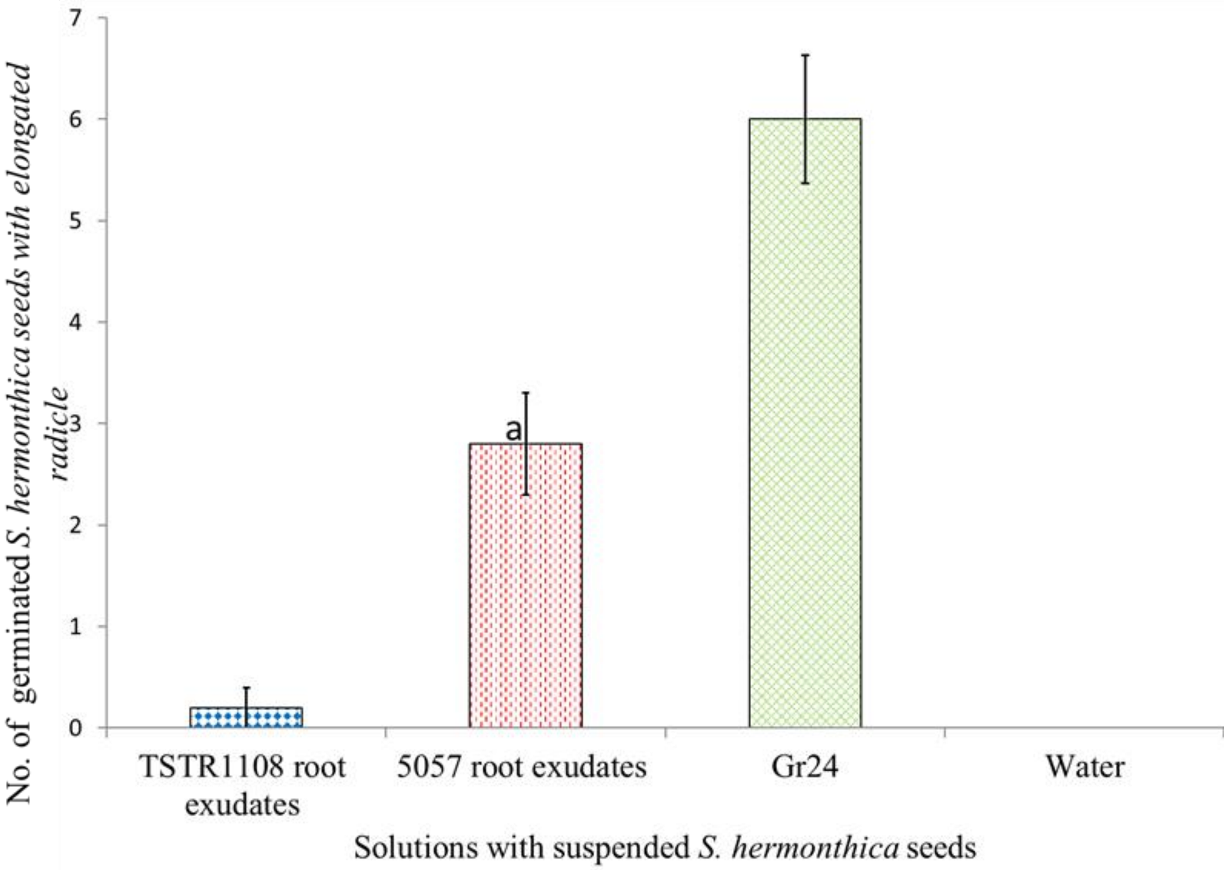


Figure 4.18 Number of *Striga hermonthica* seeds with elongated radicle after germination and continued suspension in GR24 and distilled water and root exudates of the maize lines.

a = significant at $\alpha_{0.05}$

The bars show Standard deviation.

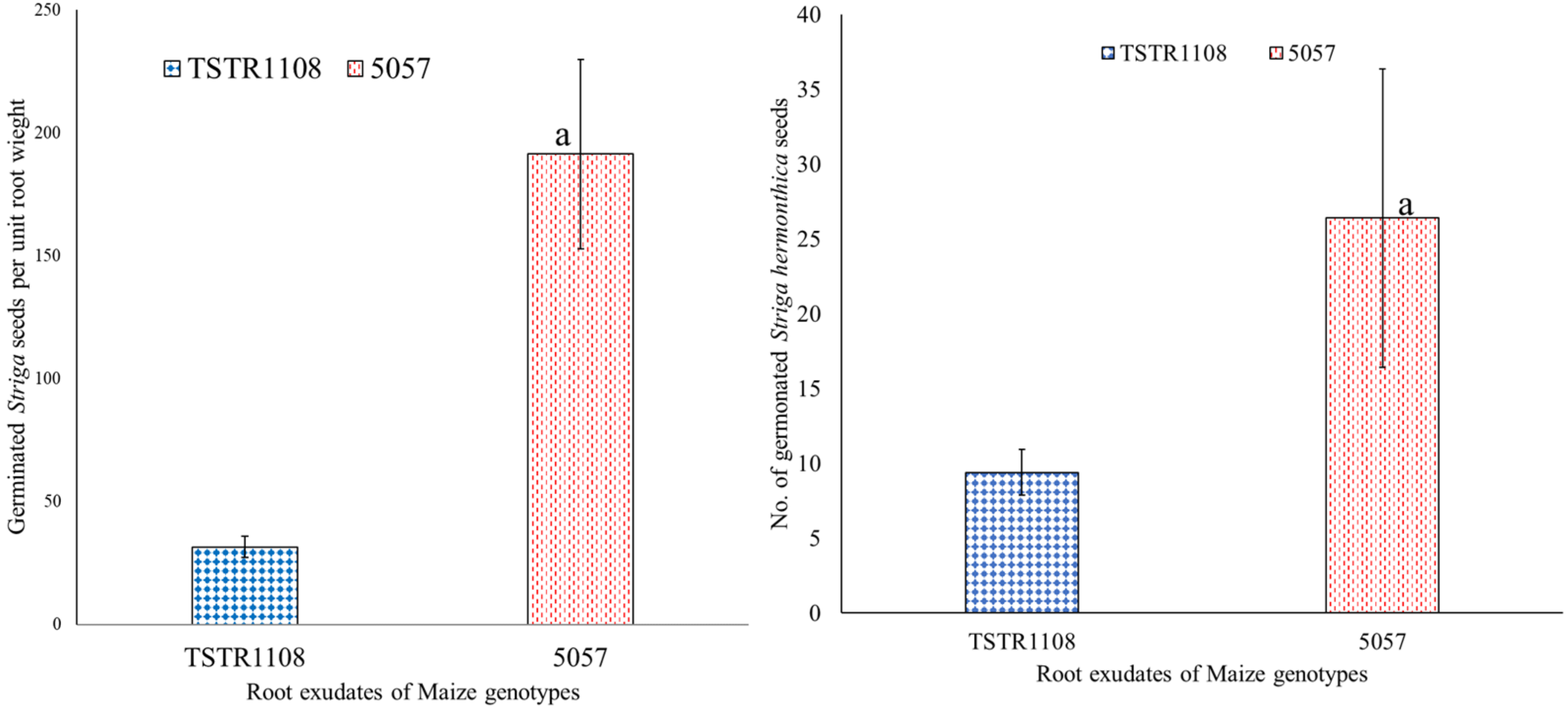


Figure 4.19. (A) A histogram of the number of *Striga hermonthica* seeds germinated by the root exudates of the two varieties after 96 hours.

(B) A histogram of the number of Striga hermonthica seeds germinated per unit root weight of both genotypes.

The different letter (a) in the graph show significant differences among groups at α0.05. The bars show Standard deviation.

4.5.2. Total length of major roots on the resistant (ZD05) and susceptible (5057) genotypes.

The total root length was defined as the sum of all the major roots in each plant. The resistant genotype (ZD05) had significantly shorter total root lengths (257 ± 36.1 cm) than the susceptible genotype (5057) (352.9 ± 36 cm) before infestation. Upon infestation the total major root length of ZD05 (284 ± 27.4 cm) was also significantly shorter than that of 5057 (382 ± 68.8 cm). There was no significant difference in total root length of infested 5057 and uninfested 5057, as well as, between infested ZD05 and uninfested ZD05 were not significant (Figure 4.20).

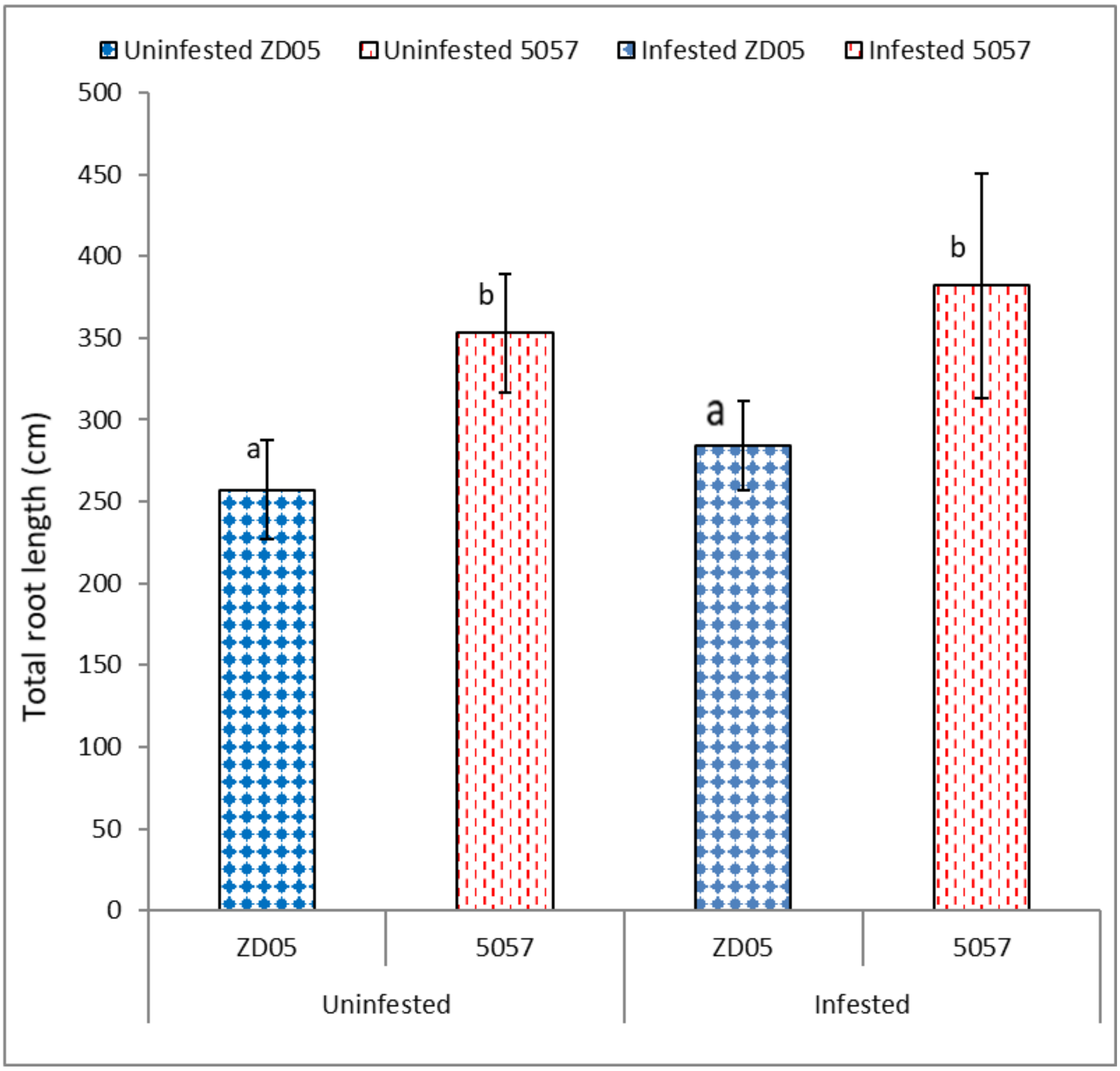


Figure 4.20. Total root length of major roots on both genotypes with and without infestation with *Striga hermonthica*.

$\alpha_{0.05}$ the different letters (a and b) in graph show significant differences among groups

The bars show Standard deviation.

4.5.3. Growth rate of attached *S. hermonthica* plants on susceptible and a resistant maize genotype.

The *Striga hermonthica* plants attached to the susceptible genotype (5057) developed faster than the plants attached to the resistant genotype (ZD05) as measured by the rate at which new leaves were formed in the parasite.

The rate of development (measured by leaf count) of *Striga* plants attached to the lateral roots of the susceptible genotype (Y = 0.85 - 20) was significantly faster than the rate of those attached to the resistant plants (Y= 0.18 - 0.87) at $\alpha_{0.000}$ (Figure 4.21 and Table 4.10). It is evident from the slopes of the regression lines that the *S. hermonthica* plants on the lateral roots of the susceptible line (5057) developed at the rate of 0.85 leaves per day. Whereas the *S. hermonthica* plants on the lateral roots of the resistant line (ZD05), developed at the rate of 0.18 leaves per day. The *S. hermonthica* plants on the lateral roots of the susceptible plant developed 4.7 times faster than the ones on the lateral roots of the resistant plant. (ZD05).

The same higher rate of development was also observed on the major roots. Y = 0.54 - 11.96 for susceptible genotype (5057) and Y = 0.29 - 4.48 for resistant genotype (ZD05) at $\alpha_{0.000}$. (Figure 4.22 and Table 4.11). In this instance, from the slopes of the regression lines, the *S. hermonthica* plants on the major roots of the susceptible line (5057) developed at the rate of 0.54 leaves per day. Whereas the *S. hermonthica* plants on the major roots of the resistant line (ZD05), developed at the rate of 0.29 leaves per day. The *S. hermonthica* plants on the major roots of the susceptible plant developed 1.8 times faster than the ones on the major roots of the resistant plant. (ZD05).

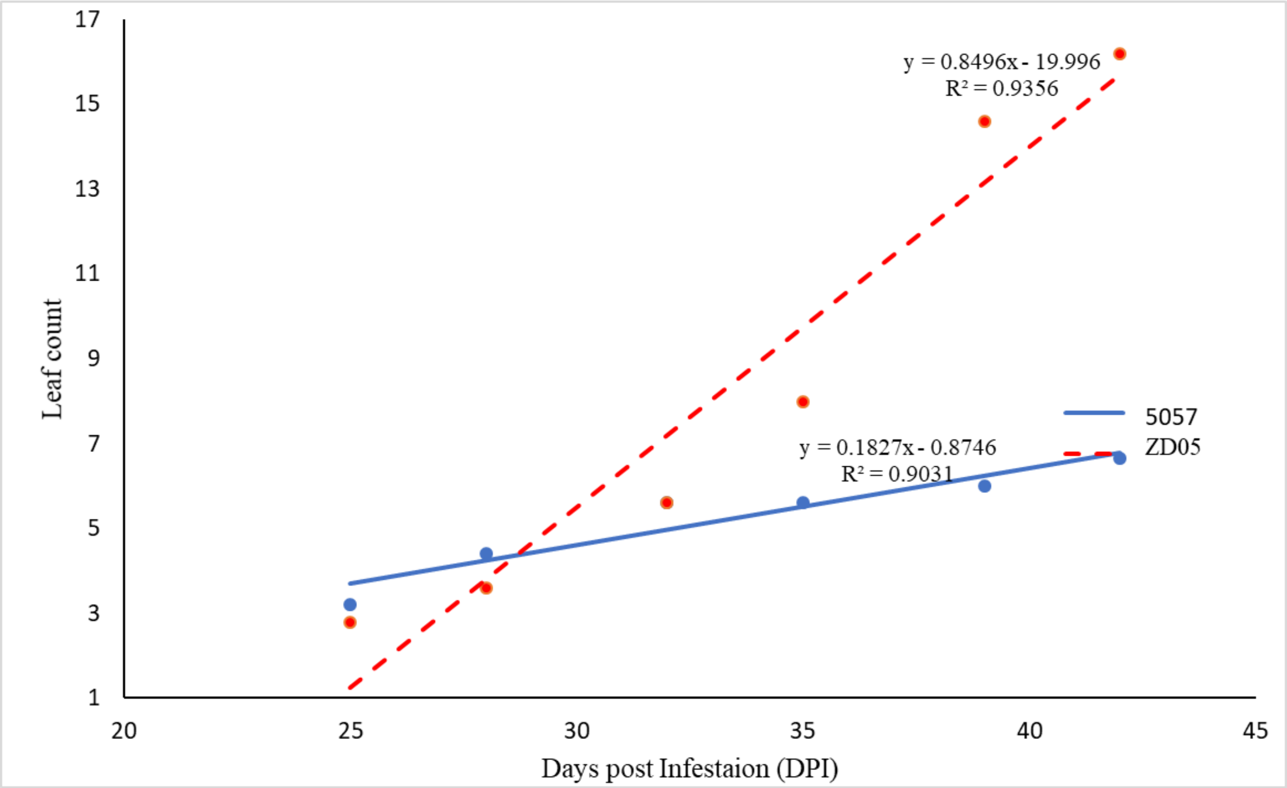


Figure 4.21. Regression lines showing the rate of development of *Striga hermonthica* plants (measured using number of leaves) of attached to the lateral roots of the two maize genotypes.

S. hermonthica rate of development on the lateral roots of 5057 = Y= 0.85x -20

S. hermonthica rate of development on the lateral roots ZD05 = Y= 0.18x -0.87

Table 4.10. ANCOVA table of the leaf count of *Striga hermonthica* plants attached to the lateral roots of the two maize genotypes.

Parameter	Df	Sum Sq	Mean Sq	F value	Pr (>F)
DPI	1	70.44	70.44	285.12	1.53e-07 ***
Treatment	1	3.2	3.2	12.97	0.006974 **
DPI: Treatment	1	6.75	6.75	27.34	0.000794 ***

DPI= Days post infestation

Treatment =5057 or ZD05

'**' = $\alpha_{0.001}$ '***'= $\alpha_{0.0}$

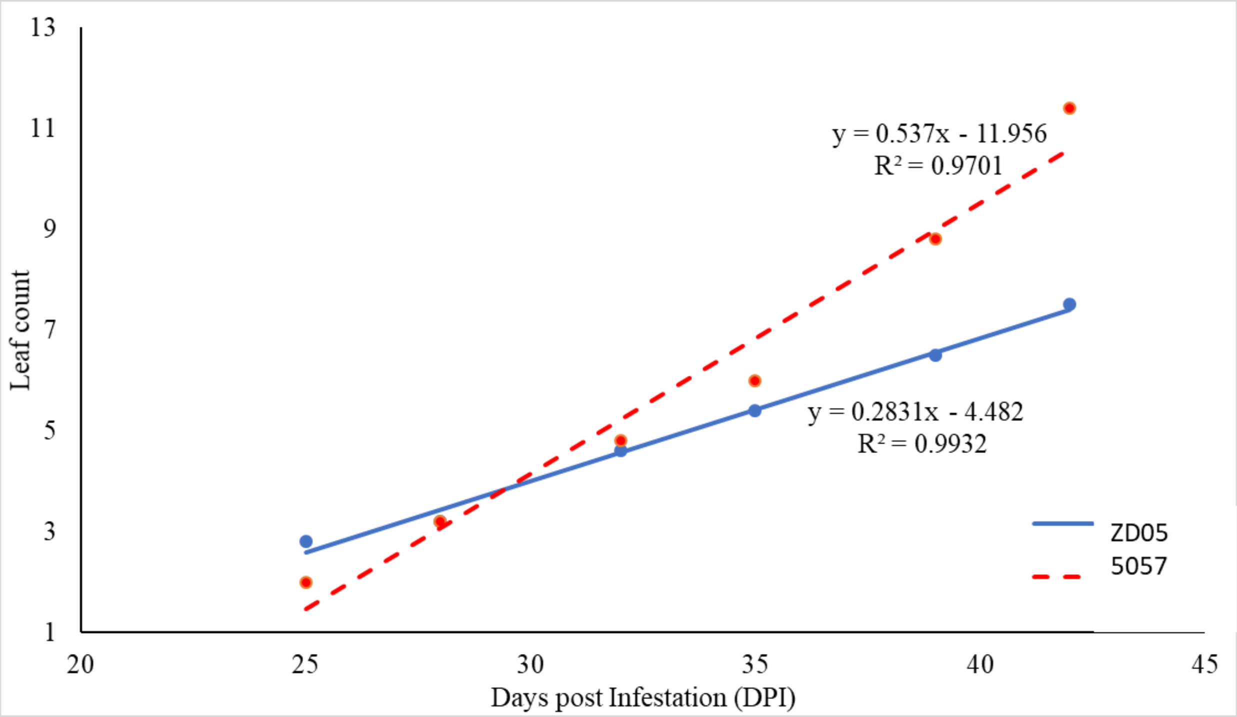


Figure 4.22. Regression lines showing the rate of development of *Striga hermonthica* plants (measured using number of leaves) of attached to the major roots of the two maize genotypes.

S. hermonthica rate of development on major roots of 5057 = Y= 0.5x -11.96

S. hermonthica rate of development on major roots of ZD05 = Y= 0.28x -4.48

DPI= Days post infestation

Table 4.11. ANCOVA table of the leaf count of *Striga hermonthica* plants attached to the major roots of the two maize genotypes.

Parameter	Df	Sum Sq	Mean Sq	F value	Pr (>F)
DPI	1	70.44	70.44	285.12	1.53e-07 ***
Treatment	1	3.2	3.2	12.97	0.006974 **
DPI: Treatment	1	6.75	6.75	27.34	0.000794 ***

DPI= Days post infestation

Treatment =5057 or ZD05

‘**’ = $\alpha_{0.001}$ ‘***’= $\alpha_{0.0}$

4.6. Differentially expressed genes by resistant and susceptible maize genotypes in response to *Striga hermonthica* infestation.

Ribonucleic acid (RNA) sequencing produced between 25 million and 40 million paired end reads per sample, with a read length of 151 nucleotides. Which were then trimmed to give an average read length of 137 nucleotides. This was mapped against the maize genome resulting between 33 to 53% of uniquely mapped reads. Cufflinks software produced 113522 (26409 multi-transcript, about 6.4 transcripts per locus) union super-loci (non-union super loci are loci that did not map to multiple regions) across all input dataset. An expression catalogue for the samples in 3 replicates containing 67205 genes, 112582 isoforms, 75598 transcription start sites, 4435530 promoters, 4989468 splicing sites was created by the Cufflinks package. Of these 67205 genes, only 19674 genes were differentially expressed across the samples compared.

4.6.1 Gene expression profile at first time point (three days post infestation)

Three days post infestation, the infested susceptible plants (5057) differentially expressed 477 when compared to the uninfested 5057 plants. While, the infested resistant plants (ZD05) differentially expressed 437 genes when compared to the uninfested ZD05 plants. The susceptible genotype (5057) upregulated 403 and down regulated 32 genes, while the resistant genotype (ZD05) upregulated 197 and downregulated 240 genes. The two genotypes had 42 differentially expressed genes in common, with the susceptible plant upregulating 40 and downregulating 2. While the resistant plant upregulated 30 and downregulated 12 genes (Figure 4.23).

The resistant genotype (ZD05) upregulated genes involved in plant defence. These include the first five genes involved in the synthesis of DIBOA (2, 4-Dihydroxy-1,4-benzoxazin-3-one) and DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one). (Fig. 4.24). Chalcone synthase gene and phenylalanine ammonia lyase were also upregulated (Fig. 4.24), while largely downregulating these genes were for heat shock proteins and factors. Catalase was the only gene involved in mopping up H_2O_2 that was upregulated at this time point the resistant genotype. At this time point this genotype also downregulated of genes like tryptophan aminotransferase related 2 (tar2), Auxin response factor 5 (ARF5), IAA9-auxin-responsive Aux/IAA family member, auxin import carrier 1, Patatin-like protein 6, auxin-induced beta-glucosidase, glutathione-s-transferase (11, 14 and 16). Auxin transporters, PIN-formed protei n4 (PIN4), was downregulated and PIN 9 and 11 were upregulated. Genes involved in cell wall reorganisation were also downregulated including pectate lyase 8, xyloglucan-6-xylosyltransferase 5 and 6, xyloglucan endotransglucosylase/ hydrolase protein 21, endoglucanase 1 and 6, α-expansin 4, β-expansin 3 and 8 (Table 4.12 and Table 4.13).

The susceptible genotype showed increased expression of heat shock factors and heat shock proteins as well as of genes involved in the metabolism of hormones. These include alternative oxidase (the action of AOX results in the production of H_2O_2), L ascorbate and Oxidative stress 3 (which are markers for oxidative stress) and peroxidases (that mop up H_2O_2). Both genotypes upregulated genes involved in plant secondary metabolism, however, the resistant genotype upregulated more of these genes and did not show downregulation of any of these genes while the susceptible genotype downregulated some of these genes. (Table 4.12 and Table 4.13) Genes involved in abscisic acid (ABA) signalling and genes induced by ABA, including ABA-responsive protein, Abscisic acid receptor PYL5, MAP kinase 3, Late embryogenesis abundant proteins and dehydrins were upregulated by the susceptible genotype. (Table 4.12 and Table 4.13),

Pathway enrichment analysis

At 3 days after infestation, pathway enrichment analysis of differentially regulated genes showed that genes in 38 pathways were upregulated in the susceptible line and genes from 6 pathways were downregulated. In the resistant genotype, genes from 28 and 25 pathways were upregulated and downregulated respectively. The susceptible genotype did not have any genes common to pathways in the upregulated and downregulated groups. This was not the case with the resistant genotype as 8 of the pathways were common to the upregulated and downregulated genes. However different were individual genes were upregulated or downregulated in the pathways (Table 4.14).

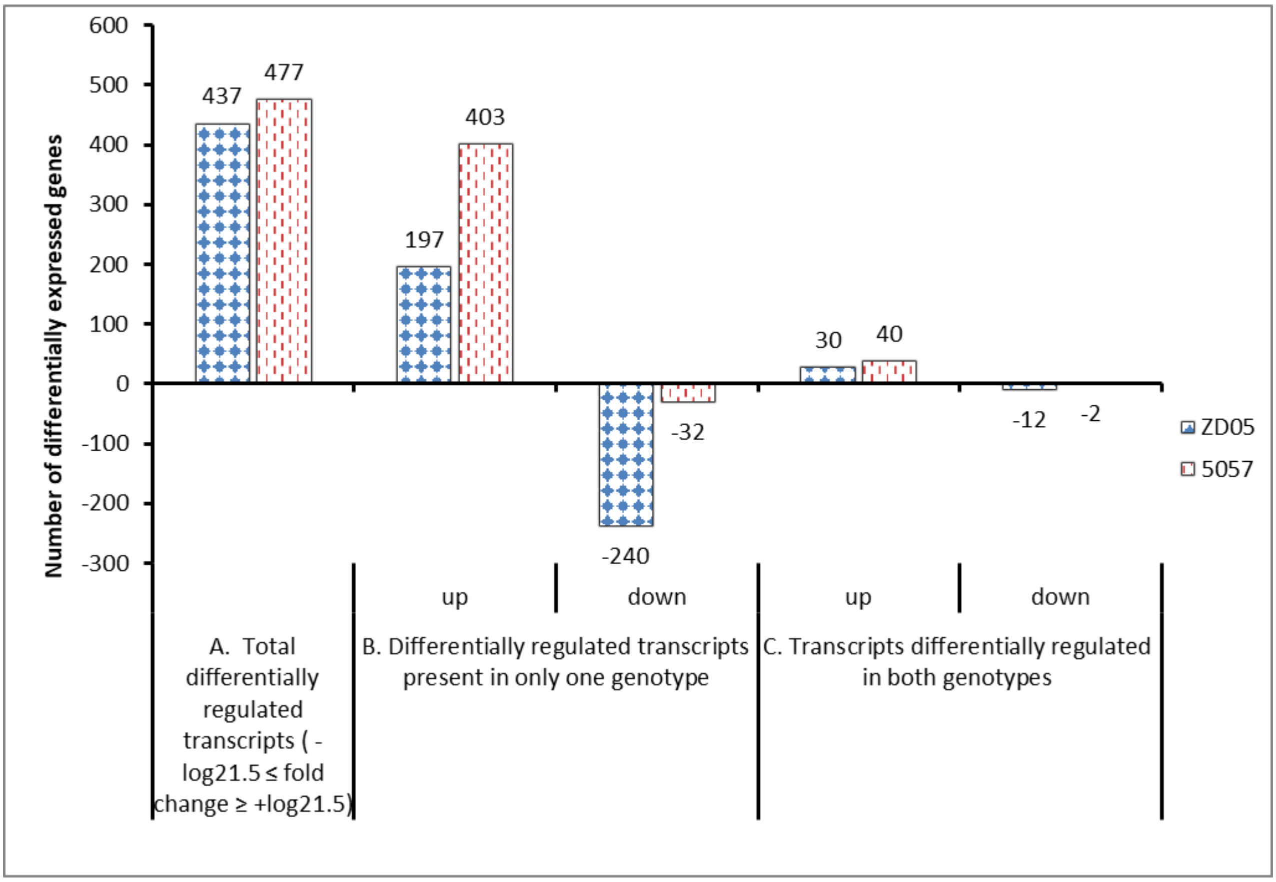


Figure 4.23. The distribution of genes in the first harvest period (3 days post infestation).

Key

A = number of genes that showed significant differences in expression levels between the infested and uninfested plants.

B = Genes that were differentially regulated in only one genotype (That is, either ZD05 or 5057).

C = Transcripts differentially regulated and present in both genotypes.

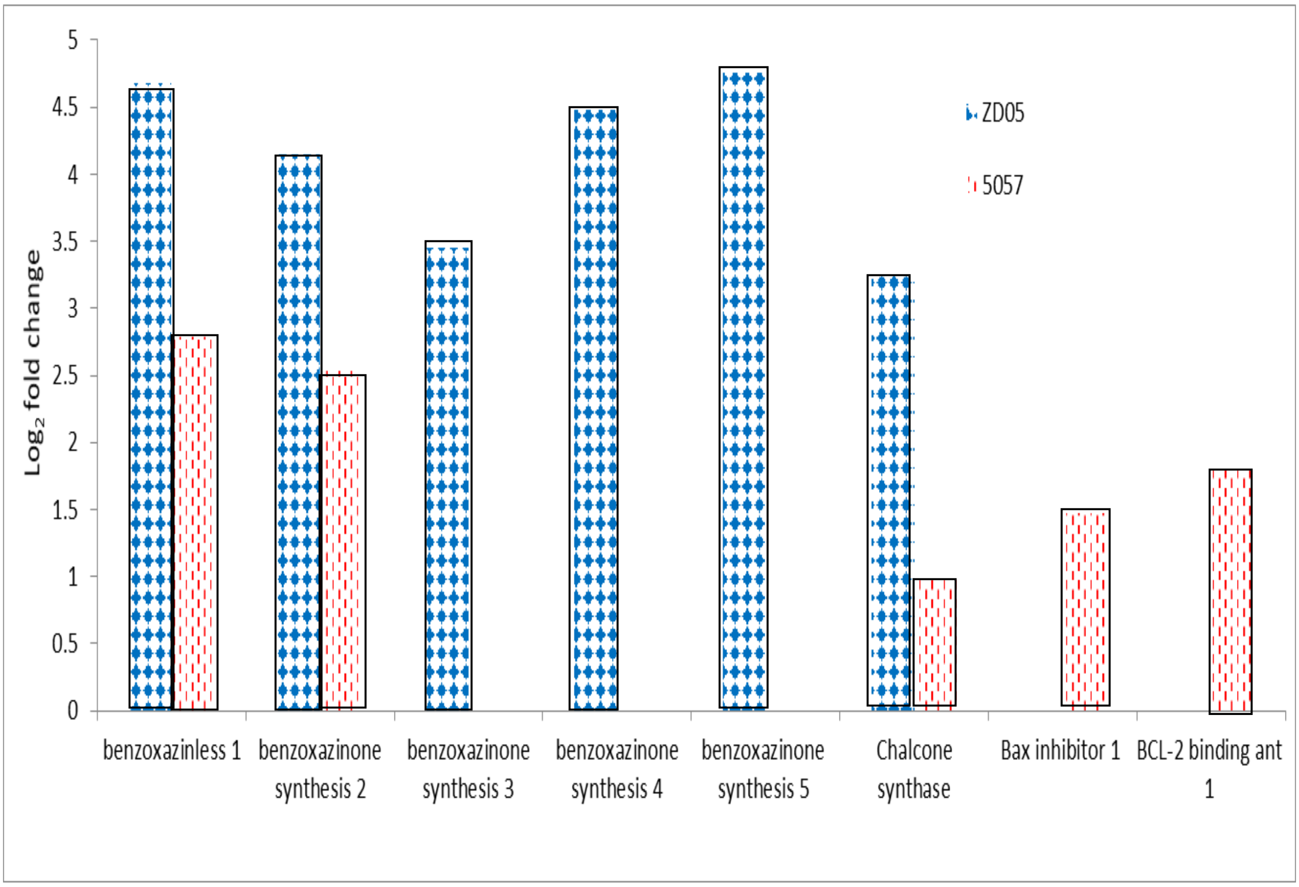


Figure 4.24. Expression levels of selected defence and secondary metabolism at the three days post infestation.

Table 4.12. Representative genes upregulated at 3 days post infestation time in both genotypes.

5057 (Susceptible)	ZD05 (Resistant)
Hormone and hormonal metabolism	Hormone and hormonal metabolism
ABA-responsive protein	Auxin response factor 2
Abscisic acid receptor PYL5	Ethylene-responsive transcription factor RAP 2-3
Auxin-responsive protein SAUR32	PIN-formed protein11 and 9
Indole-3-acetate beta-glucosyltransferase	WAT1-related protein
Gibberellin receptor GID1L2	
1-aminocyclopropane-1-carboxylate oxidase 7	
S-adenosylmethionine synthase 1	
Indole-3-acetate beta-glucosyltransferase	
Ethylene-responsive transcription factor 1A	
Ethylene-responsive transcription factor ERF035	
allene oxide synthase 1	
jasmonate-regulated gene 21	
Defence	Defence
Dehydration responsive element binding protein	Osmotin-like protein OSM34
Dehydrin DHN1	Beta-glucosidase 17
Protein DOWNY MILDEW RESISTANCE 6	hypersensitive induced reaction 2 and 3
Wound/stress protein	Disease resistance gene analog PIC17
Xyloglucan endotransglucosylase/hydrolase protein 2	trehalose-6-phosphate phosphatase 2 and 8
stearoyl-acyl-carrier-protein desaturase9	
lipoxygenase 5	
H_2O_2 maetabolism and oxidative stress	H_2O_2 maetabolism and oxidative stress
alternative oxidase 2 and 3	Catalase isozyme 3
glutathione transferase 5 and 42	
Peroxidase 1, 2, 24, 52, 70, R15	
Glutathione S-transferase GSTU6	

oxidative stress 3	
Plant L-ascorbate oxidase	
Transporters	Transporters
Symbiotic ammonium transporter	Equilibrative nucleotide transporter 2
ABC transporter G family member 6	Sugar transport protein 3
Sulfate transporter 3.1	potassium channel 6
Symbiotic ammonium transporter	Major facilitator superfamily protein
phosphate transporter protein 13	Methionine aminopeptidase
Amino acid permease 2	calcium exchanger3
Amino acid transporter ANTL2	ABC transporter B family member 9
Aquaporin PIP1-6., 1-1, 2-2, 2-3	ABC transporter G family member 34
	ammonium transporter2
Secondary metabolism	Secondary metabolism
Naringenin2-oxoglutarate 3-dioxygenase	Chalcone synthase C2
phenylalanine ammonia lyase 8	bx 3, 4 and 5
chalcone flavanone isomerase1	Dihydroflavonol-4-reductase
DIBOA-glucoside dioxygenase BX6	Flavonoid 3'-monooxygenase
Polyketide cyclase/dehydrase and lipid transport superfamily protein	kaurenoic acid oxidase 2
	Phenylalanine ammonia-lyase 1
	Polyketide cyclase/dehydrase and lipid transport superfamily protein
	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase
Heat Shock proteins and heat shock factors	
17.4 kDa class I heat shock protein 3	
17.4 kDa class III heat shock protein	
Activator of heat shock protein ATPase	
Heat shock 70 kDa protein 5 and 8	
Heat shock factor protein 7	

Heat stress transcription factor A-6b	
25.3 kDa heat shock protein chloroplastic	
heat shock protein 17.2	
HSP40/DnaJ peptide-binding protein	
Chaperone DnaJ-domain superfamily protein	
Chaperone protein ClpB1	
Chaperone protein dnaJ 3	
Histone H1, H2A, H3, H4	
Heat shock protein, 90 kDa	
Anti-apoptotic genes	
Bax inhibitor 1	
BCL-2 binding anthanogene-1	

Table 4.13 Representative genes downregulated at 3 days post infestation time in both genotypes.

