IDENTIFICATION OF STRIGA RESISTANCE IN SORGHUM LANDRACES FROM ERITREA USING MOLECULAR AND INVITRO METHODS

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I dedicate this piece of work to all Eritrean journalists, prisoners of conscience, and change seekers who are in jail in Eritrea for more than 20 years with out due process of law.

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ABBREVIATIONS AND ACRONYMS

BecA	Bioscience in east and central Africa
cM	centimorgan
CTAB	Cetyl trimethyl-ammonium bromide
DEGs	Differentially expressed genes
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
EDTA	Ethylene diamine tetra-acetic acid
GO	Gene Ontology
Ha	Hectare
ICRISAT	International crop research institute for semi-arid tropics
ILRI	International livestock research Institute
KALRO	Kenya Agricultural and Livestock Research Organization
MoA	Ministry of Agriculture
MAS	Marker assisted selection
NGS	Next generation sequencing
NARI	National Agricultural research institute
nm	nanometer
PCR	Polymerase chain reaction
pH	Potential Hydrogen
QTL	Quantitative trait loci
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	Rotations per minute

SNPs	Single nucleotide polymorphisms
SPSS	Statistical product and service solutions
SSA	Sub Saharan Africa
SSRs	Simple sequence repeats
TBE	Tris base boric acid and EDTA
TE	Tris - EDTA
w/v	Weight per volume
µM	Micro molar
µg	Microgram
µl	Microlitre

ABSTRACT

Striga hermonthica is the main biological constriant hampering sorghum output in several regions of sub-Saharan Africa including Eritrea. *Striga hermonthica*, endemic parasitic weed of sub-Saharan Africa is steadily increasing its geographic distribution and level of infestation, and thereby reducing crop yield. *Striga* attaches itself to the host crop's roots, causing serious damage and reduced yield. Utilization of sorghum genotypes which are resistant to *Striga* is the best practical and economical method of dealing with the *Striga* problem.

The study's aims were as follows: 1) To determine farmers' views on sorghum production constraints; opportunities; *Striga* incidence and extent in sorghum growing area of Eritrea. 2) To find out the levels of stimulants for *Striga* germination in sorghum landraces and their derivatives. 3) Select for *Striga* resistance in sorghum landraces from Eritrea using polymorphic SSR markers. 4) To determine at gene level, the differential expression of *Striga* resistance in a *Striga* vulnerable and resistant genotypes of sorghum.

In order to achieve the first objective, 136 farmers from the Eritrean subzones of Golij, Tesenei, and Hamelmalo were interviewed. The interviews were conducted utilizing semi structured questionnaire and small-group discussions in order to understand the difficulties that the research area's sorghum production faces. Crops such as sorghum, sesame, pear millet, and groundnut were the major significant cereal crops in the research area. More than 80% of the respondents in the surveyed area indicated that drought stress as the most significant limitation to sorghum output, followed by infestation of *Striga*. Most of the interviewed farmers (81.6%) stated that *Striga* affected their sorghum farm and the degree of damage it inflicted varied from one subzone to another raging from mild (10%) to severe (70% and above). When it came to choosing

sorghum cultivars, local farmers said increased crop output, tolerance to drought, and resistance to *Striga* were the major essential factors. In the research area, up to 31 diverse landraces were identified as the most prevalent. Local varieties saved by farmers from past harvests were the most common source of seed for production.

To determine the levels of stimulants for *Striga* germination in sorghum from Eritrea, the resilience of 111sorghum local varieties and their derivatives were evaluated using the capability of genotypes of sorghum to effect germination of *Striga* seed as a measure of the amount of germination stimulant generated. The number of germinated *Striga* seeds was counted, and the germination percentage for *Striga* was calculated. Sorghum accessions EG830, EG1076, EG473, EG1261, EG546, and EG746 were reported to produce low levels of *Striga* germination, with 11.85 %, 13.05 %, 14.68 %, 15.32 %, 15.74 %, and 16.5 % germination percentages, respectively, when compared to controls, IS9830, SRN39, and Framida, which had 22.46%, 22.67%, and 23.27%. Despite the fact that these accessions did not demonstrate total resistance to *Striga* seed germination, the low amount of stimulant production showed that they had a high level of *Striga* resistance. The findings suggested that the germplasms identified could be exploited in sorghum breeding efforts as viable options of *Striga* infection resistance.

Laboratory studies using SSR markers aimed at investigating the presence of *Striga* resistance QTLs in 92 landrace sorghum accessions indicated that 8 genotypes have shown one to three *Striga* resistance QTLs. Accessions containing one or more *Striga* resistance QTLs were further evaluated in pot experiment. The results indicated that accessions EG1075, EG1168, and EG1239 have shown lesser number of *Striga* count and better grain yield compared to the other genotypes tested implying better resistance to *Striga*.

In the gene expression analysis, transcriptome of sorghum varieties, N13 (resistant) and Hugurtay (susceptible) to *Striga*, was analysed at two developmental stages of *Striga* infection. Expressed transcripts in the two developmental phases of *Striga hermonthica* infection (attachment and in host development stages) were presented, and transcript levels in both developmental stages were compared. The findings demonstrated that 15 genes were directly expressed in response to a stimulus and to carry out signaling within cell. There was overrepresentation of SORBI_3004G065900, SORBI_3001G482800, SORBI_3001G482700, SORBI_3001G077400 and SORBI_3001G259700 as a response to *Striga* wounding. In N13, resistance against Striga is mediated by signaling genes and pathways. Additionally, genes and processes for wound repair prohibit *Striga* from penetrating, rendering N13 resistant to infestation. These pathways were suppressed or barely expressed in Hurgutay, which may have contributed to its demise from *Striga*. Overall the study identified farmers' preferred traits, selected *Striga* resistant landrace accessions, and discovered genes and molecular pathways that contribute to *Striga* resistance in N13 (resistant variety). The genes and molecular pathways identified may provide a strong basis for a better understanding of *Striga* resistance in sorghum breeding program.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Sorghum is a key cereal crop in many parts of the world. Ninety percent of the globe's sorghum-growing land is in less developed countries. The primary demand for sorghum is for food in Africa, especially in the dry land regions. The pattern of expanding sorghum acreage in Africa over the last five decades reflects this continued demand, but productivity has not kept up with this rising demand (FAOSTAT, 2019). Grain yields are especially low in Eastern Africa countries such as Eritrea as compared to yields in the world average and well below the genetic potential (FAOSTAT, 2019). These low yields are attributed to a number of biotic and abiotic stress, low agricultural input and a lag in crop improvement efforts.

In Africa, *Striga hermonthica* plays a major significant role in limiting production of sorghum (Mbuvi et al., 2017; Runo and Kuria, 2018; Kavuluko et al., 2021). *Striga* infestation is a challenging task because it causes complicated connections between the host and the parasite, generates a huge quantity of seeds with a lengthy lifespan, and has unique germination and growth requirements (Mohamed, 2002). *Striga* is a parasite that affects the living condition of many small - scale farming communities in many parts of the world. According to Parker (1991), continuous farming and the expansion of farming to marginal land, has contributed to the dissemination and exacerbation of the *Striga* threat. *Striga* infests 100 million hectares of African grassland (Ejeta, 2007). *Striga* problem is frequently linked to poor nutrient content of soil and increased farming of cereal crops in marginal areas (Ransom, 2000).

Farmers have been employing different *Striga* management options such as hand-pulling, use of *Striga* free seeds, intercropping cereals with legumes, use of trap crops, rotation of cereals with legumes, use of nitrogen fertilizer and manure (Ejeta and Gressel, 2007). The use of resistant cultivars to combat the weed may be an effective strategy.

Identification of genotypes that are resistant to *Striga* infection in the field is complicated by the unpredictable environmental factors that make it difficult to select for resistance. Recent developments in molecular breeding, particularly the discovery of molecular markers associated to *Striga* resilience QTL, have enabled precise breeding against *Striga* (Ejeta, 2007). Thus it is evident that sorghum improvement efforts using molecular and invitro techniques combined with improved agronomic practices is crucial for the crop in Africa, especially in view of the changing climate

1.2 Statement of the problem

Sorghum is a significant staple crop for many households across Africa, however *Striga hermonthica* is a serious impediment to its cultivation and yield enhancement (Kavuluko et al., 2021*)*. *Striga* is a parasite plant which threatens the cultivation of economically significant grains in SSA (Ejeta & Gressel, 2007). The weed clings to the host plant's roots, depleting the host's carbon assimilates, moisture, and minerals, resulting in severe stunting, wilting, chlorosis, reduces panicle weight and grain yield (Ejeta, 2007). A single *Striga* plant inflicts about a 5% decrease in grain output per host plant and heavy infestations can result in total crop failure (Mutuku et al., 2019).

Striga's wide geographical distribution and its adaptation to various hosts and environments has enabled the parasite to colonize more farm lands and makes difficult to control which leads to a

devastating effects on farming in SSA (Ejeta and Gressel, 2007). *Striga* infests 57 percent of the total cropland in SSA used for grain production as a result of its efficient capacity to disperse *Striga* seeds and a lack of knowledge and resources to manage the parasite (Sauerborn, 1991). A significant rise in spatial coverage and infection extent, especially in SSA have been reported (Ejeta & Gressel, 2007). Striga's geographic occurrence and aggressive invading capability may be exacerbated by climate change, as habitats conducive to the parasite's growth are expected to rise (Mohamed et al., 2006).

Striga has the potential to cut crop yields by 20 percent to 100 percent for about 40 million African families per year (Atera et al., 2013; Scholes and Press, 2008). According to estimates, *Striga* invasion costs about US $ 7 billion each year in Africa (Ejeta and Gressel, 2007).

Striga is prevalent in the Gash-Barka region of Eritrea, where sorghum is the primary cereal crop (Yohannes et al., 2015). According to reports from Eritrea's National Agricultural Research Institute (NARI), in the Gash-Barka region, the average number of *Striga* plants per square meter vary from 71-335. The amount of sorghum yield loss caused by *Striga* infestation varies depending on the infestation level. In Eritrea, moderate to heavy infestations resulted in 60 percent yield reductions on average (AATF, 2011). The continental average yield loss is 40% (Lagoke et al., 1991). According to reports from Eritrea's National Agricultural Research Institute (NARI), *Striga* is rapidly spreading in various parts of Eritrea, including the mid-highlands, where it was not previously a problem.

Lack of availability of *Striga* resistant varieties of sorghum have contributed to the low production of sorghum in Eritrea. Using resistant cultivars is perhaps a competent way to tackle the weed. Even though Eritrea has rich sorghum diversity (Abraha et al., 2014; Ghebru et al., 2002), there has been no studies using in-vitro and molecular techniques on the available

sorghum landraces for potential *Striga* resistance. Furthermore, there is a scarcity of data on sorghum productivity and production challenges.

1.3 Justification

In Eritrea sorghum productivity is 0.58 tons per hectare, which is significantly less than the 1.44 tons per hectare average for the world. (FAOSTAT, 2019). A significant biotic constraint is *Striga* weed, which may produce thousands of seeds per plant and can live for years in the soil (Gurney et al., 2006). There is need therefore to develop an effective management control strategy that could reduce the number of *Striga* seeds in infested soils and prevent further multiplication. Despite major efforts to combat the parasite, *Striga* continues to cause significant crop losses on sorghum, worsening the food insecurity in many rural communities. Several *Striga* management options that include depleting the *Striga* seed bank or inhibiting the germination of the parasite seeds have been suggested (Berner et al. 1996, 1997; Gamar and Mohamed 2013). However, use of *Striga* resilient genotypes provide the best economical and practical method to control *Striga* (Gamar and Mohamed 2013).

Screening germplasm against *Striga* is the first step toward the identification of *Striga*-resistant genotypes (Muchira et al., 2021). Screening genotypes in *Striga* infested fields is often less efficient due to the host complexity, parasite and environment interactions. Besides, field screening is less accurate, slow and time consuming to identify *Striga* resistant genotype. Molecular markers are useful selection tools in crop breeding efforts, especially when linked to resistance QTLs (Lammerts Van Bueren et al., 2010; Mohamed et al., 2014; Ngugi et al., 2015; Yohannes et al., 2015).

Molecular markers that tag gene(s) conferring resistance to *Striga* in N13 sorghum cultivar were identified. Five chromosomal areas (QTLs) related with resistance to *Striga* in sorghum are known (Haussmann et al., 2004). SSR markers that tag these QTLs have been identified and utilized in sorghum improvement programs for *Striga* resistance (Gemar and Mohammed, 2013).

One resistance mechanisms to *Striga* in sorghum is production of low *Striga* stimulant (Gobena et al., 2017). Exudates necessary for *Striga* seed germination are in short supply in genotypes with limited germination stimulant production (Mohammed, 2002). *Striga* resistance conferred by reduced *Striga* germination stimulant activity allows for crop control and permits economic production (Perez-Vich et al., 2013). Seeds of *Striga* can not germinate until they get an exudate signal from the host (Rich and Ejeta, 2007). Thus, one strategy for reducing the threat posed by *Striga* would be to seek out cultivars that release little germination stimulant.

Low *Striga* stimulant production in maize has been identified utilizing in-vitro methods (Karaya et al., 2012). Similar technique can be applied to identify low *Striga* germination stimulant producers of sorghum. Sorghum genotypes that produce less amounts of *Striga* germination stimulant could help to reduce the quantity of seeds stored in the soil by reducing seed multiplication and dispersal (Mohammed, 2002). New source of resistance from landrace cultivars would give breeders more options in their breeding program.

Transcriptome analysis is crucial for identifying and characterizing important traits in organisms. It is essential in interpreting the functional elements of the genome and variations brought about by stress (Johnson et al., 2014). Analysis of sorghum transcriptome is an important tool for quantification of the genes necessary for resistance to *Striga* at different stages of growth. Transcripome analysis can reveal the various gene networks involved in resistance to *Striga*. It helps to identify transcripts induced or suppressed upon exposure to *Striga* in different

genotypes. The differentially expressed genes between different genotypes can be further analyzed to identify any genic single nucleotide polymorphisms (SNPs) that can be used for future characterization of sorghum.

1.4 Objectives

1.4.1 General objective

The overall objective was to improve sorghum productivity in Eritrea by identifying *Striga* resistant varieties.

1.4.2 Specific objectives

1) To determine farmers' views on sorghum production constraints; opportunities; *Striga* incidence and extent in sorghum growing area of Eritrea
2) To find out the levels of stimulants for *Striga* germination in sorghum landraces and their derivatives.
3) To select for *Striga* resistance in sorghum landraces from Eritrea with the help of SSR markers.
4) To determine at the gene level, the differential expression of *Striga* resistance in resistant and susceptible sorghum genotypes

1.5 Hypothesis

1. Smallholder farmers in the sorghum-growing sub-zones of Eritrea face a variety of social, cultural, and economic challenges and possibilities that might affect sorghum production.
2. The amount of *Striga* germination stimulant production varies among Eritrean landrace sorghum accessions.
3. Among the Eritrean sorghum accessions, there exist genetically superior sorghum landraces that confer *Striga* resistance QTLs.
4. After *Striga* infestation, there are nucleotide variations in the transcripts of resistant and susceptible sorghum genotypes.

CHAPTER TWO: LITERATURE REVIEW

2.1 Sorghum biology and domestication

Sorghum which has 10 chromosomes (2n=20) is an inbreeding crop with about 6% out-crossing (Pedersen et al., 1998). The amount of outcrossing varies depending on the cultivar's panicle type, and is often higher in sorghum with loose-panicles grassy and lower in domesticated sorghum with compact panicles. Descriptions of S. *bicolor's* flowering and pollination is well documented by Singh et al., (1997). In tropical climates, sorghum typically blooms 55 to 70 days after germination, however depending on the genotype and weather condition, flowering may take place 30 to 100 days after germination. Flowering begins at the tip of the inflorescence and progresses downwards within 4 to 5 days. A single panicle may produce up to 6,000 florets (Quinby and Karper 1947). Not all sorghum heads bloom at the same time, therefore pollen is often present for 10 to 15 days. Depending on the genotype and temperature, flowering can begin at any time between midnight and midday, reaching its peak around sunrise.

Sorghum has been domesticated in the Eastern African region, which has been described as a hub of diversification and domestication (Vavilov, 1992). Several wild relatives of farmed sorghum can be found in Africa, both as weeds in farmers' fields and in natural settings. In and near sorghum fields in Africa, spontaneous, morphologically intermediate plants between cultivated sorghum and its wild families have been reported (Dogget and Prasada Rao 1995; Tesso et al., 2008). Some wild sorghum families, such as sorghum *verticilliforum* and sorghum *aethiopicum*, have been identified in Eritrea (ICRISAT, 2002).

Sorghum, both wild and farmed, is interfertile and grows in many agroecosystems in Sub-Saharan Africa (Mutegi et al., 2010). Tesso et al. (2008) revealed a similarity in incidence and blooming patterns among the domesticated and non-domesticated sorghum, implying the likelihood of hybridization. This is mainly due to overlapping geographic distributions (Mutegi et al., 2010). There has been reports of gene transfer between wild and cultivated sorghum (Morrell et al., 2005). Such wild-to-cultivated sorghum crossbreeding could be useful in breeding efforts to combat biotic and abiotic stress.

More than 35% of sorghum is used as a food grain and the stover is predominantly utilized for livestock, alcohol extraction, and industrial applications. Sorghum is a significant cereal crop in Africa in general (FAOSAT, 2019; Ngugi et al., 2015), and the second preferred cereal for preparing 'enjera,' the staple meal in Eritrea and Ethiopia, after teff (Eragrostis tef (Zucc.) Trotter).

2.2 Sorghum production in Eritrea

In Eritrea, a diverse types of crops are frequently grown, however sorghum is the most prevalent in terms of harvest and area. Sorghum is often farmed by resource poor subsistence farmers with hardly any capital inputs under rain-fed circumstances (Abraha et al., 2013). Sorghum is cultivated in eastern and western lowland parts of the country where they use diversion canal to irrigate. Sorghum accounts up a significant portion of Eritrea's total crop production. From 1998 to 2019, the average area of sorghum farming was around 219,903 hectares of land (Table 2.1).