5057 (Susceptible)	ZD05 (Resistant)
Plant defence	Plant defence
Pathogenesis-related protein 10	Disease resistance protein RPM1
Pollen Ole e 1 allergen and extensin family protein	Disease resistance protein RPS2
Protein DOWNY MILDEW RESISTANCE 6	Disease resistance RPP13-like protein 4
trehalose-6-phosphate synthase 9	Endoglucanase 1 and 6
Hormone and hormonal metabolism	Hormone and hormonal metabolism
WAT1-related protein	ABA induced plasma membrane protein PM 19
	ABC transporter A family member 7
	gibberellin 2-oxidase 3
	cyclin 5 and D2-1
	auxin import carrier 1
	Auxin-induced beta-glucosidase 3B
	tryptophan aminotransferase related 2
	Auxin response factor 5
	IAA9-auxin-responsive Aux/IAA family member
	Jasmonate-induced protein
	jasmonate-regulated gene 21
	Pin 4
Secondary metabolites	Heat Shock proteins and heat shock factors
Terpene synthase 6	Heat stress transcription factor B-4
Alpha-humulene/(-)-(E)-beta-caryophyllene synthase	Hsp20/alpha crystallin family protein
	HSP20-like chaperones superfamily protein
	H_2O_2 metabolism and oxidative stress
	glutathione transferase 11, 14 and 16

Table 4.14. Pathways with upregulated genes and downregulated genes at 3 days post infestation time in both resistant and susceptible genotypes

Upregulate	
5057 (Susceptible)	ZD05 (Resistant)
zma00010: Glycolysis / Gluconeogenesis	zma00061: Fatty acid biosynthesis
zma00052: Galactose metabolism	zma00220: Arginine biosynthesis
zma00053: Ascorbate and aldarate metabolism	zma00230: Purine metabolism
zma00061: Fatty acid biosynthesis	zma00280: Valine leucine and isoleucine degradation
zma00100: Steroid biosynthesis	zma00330: Arginine and proline metabolism
zma00190: Oxidative phosphorylation	zma00402: Benzoxazinoid biosynthesis
zma00270: Cysteine and methionine metabolism	zma00460: Cyanoamino acid metabolism
zma00360: Phenylalanine metabolism	zma00480: Glutathione metabolism
zma00402: Benzoxazinoid biosynthesis	zma00500: Starch and sucrose metabolism
zma00480: Glutathione metabolism	zma00520: Amino sugar and nucleotide sugar metabolism
zma00591: Linoleic acid metabolism	zma00902: Monoterpenoid biosynthesis
zma00940: Phenylpropanoid biosynthesis	zma00904: Diterpenoid biosynthesis
zma00941: Flavonoid biosynthesis	zma00910: Nitrogen metabolism
zma01040: Biosynthesis of unsaturated fatty acids	zma00941: Flavonoid biosynthesis
zma03010: Ribosome	zma02010: ABC transporters
zma03013: RNA transport	zma03010: Ribosome
zma03040: Spliceosome	zma04626: Plant-pathogen interaction
zma04075: Plant hormone signal transduction	zma01040: Biosynthesis of unsaturated fatty acids
zma04141: Protein processing in endoplasmic reticulum	zma00250: Alanine aspartate and glutamate metabolism
zma04144: Endocytosis	zma01110: Biosynthesis of secondary metabolites
zma00071: Fatty acid degradation	zma01100: Metabolic pathways
zma01100: Metabolic pathways	zma00944: Flavone and flavonol biosynthesis
zma00073: Cutin, suberine and wax	zma01212: Fatty acid metabolism

biosynthesis, zma01110: Biosynthesis of secondary metabolites zma00195: Photosynthesis zma00260: Glycine, serine and threonine metabolism zma00400: Phenylalanine, tyrosine and tryptophan biosynthesis zma01212: Fatty acid metabolism zma04931: Insulin resistance zma00350: Tyrosine metabolism zma00310: Lysine degradation zma00750: Vitamin B6 metabolism zma01230: Biosynthesis of amino acids zma01200: Carbon metabolism zma01220: Degradation of aromatic compounds zma04146: Peroxisome zma01130: Biosynthesis of antibiotics zma04626: Plant-pathogen interaction	zma00940: Phenylpropanoid biosynthesis zma00630: Glyoxylate and dicarboxylate metabolism zma04712: Circadian rhythm - plant zma01130: Biosynthesis of antibiotics zma01230: Biosynthesis of amino acids
Downregulated	
5057 (Susceptible)	ZD05 (Resistant)
zma00195: Photosynthesis zma00500: Starch and sucrose metabolism zma00902: Monoterpenoid biosynthesis zma00909: Sesquiterpenoid and triterpenoid biosynthesis zma01100: Metabolic pathways zma01110: Biosynthesis of secondary metabolites	zma00053: Ascorbate and aldarate metabolism zma00230: Purine metabolism zma00250: Alanine aspartate and glutamate metabolism zma00380: Tryptophan metabolism zma00480: Glutathione metabolism zma00500: Starch and sucrose metabolism zma00564: Glycerophospholipid metabolism zma00591: Linoleic acid metabolism

	zma00904: Diterpenoid biosynthesis zma00910: Nitrogen metabolism zma03008: Ribosome biogenesis in eukaryotes zma03060: Protein export zma03440: Homologous recombination zma04075: Plant hormone signal transduction zma04141: Protein processing in endoplasmic reticulum zma04144: Endocytosis zma04626: Plant-pathogen interaction zma01100: Metabolic pathways zma00410: beta-Alanine metabolism zma03020: RNA polymerase zma00430: Taurine and hypotaurine metabolism zma00650: Butanoate metabolism zma01110: Biosynthesis of secondary metabolites zma00240: Pyrimidine metabolism zma00592: alpha-Linolenic acid metabolism

4.6.2. Gene expression profile at second time point (9 days post infestation)

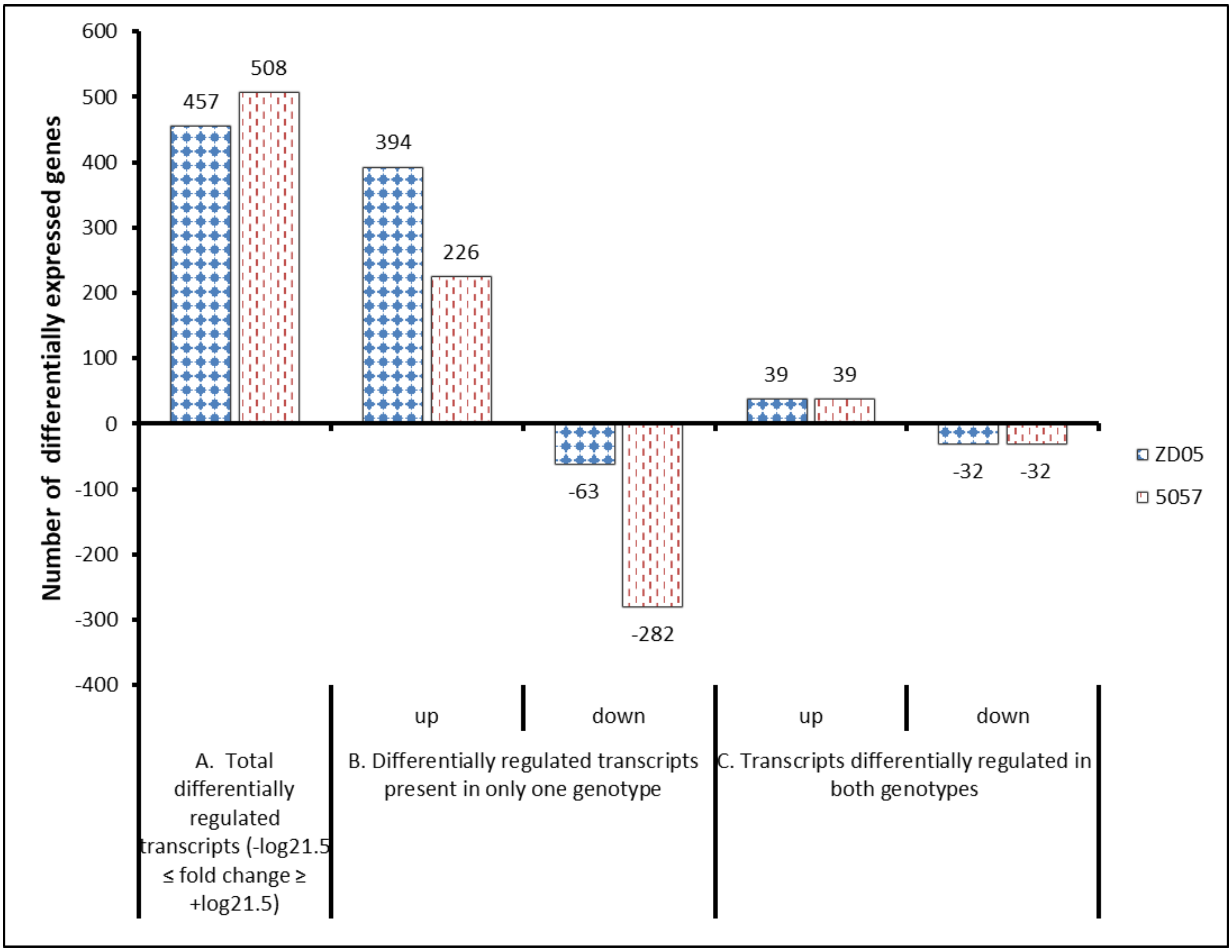
The results at nine days post infestation (9 DPI) show that, upon infestation the susceptible plants (5057) differentially expressed a total of 508 when compared to the uninfested 5057 plants. While, the infested resistant plants (ZD05) differentially expressed 475 genes when compared to the uninfested ZD05 plants. The two maize lines, 5057 and ZD05, upregulated 226 and 394 genes respectively and 282 and 63 genes were downregulated in 5057 and ZD05 respectively. The two genotypes had 71 differentially expressed genes in common, with both the susceptible and resistant plants having 39 upregulated and 32 downregulated genes (Figure 4.25).

At nine DPI, the resistant genotype shows upregulation of more plant defence and pathogenesis-related proteins than the susceptible genotype. Genes involved in hormones and metabolism of hormones, plant defence and pathogenesis-related proteins and in secondary metabolism were upregulated resistant genotype. It also upregulated abscisic acid 8'-hydroxylase 1, S-adenosylmethionine synthase 1 and 1-aminocyclopropane-1-carboxylate (ACC) oxidases, ethene-responsive transcription factors (ERF014, ERF053 and RAP2-11) and endoglucanase 11 and 25 were also observed in the resistant genotype.

Many of heatshock proteins and factors were again upregulated in the susceptible genotype. Genes involved in hormones and metabolism of hormones, plant defence and pathogenesis-related proteins and in secondary metabolism were majorly downregulated in the susceptible genotype. The susceptible genotype also upregulated anti-apoptotic proteins at 9 DPI. (Figure 4.26, Table 4.15 and Table 4.16).

Pathway Enrichment analysis.

Pathway enrichment analysis of differentially regulated genes at the second time point shows that the resistant genotype upregulated and downregulated genes in 41 and 30 pathways respectively. While the susceptible genotype upregulated and downregulated genes in 22 and 38 pathways respectively. (Table 4.17)



4.21. The distribution of genes in the second time point (9 days post infection).

Figure Key

A) Number of genes that showed significant differences in expression levels between the infested and uninfested plants.

B) Genes that were differentially regulated in only on genotype (That is, either ZD05 or 5057).

C) Transcripts differentially regulated and present in both genotypes.

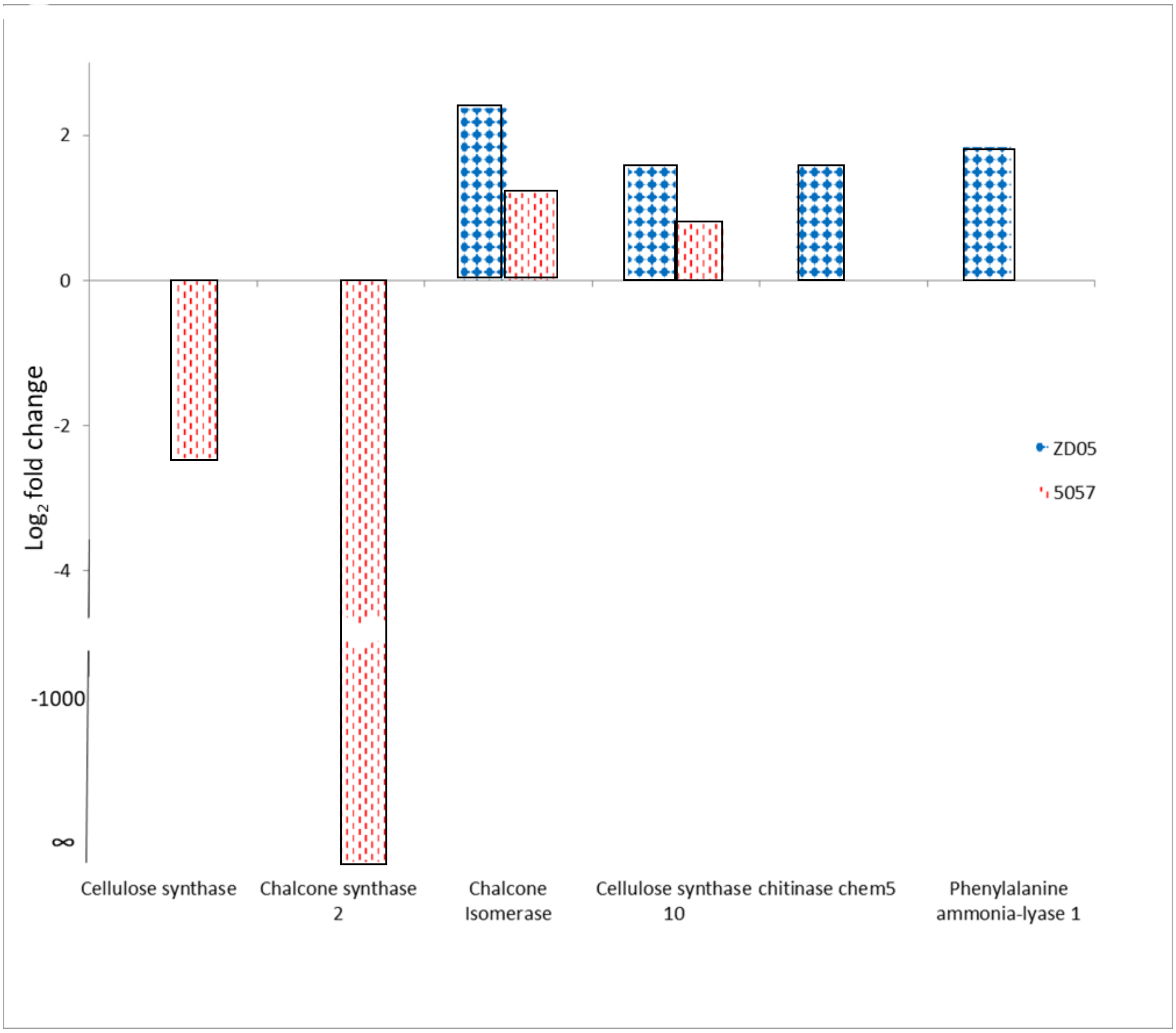


Figure 4.22. Expression levels of selected defence and secondary metabolism on both genotypes at 9 DPI

Table 4.15. Upregulated genes at 9 days post infestation time in both genotypes.

Upregulated

5057 (Susceptible)	ZD05 (Resistant)
Hormone and hormonal metabolism	Hormone and hormonal metabolism
Aux/IAA-transcription factor 5	Abscisic acid 8'-hydroxylase1
Protein SAR DEFICIENT 1	Abscisic acid receptor PYL9
1-aminocyclopropane-1-carboxylate synthase7	Ethylene response factor
Ethylene-responsive transcription factor ERF014 and ERF053	Ethylene-responsive transcription factor RAP2-2 and RAP2-3
	1-aminocyclopropane-1-carboxylate oxidase 2
	reversion-to-ethylene sensitivity1 like3
	ethylene receptor homolog 2
	S-adenosylmethionine synthase 1
	cytokinin oxidase 3
	Auxin response factor 5
	PIN-formed protein 4 and 9
	Gibberellin 20 oxidase 2
	Gibberellin receptor GID1L2
Secondary metabolism	Secondary metabolism
Chalcone synthase C2 and WHP1	cellulose synthase10
Alpha-humulene/(-)-(E)-beta-caryophyllene synthase	chalcone flavanone isomerase 1
terpene synthase 6	DIBOA-glucoside dioxygenase BX6
Dihydroflavonol-4-reductase	Nana2-like1
	Phenylalanine ammonia-lyase 1
Plant defence and pathogenesis-related proteins	Plant defence and pathogenesis-related proteins
Disease resistance gene analog PIC17	Chitinase chem5
Xyloglucan endotransglucosylase/hydrolase protein 14	Xyloglucan endotransglucosylase/hydrolase protein 32

Pathogen-related protein	trehalose-6-phosphate phosphatase8
Stress responsive protein	trehalose-6-phosphate synthase5
Endoglucanase 25	Glucan endo-1,3-beta-glucosidase homolog 1
	Protein DOWNY MILDEW RESISTANCE 6
	Stress responsive protein
Transporters	Transporters
Heavy metal transport/detoxification superfamily protein	ABC transporter B family member 15
High affinity nitrate transporter	ABC transporter G family member 6, 34 and 40
Sulfate transporter 1.2	Amino acid permease 2, 6 and BAT1
Major facilitator superfamily protein	Ammonium transporter 1 and 2
Mechanosensitive ion channel protein 6	GDU1
Lysine histidine transporter-like 7	High affinity nitrate transporter 2.5
ABC transporter A family member 7	Major facilitator superfamily protein
	Metal transporter Nramp6
	Major facilitator superfamily defence 1
	Nitrate transport 2
	Sugar transport protein 3, 5 and 14
	Phosphate transporter protein 2, 7 and 9
Antiapoptotic proteins	Antiapoptotic proteins
BAG family molecular chaperone regulator 1	hemoglobin1
Bax inhibitor-1 family protein	
BCL-2 binding anthanogene-1	
Heat shock proteins and factors	H_2O_2 metabolism and oxidative stress
16.9 kDa class I heat shock protein 1	Glutathione transferase 7 and 21:
17.0 kDa class II heat shock protein	Peroxidase 42, 52, 53 and 64
17.4 kDa class I heat shock protein	
17.5 kDa class II heat shock protein	

25.3 kDa heat shock protein chloroplastic	
Heat shock 70 kDa protein 5,6 and 8	
Heat shock factor protein 7	
Heat stress transcription factor A-6b	
heat shock protein17.2	
18 kda heat shock protein18a	
heat shock protein 26	
heat shock protein, 90 kDa	
HSP40/DnaJ peptide-binding protein	
Activator of heat shock protein ATPase	

Table 4.16. Downregulated genes at 9 days post infestation time in both genotypes.

Downregulated	
5057 (Susceptible)	ZD05 (Resistant)
Heat shock proteins and factors	Heat shock proteins and factors
Heat shock protein 90-2	Heat stress transcription factor A-6b
Transporters	Transporters
Inositol transporter 4	Potassium transporter 5
Major facilitator superfamily protein	Symbiotic ammonium transporter
multidrug and toxic compound extrusion 2	Phosphatidylinositol/phosphatidylcholine transfer protein SFH10
nucleotide sugar transporter-KT 1	Mechanosensitive ion channel protein 6
Organic cation/carnitine transporter 3	Protein DETOXIFICATION 40
Protein DETOXIFICATION 40	
S-type anion channel SLAH3	
Sugar carrier protein C	
Sugar transport protein 13	
sugars will eventually be exported transporter17b	
ABC transporter B family member 21	
ABC transporter G family member 40	
Copper transport protein CCH	
Aquaporin PIP1-6	
H_2O_2 metabolism and oxidative stress	H_2O_2 metabolism and oxidative stress
L-ascorbate peroxidase 2 cytosolic	Peroxidase R15
peroxidase 3, 12 and 70	
Plant defence and pathogenesis-related proteins	Plant defence and pathogenesis-related proteins
Stress responsive protein	trehalose-6-phosphate phosphatase 11
trehalose-6-phosphate synthase 9 and 11	
lipoxygenase2	
Pathogenesis-related protein 1	

Pathogenesis-related thaumatin superfamily protein
pectin methylesterase inhibitor 1
late embryogenesis abundant protein-related / LEA protein-related
Bowman-Birk type wound-induced proteinase inhibitor WIP1
Acidic endochitinase
Basic endochitinase B

Hormone and hormonal metabolism
1-aminocyclopropane-1-carboxylate synthase2
allene oxide synthase 1
Auxin-responsive protein IAA26
Benzoate carboxyl methyltransferase
tryptophan aminotransferase related 2
Salicylate/benzoate carboxyl methyltransferase
scarecrow-like 1
Jasmonate-induced protein
jasmonate-regulated gene 21
Jasmonic acid-amido synthetase JAR1
Indole-3-glycerol phosphate synthase chloroplastic
Ethylene-responsive transcription factor ERF035
IAA-amino acid hydrolase ILR1-like 4
gibberellin 2-oxidase 3 and 10

Secondary metabolism
Chalcone synthase 2
benzoxazinone synthesis 11 and 14
terpene synthase 2, 8 and 23

Table 4.17. Pathways with upregulated genes and downregulated genes at 9 days post infestation time in both resistant and susceptible genotypes

Upregulated	
5057 (Susceptible)	ZD05 (Resistant)
zma00052: Galactose metabolism	zma00010: Glycolysis / Gluconeogenesis
zma00053: Ascorbate and aldarate metabolism	zma00052: Galactose metabolism
zma00061: Fatty acid biosynthesis	zma00053: Ascorbate and aldarate metabolism
zma00073: Cutin, suberine and wax biosynthesis	zma00130: Ubiquinone and other terpenoid-quinone biosynthesis
zma00196: Photosynthesis - antenna proteins	zma00270: Cysteine and methionine metabolism
zma00230: Purine metabolism	zma00360: Phenylalanine metabolism
zma00270: Cysteine and methionine metabolism	zma00380: Tryptophan metabolism
zma00330: Arginine and proline metabolism	zma00400: Phenylalanine, tyrosine and tryptophan biosynthesis
zma00402: Benzoxazinoid biosynthesis	zma00402: Benzoxazinoid biosynthesis
zma00860: Porphyrin and chlorophyll metabolism	zma00460: Cyanoamino acid metabolism
zma00902: Monoterpenoid biosynthesis	zma00480: Glutathione metabolism
zma00906: Carotenoid biosynthesis	zma00520: Amino sugar and nucleotide sugar metabolism
zma00940: Phenylpropanoid biosynthesis	zma00905: Brassinosteroid biosynthesis
zma03010: Ribosome	zma00940: Phenylpropanoid biosynthesis
zma04075: Plant hormone signal transduction	zma00941: Flavonoid biosynthesis
zma04141: Protein processing in endoplasmic reticulum	zma01040: Biosynthesis of unsaturated fatty acids
zma04626: Plant-pathogen interaction	zma03010: Ribosome
zma01212: Fatty acid metabolism	zma03040: Spliceosome
zma01100: Metabolic pathways	zma03060: Protein export

zma01110: Biosynthesis of secondary metabolites	zma04070: Phosphatidylinositol signalling system
zma01040: Biosynthesis of unsaturated fatty acids	zma04075: Plant hormone signal transduction
zma00740: Riboflavin metabolism	zma04141: Protein processing in endoplasmic reticulum
	zma04626: Plant-pathogen interaction
	zma00071: Fatty acid degradation
	zma00500: Starch and sucrose metabolism
	zma01100: Metabolic pathways
	tyrosine and tryptophan biosynthesis
	zma01110: Biosynthesis of secondary metabolites
	zma01212: Fatty acid metabolism
	zma00350: Tyrosine metabolism
	zma00945: Stilbenoid diarylheptanoid and gingerol biosynthesis
	zma04144: Endocytosis
	zma04145: Phagosome
	zma01230: Biosynthesis of amino acids
	zma04712: Circadian rhythm - plant
	zma00400: Phenylalanine tyrosine and tryptophan biosynthesis
	zma01130: Biosynthesis of antibiotics
	zma01200: Carbon metabolism
	zma00950: Isoquinoline alkaloid biosynthesis
	zma01220: Degradation of aromatic compounds
	zma00960: Tropane piperidine and pyridine alkaloid biosynthesis

Downregulated

5057 (Susceptible)	ZD05 (Resistant)
zma00010: Glycolysis / Gluconeogenesis	zma00010: Glycolysis / Gluconeogenesis
zma00040: Pentose and glucuronate interconversions	zma00061: Fatty acid biosynthesis
zma00100: Steroid biosynthesis	zma00073: Cutin
zma00240: Pyrimidine metabolism	zma00430: Taurine and hypotaurine metabolism
zma00460: Cyanoamino acid metabolism	zma00500: Starch and sucrose metabolism
zma00906: Carotenoid biosynthesis	zma00561: Glycerolipid metabolism
zma00910: Nitrogen metabolism	zma00902: Monoterpenoid biosynthesis
zma00940: Phenylpropanoid biosynthesis	zma00905: Brassinosteroid biosynthesis
zma00960: Tropane piperidine and pyridine alkaloid biosynthesis	zma00906: Carotenoid biosynthesis
zma01200: Carbon metabolism	zma00909: Sesquiterpenoid and triterpenoid biosynthesis
zma00030: Pentose phosphate pathway	zma00940: Phenylpropanoid biosynthesis
zma01100: Metabolic pathways	zma00941: Flavonoid biosynthesis
zma00760: Nicotinate and nicotinamide metabolism	zma03040: Spliceosome
zma00500: Starch and sucrose metabolism	zma04075: Plant hormone signal transduction
zma00051: Fructose and mannose metabolism	zma04141: Protein processing in endoplasmic reticulum
zma01110: Biosynthesis of secondary metabolites	zma04626: Plant-pathogen interaction
zma00710: Carbon fixation in photosynthetic organisms	zma00030: Pentose phosphate pathway
zma01130: Biosynthesis of antibiotics	zma00260: Glycine serine and threonine metabolism
zma01110: Biosynthesis of secondary metabolites	zma01100: Metabolic pathways
	suberine and wax biosynthesis

	zma00564: Glycerophospholipid metabolism zma01110: Biosynthesis of secondary metabolites zma00944: Flavone and flavonol biosynthesis zma00051: Fructose and mannose metabolism zma01212: Fatty acid metabolism zma04144: Endocytosis zma00052: Galactose metabolism zma04712: Circadian rhythm - plant zma01130: Biosynthesis of antibiotics zma01200: Carbon metabolism zma01230: Biosynthesis of amino acids zma03018: RNA degradation

4.6.3 Gene expression profile at Third time point (Twenty-two days post infestation)

At 22 days post infestation (22DPI) a total of 436 genes were differentially regulated in the susceptible genotype when compared to its uninfested state. The resistant genotype upregulated 384 genes upon infestation. Upon infestation, three hundred and twenty-two and two hundred and ninety-nine genes were upregulated in ZD05 and 5057 respectively, while 136 and 61 genes were downregulated in 5057 and ZD05 respectively. The two genotypes had 42 differentially expressed genes in common, with the susceptible upregulating 40 of these genes and downregulating two. While, the resistant genotype upregulated and down regulated 32 and 10 of these genes respectively (Fig. 4.27).

The resistant genotype also began to show upregulation of more heat shock factors and heatshock proteins than the susceptible genotype. At 22 DPI the transcriptomic profile of the resistant genotype was approximately 50% similar to the profile of the susceptible genotype at the first time point (3 days post infestation) (see bax inhibitor, fig. 4.28). This indicates that the resistant genotype was gradually beginning to show the effects of parasitization. Also, genes involved in hormone and hormonal metabolism were upregulated in the resistant genotype. The susceptible genotype, on the other hand downregulated some of these genes and upregulated others. The resistant genotype also upregulated more genes involved in secondary metabolism, transporters and plant defence and pathogenesis-related protein genes than the susceptible genotype (Table 4.18 and 4.19).

Pathway Enrichment at 22 DPI

Pathway enrichment analysis of differentially regulated genes at the third time point revealed that the resistant genotype upregulated and downregulated genes in 40 and 31 pathways respectively. The susceptible genotype upregulated and downregulated genes in 22 and 19 pathways respectively. (Table 4.20).

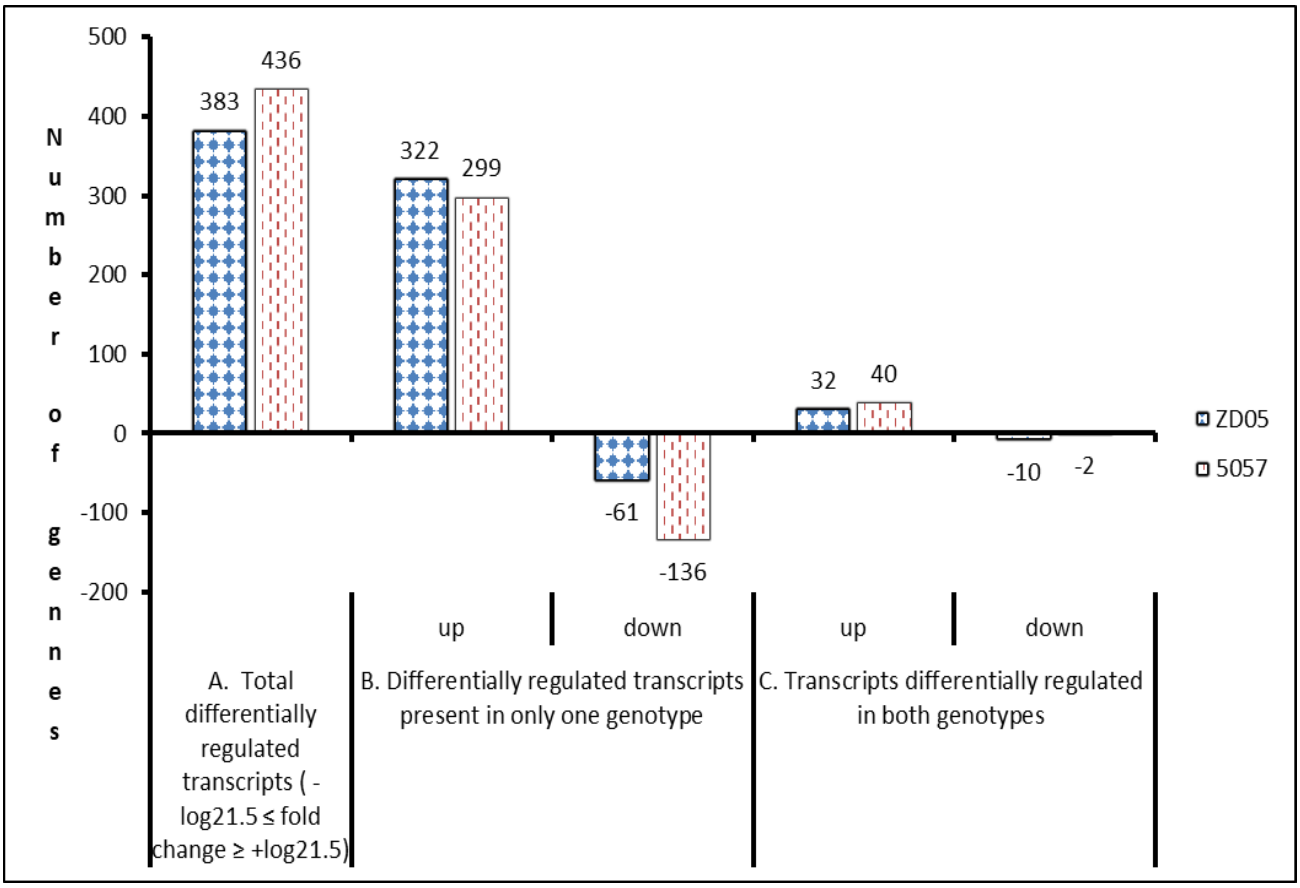


Figure 4.27. The distribution of genes in the third time point (22 days post infection).

Key

A = number of genes that showed significant differences in expression levels between the infested and uninfested plants.

B) Genes that were differentially regulated in only on genotype (That is, either ZD05 or 5057).

C) Transcripts differentially regulated and present in both genotypes.

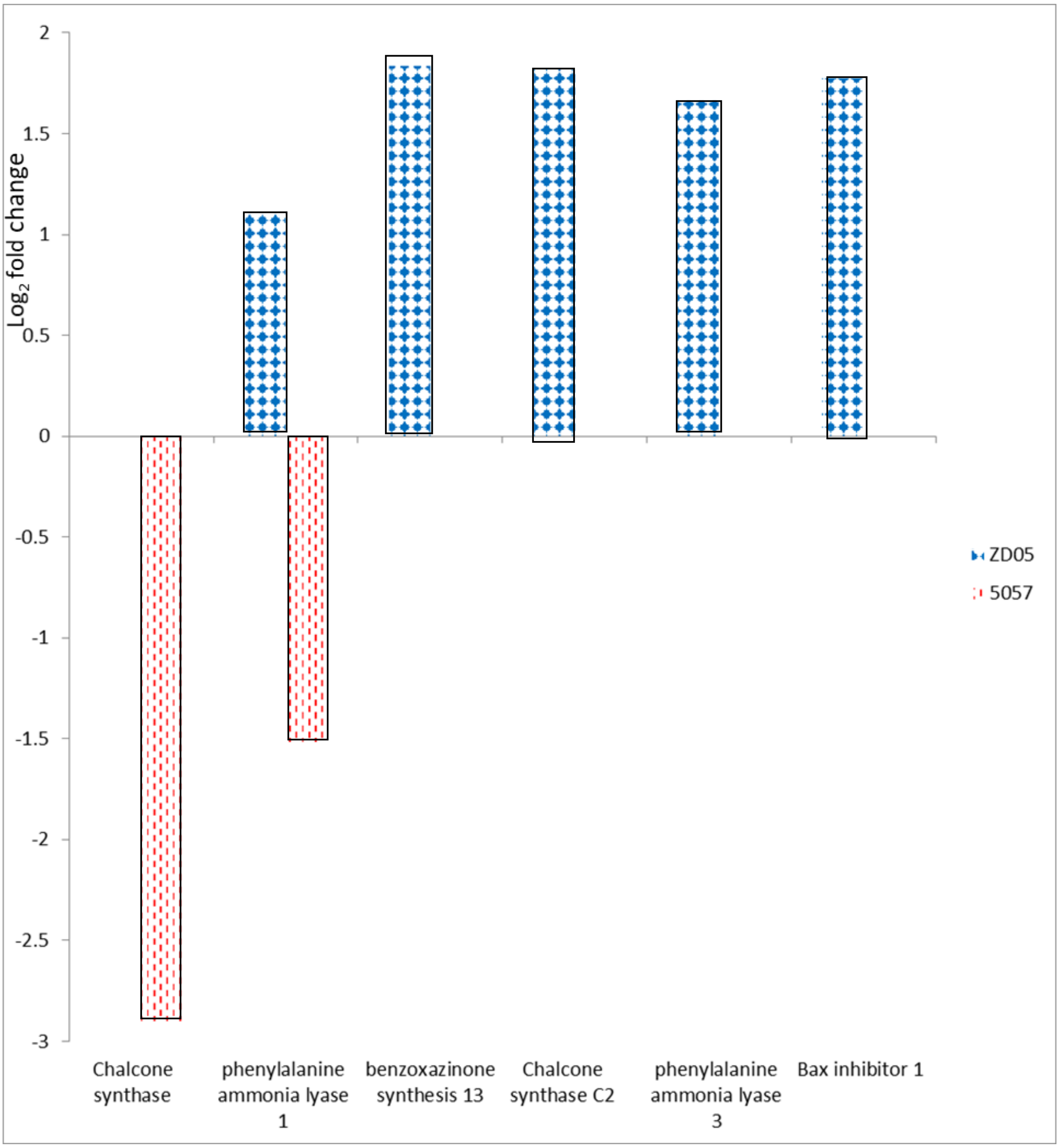


Figure 4.28. Expression levels of selected defence and secondary metabolism on both genotypes at the third time point (22 days post infestation).

Table 4.18. Upregulated genes at 22 days post infestation time in both genotypes.

5057 (Susceptible)	ZD05 (Resistant)
Heat shock proteins and factors	Heat shock proteins and factors
16.9 kDa class I heat shock protein 1	17.4 kDa class I heat shock protein
17.4 kDa class I heat shock protein	17.4 kDa class III heat shock protein
17.5 kDa class II heat shock protein	23.6 kDa heat shock protein mitochondrial
18 kda heat shock protein18a	65-kDa microtubule-associated protein 6
HSP40/DnaJ peptide-binding protein	Heat shock 70 kDa protein
	Heat shock protein 90-2
	Heat stress transcription factor A-6b
	HSP40/DnaJ peptide-binding protein
Hormone and hormonal metabolism	Hormone and hormonal metabolism
abscisic acid 8'-hydroxylase 4	S-adenosylmethionine synthase 1
1- aminocyclopropane-1-carboxylate oxidase 1	Salicylate/benzoate carboxyl methyltransferase
Ethylene-responsive transcription factor RAP2-3	ABA induced plasma membrane protein PM 19
Auxin response factor 3 WAT1-related protein	aasr3; abscisic acid stress ripening3:
cyclin-dependent kinase inhibitor 8	SAUR-like auxin-responsive protein family
	Auxin response factor 16
	IAA-amino acid hydrolase ILR1-like 4
	Ethylene-responsive transcription factor 4
	Ethylene-responsive transcription factor ERF035, ERF118 and RAP2-2
	Gibberellin-regulated protein 10
	dwarf plant 8
	Protein SCARECROW
	Jasmonate-induced protein
Secondary metabolism	Secondary metabolism
benzoxazinone synthesis 4	Acidic endochitinase
Cellulose synthase 5, 7 and 11	aos1; allene oxide synthase 1

Dihydroflavonol-4-reductase Endoglucanase 2, 11 and 25	bx2; benzoxazinone synthesis 2 Cell envelope integrity inner membrane protein TolA Chalcone synthase C2 Glucan endo-13-beta-glucosidase 3 Phenylalanine ammonia lyase 3, 5, 6 and 8 Delta (12)-fatty-acid desaturase DIBOA-glucoside dioxygenase BX6 Dihydroflavonol-4-reductase Lipoxygenase 2 chloroplastic
Transporters Protein DETOXIFICATION 16 Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein ABC transporter G family member 29	Transporters Mechanosensitive ion channel protein 6 Sweet1b; sugars will eventually be exported transporter1b Sugar transport protein 5 Sulfate transporter 3.1 Oligopeptide transporter 7 Vacuolar iron transporter 1 Plant Cation-Chloride Cotransporters (CCC) Protein DETOXIFICATION 21 ABC transporter G family member 25 Amino acid permease 2 and 6 Aluminum-activated malate transporter 10 Amino acid transporter ANTL2 Aquaporin PIP1-2, PIP1-3, PIP1-4, PIP1-5, PIP1-6 and PIP1-7 Heavy metal transport/detoxification superfamily protein
H_2O_2 metabolism and oxidative stress Glutathione S-transferase L2 chloroplastic L-ascorbate oxidase	H_2O_2 metabolism and oxidative stress Peroxidase 2, 12, 24, 52, 54, 70 L-ascorbate oxidase

Peroxidase 1, 11, 42 and 67	Glutathione transferase 5
Glutathione transferase 38	
Plant defence and pathogenesis-related proteins	Plant defence and pathogenesis-related proteins
Barley mlo defence gene homolog 2	Stress responsive protein
Hypoxia-responsive family protein	THAUMATIN-LIKE PROTEIN 1
	Wound/stress protein
	Xyloglucan endo-transglycosylase/hydrolase1
	Protein DOWNY MILDEW RESISTANCE 6
Antiapoptotic proteins	Antiapoptotic proteins
BAG family molecular chaperone regulator 1 and 6	Bax inhibitor 1
	Apoptotic proteins
	DCD (Development and Cell Death) domain protein

Table 4.20. Downregulated genes at 22 days post infestation in resistant and susceptbile genotypes.

5057 (Susceptible)	ZD05 (Resistant)
Transporters	Transporters
Amino acid permease 6	ABC transporter B family member 9 and 15
S-type anion channel SLAH3	ABC transporter G family member 37 and 40
Potassium channel AKT1 and SKOR	Potassium transporter 5
nrt2; nitrate transport 1 and 2	multidrug resistance-associated protein 11
Symbiotic ammonium transporter	Divalent ion symporter
Glucose-6-phosphate/phosphate translocator 2	
Aluminum-activated malate transporter 10	
phosphate transporter protein 9	
Secondary metabolism	Secondary metabolism
Flavonoid 3-monooxygenase	benzoxazinone synthesis 14
Chalcone synthase 2	trehalose-6-phosphate synthase 5
Phenylalanine ammonia-lyase 1	
terpene synthase17:	
Polyketide cyclase/dehydrase and lipid transport superfamily protein	
Plant defence and pathogenesis-related proteins	Plant defence and pathogenesis-related proteins
Disease resistance protein RPM1	Pathogenesis-related protein 10
	Disease resistance RPP13-like protein 4
	Bowman-Birk type wound-induced proteinase inhibitor WIP1
	trehalose-6-phosphate phosphatase 11
H_2O_2 metabolism and oxidative stress	H_2O_2 metabolism and oxidative stress
Peroxidase 64	Peroxidase 59
Hormone and hormonal metabolism	
cytokinin oxidase 4b:	

Auxin responsive protein Putative auxin efflux carrier WAT1-related protein 1-aminocyclopropane-1-carboxylate synthase1 jasmonate-regulated gene 21 Scarecrow-like protein 26	

Table 4.21. Pathways with upregulated genes and downregulated genes at 22 days post infestation in both resistant and susceptible genotypes

Upregulated Pathway

5057 (Susceptible)	ZD05 (Resistant)
zma00052: Galactose metabolism	zma00010: Glycolysis / Gluconeogenesis
zma00053: Ascorbate and aldarate metabolism	zma00052: Galactose metabolism
zma00061: Fatty acid biosynthesis	zma00053: Ascorbate and aldarate metabolism
zma00073: Cutin suberine and wax biosynthesis	zma00130: Ubiquinone and other terpenoid-quinone biosynthesis
zma00196: Photosynthesis - antenna proteins	zma00270: Cysteine and methionine metabolism
zma00230: Purine metabolism	zma00360: Phenylalanine metabolism
zma00270: Cysteine and methionine metabolism	zma00380: Tryptophan metabolism
zma00330: Arginine and proline metabolism	zma00400: Phenylalaninetyrosine and tryptophan biosynthesis
zma00402: Benzoxazinoid biosynthesis	zma00402: Benzoxazinoid biosynthesis
zma00860: Porphyrin and chlorophyll metabolism	zma00460: Cyanoamino acid metabolism
zma00902: Monoterpenoid biosynthesis	zma00480: Glutathione metabolism
zma00906: Carotenoid biosynthesis	zma00520: Amino sugar and nucleotide sugar metabolism
zma00940: Phenylpropanoid biosynthesis	zma00905: Brassinosteroid biosynthesis
zma03010: Ribosome	zma00940: Phenylpropanoid biosynthesis
zma04075: Plant hormone signal transduction	zma00941: Flavonoid biosynthesis
zma04141: Protein processing in endoplasmic reticulum	zma01040: Biosynthesis of unsaturated fatty acids
zma04626: Plant-pathogen interaction	zma03010: Ribosome
zma01100: Metabolic pathways	zma03040: Spliceosome
zma01040: Biosynthesis of unsaturated fatty acids	zma03060: Protein export

zma00740: Riboflavin metabolism	zma04070: Phosphatidylinositol signalling system
zma01110: Biosynthesis of secondary metabolites	zma04075: Plant hormone signal transduction
zma01212: Fatty acid metabolism	zma04141: Protein processing in endoplasmic reticulum
	zma04626: Plant-pathogen interaction
	zma00071: Fatty acid degradation
	zma01220: Degradation of aromatic compounds
	zma00500: Starch and sucrose metabolism
	zma01100: Metabolic pathways
	tyrosine and tryptophan biosynthesis
	zma01230: Biosynthesis of amino acids
	zma01110: Biosynthesis of secondary metabolites
	zma00960: Tropanepiperidine and pyridine alkaloid biosynthesis
	zma01130: Biosynthesis of antibiotics
	zma01212: Fatty acid metabolism
	zma01200: Carbon metabolism
	zma00950: Isoquinoline alkaloid biosynthesis
	zma04144: Endocytosis
	zma04145: Phagosome
	zma00400: Phenylalanine tyrosine and tryptophan biosynthesis
	zma04712: Circadian rhythm - plant
	zma00350: Tyrosine metabolism
	zma00945: Stilbenoid diarylheptanoid and gingerol biosynthesis
Downregulated Pathways	
5057 (Susceptible)	ZD05 (Resistant)
zma00010: Glycolysis / Gluconeogenesis	zma00010: Glycolysis / Gluconeogenesis
zma00040: Pentose and glucuronate interconversions	zma00061: Fatty acid biosynthesis
zma00100: Steroid biosynthesis	zma00073: Cutin

zma00240: Pyrimidine metabolism
zma00460: Cyanoamino acid metabolism
zma00906: Carotenoid biosynthesis
zma00910: Nitrogen metabolism
zma00940: Phenylpropanoid biosynthesis
zma00960: Tropane piperidine and pyridine alkaloid biosynthesis

zma00030: Pentose phosphate pathway
zma01100: Metabolic pathways
zma00760: Nicotinate and nicotinamide metabolism
zma00500: Starch and sucrose metabolism
piperidine and pyridine alkaloid biosynthesis
zma00051: Fructose and mannose metabolism
zma01110: Biosynthesis of secondary metabolites
zma00710: Carbon fixation in photosynthetic organisms

zma01130: Biosynthesis of antibiotics
zma01200: Carbon metabolism

zma00430: Taurine and hypotaurine metabolism
zma00500: Starch and sucrose metabolism
zma00561: Glycerolipid metabolism
zma00902: Monoterpenoid biosynthesis
zma00905: Brassinosteroid biosynthesis

zma00906: Carotenoid biosynthesis
zma00909: Sesquiterpenoid and triterpenoid biosynthesis
zma00940: Phenylpropanoid biosynthesis

zma00941: Flavonoid biosynthesis

zma03040: Spliceosome

zma04075: Plant hormone signal transduction
zma04141: Protein processing in endoplasmic reticulum

zma04626: Plant-pathogen interaction

zma00030: Pentose phosphate pathway
zma00260: Glycine serine and threonine metabolism
zma01100: Metabolic pathways
suberine and wax biosynthesis
zma00564: Glycerophospholipid metabolism
zma01110: Biosynthesis of secondary metabolites
zma00944: Flavone and flavonol biosynthesis
zma00051: Fructose and mannose metabolism
zma01212: Fatty acid metabolism
zma04144: Endocytosis
zma00052: Galactose metabolism

	zma04712: Circadian rhythm - plant zma01130: Biosynthesis of antibiotics zma01200: Carbon metabolism zma01230: Biosynthesis of amino acids zma03018: RNA degradation

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CHAPTER 5

DISCUSSION

Crop varieties that are resistant to *Striga hermonthica* are central to any effective *S. hermonthica* control strategy for resource-poor small holder farmers (Kim, 1994). This is because all other control methods are not within the reach of these farmers. They also do not achieve complete control of the parasite when used separately and therefore, have to be combined. *Striga hermonthica* control options are further threatened by its self-incompatible out breeding nature. This is because of the likely high amounts of diversity within its populations (Hearne, 2009). It is therefore imperative to account for the possible geographical variability of *S. hermonthica* when selecting representative testing sites for resistance screening and the evaluation of the viability of other control options against the parasite. It is also important to consider pyramiding several mechanisms of resistance into one host plant to create maize plants with stable resistance to the parasite across geographical locations and over time.

5.1 Discussion

5.1.1 Genetic diversity of *Striga hermonthica* populations in Nigeria and Kenya

Outbreeding and weedy annual species are expected to have high amounts of genetic variation (Loveless and Hamrick 1984; Hamrick and Godt 1996). This is because there is a constant mixing of genetic material between the plants. *S. hermonthica* is an obligate out-breeder that achieves cross pollination through insect vectors (Musselman *et al.*, 1983), the amount of genetic variation in its population in Nigeria and Kenya was the subject of this study.

The *S. hermonthica* populations sampled from both countries exhibited high levels of genetic diversity, with the greater genetic diversity among the plants collected in Kenya. The level of genetic diversity within a population can affect the productivity, growth and stability of that population, as well as interspecific interactions within its communities. It is directly related to the evolutionary potential of the population (Hughes *et al.,* 2008). Therefore, the high level of genetic diversity observed in Nigerian and Kenyan *S. hermonthica* population may be among the factors that enable *S. hermonthica* to overcome host plant resistance and other control methods, leading to seasonal and geographical variability in the effectiveness of *S. hermonthica* control achieved as observed by Koyama (2000) and Hearne, (2009).

A comparison of the Allele richness and effective number of alleles in both populations show that both populations have a few alleles with low frequencies this is particularly evident in the Nigerian population. Allele richness is a strong indicator of the evolutionary potential of a population (Calbarello and Garcia-Dorado, 2013). It differs from effective number of alleles in that alleles with low frequencies have little contribution to the effective number of alleles. Although not all variation is related to the adaptive potential, the presence of diverse alleles increases the probability of a population to adapt to changing abiotic and biotic conditions (Hughes *et al.*, 2008) thus indicating that the Nigerian population may have higher adaptability than the Kenyan population. This is also seen as the Nigerian population has a higher number of rare alleles. Hamrick *et al.* (1979) and Loveless and Hamrick (1984) indicated that the heterozygosity for annuals is 0.116; for dicot species is 0.113; for outcrossed species, 0.185; and for weedy species is 0.116. *S. hermonthica* is obligate out-crossing weedy species and the results show the presence of a higher amount of heterozygosity thus indicating a high amount of diversity (Kenya = 0.282 and Nigeria = 0.209) in both Nigerian and Kenyan *S. hermonthica* populations and thus genetic diversity.

5.1.2. Genetic differentiation and population structure in Kenya and Nigerian ***Striga hermonthica*** populations

This study had established the high level of diversity in *S. hermonthica* populations in both Nigeria and Kenya. It was therefore was pertinent to the presence of partitioning within both populations and, the factors driving this partitioning. The presence or absence of subpopulations within the *S. hermonthica* populations in both countries as well as the factors driving the formation of these subpopulations was also investigated.

Results obtained using various multivariate methods found the presence of two genetically distinct groups Nigeria and Kenya indicating that the two populations had limited exchange of genes, possibly due to the geographic isolation of the populations originating from the two countries. This was consistent with the results presented by Bozkurt *et al* (2014) and Koyama *et al* (2000). A large part of the genetic variance was observed within *S. hermonthica* populations, with smaller fractions occurring between populations and between the origins of the populations. This was consistent with the structure of populations observed in out-crossing species (Hamrick 1982, Hamrick and Godt, 1996, Linhart and Grant, 1996).

The Kenyan *Striga hermonthica* population showed little or no population structure and a low-level genetic differentiation that correlated with the distance between the sampling sites. This could suggest that the sampled *S. hermonthica* populations in Kenya are interconnected by stepwise exchange of genetic material among adjacent populations resulting in an isolation-by-distance pattern. When host plants (Sorghum, Rice and Maize) are used as the basis of clustering, there is indication that some amount of differentiation exists between the samples with maize and sorghum as hosts on the one hand and rice on the other. This study suggests the presence of two biotypes in Kenya, one adapted to rice and the other to both maize and sorghum. This is, however, not conclusive as the observed differentiation might be due to isolation by distance.

The results show that Nigerian samples are divided into three subpopulations that correspond largely to the three regions in Northern Nigeria. This latitudinal stratification is also observed in that a plot of coordinates of the sample collection sites shows that the distinct *S. hermonthica* populations are found in three separate areas within Nigeria. This is similar in distribution to the distribution of three *Striga gesnerioides* biotypes observed in Nigeria by Lane *et al*. (1996). The sub-populations observed in Nigeria did not consist of purely genetically homogeneous groups of individuals; samples from the one observed population had partial membership in other populations. It is thus possible that these individuals have ancestral relationships with plants from other subpopulations. This may be due to some amount of gene flow arising as a result of movement of genetic material from one farm to another farm in northern Nigeria. *Striga* plants attached to maize, pearl millet, and sorghum collected in Nigeria did not show clear differentiation. This implies that host plants are not the main factor driving the differentiation of the Nigerian *S. hermonthica* populations.

In studies on other parasitic plants like *Striga generioides* (Lane *et al.* 1996), *Viscum album* L. (Zuber and Widmer, 2000), and *Arceuthobium americanun* (Jerome and Ford, 2002), host adaptation was suggested to drive race formation. However, Botanga and Timko (2014) suggested that in addition to host adaptation, geographic isolation is also a critical factor in race formation. Geography appeared to be the major element structuring genetic variation and differentiation in this study. The results suggested that *S. hermonthica* populations retain a rather broad host range. As stated by Huang *et al.* (2012), the rotation of crop cultivars and species (through mixed cropping, relay cropping, and crop rotation systems) that is common in *Striga* infested areas in Nigeria and Kenya (Elemo *et al.,* 1988; Ajeigbe *et al.,* 2010), could provide an explanation for the maintenance of a broad range of hosts by *S. hermonthica* populations. This is because the continuous changing of crop varieties and species planted in a particular location often will prevent tight adaptation of *Striga* to any one of them (Huang *et al.* 2012). The different populations and subpopulations observed may therefore have resulted from differential adaptation to environmental conditions prevalent across the locations where they were found not necessarily the host crop species at all instances.

5.1.3. Identification of potential loci under selection within the *Striga hermonthica* populations

A number of ways to identify the genetic signatures of local adaptation within plant populations including outlier tests. Outlier tests indicate whether some loci show genetic differentiation. In the present study, loci undergoing selection were found among the Nigerian and Kenyan samples, between the three subpopulations found in Nigeria. Also, these loci where also found between *S. hermonthica* samples from Kenya with maize and sorghum as hosts on one hand and those with rice as host. In all these cases it was observed that positive selection is occurring within these populations, indicating that new alleles are being fixed in these populations thus giving rise to new phenotypes. Thus, strongly suggesting that positive selection played a role in the divergence of the Kenyan and Nigerian populations of *S. hermonthica* and also in the divergence of the Nigerian subpopulations. Information on the Kenyan maize, sorghum, and rice populations is however not conclusive, because while outlier tests indicated diversifying selection (positive selection), Mantel’s tests also indicated the presence of isolation by distance.

Positive selection is the primary mechanism of adaptation (that is, the genesis of phenotypes that is apt for a specific environment or niche) (Vitti *et al*., 2013). This implies that the *Striga hermonthica* populations are adapting to certain conditions that are prevalent in the local regions where they are found. The environment where parasites are found pose challenges for the parasite. These include strategies used by the host to resist parasitic plants (Yoder and Scholes, 2010). So, parasites will probably have to battle their hosts on multiple fronts and thus many parts of parasite physiology and development, and consequently many different genes, will be under the strong selective pressure to overcome these challenges. (Bromham 2013). The observed new fixation of new alleles in *Striga hermonthica* populations in these regions can lead to the failure of control efforts. It is therefore essential that they are tested across these regions before deployment. In addition, there is the potential for these ecotypes to invade areas with conditions that are similar to the conditions driving selection in these ecotypes.

5.1.4. Effects of *S. hermonthica* infestation on a susceptible and a resistant maize genotype.

Striga hermonthica infestation causes a plethora of deleterious effects on its host. These effects are expected to be reduced in the resistant plant when compared to the susceptible plant. This study investigated three effects of *Striga hermonthica* infestation on two maize genotypes. The results show that resistant genotype (ZD05) supported growth of fewer *S. hermonthica* plants than the susceptible genotype (5057) at all the developmental stages evaluated. It also supported the growth of fewer total number of attached *S. hermonthica.* It therefore has the ability to either stimulate the growth and attachment of fewer *S. hermonthica* plants than the susceptible variety (5057) and/or upon attachment, it has the ability to limit their growth. This agrees with the field testing results of Menkir *et al*., (2006). The large capacity for seed production coupled with its lengthy viability (Parker and Riches, 1993) creates conditions of high numbers of *S. hermonthica* seed in the soil seed bank in areas where the plant is endemic. This can lead to up a hundred *Striga* plants on a single host plant. *Striga hermonthica* extracts photosynthates, solutes and water from its host and a single plant of *S. hermonthica* can inflict approximately five per cent loss in yield in a host plant (Parker and Riches, 1993). Therefore, the higher the number of attached plants, the more the deleterious effects of the parasite. The ability to reduce attachment is therefore an important trait in the fight for survival by the host.

It was also observed that the infested resistant maize plants were significantly taller than the infested susceptible plants. Since both maize lines did not differ significantly in their heights without *S. hermonthica,* it implies that the susceptible plant was significantly affected by infestation. There were also indications that that the deleterious effect of *Striga* on the host plant height depends on the number of parasitic seeds attached as well as some inherent biochemical and physiological property of the host. This was observed as treatment infestation severely altered the plant height regardless of the *Striga hermonthica* seeds. This alteration in height leads to increased self-shading because the plants are stunted (Gurney *et al.*, 1995).

There were disturbances in host plant allometry induced by infestation of *Striga hermonthica,* the resistant plants (ZD05) had a lower root/shoot ratio by weight than the infested 5057. This shows that the physiology and the biochemistry of the susceptible plants could have been significantly disrupted leading to the preferential allocation of biomass to the roots of the host rather than to the shoot. It has been suggested that the elevation of xylem and foliar Absisic acid (ABA) resulted in lowered internode elongation and hence stunting (Taylor *et al.*, 1996). A study of the impact of Absisic acid (ABA) on spring wheat by Quarrie (1982), showed that ABA accumulation is linked to reduced plant height. Absisic acid accumulation has also been linked to perturbations in the root:shoot ratios of plants. Root growth has been shown to continue in droughted maize even when shoot growth is inhibited (Sharp *et al.,*1988), and this is maintained through the action of ABA (Saab *et al.,*1990). The role of ABA in stunting of *S. hermonthica* infected plants is still unclear. Evidence from a study of *S. hermonthica* infected sorghum by Taylor (2001) however suggests that no ABA accumulation occurs in the shoot of infected sorghum.

Plant biomass parameters were used to measure the tolerance of maize inbred lines to *Striga* asiatica infestation (Nyakurwa *et al*., 2018, Gasura *et al.,* 2019). According to Gasura *et al.* (2019) tolerant maize inbred lines can be regarded as maize lines that with root:shoot ratios not affected by *Striga* and that higher emerged *Striga* and haustorial root attachment values led to increased root biomass. The parasite may achieve this through stimulation of lignification of host tissues or root growth can be a result of *Striga* creating a sink which competes with above ground parts for assimilates (Joel *et al*. 2013). The above results, when taken together, show that the resistant genotype, ZD05, successfully resists infestation by the parasite and upon infestation is able to limit the negative effects of infestation to levels that are comparable to the uninfested state. The susceptible plants, 5057, on the other hand, shows all the deleterious effects of parasitization.

5.1.5. Physiological and biochemical mechanisms of resistance to *Striga hermonthica* by maize.

Evidence has shown that mechanisms of resistance in maize could be expressed through low stimulation of *Striga* seed germination, low haustorial induction, avoidance through root architecture (fewer and/or thinner branches), escape by early maturity, resistance to parasite attachment, and failure to support attached parasites (incompatibility) (Rich and Ejeta, 2008). The resistant inbred line (ZD05) tested in this study induced the germination and radicle elongation of fewer number of *Striga hermonthica* seeds compared to the susceptible inbred (5057). It appears to have a lower ability to stimulate the germination of *Striga hermonthica* when compared to the susceptible genotype (5057). This can be due to the quantity of germination stimulant and/or haustorial inducing factor or the composition of germination stimulant or haustorial inducing factor. Rich *et al*., (2004) reported that some wild sorghum varieties show low germination stimulant production. Lower stimulation of germination or lower induction of haustorial formation means fewer *Striga hermonthica* seeds will germinate, form haustoria and attach to the host plant thus reducing the effect of the parasite on its host.

The lower total major root length observed in the resistant plant when compared to the susceptible plant (5057). Indicate that both genotypes have different root architecture. Amusan *et al*., (2008) observed differences in root morphology between ZD05 and 5057 but could not determine if this difference was due to *S. hermonthica* infestation. The apparent differences in root architecture between the two maize inbred lines may account for an additional mechanism of avoidance in the resistant inbred, this is because *S. hermonthica* is a root parasite and the fewer the roots a host possesses the less the likelihood of *Striga*-host root interactions. Cherif-Ari *et al*. (1990) showed that low root length density might be one mechanism adopted by certain sorghum varieties to avoid *Striga* parasitism.

There was significant retardation in the growth of *S. hermonthica* plants attached to the resistant maize genotype when compared to the growth of *S. hermonthica* attached susceptible genotype. The parasites attached to the susceptible genotype grew and developed faster as indicated by the rate of emergence of leaves. This is similar to what was observed by Amusan *et al.* (2008). In their study, *Amusan et al*., (2008) observed that the development of *S. hermonthica* plants on the resistant ZD05 was retarded and the parasites often died often early in contrast to the rapid shoot development of parasites on the roots of 5057. In our laboratory, coculture method ZD05. In field trials by Menkir (2006) ZD05 was seen to have inbred has fewer emerged *Striga* per plot than other 5057. This may indicate an induced physiological or biochemical defence response from ZD05, which leads to the observe phenotype. These results show that ZD05 resists infestation by multiple methods that include biochemical and morphological means and exhibits both pre-attachment and post-attachment resistant.

5.1.6. Molecular responses of a susceptible and a resistant maize genotype to *S. hermonthica* infestation.

A number of genes have been shown to be involved in differential responses to parasite infestation in maize (Kakumanu *et al.,* 2012) and other crops (Hiroka *et al.,* 2008). In the present study, the molecular response of both the resistant and susceptible genotypes to parasitization was investigated at three different time eperiods.

5.1.6.1 Molecular responses of both genotypes at the first period (3DPI)

At three days after infestation, when the plant first comes in contact with the parasite, the pattern of gene expression by the resistant genotype appears to show a downregulation of genes involved in and induced by increased auxin concentration in the roots of the plants. These genes include tryptophan aminotransferase related 2 (tar2) which is involved in auxin biosynthesis (Ma *et al.,* 2014), Auxin response factor 5 (ARF5), IAA9-auxin-responsive Aux/IAA family member, auxin import carrier 1, Patatin-like protein 6, auxin-induced beta-glucosidase, glutathione-s-transferase (11, 14 and 16). Auxin transporters, PIN-formed protein4 (PIN4), was downregulated and PIN 9 and 11 were upregulated. This indicates that there may be a reduction in auxin concentration in some regions of the infested ZD05 plant root when compared to the uninfested plant. Auxins have been implicated in plant roots growth response by inducing cell elongation and expansion, and lateral root formation (Davies *et al*., 1995). Auxin response factor 5 (ARF5), which was downregulated, activates transcription while Auxin response factor 2 (ARF2), which was upregulated, results in the repression of target transcription (Tiwari *et al*. 2001; Hagen and Guilfoyle 2002). Ma *et al*., (2014) suggested that the overexpression of *tar2* increased total lateral roots in Arabidopsis length by increasing the number of visible lateral roots. In addition to transcriptional regulations of genes, auxin signals are transduced to regulate some ion-channels in the cell membrane. It activates proton pumps resulting in the acidification of apoplast (which is necessary to incorporate IAA and other ions by the proton co-transport system). This acidification of cell walls accelerates cell wall loosening and expansion of root cells (Tanimoto 2005). This acid-induced cell wall loosening is mediated, at least partially, by the apoplastic protein(s) such as expansins (Cosgrove *et al*., 2002). It was observed that a number of genes involved in cell wall reorganisation were downregulated including pectate lyase 8, xyloglucan-6-xylosyltransferase 5 and 6, xyloglucan endotransglucosylase/ hydrolase protein 21, endoglucanase 1 and 6, α-expansin 4, β-expansin 3 and 8 (Xyloglucan endotransglucosylase activity loosens a plant cell wall. (Cosgrove *et al*., 2002, Marín-Rodríguez *et al*., 2002, Van Sandt *et al.*, 2007). Thus, there appears to be an attempt to reduce the breakdown of the cell wall in the resistant genotype. Some of the detrimental effects of the parasite are attributed to increased levels of abscisic acid (Frost et al., 1997). The susceptible genotype upregulated genes involved in abscisic acid (ABA) signalling and genes induced by ABA, including ABA-responsive protein, Abscisic acid receptor PYL5, MAP kinase 3, Late embryogenesis abundant proteins and dehydrins. These genes are also involved in response to drought; however, the susceptible plant is increasing the amounts of abscisic acid and hence its effects.

Catalase isozyme 3 was upregulated by the resistant genotype, while the susceptible genotype upregulated various peroxidases. The activities of both enzymes maintain very low levels of H_2O_2 in roots (Salguero *et al.*, 1995). However, while catalase catalyses the disproportionation of H_2O_2 to give water and gaseous oxygen, peroxidases catalyse the hydrogen peroxide mediated oxidation of a wide variety of organic and inorganic substrates and this gives water and the oxidized substrates. In plant cells, when cell wall components are oxidized there is a release of 2, 4-dimethoxy-p-benzoquinone (DMBQ), which in turn induces haustorial development in the parasite (Yoder and Scholes, 2010) thus increasing attachment by the parasite. A number of marker genes that are known to be highly responsive to H_2O_2 were also upregulated by the susceptible genotype, indicating that this genotype was responding to reactive oxygen species. These include glutathione-S-transferase 5, 6, 34 and 42, plant L-ascorbate oxidase (Kapova *et al* 2002) and alternative oxidase 2 and 3. Alternative oxidases are often used as a general marker of mitochondrial dysfunction and/or cellular oxidative stress (Vanlerberghe, 2013). Alternative oxidases (AOX) are one of the terminal oxidases of the plant mitochondrial electron transport chain. It acts as a means to relax the highly coupled and tensed electron transport process in mitochondria thus providing and maintaining the much-needed metabolic homeostasis by directly reducing oxygen to water while releasing heat (Miller *et al*., 2011, Saha *et al*., 2016).

Genes involved in the synthesis of phytoalexins and benzazinoids including phenylalanine ammonia-lyase 1, Chalcone synthase C2, Dihydroflavonol-4-reductase and benzoxazinone synthesis 2, 3, 4 and 5 (three of the four genes that catalyse four consecutive hydroxylations form the defence compound DIMBOA. Frey *et al.,* (1995), Poloni *et al* (2014)) were all upregulated in the resistant genotype. So also, was Alpha-humulene/ (-) -(E)-beta-caryophyllene synthase which catalyses the synthesis of (E)-b-caryophyllene, which is released in response to attack by root herbivores attracting their natural enemies (Rasman et al., 2005). The above genes are all involved in plant defence against pathogens and hebivory. Stearoyl-acyl-carrier-protein desaturase, hypersensitive induced reaction 3, and hypersensitive-induced response protein 2 were also upregulated even though a hypersensitive reaction has not been intensively observed in *Striga* infestation in maize. The susceptible genotype downregulated some of these genes (e.g. Alpha-humulene/(-)-(E)-beta-caryophyllene synthase). It also downregulated trehalose-6-phosphate synthase 9 which is involved in the synthesis of trehalose which has been implicated in sugar metabolism and plant defence (Schluepmann *et al*., 2003, Fernandez *et al*., 2010), this is important because trehalose-6-phosphate phosphatase the last enzyme in the trehalose pathway was upregulated by

ZD05. 5057 upregulated BCL-2 binding anthanogene-1 and Bax inhibitor-1 (BI-1), both of which are involved in inhibiting programmed cell death. Transcription of plant BI-1 is highly upregulated under stressful conditions and overexpression of plant BI-1 leads to enhanced tolerance to not only Bax, but also H_2O_2 and other ROS-generating chemicals (Ishikawa *et al.*, 2009), suggesting that plant BI-1 acts as a universal suppressor of ROS mediated cell death under various environmental stresses (Ishikawa *et al.*, 2011). A number of heat shock proteins and heat shock factors, which are induced in response to various environmental stresses, were upregulated by 5057 genotype. This indicates that even at this early time point the 5057 (the susceptible genotype) was already under immense stress while ZD05 (the resistant genotype) was not.

Both the resistant and susceptible genotypes mobilized comprehensive responses to the presence of the parasite at this time point. However, the susceptible genotype, which upregulated a higher number of genes, was already under a lot of stress and appeared to be responding to the presence of reactive oxygen species, heat, drought and pathogens. Its responses in some cases would have increased the production of reactive oxygen species and heat. Heat would increase stress on the plant and ROS will increase the attachment of the parasite leading to a vicious cycle. The resistant genotype, on the other hand, responded to reactive oxygen species and the presence of pathogen/root herbivores. It can be inferred from the results that the attack by the parasite is more effective against the susceptible genotype as the upregulated genes show it was undergoing stress (e. g. heat stress and oxidative stress) while the resistant genotype was differentially regulating genes that will mitigate the parasite’s attack.

5.1.6.2. Molecular responses of both genotypes at the second period (9DPI).

At nine days post infestation, the susceptible maize genotype (5057) showed a wholesale downregulation of genes including genes involved in hormone and hormonal metabolism. Indicating a reduction in jasmonate, salicylic acid and auxin activity. While ZD05 upregulated abscisic acid 8'-hydroxylase 1 that catalyses the catabolism of Abscisic acid, indicating decreased abscisic acid activity. The resistant genotype upregulated S-adenosylmethionine synthase 1 and 1-aminocyclopropane-1-carboxylate (ACC) oxidases, enzymes in the ethene biosynthesis pathway and ethene-responsive transcription factors (ERF014, ERF053 and RAP2-11). This indicates that there was an increase in ethene synthesis and concentration at this time point. Ethene (ET), alone and in combination with other hormones like jasmonates, has been implicated as one of the key players in the determination of the most suitable genetic defence response (Adie *et al*., 2007). It has also been implicated in the transcriptional induction of the phytoalexin elicitor-releasing factor, b-1, 3-endoglucanase, in soybean. An upregulation of Endoglucanase 11 and 25 were also observed in the resistant genotype.

A host of genes involved in plant defence and secondary metabolism including Chalcone synthase 2, benzoxazinone synthesis 11 and 14, and terpene synthase 2, 8 and 23 were downregulated in the susceptible genotype but were upregulated in the resistant genotype. These genes control cell wall modification and defence response synthesis of phytoalexins, both of which are means through which the plant protects itself from invading pathogens.

A number of heat shock proteins and heat shock factors, as well as anti-apoptotic proteins (Bax inhibitor-1 family protein and BCL-2 binding anthanogene-1) were again upregulated by the susceptible genotype.

From the above, the progression of parasitization is evident in both genotypes. The resistant variety is responding by up-regulating genes involved in plant defence and secondary metabolite production. While the susceptible genoptype appears to be responding to damage by the parasite. At this time point the resistant genotype is upregulating genes that will effectively combat and/or mitigate the effects of infestation by the parasite, while the susceptible genotype is downregulating these genes and thus appears to be succumbing to infestation.

5.1.6.3. Molecular responses of both genotypes at the third time point (22DPI)

At 22 days after infestation, about 50 per cent of the genes upregulated by the resistant genotype were identical to about 50 per cent of the genes upregulated by the susceptible genotype at the first time point. This indicates that the resistant genotype reached the same level of damage after 22 days that the susceptible genotype reached after three days. Genes upregulated by ZD05 at this time point include heat shock proteins and genes involved in H_2O_2 metabolism, this indicates that the plant is now undergoing heat stress and oxidative stress. Similar suits of genes were also upregulated by the susceptible genotype; these genes have been upregulated in this genotype throughout the period of this experiment. Genes involved in secondary metabolism and plant defence were upregulated by both genotypes, however, ZD05 upregulated more of these genes. Genes involved in the metabolism of hormones were also upregulated in ZD05. These include IAA-amino acid hydrolase ILR1-like 4, SAUR-like auxin-responsive protein family and the Auxin response factor 16. IAA-amino acid hydrolase ILR1-like 4 regulates the levels of the auxin indole-3-acetic acid (IAA) by hydrolyzing of amide-linked conjugates that act as storage or inactivated forms of the hormone conjugates to free IAA in vitro (Rampey *et al*., 2005). SAUR-like auxin-responsive protein family is an early auxin responsive gene and the Auxin response factor 16 is positively regulated by auxins (Wang *et al*., 2005). The presence of increased amounts of these three genes in the resistant genotype at this time point indicates an increase in the action of Auxins. Jasmonate-induced protein, allene oxide synthase1, and Salicylate/benzoate carboxyl methyltransferase were upregulated; these genes are induced by methyl jasmonate and wounding indicating an increase in jasmonic acid (Koo *et al.,* 2007). Salicylate/benzoate carboxyl methyltransferase converts salicylic acid to methyl salicylate, which is volatile and can evaporate. It serves the purpose of attracting insect pollinators and predators that capture herbivorous insects that may have inflicted wounds on the plant (Knudsen *et al*., 1993; Van Poecke *et al*., 2001) but it also depletes the salicylic acid pool within the organism. This indicates that there is an increase in jasmonate activity and a reduction in the activity of salicylate. Abscisic acid 8'-hydroxylase 4, an enzyme that catalyses the irreversible degradation of Abscisic acid was upregulated by the susceptible genotype. This indicates a reduction in abscisic acid activity while the resistant genotype upregulated abscisic acid stress ripening 3 and ABA induced plasma membrane protein PM 19. This pattern was observed at 3 days post infestation in the susceptible genotype. This again indicates that ZD05 was in the state the 5057 was at three days post infestation. These results show that although the resistant genotype is beginning to show the deleterious effects of *Striga hermonthica* infestation, it is however responding more robustly and vigorously to the parasite than the susceptible genotype.

Across the three time periods root tissue was harvested from the plants, genes including those involved in secondary metabolism, plant defence, and cellular transport were upregulated in the resistant genotype ZD05. Secondary metabolites are compounds present in specialized cells. These compounds are not necessary for the cells' survival but are thought to be required for the plant's survival in the environment (Kliebenstein, 2004). Secondary metabolites mostly act as analogues of cellular signal compounds or substrates thus; they can affect and derail various physiological processes and constituent parts of pathogens like their biomembranes, enzymes, estrogenic properties and DNA alkylation (Morrissey, 2009). Most secondary metabolites can interact with proteins in one or another way by binding, complexing, denaturing, thereby leading to conformational changes in the protein and loss of activity or altered protein turnover (Wink, 2008; Goyal *et al.,* 2012).

Plant defence and defence related proteins include Resistance proteins and Pathogenesis-related proteins (PR). These proteins can be produced and accumulate both locally in the infected and surrounding tissues and in remote uninfected tissues. This production and accumulation in uninfected parts of plants can prevent the affected plants from further infection (Bowles 1990, Ryals *et al.,* 1996). Thus, making pathogenesis-related proteins very important in plants' response to invading pathogen and/or stress situation.

Cellular transporters like the ABC binding cassette family of transporters actively transport a diverse array of compounds across biological membranes, including toxins and secondary metabolites (Swarbrick *et al.,* 2008). Some of the detrimental effects of *Striga hermonthica* on the growth of its host may result from the production of a toxic metabolite by the parasite which has not yet been identified. It is therefore possible that one or more proteins will be involved in the transport or detoxification of such a *Striga*-derived metabolite. There is also increasing evidence of the involvement of ABC transporters in plant defence (Swarbrick *et al.,* 2008). Also, besides the synthesis of secondary metabolic defence compounds, their storage and transport may require cellular transporters like the ATP-binding cassette transporters (ABC-transporter) (Swarbrick *et al.,* 2008, Wink *et al.,* 2010). From the foregoing, it is evident that a combination of these genes will no doubt improve the survival of the resistant genotype in the event of an attack by the parasite.

CHAPTER 6

Conclusions and Recommendations

6.1. Conclusions

This study was carried out to quantify amount of diversity and population structure in *Striga hermonthica* weed populations in Nigeria and Kenya. The molecular responses of maize lines to infestation by the parasite was also investigated in a bid to elucidate the physiological, biochemical and molecular interactions between *Striga hermonthica* and infested maize plants. This study showed that;

Striga hermonthica populations had high amounts of genetic diversity. The Kenyan and Nigerian populations of *Striga hermonthica* represent distinct *Striga hermonthica* ecotypes. The Nigerian population is divided into three genetic groups; these three groups exist in different sub-regions within Nigeria.

The divergence of the Kenyan and Nigerian *S. hermonthica* populations as well as the divergence of the subpopulations within the Nigerian population was due to positive selection. This selection is mostly due to geographical location and not the host plants of the parasite.

ZD05 (the resistant genotype) showed little or no distortion in allometry due to parasitization by *Striga hermonthica*, while the susceptible genotype shows significant distortions in its allomertry.

ZD05 (the resistant genotype) resists/tolerates infestation by producing root exudates that induce the germination of fewer seeds of the parasite. It also had smaller total major roots length thus limiting the opportunity for attachment by the parasite. In addition, it retards and restricts the growth and development of attached parasites.

On a molecular level, the resistant (ZD05) genotype mobilizes a more comprehensive response to infestation by the parasite by up-regulating more genes involved in plant secondary metabolism, defence and cellular transport while the susceptible genotype (5057) responds to infestation by down-regulating some of these genes and up-regulating some abiotic stress genes..

6.2. Contributions to knowledge

The findings of this present study show that;

1) The parasitic plant *Striga hermonthica* exhibits high amounts of diversity and regionally adapted populations (ecotypes) of the parasite exist.

2) The parasitic plant is evolving and certain alleles in the genome of the plant are being selected for based on the location of the parasite within Africa.

3) The maize line ZD05 is resistant to *Striga hermonthica* infestation. The line has the capability to resist infestation by the parasite both before and after attachment.

4) The resistant plants resist infestation through multiple biochemical and physiological means. These include the production of root exudates with a lower ability to stimulate the germination of the parasite, a root architecture that reduces the probability of attachment and ability support the attachment of fewer parasites.

5) Multiple genes including defence genes, genes involved in secondary metabolism, and pathogenesis related genes control the resistance of maize to *Striga hermonthica* (That is, multigenic resistance).

6) Maize plants mobilize genes involved in plant secondary metabolism, defence and cellular transport in response to *Striga hermonthica* infestation including *phenylalanine ammonia lyase, Chalcone Synthase, Alpha-humulene/(-) -(E)-beta-caryophyllene synthase, Catalase 3, Chitinase, Cellulose synthase* and genes involved in the benzoxazinoid synthesis pathway.

6.2. Recommendations

This study strongly suggests that the testing of *S. hermonthica* control technologies in Nigeria should be done at sites representing the areas of collections of the three subpopulations.

As ZD05 shows resistance to the parasite, it can be used as germplasm and stock material upon which other resistance genes can be pyramided to develop maize varieties that will resistant *Striga hermonthica* from every region and host crop.

Further studies are recommended to characterize the *Striga hermonthica* subpopulations in Nigeria phenotypically to determine if they exhibit variations in their virulence characteristics.

In addition, gene products and metabolites from the genes observed to be involved in the resistance response of maize should be identified and characterized.

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APPENDICES

Appendix 1. Preparation of reagents

1. Reagents for DNA extraction

a) CTAB extraction buffer

The CTAB extraction buffer was prepared for 110 samples by mixing 1.5g of CTAB (Cetyl triammonium bromide), 15.5mL of 1.0M Tris-HCl (pH 7.5), 7.7mL of 0.5M EDTA (pH 8.0) and 30.8mL of 5.0M NaCl were mixed in a beaker and dissolved in the solution. 22.3 mL of double distilled water was added to the solution and then the beaker heated in a water bath to completely dissolve the CTAB. 800 uL of Mercaptoethanol was added immediately prior to use.

TRIS-HCl (1 M), 0.5 M Ethylenediaminetetraacetic acid (EDTA), 5 M Sodium Chloride (NaCl) were prepared as described below

- 1.0M TRIS-HCl

  121.1 g of TRIZMA base was dissolved in 500ml of water and then 40ml of concentrated HCl was added. The pH of the resulting solution was adjusted to 8.0 and the solution made up to 1000ml and autoclaved.

- 0.5 M EDTA

  146.1 g of Ethylenediaminetetraacetic acid was added to 500ml of water, the pH of the resulting solution adjusted to 7.5, the solution made up to 1000 ml and autoclaved.

- 5.0 M NaCl

  292 g of NaCl was added to 700 ml of water and the solution made up to 1000 ml and autoclaved

b) Chloroform Isoamyl alcohol (24:1).

94 ml of chloroform was mixed with 4ml of iso-amyl alcohol.

c) 70% Ethanol

30 ml of double-distilled water was added to 70ml of Ethanol

d) RNASE

RNase was obtained in its lyophilized form and was then reconstituted by adding to the entire contents of the bottle, 250 ul of TRIS buffer, 275 ul of 5 M NaCl and making up to 25ml, then heating the solution at 65°C for 5 minutes.

2. Reagents for gel electrophoresis

10 x Tris-Boric acid-EDTA (TBE) Buffer for running gel

216 g of trizma base, 110 g of boric acid and 16.6 g of EDTA were weighed into a beaker and distilled water added to make the solution up to 2 litres, after which it was autoclaved. The buffer was diluted to 1x before use.

3. Reagents for Genotyping by sequencing

a) PicoGreen

Working PicoGreen solution was prepared by adding 200 ml of low salt Tris-EDTA buffer to 1ml of DMSO-PicoGreen stock solution as indicated by the manufacturer.

b) Lambda DNA solution.

500m g/ul lambda DNA solution was diluted serially from 0 to 200 ng/ul with low-salt Tris-EDTA buffer.

c) Low-salt Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

The buffer was prepared by adding 1.21 g of trizma base and 0.29 g of EDTA in 500 ml of water. The PH was adjusted to 8 and the solution was made up to 1000 ml.

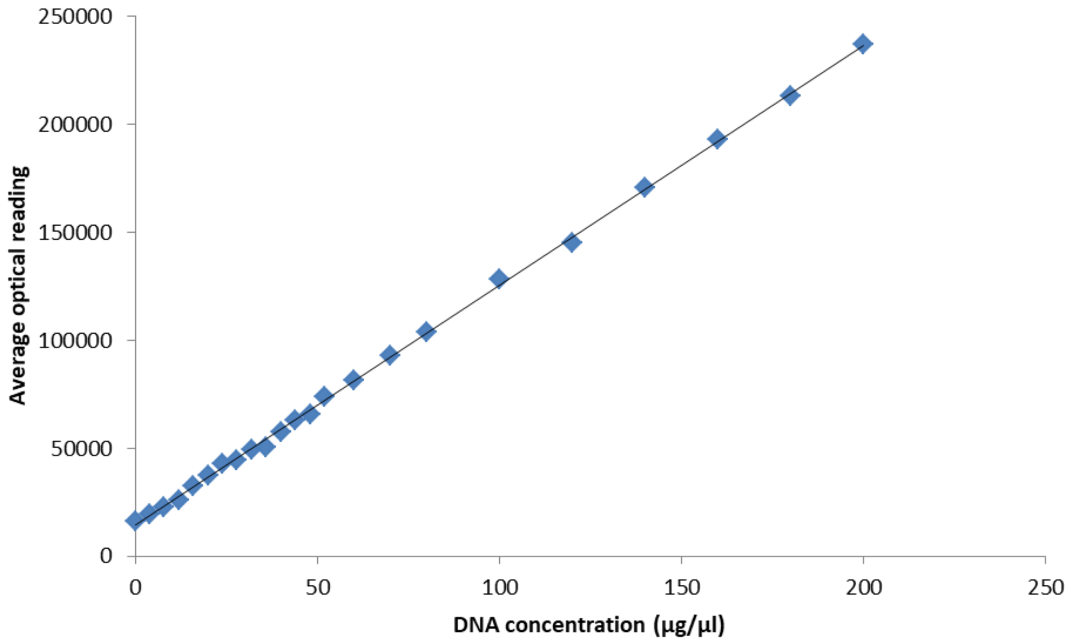
4. Reagents for RNA extraction

a) CTAB (Cetyl trimethyl ammonium bromide, hexadecyl trimethyl ammonium bromide) buffer (0.1 M Tris-HCl PH 8, 20 mM EDTA, 1.4M NaCl, 2 % polyvinyl pyrrolidone)

The CTAB extraction buffer was prepared for 110 samples by mixing 1.5g of CTAB (Cetyl triammonium bromide), 15.5mL of 1.0M Tris-HCl (pH 7.5), 7.7mL of 0.5M EDTA (pH 8.0), 30.8mL of 5.0M NaCl and 2 g polyvinyl pyrrolidone with 22.3 mL of double distilled water.

b) DNASE

DNASE enzyme reconstituted by adding to the entire content of the bottle (50 µg of lyophilised DNase powder), 250 ul of low salt TE buffer, 5ul of 5 M NaCl and making up to 25 ml with double distilled water, then heating the solution at 65 °C for 5minutes.