Table 2. 1: Sorghum production in Eritrea between 1998 and 2019

Year	Area (ha)	Production (tonnes)	Productivity (t ha^{-1})
1998	236,231	269,771.90	1.14
1999	236,371	207,196.90	0.88
2000	150,558	62,004.70	0.41
2001	165,821	78,758.50	0.47
2002	182,051	28,433.60	0.16
2003	200,933	64,061	0.32
2004	211,756	56,745	0.27
2005	233,134	184,271	0.79
2006	282,203	222,685.10	0.79
2007	202,909	302,515.40	1.07
2008	249,286	67,981	0.27
2009	250971	67,981	0.24
2010	255332	135164.5	0.53
2011	218500	106497.2	0.49
2012	217331	115694.895	0.53
2013	220876	83038	0.38
2014	234254	244344.5	1.04
2015	218233	32091.3	0.15
2016	221488	113310.14	0.51
2017	202914.3	46350.1	0.23
2018	230512	113798.45	0.49
2019	216210	218686.3	1.01
Avarage	**219903**	**128244.56**	**0.58**

Source: Ministry of Agriculture, Eritrea 2020

In Eritrea, sorghum is grown in various zones (Figure 2.1), which varies in length of growing season, rainfall, temperature, and altitude. Over 80% of the crop is grown in the dry and hot lowlands of the country's east and west, such as the Gash-Barka and Northern Red Sea regions. A substantial amount is also produced in the midlands like Debub and Anseba regions. Short rainy seasons with minimal and irregular rainfall define the arid and hot lowland areas of the country. Over 90% of farmers grow landrace sorghum types that have been saved from past harvests (Abraha et al., 2013).

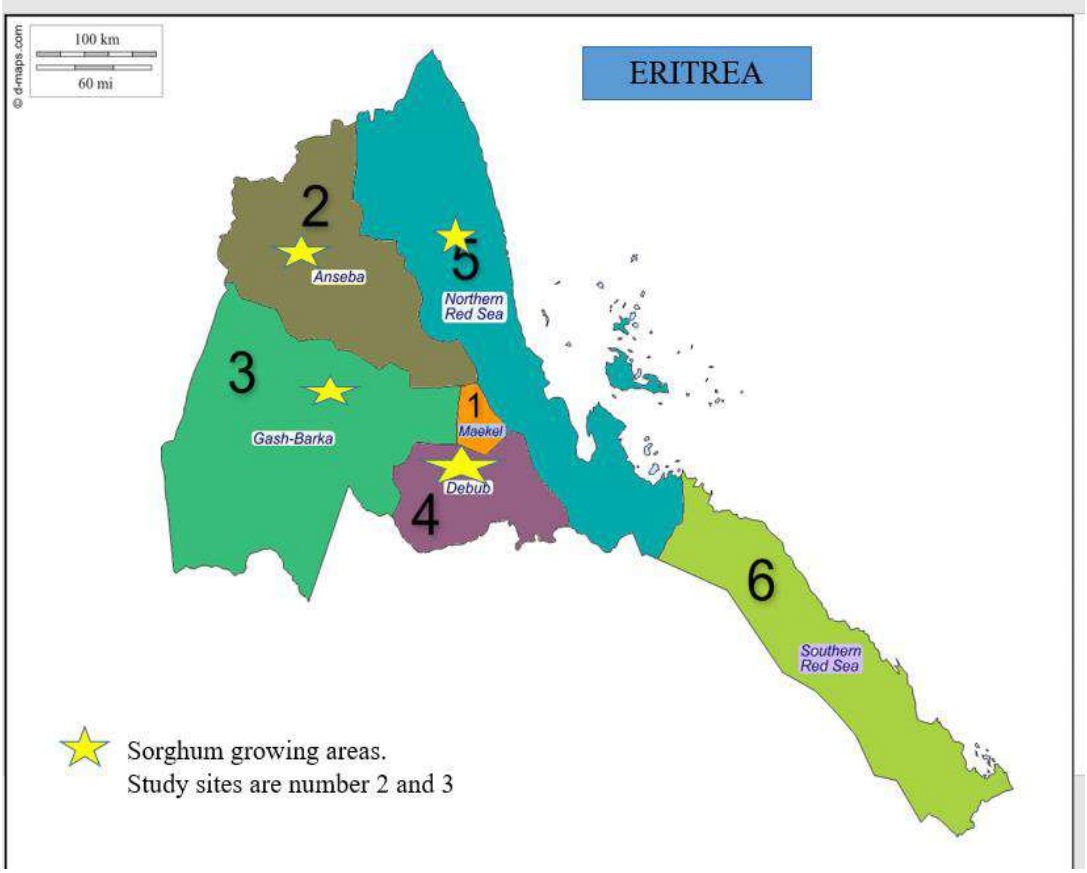

Figure 2. 1: Sorghum growing regions of Eritrea

Cropping practices in sorghum-growing regions of the country vary by location, but mono cropping (the most prevalent approach in the lowlands), intercropping, inter-cultivation, and crop rotation are all common. Depending on the crop variety and agro-ecology, the rotation cycle varies from one sub region to the next. Abraha et al. (2013) reported that in the Hamelmalo sub region, they rotate sorghum-groundnut-pearl millet, whereas in the Segeneiti sub region, they rotate sorghum-barley-teff-chickpea-finger millet.

2.3 Sorghum production constraints

Sorghum productivity has been constrained by several factors such as drought, pests and diseases (Sleper and Poehlman, 2006). Moisture stress, *Striga*, access to labor, access to financing, access to land, access to fertilizer, and access to market are the key production obstacles in Eritrea (Abraha et al., 2013). Drought is a common occurrence in Eritrea, and it happens when rainfall is irregular and low. Similarly drought is a significant agronomic issue that causes damage to the crop in many nations in the east Africa region. *Striga* has a severe influence on drought, especially in the western half of the nation. Sorghum grain output is also affected by bird damage, inadequate availability of macro and micro nutrients (NARI, 2010).

In terms of material and labor inputs, sorghum is given a lesser priority than other grains. Because the crop is cultivated with little inputs, yields are low in general. Reduced soil fertility levels have had an impact on African yields, which are generally low (DeVeries and Toenniesen, 2001). The challenge of *Striga* infestation is made worse by the fact that the majority of small-holder farmers in many parts of Africa use either very little or no capital inputs.

2.4 The parasitic weed *Striga*

Striga is a parasitic plant genus with approximately 30 species found all over the world (Muchira et al., 2021). Several economically significant parasitic species of *Striga* has been reported in Africa (Parker, 2012). In comparism to other species of *Striga*, the greatest destructive and widely spread is *S. hermonthica*.

Striga seeds are tiny, measuring 200 X 300 microns in diameter and weighing 5 X 10^{-6} g each (Berner et al., 1997). *Striga* seeds can survive in the soil for more than a decade without losing viability (Yoder and Scholes, 2010). In the presence of a suitable host, only a small percentage of *Striga* seeds germinate in any season (Runo and Kuria, 2018). *Striga* plants have a robust stem

with long and wide leaves (Berner et al., 1997). Blossoms are normally bright pink, however there are various distinctions, and entirely white flowers have been seen on occasion. A single *Striga* plant can generate anywhere between 10,000 and 100,000 seeds (Parker and Riches, 1993).

Striga parasitizes several cereal crop plants such as sorghum and corn (Rodenburg, 2005; Runo and Kuria, 2018). *Striga* dehydrates, nutrient-depletes, and assimilates its host. *Striga* causes alterations in enzymes and plant hormones, creating a disruption in the interaction of hydrocarbon capture of the host (Press et al., 1996). It is one of Africa's most severe biological barriers to food production, affecting almost 100 million hectares of African grassland each year (Ejeta and Gressel, 2007). Depending on the crop variety and the intensity of the infection, yield loss from *Striga* can range from mild to complete crop failure (Rodenburg, 2005). *Striga* infestation is frequently linked to low soil fertility and high cropping frequencies in marginal areas, a circumstance that is typical among resource-poor farmers (Ransom, 2000).

2.4.1 The lifecycle of *Striga*

To promote effective multiplication, *Striga* as a parasitic plant have advanced and well developed strategies. Some of its tactics include: longer lifespan of seeds, small and very light weight of seeds for easier dispersal, production of huge number of seeds that remain viable for many years (Runo and Kuria, 2018). *Striga* reproduction system follows a sequence of growing phases as illustrated in Figure 2.2. According to Parker and Riches (1993), *Striga* seeds need to be preconditioned for roughly two weeks of humid and warm temperatures in order to germinate (1993). *Striga* seeds germinate in the presence of metabolites (xenognosins), derived from the

host root as described by Yoder (2001). The germination stimulants released by the host plant assist in directing the *Striga* radicle in the direction of the host root. Parasitism is already established once it attaches to the host root and begins sucking water and nutrients from the host (Kuijt, 1977). The *Striga* then emerge to the surface and continue with vegetative, flowering, and seed production (Doggett, 1988).

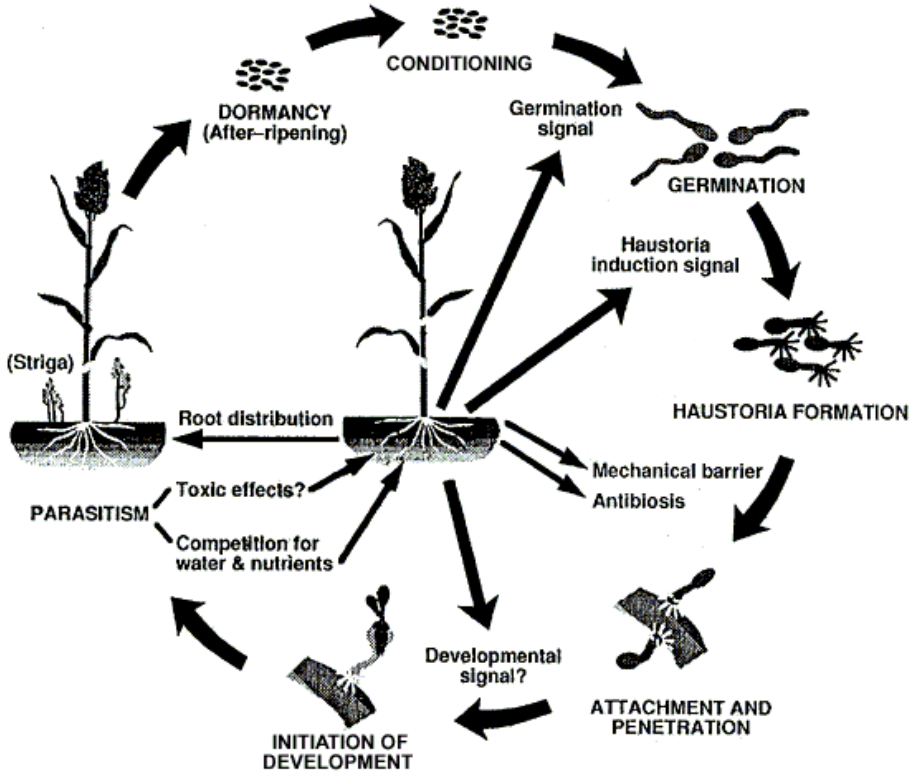

Figure 2. 2: Developmental stages of *Striga* (source: Ejeta and Butler 1993)

2.4.2 *Striga* effects on its host

Striga plays a negative role on its sorghum host. Long before *Striga* develops and becomes apparent above surface, *Striga* causes harm to its hosts thereby affecting yield significantly. Frost et al. (1997) reported that *Striga* lowered biomass in the shoots and roots, reduced leaf area,

slowed growth, and reduced yield in the sorghum host. Besides, *Striga* destabilizes concentration of important growth hormones of its host. *Striga's* chlorophyll content is substantially lower than that of similar non-parasitic plants, implying that *Striga* is less efficient in photosynthesizing its food (Tuquet et al., 1990). *Striga* has a higher rate of transpiration than its host as reported by Ackroyd and Graves (1997), resulting in a continuous syphoning of water from the host (Pageau et al., 2003). Through this continuous movement of water from the host, the parasite can ingest hydrocarbons and other nutrients from the host (Pageau et al., 2003).

2.4.3 *Striga* resistance mechanisms

In general, plants have some form of defense mechanisms against attack. One of the methods is the detection of infections and activation of host defense by receptors that operate as a monitoring program (Dodds and Rathjen, 2010). To control the expression of defense genes, multiple transcriptional factors are triggered (Dodds and Rathjen, 2010). This elicits a variety of host defense, including cell wall thickening, phytoalexin production, and protein synthesis (Van Loon, 2006). The attacking organisms, on the other hand, have a specialized ways for attacking and penetrating the plant.

Different defense mechanisms to *Striga* in sorghum have been reported by Haussman et al., (2004). These mechanisms can be observed at various phases of *Striga* development. Pre attachment defense mechanism includes production of little germination stimulant and low haustoria production as reported by Mohammed et al. (2003). Post attachment defense mechanisms are such as oversensitive reaction, incompatibility reaction (Mohammed et al., 2003). Therefore, interrupting *Striga's* developmental phases at any stages of its growing phases can result in the parasite's demise (Ejeta and Butler, 1993).

Low production of germination stimulant type of resistance mechanism is one of the widely researched mechanism. In such type of resistance mechanism the host releases inadequate amount of the stimulant needed by the *Striga* to germinate. For example in sorghum cultivars such as IS9830 and SRN39 low levels of production of the stimulant have been reported (Mohamed, 2002). Similarly, in maize genotypes, Karaya et al. (2012) reported minimal production of *Striga* germination stimulant resistance mechanisms. In sorghum genotypes which are characterized by less haustoria production, they fail to form a connection at the point of contact with their possible host (Mohamed, 2002; Mallu et al., 2021). As a result, parasitism will not develop, and the germinated *Striga* will eventually run out of reserve energy and perish.

Resistance founded on oversensitive reaction is characterized by an intense hypersensitive reaction at the point of contact (Mohamed, 2002). In such type of sorghum genotypes, the germinated *Striga* seedling fail to form an association with the likely host (Kavuluko et al., 2021) and leads to its ultimate termination.

In sorghum cultivar N13, a mechanical type of *Striga* resistance has been discovered (Maiti et al., 1984). A similar type of resistance mechanism has been reported recently by Kavuluko et al. (2021) on sorghum cultivar named IS10978. In such type of resistance, a mechanical barrier prevents haustoria penetration in to the host resulting no vegetative growth of *Striga*. Cissoko et al. (2011) reported a sort of resistance in rice where a physical barrier stopped the germinated *Striga* from attaching to the rice root.

Kavuluko et al. (2021) reported a resistance mechanism which was displayed by a sorghum genotype IS9830 from ICRISAT collections, in which *Striga* connected, expanded past the cortex, endodermis, and exited at the opposite side without creating vascular connections with the host.

In incompatible reaction, the *Striga* root can penetrate the host but do not develop a parasitism relationship (Mohamed, 2002). When the parasitic weed attaches, it produces a poison that some sorghum genotypes do not respond to, and as a result, *Striga* growth stops immediately after the first penetration (Grenier et al., 2001). Some *Striga* plants tend to progress properly at first but then inhibited growth was observed (Ejeta, 2007)

Sorghum plants have biochemical compounds such as strigolactone in their root exudates that stimulate *Striga* germination (Pieterse and Pesch, 1983). To adhere to its hosts, the germinated *Striga* seeds develop a haustorium. This haustorium development is brought on by haustorial initiation factors, which are chemical stimulants released by the host (Riopel and Musselman, 1979). *Striga* that germinate close to the roots of sorghum but lack haustorial initiation factors typically do not produce haustoria and perish because they are unable to adhere to their possible host (Yoder, 1999).

2. 4. 4 *Striga* management options

Striga is difficult to control because the weed inflicts its significant harm when it is under the surface. *Striga* control methods have been extensively researched and developed, including cultural, chemical, genetic, and biological possibilities (Ejeta and Gressel, 2007). In the majority of cases, these control methods have limited success. Despite the tremendous promise of several of these options, no one option on its own is appropriate in subsistence farming the use of a combination of *Striga* management strategies has been suggested (Runo and Kuria, 2018).

In most parts of Africa, hand weeding is one of the most popular practices in managing *Striga*. This is crucial in preventing seed production and dissemination. Nevertheless, the practice of hand weeding is costly in terms of time and labor needed as reported by Parker and Riches (1993).

Crop rotation with non-host crops is the most straightforward approach for controlling parasitic weeds. Rotation with non-host helps to prevent production of more *Striga* seeds, resulting in a decrease in seed quantity in the soil. For instance in Ethiopia, it has been reported that two years of cropping to non-host crop reduced *Striga* infestation by 50% (Shank, 2002). Selection of the rotational crop varies from place to place (Parker and Riches, 1993).

Trap crop reduces the accumulation of *Striga* seeds in the field by causing them to germinate and eventually die because the germinated seeds does not form haustoria and cannot attach to form connection with the host. Since most of the trap crops are legumes, they improve fertility of the soil thereby enhancing the host's growth.

In many regions of Africa, intercropping legumes with cereal crops is a common cultural practice. Intercropping is potentially viable, low cost technology and improves soil fertility. Fasil (2002) reported that intercropping of sorghum with cowpea enhanced crop yield. Application of nitrogen fertilizers also delays emergence of *Striga* thereby stronger crop growth.

Different herbicides such as 2, 4-D, 2-methyl-4-chlorophenoxyaceti acid (MCPA), Glufosinate and Oxyflourfen have been used against *Stiga*. Runo and Kuria (2018) reported that chemical compounds have been used to deplete the *Striga* seedbank. These chemical compounds mimic strigolactone function by inducing the *Striga* seed to germinate without a host, a process known as suicidal germination (Zwanenburg et al., 2016).

For resource-strapped farmers, crop plants possessing resistance might be the best option. But to date, there hasn't been any report of a variety with complete *Striga* resistance. Sorghum cultivars have been identified that have a distinctive form of resistance mechanism (Bellis et al., 2020; Mallu et al., 2021). Combining diverse mechanisms of resistance to generate more persistent and consistent cultivar can be made easier by identifying donor germplasm with distinct resistance mechanisms. Striga attachment and development can be minimized by resistant genotypes, enabling the crop to flourish and produce in *Striga*-infested situations.

2.5 Molecular Markers

In conventional plant breeding, visual selection is used to determine the genetic variability. However, with the advancement of molecular biology, it is now possible to identify the desired trait at molecular level (Xu, 2010). The invention of genetic markers was one of the major discoveries of the molecular breeding era. Molecular markers are the most commonly employed among genetic markers, owing to their abundance. Genetic markers are easy-to-score entities that are heritable as basic Mendelian features (Schulman et al., 2004). Molecular markers have a high heritability and are unaffected by environmental or developmental factors (Xu, 2010).

DNA markers are critical for improving the effectiveness of traditional plant breeding operations and enhancing crop production. They posses the capability of enhancing the performance in crop improvement program operations in many ways, including for genetic diversity study, germplasm fingerprinting, and in selection of complex characters as reported by Patil et al. (2010). For instance in Eastern Africa DNA markers were utilized to introgress *Striga* resistance

traits in to a local adapted varieties. Various forms of DNA markers have been developed, with SSR being among the most often used markers (Xu, 2010).

2.5.1 SSR Markers

SSRs are PCR-based markers that necessitate prior sequence structure knowledge before they can be used as a genetic marker. SSR markers can be developed in one of two ways: (1) after screening for microsatellite repeat arrays, the needed genome must be sequenced; or (2) initial sequenced genome databases can be explored by means of a variety of in silico bioinformatics tools (Hodel et al., 2016). Microsatellites can be utilized for a variety of reasons, including parentage analysis, plant varieties and germplasm DNA barcoding, gene flow and seed integrity testing, marker assisted breeding, and genetic diversity study (White et al., 2007).