Appendix 2. Standard curve for Determining DNA concentration

Appendix 3. List of differentially expressed genes at the first time period (3 days post infestation)

Genes Present in both genotypes

Gene Id	SAI	SAU	log2 fold change	P value	Gene Id.1	RAI	RAU	log2 fold change	P value
Zm00001d047441	12.15	3.21	-1.9202	0.0004	Zm00001d047441	2.268	51.3	4.4978	0.00005
Zm00001d018064	4.881	0.98	-2.31171	0.0002	Zm00001d018064	0.845	3.46	2.0355	0.0006
Zm00001d004243	40.68	9.17	-2.14895	0.00005	Zm00001d004243	3.281	11.3	1.7784	0.00005
Zm00001d028887	72.56	18.3	-1.9888	0.00005	Zm00001d028887	11.09	30.8	1.4739	0.0001
Zm00001d033478	7.645	1.96	-1.96292	0.00805	Zm00001d033478	12.14	33.8	1.4777	0.0008
Zm00001d028888	69.4	18	-1.94669	0.00005	Zm00001d028888	9.789	27.4	1.4826	0.0002
Zm00001d045470	22.06	7.88	-1.48525	0.00065	Zm00001d045470	5.893	21	1.8361	0.00005
Zm00001d031168	415.8	129	-1.69008	0.00005	Zm00001d031168	445.3	1243	1.4808	0.00005
Zm00001d018335	5.592	1.92	-1.54505	0.00435	Zm00001d018335	6.413	18.7	1.5431	0.0001
Zm00001d033794	6.968	2.44	-1.51347	0.002	Zm00001d033794	2.657	7.39	1.4761	0.003
Zm00001d031971	31.65	1.87	-4.0823	0.00005	Zm00001d031971	16	4.28	-1.903	0.00105
Zm00001d024522	43.71	4.23	-3.36997	0.00005	Zm00001d024522	51.15	15.4	-1.728	0.00005
Zm00001d030028	10.41	1.01	-3.36035	0.0008	Zm00001d030028	10.91	3.23	-1.756	0.0021
Zm00001d043929	23.69	1.62	-3.86684	0.0011	Zm00001d043929	18.29	3.75	-2.287	0.001
Zm00001d053220	11.24	1.14	-3.29889	0.0021	Zm00001d053220	18.97	5.56	-1.771	0.00095
Zm00001d019605	8.701	0.94	-3.21324	0.00005	Zm00001d019605	8.546	2.45	-1.801	0.0004
Zm00001d048819	10.37	1.05	-3.30533	0.00005	Zm00001d048819	9.442	1.89	-2.319	0.00005
Zm00001d045101	9.1	1.39	-2.71237	0.00315	Zm00001d045101	48.35	12.3	-1.979	0.00005
Zm00001d053746	30.95	3.81	-3.02181	0.00005	Zm00001d053746	38.66	7.79	-2.311	0.00005
Zm00001d006211	4.681	0.75	-2.64783	0.00435	Zm00001d006211	4.926	1.27	-1.961	0.00445
Zm00001d029906	18.05	3.02	-2.58191	0.00005	Zm00001d029906	8.256	2.2	-1.905	0.00155
Zm00001d032869	3.619	0.78	-2.21235	0.0066	Zm00001d032869	3.967	1.31	-1.602	0.0065
Zm00001d034217	15	3.78	-1.98927	0.00005	Zm00001d034217	12.95	4.34	-1.576	0.00095
Zm00001d025059	8.361	1.47	-2.5114	0.0006	Zm00001d025059	24.16	5.52	-2.13	0.00015
Zm00001d039010	22.28	4.47	-2.3181	0.0006	Zm00001d039010	14.21	2.99	-2.247	0.0026
Zm00001d008251	1.589	5.15	1.69702	0.00365	Zm00001d008251	2.603	8.43	1.6955	0.00115
Zm00001d013635	6.702	2.08	-1.68843	0.00555	Zm00001d013635	9.136	2.72	-1.747	0.00115
Zm00001d000556	1.93	0.58	-1.72805	0.0039	Zm00001d000556	4.689	1.32	-1.832	0.00345
Zm00001d037384	24.92	6.8	-1.87299	0.00025	Zm00001d037384	19.53	4.94	-1.983	0.0001
Zm00001d006596	13.44	3.86	-1.8004	0.00005	Zm00001d006596	17.2	4.44	-1.953	0.00005
Zm00001d008604	10.96	3.96	-1.46883	0.0003	Zm00001d008604	15.74	4.41	-1.835	0.00005
Zm00001d008837	9.797	2.47	-1.98729	0.00235	Zm00001d008837	15.26	2.63	-2.539	0.00005
Zm00001d022524	8.626	40.8	2.24018	0.00005	Zm00001d022524	6.446	20.6	1.6737	0.0001

Zm00001d031769	8.419	1.59	-2.40694	0.00065	Zm00001d031769	10.47	1.22	-3.101	0.00005
Zm00001d003866	4.231	1.41	-1.58605	0.00565	Zm00001d003866	4.453	0.72	-2.626	0.00285
Zm00001d018744	56.98	15.3	-1.89219	0.0022	Zm00001d018744	507.8	44.5	-3.512	0.00005
Zm00001d048710	1.675	6.36	1.92452	0.0029	Zm00001d048710	42.88	6.2	-2.789	0.00005
Zm00001d048709	6.179	30.8	2.31864	0.00005	Zm00001d048709	106.1	18.3	-2.533	0.00005
Zm00001d014032	1.788	0	-	0.00725	Zm00001d014032	3.624	0	-	0.0024
Zm00001d020332	2.422	0	-	0.00005	Zm00001d020332	4.269	0.67	-2.681	0.00405
Zm00001d022390	1.769	0	-	0.00005	Zm00001d022390	1.866	0	-	0.00005
Zm00001d053965	1.919	0	-	0.00475	Zm00001d053965	3.038	0	-	0.0005

Genes upregulated in the susceptible genotype					Genes upregulated in the resistant genotype				
Gene Id	SAI	SAU	log2 fold change	P value	Gene Id	RAI	RAU	log2 fold change	P value
Zm00001d001766	3.448	0	-	0.00005	Zm00001d000659	1.977	0	-	0.00005
Zm00001d001774	4.03	0	-	0.00005	Zm00001d001650	4.687	0	-	0.00015
Zm00001d001960	2.664	0	-	0.00005	Zm00001d003020	4.017	0	-	0.00005
Zm00001d003709	1.671	0	-	0.00725	Zm00001d005790	3.096	0	-	0.00005
Zm00001d005146	3.392	0	-	0.0008	Zm00001d005841	3.766	0	-	0.00005
Zm00001d005860	1.857	0	-	0.00005	Zm00001d006196	6.083	0	-	0.00205
Zm00001d006256	2.468	0	-	0.0001	Zm00001d006821	5.743	0	-	0.00005
Zm00001d006815	3.704	0	-	0.00005	Zm00001d007957	3.01	0	-	0.0064
Zm00001d007076	3.378	0	-	0.00005	Zm00001d008793	1.884	0	-	0.0002
Zm00001d007411	3.908	0	-	0.00005	Zm00001d010588	2.477	0	-	0.00005
Zm00001d007898	2.825	0	-	0.00005	Zm00001d016342	4.146	0	-	0.00025
Zm00001d009943	3.235	0	-	0.00045	Zm00001d017412	1.715	0	-	0.00035
Zm00001d010814	4.175	0	-	0.00015	Zm00001d018629	1.945	0	-	0.00245
Zm00001d011133	2.574	0	-	0.00025	Zm00001d018917	3.602	0	-	0.00045
Zm00001d011919	2.389	0	-	0.00025	Zm00001d020028	162.5	0	-	0.00195
Zm00001d012304	3.124	0	-	0.00005	Zm00001d020658	1.729	0	-	0.00015
Zm00001d014733	7.194	0	-	0.00005	Zm00001d020773	2.308	0	-	0.00005
Zm00001d014774	1.767	0	-	0.00815	Zm00001d021439	2.625	0	-	0.00625
Zm00001d017002	5.697	0	-	0.00005	Zm00001d022197	3.416	0	-	0.0002
Zm00001d017462	2.694	0	-	0.00045	Zm00001d022593	4.103	0	-	0.00005
Zm00001d017477	1.69	0	-	0.00075	Zm00001d025957	2.645	0	-	0.00005
Zm00001d017645	2.986	0	-	0.0004	Zm00001d027290	3.005	0	-	0.0001
Zm00001d017991	4.973	0	-	0.00465	Zm00001d027313	2.051	0	-	0.00005
Zm00001d019463	1.923	0	-	0.00005	Zm00001d027456	5.139	0	-	0.00005
Zm00001d019750	2.767	0	-	0.00005	Zm00001d028839	3.716	0	-	0.00005
Zm00001d020736	2.73	0	-	0.00005	Zm00001d029028	4.027	0	-	0.0002

Zm00001d020797	3.328	0	-	0.00005
Zm00001d021315	2.872	0	-	0.00005
Zm00001d021329	1.841	0	-	0.00015
Zm00001d021591	3.543	0	-	0.00005
Zm00001d021666	1.702	0	-	0.00005
Zm00001d022416	9.183	0	-	0.00005
Zm00001d023040	42.57	0	-	0.00005
Zm00001d023419	3.309	0	-	0.00135
Zm00001d023856	5.873	0	-	0.00005
Zm00001d023888	2.303	0	-	0.0002
Zm00001d025333	4.31	0	-	0.00005
Zm00001d026447	2.523	0	-	0.0008
Zm00001d026662	8.084	0	-	0.00005
Zm00001d027901	2.966	0	-	0.0008
Zm00001d027929	4.397	0	-	0.00005
Zm00001d027944	3.669	0	-	0.00045
Zm00001d028389	3.494	0	-	0.00005
Zm00001d028793	2.251	0	-	0.0054
Zm00001d028941	2.487	0	-	0.00185
Zm00001d031184	1.994	0	-	0.0002
Zm00001d031278	3.183	0	-	0.00005
Zm00001d031423	6.102	0	-	0.00005
Zm00001d031805	1.879	0	-	0.00045
Zm00001d032036	1.904	0	-	0.0002
Zm00001d032087	2.255	0	-	0.00475
Zm00001d033531	19.73	0	-	0.00005
Zm00001d033793	1.964	0	-	0.0035
Zm00001d034145	3.286	0	-	0.00005
Zm00001d035095	4.573	0	-	0.00005
Zm00001d035963	1.813	0	-	0.0005
Zm00001d036973	1.839	0	-	0.00005
Zm00001d038117	1.462	0	-	0.0035
Zm00001d040324	2.063	0	-	0.00065
Zm00001d042035	2.214	0	-	0.00005
Zm00001d042765	2.758	0	-	0.00475
Zm00001d043610	3.785	0	-	0.00285
Zm00001d043974	2.641	0	-	0.00045
Zm00001d044227	2.208	0	-	0.00725
Zm00001d044700	2.176	0	-	0.0009
Zm00001d044861	4.41	0	-	0.0009
Zm00001d030698	2.757	0	-	0.00205
Zm00001d033595	1.718	0	-	0.00035
Zm00001d035759	8.266	0	-	0.00195
Zm00001d037435	5.658	0	-	0.00005
Zm00001d040210	13.7	0	-	0.00005
Zm00001d040674	3.379	0	-	0.00015
Zm00001d047115	1.803	0	-	0.00005
Zm00001d047139	1.774	0	-	0.00005
Zm00001d048161	2.613	0	-	0.00005
Zm00001d049016	2.509	0	-	0.00085
Zm00001d051519	2.374	0	-	0.00595
Zm00001d051887	2.395	0	-	0.00005
Zm00001d052122	5.861	0	-	0.0017
Zm00001d052662	1.641	0	-	0.00005
Zm00001d052743	5.744	0	-	0.00145
Zm00001d052872	3.05	0	-	0.00005
Zm00001d053988	7.128	0	-	0.00015
Zm00001d054067	1.93	0	-	0.00005
zma-MIR164d	7.828	0	-	0.00145
Zm00001d028952	12.08	4.42	-1.449	0.00005
Zm00001d038447	16.1	5.88	-1.453	0.00025
Zm00001d009506	15.83	5.77	-1.456	0.00045
Zm00001d016933	14.64	5.33	-1.457	0.0011
Zm00001d041472	7.962	2.9	-1.457	0.00845
Zm00001d048050	100.9	36.6	-1.461	0.00005
Zm00001d038003	59.8	21.7	-1.463	0.0002
Zm00001d033286	124.3	45	-1.464	0.00005
Zm00001d007187	30.7	11.1	-1.464	0.00015
Zm00001d044533	86.86	31.4	-1.467	0.00005
Zm00001d016878	3.636	1.31	-1.47	0.0071
Zm00001d044008	3.792	1.36	-1.479	0.00005
Zm00001d017613	11.55	4.14	-1.482	0.002
Zm00001d007783	3.338	1.19	-1.485	0.00195
Zm00001d043766	20.3	7.22	-1.492	0.0008
Zm00001d002174	3.987	1.41	-1.5	0.00255
Zm00001d042731	18.07	6.39	-1.5	0.0001
Zm00001d028599	12.8	4.47	-1.518	0.0003
Zm00001d038578	15.13	5.24	-1.53	0.0012
Zm00001d021935	17.96	6.22	-1.531	0.00175
Zm00001d022547	2.986	1.03	-1.532	0.00715

Zm00001d045535	2.015	0	-	0.00125
Zm00001d046253	3.558	0	-	0.00005
Zm00001d046471	8.267	0	-	0.00005
Zm00001d047420	2.109	0	-	0.007
Zm00001d047535	1.705	0	-	0.00725
Zm00001d047913	3.919	0	-	0.00005
Zm00001d047932	2.056	0	-	0.00005
Zm00001d048208	3.561	0	-	0.00005
Zm00001d048533	4.342	0	-	0.00005
Zm00001d049103	2.632	0	-	0.0001
Zm00001d049189	2.099	0	-	0.00075
Zm00001d049768	2.101	0	-	0.00005
Zm00001d050864	5.578	0	-	0.0009
Zm00001d051939	2.46	0	-	0.00005
Zm00001d052242	3.257	0	-	0.00005
Zm00001d052530	2.967	0	-	0.00005
Zm00001d053736	3.263	0	-	0.00005
Zm00001d048021	40.84	15	-1.44504	0.0001
Zm00001d022458	12.31	4.5	-1.45075	0.00015
Zm00001d005190	7.014	2.56	-1.45287	0.0008
Zm00001d042276	43.84	16	-1.45396	0.00005
Zm00001d004472	13.93	5.08	-1.4543	0.00005
Zm00001d007604	24.16	8.82	-1.45435	0.0003
Zm00001d002342	8.612	3.14	-1.45611	0.0052
Zm00001d007700	20.53	7.47	-1.4585	0.00325
Zm00001d002584	27.01	9.82	-1.46024	0.0016
Zm00001d051174	109.3	39.7	-1.4607	0.00005
Zm00001d052224	17.65	6.4	-1.46275	0.0041
Zm00001d027652	476.1	173	-1.4644	0.00005
Zm00001d015091	643.1	232	-1.47233	0.00005
Zm00001d024294	30.34	10.9	-1.47557	0.001
Zm00001d027645	6.521	2.34	-1.47758	0.003
Zm00001d043019	17.61	6.31	-1.48114	0.00055
Zm00001d048787	189.7	67.9	-1.48224	0.0004
Zm00001d021695	9.425	3.37	-1.48551	0.00135
Zm00001d030342	11.35	4.05	-1.48693	0.0066
Zm00001d032692	10.04	3.58	-1.48766	0.00035
Zm00001d028588	5.18	1.85	-1.48902	0.00405
Zm00001d017099	19.72	6.99	-1.49639	0.00145
Zm00001d004506	5.814	2.05	-1.5022	0.00355
Zm00001d020971	3.56	1.23	-1.533	0.0077
Zm00001d018742	8.739	3.01	-1.536	0.0026
Zm00001d028214	20.45	7.02	-1.542	0.00005
Zm00001d036833	4.401	1.51	-1.544	0.00075
Zm00001d009010	18.36	6.28	-1.547	0.00055
Zm00001d052726	72.58	24.8	-1.547	0.00005
Zm00001d021006	16.32	5.58	-1.548	0.00125
Zm00001d006199	142.9	48.7	-1.554	0.00005
Zm00001d013055	8.732	2.96	-1.559	0.00025
Zm00001d003048	2.62	0.89	-1.56	0.0008
Zm00001d009586	6.765	2.29	-1.565	0.0067
Zm00001d053593	271.6	91.5	-1.569	0.00475
Zm00001d007072	10.55	3.56	-1.569	0.00085
Zm00001d028472	6.809	2.29	-1.57	0.00005
Zm00001d028219	14.71	4.92	-1.579	0.00005
Zm00001d026156	4.846	1.62	-1.58	0.00405
Zm00001d026628	10.86	3.63	-1.581	0.0056
Zm00001d016995	2.904	0.97	-1.581	0.00095
Zm00001d047853	2.287	0.76	-1.582	0.0027
Zm00001d008925	43.79	14.5	-1.59	0.00005
Zm00001d020915	12.19	4.03	-1.597	0.00785
Zm00001d030222	18.2	6.02	-1.597	0.0006
Zm00001d043179	6.647	2.2	-1.598	0.0016
Zm00001d054060	20.31	6.7	-1.601	0.00045
Zm00001d039283	158.6	52.3	-1.602	0.00005
Zm00001d022270	14.31	4.7	-1.607	0.0064
Zm00001d030171	3.857	1.26	-1.609	0.0067
Zm00001d032467	28	9.17	-1.611	0.0001
Zm00001d040670	6.408	2.09	-1.616	0.0002
Zm00001d054044	47.91	15.6	-1.618	0.00005
Zm00001d025012	14.76	4.81	-1.618	0.00005
Zm00001d024891	21.6	7.01	-1.623	0.00005
Zm00001d010700	7.259	2.36	-1.624	0.00035
Zm00001d010521	17.29	5.61	-1.624	0.0003
Zm00001d040555	6.055	1.96	-1.626	0.00215
Zm00001d027546	8.733	2.81	-1.635	0.0016
Zm00001d015410	6.282	2.02	-1.635	0.0039
Zm00001d017714	5.968	1.92	-1.636	0.0017
Zm00001d021753	6.031	1.93	-1.643	0.0002
Zm00001d008746	19.74	6.32	-1.644	0.0001

Zm00001d041327	2.72	0.96	-1.50275	0.00755	Zm00001d043084	4.761	1.52	-1.644	0.00015
Zm00001d023215	37.78	13.3	-1.50308	0.00005	Zm00001d052164	13.09	4.16	-1.653	0.00585
Zm00001d042123	3.517	1.24	-1.50324	0.0053	Zm00001d037680	23.03	7.32	-1.654	0.0009
Zm00001d033718	7.051	2.47	-1.51105	0.0025	Zm00001d032250	9.03	2.85	-1.661	0.0023
Zm00001d002564	7.447	2.6	-1.51969	0.00285	Zm00001d020272	8.578	2.71	-1.665	0.0029
Zm00001d012477	7.007	2.43	-1.52595	0.0029	Zm00001d003414	6.58	2.07	-1.665	0.0064
Zm00001d028711	4.318	1.5	-1.52797	0.00575	Zm00001d031186	8.257	2.58	-1.677	0.003
Zm00001d042993	20.48	7.08	-1.53138	0.0001	Zm00001d044254	9.528	2.98	-1.679	0.0003
Zm00001d020555	7.292	2.52	-1.53277	0.00635	Zm00001d046423	6.351	1.98	-1.68	0.0025
Zm00001d029391	5.468	1.89	-1.53367	0.008	Zm00001d005658	36.63	11.4	-1.686	0.0001
Zm00001d048054	11.12	3.84	-1.53532	0.0002	Zm00001d039387	32.61	10.1	-1.694	0.00035
Zm00001d003144	6.04	2.08	-1.53595	0.00545	Zm00001d049910	3.288	1.01	-1.701	0.0017
Zm00001d032923	52.79	18.1	-1.54336	0.00005	Zm00001d020982	15.56	4.74	-1.714	0.0012
Zm00001d026060	14.57	4.98	-1.54766	0.00055	Zm00001d007394	6.354	1.93	-1.717	0.0068
Zm00001d013493	42.26	14.4	-1.5488	0.00005	Zm00001d007427	2.217	0.67	-1.73	0.00115
Zm00001d040697	193	65.9	-1.54968	0.00005	Zm00001d023659	10.2	3.07	-1.731	0.00005
Zm00001d043787	5.336	1.82	-1.55242	0.0063	Zm00001d040720	12.25	3.69	-1.732	0.0018
Zm00001d042906	12.14	4.13	-1.55559	0.0012	Zm00001d034022	19.35	5.82	-1.734	0.0003
Zm00001d009765	5.427	1.84	-1.55672	0.0002	Zm00001d027755	12.55	3.77	-1.735	0.0005
Zm00001d021263	25.28	8.59	-1.55816	0.0002	Zm00001d034518	9.366	2.81	-1.739	0.00215
Zm00001d003298	7.719	2.61	-1.5655	0.0084	Zm00001d031155	18.74	5.61	-1.741	0.0076
Zm00001d045000	65.17	22	-1.56634	0.00085	Zm00001d022126	16.42	4.87	-1.753	0.00015
Zm00001d020383	79.2	26.7	-1.56776	0.00005	Zm00001d002532	14.42	4.25	-1.763	0.00005
Zm00001d052978	13.93	4.7	-1.56907	0.0032	Zm00001d017333	13.89	4.08	-1.768	0.00055
Zm00001d025166	25.25	8.51	-1.56961	0.0001	Zm00001d011813	913.1	266	-1.78	0.00005
Zm00001d034745	12.68	4.22	-1.58783	0.00755	Zm00001d029847	2.819	0.81	-1.808	0.0051
Zm00001d038870	13.68	4.54	-1.59033	0.0023	Zm00001d006570	5.146	1.47	-1.811	0.00375
Zm00001d042767	16.64	5.52	-1.59195	0.00165	Zm00001d010210	10.6	3	-1.822	0.00055
Zm00001d029903	23.62	7.83	-1.59283	0.0009	Zm00001d028649	5.212	1.47	-1.822	0.0005
Zm00001d033957	3.056	1.01	-1.59366	0.0006	Zm00001d003037	1.972	0.55	-1.851	0.00655
Zm00001d039241	11.54	3.82	-1.59612	0.0049	Zm00001d028260	172.2	47.5	-1.86	0.00005
Zm00001d040148	14.21	4.7	-1.59664	0.0005	Zm00001d014091	3.796	1.04	-1.863	0.0036
Zm00001d038598	37.16	12.3	-1.5971	0.00025	Zm00001d011328	10.18	2.77	-1.879	0.00095
Zm00001d002253	51.41	17	-1.60036	0.0008	Zm00001d046697	19.31	5.25	-1.88	0.00005
Zm00001d017984	16.47	5.4	-1.60886	0.00465	Zm00001d053410	57.49	15.6	-1.885	0.00045
Zm00001d045519	10.73	3.51	-1.61399	0.00035	Zm00001d027435	8.041	2.17	-1.89	0.0012
Zm00001d033187	6.298	2.06	-1.61545	0.00225	Zm00001d048705	214.6	57.6	-1.899	0.00005
Zm00001d020887	12.63	4.1	-1.62399	0.00135	Zm00001d011363	23.84	6.33	-1.914	0.00005
Zm00001d038288	18.99	6.14	-1.62985	0.00215	Zm00001d032719	3.087	0.82	-1.916	0.0054
Zm00001d035659	41.71	13.4	-1.63446	0.00005	Zm00001d004689	306.9	81.1	-1.921	0.00005

Zm00001d030348	15.16	4.88	-1.63635	0.0002	Zm00001d002848	7.272	1.91	-1.927	0.00215
Zm00001d007705	26.01	8.35	-1.63919	0.0006	Zm00001d039173	21.2	5.56	-1.932	0.00005
Zm00001d053394	258.8	83	-1.64056	0.00005	Zm00001d001984	7.432	1.94	-1.936	0.0018
Zm00001d017455	17.88	5.72	-1.64348	0.0018	Zm00001d051859	6.204	1.62	-1.938	0.00455
Zm00001d051800	27.27	8.72	-1.64482	0.0012	Zm00001d047830	5.411	1.41	-1.943	0.0007
Zm00001d021973	8.587	2.74	-1.6453	0.0041	Zm00001d041951	6.65	1.73	-1.946	0.0002
Zm00001d023664	42.12	13.4	-1.64768	0.00005	Zm00001d036023	1.969	0.51	-1.951	0.004
Zm00001d013489	24.89	7.94	-1.6485	0.00025	Zm00001d005951	16.25	4.19	-1.954	0.00005
Zm00001d010617	9.943	3.16	-1.65604	0.00695	Zm00001d038971	7.501	1.93	-1.956	0.00215
Zm00001d037828	8.683	2.74	-1.66586	0.0056	Zm00001d020961	3.821	0.98	-1.965	0.00305
Zm00001d006947	5.897	1.86	-1.66841	0.003	Zm00001d038355	2.67	0.68	-1.974	0.00505
Zm00001d007341	23.1	7.26	-1.66904	0.0002	Zm00001d017536	5.879	1.49	-1.985	0.0003
Zm00001d045302	29.42	9.24	-1.67097	0.00015	Zm00001d041787	1.867	0.47	-1.986	0.0001
Zm00001d012420	423.4	133	-1.67155	0.00005	Zm00001d035682	17.21	4.34	-1.987	0.00005
Zm00001d020552	15.63	4.9	-1.67412	0.0005	Zm00001d018364	5.02	1.27	-1.987	0.00385
Zm00001d005148	10.43	3.27	-1.67503	0.0023	Zm00001d026695	5.308	1.33	-1.993	0.0042
Zm00001d017276	84.03	26.3	-1.67663	0.00005	Zm00001d027925	90.08	22.6	-1.995	0.00005
Zm00001d050119	10.44	3.25	-1.68176	0.0012	Zm00001d002362	1.757	0.44	-2	0.0001
Zm00001d034601	46.44	14.4	-1.69134	0.00005	Zm00001d040544	63.59	15.8	-2.006	0.00005
Zm00001d044685	14.6	4.49	-1.7005	0.0061	Zm00001d031453	6.406	1.59	-2.012	0.00125
Zm00001d026163	17.41	5.35	-1.70222	0.0022	Zm00001d048865	3.95	0.97	-2.023	0.00775
Zm00001d003751	55.23	17	-1.70421	0.00005	Zm00001d020960	1.88	0.46	-2.04	0.00005
Zm00001d051543	92.61	28.4	-1.70462	0.00005	Zm00001d031945	2.536	0.61	-2.064	0.0063
Zm00001d021981	7.34	2.25	-1.70606	0.00805	Zm00001d015513	6.387	1.5	-2.092	0.00155
Zm00001d013811	8.595	2.63	-1.70767	0.00435	Zm00001d006561	9.612	2.24	-2.101	0.00755
Zm00001d046599	30.33	9.28	-1.70807	0.00155	Zm00001d045395	6.289	1.44	-2.128	0.0076
Zm00001d008266	9.085	2.78	-1.71003	0.00595	Zm00001d050872	33.13	7.43	-2.156	0.00005
Zm00001d052651	13.55	4.14	-1.71012	0.00415	Zm00001d033607	11.33	2.53	-2.161	0.0006
Zm00001d041611	31.95	9.74	-1.71387	0.00645	Zm00001d042275	3.735	0.8	-2.223	0.0057
Zm00001d027548	15.28	4.66	-1.71406	0.0042	Zm00001d005901	6.555	1.39	-2.234	0.00165
Zm00001d035054	11.11	3.39	-1.71448	0.0077	Zm00001d039698	23.44	4.96	-2.24	0.00005
Zm00001d023596	12.74	3.88	-1.71498	0.00125	Zm00001d028056	3.931	0.82	-2.253	0.0039
Zm00001d005023	56.3	17.1	-1.71809	0.0007	Zm00001d006082	10.24	2.1	-2.284	0.0011
Zm00001d046330	3.778	1.14	-1.72653	0.00155	Zm00001d014239	1.704	0.35	-2.286	0.00525
Zm00001d020970	5.429	1.64	-1.72971	0.00185	Zm00001d033619	2.106	0.43	-2.3	0.00725
Zm00001d046945	13.43	4.04	-1.73489	0.0002	Zm00001d048702	182.3	34.5	-2.402	0.00005
Zm00001d036066	12.35	3.71	-1.73492	0.0008	Zm00001d048703	139.1	26.2	-2.409	0.00005
Zm00001d004840	4.03	1.21	-1.73573	0.00425	Zm00001d026070	3.614	0.67	-2.439	0.00005
Zm00001d007462	4.628	1.39	-1.73836	0.00305	Zm00001d029696	6.957	1.28	-2.446	0.0048
Zm00001d010451	17.79	5.33	-1.74002	0.0012	Zm00001d030037	1.914	0.34	-2.483	0.0053

Zm00001d052793	4.515	1.35	-1.74334	0.00175
Zm00001d034421	2.71	0.81	-1.74917	0.0018
Zm00001d045205	18.81	5.59	-1.75079	0.004
Zm00001d040581	29.69	8.79	-1.7554	0.00005
Zm00001d039000	60.12	17.8	-1.75617	0.00005
Zm00001d016019	9.504	2.81	-1.75868	0.00795
Zm00001d006591	7.914	2.34	-1.75957	0.00225
Zm00001d011912	4.738	1.4	-1.76094	0.0057
Zm00001d009556	37.28	11	-1.76383	0.00005
Zm00001d047764	12.08	3.56	-1.7642	0.0007
Zm00001d006230	8.419	2.47	-1.76853	0.00015
Zm00001d025507	7.408	2.17	-1.77027	0.00005
Zm00001d028144	6.958	2.04	-1.7704	0.0033
Zm00001d037894	6.539	1.91	-1.77589	0.0079
Zm00001d010038	16.66	4.86	-1.77667	0.00335
Zm00001d038274	4.072	1.19	-1.77829	0.00775
Zm00001d048634	15.43	4.49	-1.78083	0.0011
Zm00001d006555	20.6	5.99	-1.78118	0.00415
Zm00001d032162	20.67	6.01	-1.78214	0.00055
Zm00001d005543	13.66	3.96	-1.78609	0.0013
Zm00001d042209	12.69	3.65	-1.79667	0.0064
Zm00001d042449	9.008	2.59	-1.79873	0.0019
Zm00001d003730	73.53	21.1	-1.79958	0.00005
Zm00001d049932	10.63	3.03	-1.8095	0.0054
Zm00001d017719	48.23	13.7	-1.81452	0.0004
Zm00001d021422	20.5	5.82	-1.81572	0.007
Zm00001d011845	9.809	2.78	-1.81665	0.00705
Zm00001d018298	103.6	29.3	-1.82341	0.00005
Zm00001d041236	8.538	2.4	-1.82905	0.0007
Zm00001d044111	9.345	2.62	-1.83698	0.00155
Zm00001d013111	45.82	12.7	-1.84904	0.00005
Zm00001d017557	19.78	5.47	-1.85469	0.00045
Zm00001d022453	13.35	3.69	-1.85569	0.00115
Zm00001d019994	13.35	3.66	-1.868	0.0006
Zm00001d002436	84.9	23.2	-1.86865	0.00005
Zm00001d039542	5.544	1.51	-1.87706	0.00675
Zm00001d003160	11.23	3.06	-1.87823	0.0031
Zm00001d051485	13.28	3.6	-1.88345	0.00055

Zm00001d030213	5.316	0.95	-2.484	0.0001
Zm00001d047736	56.88	10.1	-2.487	0.00005
Zm00001d025964	4.458	0.79	-2.505	0.0056
Zm00001d004687	75.08	13.2	-2.508	0.00005
Zm00001d012221	10.51	1.68	-2.648	0.00055
Zm00001d003208	3.987	0.61	-2.717	0.00235
Zm00001d053409	7.685	1.04	-2.883	0.0003
Zm00001d017249	8.548	1.16	-2.885	0.0065
Zm00001d012801	1.876	0.22	-3.089	0.0008
Zm00001d052673	11.78	1.25	-3.238	0.00015
Zm00001d035713	7.825	0.52	-3.905	0.00105

Genes downregulated in the resistant genotype

gene_id.1	RAI	RAU	log2 fold change	P value
Zm00001d006495	0	3.13	∞	0.00225
Zm00001d012443	0	40.6	∞	0.0068
Zm00001d012848	0	4.84	∞	0.00005
Zm00001d019002	0	5.59	∞	0.0068
Zm00001d021504	0	8.01	∞	0.00005
Zm00001d022084	0	3.61	∞	0.00005
Zm00001d025423	0	3.64	∞	0.00535
Zm00001d035222	0	2.73	∞	0.0001
Zm00001d037167	0	5.39	∞	0.00005
Zm00001d038403	0	14.8	∞	0.00015
Zm00001d038997	0	7.31	∞	0.00055
Zm00001d039170	0	4.56	∞	0.00535
Zm00001d041356	0	1.74	∞	0.00005
Zm00001d050396	0	1.88	∞	0.00005
zma-MIR169i	0	29.4	∞	0.00005
Zm00001d019137	53.53	1211	4.4998	0.00005
Zm00001d001139	165.1	3435	4.3794	0.00005
Zm00001d050295	0.685	8.77	3.6796	0.0008
Zm00001d050682	2.58	30	3.5416	0.00005
Zm00001d048760	1.532	17.1	3.4759	0.00265
Zm00001d022883	8.921	87	3.2865	0.00015
Zm00001d019615	0.44	3.97	3.1739	0.0009
Zm00001d010446	0.924	8.28	3.1636	0.00045
Zm00001d021492	2.509	21.2	3.0767	0.00205

Zm00001d015092	12.87	3.47	-1.88876	0.0051	Zm00001d029195	0.95	7.59	2.9985	0.00005
Zm00001d022636	6.317	1.71	-1.88921	0.0021	Zm00001d012246	1.653	12.9	2.9672	0.00105
Zm00001d028151	12.36	3.3	-1.90371	0.00035	Zm00001d001563	38.81	303	2.9632	0.00005
Zm00001d046029	22.15	5.91	-1.90531	0.00005	Zm00001d036940	0.391	2.79	2.8311	0.0013
Zm00001d010667	2.447	0.65	-1.90571	0.008	Zm00001d032083	1.255	8.61	2.7784	0.00005
Zm00001d033872	5.692	1.52	-1.90838	0.0006	Zm00001d049805	4.284	29	2.7594	0.00005
Zm00001d012091	56.43	15	-1.9087	0.00005	Zm00001d050667	1.035	6.73	2.7021	0.00255
Zm00001d027749	29.79	7.91	-1.91272	0.00005	Zm00001d028746	0.671	4.26	2.6677	0.00435
Zm00001d006535	5.217	1.38	-1.91453	0.0026	Zm00001d045676	1.525	9.6	2.6543	0.00315
Zm00001d020261	6.477	1.71	-1.91885	0.00475	Zm00001d009097	0.905	5.53	2.6109	0.00275
Zm00001d003725	78.7	20.8	-1.922	0.0006	Zm00001d010049	1.275	7.74	2.6008	0.0005
Zm00001d027757	10.51	2.77	-1.9257	0.00055	Zm00001d004509	0.721	4.26	2.563	0.0003
Zm00001d013370	16.83	4.37	-1.94461	0.00005	Zm00001d024926	3.899	22.9	2.5539	0.00285
Zm00001d022569	21.26	5.51	-1.94773	0.00095	Zm00001d018414	1.031	6.01	2.5449	0.00305
Zm00001d013979	4.987	1.29	-1.94879	0.0056	Zm00001d031940	9.393	54.6	2.5383	0.00005
Zm00001d014971	5.852	1.52	-1.94933	0.003	Zm00001d029801	2.124	11.9	2.488	0.00085
Zm00001d029699	22.44	5.79	-1.9536	0.0003	Zm00001d023903	5.609	30.2	2.4298	0.00005
Zm00001d040067	18.4	4.75	-1.95462	0.00005	Zm00001d006365	1.354	7.23	2.4174	0.0007
Zm00001d028574	3.915	1.01	-1.96017	0.00395	Zm00001d023905	5.559	29.7	2.4162	0.00005
Zm00001d027708	6.927	1.77	-1.96716	0.0011	Zm00001d026872	67.87	359	2.4048	0.00005
Zm00001d010234	5.445	1.39	-1.96822	0.00335	Zm00001d007788	2.864	15.2	2.4038	0.00005
Zm00001d023294	2.782	0.71	-1.97567	0.0076	Zm00001d041656	3.31	17.4	2.3919	0.00005
Zm00001d027996	4.366	1.11	-1.97683	0.00165	Zm00001d041670	4.057	20.9	2.3642	0.0005
Zm00001d017840	63.7	16.2	-1.97888	0.00005	Zm00001d045368	44.91	230	2.3589	0.00005
Zm00001d007345	6.181	1.56	-1.98236	0.0039	Zm00001d021481	0.517	2.63	2.3485	0.00195
Zm00001d018601	4.431	1.12	-1.98265	0.0083	Zm00001d039240	8.969	45.6	2.3473	0.00005
Zm00001d004208	18.77	4.73	-1.98815	0.0078	Zm00001d012902	1.156	5.83	2.3361	0.0021
Zm00001d020628	3.508	0.88	-1.98953	0.0083	Zm00001d023393	1.117	5.61	2.3277	0.00005
Zm00001d042826	15.74	3.93	-2.00069	0.00045	Zm00001d050674	0.693	3.3	2.2526	0.00145
Zm00001d023669	28.89	7.16	-2.01202	0.0002	Zm00001d003781	1.185	5.64	2.2516	0.0027
Zm00001d005410	24.38	6.03	-2.01629	0.00175	Zm00001d001989	1.906	9.08	2.2515	0.00575
Zm00001d002830	3.767	0.93	-2.02001	0.00265	Zm00001d005043	0.688	3.27	2.2482	0.00295
Zm00001d048073	11.72	2.88	-2.02434	0.00005	Zm00001d005044	0.688	3.27	2.2482	0.00295
Zm00001d010515	22.29	5.46	-2.02874	0.0029	Zm00001d030834	1.722	8.15	2.2419	0.00125
Zm00001d015921	6.441	1.57	-2.03807	0.00065	Zm00001d036455	1.596	7.55	2.2416	0.00115
Zm00001d033574	16.44	4	-2.0383	0.00365	Zm00001d053876	0.376	1.76	2.2251	0.0001
Zm00001d001970	4.971	1.21	-2.0398	0.0052	Zm00001d038910	40.85	190	2.2182	0.00005
Zm00001d012090	59.23	14.4	-2.04115	0.00005	Zm00001d006068	3.817	17.7	2.2153	0.0025
Zm00001d008545	20.76	5.02	-2.04698	0.0024	Zm00001d050104	0.346	1.6	2.2147	0.0009
Zm00001d027892	8.917	2.15	-2.04887	0.00035	Zm00001d053279	1.514	6.95	2.1985	0.00715

Zm00001d015515	53.36	12.7	-2.07284	0.00005	Zm00001d018600	0.531	2.4	2.1786	0.0082
Zm00001d025141	22	5.2	-2.08152	0.00005	Zm00001d020886	2.242	10	2.1621	0.0013
Zm00001d042922	179.5	42.1	-2.09189	0.00005	Zm00001d011165	0.358	1.59	2.1483	0.0073
Zm00001d020137	27.43	6.43	-2.09373	0.00005	Zm00001d002422	1.549	6.85	2.1458	0.00005
Zm00001d042886	8.348	1.94	-2.10395	0.0014	Zm00001d012602	8.852	39.2	2.1457	0.00005
Zm00001d018282	20.92	4.85	-2.10765	0.0001	Zm00001d004117	1.15	5.05	2.1339	0.0057
Zm00001d038780	6.593	1.52	-2.11565	0.00615	Zm00001d020722	3.154	13.8	2.1296	0.00405
Zm00001d016982	6.296	1.45	-2.11694	0.00215	Zm00001d011840	14.07	61.4	2.1248	0.00005
Zm00001d034501	26.71	6.11	-2.12878	0.00005	Zm00001d018088	1.419	6.16	2.1171	0.00265
Zm00001d005208	6.584	1.5	-2.1352	0.0019	Zm00001d048109	7.615	32.9	2.1103	0.00005
Zm00001d023468	23.87	5.43	-2.13637	0.0013	Zm00001d011431	17.22	74	2.1034	0.00005
Zm00001d043043	5.187	1.18	-2.14055	0.0043	Zm00001d041712	22.46	96.5	2.1026	0.00005
Zm00001d046596	15.42	3.48	-2.14819	0.00395	Zm00001d045512	1.206	5.18	2.1021	0.00005
Zm00001d027471	12.77	2.88	-2.14955	0.00105	Zm00001d028874	1.764	7.4	2.0685	0.00475
Zm00001d034635	12.78	2.87	-2.1525	0.00145	Zm00001d047738	8.343	34.8	2.0595	0.00005
Zm00001d036151	41.72	9.33	-2.16004	0.0002	Zm00001d005867	3.403	14.2	2.057	0.001
Zm00001d038904	7.56	1.69	-2.1605	0.00505	Zm00001d049244	5.069	21	2.0516	0.0005
Zm00001d031736	7.828	1.75	-2.16317	0.00095	Zm00001d048669	1.072	4.39	2.0347	0.00755
Zm00001d005577	6.668	1.49	-2.1643	0.00165	Zm00001d048434	1.087	4.45	2.0346	0.00005
Zm00001d020163	5.469	1.19	-2.19769	0.0046	Zm00001d039691	1.653	6.77	2.0339	0.0002
Zm00001d034453	16.21	3.53	-2.19815	0.00005	Zm00001d017377	4.335	17.7	2.0301	0.00005
Zm00001d052010	3.885	0.85	-2.19896	0.0036	Zm00001d019053	1.175	4.7	1.9992	0.00615
Zm00001d028689	7.388	1.61	-2.20188	0.00095	Zm00001d017786	14.46	57.7	1.9977	0.00005
Zm00001d014530	7.896	1.71	-2.20659	0.00375	Zm00001d047119	7.623	30.3	1.9917	0.003
Zm00001d020954	45.73	9.87	-2.21252	0.00005	Zm00001d044119	3.087	12.3	1.9895	0.0004
Zm00001d035881	5.03	1.07	-2.23584	0.00785	Zm00001d027801	1.063	4.2	1.9824	0.0007
Zm00001d028686	10.7	2.26	-2.24355	0.00015	Zm00001d002412	2.075	8.11	1.9672	0.00255
Zm00001d020976	9.813	2.07	-2.24417	0.0014	Zm00001d036255	29.25	114	1.9619	0.00005
Zm00001d034461	24.17	5.1	-2.24449	0.00005	Zm00001d035363	1.695	6.59	1.9595	0.0041
Zm00001d047235	8.35	1.76	-2.25029	0.0009	Zm00001d015783	8.373	32.4	1.9536	0.00005
Zm00001d012641	6.149	1.29	-2.25237	0.00795	Zm00001d004822	1.556	6.01	1.9487	0.0053
Zm00001d031270	5.853	1.23	-2.25619	0.00565	Zm00001d027539	4.717	18.2	1.9476	0.0011
Zm00001d027900	27.79	5.77	-2.26757	0.00005	Zm00001d040991	1.706	6.56	1.9423	0.008
Zm00001d028557	312.5	64.7	-2.2718	0.00005	Zm00001d052874	3.125	12	1.938	0.0047
Zm00001d029304	37.71	7.81	-2.27228	0.00005	Zm00001d013127	32.86	126	1.9336	0.00005
Zm00001d032691	16.28	3.37	-2.27251	0.00005	Zm00001d041204	11.34	43.2	1.9293	0.00005
Zm00001d032608	5.776	1.2	-2.27282	0.00455	Zm00001d033919	3.213	12.2	1.9226	0.0051
Zm00001d039935	410	84.8	-2.27323	0.00005	Zm00001d035428	54.99	208	1.9191	0.00005
Zm00001d008594	65.45	13.4	-2.28507	0.00005	Zm00001d012474	0.954	3.6	1.9144	0.008
Zm00001d032458	8.1	1.66	-2.28879	0.0013	Zm00001d013703	0.418	1.58	1.9139	0.00435

Zm00001d009328	5.303	1.08	-2.29185	0.00695	Zm00001d043737	21.33	80.1	1.9085	0.00005
Zm00001d027852	3.269	0.67	-2.29192	0.00655	Zm00001d018964	5.138	19.3	1.9063	0.00005
Zm00001d045036	23.75	4.83	-2.29774	0.0001	Zm00001d014721	1.231	4.61	1.904	0.0067
Zm00001d011006	6.277	1.28	-2.29812	0.0029	Zm00001d053766	3.173	11.9	1.9008	0.0006
Zm00001d011650	23.11	4.7	-2.2994	0.00055	Zm00001d025556	0.603	2.25	1.8983	0.0047
Zm00001d020429	4.795	0.97	-2.30249	0.0033	Zm00001d026139	16.1	59.9	1.8968	0.00005
Zm00001d034781	7.035	1.42	-2.31099	0.00045	Zm00001d019582	2.324	8.63	1.8918	0.00005
Zm00001d051389	4.259	0.86	-2.31174	0.00615	Zm00001d037898	0.932	3.44	1.8856	0.0004
Zm00001d026343	95.12	19.1	-2.3174	0.00005	Zm00001d035097	0.972	3.58	1.8826	0.00345
Zm00001d028555	352.3	69.3	-2.34541	0.00005	Zm00001d035434	51.04	188	1.8771	0.00005
Zm00001d004443	6.013	1.18	-2.34666	0.00265	Zm00001d040171	27.93	103	1.8766	0.00005
Zm00001d017713	14.99	2.94	-2.35066	0.0001	Zm00001d048493	4.973	18.2	1.8718	0.00005
Zm00001d008546	36.02	7.05	-2.35245	0.00005	Zm00001d015101	46.68	170	1.8688	0.00005
Zm00001d029913	19.17	3.74	-2.35848	0.00005	Zm00001d023785	2.272	8.27	1.8644	0.00155
Zm00001d045495	12.76	2.46	-2.37212	0.0032	Zm00001d007603	6.876	25	1.863	0.00005
Zm00001d039770	13.74	2.62	-2.39287	0.0072	Zm00001d050475	0.736	2.67	1.8595	0.00665
Zm00001d032443	20.4	3.88	-2.39454	0.0032	Zm00001d021682	1.297	4.71	1.8591	0.00505
Zm00001d020492	3.824	0.73	-2.39467	0.002	Zm00001d048366	3.641	13.1	1.8431	0.00035
Zm00001d025132	15.84	2.99	-2.40769	0.00685	Zm00001d010511	4.628	16.5	1.8344	0.0044
Zm00001d047744	6.242	1.17	-2.41303	0.0034	Zm00001d051479	0.475	1.69	1.83	0.0003
Zm00001d052480	6.538	1.22	-2.42683	0.008	Zm00001d047339	1.65	5.86	1.8291	0.00235
Zm00001d047072	2.472	0.46	-2.43044	0.0045	Zm00001d025047	57.97	205	1.8233	0.00005
Zm00001d035055	12.75	2.33	-2.45063	0.0059	Zm00001d013641	6.982	24.7	1.8216	0.0008
Zm00001d049201	5.102	0.93	-2.45354	0.00655	Zm00001d003522	5.509	19.4	1.8198	0.00005
Zm00001d044153	18.17	3.31	-2.45653	0.00005	Zm00001d019069	6.316	22.2	1.8163	0.0064
Zm00001d038806	194.6	35.2	-2.4685	0.00005	Zm00001d033412	2.804	9.84	1.8114	0.00265
Zm00001d014116	36.51	6.6	-2.46864	0.00005	Zm00001d046781	6.807	23.9	1.8097	0.00005
Zm00001d026094	7.471	1.35	-2.4695	0.00435	Zm00001d044605	2.353	8.17	1.7956	0.00015
Zm00001d004664	6.592	1.18	-2.47665	0.00685	Zm00001d028051	1.347	4.65	1.7866	0.0077
Zm00001d034559	7.325	1.32	-2.47704	0.0012	Zm00001d031878	1.343	4.63	1.7845	0.0078
Zm00001d011420	4.19	0.75	-2.48183	0.00365	Zm00001d010062	1.293	4.44	1.7797	0.0036
Zm00001d016723	45.66	8.15	-2.48612	0.0004	Zm00001d009932	18.51	63.1	1.7704	0.00005
Zm00001d010529	64.85	11.5	-2.5011	0.00005	Zm00001d008221	0.784	2.67	1.769	0.0042
Zm00001d047639	33.36	5.87	-2.50689	0.00005	Zm00001d014455	21.26	72.4	1.7673	0.00005
Zm00001d024379	36.38	6.31	-2.52767	0.00005	Zm00001d021470	0.766	2.6	1.7642	0.00235
Zm00001d022496	5.85	1.01	-2.53956	0.00365	Zm00001d052340	1.642	5.57	1.7617	0.0008
Zm00001d048356	8.323	1.42	-2.55298	0.00665	Zm00001d023553	1.987	6.73	1.7593	0.00005
Zm00001d049584	16.11	2.74	-2.55574	0.00005	Zm00001d041663	5.628	19	1.7571	0.0003
Zm00001d020339	7.016	1.19	-2.56173	0.00185	Zm00001d047245	7.021	23.7	1.7549	0.00115
Zm00001d002434	2.79	0.47	-2.56902	0.00095	Zm00001d042636	3.024	10.2	1.7523	0.0014

Zm00001d011649	11.98	2.01	-2.5743	0.00155
Zm00001d032003	7.883	1.32	-2.57692	0.0082
Zm00001d047093	11.52	1.92	-2.58398	0.00045
Zm00001d053916	16.58	2.76	-2.58833	0.00005
Zm00001d008715	3.644	0.59	-2.62428	0.00665
Zm00001d051791	14.11	2.24	-2.65379	0.00005
Zm00001d027283	14.18	2.24	-2.65996	0.0006
Zm00001d048538	4.171	0.66	-2.6609	0.00125
Zm00001d022516	10.14	1.6	-2.66402	0.00565
Zm00001d044664	87.85	13.8	-2.67355	0.00005
Zm00001d042813	5.83	0.9	-2.69098	0.0062
Zm00001d014996	3.563	0.55	-2.69869	0.00755
Zm00001d040457	36.77	5.64	-2.70505	0.00115
Zm00001d023216	8.374	1.27	-2.72599	0.0017
Zm00001d022053	11.23	1.7	-2.72635	0.0034
Zm00001d041246	20.67	3.11	-2.73252	0.0061
Zm00001d028931	4.98	0.75	-2.73718	0.00105
Zm00001d006106	4.942	0.74	-2.74918	0.0033
Zm00001d031201	4.407	0.66	-2.74943	0.00095
Zm00001d044373	4.468	0.66	-2.75517	0.0061
Zm00001d014734	9.657	1.42	-2.76344	0.00695
Zm00001d032822	9.394	1.36	-2.78466	0.00825
Zm00001d019712	7.04	1.02	-2.78536	0.0085
Zm00001d005823	5.27	0.76	-2.79999	0.00085
Zm00001d045478	23.46	3.23	-2.86184	0.0004
Zm00001d052221	2.107	0.28	-2.89064	0.00345
Zm00001d028998	111.6	15	-2.89686	0.00005
Zm00001d034356	75.91	10.1	-2.90287	0.00005
Zm00001d022044	3.527	0.46	-2.93678	0.00035
Zm00001d018388	18.4	2.4	-2.94085	0.0087
Zm00001d050346	13.4	1.74	-2.94476	0.00355
Zm00001d007351	5.955	0.77	-2.9565	0.0044
Zm00001d008377	10.88	1.39	-2.96882	0.0058
Zm00001d042730	17.31	2.2	-2.97916	0.00865
Zm00001d037514	10.52	1.32	-2.99181	0.00115
Zm00001d003830	10.01	1.25	-2.99842	0.00305
Zm00001d027323	40.68	4.84	-3.07078	0.0007
Zm00001d020585	29.29	3.48	-3.07503	0.0074
Zm00001d011787	7.52	0.85	-3.14175	0.00355
Zm00001d034564	4.26	0.48	-3.15915	0.00075

Zm00001d012797	1.035	3.48	1.7514	0.00005
Zm00001d038619	1.481	4.97	1.746	0.00635
Zm00001d011631	33.63	112	1.739	0.00005
Zm00001d051837	34.3	114	1.7337	0.00005
Zm00001d009969	81.7	270	1.7252	0.00005
Zm00001d004448	1.454	4.79	1.7201	0.00425
Zm00001d011241	15.06	49.5	1.7172	0.00005
Zm00001d024396	0.993	3.25	1.7115	0.00035
Zm00001d036647	0.481	1.57	1.7099	0.008
Zm00001d023273	9.553	31.1	1.7034	0.00005
Zm00001d050714	4.612	15	1.7007	0.00005
Zm00001d030310	5.178	16.8	1.6976	0.00005
Zm00001d032142	0.854	2.77	1.6966	0.00435
Zm00001d031807	1.952	6.32	1.695	0.0004
Zm00001d046134	18.07	58.4	1.6924	0.00005
Zm00001d050260	0.6	1.94	1.692	0.00865
Zm00001d028980	1.229	3.97	1.6902	0.00395
Zm00001d044566	13.62	43.7	1.6817	0.00005
Zm00001d032893	31.57	101	1.6805	0.00005
Zm00001d035562	7.856	25.1	1.6767	0.0001
Zm00001d037792	1.42	4.51	1.6658	0.00835
Zm00001d043578	11.85	37.5	1.6637	0.0039
Zm00001d043411	3.155	9.97	1.6604	0.00125
Zm00001d022725	322.7	1014	1.6511	0.00005
Zm00001d047087	13.58	42.5	1.6464	0.00035
Zm00001d039391	3.04	9.51	1.6451	0.00225
Zm00001d008408	18.65	58.3	1.6434	0.00005
Zm00001d043232	18.21	56.9	1.643	0.0002
Zm00001d040144	2.046	6.37	1.6388	0.0003
Zm00001d015485	1.782	5.55	1.6387	0.00345
Zm00001d011657	33.42	104	1.6368	0.00005
Zm00001d035211	1.996	6.2	1.6359	0.0055
Zm00001d042386	4.196	13	1.6335	0.0041
Zm00001d014386	2.908	9.01	1.6307	0.00515
Zm00001d007391	1.751	5.39	1.6225	0.0002
Zm00001d011753	34.55	106	1.618	0.00005
Zm00001d047483	2.341	7.18	1.6163	0.00145
Zm00001d039169	33.28	102	1.6139	0.00005
Zm00001d028457	9.605	29.4	1.6139	0.00005
Zm00001d040372	7.567	22.9	1.598	0.00005

Zm00001d033987	48.68	5.44	-3.16065	0.00005
Zm00001d027932	37.17	4	-3.21551	0.00005
Zm00001d019045	34.05	3.55	-3.2621	0.0023
Zm00001d047658	11.69	1.21	-3.27375	0.00505
Zm00001d017025	3.9	0.4	-3.28961	0.00825
Zm00001d027387	5.164	0.52	-3.31524	0.0055
Zm00001d031325	21.18	2.08	-3.34959	0.00005
Zm00001d039908	57.16	5.49	-3.38023	0.00005
Zm00001d047799	17.61	1.66	-3.40918	0.00005
Zm00001d047651	12.65	1.18	-3.42762	0.00305
Zm00001d006213	27.28	2.35	-3.53633	0.00825
Zm00001d029859	28.09	2.4	-3.54908	0.0052
Zm00001d047017	8.888	0.72	-3.62002	0.00255
Zm00001d033990	8.316	0.66	-3.66567	0.00125
Zm00001d024903	33.15	2.49	-3.73252	0.00005
Zm00001d009567	46.47	3.32	-3.80694	0.00005
Zm00001d024843	7.93	0.54	-3.88472	0.00265
Zm00001d028561	30.85	2.08	-3.89212	0.00635
Zm00001d039936	214.6	13.9	-3.95278	0.00005

Genes downregulated in the susceptible genotype

gene_id	SAI	SAU	$\log_2$ fold change	P value
Zm00001d008061	0	4.88	Inf	0.0002
Zm00001d003267	0.89	13.6	3.9316	5E-05
Zm00001d024207	0.42	4.31	3.35109	5E-05
Zm00001d028816	19.8	141	2.83226	5E-05
Zm00001d024210	1.95	13.1	2.74051	5E-05
Zm00001d010459	0.37	2.34	2.66426	0.0035
Zm00001d033710	4.42	26.5	2.58317	5E-05
Zm00001d002847	0.61	3.49	2.52344	0.0063
Zm00001d024211	0.53	2.97	2.4975	0.0003
Zm00001d024208	1.97	10.8	2.4625	5E-05
Zm00001d052518	2.01	10.5	2.38699	0.0032
Zm00001d022233	1.99	8.86	2.1568	0.0004
Zm00001d041726	3.26	14.4	2.14593	5E-05
Zm00001d047323	1.09	4.79	2.13597	0.0074
Zm00001d038352	1.91	8.06	2.07828	0.0005
Zm00001d008548	8.43	35.5	2.07199	0.0003
Zm00001d022457	3.13	12.7	2.0235	0.0007
Zm00001d011847	4.19	15.8	1.91271	5E-05

Zm00001d041660	6.697	20.3	1.5979	0.00245
Zm00001d024875	3.049	9.22	1.5972	0.0022
Zm00001d020312	1.301	3.93	1.5968	0.00005
Zm00001d023399	2.621	7.89	1.5908	0.0051
Zm00001d007602	1.082	3.26	1.5907	0.0013
Zm00001d022032	1.945	5.86	1.5905	0.0054
Zm00001d011874	1.316	3.95	1.5875	0.00555
Zm00001d037452	52.85	159	1.5863	0.00005
Zm00001d018930	1.266	3.8	1.5852	0.00085
Zm00001d042511	4.955	14.9	1.5843	0.0002
Zm00001d022265	1.392	4.14	1.5718	0.007
Zm00001d035824	22.58	67.1	1.5716	0.00005
Zm00001d007491	30.75	91.1	1.567	0.00005
Zm00001d023710	1.028	3.03	1.5624	0.0007
Zm00001d026406	10.3	30.4	1.5612	0.0004
Zm00001d016066	9.954	29.3	1.5598	0.00015
Zm00001d029397	13.01	38.2	1.5539	0.00005
Zm00001d031222	19.38	56.8	1.5503	0.00005
Zm00001d024519	2.938	8.6	1.5499	0.00785

Zm00001d038718	22.6	81.3	1.84844	5E-05
Zm00001d011088	1.38	4.81	1.80132	0.0042
Zm00001d052269	4.17	14	1.74785	5E-05
Zm00001d026288	0.87	2.87	1.71833	0.0006
Zm00001d020069	6.83	21.7	1.66535	0.0001
Zm00001d050293	2.13	6.55	1.62166	5E-05
Zm00001d024667	3.77	11.4	1.59346	0.0007
Zm00001d027422	2.01	6.03	1.5853	0.0026
Zm00001d024669	9.33	27.8	1.57502	0.0006
Zm00001d002343	2.18	6.46	1.57019	0.0012
Zm00001d028986	1.84	5.39	1.55164	0.0012
Zm00001d033459	3.28	9.55	1.54129	0.0083
Zm00001d026295	0.59	1.66	1.48521	0.0001
Zm00001d007962	3.45	9.44	1.45204	0.0039

Appendix 4: List of differentially expressed genes at the second time point (9 days post infestation)

Genes present in both genotypes

Gene_id	SBI	SBU	log2 fold change	P value	Gene id	RBI	RBU	log2 fold change	P value
Zm00001d002158	10.57	0	-100	0.00005	Zm00001d002158	3.934	0	-100	0.0063
Zm00001d023040	8.478	0	-100	0.00005	Zm00001d023040	45.46	0	-100	0.00005
Zm00001d021208	36.21	3.274	-3.46735	0.0001	Zm00001d030369	5.348	0	-100	0.00005
Zm00001d026872	193.5	18.02	-3.42408	0.00005	Zm00001d010671	32.91	1.853	-4.15003	0.0001
Zm00001d007700	33.6	4.015	-3.06486	0.00005	Zm00001d029913	38.58	3.242	-3.5728	0.00005
Zm00001d037894	15.27	2.524	-2.59717	0.0002	Zm00001d014852	2.173	0.262	-3.05319	0.00865
Zm00001d007705	44.07	7.309	-2.59212	0.00005	Zm00001d019137	222.7	30.38	-2.87356	0.00005
Zm00001d013111	76.32	14.23	-2.42352	0.00005	Zm00001d007700	14.43	1.98	-2.86479	0.00035
Zm00001d023859	14.77	2.907	-2.34539	0.00005	Zm00001d020496	6.563	0.951	-2.7868	0.00855
Zm00001d029906	44.92	9.801	-2.19654	0.00005	Zm00001d001139	544.1	79.6	-2.77297	0.00005
Zm00001d018298	133.7	29.34	-2.18813	0.00005	Zm00001d025803	5.028	0.761	-2.72473	0.00005
Zm00001d000874	274.8	60.8	-2.17596	0.00005	Zm00001d027987	28.6	4.384	-2.70581	0.00005
Zm00001d005543	13.46	3.35	-2.00688	0.0009	Zm00001d007705	12.79	2.027	-2.65784	0.00025
Zm00001d006027	3.13	0.793	-1.98087	0.0014	Zm00001d039770	20.93	3.339	-2.64763	0.0008
Zm00001d010586	24.1	6.242	-1.94893	0.0017	Zm00001d028887	165.6	26.49	-2.64407	0.00005
Zm00001d039000	91.92	24.36	-1.91598	0.00005	Zm00001d028888	144.8	23.44	-2.62707	0.00005
Zm00001d019704	16.08	4.364	-1.8816	0.00185	Zm00001d029391	6.193	1.013	-2.6117	0.00045
Zm00001d044683	55.45	15.34	-1.8541	0.00005	Zm00001d029906	21.42	4.221	-2.34328	0.00005
Zm00001d038471	46.79	13.15	-1.83106	0.00005	Zm00001d035455	8.213	1.68	-2.2895	0.00425
Zm00001d054060	95.08	26.86	-1.82378	0.00005	Zm00001d025388	2.069	0.452	-2.19583	0.0043
Zm00001d025803	6.94	1.977	-1.81167	0.00005	Zm00001d047639	22	5.091	-2.11162	0.0004
Zm00001d004840	5.202	1.5	-1.79463	0.00165	Zm00001d047093	19.04	4.468	-2.09157	0.00015
Zm00001d052194	484.3	141.1	-1.77951	0.00005	Zm00001d044683	24.4	5.979	-2.02919	0.00115
Zm00001d001139	254.6	74.74	-1.7682	0.00005	Zm00001d054060	82.64	20.37	-2.02004	0.00005
Zm00001d035455	23.78	7.044	-1.75559	0.0006	Zm00001d023859	18.4	4.84	-1.92641	0.00005
Zm00001d029913	43.67	13.34	-1.71065	0.00005	Zm00001d000874	376.9	100.4	-1.90842	0.0001
Zm00001d047093	24.02	7.381	-1.70234	0.00055	Zm00001d021208	22.31	6.351	-1.81249	0.0027
Zm00001d029391	14.25	4.39	-1.6987	0.00035	Zm00001d042826	93.37	27.49	-1.76428	0.00005
Zm00001d039770	77.77	24.59	-1.66141	0.0001	Zm00001d002287	20.01	5.909	-1.75983	0.00005
Zm00001d006199	65.79	20.87	-1.65642	0.00005	Zm00001d037384	26.31	7.876	-1.7403	0.00005
Zm00001d025388	4.023	1.294	-1.63681	0.00155	Zm00001d007962	34.35	10.41	-1.72229	0.00005
Zm00001d053746	270.7	87.49	-1.62942	0.00005	Zm00001d038352	29.29	9.152	-1.67835	0.00005
Zm00001d022391	9.468	3.116	-1.60319	0.00005	Zm00001d006027	3.696	1.186	-1.63926	0.00215
Zm00001d020496	10.01	3.328	-1.58847	0.0061	Zm00001d038471	7.649	2.537	-1.59221	0.00875

Zm00001d027987	8.352	2.833	-1.55999	0.00075	Zm00001d017249	28.35	9.694	-1.54823	0.00085
Zm00001d017249	37.03	12.81	-1.53152	0.0001	Zm00001d026872	99.11	34.43	-1.52547	0.00005
Zm00001d047187	186.1	64.4	-1.531	0.00005	Zm00001d022391	11.15	4.07	-1.45407	0.00005
Zm00001d037384	58.71	21.22	-1.46803	0.00005	Zm00001d006199	112.2	41.04	-1.4509	0.00005
Zm00001d019137	51.59	18.77	-1.45837	0.00015	Zm00001d026012	38.19	14	-1.44795	0.0014
Zm00001d010671	5.029	13.7	1.44576	0.00535	Zm00001d048099	8.344	23.53	1.49558	0.00025
Zm00001d027422	1.875	5.341	1.51001	0.00605	Zm00001d027422	1.575	4.475	1.50615	0.00845
Zm00001d042826	49.31	140.5	1.51063	0.00005	Zm00001d033718	5.047	14.48	1.52018	0.00065
Zm00001d047639	61.56	182	1.56339	0.00005	Zm00001d052651	5.503	15.79	1.52055	0.00335
Zm00001d028887	61.42	187.6	1.61111	0.00005	Zm00001d013111	18.16	54.31	1.58063	0.00005
Zm00001d041984	11.04	33.76	1.61242	0.0002	Zm00001d021736	2.344	7.252	1.62912	0.0006
Zm00001d028888	57.35	176.1	1.61816	0.00005	Zm00001d031344	50.31	159.5	1.66457	0.00005
Zm00001d007962	5.805	18.11	1.6412	0.0009	Zm00001d013493	23.98	76.73	1.67812	0.00015
Zm00001d033718	15	47.7	1.66855	0.00005	Zm00001d053746	9.695	32.08	1.72648	0.00075
Zm00001d026012	10.5	34.11	1.70038	0.0007	Zm00001d053163	4.369	14.72	1.75238	0.0002
Zm00001d011642	8.944	29.98	1.74497	0.00005	Zm00001d041984	3.803	12.98	1.77161	0.00195
Zm00001d048099	4.512	15.24	1.7558	0.0004	Zm00001d044763	1.249	4.321	1.79096	0.00315
Zm00001d002287	4.019	13.62	1.761	0.00005	Zm00001d004506	2.789	10.37	1.89491	0.00055
Zm00001d052651	4.107	14.01	1.77074	0.0009	Zm00001d027855	5.964	22.49	1.91487	0.0002
Zm00001d014852	0.841	2.976	1.82335	0.00785	Zm00001d037894	6.063	23.36	1.9461	0.00005
Zm00001d038352	5.997	21.83	1.86403	0.00005	Zm00001d044640	0.737	2.947	1.99986	0.0009
Zm00001d044640	0.634	2.421	1.93285	0.00115	Zm00001d039000	7.98	32.13	2.00941	0.00005
Zm00001d053163	6.16	25.25	2.03556	0.00005	Zm00001d047187	9.527	40.11	2.07393	0.00005
Zm00001d031344	42.32	174.3	2.04205	0.00005	Zm00001d004840	0.808	3.404	2.07533	0.0047
Zm00001d013493	47.84	201	2.07133	0.00005	Zm00001d005543	3.151	13.56	2.10547	0.001
Zm00001d050201	5.653	24.22	2.09904	0.00005	Zm00001d034453	5.03	21.7	2.10872	0.00005
Zm00001d025873	5.651	25.69	2.18481	0.0002	Zm00001d050201	3.291	14.47	2.13643	0.0003
Zm00001d021736	1.448	6.941	2.26107	0.0001	Zm00001d018298	21.12	93.7	2.14968	0.00005
Zm00001d004506	3.157	15.7	2.31368	0.00005	Zm00001d011642	7.84	38.6	2.29962	0.00005
Zm00001d027855	2.606	14.06	2.43142	0.0003	Zm00001d010586	5.935	30.17	2.34574	0.0002
Zm00001d044763	1.634	9.215	2.49588	0.00005	Zm00001d034461	5.698	29.1	2.3525	0.00005
Zm00001d034453	9.768	79.53	3.02537	0.00005	Zm00001d033413	5.507	28.24	2.35845	0.00005
Zm00001d034461	13.94	129.5	3.21651	0.00005	Zm00001d003994	0.845	4.769	2.49717	0.00125
Zm00001d003994	6.954	71.73	3.36676	0.00005	Zm00001d019704	2.011	11.75	2.54613	0.00675
Zm00001d004509	1.26	14.32	3.50634	0.00005	Zm00001d052194	9.626	82.42	3.09801	0.00005
Zm00001d033413	3.731	74.47	4.31893	0.00005	Zm00001d004509	0.536	5.246	3.2907	0.00045
Zm00001d030369	0	3.959	100	0.0002	Zm00001d025873	2.518	32.55	3.69231	0.00035
Genes upregulated in the susceptible genotype					Genes upregulated in the resistant genotype				
Gene_id	SBI	SBU	Log$_2$ fold change	P value	Gene_id.1	RBI	RBU	log$_2$ fold change	P value

Zm00001d001180	2.392	0	-100	0.00275	Zm00001d005753	2.537	0	-100	0.003
Zm00001d010449	1.883	0	-100	0.0013	Zm00001d007285	2.62	0	-100	0.00005
Zm00001d012041	4.777	0	-100	0.0001	Zm00001d007781	2.287	0	-100	0.00005
Zm00001d017008	2.365	0	-100	0.0051	Zm00001d008022	2.023	0	-100	0.00005
Zm00001d018191	3.903	0	-100	0.0001	Zm00001d008194	3.58	0	-100	0.0072
Zm00001d019116	2.149	0	-100	0.00005	Zm00001d008562	2.501	0	-100	0.001
Zm00001d021205	6.699	0	-100	0.00005	Zm00001d008905	2.71	0	-100	0.00005
Zm00001d024324	3.996	0	-100	0.0016	Zm00001d011914	2.157	0	-100	0.00005
Zm00001d044783	1.715	0	-100	0.0045	Zm00001d016857	4.418	0	-100	0.00055
Zm00001d053046	3.701	0	-100	0.00265	Zm00001d017378	2.927	0	-100	0.0002
Zm00001d028561	169.5	4.893	-5.11452	0.00005	Zm00001d021538	2.411	0	-100	0.0016
Zm00001d033990	14.93	1.001	-3.89813	0.0001	Zm00001d023200	3.398	0	-100	0.001
Zm00001d028408	38.32	2.896	-3.72563	0.00005	Zm00001d026390	8.621	0	-100	0.00145
Zm00001d039936	318.5	24.55	-3.69739	0.00005	Zm00001d027679	1.959	0	-100	0.00005
Zm00001d002590	35.49	2.744	-3.69284	0.00005	Zm00001d029506	5.686	0	-100	0.00005
Zm00001d022435	38.72	3.224	-3.58586	0.00005	Zm00001d030919	2.857	0	-100	0.00845
Zm00001d009309	19.28	1.649	-3.54762	0.0019	Zm00001d031158	3.418	0	-100	0.0013
Zm00001d024903	96.52	8.542	-3.49821	0.00005	Zm00001d031161	1.948	0	-100	0.00775
Zm00001d010956	17.34	1.733	-3.32272	0.00145	Zm00001d031805	1.659	0	-100	0.0018
Zm00001d039935	784.7	78.86	-3.31467	0.00005	Zm00001d031852	4.276	0	-100	0.0063
Zm00001d044122	27.48	2.852	-3.26865	0.00005	Zm00001d033846	4.059	0	-100	0.00245
Zm00001d049244	31.15	3.297	-3.24005	0.00005	Zm00001d034572	1.693	0	-100	0.00325
Zm00001d011133	19.18	2.13	-3.17049	0.0034	Zm00001d036902	3.243	0	-100	0.001
Zm00001d009328	56.17	6.259	-3.16581	0.00005	Zm00001d037769	4.072	0	-100	0.00005
Zm00001d052243	3.266	0.371	-3.13964	0.0048	Zm00001d039648	4.13	0	-100	0.001
Zm00001d039566	47.21	5.58	-3.08071	0.00085	Zm00001d041604	2.735	0	-100	0.00775
Zm00001d031325	20.16	2.476	-3.02585	0.00005	Zm00001d042875	3.324	0	-100	0.0018
Zm00001d039933	67.72	8.463	-3.00025	0.00005	Zm00001d046148	3.819	0	-100	0.00005
Zm00001d048189	2.51	0.32	-2.97339	0.00045	Zm00001d047088	16.2	0	-100	0.00005
Zm00001d047799	35.5	4.606	-2.9462	0.00005	Zm00001d048307	4.458	0	-100	0.00665
Zm00001d029290	4.329	0.572	-2.92054	0.0029	Zm00001d051833	1.858	0	-100	0.0003
Zm00001d047553	30.83	4.275	-2.85041	0.0022	Zm00001d052545	6.897	0	-100	0.00005
Zm00001d039524	33.93	4.825	-2.81393	0.00005	Zm00001d001924	46.56	0.597	-6.28537	0.00005
Zm00001d047548	29.4	4.275	-2.78192	0.0026	Zm00001d023090	27.88	0.929	-4.90725	0.00005
Zm00001d048073	34.42	5.087	-2.75835	0.00005	Zm00001d011679	23.28	0.818	-4.83035	0.0011
Zm00001d041282	7.871	1.182	-2.73475	0.00005	Zm00001d051773	5.617	0.281	-4.32024	0.00685
Zm00001d004243	156.4	23.89	-2.71048	0.00005	Zm00001d037684	115.5	6.495	-4.15303	0.0039
Zm00001d049932	16.75	2.612	-2.6806	0.0001	Zm00001d042143	17.18	1.181	-3.86223	0.00045
Zm00001d027757	18.69	2.918	-2.67926	0.00005	Zm00001d022557	12.1	0.853	-3.82668	0.00835
Zm00001d009599	11.44	1.794	-2.6733	0.00145	Zm00001d031769	50.98	3.653	-3.80283	0.00005

Zm00001d007175	17.79	2.795	-2.67001	0.00005
Zm00001d049421	14.87	2.398	-2.63286	0.00005
Zm00001d028718	6.615	1.07	-2.62857	0.0046
Zm00001d048608	8.652	1.451	-2.57585	0.00215
Zm00001d020976	17.61	2.989	-2.55865	0.00005
Zm00001d028555	811.1	141.7	-2.51703	0.00005
Zm00001d028374	9.133	1.606	-2.50735	0.00215
Zm00001d037398	4.583	0.816	-2.48934	0.00005
Zm00001d028557	660.8	120.9	-2.45078	0.00005
Zm00001d025507	12.17	2.266	-2.42531	0.00005
Zm00001d025823	9.258	1.769	-2.38811	0.00325
Zm00001d034552	4.414	0.868	-2.34678	0.004
Zm00001d012326	5.874	1.164	-2.33539	0.0063
Zm00001d029304	50.55	10.03	-2.33338	0.00005
Zm00001d006563	6.097	1.226	-2.31421	0.0005
Zm00001d034130	12.96	2.63	-2.30162	0.00095
Zm00001d038806	402.4	81.65	-2.30107	0.00005
Zm00001d038971	22.07	4.482	-2.29973	0.00005
Zm00001d004401	12.71	2.587	-2.29636	0.00005
Zm00001d032719	5.594	1.141	-2.2939	0.0025
Zm00001d003052	5.299	1.081	-2.29318	0.00395
Zm00001d047765	21.84	4.474	-2.287	0.00005
Zm00001d026662	56.28	11.56	-2.28344	0.00005
Zm00001d028752	48.82	10.26	-2.25065	0.00005
Zm00001d004624	15.12	3.178	-2.24994	0.00005
Zm00001d005790	15.68	3.297	-2.24981	0.00335
Zm00001d033987	48.58	10.24	-2.24584	0.00005
Zm00001d013289	7.076	1.54	-2.20002	0.00665
Zm00001d044300	22.18	4.837	-2.19678	0.0001
Zm00001d005622	20.84	4.575	-2.1871	0.00045
Zm00001d045036	33.32	7.339	-2.18244	0.00005
Zm00001d003298	9.277	2.044	-2.18241	0.00155
Zm00001d003048	15.11	3.332	-2.18065	0.00005
Zm00001d051887	5.87	1.295	-2.18014	0.0034
Zm00001d039963	34.49	7.687	-2.16569	0.00005
Zm00001d052585	18.66	4.203	-2.15078	0.00005
Zm00001d027924	10.8	2.44	-2.14566	0.00315
Zm00001d010903	109.1	24.75	-2.14026	0.0025
Zm00001d047402	7.332	1.666	-2.13804	0.0007
Zm00001d013717	5.677	1.301	-2.12557	0.00745
Zm00001d031155	35.44	2.574	-3.78304	0.00545
Zm00001d013896	9.935	0.726	-3.77367	0.00605
Zm00001d027283	34.68	2.659	-3.70493	0.00005
Zm00001d031157	33.19	2.574	-3.6886	0.0057
Zm00001d039826	14.74	1.2	-3.61804	0.0062
Zm00001d012088	98.28	8.047	-3.61042	0.00005
Zm00001d032552	47.28	3.96	-3.5774	0.00005
Zm00001d006947	14.52	1.305	-3.4763	0.00005
Zm00001d050079	28.89	2.626	-3.45964	0.0062
Zm00001d043723	22.05	2.159	-3.35278	0.00055
Zm00001d049995	45.61	4.512	-3.3376	0.00005
Zm00001d008377	20.71	2.115	-3.29163	0.0019
Zm00001d047981	101.5	10.43	-3.28362	0.00005
Zm00001d037018	16.68	1.791	-3.21986	0.00785
Zm00001d017762	175.8	20.08	-3.13037	0.00005
Zm00001d033279	8.513	0.986	-3.10941	0.00275
Zm00001d014341	6.821	0.811	-3.07262	0.0026
Zm00001d023950	16.29	2.017	-3.0139	0.003
Zm00001d025727	14.77	1.862	-2.98783	0.00005
Zm00001d006754	20.43	2.649	-2.94747	0.0004
Zm00001d050682	10.73	1.391	-2.94685	0.0048
Zm00001d007180	6.699	0.899	-2.8983	0.00305
Zm00001d026632	34.3	4.628	-2.8899	0.00265
Zm00001d042864	50.79	6.88	-2.88415	0.00005
Zm00001d022420	33.67	4.678	-2.84732	0.00065
Zm00001d028471	2.248	0.316	-2.83041	0.0008
Zm00001d005391	7.714	1.097	-2.81451	0.00875
Zm00001d038291	132	18.87	-2.80658	0.00005
Zm00001d042202	8.316	1.199	-2.79429	0.0005
Zm00001d052139	8.203	1.202	-2.77039	0.0015
Zm00001d052165	19.07	2.808	-2.76368	0.00215
Zm00001d012494	9.921	1.478	-2.74678	0.00035
Zm00001d009608	6.253	0.953	-2.71388	0.0056
Zm00001d024265	21.27	3.259	-2.70607	0.00025
Zm00001d044147	9.232	1.435	-2.68576	0.00055
Zm00001d031882	4.314	0.674	-2.67836	0.007
Zm00001d045495	15.37	2.404	-2.67714	0.00155
Zm00001d049630	5.319	0.834	-2.6738	0.00015
Zm00001d001858	4.623	0.725	-2.67237	0.00355
Zm00001d047656	14.33	2.249	-2.6713	0.00095