SSR markers are valuable as microsatellite tools since they are simple to generate, indicate expressed transcripts, and a hypothesized functionality can frequently be derived using a homologous investigation as described by Varshey et al. (2005). SSR markers are known for their high diversity, reproducibility, loci uniqueness, and unsystematic spreading across most genomes (Xu, 2010). Prior to the introduction of SNPs, microsatellites were the preferred markers. SSR markers have been produced and characterized in a variety of different crops and they are beneficial for marker-assisted selection, particularly when the markers are located in the genes that control phenotypic characters.

SSRs have a number of significant advantages: PCR grounded; robust and widely spread throughout a genome; produce a vast amount of information; co-dominant, proper in identifying heterozygotes, and multiple-allele; repeatable in experiments; transferable among interrelated biological classifications; simple and efficient; SSRs can amplify DNAs of low quality and

quantity; and are apparently neutral (White et al., 2007). SSRs are preferred markers for small-scale genetic investigations with minimal costs, as they have the capability to detect vast amounts of genetic data and physiological factors in a genome (Finkeldey and Ziehe, 2004), and don't necessitate a large number of markers.

Because SSRs are extremely variable and cover a huge proportion of the genome, SSRs have emerged as the marker of preference for many applications in plants (Gupta et al., 1999). SSRs are useful to evaluate genetic distinction at the molecular level in a germplasm pool so that the right parents can be selected for hybrid breeding programs (Kalia et al., 2011). SSR markers have developed into an efficient method for estimating the genetic diversity and phylogenetic relationships of species. SSR markers are increasingly being used in sorghum improvement programs for genetic analysis studies (Abraha et al., 2014; Ramu et al., 2013; Ng'uni et al., 2011) and in manipulation of critical traits such as resistance to *Striga* (Haussmann et al., 2004).

2.6 Marker Assisted selection (MAS)

In most crop researches around the world, molecular markers are a noble supplement to traditional breeding approaches. Data from molecular marker analysis along with phenotypic information can considerably simplify selection process in plant breeding programs. Molecular markers are invaluable tools in crop improvement research when dealing with biological concerns of a crop. Several molecular marker applications in crop research programs have been reported such as MAS, gene pyramiding, QLT mapping (Xu, 2010).

The process of selecting cultivars that possess a targeted gene is known as marker-assisted selection (MAS) (Lammerts, 2010). When agronomically essential traits are difficult to assess

due to environmental interactions, MAS can help with selection. MAS aids in QTL introgression and backcross breeding by speeding up the recovery of the recurrent parent genome (Hospital and Charcosset, 1997). With the ultimate goal of developing agricultural cultivars with more desirable traits, MAS can be used to pyramid important genes such as disease and pest resistance genes (Mohan et al., 1997). MAS is effective for achieving the same breeding progress in a considerably shorter time than traditional breeding, as well as pyramiding combinations of genes that are difficult to combine using other methods (Xu and Crouch, 2008).

MAS entails rating individuals for the presence or absence of specific traits in order to enable indirect selection using DNA banding patterns of linked markers on a gel, autoradiogram, or sequencer output, depending on the marker system utilized. This can help enhance screening efficiency, especially for complicated features. In sorghum, MAS has been employed successfully in Eritrea (Yohannes et al., 2015), Kenya (Ngugi, 2012), and Sudan (Mohamed et al., 2014) to transfer QTLs with *Striga* resistance from *Striga* resistant N13 to farmer favored sorghum cultivars.

2.7 Transcriptome analysis

Wang et al. (2009) defined Transcriptome as a comprehensive list of transcripts in a cell, as well as their abundance, for a certain growth condition or physiological situation. It is crucial to understand transcriptome in order to identify and characterize significant features in organisms. Transcriptome analysis is critical for identifying the functional parts of the genome as well as stress-related changes (Johnson et al., 2014). Analysis of sorghum transcriptome is an important tool for quantification of the genes necessary for resistance to *Striga* at different stages of

growth. *Striga* causes significant damage to sorghum, resulting in lower yields (Ejeta, 2007). However, sorghum genotypes have showed variable levels of resistance and susceptibility to *Striga* (Mohammed et al., 2010). Understanding the transcriptome of genotypes with different *Striga* responses can show gene networks involved in *Striga* resistance and/or susceptibility. The transcriptome of sorghum after *Striga* infestation enable researchers to identify transcripts that are activated or inhibited in distinct genotypes after exposure to *Striga*. The differentially expressed genes between genotypes can then be studied further to see whether there are any genic single nucleotide polymorphisms (SNPs) that can be utilized to characterize sorghum in the future.

Plants adjust their biochemical and molecular machinery to adapt to changes in their environment when they are faced with harsh conditions. For instance sorghum has undergone physiological changes as a result of exposure to heat and/or drought (Johnson et al., 2014). Similarly, when Zea diploperennis was exposed to *Striga hermonthica*, alterations in root growth were seen (Amusan et al., 2008), with resistant lines displaying a developmental barrier and incompatible response to *Striga*. One of the most important approaches for determining the amount of infection in cereal crops such as rice, maize, and wheat is to look for transcriptional alterations within the genome (Soós et al., 2012). Xin et al., (2012) reported transcriptome studies on resistant and susceptible wheat genotypes. The authors looked at the transcriptome to see if there were any alterations in molecular pathways as a result of exposure to powdery mildew.

Transcriptome analysis reveals major changes in the expressed genes that would not otherwise be visible at the genomic level. Johnson et al., (2014) revealed that analyzing variations in transcript levels can help find new signaling proteins and metabolic pathways that are critical for

plant stress tolerance. Transcriptome analysis also provides a data set that may be utilized to explore gene regulatory networks and develop gene regulation prediction models in various organisms (Tulin et al., 2013). To predict a comprehensive control system for the entire organism, all of the genes whose products make up the regulatory network must be identified (Tulin et al., 2013).

Transcriptome sequencing using Next Generation Sequencing (NGS) is beneficial for determining links between genetic variants and examining functional implications of genetic variability (Duitama et al., 2012). The current study helps in addressing research gap in transcriptome analysis in sorghum under *Striga* infestation using NGS. This can be achieved by comparing the transcriptome sequence data of known *Striga* resistant and susceptible sorghum genotypes. The identified transcripts could help to discover SNPs for further *Striga*-sorghum research. Besides, Identification of *Striga* resistant varieties generated from this study is expected to be included in breeding programs to increase sorghum production in *Striga* infested areas of the country.

CHAPTER THREE

A DIAGNOSTIC APPRAISAL OF SORGHUM FARMING IN STRIGA ENDEMIC AREAS OF ERITREA

Abstract

Many challenges impede sorghum production, including farmer perceptions, a lack of suitable varieties, underdeveloped seed systems, drought stress, and *Striga* infection. The objective of this study was to learn more about sorghum smallholder livelihoods, farming practices, *Striga* incidence and infestation levels, and the sorts of varieties planted in *Striga*-infested sorghum-growing areas. Using a semi-structured questionnaire and focused group discussions, 136 randomly chosen farmers from the three *Striga* endemic sub-regions of Eritrea—Hamelmalo, Goluj, and Tesseney—were interviewed and the information gathered were analysed using SPSS software. The results showed that the most significant cereal crops in the research area were sorghum, pear millet, ground nut, and sesame. Over 80% of the respondents in the study area indicated that the major constraints on sorghum output were drought stress, followed by *Striga* infestation. Most of the responders (81.6%) stated that *Striga* had infested their sorghum, with infestation levels ranging from light (10%) to severe (70% and higher). High grain production, drought tolerance/resistance, and *Striga* resistance were listed as the small-holder farmers' top priority selection criteria for sorghum varieties. The most common landraces in the research area were listed as being up to 31 different varieties. The native varieties that farmers had saved from past harvests served as the primary source of seed for production. The information documented from this study may be used in future as a basis for a participatory farmer-oriented sorghum breeding program.

Keywords: Eritrea, landraces, production constraint, sorghum, Striga

3.1 Introduction

In Eritrea, sorghum is mainly grown as a rainfed crop in the highland and in spate irrigated areas of eastern lowland such as Sheeb and Wadilabka (Van Steenberger et al., 2010). Sorghum forms an important dietary component prepared as 'injera' (leavened bread) or as thick porridge and contains 68-74% carbohydrate, 8-15% protein, 2-5% fat, 8-16% water, 1-3% fiber and 1.5-2% ash (Perseglove, 1975). In this regard, sorghum covers the majority of the human body's primary dietary requirements, and its consumption is highest in the world's poorest and most food-insecure countries (Ejeta & Knoll, 2007). However, the grain yield of sorghum is low due to not only biotic and abiotic stresses, but also to socio-economic factors such as farmer preference of what commodities to invest in a traditional farming system basically made of crops and livestock.

From 1998 to 2019, sorghum occupied an average of 219,903 ha of arable land in Eritrea and produced an average of 128,244.56 metric tons of grain annually (MoA, 2020).This productivity is low (0.58 t/ha) compared to that in the east and central Africa (ECA) region (FAOSTAT, 2020), that has an average productivity of 0.93 t/ha in the same period. Soil fertility, drought stress, pests and diseases were listed by Wortmann et al. (2006) as the major constraints and their relative importance varied between agro-zones.

Increasing the productivity of sorghum in Eritrea will help improve household income, reduce poverty and food insecurity because the crop accounts for about 50% of total cereal production (MoA, 2020). Furthermore, because sorghum is well adapted to dry areas and gives reasonable yields in droughted seasons compared to other cereals in the region, farmers have used the crop in these agro-ecological zones where rainfall is scarce and unreliable.

Striga is one of the most severe constraints to cereal production in sub-Saharan Africa (Oswald & Ransom, 2004). The increasing incidence of *Striga* has been attributed to poor soil fertility and structure, intensification of land-use through continuous cultivation and an expansion of cereal production (Rodenburg et al., 2005). In Eritrea, *Striga* mainly affects western part of the country where sorghum mono-cropping system is practiced. The African Agricultural Technology Foundation, AATF (2011) estimated that 64,000 ha of sorghum fields in Eritrea are affected by *Striga*. The extent to which *Striga* reduces the growth of its host is highly variable and depends on factors such as host plant genotype, parasite infestation level, and environment (van Ast et al., 2005).

The results of this study are likely to provide future insights into possible interventions needed to mitigate *Striga* infestation and the agronomic control measures that small-scale farmers would adopt in a cost effective manner.

3.2 Methodology

3.2.1 Study area description

A baseline survey was conducted in three sub-regions of Eritrea namely: Goluj, Tesseney and Hamelmalo where sorghum is the major crop and *Striga hermonthica* is the commonest threat to its production. Agro-ecologically, Goluj (140º74′N, 360º72′E) and Tesseney (150º11′N, 360º66′E) sub-regions fall in the South Western Lowland Zone (SWLZ) of the country. The altitude of the agro-ecological zone ranges between 600 and 700 meters above sea level. The zone has hot-semiarid climate with an erratic rainfall that ranges between 300 and 700

mm/annum. According to reports from NARI (2010), various soil types exist in the study area, but vertisols are the dominant ones. The sub-region Hamelmalo (160º01′N, 380º20′E) is characterized by an altitude of 1280 meters above sea level with average annual rainfall of 479.2 mm and sandy and sandy loam soils.

3.2.2 Data collection and analysis

A semi structured questionnaire (Appendix 1) and focused group discussions were employed to gather information to determine the following major factors: production constraints, seed system; structure of a family, *Striga* infection rates; damage caused by *Striga* and presence/absence of tolerant/resistant varieties. A total of 136 farmers were randomly selected for the interview working in collaboration with the extension service of the ministry of agriculture and with village administrators of the three respective sub-regions.

The sampling strategy used was proportional sampling, as described by Cochrane (1977), and the sample size was calculated as given in Table 3.1. Sampling by proportion allows that sub-regions with many farmers growing sorghum under *Striga* infestation get higher sample size (Table 3.1). Farmers for the survey were chosen using simple random sampling, as indicated in Table 3.1, by determining the sampling interval for every sub-region. Accordingly, the sampling interval was approximately 7 for all sub-regions; hence every 7^{th} farmer in a list of names arranged alphabetically was selected for the interview in each sub-region.

Each sub zone of the study area had three focused group didcussions. Farmers, extension staff, researchers, and village administration participated in the group sessions in each sub zone. Secondary data/information was reviewed to reinforce the study.

Table 3. 1: Sample size and sampling interval determination for the surveyed area

Sub region	No of sorghum farmers (A)	Sample size (B)	Sampling Interval (A/B)
Goluj (G)	309	(G/D) × 136 = (309/1001)*136 = 42	309/42 = 7.3
Hamelmalo (H)	324	(H/D) × 136 = (324/1001)*136 = 44	324/44 = 7.4
Teseney (T)	368	(T/D) × 136 = (300/1000)*136 = 50	368/50 = 7.4
TOTAL (D)	1001	136	

H=Hamelmalo, G=Goluj, T=Tesenei, D=Total

Data collected from the diagnostic baseline survey were analyzed for means, descriptive statistics and tables, using the Statistical Product and Service Solutions (SPSS) software package.

3.3. Results and discussion

3.3.1 Characteristics of sampled households

There were similarities of responses among the farmers across the sub-regions in relation to age, sex and size of the households. The study revealed that almost all the sampled households were headed by men (Table 3.2). This may be attributed to the fact that in Eritrea, the husband is the household's primary authority figure. The average age of household head interviewed in the study area ranged between 53 to 55 years (Table 3.2).

Household size determines the availability of household labor supply and the results indicated large household size for all three sub-regions studied (Table 3.2). Large household size tends to be allied with rural areas characterized by the advocacy or support of a high birth rate and extended family relations. Biniam et al. (2014) found comparable results in their study in other sections of the country, where family sizes ranged from 5.4 to 7.8.

Table 3. 2: Characteristics of the sampled households

Characteristics	Hamelmalo	Tesseney	Goluj
Male household head (%)	100	90	97.6
Average age of household head (years)	53.7	54.4	55
Average household size (number)	7.2	5.7	8

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All the households interviewed indicated to have owned their farm fields (Table 3.3). In Eritrea, land belongs to the government and each household in the farming community is entitled to have land based on their family size. Reports from ministry of agriculture (MoA) and local government of zone Gashbarka offices indicated that there are two types of tenure systems; where every farming household is entitled to own about two hectares of land for subsistence agriculture but the rest of the cultivable farmland is reserved for commercial rain-fed agriculture

(NARI, 2010). The average area of land available for farming was different in each sub-region as shown in Table 3.3. The average available land (farmer owned land) was by far larger in Goluj (5.4 ha) compared to Hamelmalo (1.5 ha) and Tesseney (2.6 ha) sub-regions. The relatively larger size of owned farmland in Goluj sub-region may be due to the existence of many commercial rain-fed farming in this sub-region. The other farm fields were either rented from farmers or share cultivated with other farmers as indicated in Table 3.3

Table 3. 3: Average farm land size in sub-regions of Hamelmalo, Goluj and Tesseney

Land tenure	Hamelmalo		Goluj		Tesseney	
	N	Ha	N	ha	N	ha
Owned land	44	1.5	42	5.4	50	2.6
Rented	23	1.6	7	3.7	9	2
Shared	13	1.7	5	7.4	0	0

N= number of respondents

Crop and livestock production were key sources of food, feed, and revenue for most of the farmers. Most farmers had a mix of livestock which included oxen, cattle, sheep, goats, donkeys and chicken. The main crops grown included: sorghum, pearl millet, sesame, ground nut and maize as shown in Table 3.4. Sorghum was listed as the most important crop in Tesseney and Goluj sub-regions where as in Hamelmalo, Pearl millet was the most preferred crop followed by sorghum. These findings corroborate with reports by Abraha et al. (2013).

Table 3. 4: Frequency of respondents to the level of importance of the crop in question

	Frequency of respondents														
	Hamelmalo					Tesseney					Goluj				
Crop type	EI	VI	MI	SI	LI	EI	VI	MI	SI	LI	EI	VI	MI	SI	LI
Sorghum	1	32	11	0	0	46	4	0	0	0	30	12	0	0	0
Maize	0	0	1	12	31	0	1	2	21	26	0	0	3	15	24
P.millet	38	5	1	0	0	0	8	36	6	0	0	2	36	4	0
Ground nut	5	7	32	0	0	0	0	0	20	30	0	0	1	25	16
Sesame	0	1	1	0	42	4	32	13	0	1	12	27	3	0	0

EI= extremely important, VI= very important, MI= moderately important, SI= slightly important, IL= less important

In selecting a variety, farmers considered different characteristics of the crop in question as shown in Table 3.5. Accordingly, high yield, drought and *Striga* resistance were reported as the most important characteristics of a good sorghum variety (Table 3.5). Such desirable traits of a crop were crucial in the selection and adoption of a variety. Other characters considered as important by the farmers were, adaptation to the local environment, plant height (medium to tall varieties more preferred), grain size and grain color as indicated in Table 3.5. Abraha et al. (2013) reported similar findings that showed that adaptability and grain yield are among the most important characteristics of a good sorghum seed in the sampled agro-ecological zones.

Table 3. 5: Frequency of respondents on characteristics of an ideal sorghum variety across the sub zones

	Hamelmalo			Tesseney			Goluj		
Trait/characteristic	**MI**	**SWI**	**LI**	**MI**	**SWI**	**LI**	**MI**	**SWI**	**LI**
High yield	39	4	1	37	11	2	33	7	2
Adaptation	1	25	18	9	40	1	7	35	0
Striga resistance	15	28	1	25	21	4	25	17	0
Drought resistance	15	27	2	25	24	1	23	17	2
Plant height	4	16	24	5	26	19	1	18	23
Panicle size	14	24	6	22	19	9	10	27	5
Grai size	5	18	21	7	30	13	0	26	16
Grain color	0	11	33	1	17	32	0	10	32
Suitability food preparation	4	25	15	6	26	18	6	21	15
Tillering	1	15	28	2	16	32	0	1	41

MI=most important, SWI=somewhat important, LI=least important

The dominant sorghum seed source (65.4%) for cultivation in the study area was from the seeds of local varieties (landraces) retained by farmers themselves from previous harvests (Figure 1). A few (4.2%) of the interviewed farmers had acquired improved varieties from ministry of agriculture offices, research centers or local markets. The study confirmed that farmers have a long-standing tradition of preserving their own varieties (landraces) for future use and, as a result, passing them down from generation to generation.