Zm00001d046169	19.89	4.583	-2.11766	0.00025
Zm00001d002584	49.89	11.6	-2.10507	0.00005
Zm00001d018819	16.45	3.845	-2.09712	0.0001
Zm00001d038465	4.253	0.998	-2.09116	0.0022
Zm00001d045647	5.624	1.358	-2.0496	0.006
Zm00001d018229	31.4	7.626	-2.04175	0.00005
Zm00001d026131	13.16	3.208	-2.03627	0.0003
Zm00001d001774	31.16	7.633	-2.02936	0.0005
Zm00001d002167	14.51	3.593	-2.01349	0.00005
Zm00001d044426	5.123	1.27	-2.0119	0.0045
Zm00001d001915	52.52	13.03	-2.01085	0.00005
Zm00001d003267	2.973	0.753	-1.98072	0.0004
Zm00001d020137	134.2	34.28	-1.96908	0.00005
Zm00001d036066	16.27	4.173	-1.96276	0.0001
Zm00001d042449	28.81	7.413	-1.9586	0.0002
Zm00001d036122	6.096	1.574	-1.95329	0.00345
Zm00001d004310	296.1	76.75	-1.94789	0.00005
Zm00001d031736	17.02	4.446	-1.93659	0.00005
Zm00001d049525	54.09	14.18	-1.93143	0.00005
Zm00001d012242	9.691	2.569	-1.9153	0.00405
Zm00001d042922	426.3	113.8	-1.90583	0.00005
Zm00001d034145	17.54	4.689	-1.90355	0.00485
Zm00001d029274	85.69	22.92	-1.90246	0.00005
Zm00001d029025	6.274	1.697	-1.88668	0.00125
Zm00001d010520	8.106	2.196	-1.88392	0.0014
Zm00001d043528	8.328	2.266	-1.87794	0.0084
Zm00001d017536	7.005	1.915	-1.87135	0.0001
Zm00001d044272	22.75	6.303	-1.85183	0.00005
Zm00001d030555	4.766	1.323	-1.84931	0.00025
Zm00001d002410	14.95	4.156	-1.84681	0.0016
Zm00001d002019	8.704	2.429	-1.84153	0.00035
Zm00001d051415	2.08	0.582	-1.83801	0.00115
Zm00001d052673	152.1	42.68	-1.83324	0.00005
Zm00001d015917	20.98	5.911	-1.8272	0.006
Zm00001d052962	23.22	6.582	-1.81877	0.00085
Zm00001d020607	19.99	5.669	-1.81798	0.00015
Zm00001d019117	41.31	11.78	-1.80968	0.00005
Zm00001d008214	48.96	13.99	-1.80701	0.00005
Zm00001d011228	3.317	0.951	-1.80228	0.00365
Zm00001d033572	2.172	0.623	-1.80074	0.0035
Zm00001d043547	5.334	0.842	-2.66269	0.00555
Zm00001d046299	24.34	3.858	-2.65767	0.00005
Zm00001d042104	17.7	2.829	-2.6455	0.00025
Zm00001d007842	4.085	0.654	-2.64247	0.00065
Zm00001d042684	21.21	3.405	-2.63888	0.00005
Zm00001d017494	16.92	2.725	-2.63408	0.00005
Zm00001d027928	23.17	3.737	-2.6319	0.0027
Zm00001d047090	11.21	1.812	-2.62876	0.00025
Zm00001d008952	3.972	0.643	-2.6271	0.0084
Zm00001d045395	23.28	3.815	-2.60959	0.00135
Zm00001d024752	10.17	1.67	-2.60601	0.00235
Zm00001d010446	4.065	0.679	-2.58242	0.0084
Zm00001d015202	5.683	0.949	-2.58239	0.00165
Zm00001d047663	68.5	11.6	-2.56156	0.00005
Zm00001d020631	7.722	1.309	-2.56082	0.002
Zm00001d036880	186.9	32.42	-2.52713	0.00005
Zm00001d016551	18.78	3.262	-2.52572	0.00005
Zm00001d045386	16.77	2.937	-2.51362	0.00005
Zm00001d009140	10.31	1.823	-2.4992	0.0008
Zm00001d049059	541.9	96.04	-2.49627	0.00005
Zm00001d022636	32.78	5.816	-2.49494	0.00005
Zm00001d029814	14.75	2.617	-2.49444	0.0017
Zm00001d049926	13.23	2.378	-2.47633	0.00025
Zm00001d031875	11.48	2.075	-2.46831	0.0004
Zm00001d032162	7.558	1.367	-2.46736	0.0069
Zm00001d008837	18.11	3.309	-2.45204	0.0001
Zm00001d043179	20.35	3.779	-2.42854	0.00005
Zm00001d047651	24.65	4.59	-2.42511	0.00015
Zm00001d047658	20.71	3.856	-2.42499	0.0005
Zm00001d018388	15.97	2.999	-2.41248	0.00875
Zm00001d027929	32.3	6.08	-2.40948	0.00235
Zm00001d052164	65.47	12.37	-2.40375	0.00005
Zm00001d034635	17.38	3.351	-2.37467	0.00155
Zm00001d005392	65.68	12.8	-2.35891	0.00005
Zm00001d051754	54.75	10.7	-2.35534	0.00005
Zm00001d022593	13.74	2.696	-2.34906	0.00355
Zm00001d044951	7.536	1.48	-2.34797	0.0002
Zm00001d054057	142.6	28.1	-2.3435	0.00005
Zm00001d008222	10.48	2.068	-2.34055	0.0008
Zm00001d048020	493.7	98.63	-2.3237	0.00005

Zm00001d037397	9.971	2.862	-1.80055	0.0001
Zm00001d045101	25.88	7.449	-1.7968	0.00015
Zm00001d028599	20.04	5.777	-1.7945	0.00005
Zm00001d031726	5.995	1.733	-1.79069	0.00615
Zm00001d006230	9.191	2.672	-1.78212	0.00005
Zm00001d013022	6.988	2.035	-1.77972	0.00845
Zm00001d022476	38.13	11.14	-1.77459	0.0008
Zm00001d024211	9.756	2.853	-1.77371	0.00005
Zm00001d010521	62.22	18.21	-1.77273	0.00005
Zm00001d037336	14.68	4.307	-1.76944	0.00095
Zm00001d048136	18.35	5.387	-1.76825	0.00315
Zm00001d014486	200.1	59.12	-1.7593	0.00005
Zm00001d042813	17.81	5.272	-1.75662	0.00095
Zm00001d002675	8.015	2.373	-1.75583	0.0023
Zm00001d026060	69.41	20.58	-1.75354	0.00005
Zm00001d042909	55.98	16.62	-1.75245	0.00005
Zm00001d017077	7.952	2.364	-1.75031	0.0032
Zm00001d045476	28.04	8.344	-1.74886	0.0085
Zm00001d029736	3.725	1.121	-1.73314	0.00855
Zm00001d013507	11.68	3.513	-1.73289	0.00005
Zm00001d052733	43.9	13.21	-1.73252	0.00005
Zm00001d029011	5.021	1.52	-1.72378	0.00035
Zm00001d017366	7.724	2.342	-1.72149	0.0071
Zm00001d051814	10.56	3.202	-1.72104	0.0017
Zm00001d043350	4.792	1.465	-1.70932	0.00285
Zm00001d026657	90.86	27.9	-1.70353	0.00005
Zm00001d029140	5.534	1.701	-1.70221	0.0003
Zm00001d017209	2.694	0.829	-1.70072	0.00375
Zm00001d010515	26.49	8.164	-1.69832	0.00425
Zm00001d040067	68.59	21.16	-1.69675	0.00005
Zm00001d040594	17.69	5.457	-1.69649	0.0078
Zm00001d053799	3.859	1.193	-1.69406	0.00025
Zm00001d024745	18.34	5.67	-1.69368	0.0012
Zm00001d042214	27.67	8.558	-1.69308	0.0019
Zm00001d049435	7.115	2.201	-1.69243	0.0015
Zm00001d012420	919.5	284.6	-1.69205	0.00005
Zm00001d019510	8.997	2.786	-1.69117	0.0001
Zm00001d013489	42.43	13.16	-1.68913	0.00005
Zm00001d034463	2.626	0.817	-1.68482	0.0047
Zm00001d038023	28.02	8.744	-1.68021	0.00025
Zm00001d033088	4.42	0.885	-2.31973	0.00715
Zm00001d027313	10.19	2.045	-2.31759	0.0023
Zm00001d034066	1.981	0.398	-2.31463	0.0016
Zm00001d018983	5.135	1.046	-2.29589	0.00185
Zm00001d003142	3.49	0.718	-2.28124	0.00815
Zm00001d012333	8.362	1.721	-2.28059	0.0087
Zm00001d004309	126.4	26.17	-2.27209	0.00005
Zm00001d042739	9.217	1.909	-2.27162	0.00085
Zm00001d024676	7.073	1.472	-2.26412	0.0051
Zm00001d032115	27.04	5.645	-2.2601	0.0026
Zm00001d031971	56.83	11.89	-2.25698	0.00005
Zm00001d018206	85.91	17.98	-2.25662	0.00005
Zm00001d046746	18.78	3.938	-2.25401	0.0003
Zm00001d038412	10.19	2.172	-2.23021	0.00045
Zm00001d039778	3.998	0.857	-2.2221	0.00775
Zm00001d025831	15.04	3.24	-2.21453	0.0005
Zm00001d003208	15.68	3.411	-2.20016	0.00005
Zm00001d005798	67.38	14.69	-2.1972	0.00005
Zm00001d047339	9.79	2.138	-2.19506	0.0001
Zm00001d029932	60.69	13.36	-2.18382	0.00005
Zm00001d005378	28	6.19	-2.17722	0.00005
Zm00001d035561	18.6	4.121	-2.17438	0.00135
Zm00001d023543	11.5	2.571	-2.16086	0.00025
Zm00001d011611	92.13	20.62	-2.15946	0.00005
Zm00001d027546	40.38	9.116	-2.14733	0.00005
Zm00001d007381	7.836	1.772	-2.14442	0.0006
Zm00001d050893	45.78	10.38	-2.14099	0.00005
Zm00001d046423	15.43	3.504	-2.13821	0.00005
Zm00001d020272	23.18	5.279	-2.13452	0.00005
Zm00001d049286	6.615	1.508	-2.1328	0.00005
Zm00001d038355	6.92	1.586	-2.1258	0.00015
Zm00001d034745	16.33	3.765	-2.1174	0.0041
Zm00001d045470	9.688	2.24	-2.11263	0.0009
Zm00001d042275	9.317	2.158	-2.11015	0.0009
Zm00001d022453	32.97	7.662	-2.10551	0.00005
Zm00001d020556	15.97	3.717	-2.10346	0.00005
Zm00001d039387	39.29	9.155	-2.10168	0.00005
Zm00001d027763	17.36	4.06	-2.09642	0.0009
Zm00001d031926	7.817	1.835	-2.09088	0.0011
Zm00001d044716	5.111	1.203	-2.08648	0.00645

Zm00001d038585	31.81	9.937	-1.67872	0.0016
Zm00001d017095	174.7	54.82	-1.67182	0.00005
Zm00001d009556	70.99	22.4	-1.66429	0.00005
Zm00001d047764	10.5	3.32	-1.66086	0.00265
Zm00001d049418	11.36	3.608	-1.65421	0.0007
Zm00001d052478	6.254	1.998	-1.6462	0.00135
Zm00001d006330	14.45	4.625	-1.64348	0.00165
Zm00001d033872	4.654	1.513	-1.62106	0.0032
Zm00001d007581	15.38	5.014	-1.61724	0.0028
Zm00001d053202	5.62	1.836	-1.61436	0.00235
Zm00001d045478	32.35	10.57	-1.61406	0.0008
Zm00001d020429	33.65	11	-1.61295	0.0001
Zm00001d051139	56.77	18.58	-1.61139	0.00005
Zm00001d017084	3.075	1.009	-1.60746	0.0022
Zm00001d032172	2.175	0.714	-1.60739	0.00165
Zm00001d049647	21.68	7.163	-1.5974	0.00785
Zm00001d033296	37.45	12.42	-1.5921	0.0007
Zm00001d012321	10.39	3.448	-1.59064	0.00425
Zm00001d044685	19.46	6.474	-1.58783	0.00275
Zm00001d044340	12.11	4.054	-1.57942	0.0007
Zm00001d049427	6.117	2.053	-1.57537	0.00455
Zm00001d045582	30.58	10.3	-1.57063	0.00005
Zm00001d050259	7.58	2.575	-1.55786	0.00095
Zm00001d021677	11.39	3.887	-1.55158	0.005
Zm00001d034421	3.373	1.151	-1.55098	0.0029
Zm00001d044253	23.37	7.977	-1.55079	0.00005
Zm00001d013208	26.89	9.254	-1.53872	0.00275
Zm00001d021303	9.944	3.425	-1.53787	0.0004
Zm00001d028588	11.03	3.801	-1.53692	0.00015
Zm00001d052180	23.88	8.233	-1.53642	0.00005
Zm00001d047192	29.82	10.31	-1.53269	0.00045
Zm00001d017984	64.62	22.34	-1.53222	0.00005
Zm00001d016604	6.467	2.238	-1.53101	0.0057
Zm00001d049359	6.78	2.35	-1.52853	0.00435
Zm00001d016558	14.91	5.175	-1.52673	0.00565
Zm00001d021296	9.228	3.211	-1.52323	0.00475
Zm00001d047208	12.66	4.421	-1.51767	0.00035
Zm00001d050119	13.31	4.654	-1.5158	0.00105
Zm00001d021680	2.49	0.872	-1.51351	0.0068
Zm00001d048588	10.06	3.533	-1.51002	0.00055
Zm00001d007379	10.44	2.46	-2.08511	0.00645
Zm00001d021698	89.01	21.04	-2.08084	0.00005
Zm00001d051525	5.319	1.258	-2.07981	0.00005
Zm00001d033411	20.59	4.896	-2.07212	0.0048
Zm00001d026649	55.08	13.14	-2.06705	0.00005
Zm00001d044460	18.12	4.352	-2.05811	0.0008
Zm00001d032850	9.415	2.27	-2.05196	0.0046
Zm00001d020938	4.538	1.095	-2.05121	0.006
Zm00001d012883	5.897	1.432	-2.04199	0.0079
Zm00001d025860	15.49	3.787	-2.03187	0.00005
Zm00001d029078	8.088	1.981	-2.02962	0.00145
Zm00001d025703	3.128	0.767	-2.02772	0.00195
Zm00001d004187	4.542	1.115	-2.02674	0.0071
Zm00001d025383	2.663	0.656	-2.0225	0.00125
Zm00001d042482	68.34	16.89	-2.01636	0.00005
Zm00001d050674	2.484	0.616	-2.01285	0.00295
Zm00001d013033	27.59	6.838	-2.01219	0.00005
Zm00001d033683	12.5	3.103	-2.01003	0.00235
Zm00001d025933	15.45	3.836	-2.00964	0.00025
Zm00001d027415	11.64	2.897	-2.00674	0.00005
Zm00001d003195	36.91	9.208	-2.00292	0.00055
Zm00001d015700	30.91	7.711	-2.00289	0.00045
Zm00001d049113	3.179	0.794	-2.00081	0.00415
Zm00001d019605	7.625	1.91	-1.99744	0.00195
Zm00001d043174	12.8	3.227	-1.98816	0.00025
Zm00001d042438	9.983	2.517	-1.98805	0.00045
Zm00001d001975	7.558	1.917	-1.97932	0.00255
Zm00001d036151	40.62	10.33	-1.97598	0.0008
Zm00001d043798	15.68	3.992	-1.97414	0.00145
Zm00001d042096	17.52	4.462	-1.9731	0.006
Zm00001d005687	266.4	68.03	-1.96952	0.00005
Zm00001d002198	2.368	0.606	-1.96609	0.00415
Zm00001d006053	33.03	8.462	-1.96495	0.0001
Zm00001d025050	52.58	13.47	-1.96466	0.00005
Zm00001d020595	153.9	39.58	-1.95912	0.00005
Zm00001d044431	17.73	4.591	-1.94888	0.00005
Zm00001d017834	7.611	1.972	-1.94848	0.00415
Zm00001d026540	1.768	0.458	-1.94788	0.00005
Zm00001d036072	5.103	1.323	-1.94771	0.0017
Zm00001d002549	21.5	5.577	-1.94691	0.0019

Zm00001d007403	9.957	3.497	-1.50968	0.00345
Zm00001d047302	42.15	14.82	-1.50778	0.00005
Zm00001d027436	9.493	3.339	-1.50755	0.00255
Zm00001d022516	41.07	14.5	-1.50213	0.0007
Zm00001d024210	31.1	10.99	-1.50075	0.00005
Zm00001d051288	5.439	1.928	-1.49593	0.0058
Zm00001d049966	56.45	20.02	-1.4953	0.0043
Zm00001d039727	15.08	5.355	-1.4934	0.00045
Zm00001d017962	3.389	1.205	-1.49144	0.00015
Zm00001d022451	21.28	7.578	-1.48974	0.0038
Zm00001d005148	31.36	11.17	-1.48897	0.00005
Zm00001d020264	16.78	6.003	-1.4834	0.00045
Zm00001d024208	26.84	9.626	-1.4795	0.00005
Zm00001d051693	13.59	4.905	-1.46988	0.00105
Zm00001d014166	13.5	4.876	-1.46916	0.0004
Zm00001d017699	20.64	7.474	-1.46519	0.00125
Zm00001d007915	18.39	6.661	-1.46497	0.0004
Zm00001d030697	22.91	8.315	-1.46201	0.0017
Zm00001d022517	75.4	27.48	-1.45644	0.00005
Zm00001d006375	3.059	1.116	-1.45519	0.00815
Zm00001d031127	59.59	21.81	-1.44996	0.00005
Zm00001d008349	2.933	1.074	-1.44921	0.0028
Zm00001d029114	3.208	1.175	-1.44919	0.0027
Zm00001d044235	27.89	10.21	-1.44917	0.00035
Zm00001d003365	16.65	6.102	-1.44862	0.0052
Zm00001d024867	5.615	2.057	-1.44862	0.00365

Genes downregulated in the susceptible genotype

Gene id	RAI	RAU	log2 fold change	p value
Zm00001d017700	10.53	28.75	1.44901	0.00005
Zm00001d046485	1.442	3.937	1.44936	0.0015
Zm00001d029075	23.86	65.15	1.44948	0.00005
Zm00001d033710	12.47	34.07	1.4499	0.0007
Zm00001d051548	1.777	4.856	1.45008	0.0077
Zm00001d020008	7.815	21.5	1.45991	0.00065
Zm00001d001936	9.046	25	1.46671	0.00005
Zm00001d040020	1.453	4.019	1.46797	0.00715
Zm00001d039542	6.646	18.41	1.47023	0.00235
Zm00001d003903	6.687	18.56	1.47251	0.00185
Zm00001d052918	1.826	5.083	1.47662	0.00155

Zm00001d019944	15.14	3.933	-1.94462	0.00005
Zm00001d006360	11.28	2.945	-1.93752	0.0026
Zm00001d011006	6.367	1.664	-1.93613	0.00445
Zm00001d013151	10.09	2.658	-1.92413	0.0036
Zm00001d005383	25.15	6.644	-1.92058	0.00005
Zm00001d042135	10.63	2.819	-1.91436	0.0011
Zm00001d039166	92.84	24.65	-1.91332	0.00005
Zm00001d042740	5.681	1.511	-1.911	0.0007
Zm00001d046599	31.56	8.392	-1.91092	0.0006
Zm00001d052937	6.622	1.776	-1.89883	0.0011
Zm00001d051749	42.71	11.47	-1.89714	0.00035
Zm00001d030171	7.595	2.041	-1.89597	0.0007
Zm00001d032142	2.161	0.581	-1.895	0.0083
Zm00001d015133	77.41	20.85	-1.89229	0.00005
Zm00001d017495	13.06	3.523	-1.89001	0.00025
Zm00001d017880	80.84	21.86	-1.88713	0.00005
Zm00001d016755	32.71	8.863	-1.88365	0.00005
Zm00001d051203	9.84	2.67	-1.88195	0.0086
Zm00001d039335	30.24	8.224	-1.87861	0.00005
Zm00001d052405	5.943	1.621	-1.87403	0.00025
Zm00001d046945	21.32	5.848	-1.86577	0.00005
Zm00001d050872	72.2	19.85	-1.8629	0.00005
Zm00001d018605	7.339	2.028	-1.85572	0.00455
Zm00001d053396	49.59	13.71	-1.85445	0.00005
Zm00001d028999	17.27	4.779	-1.8537	0.0043
Zm00001d019422	5.909	1.636	-1.85314	0.0063
Zm00001d022456	55.24	15.37	-1.8456	0.0002
Zm00001d033286	178.4	49.94	-1.83707	0.00005
Zm00001d033815	16.27	4.567	-1.83325	0.00125
Zm00001d035343	10.09	2.834	-1.83137	0.0013
Zm00001d052442	5.575	1.568	-1.83026	0.004
Zm00001d002026	4.18	1.181	-1.82348	0.0016
Zm00001d051896	20.44	5.83	-1.80994	0.00165
Zm00001d048634	11.56	3.298	-1.80897	0.00375
Zm00001d034991	32.53	9.308	-1.80517	0.0015
Zm00001d045063	7.357	2.106	-1.80448	0.0058
Zm00001d003866	6.411	1.84	-1.80084	0.002
Zm00001d052612	51.39	14.77	-1.79921	0.00005
Zm00001d031201	5.508	1.586	-1.7959	0.0034

Zm00001d040621	2.689	7.512	1.4819	0.00255	Zm00001d002550	8.789	2.542	-1.78955	0.00195
Zm00001d048021	50.95	142.6	1.48518	0.00005	Zm00001d016518	1.823	0.528	-1.78876	0.00005
Zm00001d027423	24.43	68.52	1.48784	0.00005	Zm00001d014919	1.996	0.578	-1.78773	0.0069
Zm00001d019692	46.23	129.8	1.48929	0.00005	Zm00001d044159	15.21	4.406	-1.7872	0.00005
Zm00001d012909	18.33	51.47	1.48979	0.00465	Zm00001d013898	5.173	1.499	-1.7871	0.0028
Zm00001d029356	27.87	78.33	1.49096	0.00005	Zm00001d028736	15.1	4.395	-1.78075	0.00485
Zm00001d025141	74.68	210.6	1.49544	0.00005	Zm00001d011890	11.94	3.476	-1.78064	0.00005
Zm00001d047367	16.34	46.15	1.49802	0.00005	Zm00001d011157	7.514	2.192	-1.77762	0.00005
Zm00001d048344	20.55	58.25	1.50351	0.00005	Zm00001d003013	7.149	2.087	-1.77654	0.0007
Zm00001d003949	6.912	19.61	1.50409	0.00005	Zm00001d031275	59.71	17.59	-1.76296	0.00005
Zm00001d038221	10.39	29.5	1.50518	0.00025	Zm00001d040468	8.291	2.445	-1.76156	0.00035
Zm00001d011422	8.98	25.53	1.50724	0.0001	Zm00001d028759	111.9	33.01	-1.76116	0.00005
Zm00001d025943	8.576	24.42	1.51001	0.00025	Zm00001d041298	4.074	1.206	-1.75646	0.0005
Zm00001d018059	5.325	15.29	1.52139	0.0009	Zm00001d012836	21.39	6.335	-1.75563	0.00005
Zm00001d033822	8.445	24.27	1.52323	0.00175	Zm00001d046745	12.53	3.728	-1.74843	0.0012
Zm00001d045088	3.364	9.681	1.52489	0.00105	Zm00001d038852	14.81	4.412	-1.74659	0.00005
Zm00001d035875	16.04	46.25	1.52769	0.00005	Zm00001d020703	7.019	2.097	-1.74296	0.0012
Zm00001d044156	6.714	19.36	1.5278	0.0007	Zm00001d033931	272.9	81.55	-1.74237	0.00005
Zm00001d006493	12.81	36.95	1.52867	0.00005	Zm00001d037810	5.898	1.764	-1.7416	0.0003
Zm00001d018966	22.5	65.06	1.53211	0.0003	Zm00001d034217	11.31	3.388	-1.7398	0.00125
Zm00001d049081	2.854	8.269	1.53492	0.00115	Zm00001d024916	4.666	1.398	-1.73896	0.00665
Zm00001d006306	1.6	4.639	1.53582	0.00825	Zm00001d025012	53.47	16.03	-1.73827	0.00005
Zm00001d052835	7.39	21.52	1.54192	0.00005	Zm00001d003468	16.98	5.11	-1.73216	0.0002
Zm00001d047446	6.702	19.53	1.54325	0.00005	Zm00001d030999	15.86	4.783	-1.72953	0.0027
Zm00001d016691	5.348	15.71	1.55429	0.0019	Zm00001d026268	8.127	2.459	-1.72468	0.00705
Zm00001d043263	15.46	45.8	1.56671	0.00005	Zm00001d014291	51.99	15.81	-1.71738	0.00005
Zm00001d009071	48.52	143.8	1.56774	0.00005	Zm00001d037080	4.411	1.343	-1.71612	0.0027
Zm00001d033407	1.35	4.014	1.57214	0.00105	Zm00001d043048	6.113	1.861	-1.71533	0.0028
Zm00001d001779	5.368	15.98	1.57338	0.00475	Zm00001d045124	1.919	0.587	-1.70997	0.00005
Zm00001d017241	1.136	3.381	1.57349	0.00075	Zm00001d030106	2.067	0.634	-1.70484	0.00005
Zm00001d040562	150.4	448.9	1.57784	0.00005	Zm00001d034345	933.6	287	-1.70155	0.00005
Zm00001d034069	1.701	5.085	1.58033	0.00685	Zm00001d050955	4.886	1.503	-1.70071	0.00005
Zm00001d016768	38.58	115.5	1.5817	0.00005	Zm00001d009118	54.74	16.86	-1.69915	0.00005
Zm00001d004664	11.17	33.5	1.58433	0.00005	Zm00001d027593	4.9	1.512	-1.69597	0.00725
Zm00001d011377	22.8	68.54	1.58797	0.00005	Zm00001d038741	19.88	6.147	-1.69327	0.00095
Zm00001d028094	5.832	17.55	1.58931	0.00005	Zm00001d035236	6.325	1.957	-1.69233	0.00545
Zm00001d037766	1.474	4.451	1.59462	0.0061	Zm00001d017138	22.31	6.91	-1.69093	0.0016
Zm00001d049203	1.185	3.588	1.59833	0.00105	Zm00001d027405	25	7.751	-1.68933	0.00065
Zm00001d026056	7.095	21.56	1.60315	0.0033	Zm00001d046803	13.02	4.045	-1.68691	0.00545
Zm00001d031093	1.547	4.7	1.60318	0.00295	Zm00001d042636	11.27	3.525	-1.67632	0.00165

Zm00001d048507	28.9	87.86	1.6041	0.00005	Zm00001d049187	243.9	76.4	-1.67478	0.00005
Zm00001d032155	4.956	15.08	1.60575	0.004	Zm00001d042619	50.8	15.92	-1.6741	0.00005
Zm00001d049129	3.255	9.937	1.61011	0.0038	Zm00001d032467	38.68	12.13	-1.67261	0.00035
Zm00001d024281	63.2	193.4	1.61382	0.00005	Zm00001d016301	33.5	10.54	-1.66857	0.0003
Zm00001d021938	17.94	55.01	1.6168	0.00005	Zm00001d049554	50.11	15.77	-1.66797	0.00005
Zm00001d021629	14.68	45.04	1.61689	0.0001	Zm00001d029410	26.51	8.368	-1.66341	0.00005
Zm00001d049201	5.897	18.1	1.6176	0.0013	Zm00001d008103	6.611	2.087	-1.66322	0.00445
Zm00001d043063	2.501	7.684	1.61923	0.0071	Zm00001d037550	22.77	7.195	-1.662	0.00025
Zm00001d020697	6.202	19.06	1.61949	0.0002	Zm00001d016242	2.604	0.823	-1.66134	0.00415
Zm00001d006903	50.12	155.2	1.63026	0.00005	Zm00001d012090	408	129.1	-1.6599	0.00005
Zm00001d029920	4.766	14.8	1.63511	0.0002	Zm00001d040697	302.6	95.99	-1.65636	0.00005
Zm00001d006059	21.7	67.42	1.63553	0.00005	Zm00001d010640	34.86	11.11	-1.64999	0.00005
Zm00001d041634	2.851	8.873	1.63781	0.00005	Zm00001d027435	7.656	2.446	-1.64638	0.002
Zm00001d024885	17.16	53.44	1.63903	0.00005	Zm00001d041308	3.935	1.264	-1.6385	0.0003
Zm00001d042541	59.87	187.3	1.64571	0.00005	Zm00001d009666	26.24	8.437	-1.6369	0.0001
Zm00001d006948	3.216	10.07	1.64623	0.00005	Zm00001d018428	15.22	4.897	-1.63564	0.00005
Zm00001d051684	2.176	6.818	1.64748	0.00005	Zm00001d003283	4.942	1.591	-1.63552	0.00775
Zm00001d052260	12.52	39.24	1.64818	0.00005	Zm00001d011428	7.127	2.297	-1.6334	0.00615
Zm00001d006921	1.259	3.948	1.64863	0.00745	Zm00001d035605	329.4	106.2	-1.63232	0.00005
Zm00001d034823	3.596	11.3	1.65158	0.0002	Zm00001d012929	5.192	1.675	-1.63221	0.002
Zm00001d000640	10.81	33.98	1.65238	0.00765	Zm00001d018901	6.881	2.23	-1.6253	0.0051
Zm00001d011687	1.228	3.866	1.6544	0.0067	Zm00001d021596	136.1	44.17	-1.62373	0.00005
Zm00001d021420	12.92	40.69	1.65488	0.00005	Zm00001d021016	40.72	13.24	-1.62147	0.00035
Zm00001d046112	42.92	136.2	1.66607	0.00005	Zm00001d052793	5.705	1.855	-1.62126	0.0032
Zm00001d043782	5.369	17.23	1.6825	0.00375	Zm00001d025015	582.9	190.4	-1.61399	0.00005
Zm00001d028400	8.445	27.12	1.68307	0.00105	Zm00001d048667	22.67	7.412	-1.61273	0.00005
Zm00001d042307	6.067	19.53	1.68639	0.0013	Zm00001d051634	7.25	2.371	-1.61255	0.00005
Zm00001d022226	2.137	6.935	1.69846	0.0046	Zm00001d025654	14.28	4.677	-1.61077	0.00245
Zm00001d026406	5.733	18.61	1.69881	0.001	Zm00001d005594	12.93	4.24	-1.6088	0.0015
Zm00001d026206	0.703	2.296	1.70662	0.00485	Zm00001d034277	8.154	2.677	-1.60679	0.0019
Zm00001d017597	4.334	14.18	1.71028	0.00005	Zm00001d037721	6.193	2.034	-1.60655	0.00825
Zm00001d043411	8.225	27.11	1.72069	0.0001	Zm00001d038675	8.608	2.833	-1.6035	0.00005
Zm00001d041670	9.252	30.53	1.72248	0.00045	Zm00001d027938	67.36	22.22	-1.60023	0.00005
Zm00001d025834	1.191	3.939	1.72595	0.0017	Zm00001d018431	7.546	2.494	-1.59692	0.0003
Zm00001d005337	2.346	7.9	1.75159	0.0049	Zm00001d046207	7.414	2.452	-1.59654	0.00595
Zm00001d032363	3.945	13.33	1.75709	0.00175	Zm00001d022965	8.81	2.914	-1.59618	0.00245
Zm00001d003494	17.43	59.44	1.77004	0.00285	Zm00001d042349	10.98	3.637	-1.59445	0.00205
Zm00001d053684	2.661	9.084	1.77151	0.00005	Zm00001d022270	27.01	8.947	-1.59402	0.00055
Zm00001d013810	62.78	214.8	1.77442	0.00005	Zm00001d043650	53.87	17.86	-1.59293	0.00005
Zm00001d047483	1.677	5.739	1.77449	0.0016	Zm00001d051847	6.016	1.995	-1.59254	0.0083

Zm00001d024489	2.117	7.313	1.78886	0.00315
Zm00001d006106	16.59	57.68	1.79772	0.00005
Zm00001d047437	2.464	8.615	1.80559	0.0038
Zm00001d015053	0.831	2.907	1.80679	0.0073
Zm00001d024234	11.95	42.15	1.81839	0.00005
Zm00001d008756	3.16	11.25	1.83216	0.0087
Zm00001d002132	11.42	41.08	1.847	0.00115
Zm00001d045420	5.723	20.73	1.8569	0.0005
Zm00001d005692	2.462	8.954	1.86298	0.00145
Zm00001d020134	1.112	4.051	1.86482	0.00005
Zm00001d044652	1.789	6.522	1.86655	0.00265
Zm00001d018342	11.89	43.38	1.86775	0.00005
Zm00001d053675	43.72	160.5	1.87605	0.00005
Zm00001d007892	20.77	76.26	1.87646	0.00005
Zm00001d028714	4.175	15.41	1.8843	0.00375
Zm00001d018704	5.239	19.51	1.89709	0.0002
Zm00001d028160	1.326	5	1.91533	0.0069
Zm00001d047441	3.233	12.2	1.91606	0.00055
Zm00001d048789	2.171	8.215	1.91981	0.00635
Zm00001d040028	92.23	352.7	1.93521	0.00005
Zm00001d034946	2.078	7.947	1.93525	0.0043
Zm00001d023210	5.028	19.24	1.93598	0.00005
Zm00001d027344	27.72	106.5	1.94138	0.00005
Zm00001d007714	0.566	2.208	1.96371	0.00835
Zm00001d041653	2.352	9.189	1.96611	0.00155
Zm00001d050293	2.808	10.98	1.96703	0.00005
Zm00001d031149	0.405	1.595	1.9756	0.0008
Zm00001d038460	124.4	489.3	1.97578	0.00005
Zm00001d005459	42.4	167.5	1.98233	0.00005
Zm00001d024886	18.41	74.05	2.00795	0.00005
Zm00001d007604	44.19	177.9	2.009	0.00005
Zm00001d047579	8.291	33.4	2.01041	0.00005
Zm00001d001076	31.5	129.3	2.0377	0.00005
Zm00001d047124	2.118	8.698	2.03813	0.0026
Zm00001d033412	6.1	25.52	2.06451	0.0002
Zm00001d053916	16.35	69.3	2.0839	0.00005
Zm00001d041544	8.899	38.12	2.09893	0.00005
Zm00001d046805	7.118	31.07	2.12603	0.00005
Zm00001d024014	4.641	20.29	2.12799	0.00005
Zm00001d038453	189.8	835.5	2.13788	0.00005
Zm00001d020378	14.82	4.917	-1.59153	0.00005
Zm00001d004918	5.107	1.697	-1.5892	0.008
Zm00001d044430	5.126	1.704	-1.58857	0.00025
Zm00001d024630	5.735	1.907	-1.58831	0.0062
Zm00001d012292	10.65	3.545	-1.58669	0.0084
Zm00001d042244	3.039	1.013	-1.58494	0.00155
Zm00001d020881	28.79	9.6	-1.58435	0.00005
Zm00001d043196	13.77	4.591	-1.58418	0.00255
Zm00001d043988	19.59	6.544	-1.58202	0.0081
Zm00001d032776	82.08	27.43	-1.58144	0.00005
Zm00001d045804	3.772	1.263	-1.57871	0.00005
Zm00001d010137	29.51	9.905	-1.57484	0.00025
Zm00001d049588	4.319	1.455	-1.56936	0.00645
Zm00001d002199	2.945	0.995	-1.56553	0.00595
Zm00001d045843	6.551	2.216	-1.56343	0.0084
Zm00001d003039	17.31	5.856	-1.56328	0.0002
Zm00001d012091	44.39	15.03	-1.56236	0.0001
Zm00001d025346	30.53	10.36	-1.55932	0.00005
Zm00001d025338	78.25	26.56	-1.55879	0.00005
Zm00001d036761	33.17	11.26	-1.55834	0.00015
Zm00001d010948	9.891	3.374	-1.5518	0.00195
Zm00001d053293	39.25	13.44	-1.54604	0.00005
Zm00001d017333	18.61	6.378	-1.54502	0.0013
Zm00001d045539	13.04	4.487	-1.53902	0.0052
Zm00001d034869	9.75	3.357	-1.53801	0.0042
Zm00001d015004	3.475	1.198	-1.53589	0.00395
Zm00001d041305	12.02	4.154	-1.53353	0.0068
Zm00001d010987	28.48	9.846	-1.53237	0.0001
Zm00001d020932	7.186	2.485	-1.53205	0.00355
Zm00001d021119	48.65	16.85	-1.52973	0.00005
Zm00001d035246	64.62	22.38	-1.52966	0.00005
Zm00001d032849	15.22	5.302	-1.52189	0.0048
Zm00001d039081	55.42	19.3	-1.52172	0.00005
Zm00001d039908	56.92	19.83	-1.5214	0.00215
Zm00001d023596	20.7	7.234	-1.51677	0.00125
Zm00001d037015	3.871	1.355	-1.51496	0.0066
Zm00001d014447	11.72	4.103	-1.51413	0.00065
Zm00001d011315	7.736	2.713	-1.51188	0.00005
Zm00001d032024	26.63	9.343	-1.51105	0.0012
Zm00001d018161	182.8	64.16	-1.51036	0.00005

Zm00001d002847	1.408	6.198	2.13829	0.00335
Zm00001d028230	13.57	60.05	2.14601	0.00005
Zm00001d041458	2.411	10.73	2.15455	0.00005
Zm00001d010061	19.6	88.11	2.16827	0.00005
Zm00001d014030	0.479	2.189	2.19392	0.0071
Zm00001d018839	2.216	10.23	2.20714	0.00235
Zm00001d008370	5.948	27.72	2.22058	0.0002
Zm00001d026542	1.401	6.553	2.22619	0.0002
Zm00001d042540	52.54	246.1	2.22772	0.00005
Zm00001d053220	17.63	82.7	2.23009	0.00005
Zm00001d024875	4.18	19.69	2.23618	0.00005
Zm00001d021781	1.847	8.746	2.24366	0.00125
Zm00001d002898	4.504	21.46	2.25214	0.00025
Zm00001d048181	0.535	2.563	2.26124	0.0084
Zm00001d028319	0.653	3.14	2.26518	0.00575
Zm00001d008695	2.504	12.1	2.27254	0.0038
Zm00001d037610	2.715	13.24	2.28579	0.00025
Zm00001d028303	0.737	3.596	2.28637	0.0057
Zm00001d016948	5.89	28.83	2.29133	0.0053
Zm00001d005456	0.954	4.691	2.29839	0.00085
Zm00001d040027	32.36	159.5	2.30142	0.00005
Zm00001d045130	2.825	14.04	2.31345	0.0001
Zm00001d042062	3.026	15.29	2.33651	0.0013
Zm00001d003395	42.86	217.9	2.34623	0.00015
Zm00001d042730	4.869	24.83	2.35046	0.00235
Zm00001d040029	62.29	318	2.35169	0.00005
Zm00001d052340	4.224	21.9	2.37427	0.00005
Zm00001d012712	1.04	5.391	2.3745	0.00235
Zm00001d052124	8.379	43.69	2.38239	0.00005
Zm00001d009060	1.193	6.232	2.38455	0.00085
Zm00001d039691	1.471	7.686	2.38504	0.0002
Zm00001d028744	27.43	144.3	2.39525	0.00005
Zm00001d019985	0.77	4.059	2.39779	0.0058
Zm00001d026163	4.103	21.74	2.4054	0.00015
Zm00001d051473	1.414	7.639	2.43345	0.0087
Zm00001d045907	0.93	5.063	2.44451	0.00015
Zm00001d032909	3.485	19.03	2.44858	0.00005
Zm00001d016237	7.109	38.89	2.45172	0.00005
Zm00001d028282	1.552	8.561	2.46351	0.00175