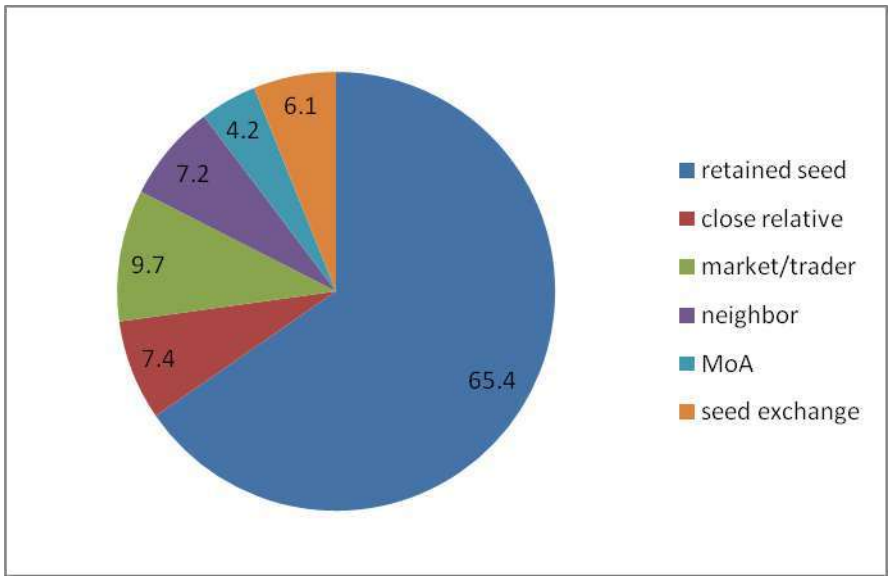


Figure 3. 1: Sorghum seed sources of the households in percentage

3.3.2 Fertilizer use

The common means of enhancing soil fertility in small farm agriculture has been to use chemical fertilizers to increase food production (Mignouna et al., 2013). Despite this fact however, results of this study revealed that majority of the respondents (79.4%) do not apply fertilizer to their sorghum farm (Table 3.6). This could be one of the explanations for the studied area's low sorghum productivity. This results is comparable to one made by Abraha et al. (2013) in a previous study which indicated that commercial fertilizer is rarely used by farmers in the Goluj and Tesseney sub-regions. Some farmers in the Hamelmalo sub-region used farm yard manure in their fields near the homestead. Despite not using fertilizers, the farmers interviewed believed that the use of fertilizer for sorghum production is important. However, unaffordable costs, unavailability of fertilizer and the moisture stress at the end of growing season precluded the farmers from the use of commercial fertilizers.

Table 3. 6: Respondents' frequency on use of fertilizer

Sub zone	Application of fertilizer		Availability of fertilizer on time		Right amount of fertilizer	
	Yes	No	Yes	No	Yes	No
Hamelmalo	20	24	30	14	21	23
Tesseney	6	44	14	36	12	38
Goluj	2	40	9	33	6	36
Total	28	108	53	83	39	97
%	20.6	79.4	39	61	28.7	71.3

3.3.3 Sorghum production constraints

Over 80% in the surveyed area reported drought stress as the most important constraint to sorghum production which was followed by *Striga* as indicated in Table 3.7 as also reported by Abraha et al. (2013). Other minor constraints to sorghum production included access to crop protection facilities, fertilizer, and labor as shown in Table 3.7.

Table 3. 7: Frequency of sorghum production constraints in sub-regions Hamelmalo, Teseney and Goluj

	Hamelmalo			Tesseney			Goluj		
Constraints	MI	SWI	LI	MI	SWI	LI	MI	SWI	LI
Drought	22	17	5	35	5	10	21	14	7
Striga infestation	17	20	7	29	10	11	34	4	4
Quality seed	0	0	42	0	4	46	0	0	42
Access to labor	0	1	43	4	7	38	4	6	32
Access to credit	0	0	44	2	4	44	4	9	29
Access to land	1	1	42	1	1	48	0	2	40
Access to irrigation	0	1	42	0	0	50	0	0	42
Access to crop protection	8	16	20	3	10	36	1	4	37
Fertilizer	3	10	31	0	0	52	0	0	42
Market	0	0	44	2	2	46	1	0	41

3.3.4 Perception of farmers to *Striga* incidence and extent in sorghum production

Farmers considered *Striga* hand weeding at its early stage as the most effective practice to reduce its impact in subsequent cropping seasons. This method could aid in limiting *Striga* plant proliferation and seed dissemination. In the past researchers indicated that even though hand weeding prevents further seed multiplication of *Striga* weed, it is a less efficient control method once the *Striga* is established (Woomer et al., 2004). Furthermore, hand weeding of *Striga* may not increase the yield of already infected plant because most of the damage (75%) occurs before the weed emerges above the ground (Ejeta, 2007).

The majority of the respondents (81.6%) in the study area reported that their sorghum farm was infested with *Striga* (Table 3.8). The level of infestation varied from mild to severe (Table 3.8). Almost 49% of the respondents reported that the proportion of their sorghum farm infested with *Striga* exceeded 70%. This may infer that the existing varieties were less resistant to *Striga* and hence need farther improvement for resistance. The intensity of *Striga* infestation was exacerbated by drought stress. A field survey on *Striga* by National Agricultural Research Institute (NARI) of Eritrea also showed that the mean number of *Striga* plants per meter square in the study area ranged 71-335 (NARI, 2001). Considering that *Striga* produces 10,000-100,000 seeds/plant (Parker & Riches, 1993), the level of soil infestation could exponentially increase every season and leads to a devastating effect on the sorghum farmers. According to reports from nearby countries such as Ethiopia (Gebretsadik et al., 2014), Sudan (Gemar & Mohamed, 2013), Kenya (Kanampiu et al., 2002), and Uganda (Olupot et al., 2005), the incidence and extent of *Striga* infestation is increasing as sorghum hectareage increases, necessitating a regional approach to mitigate the problem.

Table 3. 8: Frequency of farmers' response on if their sorghum farm was infested with *Striga* and the proportion of infestation

Sub-zone	Response		Proportion and number of respondents			
	Yes	No	<10%	11-40%	41-70%	>70%
Hamelmalo	36	8	0	5	20	19
Tesesney	42	8	7	13	10	20
Goluj	33	9	0	3	11	28
Total	111	25	7	21	41	67
%	81.6	18.4	5.1	15.4	30.1	49.3

3.3.5 Commonly grown Sorghum varieties and their preferred traits

A wide variety of sorghum landraces which have been grown in the study area were mentioned by farmers (Table 3.9). Some of the landraces were commonly recognized by most farmers across the three sub-regions studied, while some were common only in one or two of the sub-regions. A large number of landraces show the existence of diverse genetic resources of sorghum which have evolved under different environmental conditions and management practices by smallholder farmers. Genetic diversity study on sorghum landraces from Eritrea by Ghebru et al. (2002) and Abraha et al. (2014) using molecular markers reported a range of genetic diversity which supports the availability of a diverse of landraces mentioned by the interviewed farmers. Such diversity could be exploited in sorghum breeding programs for further improvement.

Only a small number of improved sorghum varieties were mentioned by farmers across the three sub-regions (Table 3.9). It was noted that farmers' preference to a particular variety was associated with certain characteristic such as drought resistance, early maturity, resistance to *Striga*, market value, and cooking quality.

Table 3. 9: List of sorghum varieties commonly grown in the study sub-zones and their associated characteristics as described by farmers

Sub-zone	Variety name	Suggested traits
Hamelmalo	Bariyay(Red), Ewarda, Delek, Shigrey, Meriro, Senadir, Red-sorghum, white sorghum, Abu-arbin,	
	Bariyay (white), Helle, Wediaker, Embulbul, Kibra, Hariray,	Early maturity
	Bazenay,	Striga resistance
Goluj	Red-Bariyay, Bazenay, Arfae gedem, Keyih,Bazenay, Feteret, , Shenedeck Karakora,Baryai, (**PP290**/**Shambuko)**, Ghedem hamam, **ICSV 111(Seare)**	
	Bariyay, Wedi-Aker, Hariray, wedi-Fereg,	Early maturity
	Bazenay, Bariyay	Striga resistance
	Hugurtay, Bariyay, Hariray	Drought resistance
	PP290/shambuko, ICSV 111(Seare)	High yield
Tesseney	Fetereta, , Hugurtay, Arfae-ghedem, , Keyih, Korakora, , Deber, Safra, , Red and white, , , Ghedem hamam, Aklomay, Nugud, Esferf, Semsem	
	Hariray, Wedi aker, Bariyay Embulbul, Wediferej, Wedi-arbaa,	Early maturity
	Bazenay, Bariyay	Striga resistance
	Hugurtay, Hariray	Drought resistance
	PP290(shambuko), ICSV 111(Seare)	High yield

Bold scripts are improved varieties

3.4 Conclusion

This study identified farmers' sorghum production opportunities, the main constraints, the use of indigenous knowledge in farming, farmers' perceptions and preferences of biological traits in varieties currently grown in Hamelmalo, Goluj and Tesseney sub-regions. The effect of *Striga* infestation was identified as the second most important constraint limiting sorghum production after drought. Farmers in the study area preferred high yield, drought resistance and *Striga* resistance traits as the most important for sorghum varietal selection criteria. But the relative

importance of constraints varied considerably within and between the three endemic *Striga* sub-regions studied. The farmers interviewed indicated that landraces have wide adaptation to the farming systems, with relatively better level of drought tolerance. The study also identified that *Striga* infestation is high in all the three sub-regions studied, but more so in the Goluj sub-region. This implies that there is need to deploy efficient strategies to limit the rapid increase in soil *Striga* seed density and spread of *Striga* to new farming lands in Eritrea. Thus, based on farmers' perceptions, sorghum improvement programs in Eritrea should focus on developing cultivars that incorporate farmers-preferred traits with emphasis on *Striga* and drought resistance.

CHAPTER FOUR

GENOTYPIC VARIATION FOR LOW STRIGA GERMINATION STIMULATION IN SORGHUM "*Sorghum bicolor* " LANDRACES FROM ERITREA

Abstract

Sorghum varieties that produce low quantities of chemical stimulants such as sorgolactones, which inhibit *Striga* seed germination and are hence considered parasite resistant, have been identified. However, the presence of sorghum genetic variation for resistance has yet to be proven, among farmers' landraces. The aim of the study was to examine how much *Striga* germination stimulants were produced by each of the 111 Eritrean landrace sorghums and their descendants. The ability of a sorghum genotype to influence germination of a *Striga* seed was used as a measure of the quantity of germination stimulant generated to assess the resilience of these germplasms. By counting the number of germinated *Striga* seeds, the data was recorded as a *Striga* germination percentage. Landraces EG830, EG1076, EG473, EG1261, EG546, and EG746 stimulated low levels of *Striga* germination percentages, with 11.85 %, 13.05 %, 14.68 %, 15.32 %, 15.74 %, and 16.5 % respectively, when compared to commercial checks IS9830, SRN39, and Framida, which had 22.46%, 22.67 %, and 23.27 %. While these variants did not entirely prevent *Striga* seed germination, their high level of *Striga* resistance was inferred by the low amount of stimulant production. These findings suggested that these germplasms could be considered as potential sources of *Striga* resistance in sorghum breeding programs.

Key words: Eritrea, landrace sorghum, *Striga hermonthica*, *Striga* germination stimulants, seed, parasitic plants

4.1 Introduction

Despite the fact that sorghum consumption is substantial in most SSA countries, farm-level grain yields are low because of biological and non-biological stressors (Mohamed et al., 2011; Mohamed and Gamar, 2011). *Striga hermonthica* impacts above 100 million people and infests over 40% of the savanna region's arable land (Parker, 2009). *Striga* is more difficult to control since it does most of its damage before it emerges from the soil (Ejeta, 2007). Because mechanical and chemical management techniques impact *Striga* after it has already connected to and harmed the host, they are less effective (Ejeta, 2007). Hand weeding, crop rotation, trap crops, catch crops, intercropping, fertilizers, and herbicides have all been advocated, albeit to varying degree of effectiveness. Many herbicides have been attempted, but they have not proven to be effective, are expensive, and SSA resource-poor farmers may not have access to these technologies.

Striga hermonthica affects the majority of Eritrean farmers, especially those in the country's western region, where continuous monoculture is practiced (Yohannes et al., 2015). A report by the African Agricultural Technology Foundation, AATF (2011) indicated that 30,000 to 90,000 tonnes of grain sorghum is lost annually due to *Striga* in Eritrea. Annual yield losses due to *Striga* in neighbouring countries such as Sudan, Ethiopia, Kenya and Uganda is estimated at 1,060,000; 500,000; 50,000 and 40,000 tonnes respectively (AATF, 2011). To minimize such yield loses there is a need to devise control measures against the parasite.

Crop development efforts in the past have primarily focused on host plant resistance as a technique of breeding against *Striga*. The utilization of resistant varieties is thought to be a more effective and practical solution for managing *Striga* infestations. However, conventional breeding against parasite has proved slow and laborious (Patrick et al., 2004). As demonstrated

in prior investigations, combining host plant resistance mechanisms with molecular marker assisted selection (MAS) would almost certainly generate positive results (Yohannes et al., 2015).

Various mechanisms of *Striga* resistance in sorghum have been identified, which may function independently or in different combination (Ejeta, 2007; Haussmann et al., 2004). Using in-vitro laboratory techniques, four unique resistance mechanisms to *Striga* were revealed in farmed sorghums and some wild germplasm, including Low production of germination stimulants, haustoria initiating factor, hypersensitive reaction, and incompatibility reaction (Ejeta, 2007; Mohamed et al., 2003). Sorghum varieties with low germination stimulants provide insufficient exudates for germination of conditioned *Striga* seed. Reducing the amount of germination stimulants produced by host plants allows for fewer seeds to germinate (Karaya et al., 2012). Low or no stimulant production by cereal roots has been reported as one mechanism of host plant resistance to *Striga hermonthica* infections (Mohamed et al., 2001). In field studies, sorghum varieties that produce low levels of germination stimulants were found to be resistant to *Striga* (Ramaiah, 1987). Parker (2009) reported that highly vulnerable sorghum varieties produced a lot of germination stimulants. This study compared the germination stimulant production reactions of Eritrean landraces and commercial cultivars, identified genotypes with low levels of germination stimulant production that may be classified as *Striga* resistant.

4.2. Materials and Methods

4. 2.1. Plant materials

Striga hermonthica seeds were obtained from Kenya Agricultural and Livestock Research Organization (KALRO) sub-station Kibos. They were collected in 2011 from sorghum growing fields at Kibos (00° 04' S, 34° 48' E, 1214 m altitude) using standard protocols (Bernner et al., 1996). Sorghum landraces were sourced from National Agricultural Research Institute (NARI) of Eritrea which was collected from sorghum growing zones of Gashbarka, Anseba, southern zone and Northern red sea regions of the country (Abraha et al., 2014). Elite backcross lines, improved varieties and commercial checks were included in the experiment as indicated in Table 4.1.

Table 4. 1: Summary of sorghum germplasm used in the study

Germplasm	Number	Source
Landraces	86	NARI
Improved varieties	5	ICRISAT-Nairobi
Elite crossed lines	17	NARI, ICRISAT
Commercial check	3	ICRISAT-Nairobi
Total	111	

NARI=National agricultural research institute, ICRISAT= International Crops Research Institute for the Semi-Arid Tropics

4.2.2. *Striga* seed conditioning

To respond to a germination stimulant, *Striga hermonthica* seeds must be conditioned by being exposed to ideal moisture and temperature (30 °C) for two weeks (Worsham, 1987). To condition *Striga* seeds, they were initially surface disinfected for 5 minutes in a mix of 1 %

sodium hypochlorite containing 0.02% (v/v) Tween 20 (Berner et al., 1995). Floating seeds and other waste materials were thrown away. The remaining seeds were rinsed using sterile distilled water and later air dried under laminar flow hood. Twin layers of Whatman No. 1 filter papers were placed in a 90 mm sterile Petri dish then the twin filter papers were moistened with 5 ml of sterile distilled water (Berner et al., 1997). The air dried *Striga* seeds were dispersed on the glass-fiber discs (Whatman GF/C) in such a way that each disc had 20-30 *Stiga* seeds, and then put in an incubator for two weeks at 30 °C (Berner et al., 1997; Karaya et al., 2012).

4.2.3. Experiment setup

The experiment was conducted in laboratory and screen house at BecA-ILRI Hub, Nairobi, Kenya. After sterilizing sand in a preheated oven for 30 minutes at 85°C, each sorghum accession was planted in a 10 cm diameter pot containing sterilized sand. Each pot contained 8-10 plants, allowing at least 1 gram of root to be harvested. To achieve synchronization for optimal stimulant synthesis in the early developmental stages of roots, planting took place on the same day that *Striga* seeds were put in an oven for conditioning (Karaya ey al., 2012). The seedlings were let to grow for two weeks before they were uprooted. The roots of the two-week-old sorghum seedlings were washed after they were gently removed from the pot (Figure 4.1c).

(a) **(b)** **(c)**

Figure 4. 1: a) Sorghum seedlings, (b) uprooted sorghum, (c) washing sorghum roots

The washed roots were chopped into small pieces of roughly 0.5 cm and 1gram was weighed to test germination of *Striga* seeds. In a 90 mm Petri dish, four radial rows of fiber-glass discs containing conditioned *Striga* seeds were put around a 1.5 cm diameter aluminum foil ring (Figure 4.2), which was centered on a double layer of Whatman no.1 filter paper wet with 3ml of double distilled water (Ahonsi and Emechebe 2005). Then, according to the recommendations, 1 gram of cut root pieces was introduced into the aluminum foil ring, followed by 3 ml of double distilled water to diffuse root exudates across the filter paper (Karaya et al., 2012; Berner et al., 1996). The positive and negative controls were GR24 and double distilled water, respectively. The petridish was then sealed with parafilm, wrapped in aluminum foil, and placed in an incubator for *Striga* germination at 30 °C for 48 hours (Berner et al., 1997). GR24 is a commercially available synthetic germination stimulant that is a chemical equivalent of strigolactones. The stock was prepared as 100mg of GR24 in 10ml of acetone and then diluted with sterile distilled water, a 1 litter stock solution (100 $mg \cdot L^{-1}$) was made and used at a final concentration of 0.01 $mg \cdot L^{-1}$.

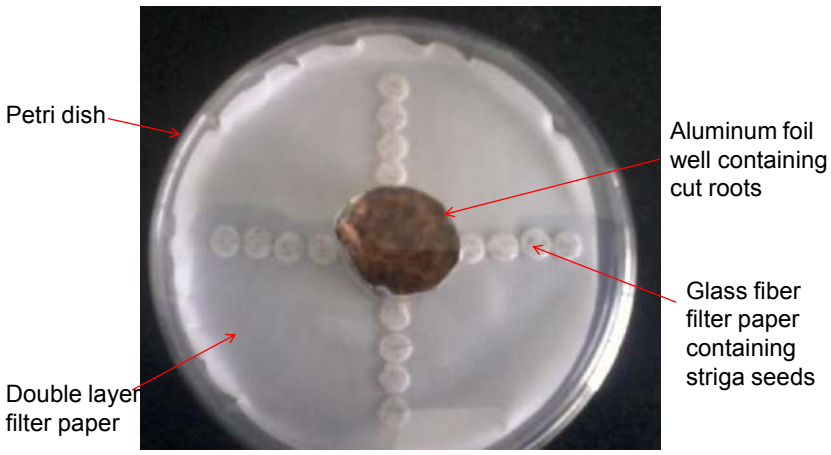


Figure 4. 2: *Striga* germination test using sorghum root exudes

4.2.4. Data recording and analysis:

Striga germination count was performed using dissecting microscope 48 hours after receiving the *Striga* germination stimulants by counting the number of *Striga* seeds in each fiber glass disc that had sprouted as specified by (Berner et al., 1997). If the radicle protruded through the seed coat as indicated in Figure 4.3, the seed was considered germinated.

For each treatment, the percentage of *Striga hermonthica* seeds that germinated was calculated. Analysis of variance (ANOVA) was carried out using Genstat®15th Edition (http://www.vsni.co.uk). The least significant difference test was used to identify treatment means at the 5% level. Statistical analysis for percent *Striga* germination data was performed after logarithmic transformations using the formula (log (X +1), where X is the original

individual observation) (Rodenburg et al., 2006). Correlations was done between the percentages of *Striga* germination and the distance from the source of *Striga* germination stimulant.

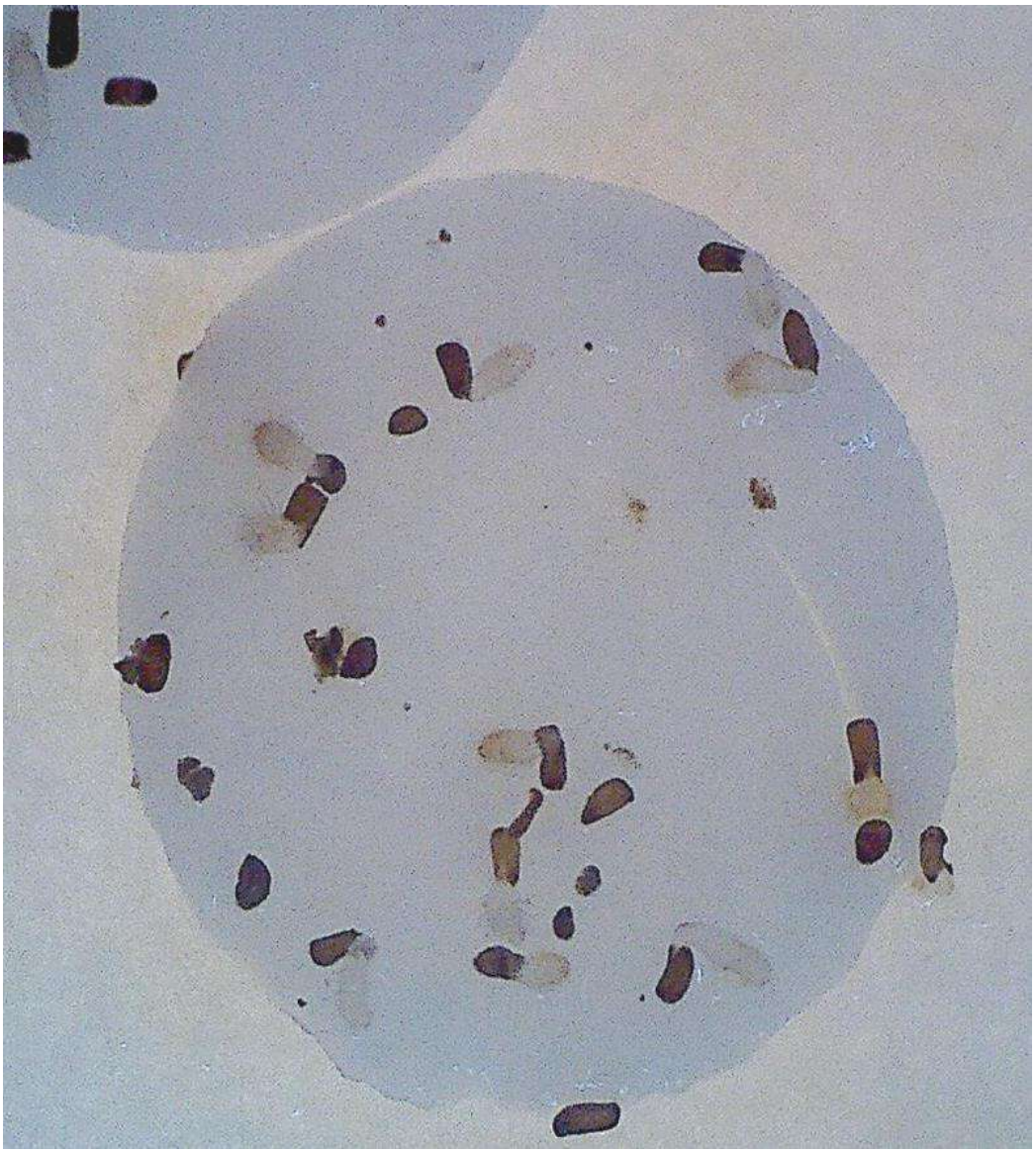


Figure 4. 3: Germinated *Striga* due to stimulant production of sorghum

4.3. Results and Discussion

All sorghum accessions used in this study germinated well in the pots. This enabled the harvesting of at least one gram of root from each accession which was required as source of *Striga* germination stimulant in the study. The term stimulant refers to a component of sorghum root exudates that helps the *Striga hermonthica* strain germinate (Ramaiah et al., 1990). Analysis of variance for *Striga* germination revealed that highly significant differences ($P < 0.001$) were observed among the sorghum accessions tested for their ability to cause *Striga* germination with a range of 11.8 to 40.6% (Table 4. 2). In all sorghum genotypes, *Striga* seeds germinated at various levels along the circumferential location in the petri dish, demonstrating the presence of varying doses of germination stimulants. This is in agreement with the work of Karaya et al. (2012), who reported the variability of *Striga* germination stimulant levels in maize.

Accession EG1168 stimulated the highest germination of *Striga* seeds (40.3%±4.9) compared to the rest of accessions. On the contrary accession EG830 induced the lowest level of *Striga* germination (11.85%±2.4). Such low *Striga* germination percent may indicate a potential for resistance to *Striga*. No *Striga* germination was observed in the negative control (double distilled water) while the positive control GR24 exhibited 43.73%, which was not significantly different from germination observed with sorghum accession EG1168 (40.6%±4.9). However, all the rest of the sorghum accessions induced significantly lower *Striga* germination compared to the GR24.

Table 4. 2: Levels of *Striga* germination percent exhibited by the sorghum accessions tested

Rank	Entry	Accession name	Accession source	*Striga* germination percent (%)
1	83	EG830	GB	11.85
2	94	EG1076	AN	13.05
3	2	EG473	GB	14.68
4	70	EG1261	GB	15.32
5	14	EG546	AN	15.74
6	86	EG898	GB	16.24
7	92	EG746	S	16.5
8	67	EG1256	GB	17.12
9	93	L2P3	NARI-cross	17.66
10	32	EG801	AN	18.22
11	85	EG1258	GB	18.23
12	73	EG2457	NRS	18.37
13	109	IESV 23010	ICRISAT	18.37
14	62	EG1208	NRS	18.39
15	111	L2P5P15	NARI-cross	18.72
16	64	EG1235	GB	19.05
17	91	ICSV111	ICRISAT	19.25
18	69	EG1259	GB	19.3
19	65	EG1237	S	19.87
20	112	L2P5P35	NARI-cross	20.19
21	88	EG806	GB	20.2
22	3	EG480	GB	20.44
23	82	EG881	GB	20.47
24	56	EG896	GB	20.74
25	81	EG1246	S	20.93
26	5	EG497	AN	21.09
27	29	EG789	GB	21.12
28	71	EG2161	NRS	21.23
29	28	EG787	GB	21.37
30	33	EG745	S	21.56
31	61	ICSV 111-2	ICRISAT	21.6
32	57	EG1224	GB	21.81
33	54	Hamelmalo	AN	21.99
34	89	EG896	GB	22.14
35	97	L3P3	NARI-cross	22.41
36	12	IS9830	ICRISAT	22.46
37	1	EG469	GB	22.65
38	101	SRN39	ICRISAT	22.67
39	78	EG786	GB	22.98
40	26	EG779	S	22.99

Continued Table 4.2,

Rank	Entry	Accession name	accession source	Striga germination percent (%)
41	47	EG873	GB	23.04
42	113	L2P7	ICRISAT	23.17
43	100	Framida	ICRISAT	23.27
44	84	EG1076-2	AN	23.39
45	30	EG791	GB	23.83
46	43	EG2456	NRS	23.87
47	51	EG889	GB	23.9
48	72	EG2453	NRS	23.92
49	76	EG794	GB	24.03
50	53	EG893	GB	24.17
51	38	EG845	GB	24.33
52	40	EG849	GB	24.33
53	87	EG864	GB	24.41
54	55	EG1075	NRS	24.44
55	105	L2P2P8	NARI-cross	24.7
56	36	EG836	AN	24.79
57	44	EG858	S	24.94
58	37	EG843	GB	25.26
59	68	EG1257	GB	25.41
60	95	L1P5	NARI-cross	25.42
61	31	EG797	GB	25.47
62	110	L2P6	NARI-cross	25.64
63	90	Kibra	AN	25.71
64	17	EG717	GB	25.88
65	13	EG554	S	26
66	104	L2P5P25	GB	26.01
67	34	EG813	GB	26.05
68	8	EG540	GB	26.07
69	39	EG846	GB	26.23
70	103	L2P5P20	S	26.26
71	15	EG557	S	26.41
72	20	EG750	S	26.41
73	108	L1P4	NARI-cross	26.41
74	66	EG1239	NRS	26.65
75	41	EG850	GB	26.66
76	98	L2P3	NARI-cross	26.72
77	4	EG494	GB	26.83
78	16	EG584	GB	26.83
79	46	EG870	GB	26.88
80	52	EG890	GB	27.14

Continued Table 4.2,

Rank	Entry	Accession name	Accession source	Striga germination percent (%)
81	63	EG1233	GB	27.16
82	19	EG855	GB	27.18
83	75	EG806	GB	27.24
84	96	Macia X IS2205	ICRISAT	27.51
85	27	EG782	GB	27.56
86	49	EG883	GB	27.91
87	77	EG532	S	28.09
88	35	EG815	GB	28.1
89	106	L2P2P24	NARI-cross	28.1
90	99	L1P2	NARI-cross	28.11
91	11	EG547	GB	28.2
92	80	EG735	GB	28.33
93	79	EG726	S	28.41
94	58	EG1157	NRS	28.64
95	7	EG537	S	28.88
96	48	EG875	GB	28.92
97	107	L3P1P4	NARI-cross	29.1
98	18	N13	ICRISAT	29.51
99	10	EG544	S	29.85
100	102	Hariray X IS2205	ICRISAT	29.95
101	74	EG538	S	31.38
102	21	EG756	AN	31.62
103	9	EG526	AN	31.67
104	6	EG519	GB	31.9
105	50	EG885	GB	31.93
106	45	EG859	S	32
107	22	EG711	NRS	32.22
108	42	EG857	GB	32.67
109	60	EG1172	NRS	32.96
110	23	EG723	AN	33.4
111	59	EG1168	NRS	40.6
	24	GR24(positive control)		43.73
	25	Water (negative control)		0
	Mean			24.45
	L.S.D			8.838
	CV(%)			26
	SIG			***

***=highly significant (P<0.001), L.S.D=least significant difference, CV=coefficient of variation, AN=Anseba, GB=Gash barka, NRS=Northern red sea, S=South

The top 9 genotypes induced less than 18% *Striga* germination (Figure 4.4), while the commercial checks, IS9830, SRN39 and Framida caused 22.46, 22.67 and 23.27% germination, respectively. There were no statistically significant differences among the commercial checks (IS9830, SRN39 and Framida). However, *Striga* germination in at least one of the tested landraces, namely, accession EG830 had significantly lower (Prob ≤ 0.05) germination than that of the commercial varieties. The five sorghum accessions with the lowest *Striga* germination were EG830, EG1076, EG473, EG 1261 and EG546 which caused *Striga* germination percentages of 11.85, 13.05, 14.68, 15.32 and 15.74, respectively. Even though these five accessions did not show total immunity against *Striga* seed germination, as there is no reported complete resistance to *Striga* so far in sorghum (Mohamed and Gamar 2011), their high level of resistance to *Striga* was indicated by their low percentage level of stimulant production. Low germination of *Striga* indicates low production of germination stimulant. The host plant's low amounts of germination stimulant may result in fewer *Striga* seeds germinating. Reduced germination may be owing to germination-inhibitory compounds released by sorghum cultivars, which may interfere with the germination response sequence of conditioned *Striga* seeds as reported by Mohamed et al. (2010).

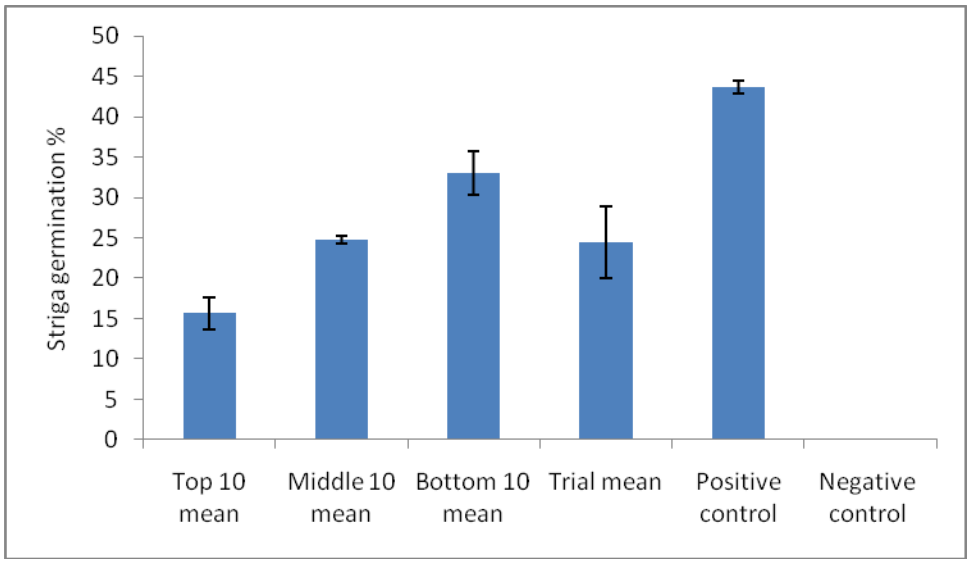


Figure 4. 4: Percent *Striga* seed germination category of sorghum accessions and their control

Figure 4.5 depicts the level of *Striga* germination as well as the distances from where stimulants were released. Germination percent was high near the stimulant source, implying that the higher the stimulant concentration, the higher the *Striga* germination percent. The germination percentage was drastically lowered to below 15% as the distance from the source of *Striga* stimulant increased. In the present study, the maximum germination was observed on discs that were closer to the stimulant source than those that were farther away. *Striga* germination and distances from the stimulant source were found to have highly significant ($P < 0.001$) and positive correlation coefficients. This is a clue indicating that the closer the *Striga* seeds are to the stimulant supply, the more seeds are stimulated to germinate, and vice versa. This result corroborates previous work on variation in *Striga* germination stimulants production in maize (Karaya et al., 2012). Reports by Hess et al. (1991) revealed that the host plant's germination stimulant is mostly released in a radius around the root surface. Support for this spatial relationship between host roots and *Striga* seed germination as a function of the distance from

the host root to where germination stimulant is active to elicit germination was documented (Fate et al., 1990).

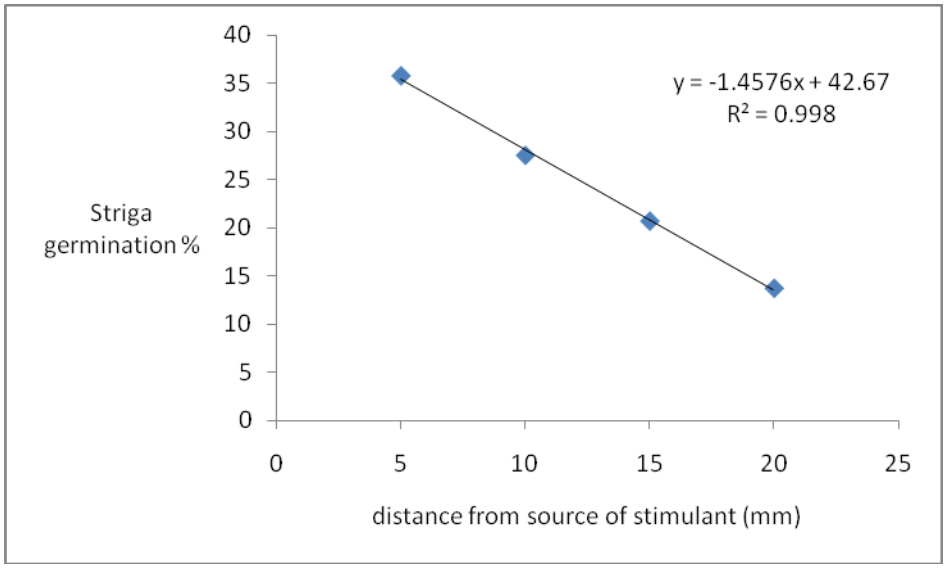


Figure 4. 5: **Correlation between *Striga* percent seed germination and the distance (mm) from the source of *Striga* germination stimulant**

The regression equation y = -1.4576x + 42.67 in Figure 4.5 implies that for every unit increase of distance from the stimulant, the germination percent of the *Striga* seed is expected to decrease by about 1.4576 percent. The negative slope of the fitted line in Figure 4.5 also suggests that decrease in *Striga* germination percent were associated with increased distance from the source of *Striga* germination stimulant. The high coefficient of determination (R^2=0.998) indicates the variation in germination percentage was almost all explained by the variation in the distance of concentration of *Striga* germination stimulants.

In sorghum, four compounds of root exudates which include sorgoleone, sorgolactone, strigol and a water-soluble compound with a quantitative biosynthetic pathway are reported as germination stimulants (Vogler et al., 1996).