Zm00001d041397	14.2	4.985	-1.51033	0.00015
Zm00001d044261	14.37	5.046	-1.51019	0.0002
Zm00001d020686	13.39	4.717	-1.50541	0.0033
Zm00001d032570	29.71	10.49	-1.50167	0.00035
Zm00001d027700	14.19	5.012	-1.50165	0.003
Zm00001d003751	43.08	15.22	-1.50089	0.00025
Zm00001d023311	111.4	39.5	-1.49575	0.00005
Zm00001d019259	12.74	4.524	-1.49375	0.0059
Zm00001d051615	5.505	1.956	-1.49278	0.0012
Zm00001d001043	41.76	14.84	-1.49273	0.00005
Zm00001d020348	46.64	16.58	-1.49193	0.00035
Zm00001d011746	5.752	2.046	-1.49104	0.0003
Zm00001d004006	124.5	44.29	-1.49095	0.00005
Zm00001d028056	10.13	3.611	-1.48745	0.00345
Zm00001d006685	82.02	29.25	-1.48735	0.00165
Zm00001d010039	25.06	8.96	-1.48371	0.00305
Zm00001d011929	45.19	16.19	-1.48066	0.00005
Zm00001d028231	25.42	9.138	-1.47618	0.001
Zm00001d021688	4.591	1.651	-1.47523	0.00295
Zm00001d038618	31.01	11.17	-1.4733	0.00005
Zm00001d038229	16.26	5.881	-1.46705	0.00005
Zm00001d013026	13.17	4.786	-1.46022	0.0001
Zm00001d020955	28.31	10.29	-1.45993	0.00005
Zm00001d029732	23.42	8.514	-1.45979	0.00115
Zm00001d012887	12.51	4.551	-1.45884	0.00305
Zm00001d028482	10.26	3.741	-1.45538	0.0076
Zm00001d040173	35.92	13.13	-1.4523	0.0001
Zm00001d047276	56.1	20.5	-1.4522	0.00005
Zm00001d021073	10.48	3.833	-1.4514	0.00485
Zm00001d026619	119.2	43.58	-1.45118	0.00005
Zm00001d040303	20.43	7.488	-1.44767	0.00005
Zm00001d009567	27.14	9.95	-1.44753	0.00815
Zm00001d027925	298.6	109.5	-1.44738	0.00005
Zm00001d046996	320	117.3	-1.44728	0.00005
Zm00001d044664	53.5	19.62	-1.44719	0.0085

Genes downregulated in the resistant genotype

Gene id	RAI	RAU	log2 fold change	P value
Zm00001d006010	94.36	257.4	1.44781	0.00005
Zm00001d052591	2.744	7.497	1.44984	0.00385

Zm00001d013753	1.592	8.793	2.46553	0.0007
Zm00001d002133	5.005	27.81	2.47412	0.00005
Zm00001d018237	1.216	6.898	2.50361	0.00005
Zm00001d009494	3.164	18.26	2.52884	0.00005
Zm00001d003226	0.634	3.724	2.55414	0.0065
Zm00001d022371	3.999	23.56	2.55886	0.00005
Zm00001d039513	11.54	68.14	2.56232	0.00005
Zm00001d010588	1.598	9.443	2.563	0.00075
Zm00001d034713	2.294	13.72	2.57998	0.00005
Zm00001d021775	16.74	100.6	2.58739	0.00005
Zm00001d007606	3.48	21.38	2.61932	0.00055
Zm00001d037637	3.74	23.79	2.66923	0.00165
Zm00001d004921	11.55	73.85	2.67683	0.00005
Zm00001d031167	7.302	46.99	2.68597	0.00005
Zm00001d048841	88.51	572.2	2.69264	0.00005
Zm00001d009646	1.141	7.431	2.70295	0.00005
Zm00001d022457	2.599	17.08	2.71659	0.0001
Zm00001d025140	1.438	9.536	2.72908	0.0014
Zm00001d002564	3.022	20.85	2.7866	0.00005
Zm00001d008587	8.831	62.22	2.81673	0.00005
Zm00001d018281	2.192	15.54	2.82556	0.00005
Zm00001d017292	3.717	26.36	2.82624	0.00045
Zm00001d018789	0.708	5.061	2.83796	0.0008
Zm00001d004486	0.618	4.573	2.88761	0.0006
Zm00001d034673	19.04	142.6	2.90551	0.00005
Zm00001d010131	0.448	3.366	2.90829	0.0085
Zm00001d016151	1.142	8.651	2.92121	0.0022
Zm00001d021702	1.829	13.98	2.9345	0.00005
Zm00001d044762	5.073	45.74	3.17244	0.00005
Zm00001d017025	1.494	13.64	3.18986	0.00005
Zm00001d038699	8.75	88.01	3.33031	0.00005
Zm00001d003304	6.312	65.7	3.3797	0.00005
Zm00001d003306	3.875	44.82	3.53165	0.00025
Zm00001d047744	1.657	20.39	3.62114	0.0001
Zm00001d012456	12.79	158.7	3.63297	0.00005
Zm00001d034382	38.73	504.2	3.70259	0.00005
Zm00001d022032	15.33	202.9	3.72619	0.00005
Zm00001d044765	0.33	4.826	3.87045	0.0018
Zm00001d029183	0.229	4.323	4.23871	0.00795
Zm00001d038288	5.612	123.4	4.45892	0.00005
Zm00001d025710	1.236	3.391	1.45601	0.0005
Zm00001d024693	0.579	1.598	1.4647	0.00375
Zm00001d031958	6.771	18.78	1.4721	0.00005
Zm00001d011401	5.222	14.53	1.47607	0.00005
Zm00001d003859	0.77	2.147	1.4804	0.00845
Zm00001d033707	32.17	90.45	1.49124	0.00005
Zm00001d045106	3.009	8.464	1.49211	0.00705
Zm00001d038301	0.883	2.49	1.4962	0.0036
Zm00001d003757	31.52	89.11	1.49945	0.00005
Zm00001d001276	16.21	46.39	1.51724	0.0022
Zm00001d006875	1.442	4.127	1.51726	0.0028
Zm00001d006365	4.448	12.91	1.53727	0.00315
Zm00001d046109	8.097	23.54	1.53975	0.00395
Zm00001d038538	7.699	22.54	1.55005	0.00005
Zm00001d026395	0.676	1.987	1.55616	0.0077
Zm00001d044153	6.002	17.66	1.55696	0.0001
Zm00001d032253	2.52	7.415	1.55698	0.0028
Zm00001d008794	1.923	5.668	1.55975	0.00785
Zm00001d028742	16.32	48.2	1.56263	0.00005
Zm00001d053702	0.934	2.798	1.58343	0.0006
Zm00001d017539	0.974	2.922	1.5849	0.00105
Zm00001d024522	37.51	112.5	1.58525	0.00005
Zm00001d041173	48.51	148.6	1.61517	0.00005
Zm00001d007768	2.335	7.18	1.62046	0.00075
Zm00001d012391	1.719	5.307	1.62661	0.00005
Zm00001d025752	2.906	9.039	1.63727	0.00425
Zm00001d053183	0.704	2.211	1.65082	0.00835
Zm00001d038065	4.341	13.7	1.65809	0.0083
Zm00001d033061	1.67	5.279	1.66078	0.00245
Zm00001d010442	0.797	2.531	1.66809	0.00115
Zm00001d017036	9.23	29.34	1.66834	0.00005
Zm00001d027355	35.49	113	1.67063	0.00005
Zm00001d053367	1.256	4.019	1.67779	0.0051
Zm00001d052029	1.957	6.261	1.67801	0.0037
Zm00001d005460	13.43	43.39	1.69163	0.00005
Zm00001d032923	9.2	30.48	1.72811	0.00005
Zm00001d017559	9.489	31.55	1.73346	0.00005
Zm00001d040629	0.971	3.24	1.73763	0.0029
Zm00001d034819	0.675	2.255	1.73953	0.00835
Zm00001d041956	0.867	2.917	1.74985	0.00475

Zm00001d014757	0	2.406	100	0.0023
Zm00001d017478	0	3.07	100	0.00005
Zm00001d018811	0	2.509	100	0.00035
Zm00001d019364	0	1.943	100	0.00625
Zm00001d022650	0	1.855	100	0.00015
Zm00001d023120	0	3.778	100	0.00005
Zm00001d027636	0	3.121	100	0.00005
Zm00001d028314	0	2.954	100	0.00005
Zm00001d029583	0	2.174	100	0.0002
Zm00001d033389	0	3.323	100	0.00505
Zm00001d033737	0	3.683	100	0.0004
Zm00001d036298	0	4.835	100	0.00005
Zm00001d037608	0	3.645	100	0.00005
Zm00001d039929	0	1.981	100	0.00045
Zm00001d041939	0	2.46	100	0.0004
Zm00001d041981	0	2.028	100	0.00085
Zm00001d046988	0	1.547	100	0.00705
Zm00001d047231	0	2.256	100	0.00055
Zm00001d048998	0	1.864	100	0.00005
Zm00001d052322	0	2.296	100	0.00505
Zm00001d052915	0	23.06	100	0.00005
Zm00001d053042	0	2.78	100	0.00545
Zm00001d013294	1.275	4.947	1.9559	0.00185
Zm00001d042323	1.722	6.698	1.95967	0.00085
Zm00001d002592	0.98	3.826	1.96456	0.0075
Zm00001d029195	1.047	4.117	1.97604	0.00295
Zm00001d049336	5.682	22.91	2.01145	0.00005
Zm00001d002823	4.786	19.34	2.01496	0.00005
Zm00001d011778	1.987	8.069	2.0219	0.00025
Zm00001d008548	21.28	86.85	2.02904	0.00005
Zm00001d048494	0.827	3.431	2.05347	0.00005
Zm00001d021095	3.449	14.43	2.06463	0.0001
Zm00001d027525	1.995	8.371	2.06902	0.0044
Zm00001d051362	100.3	422.6	2.0756	0.00005
Zm00001d031332	120.1	513.4	2.09584	0.00005
Zm00001d003059	1.958	8.415	2.10379	0.00025
Zm00001d027335	0.922	3.974	2.10741	0.00245
Zm00001d040589	4.15	18.14	2.12798	0.00005
Zm00001d022458	2.718	12.01	2.14374	0.00005
Zm00001d019312	15.14	67.2	2.14968	0.00005
Zm00001d010912	2.209	7.434	1.75074	0.00695
Zm00001d012757	1.733	5.868	1.75928	0.0028
Zm00001d016601	0.649	2.226	1.77822	0.0034
Zm00001d009716	1.99	6.863	1.78623	0.00005
Zm00001d048469	1.236	4.298	1.79798	0.00835
Zm00001d020443	12.63	43.96	1.79943	0.00005
Zm00001d033005	2.77	9.704	1.80883	0.00075
Zm00001d003368	1.409	4.968	1.81828	0.00005
Zm00001d047110	9.966	35.74	1.84247	0.00005
Zm00001d034678	2.011	7.269	1.8541	0.0004
Zm00001d041168	3.277	11.86	1.85578	0.00005
Zm00001d017874	2.489	9.062	1.86438	0.00005
Zm00001d031569	29.05	106.7	1.87691	0.00005
Zm00001d013370	3.866	14.28	1.88474	0.00005
Zm00001d002295	9.231	34.16	1.88787	0.00005
Zm00001d038598	5.68	21.09	1.89266	0.00145
Zm00001d031993	1.179	4.407	1.90183	0.00005
Zm00001d040379	2.902	10.92	1.91252	0.00525
Zm00001d029997	3.995	15.32	1.93945	0.0002
Zm00001d027932	7.062	27.11	1.94051	0.00205
Zm00001d011782	1.461	5.62	1.9433	0.0007

Zm00001d041663	3.323	14.76	2.15065	0.00245
Zm00001d017908	0.82	3.673	2.16314	0.0006
Zm00001d014758	4.124	18.9	2.19647	0.00085
Zm00001d020383	33.2	152.5	2.19972	0.00005
Zm00001d020898	30.96	143.4	2.21115	0.00005
Zm00001d002409	1.293	6.051	2.22643	0.0014
Zm00001d005898	10.55	50.65	2.26326	0.00005
Zm00001d018056	1.318	6.328	2.26362	0.0038
Zm00001d043741	1.469	7.075	2.268	0.00415
Zm00001d027900	3.818	18.43	2.27112	0.00025
Zm00001d001575	4.315	20.85	2.27271	0.00005
Zm00001d024982	1.21	6.003	2.31091	0.0031
Zm00001d033063	2.488	12.58	2.33797	0.00005
Zm00001d026412	1.776	9.522	2.42268	0.0031
Zm00001d015227	6.79	37.37	2.46058	0.003
Zm00001d041985	0.977	5.515	2.49745	0.0084
Zm00001d040308	1.075	6.595	2.61744	0.00645
Zm00001d015921	0.874	5.435	2.63605	0.00155
Zm00001d012710	3.166	20.42	2.68914	0.00005
Zm00001d026413	3.741	25.55	2.772	0.00005
Zm00001d018621	1.968	13.82	2.81229	0.00035
Zm00001d002738	3.137	23.6	2.91145	0.0021
Zm00001d053079	0.775	6.147	2.98784	0.00005
Zm00001d015560	1.23	9.87	3.00396	0.00005
Zm00001d016189	5.406	46.7	3.11097	0.0021
Zm00001d027443	1.578	15.74	3.31854	0.00625
Zm00001d032000	1.069	10.75	3.32989	0.00035
Zm00001d053304	0.266	3.483	3.7098	0.0002
Zm00001d029038	0.986	13.42	3.76785	0.0014
Zm00001d022906	0	1.926	100	0.0079
Zm00001d032287	0	2.312	100	0.00005
Zm00001d043837	0	4.054	100	0.00005

Appendix 5: List of differentially expressed genes at the third time period (22 days post infestation)

Genes Present in both genotypes

Gene id	SCI	SCU	log2 fold change	P value	Gene id	RCI	RCU	log2 fold change	P value
Zm00001d018742	24.098	2.029	-3.56976	5.00E-05	Zm00001d018742	20.142	7.10411	-1.50351	0.0015
Zm00001d003059	0.6181	1.932	1.64432	0.0056	Zm00001d003059	0.4333	4.6513	3.42406	1.00E-04
Zm00001d033710	29.021	117	2.01153	5.00E-05	Zm00001d033710	1.126	13.5314	3.58708	0.0065
Zm00001d048709	14.323	1.488	-3.2665	5.00E-05	Zm00001d048709	12.177	3.67425	-1.72866	0.0052
Zm00001d016237	1.755	13.51	2.94416	5.00E-05	Zm00001d016237	0.9511	16.2028	4.09052	5.00E-05
Zm00001d010521	17.665	3.687	-2.26044	5.00E-05	Zm00001d010521	9.5971	3.4893	-1.45966	0.004
Zm00001d001043	2.9343	9.152	1.64105	0.0047	Zm00001d001043	2.7185	14.3606	2.40125	5.00E-05
Zm00001d049525	15.784	3.426	-2.20364	2.00E-04	Zm00001d049525	11.233	4.05447	-1.47016	0.00395
Zm00001d037550	20.197	4.595	-2.13599	5.00E-05	Zm00001d037550	20.494	7.4809	-1.4539	0.00055
Zm00001d039935	443.25	40.74	-3.44376	5.00E-05	Zm00001d039935	143.09	19.6759	-2.86239	5.00E-05
Zm00001d027305	4.0241	11.54	1.51955	0.00175	Zm00001d027305	5.2498	22.0069	2.06763	5.00E-05
Zm00001d052733	37.505	9.003	-2.05867	5.00E-05	Zm00001d052733	16.899	5.87324	-1.5247	0.00015
Zm00001d023225	124.76	31.86	-1.96945	5.00E-05	Zm00001d023225	45.603	16.6893	-1.45021	5.00E-05
Zm00001d047799	17.839	3.544	-2.33156	5.00E-05	Zm00001d047799	19.573	5.43867	-1.84755	1.00E-04
Zm00001d032776	94.197	20.65	-2.18976	5.00E-05	Zm00001d032776	56.412	17.2346	-1.71071	5.00E-05
Zm00001d029814	5.8628	0.867	-2.75759	0.00735	Zm00001d029814	11.601	2.32892	-2.31652	0.00495
Zm00001d009351	2.6643	8.457	1.66632	1.00E-04	Zm00001d009351	1.5118	6.20227	2.03657	5.00E-05
Zm00001d020257	10.738	2.865	-1.90585	0.00015	Zm00001d020257	7.1441	2.45001	-1.54397	0.00525
Zm00001d029478	13.512	3.855	-1.80938	5.00E-05	Zm00001d029478	12.015	4.35118	-1.46541	0.00295
Zm00001d005775	85.915	20.4	-2.07443	5.00E-05	Zm00001d005775	58.362	17.5566	-1.733	5.00E-05
Zm00001d020531	72.686	16.64	-2.12709	5.00E-05	Zm00001d020531	46.529	13.3934	-1.79662	5.00E-05

Zm00001d009494	5.0081	14.65	1.54898	0.00015	Zm00001d009494	1.6886	6.19391	1.87499	3.00E-04
Zm00001d049204	5.4166	15.79	1.54394	0.00045	Zm00001d049204	4.5029	16.222	1.84902	5.00E-05
Zm00001d011840	8.0856	2.308	-1.80865	0.0028	Zm00001d011840	12.085	4.103	-1.55842	0.0052
Zm00001d031127	61.098	14.61	-2.06429	5.00E-05	Zm00001d031127	11.166	3.16947	-1.8168	0.00475
Zm00001d020378	6.6036	18.2	1.46296	5.00E-05	Zm00001d020378	3.9912	12.6644	1.66586	1.00E-04
Zm00001d043019	29.303	8.17	-1.84256	5.00E-05	Zm00001d043019	10.878	3.40789	-1.6745	0.00085
Zm00001d042143	4.2399	14.71	1.79485	0.00155	Zm00001d042143	9.109	35.2938	1.95405	5.00E-05
Zm00001d028599	12.24	2.811	-2.12251	5.00E-05	Zm00001d028599	7.6577	1.95661	-1.96856	0.00045
Zm00001d009726	0.728	5.456	2.90575	5.00E-05	Zm00001d009726	0.5806	4.77039	3.03842	0.00155
Zm00001d024734	43.856	11.52	-1.92878	5.00E-05	Zm00001d024734	7.3229	2.06325	-1.8275	0.0054
Zm00001d024784	27.092	8.606	-1.65449	5.00E-05	Zm00001d024784	13.701	4.65315	-1.558	0.00015
Zm00001d047201	6.072	19.84	1.70803	5.00E-05	Zm00001d047201	3.0357	10.4883	1.78868	5.00E-05
Zm00001d042906	18.45	5.103	-1.85408	5.00E-05	Zm00001d042906	8.1181	2.33889	-1.79531	0.00075
Zm00001d048705	117.21	40.03	-1.55005	5.00E-05	Zm00001d048705	129.21	44.9411	-1.52366	0.00015
Zm00001d009506	19.928	5.493	-1.85909	5.00E-05	Zm00001d009506	15.173	4.20308	-1.85198	5.00E-05
Zm00001d038296	1.3979	4.115	1.5577	0.00785	Zm00001d038296	1.362	4.02178	1.56215	0.0022
Zm00001d051442	5.3324	1.879	-1.50459	0.00555	Zm00001d051442	5.3983	1.85288	-1.54272	0.00255
Zm00001d029391	7.974	1.742	-2.19425	0.00025	Zm00001d029391	3.8179	0.80381	-2.24786	0.0026
Zm00001d038702	49.207	171.1	1.79786	5.00E-05	Zm00001d038702	68.575	222.945	1.70093	5.00E-05
Zm00001d038703	73.873	256.3	1.79462	5.00E-05	Zm00001d038703	101.13	325.099	1.68461	5.00E-05
Zm00001d009646	0.7139	3.218	2.17224	0.0039	Zm00001d009646	0.4939	2.03162	2.04044	0.0082
Zm00001d041712	8.6843	2.986	-1.54017	0.00395	Zm00001d041712	18.495	5.53655	-1.74006	0.00025
Zm00001d005570	73.778	24.73	-1.57699	5.00E-05	Zm00001d005570	31.928	9.27957	-1.78268	5.00E-05
Zm00001d048073	17.052	3.198	-2.41468	5.00E-05	Zm00001d048073	4.3173	0.68331	-2.65951	5.00E-05
Zm00001d002295	6.9223	32.56	2.23394	5.00E-05	Zm00001d002295	15.333	57.6954	1.91179	5.00E-05
Zm00001d033516	19.906	7.009	-1.50582	1.00E-04	Zm00001d033516	7.0771	1.8992	-1.89777	0.00145
Zm00001d040173	27.33	9.157	-1.57753	5.00E-05	Zm00001d040173	15.075	3.73662	-2.01237	0.00025
Zm00001d048050	31.207	135.7	2.12085	5.00E-05	Zm00001d048050	48.685	151.244	1.63533	5.00E-05

Zm00001d003016	129.91	43.61	-1.57486	5.00E-05	Zm00001d003016	58.981	14.0157	-2.0732	5.00E-05
Zm00001d031275	45.995	12.37	-1.89436	5.00E-05	Zm00001d031275	23.2	4.38209	-2.40444	5.00E-05
Zm00001d004243	69.455	7.581	-3.19564	5.00E-05	Zm00001d004243	6.7318	0.50974	-3.72316	5.00E-05
Zm00001d041656	6.8599	32.72	2.25386	5.00E-05	Zm00001d041656	9.9269	31.5481	1.66814	0.00055
Zm00001d028693	36.442	166.3	2.19009	5.00E-05	Zm00001d028693	5.8747	17.2577	1.55465	5.00E-05
Zm00001d047830	0.4016	2.182	2.44164	0.00165	Zm00001d047830	1.848	5.8693	1.66719	0.0016
Zm00001d027313	7.8746	1.392	-2.49958	0.0021	Zm00001d027313	14.662	1.44766	-3.34025	3.00E-04
Zm00001d003994	1.6493	14.98	3.1834	5.00E-05	Zm00001d003994	1.3939	5.71634	2.03599	0.0037
Zm00001d039936	171.3	16.97	-3.33569	5.00E-05	Zm00001d039936	53.614	1.9288	-4.79682	0.00485
Zm00001d014617	18.289	6.335	-1.52949	0.0086	Zm00001d014617	19.88	2.18365	-3.18652	0.00035
Zm00001d047736	8.2096	117	3.83259	5.00E-05	Zm00001d047736	11.297	49.1034	2.11989	5.00E-05
Zm00001d024903	24.612	7.23	-1.76731	5.00E-05	Zm00001d024903	20.884	1.12182	-4.21846	5.00E-05
Zm00001d004413	11.619	35.14	1.5965	3.00E-04	Zm00001d004413	25.363	6.86872	-1.88459	5.00E-05
Zm00001d034839	2.2029	6.258	1.50633	0.0041	Zm00001d034839	3.4675	0.86655	-2.00053	0.00775
Zm00001d032003	1.6503	6.828	2.04882	0.00525	Zm00001d032003	11.001	3.66552	-1.58548	0.0077
Zm00001d047658	6.1049	16.84	1.46373	0.0069	Zm00001d047658	26.689	1.91057	-3.80418	0.00045
Zm00001d009765	1.3196	4.027	1.6095	0.00015	Zm00001d009765	25.174	1.01928	-4.6263	5.00E-05
Zm00001d047548	10.934	2.001	-2.4499	0.00615	Zm00001d047548	5.2223	0	-	5.00E-05
Zm00001d047841	20.304	2.777	-2.87034	0.00035	Zm00001d047841	2.7202	0	-	1.00E-04

Genes upregulated in the susceptible genotype					Genes upregulated in the resistant genotype				
Gene_id	SBI	SBU	log2 fold change	P value	Gene_id.1	RBI	RBU	log2 fold change	P value
Zm00001d003767	2.3978	0	-	5.00E-05	Zm00001d002347	2.256	0	-	0.005
Zm00001d004894	2.6126	0	-	5.00E-05	Zm00001d003549	2.6455	0	-	5.00E-05
Zm00001d005446	2.1628	0	-	5.00E-05	Zm00001d005029	3.0561	0	-	5.00E-05
Zm00001d006111	3.4382	0	-	5.00E-05	Zm00001d005037	2.0548	0	-	0.00775
Zm00001d008814	1.7818	0	-	5.00E-05	Zm00001d005375	1.8008	0	-	3.00E-04
Zm00001d009589	3.9626	0	-	5.00E-05	Zm00001d005381	2.9139	0	-	5.00E-05

Zm00001d010102	15.226	0	-	5.00E-05	Zm00001d005766	2.5864	0	-	0.00205
Zm00001d011285	3.2912	0	-	5.00E-05	Zm00001d005819	3.23	0	-	6.00E-04
Zm00001d018913	28.657	0	-	0.0021	Zm00001d006119	1.9953	0	-	0.00415
Zm00001d018914	3.9221	0	-	0.0017	Zm00001d006882	2.8924	0	-	0.0075
Zm00001d020364	2.1836	0	-	6.00E-04	Zm00001d008397	2.3134	0	-	0.00205
Zm00001d020877	1.8581	0	-	5.00E-05	Zm00001d009446	3.8415	0	-	5.00E-05
Zm00001d021906	1.9888	0	-	5.00E-05	Zm00001d010575	3.0588	0	-	0.00495
Zm00001d023559	1.6828	0	-	5.00E-05	Zm00001d011050	4.1598	0	-	0.00285
Zm00001d028432	4.942	0	-	5.00E-05	Zm00001d011543	2.3027	0	-	0.00435
Zm00001d028880	114.64	0	-	5.00E-05	Zm00001d013208	2.0553	0	-	0.00015
Zm00001d033008	2.7007	0	-	0.0027	Zm00001d013517	2.2425	0	-	0.00045
Zm00001d039942	3.7486	0	-	5.00E-05	Zm00001d016379	3.2255	0	-	0.00025
Zm00001d041819	5.058	0	-	5.00E-05	Zm00001d016697	4.607	0	-	5.00E-05
Zm00001d043299	2.2506	0	-	2.00E-04	Zm00001d017477	2.7317	0	-	5.00E-05
Zm00001d044925	22.72	0	-	5.00E-05	Zm00001d018326	1.8189	0	-	5.00E-05
Zm00001d046786	2.1677	0	-	5.00E-05	Zm00001d018433	14.288	0	-	5.00E-05
Zm00001d051894	1.6253	0	-	1.00E-04	Zm00001d019216	1.8918	0	-	4.00E-04
Zm00001d034124	4.0285	1.478	-1.44644	0.00655	Zm00001d020195	1.6608	0	-	0.00355
Zm00001d017785	7.6951	2.819	-1.44858	0.0015	Zm00001d021591	2.617	0	-	5.00E-05
Zm00001d021304	75.091	27.48	-1.45042	5.00E-05	Zm00001d022210	4.5963	0	-	5.00E-05
Zm00001d028363	23.491	8.579	-1.45326	5.00E-05	Zm00001d022637	1.9706	0	-	3.00E-04
Zm00001d019944	11.921	4.318	-1.46518	1.00E-04	Zm00001d024200	1.8742	0	-	9.00E-04
Zm00001d019507	127.11	45.95	-1.46797	5.00E-05	Zm00001d025406	3.6124	0	-	0.00225
Zm00001d032715	6.5302	2.36	-1.46845	6.00E-04	Zm00001d026262	2.2336	0	-	9.00E-04
Zm00001d039144	90.627	32.74	-1.46876	3.00E-04	Zm00001d027345	2.939	0	-	2.00E-04
Zm00001d034175	13.417	4.841	-1.4707	6.00E-04	Zm00001d027387	2.8392	0	-	5.00E-05
Zm00001d002358	21.569	7.756	-1.47566	5.00E-05	Zm00001d029028	3.6037	0	-	0.00015
Zm00001d011274	16.567	5.949	-1.47754	9.00E-04	Zm00001d029599	1.6488	0	-	0.0012
Zm00001d031822	63.477	22.77	-1.47907	1.00E-04	Zm00001d030698	3.4757	0	-	4.00E-04
Zm00001d010066	6.7445	2.417	-1.48028	0.0023	Zm00001d031423	3.0012	0	-	0.00095
Zm00001d045833	26.77	9.582	-1.48218	0.0024	Zm00001d031431	4.5102	0	-	0.00045
Zm00001d024825	2.0436	0.731	-1.48291	0.00035	Zm00001d031806	2.4581	0	-	0.00345
Zm00001d034779	2.3358	0.833	-1.48761	0.00285	Zm00001d031852	3.808	0	-	0.00775
Zm00001d052030	1.7794	0.633	-1.49178	0.00505	Zm00001d033531	5.8802	0	-	5.00E-05
Zm00001d045582	5.2714	1.872	-1.49395	0.0067	Zm00001d033680	2.3061	0	-	5.00E-05
Zm00001d053356	8.3698	2.969	-1.49519	5.00E-05	Zm00001d035559	2.3651	0	-	5.00E-05
Zm00001d016683	7.2727	2.575	-1.49798	0.00545	Zm00001d035560	1.8863	0	-	5.00E-05
Zm00001d011088	8.6314	3.043	-1.50391	0.0034	Zm00001d035759	8.0999	0	-	0.00105
Zm00001d042078	6.4744	2.277	-1.50747	0.0071	Zm00001d036597	2.1942	0	-	0.00325
Zm00001d039946	12.529	4.389	-1.51322	0.00085	Zm00001d037361	2.6795	0	-	2.00E-04

Zm00001d052335	69.131	24.22	-1.5134	5.00E-05	Zm00001d039367	5.994	0	-	0.00105
Zm00001d010805	10.067	3.525	-1.51385	0.00675	Zm00001d039842	4.9548	0	-	5.00E-05
Zm00001d040205	5.0055	1.752	-1.51468	0.0027	Zm00001d040064	2.7615	0	-	5.00E-05
Zm00001d034553	49.357	17.27	-1.51491	5.00E-05	Zm00001d040445	2.2341	0	-	5.00E-05
Zm00001d038301	2.0708	0.723	-1.51784	0.0048	Zm00001d043984	3.1706	0	-	0.001
Zm00001d016070	838.55	292.6	-1.51922	0.00385	Zm00001d044227	3.112	0	-	0.00095
Zm00001d042899	35.803	12.49	-1.51945	5.00E-05	Zm00001d044923	2.248	0	-	0.005
Zm00001d046697	23.388	8.136	-1.52328	5.00E-05	Zm00001d045361	1.8225	0	-	0.00575
Zm00001d031732	125.23	43.56	-1.52333	5.00E-05	Zm00001d046404	1.8293	0	-	0.00775
Zm00001d030133	24.158	8.367	-1.52973	5.00E-05	Zm00001d047775	2.3614	0	-	5.00E-05
Zm00001d038533	32.557	11.23	-1.53604	5.00E-04	Zm00001d048991	3.6555	0	-	5.00E-05
Zm00001d005246	3.5044	1.205	-1.5407	0.00495	Zm00001d050697	3.5406	0	-	3.00E-04
Zm00001d026006	1.7832	0.612	-1.54339	0.0051	Zm00001d053689	3.824	0	-	0.00075
Zm00001d012176	1.7734	0.608	-1.54357	0.00715	Zm00001d020898	528.58	194.001	-1.44605	5.00E-05
Zm00001d002122	32.191	11.04	-1.54446	5.00E-05	Zm00001d048431	23.469	8.60754	-1.44707	0.0042
Zm00001d012808	39.986	13.67	-1.54819	5.00E-05	Zm00001d036993	16.815	6.15035	-1.451	0.00135
Zm00001d049692	43.588	14.9	-1.54886	0.00025	Zm00001d001977	37.192	13.5896	-1.4525	0.00015
Zm00001d030851	6.2815	2.142	-1.55231	0.00225	Zm00001d039169	19.833	7.235	-1.4548	0.00145
Zm00001d029707	11.249	3.818	-1.55887	0.00735	Zm00001d045000	39.657	14.4249	-1.45901	0.0028
Zm00001d009693	59.742	20.26	-1.56013	5.00E-05	Zm00001d046655	5.0263	1.82592	-1.46086	0.00205
Zm00001d043879	30.903	10.46	-1.56235	5.00E-05	Zm00001d017125	12.156	4.40397	-1.46478	0.00065
Zm00001d009118	9.4263	3.188	-1.56399	0.00565	Zm00001d053675	85.055	30.8071	-1.46514	5.00E-05
Zm00001d041082	13.728	4.634	-1.5669	5.00E-05	Zm00001d047211	4.7089	1.70261	-1.46765	0.0054
Zm00001d027816	16.313	5.495	-1.56994	0.0017	Zm00001d004931	5.3381	1.9301	-1.46766	0.006
Zm00001d021069	4.5264	1.523	-1.57143	0.00255	Zm00001d051415	1.9006	0.68573	-1.47072	0.0045
Zm00001d012602	11.447	3.851	-1.57159	0.0033	Zm00001d044079	12.557	4.53035	-1.47073	0.0078
Zm00001d013582	15.088	5.072	-1.57272	5.00E-05	Zm00001d028151	11.478	4.13642	-1.47243	0.00255
Zm00001d036835	16.25	5.456	-1.57457	3.00E-04	Zm00001d013222	10.174	3.66604	-1.47258	0.00765

Zm00001d051704	6.8055	2.28	-1.57795	5.00E-05	Zm00001d023277	4.6643	1.67785	-1.47506	0.00275
Zm00001d032971	4.5685	1.526	-1.58187	0.00195	Zm00001d030199	30.155	10.8061	-1.48057	0.00135
Zm00001d044594	9.5452	3.184	-1.584	1.00E-04	Zm00001d018744	616.31	219.681	-1.48824	5.00E-05
Zm00001d053011	9.9601	3.309	-1.58997	5.00E-05	Zm00001d043615	19.89	7.05773	-1.49479	6.00E-04
Zm00001d037939	9.0752	3.01	-1.59211	5.00E-05	Zm00001d003712	148.37	52.4899	-1.49904	5.00E-05
Zm00001d017820	14.664	4.851	-1.59578	5.00E-05	Zm00001d040697	221.86	78.4645	-1.49954	5.00E-05
Zm00001d049541	343.25	113.2	-1.60035	5.00E-05	Zm00001d032461	21.272	7.52102	-1.49997	9.00E-04
Zm00001d009586	5.5583	1.833	-1.60072	0.0053	Zm00001d052651	12.921	4.54428	-1.50764	0.00415
Zm00001d047763	20.752	6.829	-1.60349	5.00E-05	Zm00001d017526	643.55	225.854	-1.51065	5.00E-05
Zm00001d047765	33.023	10.85	-1.6052	5.00E-05	Zm00001d015589	10.221	3.5604	-1.5214	0.0039
Zm00001d043737	18.79	6.168	-1.60714	5.00E-05	Zm00001d049510	54.788	19.0505	-1.52402	5.00E-05
Zm00001d047981	24.175	7.932	-1.60775	5.00E-05	Zm00001d019280	56.203	19.5191	-1.52577	1.00E-04
Zm00001d031619	22.505	7.378	-1.60885	5.00E-05	Zm00001d024382	42.643	14.8082	-1.52591	5.00E-05
Zm00001d005394	106.09	34.78	-1.60897	5.00E-05	Zm00001d041663	28.223	9.78755	-1.52787	0.00035
Zm00001d015124	4.3429	1.422	-1.61075	0.00055	Zm00001d013003	23.801	8.24803	-1.52892	8.00E-04
Zm00001d032172	2.5141	0.823	-1.61094	0.0039	Zm00001d019560	10.968	3.78345	-1.53556	0.0012
Zm00001d038282	19.633	6.415	-1.61374	5.00E-05	Zm00001d028486	7.2853	2.49859	-1.54386	0.00635
Zm00001d032955	32.396	10.55	-1.61889	5.00E-05	Zm00001d018028	6.8755	2.35699	-1.54452	0.0064
Zm00001d044821	15.492	5.031	-1.62274	5.00E-04	Zm00001d028386	10.376	3.55371	-1.54583	7.00E-04
Zm00001d047806	17.713	5.74	-1.62563	5.00E-05	Zm00001d014507	6.0763	2.07334	-1.55123	0.0015
Zm00001d053313	2.9882	0.965	-1.63046	5.00E-05	Zm00001d039914	66.261	22.5263	-	5.00E-05

								1.55655	
Zm00001d022082	11.738	3.787	-1.63217	2.00E-04	Zm00001d026224	18.443	6.2493	-1.56129	0.0052
Zm00001d045695	58.254	18.7	-1.63958	5.00E-05	Zm00001d045302	24.813	8.40394	-1.56195	4.00E-04
Zm00001d009969	26.411	8.46	-1.64243	5.00E-05	Zm00001d048710	29.089	9.80773	-1.56847	0.0011
Zm00001d053545	1.8269	0.584	-1.64449	0.00365	Zm00001d009029	7.3154	2.46128	-1.57153	9.00E-04
Zm00001d029285	24.157	7.708	-1.64793	5.00E-05	Zm00001d034015	290.44	97.416	-1.57598	5.00E-05
Zm00001d039006	17.146	5.466	-1.64934	5.00E-05	Zm00001d005917	5.4224	1.81507	-1.57892	0.00445
Zm00001d017441	26.888	8.531	-1.65612	0.00055	Zm00001d013243	6.0923	2.03704	-1.58052	0.00525
Zm00001d017845	6.832	2.167	-1.65689	0.0012	Zm00001d022264	218.91	72.9393	-1.58554	5.00E-05
Zm00001d029651	3.6894	1.169	-1.65756	0.00665	Zm00001d017485	355.69	118.263	-1.58862	5.00E-05
Zm00001d027763	13.94	4.399	-1.66417	0.00185	Zm00001d049643	29.588	9.82599	-1.59034	0.00045
Zm00001d029806	13.257	4.178	-1.66584	0.001	Zm00001d017841	31.518	10.4661	-1.59046	6.00E-04
Zm00001d013319	300.15	94.54	-1.66675	5.00E-05	Zm00001d008700	48.616	15.8501	-1.61694	0.0015
Zm00001d033778	7.1699	2.254	-1.66979	0.00485	Zm00001d018282	28.457	9.26145	-1.61946	2.00E-04
Zm00001d029499	19.989	6.248	-1.67775	5.00E-05	Zm00001d044302	3.7624	1.22348	-1.62066	0.0065
Zm00001d040171	7.1893	2.246	-1.67818	0.0024	Zm00001d042276	53.064	17.2317	-1.62266	5.00E-05
Zm00001d021420	17.29	5.397	-1.67971	5.00E-05	Zm00001d022254	56.222	18.1752	-1.62915	5.00E-05
Zm00001d015101	17.177	5.353	-1.68192	7.00E-04	Zm00001d045206	89.991	29.0438	-1.63156	8.00E-04
Zm00001d048137	15.379	4.78	-1.68601	5.00E-05	Zm00001d006626	14.83	4.77624	-1.6346	0.00155
Zm00001d039387	98.95	30.72	-1.6877	5.00E-05	Zm00001d036255	26.044	8.37712	-1.63642	5.00E-05
Zm00001d048703	96.377	29.71	-1.69787	5.00E-05	Zm00001d044520	4.1476	1.33321	-1.63736	0.0072

Zm00001d028219	34.033	10.46	-1.70236	5.00E-05	Zm00001d030342	12.697	4.07189	-1.64076	0.00415
Zm00001d020731	2.6822	0.824	-1.7026	0.0044	Zm00001d017276	30.023	9.61075	-1.64335	5.00E-05
Zm00001d013255	8.4277	2.587	-1.70371	0.00065	Zm00001d032070	41.014	13.1133	-1.64508	5.00E-04
Zm00001d007475	2.9199	0.896	-1.70441	5.00E-05	Zm00001d016604	5.8661	1.86785	-1.65102	0.00615
Zm00001d022053	20.71	6.353	-1.7049	0.00025	Zm00001d042123	3.5278	1.12232	-1.65227	0.0068
Zm00001d003212	3.5325	1.079	-1.7116	0.00275	Zm00001d019565	209.15	66.1286	-1.66119	5.00E-05
Zm00001d034394	7.0441	2.147	-1.71392	0.002	Zm00001d008620	220.88	69.7013	-1.66398	5.00E-05
Zm00001d012477	10.602	3.222	-1.71832	6.00E-04	Zm00001d051161	261.71	82.5881	-1.66398	5.00E-05
Zm00001d020670	11.415	3.462	-1.72124	0.00105	Zm00001d011422	5.9578	1.87903	-1.6648	0.0054
Zm00001d035659	25.991	7.879	-1.72196	5.00E-05	Zm00001d034781	5.2323	1.63493	-1.67821	0.0052
Zm00001d016733	10.865	3.293	-1.7223	0.0086	Zm00001d053281	36.665	11.4313	-1.6814	5.00E-05
Zm00001d029768	10.142	3.052	-1.73261	0.00025	Zm00001d052224	18.453	5.75115	-1.6819	0.00155
Zm00001d005451	57.123	16.97	-1.75085	5.00E-05	Zm00001d049181	19.06	5.92579	-1.6855	0.00035
Zm00001d032274	2.9553	0.876	-1.75368	2.00E-04	Zm00001d010840	32.473	10.0767	-1.68822	5.00E-05
Zm00001d022496	7.6146	2.252	-1.75761	0.00265	Zm00001d008693	16.603	5.12805	-1.69493	0.00235
Zm00001d029274	45.897	13.56	-1.7587	5.00E-05	Zm00001d020395	9.2808	2.86506	-1.69568	0.00345
Zm00001d025752	17.821	5.265	-1.75904	0.00195	Zm00001d021296	6.9893	2.14894	-1.70152	0.00045
Zm00001d034022	61.667	18.09	-1.76893	5.00E-05	Zm00001d011144	40.809	12.4455	-1.71324	5.00E-05
Zm00001d042653	10.439	3.06	-1.77046	0.00145	Zm00001d020260	14.944	4.55345	-1.7145	0.0014
Zm00001d026268	4.8667	1.424	-1.77265	0.00755	Zm00001d007233	8.6139	2.61208	-1.72146	0.00035
Zm00001d023735	311.75	91.18	-1.77366	5.00E-05	Zm00001d008546	32.479	9.83298	-1.72379	0.00065
Zm00001d052318	49.347	14.43	-1.77386	5.00E-05	Zm00001d024535	8.7366	2.63609	-1.72868	0.00145
Zm00001d002186	55.315	16.16	-1.77534	5.00E-05	Zm00001d028317	12.532	3.77176	-	0.00015