Low *Striga* germination levels observed in some of the accessions tested in this study may be due to low production of germination stimulant, which is one of the best known mechanisms of resistance in *Striga* (Mohamed et al., 2003). This low germination stimulant production is of special interest in breeding for resistance to *Striga* in sorghum. Low seed germination induction has been effectively employed in sorghum breeding for *Striga hermonthica* resistance (Haussmann et al., 2000). Ejeta and coworkers selected sorghum lines with reduced induction of germination in their breeding programs (Mohamed et al., 2001). A wide range of sorghum of low stimulant lines has shown resistance in the field which indicates the usefulness of low stimulant form of resistance (Ramaiah et al., 1990). Identification of genotypes with low germination stimulant from the current study will play a crucial role in the improvement of sorghum cultivars for *Striga* resistance. Since the identified accessions are landraces which are adapted to the local environmental conditions of the country, they can be included directly in the sorghum breeding program for *Striga* resistance.

Conclusion

The accessions with low *Striga* germination stimulant producers identified in this study, namely EG830, EG1076, EG473, EG1261 and EG546 caused lower germination percent of *Striga* compared with the commercial controls. These accessions may be useful potential sources of resistance to *Striga* as such or in a backcross breeding program. It would be interesting to see if the genotypes with low stimulant production have the mechanical form of *Striga* resistance that has been mapped using Quantitative Trait Loci (QTL) and described elsewhere (Haussmann et al., 2004). In order to consolidate this resistance, these accessions of low stimulant production could be crossed with the already identified backcrosses with introgressed *Striga* resistance QTL

from a previous study (Yohannes et al., 2015). Such resistance to *Striga* in sorghum, resulting from a combination of two mechanisms, would be more durable and stable across ecological zones than one based on single gene resistance sources.

CHAPTER FIVE

DETERMINATION OF EXPRESSED TRANSCRIPTS BASED ON COMPARATIVE TRANSCRIPTOME SEQUENCE ANALYSIS OF KNOWN STRIGA RESISTANT AND SUSCEPTIBLE SORGHUM GENOTYPES.

Abstract

With the advancement of sequencing technology, it is possible to examine the transcriptomes and genomes of plants and animals effectively. The current study aimed to identify differentially expressed genes and elucidate their probable functions by comparing the transcriptomes of a sorghum cultivar N13 that was resistant to *Striga hermonthica* with a susceptible variety called Hugurtay. Using Illumina short reads, transcriptomes of two sorghum varieties were investigated for which limited data was previously available. Information on gene transcripts expressed in two growing phases of *Striga hermonthica* infection (attachment and in host development stages) was presented, and the transcript levels were compared between these growing phases. The results indicated that 15 genes are expressed as a direct response to stimulus and to execute signaling within cells. Further analysis revealed overrepresentation of SORBI_3004G065900, SORBI_3001G482800, SORBI_3001G482700, SORBI_3001G077400 and SORBI_3001G259700 as a response to *Striga* wounding. The study revealed that signaling genes and pathways play a crucial role in mediating defense against *Striga* in N13. Wound repair genes and mechanisms prevent *Striga* penetration making N13 resist infestation. These pathways were repressed or minimally expressed in Hurgutay possibly causing it to succumb to *Striga*.

Key words: genes, next generation sequencing, Sorghum*, Striga hermonthica*, transcriptome,

5.1 Introduction

Striga hermonthica affects many C4 and C3 cereal crops including maize, sorghum and rice (Runo and Kuria 2018). Security of food in Sub-Saharan Africa is gravely affected by *Striga* weeds, which are widespread, have broad host ranges, and are difficult to control (Mallu et al., 2021). *Striga* seeds can stay in the soil for years without losing viability and germinate following detection of strigolactone hormones produced by host plants in rhizosphere (Tsuchiya et al., 2015). Upon germination, the parasite develops a haustoria which penetrates host vascular cells establishing a feeding site (Spallek et al., 2013; Teka, 2014). Successful infection causes nutrient flow to the parasite resulting in reduced photosynthesis, stunted growth and reduced host biomass (Graves et al., 1989; Frost et al., 1997). Current *Striga hermonthica* management strategies include cultural practices such as legume intercropping to improve soil fertility, and chemical or mechanical removal from infested fields (Teka, 2014; Kountche, 2019). Resistant genotypes identified from wild accessions or landraces have been implemented to augment *Striga* management (Gebretsadik et al., 2014; Abate et al., 2014; Mbuvi et al., 2017).

Striga resistant genotypes exhibit various modes of *Striga* resistance which include low production of *Striga* germination stimulants or haustoria initiation factors, hypersensitive response and incompatibility to parasite invasion (Mohamed et al., 2003; Ejeta, 2005). Due to the added advantage of reducing *Striga* seed bank in the soil, initial breeding efforts for *Striga* control focused on genotypes with low germination stimulants or haustoria initiation factors. For instance, the resistant cultivar SRN-39 contains a single recessive mutation "Low Germination Stimulant 1 (LGS1) gene" that produce a weak *Striga* germination stimulant, 1-0-orobancol (Vogler et al., 1996; Gobena et al., 2017). For effective and durable resistance, it is important to combine genes of various modes of action against *Striga* (Mohemed et al., 2018). The resistant

sorghum variety N13, combines both *Striga* seed reduction and a mechanical barrier preventing haustoria penetration and offers a better alternative to *Striga* management (Mohamed et al., 2003; Mohemed et al., 2018). *Striga* resistance in N13 is due to at least five genomic QTLs that have been characterized and associated molecular markers developed. As a result, national breeding programs across Eastern Africa have utilized these markers to successfully introgress N13 *Striga* resistance QTLs to farmer preferred varieties (Mohamed et al., 2014; Ngugi et al., 2015; Yohannes et al., 2015). To date, it is currently unknown what genes and molecular pathways contribute to *Striga* resistance in N13.

RNA sequencing is offering a rapid method of characterizing genomic regions and pathways responsible for several traits in plants. The identification of transcriptional alterations within the genome is one of the most important methods for understanding the extent of infection in crops such as rice, maize, and wheat (Soós et al., 2010). Transcriptome studies on resistant and susceptible wheat genotypes were published by Xin et al. (2012). The authors analyzed transcriptome to evaluate changes in the molecular pathways as a result of response to exposure to powdery mildew. Transcriptome analysis in rice indicated the importance of jasmonic signaling and structural integrity of lignin as crucial factors for *Striga* resistance (Mutuku et al., 2015, 2019). In the present study, the transcriptome of N13 and a susceptible sorghum variety Hurgutay was sequenced and analyzed to identify differentially expressed genes and describe their putative function. The genes and molecular pathways identified will provide a strong basis for a better understanding of *Striga* resistance in sorghum.

5. 2. Methodology

5.2.1 Plant material

Striga susceptible sorghum variety Hugurtay and *Striga* resilient cultivar N13 were sourced from NARI-Eritrea and ICRISAT-Nairobi respectively. The *S. hermonthica* seeds used in this study were provided by the Kenya Agricultural and Livestock Research Organization. *Striga* seeds were collected in Kibos, Kenya as illustrated by Berner et al. (1996) procedure. Following Berner et al. (1997) procedure, seeds of sorghum varieties and *S. hermonthica* seeds were surface sterilized using 1% NaOCl (sodium hypochloride) solution. The *Striga* seeds were then conditioned for two weeks. The conditioned *Striga* seeds were triggered to germinate using GR24.

5.2.2 Experiment setup

Three sorghum seeds were germinated in each of the double-layered petri plates using filter paper that had a 9 cm diameter and had been moistened with sterile water. After 3 days of germination, the roots of each seedling were inoculated with germination-triggered *S. hermonthica* seeds. Sorghum seedlings without *Striga* inoculum were used as check. Every treatment was replicated three times. Plant materials were then collected for RNA extraction at two stages of *Striga* development, at attachment stage and compatibility stage, as shown in Table 5. 1.

Table 5. 1: Design and layout of the experiment

Replications	Striga development stage	Variety Names			
		Hugurtay(susceptible)		N13 (resistant)	
		With Striga	Without Striga	With Striga	Without Striga
Rep I	Attachment	Hugurtay + striga	Hugurtay	N13 + striga	N13
	Compatibility	Hugurtay + striga	Hugurtay	N13 + striga	N13
Rep II	Attachment	Hugurtay + striga	Hugurtay	N13 + striga	N13
	Compatibility	Hugurtay + striga	Hugurtay	N13 + striga	N13
Rep III	Attachment	Hugurtay + striga	Hugurtay	N13 + striga	N13
	Compatibility	Hugurtay + striga	Hugurtay	N13 + striga	N13

5.2.3 RNA extraction, quantification and quality check

Plant tissues from sorghum plants were taken for RNA isolation at two phases of *Striga* infection: attachment and in host development. The harvested plant tissues were kept at negative 80°C until used for RNA isolation. RNA was extracted using commercially available kit (Qiagen) as per manufacturer procedures. Briefly, frozen tissues were pulverized using a multibead shocker (Yasuikikai, Japan), then homogenized in the presence of guanidine isothiocyanate as a buffer. The RNA was eluted using RNase-free water after homogenate was placed to an RNeasy slip column and rinsed. Using a 2100 Bioanalyzer and RNase-free 1% agarose gel electrophoresis, the integrity of the RNA was confirmed. A RNA

Nanodrop®spectrophotometer (Thermo Fisher Scientific, Waltham, MA) was used to measure the RNA concentration once more.

5.2.4 cDNA library preparation and sequencing

RNA sampled were administered for sequencing utilizing the Truseq RNA Libraries formulation kit V2 and sequenced utilizing the Miseq 2 sequencer (Illumina, San Diego, USA). Briefly, messenger RNA was selected from each sample using PolyA RNA purification beads and fragmented. Random hexamers and a Superscript II reverse transcriptase (Invitrogen, USA) were used to make first strand cDNA. This was immediately followed up by second strand synthesis (with a DNA polymerase), 3' overhang filling and adapter ligation. Libraries were then enriched by PCR, purified and analyzed to confirm presence of approximately 250bp fragments using a Bioanalyzer (Agilent Technologies, California USA). After normalization, library were pooled and sequenced making use of 101bp paired end sequencing.

5.2.5 Data analysis

The Trimmomatic tool was used to preprocess generated sequence reads, removing low-quality reads and trimming adapters and low-quality bases (Bolger et al., 2014). HISAT2 was used to map each library's readings to the sorghum reference genome (Kim et al., 2015). Samtools was used to convert alignment files to BAM format (Li et al., 2009). Using the sorghum genome annotation file, transcript counts were performed by the function *featureCounts* of Bioconductor package *Rsubread*.

Analysis of differential expression

Differential expression between the samples (Mock vs Inoculated, and Resistant vs Susceptible) was performed using Deseq2 after normalization using variance stabilizing transformation (Love et al., 2014). The following criteria were used to identify putative differentially expressed genes (DEGs) in each comparison category: (i) fold change between samples more than two fold (absolute value of log2foldchange $\geq$ 1) and (ii) a false discovery rate adjustment with a significance level of 0.05. Finally, in the protein database, DEGs were annotated with Gene Ontology (GO) concepts www.pantherdb.org and statistical overrepresentation and enrichment tested with Fischer's Exact test (Mi et al., 2021).

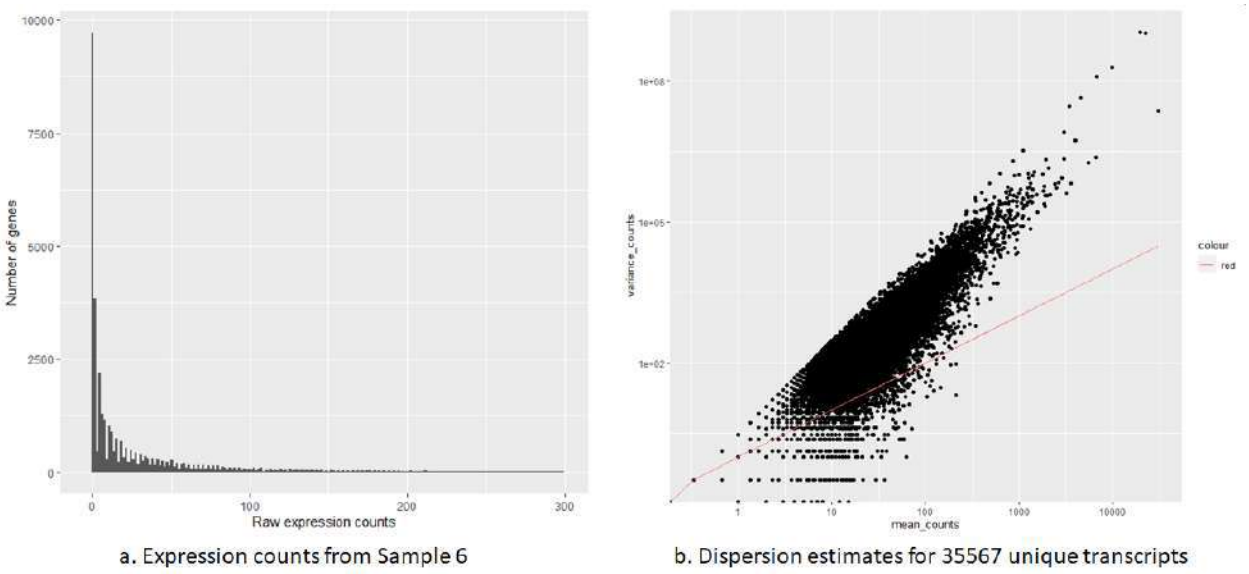
5.3. Results

A total of 40.3 million reads were obtained from 24 libraries representing an average of 1.6 million reads per library (Table 5.2).

Table 5. 2*:* Total reads for each library and alignment rate on the sorghum reference genome.

Read_File	Sample	Total reads	Overall alignment rate
10_S6_L001_R1_001.fastq.gz	10	1267925	64.29%
11_S7_L001_R1_001.fastq.gz	11	1052853	51.84%
12_S8_L001_R1_001.fastq.gz	12	2113957	59.75%
13_S5_L001_R1_001.fastq.gz	13	1158131	42.76%
14_S6_L001_R1_001.fastq.gz	14	2181830	55.80%
15_S7_L001_R1_001.fastq.gz	15	1714746	69.80%
16_S8_L001_R1_001.fastq.gz	16	1353570	65.86%
17_S9_L001_R1_001.fastq.gz	17	1528644	50.67%
18_S10_L001_R1_001.fastq.gz	18	1599895	60.54%
19_S11_L001_R1_001.fastq.gz	19	1900632	55.82%
1_S1_L001_R1_001.fastq.gz	1	884317	61.92%
20_S12_L001_R1_001.fastq.gz	20	1968230	53.55%
21_S9_L001_R1_001.fastq.gz	21	1670597	63.20%
22_S10_L001_R1_001.fastq.gz	22	2314929	68.52%
23_S11_L001_R1_001.fastq.gz	23	2754129	61.14%
24_S12_L001_R1_001.fastq.gz	24	1986660	65.05%
2_S2_L001_R1_001.fastq.gz	2	668883	70.37%
3_S3_L001_R1_001.fastq.gz	3	1734670	64.16%
4_S4_L001_R1_001.fastq.gz	4	1618849	55.96%
5_S1_L001_R1_001.fastq.gz	5	1639489	61.71%
6_S2_L001_R1_001.fastq.gz	6	1840473	66.51%
7_S3_L001_R1_001.fastq.gz	7	1761029	68.53%
8_S4_L001_R1_001.fastq.gz	8	2384624	69.75%
9_S5_L001_R1_001.fastq.gz	9	1278915	51.25%
Total		**40377977**	**14.5875**
Mean		**1682415.70**	**0.6078125**

With a 60% alignment rate, all generated sequences were successfully matched to the reference genome of sorghum. After mapping the reads to the sorghum reference transcriptome, a total of 35,567 transcripts uniquely mapped to single genes more than once. Quality control during differential expressed genes analyses indicated normal dispersion measures and expected distribution of transcripts per sample as shown in Figure 5.1.



a. Expression counts from Sample 6

b. Dispersion estimates for 35567 unique transcripts

Figure 5. 1: Expression count distribution across sample 6 (Hugurtay control at stage II) in (a) and dispersion estimates for transcripts mapping severally to unique single genes in (b).

In addition, correlation values, among the replicates were consistent with each unique sample indicating minimal technical and experimental errors (Figure 5.2).

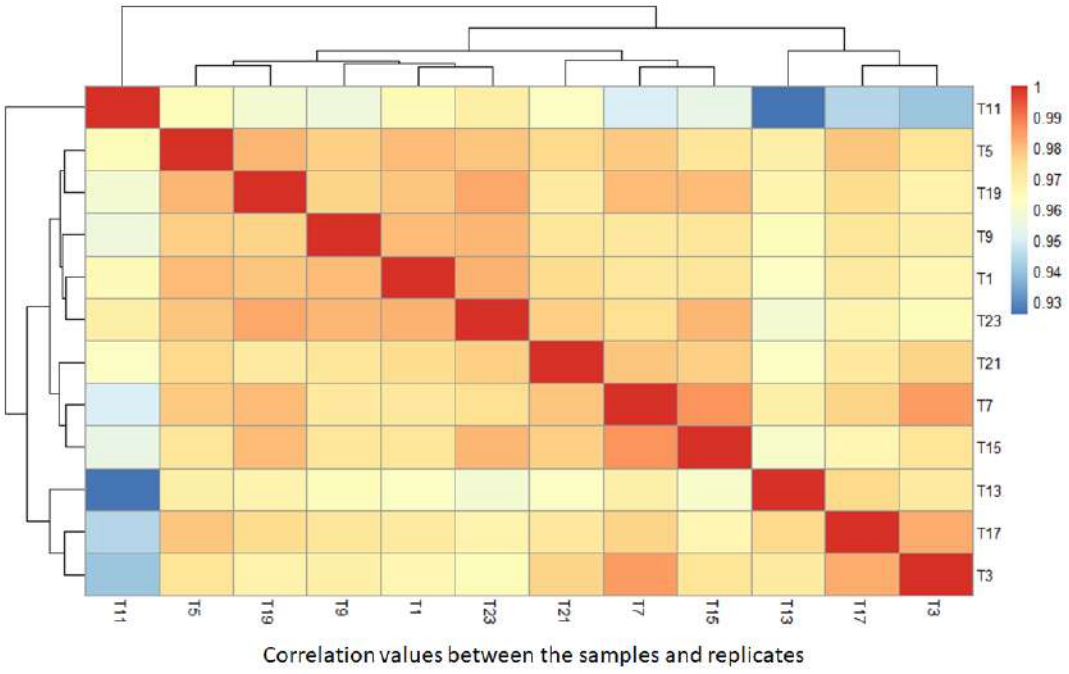


Figure 5. 2: Heatmap of Pearson correlation values among replicates of both N13 and Hugurtay samples infected with *Striga hermonthica*.

Differential analysis of 35,567 transcripts contrasting the resistant and susceptible genotypes revealed 163 significant differentially expressed genes. Of these, 155 transcripts were upregulated while 8 were down regulated as shown in Figure 5.3.

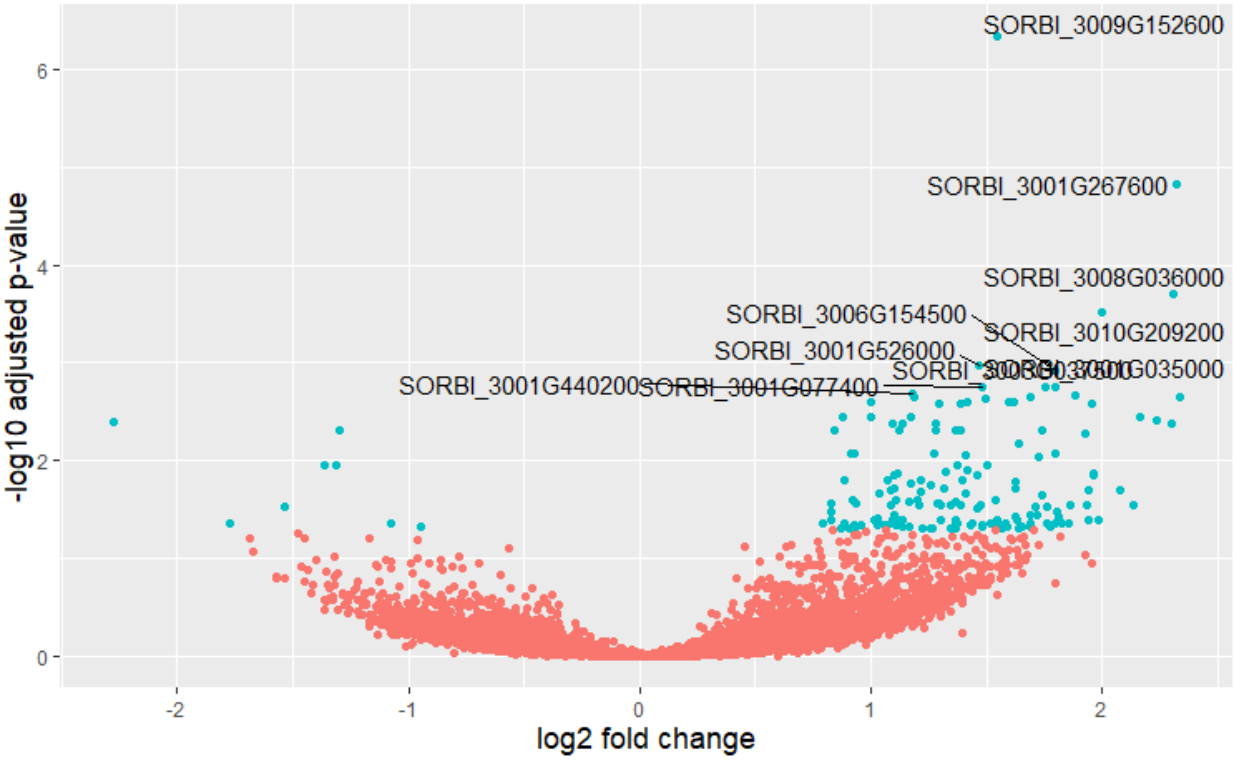


Figure 5. 3: Significantly expressed genes between resistant (N13) and susceptible (Hugurtay) samples infected with *Striga*.

Gene expression between the inoculated and non-inoculated susceptible samples showed 65 upregulated genes while only 2 were downregulated (Figure 5.4). When compared between the two infection stages, no significantly expressed genes could be identified in the susceptible cultivar.

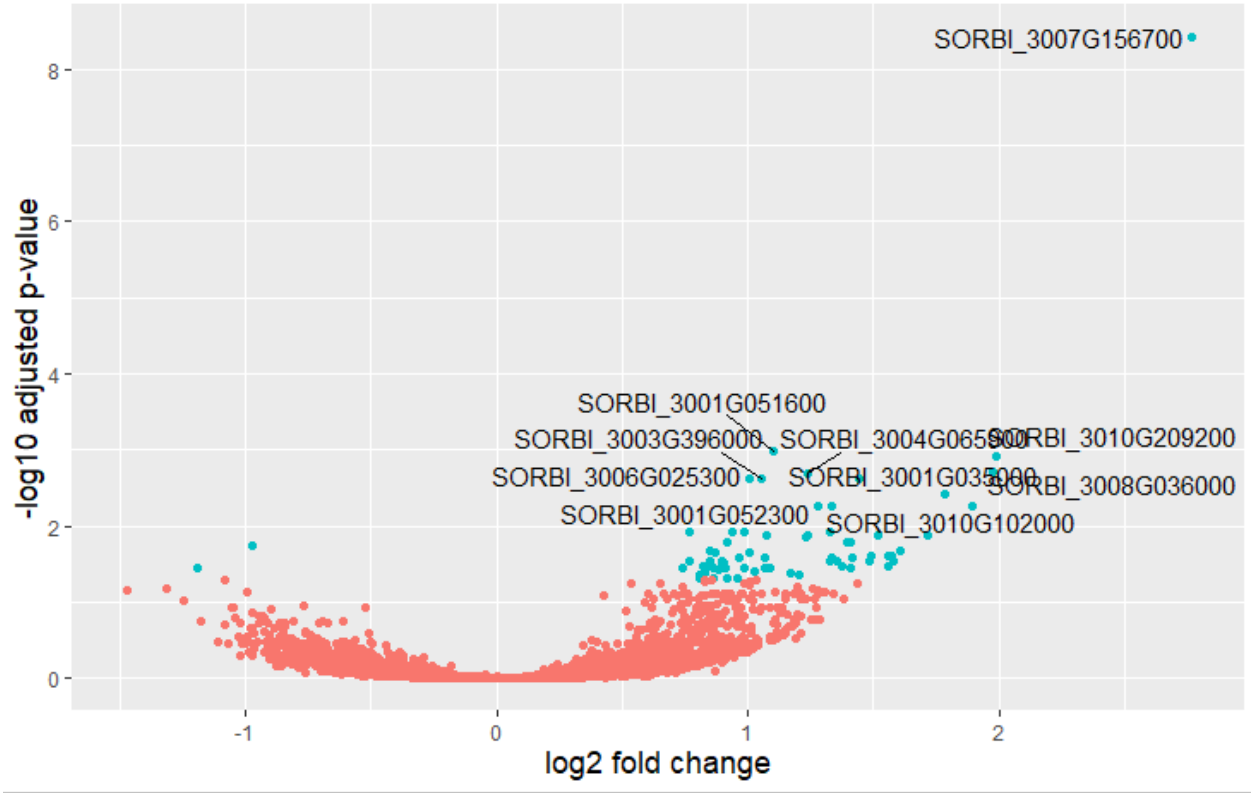


Figure 5. 4: Significant DEGs between inoculated and mock-inoculated susceptible samples

On the other hand, a total of 89 significant DEGs were expressed between mock and inoculated resistant cultivar samples. Most of these DEGs were downregulated approximately two thirds of these were downregulated (59) whereas the rest were upregulated (Figure 5.5).

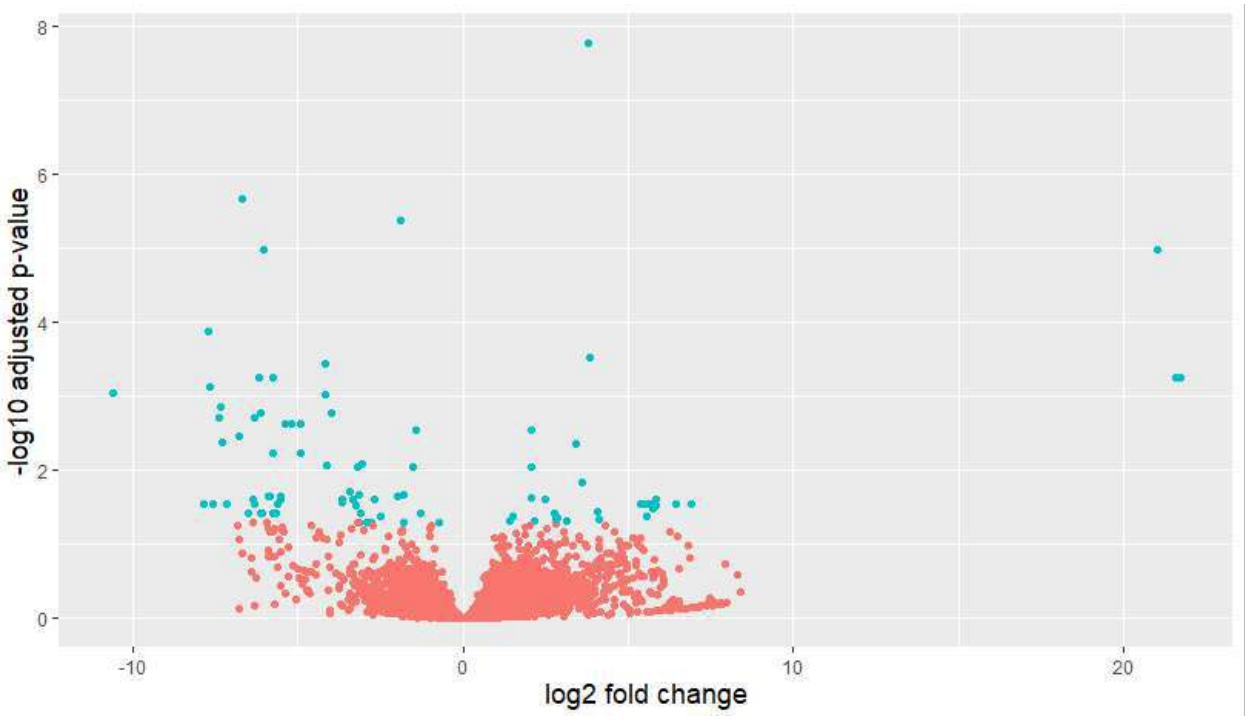


Figure 5. 5: **Significant DEGs between innoculated and mock-innoculated resistant samples**

Deferentially expressed genes (DEGs) were also compared according to the stage of *Striga* infection in the two cultivars. Only the resistant cultivar had significant DEGs as shown below in Figure 5.6.

Figure 5. 6: Significant DEGs between *Striga* infection stage I and stage II in the innoculated resistant samples N13

According to the findings, there were significant DEGs expressed between the two cultivars. The most significant of these DEGs showed a clear differentiation of the samples using normalized counts correctly indicating the resistant and susceptible sample libraries (Figure 5.7).

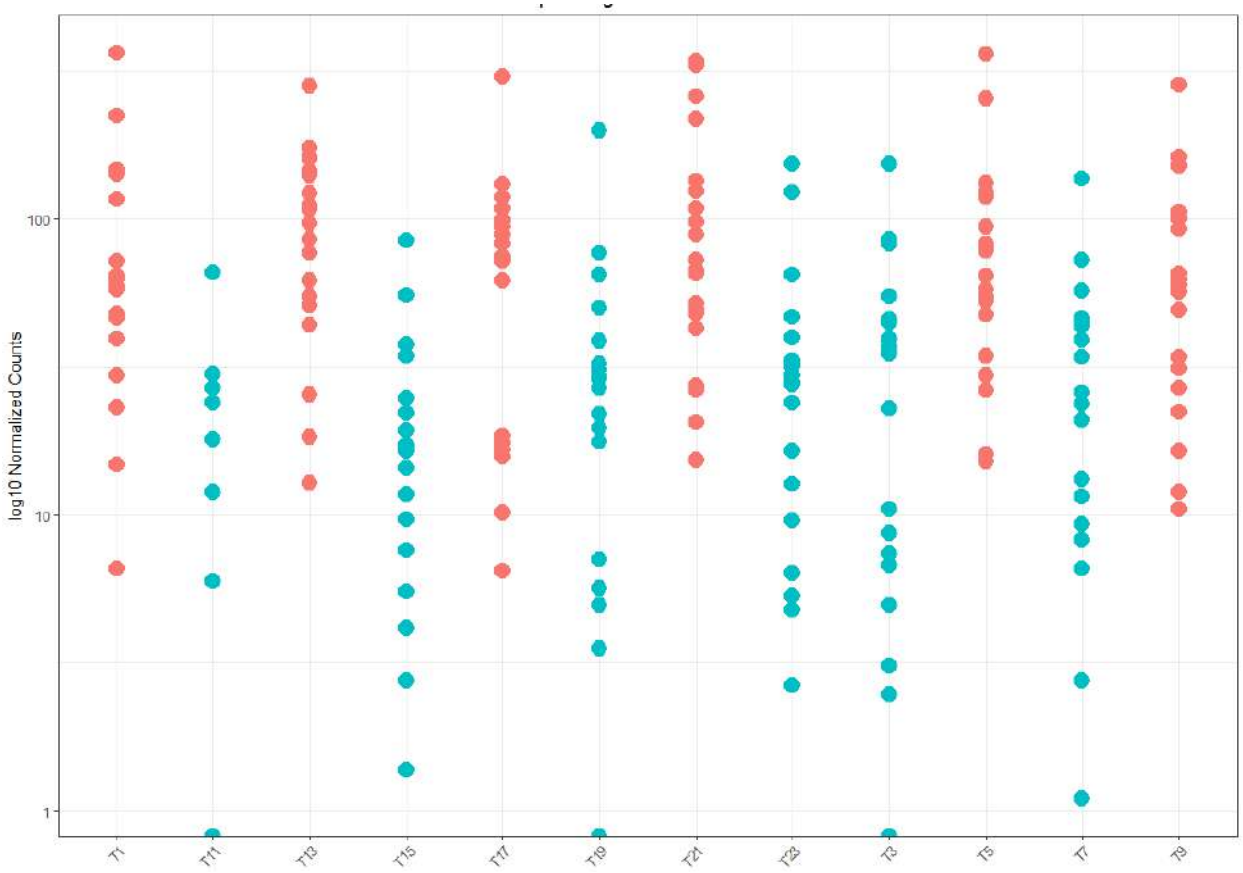


Figure 5. 7: Differentiation of resistant (blue) and susceptible (red) samples using normalized counts of 20 most significant DEGs.

Overrepresentation enrichment tests within the protein database (www.pantherdb.org) indicated several DEGs with Gene Ontology (GO) terms involved in pathogen or stress response, signaling, response to stimulus, regulation of nucleic acids, starch metabolism and cell wall synthesis. The following figure (Figure 5.8) shows overrepresentation results for significant DEGs displayed as GO terms.

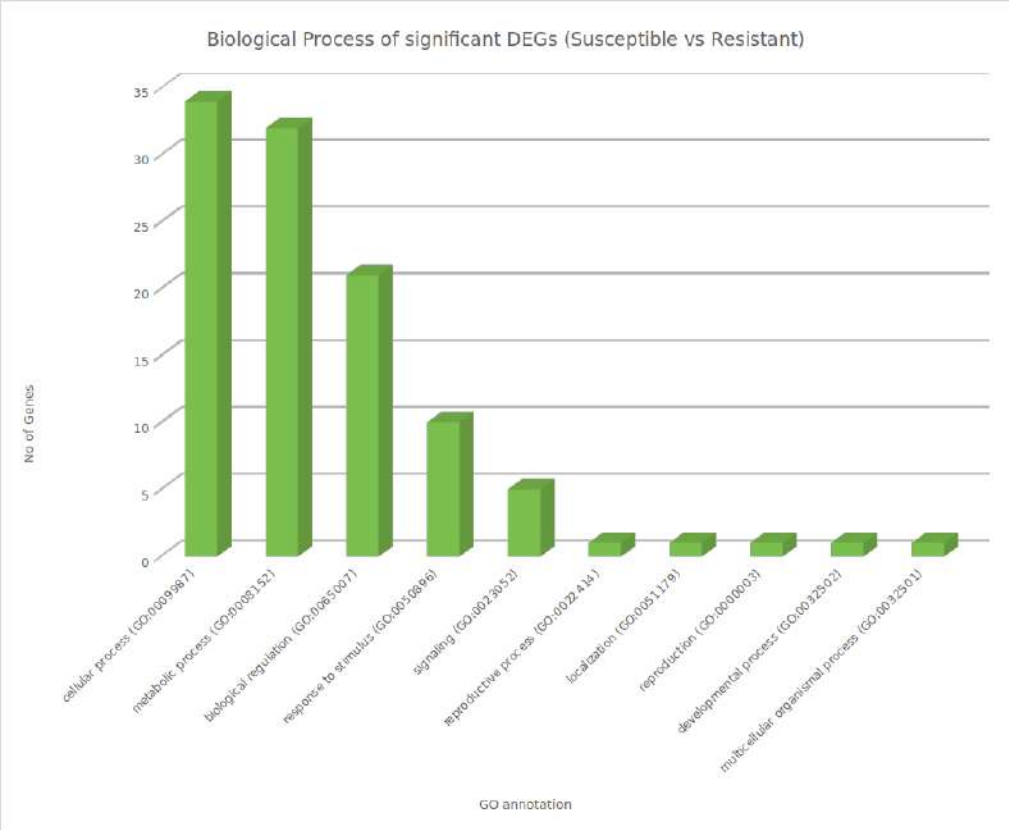


Figure 5. 8: Overrepresented biological process GO terms between susceptible and resistant samples

5.4 Discussion

Resistance to *Striga* is an important trait for sorghum cultivated in East Africa. Despite utilization of N13 to introgress this resistance into several farmer preferred varieties across East Africa, genes responsible for this resistance are to date unknown. Fifteen genes were expressed as a direct response to stimulus and to execute signaling within cells (Figure 5. 8). Several significant DEGs with putative functions in inhibiting *Striga* infection or mitigate plant damage were expressed. Significantly expressed genes between resistant N13 and susceptible Hurgutay samples are thought to be mainly involved in binding catalytic or transport activities and regulation of several molecular functions. The majority of these genes are thought to play a role in a variety of cellular and metabolic processes. There was overrepresentation of SORBI_3004G065900, SORBI_3001G482800, SORBI_3001G482700, SORBI_3001G077400 and SORBI_3001G259700 as a response to *Striga* wounding (Appendix 4). Candidate gene transcript SORBI_3004G065900 has a WRKY domain transcription factor expressed in the nucleus to regulate defense response to microbial and other pathogens while improving plant biological and non-biological tolerance for stress (Chen et al., 2017). SORBI_3001G482800, SORBI_3001G482700 and SORBI_3001G259700 are TIFY domain proteins also active in the cell nucleus, expressed mainly as a defense response to wounding and to regulate signal transduction within the hormone-mediated signaling pathways (Zhang et al., 2015). SORBI_3001G077400 is a characterized protein belonging to the ALLEN OXIDE SYNTHASE family active in chloroplast and mitochondria. ALLEN OXIDE SYNTHASES are oxidoreductases important in preventing cell death from oxidative bursts.