								1.73231	
Zm00001d021006	15.531	4.519	-1.78103	0.00015	Zm00001d027454	17.875	5.3774	-1.73296	0.00475
Zm00001d006454	17.518	5.011	-1.80563	5.00E-05	Zm00001d017025	4.1038	1.23454	-1.73299	0.00835
Zm00001d047582	14.441	4.124	-1.80795	2.00E-04	Zm00001d041305	11.856	3.56594	-1.73331	0.00425
Zm00001d005779	4.479	1.275	-1.81229	5.00E-05	Zm00001d051163	75.736	22.7717	-1.73374	5.00E-05
Zm00001d015092	21.19	6.012	-1.81735	0.00045	Zm00001d006511	8.4015	2.52165	-1.73627	0.0063
Zm00001d020238	26.003	7.374	-1.81815	5.00E-05	Zm00001d002939	9.0493	2.71466	-1.73704	6.00E-04
Zm00001d017786	5.5781	1.579	-1.82092	6.00E-04	Zm00001d045386	6.1101	1.83102	-1.73856	0.0024
Zm00001d027850	2.1719	0.615	-1.8213	0.00355	Zm00001d003015	16.666	4.97474	-1.74423	5.00E-05
Zm00001d027593	11.043	3.119	-1.82406	5.00E-05	Zm00001d048021	58.413	17.4111	-1.74629	5.00E-05
Zm00001d027987	5.4174	1.525	-1.82861	5.00E-05	Zm00001d041662	42.742	12.7184	-1.74873	5.00E-05
Zm00001d006627	1.6801	0.471	-1.83576	0.00835	Zm00001d051553	2.0499	0.6088	-1.7515	0.0015
Zm00001d052103	21.529	6.026	-1.83709	5.00E-05	Zm00001d017288	83.373	24.7324	-1.75318	5.00E-05
Zm00001d032283	7.9459	2.223	-1.83778	1.00E-04	Zm00001d034991	15.784	4.67575	-1.75516	0.0074
Zm00001d032613	5.5901	1.562	-1.83964	0.00065	Zm00001d023596	15.467	4.5281	-1.77221	5.00E-04
Zm00001d027732	5.8421	1.632	-1.84016	0.00065	Zm00001d015091	172.19	50.2899	-1.77565	5.00E-05
Zm00001d028725	60.817	16.98	-1.84089	5.00E-05	Zm00001d035881	8.8705	2.59056	-1.77575	0.00435
Zm00001d026413	24.396	6.8	-1.84296	7.00E-04	Zm00001d027619	9.5965	2.79279	-1.7808	0.00175
Zm00001d036986	21.274	5.893	-1.85201	5.00E-05	Zm00001d031427	3.6633	1.06161	-1.78689	0.00175
Zm00001d017886	2.3788	0.658	-1.85339	0.0031	Zm00001d002584	16.24	4.6777	-1.79571	0.0023
Zm00001d028751	28.45	7.847	-1.85817	5.00E-05	Zm00001d017719	31.157	8.92442	-1.80374	0.00105
Zm00001d047723	31.209	8.597	-1.86008	5.00E-05	Zm00001d027727	25.537	7.27445	-	0.00105

								1.81168	
Zm00001d037248	4.6871	1.289	-1.8626	0.0029	Zm00001d027749	36.994	10.506	-1.81609	5.00E-05
Zm00001d027946	8.5995	2.363	-1.86338	6.00E-04	Zm00001d052673	8.0778	2.28931	-1.81905	0.0016
Zm00001d006659	20.181	5.522	-1.86987	5.00E-05	Zm00001d016982	7.07	1.99528	-1.82512	0.00275
Zm00001d017199	8.4322	2.299	-1.87475	0.00215	Zm00001d038125	4.7327	1.33544	-1.82533	0.00815
Zm00001d030339	18.185	4.958	-1.8749	0.0058	Zm00001d028243	3.0321	0.85162	-1.83203	0.00835
Zm00001d007045	3.1783	0.866	-1.87621	0.00175	Zm00001d007718	209.83	58.8343	-1.83445	5.00E-05
Zm00001d034621	114.2	31.07	-1.87803	5.00E-05	Zm00001d018696	12.941	3.57893	-1.85429	2.00E-04
Zm00001d027456	13.6	3.69	-1.88203	0.0052	Zm00001d011314	29.39	8.1054	-1.85836	0.00455
Zm00001d038564	6.3313	1.716	-1.88374	0.00085	Zm00001d032608	8.1698	2.25097	-1.85976	0.0033
Zm00001d052793	2.3577	0.637	-1.88906	0.0046	Zm00001d048787	338.42	93.1447	-1.86128	5.00E-05
Zm00001d012167	25.863	6.981	-1.8893	5.00E-05	Zm00001d046330	2.5102	0.6908	-1.86144	0.00175
Zm00001d034096	4.1693	1.114	-1.90403	0.00375	Zm00001d022569	17.109	4.70624	-1.86208	0.0022
Zm00001d007345	9.2724	2.442	-1.92509	0.00055	Zm00001d030345	5.5346	1.51766	-1.86662	0.00585
Zm00001d049288	11.601	3.052	-1.92633	7.00E-04	Zm00001d034547	10.402	2.84501	-1.87033	8.00E-04
Zm00001d029913	35.164	9.218	-1.93162	5.00E-05	Zm00001d024386	56.288	15.3904	-1.87081	5.00E-05
Zm00001d036839	74.71	19.52	-1.93655	5.00E-05	Zm00001d003064	13.716	3.74142	-1.87415	0.0053
Zm00001d005148	13.672	3.562	-1.94061	5.00E-05	Zm00001d051543	60.127	16.293	-1.88375	2.00E-04
Zm00001d041725	107.37	27.96	-1.94105	5.00E-05	Zm00001d051102	7.4527	2.01375	-1.88787	0.00425
Zm00001d007231	3.2526	0.846	-1.9437	0.00645	Zm00001d005543	30.381	8.18914	-1.89139	5.00E-05
Zm00001d053210	5.9846	1.548	-1.95051	0.00315	Zm00001d005421	53.531	14.4224	-	5.00E-05

								1.89207	
Zm00001d043232	11.628	2.992	-1.95863	0.001	Zm00001d008266	11.54	3.10227	-1.89524	0.00475
Zm00001d022088	19.14	4.917	-1.96088	5.00E-05	Zm00001d026712	31.466	8.38385	-1.90809	5.00E-05
Zm00001d024208	2.9755	0.763	-1.96243	6.00E-04	Zm00001d002801	5.038	1.33952	-1.91113	0.0024
Zm00001d027938	19.19	4.915	-1.96508	5.00E-05	Zm00001d010451	6.5784	1.73002	-1.92695	0.00665
Zm00001d020371	7.1473	1.828	-1.96688	0.00305	Zm00001d048843	9.0171	2.36418	-1.93133	0.00435
Zm00001d032888	12.304	3.134	-1.9731	0.00115	Zm00001d013489	11.483	3.00814	-1.93252	5.00E-04
Zm00001d048837	11.37	2.893	-1.97436	0.00015	Zm00001d031168	1399.3	363.923	-1.94297	5.00E-05
Zm00001d043921	7.9713	2.022	-1.97876	1.00E-04	Zm00001d009556	19.767	5.13425	-1.94487	1.00E-04
Zm00001d037941	11.176	2.834	-1.97937	0.00075	Zm00001d003671	4.0027	1.03591	-1.95009	0.00515
Zm00001d027330	82.836	20.98	-1.98139	5.00E-05	Zm00001d042636	7.0323	1.81451	-1.95441	0.00225
Zm00001d038878	6.0106	1.519	-1.98415	0.00415	Zm00001d018966	15.844	4.07285	-1.95978	0.0049
Zm00001d017380	3.6552	0.924	-1.98416	0.00165	Zm00001d051896	19.333	4.96441	-1.96135	0.0018
Zm00001d014965	23.457	5.901	-1.99112	5.00E-05	Zm00001d018461	51.561	13.2211	-1.96344	5.00E-05
Zm00001d046897	6.7447	1.685	-2.00073	0.004	Zm00001d032570	25.809	6.56341	-1.97536	5.00E-05
Zm00001d012604	5.1892	1.296	-2.002	0.0015	Zm00001d043174	11.701	2.96883	-1.9786	0.00045
Zm00001d025338	81.804	20.4	-2.00381	5.00E-05	Zm00001d045368	57.079	14.4651	-1.98037	5.00E-05
Zm00001d009103	28.072	6.995	-2.00461	5.00E-05	Zm00001d045036	20.536	5.16208	-1.99214	0.00045
Zm00001d026012	9.0118	2.244	-2.00575	8.00E-04	Zm00001d028696	5.2071	1.30273	-1.99893	0.0057
Zm00001d028967	9.532	2.371	-2.00743	5.00E-05	Zm00001d017840	133.59	33.4001	-1.99986	5.00E-05
Zm00001d042813	4.1966	1.02	-2.04076	0.0052	Zm00001d013108	24.088	6.02098	-2.00022	5.00E-05

Zm00001d049630	3.9944	0.971	-2.04101	6.00E-04	Zm00001d028360	33.783	8.43768	-2.00139	1.00E-04
Zm00001d031971	6.9582	1.69	-2.04194	0.00185	Zm00001d005454	5.7534	1.43166	-2.00671	0.0044
Zm00001d012744	3.2033	0.777	-2.04421	0.00085	Zm00001d040059	8.115	2.00562	-2.01655	1.00E-04
Zm00001d004646	41.332	9.968	-2.05193	5.00E-05	Zm00001d022458	6.7487	1.6651	-2.01899	1.00E-04
Zm00001d003757	26.575	6.402	-2.05342	0.0027	Zm00001d002156	14.611	3.57085	-2.03266	0.0049
Zm00001d014136	13.599	3.275	-2.05368	5.00E-05	Zm00001d027652	584.97	142.813	-2.03424	5.00E-05
Zm00001d010038	23.143	5.543	-2.06172	1.00E-04	Zm00001d036840	12.331	3.00383	-2.03737	0.00105
Zm00001d011208	42.26	10.11	-2.06333	5.00E-05	Zm00001d006180	4.4126	1.06968	-2.04444	0.0021
Zm00001d049678	3.2099	0.766	-2.06748	0.0019	Zm00001d048634	11.355	2.74562	-2.04808	0.00345
Zm00001d026511	142.33	33.87	-2.07135	5.00E-05	Zm00001d002139	6.9461	1.67316	-2.05364	0.00285
Zm00001d013216	9.9344	2.354	-2.07755	0.00015	Zm00001d045519	11.381	2.71284	-2.06871	1.00E-04
Zm00001d033585	20.243	4.738	-2.0951	5.00E-05	Zm00001d027996	13.258	3.14025	-2.07787	5.00E-05
Zm00001d014134	27.011	6.256	-2.11019	5.00E-05	Zm00001d026343	51.612	12.182	-2.08295	5.00E-05
Zm00001d045463	31.688	7.279	-2.12214	5.00E-05	Zm00001d021690	4.8918	1.15298	-2.08499	0.00355
Zm00001d009913	15.012	3.437	-2.12696	0.0016	Zm00001d006509	15.915	3.74048	-2.08906	0.00015
Zm00001d024700	12.057	2.741	-2.13718	3.00E-04	Zm00001d017984	12.31	2.88858	-2.09139	0.0042
Zm00001d002773	4.5945	1.043	-2.13941	0.0022	Zm00001d035629	15.334	3.55467	-2.10896	0.00025
Zm00001d043872	7.8364	1.778	-2.14002	1.00E-04	Zm00001d015224	3.4003	0.7858	-2.11344	0.00375
Zm00001d044683	23.902	5.387	-2.1495	0.00015	Zm00001d039000	53.978	12.4663	-2.11434	5.00E-05
Zm00001d022456	33.878	7.629	-2.1508	5.00E-05	Zm00001d025572	13.17	3.0364	-2.1168	0.00055
Zm00001d038020	10.181	2.244	-2.18154	5.00E-05	Zm00001d023210	6.3617	1.46244	-	0.0027

								2.12102	
Zm00001d042905	6.6442	1.451	-2.19536	0.00015	Zm00001d043701	9.7214	2.22484	-2.12746	0.00725
Zm00001d034415	12.381	2.693	-2.20082	0.00015	Zm00001d028686	9.6497	2.19419	-2.13679	0.00015
Zm00001d039566	18.706	4.033	-2.21353	9.00E-04	Zm00001d020620	3.74	0.84397	-2.14778	0.00745
Zm00001d046489	45.963	9.821	-2.22646	5.00E-05	Zm00001d036151	29.732	6.69159	-2.15161	0.0025
Zm00001d015307	65.777	13.9	-2.24247	5.00E-05	Zm00001d025920	5.8052	1.29309	-2.16652	0.0048
Zm00001d029648	5.6908	1.202	-2.2435	5.00E-05	Zm00001d005023	38.634	8.56668	-2.17305	0.00175
Zm00001d016408	18.141	3.819	-2.24805	5.00E-05	Zm00001d008173	45.085	9.98449	-2.17488	5.00E-05
Zm00001d035310	7	1.448	-2.27357	0.0025	Zm00001d052194	233.44	51.5433	-2.1792	5.00E-05
Zm00001d039101	3.3284	0.687	-2.27624	0.0013	Zm00001d028557	202.28	44.6118	-2.18083	5.00E-05
Zm00001d040126	143.08	29.4	-2.28292	5.00E-05	Zm00001d003751	30.898	6.70262	-2.2047	5.00E-05
Zm00001d013979	3.8559	0.782	-2.30241	0.00085	Zm00001d048945	7.3444	1.59249	-2.20535	0.00015
Zm00001d031841	7.0187	1.423	-2.30258	0.00215	Zm00001d036608	15.071	3.25732	-2.21003	5.00E-05
Zm00001d018193	2.6946	0.541	-2.31522	0.0032	Zm00001d002899	23.219	4.87802	-2.25093	0.00035
Zm00001d007820	9.8541	1.968	-2.32421	0.00115	Zm00001d004762	4.835	1.0086	-2.26116	0.00675
Zm00001d010751	3.8116	0.761	-2.32508	0.0059	Zm00001d008594	41.201	8.48166	-2.28027	0.00015
Zm00001d038371	57.066	11.2	-2.3493	5.00E-05	Zm00001d003830	13.782	2.80404	-2.29717	0.00195
Zm00001d024946	4.9769	0.974	-2.35259	0.00395	Zm00001d033044	15.483	3.13646	-2.30351	5.00E-05
Zm00001d032443	25.261	4.933	-2.35624	0.00205	Zm00001d042826	41.089	8.25118	-2.31609	5.00E-05
Zm00001d043477	143.06	27.79	-2.36399	5.00E-05	Zm00001d027405	16.271	3.25053	-2.32354	9.00E-04
Zm00001d051389	3.4154	0.66	-2.37214	0.0019	Zm00001d040581	30.655	6.09692	-2.32995	5.00E-05
Zm00001d023859	17.437	3.363	-2.37442	5.00E-05	Zm00001d035907	6.8269	1.34337	-	0.00055

								2.34538	
Zm00001d010948	6.1556	1.18	-2.38301	0.00055	Zm00001d028555	238.64	46.8184	-2.34968	5.00E-05
Zm00001d042901	23.009	4.398	-2.38715	5.00E-05	Zm00001d041246	78.929	15.4233	-2.35545	6.00E-04
Zm00001d008230	6.6021	1.258	-2.39172	5.00E-05	Zm00001d043276	9.912	1.91775	-2.36976	0.0017
Zm00001d039004	8.1446	1.53	-2.41209	1.00E-04	Zm00001d018298	64.793	12.5282	-2.37065	5.00E-05
Zm00001d004208	21.638	4.044	-2.41957	0.00185	Zm00001d021757	4.4704	0.84538	-2.40272	0.00635
Zm00001d051548	1.6533	0.307	-2.43115	0.00675	Zm00001d014722	30.393	5.74689	-2.4029	5.00E-05
Zm00001d043728	9.6567	1.774	-2.44452	0.00035	Zm00001d028797	6.6867	1.24633	-2.42359	0.00105
Zm00001d025081	14.38	2.619	-2.45721	3.00E-04	Zm00001d022669	10.533	1.94461	-2.43737	0.00475
Zm00001d038355	2.7743	0.496	-2.48334	0.001	Zm00001d043205	16.253	2.99489	-2.44016	0.0041
Zm00001d046246	1.6756	0.299	-2.4853	0.0068	Zm00001d023213	21.347	3.91644	-2.44644	5.00E-05
Zm00001d018097	12.882	2.273	-2.50287	5.00E-05	Zm00001d041670	23.547	4.31664	-2.44756	5.00E-05
Zm00001d037359	58.258	10.25	-2.50709	5.00E-05	Zm00001d011650	18.053	3.28487	-2.45834	0.00145
Zm00001d053799	5.6351	0.99	-2.50876	5.00E-05	Zm00001d028931	16.182	2.92416	-2.4683	5.00E-05
Zm00001d047276	104.84	18.42	-2.5089	5.00E-05	Zm00001d002958	14.049	2.51834	-2.47993	3.00E-04
Zm00001d012221	17.319	3.03	-2.51478	5.00E-05	Zm00001d001802	13.584	2.43212	-2.48165	0.0036
Zm00001d025803	15.396	2.659	-2.53351	5.00E-05	Zm00001d020903	44.289	7.90828	-2.48551	5.00E-05
Zm00001d041315	2.782	0.478	-2.5412	1.00E-04	Zm00001d045478	17.935	3.16579	-2.50214	0.00565
Zm00001d039770	38.555	6.541	-2.55922	5.00E-05	Zm00001d038226	9.447	1.65969	-2.50894	0.00025
Zm00001d018229	19.775	3.342	-2.56482	5.00E-05	Zm00001d040075	4.7191	0.8271	-2.51238	0.00105
Zm00001d042864	19.215	3.196	-2.58807	2.00E-04	Zm00001d008545	52.52	9.20162	-2.5129	5.00E-05
Zm00001d032744	2.2075	0.367	-2.59032	0.0032	Zm00001d031769	4.2275	0.72739	-2.53902	0.00575

Zm00001d007765	7.8709	1.293	-2.60628	0.00035	Zm00001d028689	3.6817	0.63333	-2.53932	0.00595
Zm00001d042814	4.5347	0.745	-2.60643	0.0023	Zm00001d007842	1.7506	0.29303	-2.5787	0.0013
Zm00001d012789	4.9856	0.812	-2.61813	0.00725	Zm00001d018335	6.7596	1.12105	-2.59209	0.0023
Zm00001d048608	5.6428	0.913	-2.62849	0.00085	Zm00001d024885	19.693	3.25786	-2.59569	5.00E-05
Zm00001d024630	11.811	1.905	-2.632	5.00E-05	Zm00001d033987	27.514	4.55089	-2.59595	5.00E-05
Zm00001d044111	6.2112	0.998	-2.6374	4.00E-04	Zm00001d019994	14.259	2.35007	-2.60104	2.00E-04
Zm00001d052683	38.339	6.153	-2.6395	5.00E-05	Zm00001d026500	6.5473	1.07744	-2.60328	0.00745
Zm00001d038599	9.5551	1.518	-2.65383	2.00E-04	Zm00001d017557	42.117	6.8824	-2.61342	5.00E-05
Zm00001d038761	6.1373	0.971	-2.66016	0.00535	Zm00001d011787	6.8738	1.10917	-2.63163	0.00445
Zm00001d047553	11.423	1.772	-2.68878	0.00575	Zm00001d037228	3.6171	0.58122	-2.63768	0.0087
Zm00001d028862	13.156	2.038	-2.69077	6.00E-04	Zm00001d037384	39.722	6.34618	-2.64598	5.00E-05
Zm00001d007132	2.0793	0.317	-2.71459	0.0087	Zm00001d032083	4.2565	0.661	-2.68695	0.0036
Zm00001d019669	22.246	3.376	-2.7204	5.00E-05	Zm00001d012420	320.25	49.6113	-2.69043	5.00E-05
Zm00001d026374	4.6797	0.706	-2.72911	5.00E-05	Zm00001d033794	4.2811	0.66064	-2.69603	0.0011
Zm00001d052333	7.0435	1.053	-2.74166	8.00E-04	Zm00001d039650	2.1729	0.3322	-2.70949	0.00535
Zm00001d007267	2.7174	0.405	-2.74619	0.0022	Zm00001d039240	11.205	1.70239	-2.71848	0.004
Zm00001d027924	6.9406	1.011	-2.77975	0.0017	Zm00001d044664	56.351	8.55382	-2.71979	5.00E-05
Zm00001d045392	81.646	11.85	-2.78476	5.00E-05	Zm00001d041236	10.55	1.58507	-2.73468	1.00E-04
Zm00001d026632	11.432	1.626	-2.81399	5.00E-05	Zm00001d039908	58.335	8.74344	-2.73808	1.00E-04
Zm00001d052684	48.824	6.888	-2.82534	5.00E-05	Zm00001d033872	2.0677	0.30438	-2.76408	0.0075
Zm00001d013022	3.55	0.493	-2.84699	0.00815	Zm00001d003581	5.5285	0.81261	-	0.00785

Zm00001d012983	4.8356	0.671	-2.84957	5.00E-04
Zm00001d006449	4.5947	0.625	-2.8788	0.0012
Zm00001d031270	6.0959	0.829	-2.87895	0.00165
Zm00001d049387	5.3852	0.73	-2.88283	0.0032
Zm00001d001826	9.4562	1.192	-2.98802	0.00125
Zm00001d028451	2.9762	0.352	-3.07822	0.00665
Zm00001d017209	2.1672	0.248	-3.12595	0.0013
Zm00001d051788	5.3231	0.592	-3.16915	0.0039
Zm00001d028561	35.515	3.196	-3.47421	5.00E-05
Zm00001d048998	5.0389	0.396	-3.66897	0.00075
Zm00001d045391	11.722	0.819	-3.83952	2.00E-04
Zm00001d028541	12.485	0.859	-3.86061	0.0034
Zm00001d020717	4.9446	0.334	-3.88898	0.00085
Zm00001d016605	19.679	1.322	-3.89639	0.00225
Zm00001d002590	10.249	0.657	-3.96278	0.0086
Zm00001d039933	37.662	2.244	-4.06924	5.00E-05

Genes downregulated in the susceptible genotype

Gene id	SAI	SAU	lo$_{g2}$ fold change	p value
Zm00001d021581	0	1.472	Inf	6.00E-04
			2.76627	
Zm00001d020954	12.957	1.8971	-2.7719	0.00295
Zm00001d010529	101.15	14.7473	-2.77799	5.00E-05
Zm00001d024875	14.69	2.12752	-2.78757	5.00E-05
Zm00001d036439	15.212	2.16097	-2.81541	0.00165
Zm00001d039542	16.75	2.37248	-2.81973	0.00065
Zm00001d042922	100.47	14.2275	-2.82004	5.00E-05
Zm00001d030028	9.6028	1.3382	-2.84317	7.00E-04
Zm00001d049201	9.5024	1.30921	-2.85959	0.00125
Zm00001d052978	11.85	1.62492	-2.86647	0.0026
Zm00001d052104	6.6284	0.90188	-2.87764	0.00395
Zm00001d047339	4.3907	0.59534	-2.88266	0.00435
Zm00001d018350	9.3006	1.21689	-2.93413	0.0036
Zm00001d047639	22.443	2.9259	-2.93931	5.00E-05
Zm00001d046029	5.2458	0.67835	-2.95106	9.00E-04
Zm00001d049303	5.4432	0.70215	-2.95461	0.0069
Zm00001d010588	8.7547	1.07795	-3.02176	0.0056
Zm00001d005715	7.876	0.96079	-3.03517	0.0023
Zm00001d017455	12.278	1.48042	-3.05204	0.0035
Zm00001d009700	35.643	4.21817	-3.07891	7.00E-04
Zm00001d032295	15.268	1.7988	-3.08539	0.0081

Zm00001d026984	0	2.879	Inf	5.00E-05
Zm00001d003485	0	3.681	Inf	0.0017
Zm00001d027498	0	4.357	Inf	5.00E-05
Zm00001d039791	0	4.617	Inf	0.00145
Zm00001d019137	27.282	1256	5.52422	5.00E-05
Zm00001d044387	0.2456	7.929	5.01259	0.00655
Zm00001d022883	4.7325	115.1	4.60424	9.00E-04
Zm00001d002675	0.5643	10.99	4.28358	0.00145
Zm00001d036530	0.1895	2.966	3.9683	5.00E-05
Zm00001d047441	1.2018	15.64	3.70164	0.00015
Zm00001d011656	0.8396	10.67	3.66743	0.0048
Zm00001d048416	0.7173	8.291	3.53077	0.00155
Zm00001d019615	0.4439	5.023	3.50021	1.00E-04
Zm00001d047656	1.2817	13.45	3.39202	0.0013
Zm00001d029183	0.3966	4.084	3.36403	0.0012
Zm00001d014099	0.2388	2.423	3.3428	0.00255
Zm00001d027932	5.3637	44.45	3.05087	5.00E-05
Zm00001d018209	0.9751	7.831	3.00566	0.0043
Zm00001d029722	0.8684	6.927	2.99575	5.00E-05
Zm00001d004721	1.2743	10.16	2.99498	0.00155
Zm00001d051167	0.6101	4.803	2.97686	0.0027

Zm00001d040457	36.275	4.19108	-3.11359	0.00155
Zm00001d007604	40.065	4.57995	-3.12893	5.00E-05
Zm00001d020958	44.177	4.74718	-3.21817	5.00E-05
Zm00001d034356	24.209	2.55932	-3.24168	0.0034
Zm00001d022420	17.006	1.7776	-3.25805	0.00655
Zm00001d024379	30.134	3.12379	-3.27002	5.00E-05
Zm00001d035562	35.203	3.25971	-3.43288	5.00E-05
Zm00001d028998	43.265	3.79872	-3.50961	5.00E-05
Zm00001d007161	22.424	1.96615	-3.51157	1.00E-04
Zm00001d043929	24.914	2.16408	-3.52512	0.0056
Zm00001d028888	96.164	7.99829	-3.58774	5.00E-05
Zm00001d011080	178.05	14.4878	-3.61935	0.003
Zm00001d028887	106.98	8.58215	-3.63991	5.00E-05
Zm00001d039010	38.565	2.90374	-3.73131	0.0016
Zm00001d038806	63.785	4.39918	-3.85792	5.00E-05
Zm00001d009567	49.311	3.18419	-3.95291	0.00025
Zm00001d020339	8.2147	0.5075	-4.01674	0.0068
Zm00001d047651	28.881	1.6972	-4.08891	0.00025
Zm00001d026700	65.044	1.94806	-5.06131	5.00E-05

Genes downregulated in the resistant genotype

Zm00001d032909	0.3045	2.387	2.97085	0.0074
Zm00001d025354	3.7343	28.8	2.9473	5.00E-05
Zm00001d018161	4.819	36.66	2.92732	5.00E-05
Zm00001d052915	8.9747	67.02	2.90062	0.00835
Zm00001d039487	0.7382	5.421	2.87642	0.00195
Zm00001d047231	4.7718	34.3	2.84542	0.0016
Zm00001d027700	2.8879	20.01	2.79266	5.00E-05
Zm00001d011393	0.448	3.064	2.77376	0.0012
Zm00001d031940	8.0875	54.44	2.751	5.00E-05
Zm00001d053320	0.9432	6.238	2.72559	0.0059
Zm00001d051945	2.2364	14.53	2.69969	5.00E-05
Zm00001d024522	7.2566	46.46	2.6785	5.00E-05
Zm00001d012641	1.2857	8.189	2.67109	0.0049
Zm00001d026156	0.5964	3.754	2.65416	0.00095
Zm00001d050674	0.7975	4.972	2.64011	2.00E-04
Zm00001d041458	2.834	16.59	2.54948	5.00E-05
Zm00001d048644	0.7233	4.163	2.5248	0.0012
Zm00001d054060	8.7124	49.83	2.51587	5.00E-05
Zm00001d002543	5.5053	30.09	2.45055	0.0036
Zm00001d010655	1.9496	9.768	2.32488	6.00E-04
Zm00001d049987	4.2415	20.98	2.30628	5.00E-05
Zm00001d054057	6.1139	29.94	2.29188	5.00E-05
Zm00001d052269	3.8313	18.49	2.27098	5.00E-05
Zm00001d033413	3.0027	14.35	2.25668	5.00E-04
Zm00001d001987	1.5978	7.59	2.24796	5.00E-05
Zm00001d027900	6.8578	32.54	2.24635	5.00E-05
Zm00001d012394	0.6789	3.188	2.23138	0.00615
Zm00001d003555	1.7691	8.068	2.18926	5.00E-05
Zm00001d002035	7.381	33.32	2.17441	5.00E-05
Zm00001d021695	5.8423	26.13	2.1613	5.00E-05
Zm00001d044596	2.2213	9.831	2.1459	1.00E-04
Zm00001d048099	2.9404	12.93	2.13674	0.001
Zm00001d053163	2.8122	12.35	2.13472	5.00E-05
Zm00001d050682	3.5944	15.63	2.12076	0.00065
Zm00001d020069	4.0466	17.45	2.10834	5.00E-05
Zm00001d007175	0.6291	2.692	2.09746	0.0025
Zm00001d040308	12.781	54.47	2.09147	5.00E-05
Zm00001d040545	1.5972	6.791	2.0882	0.00615

Gene id	RAI	RAU	$\log_2$ fold change	p value
Zm00001d004921	1.3578	12.9133	3.24957	0.00045
Zm00001d013244	0.8982	7.76733	3.11237	0.00245
Zm00001d028816	7.4676	64.2046	3.10395	5.00E-05
Zm00001d035854	1.361	10.1849	2.90365	5.00E-05
Zm00001d049113	0.3781	2.7457	2.86025	0.00715
Zm00001d026542	0.7963	5.27	2.7265	4.00E-04
Zm00001d006947	1.1855	7.43205	2.64829	2.00E-04
Zm00001d028349	1.1337	7.07862	2.64248	0.00165
Zm00001d037198	0.4481	2.22001	2.30868	0.0013
Zm00001d021425	0.3678	1.68137	2.19276	0.00335
Zm00001d025015	24.858	108.491	2.12578	5.00E-05
Zm00001d004331	8.3261	35.2781	2.08307	5.00E-05
Zm00001d011644	1.6596	6.98312	2.07308	5.00E-05
Zm00001d020340	2.5806	10.5277	2.02844	1.00E-04
Zm00001d018037	5.3552	21.6245	2.01367	5.00E-05
Zm00001d049435	0.8547	3.44462	2.01081	0.0039
Zm00001d053706	5.9089	23.0099	1.96129	5.00E-05
Zm00001d029373	3.111	11.5665	1.89452	5.00E-05
Zm00001d045295	3.7586	13.8	1.8764	5.00E-05
Zm00001d033071	0.8967	3.23387	1.85055	0.0014
Zm00001d016601	0.4887	1.73989	1.83205	0.0022
Zm00001d037182	1.2564	4.43893	1.82095	0.0039
Zm00001d031926	1.2288	4.11994	1.74541	0.0057
Zm00001d005687	16.276	54.2106	1.73585	5.00E-05
Zm00001d029842	6.8249	22.6972	1.73364	5.00E-05
Zm00001d020521	0.7699	2.55343	1.72978	0.0031
Zm00001d031315	2.4512	8.12672	1.72921	0.00015
Zm00001d008548	43.933	144.948	1.72217	5.00E-05
Zm00001d011610	36.437	119.613	1.7149	5.00E-05
Zm00001d028445	8.6626	28.1943	1.70253	0.00015
Zm00001d025984	8.3148	26.9709	1.69765	5.00E-05
Zm00001d028814	6.7776	21.9794	1.69731	3.00E-04
Zm00001d032724	1.8861	6.10704	1.69509	5.00E-05
Zm00001d019497	10.991	35.4984	1.69138	5.00E-05
Zm00001d020400	2.8121	9.05964	1.68781	0.00555
Zm00001d047110	13.347	42.9305	1.68549	5.00E-05
Zm00001d049436	3.1596	10.0721	1.67254	0.00055

Zm00001d024667	0.9427	4.004	2.08668	0.00165
Zm00001d009899	5.7709	24.25	2.07123	5.00E-05
Zm00001d039698	1.3038	5.448	2.06289	0.00085
Zm00001d053746	9.5073	38.54	2.01935	5.00E-05
Zm00001d041657	8.4412	33.85	2.00346	5.00E-05
Zm00001d033543	4.7927	19.2	2.00216	0.00455
Zm00001d035214	1.3621	5.422	1.99304	0.0034
Zm00001d030172	0.6695	2.64	1.97954	0.00345
Zm00001d035756	8.153	31.94	1.97009	5.00E-04
Zm00001d039695	3.2087	12.56	1.96868	0.00015
Zm00001d013370	1.854	7.244	1.96624	1.00E-04
Zm00001d017570	6.4319	24.97	1.95708	5.00E-05
Zm00001d019044	1.3994	5.383	1.94364	0.0027
Zm00001d028260	117.51	448.9	1.93349	5.00E-05
Zm00001d032000	2.5962	9.858	1.92494	0.0018
Zm00001d028367	4.9595	18.51	1.90032	5.00E-05
Zm00001d006933	3.3316	12.3	1.88407	0.00125
Zm00001d043731	2.0935	7.683	1.87574	0.0014
Zm00001d040621	0.8771	3.207	1.87034	0.0057
Zm00001d014244	2.2477	8.182	1.86403	0.0027
Zm00001d014555	1.3205	4.77	1.85287	0.00385
Zm00001d017571	4.7911	17.28	1.85046	0.00025
Zm00001d021988	1.9832	7.147	1.84945	0.0057
Zm00001d047579	2.2917	8.238	1.84585	0.0011
Zm00001d001139	173.57	622.1	1.8416	0.00015
Zm00001d024220	1.1434	4.088	1.83793	0.00285
Zm00001d044738	1.0821	3.865	1.83659	5.00E-05
Zm00001d005823	2.3534	8.399	1.83537	0.00105
Zm00001d021093	3.0804	10.98	1.83334	0.002
Zm00001d003995	0.9217	3.237	1.81212	5.00E-05
Zm00001d053684	5.3137	18.64	1.81054	5.00E-05
Zm00001d011847	2.6812	9.235	1.78423	5.00E-05
Zm00001d026873	3.3694	11.52	1.774	0.00065
Zm00001d008587	4.9062	16.78	1.77385	2.00E-04
Zm00001d033566	1.0097	3.444	1.77016	0.00255
Zm00001d031813	3.4267	11.64	1.76417	9.00E-04
Zm00001d011473	1.1711	3.974	1.76266	5.00E-05
Zm00001d038382	4.0447	13.71	1.76137	0.0042
Zm00001d053304	0.6237	2.087	1.7426	0.0068
Zm00001d044541	3.956	13.11	1.72904	0.0026
Zm00001d010662	6.1493	19.5763	1.6706	0.00015
Zm00001d037766	1.026	3.25237	1.66451	0.00775
Zm00001d038460	71.418	226.201	1.66325	5.00E-05
Zm00001d042851	6.6875	21.1692	1.66242	5.00E-05
Zm00001d053156	3.5126	11.0763	1.65687	0.0011
Zm00001d004401	3.0606	9.5469	1.64121	0.00105
Zm00001d052435	5.3666	16.4452	1.61558	5.00E-05
Zm00001d041590	12.941	39.2386	1.60032	5.00E-05
Zm00001d045097	1.8198	5.48035	1.59046	5.00E-05
Zm00001d038453	65.861	198.029	1.58823	0.00035
Zm00001d044442	13.057	39.2366	1.58737	5.00E-05
Zm00001d011789	27.084	80.4619	1.57088	5.00E-05
Zm00001d030222	9.0628	26.8937	1.56924	5.00E-05
Zm00001d029546	130.03	385.595	1.5682	5.00E-05
Zm00001d002167	2.9319	8.54336	1.54297	0.00035
Zm00001d044138	12.286	35.0478	1.51231	5.00E-05
Zm00001d017386	1.8407	5.21566	1.5026	0.0047
Zm00001d012544	1.8758	5.28817	1.49523	0.00295
Zm00001d020134	2.3788	6.66849	1.48711	1.00E-04
Zm00001d018603	3.2667	9.10739	1.47922	1.00E-04
Zm00001d018064	2.2276	6.20696	1.47843	0.0031
Zm00001d053293	6.8614	19.1035	1.47726	0.00025
Zm00001d027415	1.1616	3.18875	1.45683	0.00165
Zm00001d023392	0.9585	2.60983	1.44518	0.00025

Zm00001d037057	1.2325	4.084	1.72851	0.00075
Zm00001d041611	24.076	78.93	1.71286	0.00305
Zm00001d035753	3.811	12.49	1.7126	0.00275
Zm00001d034421	0.7406	2.391	1.69098	0.0051
Zm00001d052443	5.9964	19.25	1.68243	0.00135
Zm00001d021653	9.0098	28.51	1.66189	0.00015
Zm00001d032822	3.5067	10.92	1.63923	0.00695
Zm00001d012229	6.1682	19.1	1.63058	0.00025
Zm00001d040755	2.074	6.414	1.62874	5.00E-04
Zm00001d044095	14.415	44.04	1.61135	5.00E-05
Zm00001d025011	10.071	30.69	1.60775	5.00E-05
Zm00001d025141	4.8491	14.69	1.59893	6.00E-04
Zm00001d023424	1.9182	5.746	1.58272	0.00165
Zm00001d028744	4.6607	13.96	1.58244	0.00065
Zm00001d030347	1.202	3.581	1.57501	0.00855
Zm00001d030121	1.4876	4.419	1.57063	0.0019
Zm00001d020491	3.2942	9.68	1.55515	5.00E-05
Zm00001d027855	9.38	27.28	1.54022	8.00E-04
Zm00001d013066	13.903	40.23	1.53282	0.00055
Zm00001d038297	3.0263	8.744	1.53081	0.0044
Zm00001d044153	4.9342	14.2	1.52531	1.00E-04
Zm00001d033286	9.8203	28.14	1.51887	5.00E-05
Zm00001d027423	5.0483	14.45	1.51704	0.003
Zm00001d021702	2.5029	7.126	1.50948	0.0016
Zm00001d012797	2.133	6.006	1.49356	5.00E-05
Zm00001d010736	5.0106	14.06	1.48855	0.00295
Zm00001d010375	2.3954	6.717	1.48751	0.00445
Zm00001d038908	1.5971	4.477	1.48703	0.0061
Zm00001d010172	7.231	20.23	1.48421	2.00E-04
Zm00001d015515	19.867	55.24	1.47539	5.00E-05
Zm00001d031344	53.527	148.6	1.47286	1.00E-04
Zm00001d022228	8.1164	22.49	1.47021	0.00365
Zm00001d004509	3.1137	8.615	1.46828	0.00395
Zm00001d006306	2.6283	7.26	1.4659	0.00555
Zm00001d031958	5.2369	14.4	1.45928	0.00015
Zm00001d008600	3.6634	10.06	1.4569	2.00E-04
Zm00001d007687	8.5464	23.33	1.44886	0.002