Overrepresentation analysis revealed 22 transcription factors that regulate DNA transcription activities such as RNA biosynthesis and metabolism, cellular macromolecule biosynthesis and nucleic acid transcription. These transcription factors included six calmodulin binding proteins, five BHLH domain proteins, four AP2/ERF domain proteins, three WRKY domain proteins, two growth regulator proteins, one NAC domain protein and one heat stress associated protein. AP2/ERF domain proteins are major plant transcription factors that activate expression of abiotic stress response genes by specifically binding to promoter regions of the dehydration response elements. AP2/ERF transcription factors also activate jasmonate and ethylene signaling pathways to induce pathogen defense genes (Pré et al., 2008). Similarly, WRKY transcription factors are also crucial in signaling pathway regulation as well as defense against biotic and abiotic stress. This indicates that N13 relies primarily on the signaling pathways to combat *Striga* infestation. Importance of WRKY-dependent signaling pathway in defense against *S. hermothica* parasitism has been reported before by (Mutuku et al., 2015). The results suggest that the AP2/ERF-dependent signaling pathway is equally vital for defense *S. hermothica* parasitism. The finding corroborates the works of Adewale et al. (2020) and Badu-Apraku et al. (2020) who found that AP2/ERFs are considerably linked to *S. hermonthica* resistance in Maize. Interestingly, the susceptible variety (hugurtay) also activates AP2/ERF signaling pathway through the highly expressed transcript SORBI-3009G145200 (Appendix 4). However, only one AP2/ERF transcript is overexpressed in Hurgutay compared to four overexpressed in N13. This is a significant variation which may explain Hurgutay's susceptibility to *Striga* parasitism. Several other genes to prevent *Striga* wounding are overexpressed in N13 but not in Hurgutay.

Significant DEGs during stage 1 and stage 2 *Striga* infestation were only expressed in N13. Overrepresentation analysis exhibited overexpression of DEGs involved in starch metabolism

and cell wall biosynthesis. SORBI-3001G293800 is a beta-amylase ortholog overexpressed in stage 1. Overexpression of beta-amylase which converts starch to maltose, an important molecule accumulated to protect proteins and membranes during abiotic stress. Two overexpressed transcripts SORBI-3001G410100 and SORBI-3005G105000 are protein kinases involved in pathogen defense through the mitogen activated protein (MAP) kinase pathway.

Conclusion;

Signaling genes and pathways play a primary role in mediating defense against *Striga* in N13. Wound repair genes and mechanisms prevent *Striga* penetration making N13 resist infestation. These pathways were repressed or minimally expressed in Hurgutay possibly causing it to succumb to *Striga*. The functional characterization of the genes and molecular pathways identified in this study will offer a strong bases for a clearer understanding of *Striga* resistance in sorghum. There is a need for further studies to functionally validate significant DEGs reported here that enhance *Striga* resistance.

CHAPTER SIX

SCREENING FOR STRIGA RESISTANCE IN SORGHUM LANDRACES FROM ERITREA BY GENOTYPING AND PHENOTYPING

Abstract

Striga resistance in cereals has been bred with much effort, and remarkable progress has been made. However, the influence of *Striga* is still significant which requires an effort to find out more resilient and durable resistant cultivar. Research on sorghum resistance to *Striga* have primarily concentrated on emerging improved crop varieties, with only a limited studies on indigenous non-improved varieties or landraces being conducted. The aim of the present study was to find out *Striga* resistant genotypes from sorghum landrace accessions from Eritrea using already mapped *Striga* resistance simple sequence repeat (SSR) markers. Ninety-two sorghum cultivars from Eritrea and one resistant check from ICRISAT were genotyped. Accessions that showed one or more *Striga* resistance QTLs in the laboratory genotyping were evaluated under artificially *Striga* infested pot experiment. Laboratory and pot experiment results indicated that accessions EG1075, EG1168, and EG1239 have shown better resistance to *Striga*. These germplasms are important genetic materials for sorghum *Striga* management.

Keywords: Sorghum; *Striga hermonthica*; landrace; SSR; genotype

6.1 Introduction

Sorghum is a popular crop among smallholder farmers in east Africa including Eritrea (AATF, 2011). In 2019, more than 29 million hectare of land was under sorghum production in Africa, accounting for 71% of the 40 million hectares planted globally in that same year (FAOSTAT, 2019). However, Africa's overall sorghum harvest in 2019 was at 28.4 million tons, accounting for 49 percent of the global total of 57.9 million tons.

Reduction in productivity in Africa are mostly caused by biotic and abiotic limitations. Water deficit and decreased soil fertility (Palé et al., 2009) are key abiotic limitations, while sorghum diseases, stem borers, and *Striga* infestation are important biotic constraints (Sleper and Poehlman, 2006). *Striga* species are the most significant biological limitations to crop output in Sub-Saharan Africa, posing a greater threat than other biotic pressures (Jemil, 2012). They are one of the highly sophisticated parasitic plants, imposing substantial damage to the hosts just after days of connection to their hosts by withdrawing moisture and nutrients, inhibiting photosynthesis, and generating a phytotoxic impact (Gurney et al., 2006).

Grassland has the largest biodiversity of *Striga*. *Striga hermonthica* however, is mostly found in farms, where it infects crops. The parasite has already wreaked damage before it even emerges to the soil surface (Ejeta and Gressel, 2007). *Striga* can lead to production reductions in crops anywhere from 15% under ideal conditions to 100% when other stress components are present, inflicting harm to millions of subsistence farmers (Ejeta, 2007).

Screening genotypes in *Striga* infested fields is often less efficient due to the complexity of the nature of the parasite, environment and the host/parasite interactions (Ejeta et al., 1992). Besides, field screening is less accurate, slow and time consuming to identify *Striga* resistant genotype. Molecular markers are effective tools in crop improvement projects to make screening more

successful (Lammerts Van Bueren et al., 2010). This entails selecting cultivars with preferred gene(s) using associated marker selection. The increased level of polymorphisms seen in simple sequence repeat markers, as well as the apparent simplicity with which these polymorphisms may be detected via PCR analysis, has contributed to simple sequence repeat markers being widely used (Karaoglu et al., 2005). SSR markers have been described and used in breeding programs to flag genes that confer essential features for resistance to *Striga* in sorghum.

Five chromosomal areas associated with resistance to *Striga*, as well as their flanking SSR markers, were discovered and made public. SSR markers have been utilized in numerous East African countries in their sorghum improvement programes. Studies on sorghum resistance to *Striga* have primarily concentrated on emerging improved crop varieties, with only a limited studies on indigenous non-improved varieties or landraces being conducted.

In order to develop a sustainable *Striga* control options and reduce its effect on the crop there is a need to identify and evaluate sorghum landraces against the parasitic threat. The current study was aimed at identifying *Striga* resistant varieties from 92 sorghum accessions from Eritrea using already mapped *Striga* resistance SSR markers. Besides, accessions that showed one or more *Striga* resistance QTLs in the laboratory genotyping were evaluated under artificially *Striga* infested pot experiment.

6.2 Methodology

6.2.1 Plant material and genomic DNA extraction

In this experiment of genotyping, 92 sorghum genotypes from Eritrea and one resistant check from ICRISAT (N13) were used (Appendix 6). A total of 93 sorghum cultivars were sown in pots for DNA extraction in a screen house. Leaf samples were then harvested from two weeks

old individual plants per accession for DNA extraction. By omitting the phenol extraction phase, genomic DNA was isolated using a protocol published by Mace et al. (2003). The quantity and quality of the DNA were measured using a Nanodrop®spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and visualized using electrophoresis on 0.8 percent agarose gels stained with GelRed (Biotium, USA).

6.2.2. PCR amplification and analysis

Eleven SSR markers (Table 6. 1) associated to *Striga* resistance QTLs were employed for this experiment as stated by (Haussmann et al., 2004). Amplification of PCR was done in an over-all of 10 µl volume, which comprised of AccuPower® PCR PreMix, 0.8 µl of each reverse and forward primer, (the forward primers were tagged with FAM, VIC, PET, or NED), 2.5 µl of diluted DNA (20 ng/l), and sterile distilled water. Amplifications of the polymerization chain reaction were performed in 96-well plates using a PCR machine with the following PCR cycle parameter settings: primary denaturation at 94°C for 3 min, then 35 cycles of denaturation at 94°C for 30 seconds, annealing temperature at 55°C for 1 min, and extension at 72°C for 2 min. Final temperature for extension at 72°C for 7 minutes.

Table 6. 1: Markers and their primer sequence used for screening sorghum genotypes.

Marker name	LG	Forward Primer	Reverse Primer	Ann.T. °C	Expected Size (bp)
Xtxp208	1	AAGGCCGTGAGGATG	AAGCAGCCAAGAGCAG	55	257
Xtxp302	1	TAGGTTCTGGACCACTTTTCTTTTTGTGTT	GAATCAACTATGTGCTTGCATTGTGCT	55	180
Xtxp050	2	TGATGTTGTTACCCTTCTGG	AGCCTATGTATGTGTTCGTCC	55	299
Xtxp201	2	GCGTTTATGGAAGCAAAAT	CTCATAAGGCAGGACCAAC	55	222
Xtxp304	2	ACATAAAAGCCCCTCTTC	CTTTCACACCCTTTATTCA	55	206
Xtxp057	6	GGAACTTTTGACGGGTAGTGC	CGATCGTGATGTCCCAATC	55	251
Xtxp145	6	GTTCCTCCTGCCATTACT	CTTCCGCACATCCAC	55	238
Xtxp303	5	AATGAGGAAAATATGAAACAAGTACCAA	AATAACAAGCGCAACTATATGAACAATAAA	55	160
Xtxp065	5	CACGTCGTCACCAACCAA	GTTAAACGAAAGGGAAATGGC	55	128
Xtxp225*	5	TTGTTGCATGTTGGTTATAG	CAAACAAGTTCAGAAGCTC	55	165
Xtxp015*	5	CACAAACACTAGTGCCTTATC	CATAGACACCTAGGCCATC	55	215

LG= Linkage group, Ann.T= Annealing temperature in ◦C, bp= base pair

After PCR amplification, 24 sets of Amplicons from every SSR marker were chosen at random and analyzed on agarose gel and observed under UV light to ensure correct amplification. A 100-bp ladder (Invitrogen, Carlsbad, CA) was used to assess the sizes of the PCR amplicons.

Capillary electrophoresis was used to analyze fragments by means of an ABI PRISM 3730 sequencer. By combining 1.5–2.5 ml of each labeled PCR product with formamide and 0.16 ml Genescan Liz 500 molecular weight standard (Applied Biosystems), three to four markers were

evaluated at the same time so as to minimize unit cost. With the help of GeneMapper version 4.1 software, the alleles were scored and the peaks were scaled for data analysis.

6.2.3 Evaluation of the selected sorghum accessions in artificially *Striga* infested pots

Plastic pots were utilized for pot experiment that was conducted in a screen house. The experimental design was RCBD with three replications. The test entries consisted of eight sorghum accessions that showed *Striga* resistance QTLs in the genotyping results. A *Striga* susceptible and *Striga* resistant sorghum variety were included as control.

The *Striga* seeds were tested for their viability before use following Berner et al. (1997) procedure. Inoculation of approximately 3000 seeds of *Striga* was done by mixing with the top 5-centimeter of soil to each pot before sowing. Four sorghum seeds were sown for every pot and one week after germination, they were thinned to leave a single plant per pot. Apart from *Striga*, other weeds emerged were immediately removed by hand weeding.

Phenotypic data collection and analysis

Variables recorded for sorghum crop in each pot were: seedling vigor, date to flowering, plant height, length and width of panicle, stover dry weight, grain weight and 100 seed mass. For *Striga* component: days to *Striga* emergence, *Striga* counts once every two weeks from the 6th week to the 12th week after sowing. Vigor of *Striga* was graded on a scale of 0-9, where 0 is resistant and 9 is susceptible (Hausmann et al., 2000).

Records on agronomic performance and *Striga* parameters were compiled in Excel and analyzed using Genstat®17th edition's analysis of variance. At a 5% probability level, Fisher's Least Significant Difference technique was used to distinguish treatment means

6.3 Results

Most of DNA extracted was good quality and the quantity was adequate for the up to 20 PCRs planned per sample, which ranged between 22 ng/µl and 554.8 ng/µl in a 100 µl volume (Appendix 2). Images of agarose (0.8% w/v) gel stained with GelRed of the extracted DNA showed clear bands of good molecular weight DNA with no smears for most samples indicating the DNA was of good quality (Figure 6.1).

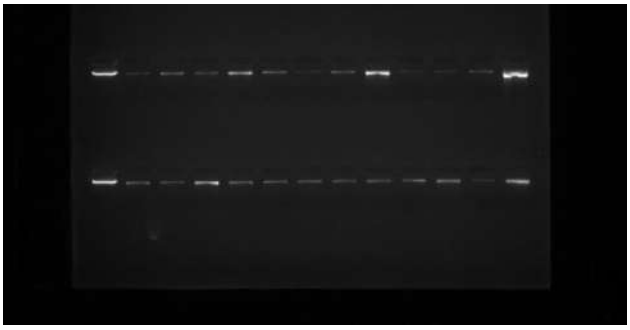


Figure 6. 1: Agarose (0.8% w/v) gel image showing the quality of the extracted DNA

PCR products of most markers showed good amplification in agarose electrophoresis as shown in Figure 6.2.

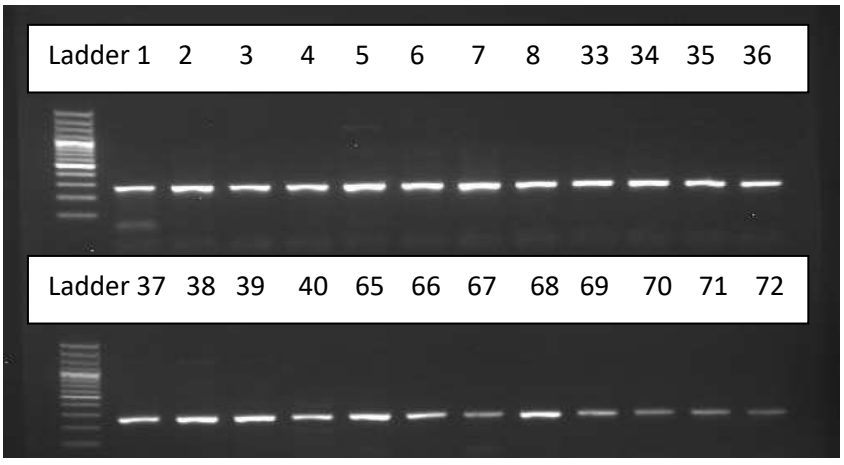


Figure 6. 2: Agarose gel image for PCR products of randomly selected samples using marker . Xtxp208

A total of 92 samples of landrace sorghum accessions and one *Striga* resistant check were genotyped to confirm the presence of *Striga* resistance QTLs using polymorphic SSR markers. From these accessions, 8 accessions have shown from one up to three *Striga* resistance QTL as shown in Table 6. 2 but 84 samples did not show any QTL.

Table 6. 2: Summary of the various combinations of QTLs in land-race sorghum accessions from Eritrea.

Accession name	QTL type	Linkage group	Collection zone
EG 1075	B	SBI02	Northern Red Sea
EG 1168	A, B, I	SBI01, SBI02, SBI06	Northern Red Sea
EG 1172	J	SBI05	Northern Red Sea
EG 1235	I	SBI06	Gash Barka
EG 1237	B	SBI02	South
EG 1239	B, I	SBI02, SBI06	Northern Red Sea
EG 2453	I	SBI06	Northern Red Sea
EG 544	B	SBI02	South

These eight accessions with *Striga* resistance QTLs from the laboratory result were further evaluated in artificially *Striga* infested pots. Analysis of variance when 10 sorghum germplasms examined under *Striga hermonthica* infestation is showed in Table 6.3.

Table 6. 3: Mean square and significance test of sorghum and *Striga hermonthica* parameters

Source of Variation	DF	DFL	PW	PL	PH	GWP	HSM	DSE	SN	SV
Replication	2	4.93	192.3	11.558	1978.6	93.07	0.2653	0.9	2.1	1.7333
Entry	9	105.34**	352.5*	28.342*	2105.8**	127.2*	0.5154	235.65**	7.574**	3.7**
Residual	18	11.53	112.5	7.836	196.9	46.29	0.2772	41.83	1.063	0.7611
Total	29									

Notes: DF = degrees of freedom; DFL = days to flowering; PW = panicle weight; PL= panicle length; PH=plant height; GYP = grain yield per plant; HSW = hundred seed weight; DSE= days to first striga emergence; SV = Striga vigor; NS = number of Striga plants and *Significant difference at 5% probability level. ** Strongly difference at 5% probability level.

The mean agronomic performance and *Striga* parameters of the sorghum accessions studied are shown in Table 6.4.

Table 6. 4: Mean agronomic characters and *Striga* parameters among 10 sorghum genotypes

Entry	DFL	GWP	HSM	PW	PH	PL	DSE	NS	SV
EG1075	66	23.4	2.767	29.6	211.7	15.17	71.7	3.33	3.67
EG1168	63	34.3	2.7	43.2	164	14.67	68.7	3.33	4.33
EG1172	66.33	14.1	2.333	14.1	208.7	16.83	67.7	7	3.67
EG1235	58	23.8	3.3	30.6	167.7	12.83	65.3	5.67	5
EG1237	72.67	16.3	2.967	20.9	171.3	16.5	60.7	4.33	4.67
EG1239	64.33	20.9	2.3	11.2	222.7	14.67	58	3.33	4.33
EG2453	53.67	16.2	2.867	22	204.3	10	75	4.33	4
EG544	69.33	17.5	2.6	8.7	248.7	14.5	67.3	4.33	4.17
N13	63	17.1	2.767	18.6	201.7	7.33	81	2.33	3.5
Hugurtay	56	15.2	3.667	33.1	206.7	10.67	49.7	7	7.33
mean	63.23	19.9	2.827	23.2	200.7	13.32	66.5	4.5	4.47
F pro	<.001	0.032	0.126	0.019	<.001	0.01	<.001	<.001	0.002
s.e.d	2.772	5.56	0.4299	8.66	11.46	2.286	5.28	0.842	0.712

Note: DFL= days to flowering; GWP =grain weight per plant; HSM = hundred seed mass; PW = panicle weight; PH=plant height; PL= panicle length; DSE= days to first striga emergence; SV = Striga vigor; NS = number of Striga plants.

6.4 Discussion

In various crop improvement studies, SSR markers have been used in marker assisted selection to detect a trait of interest. Marker-assisted selection is the technique of choosing agriculturally relevant features in breeding programs utilizing markers as indirect screening process. To identify *Striga* resistance QTLs from 92 sorghum land races, polymorphic flanking SSR markers were utilized for the five targeted loci. This process helped to improve the effectiveness of selection for the traits of interest that is *Striga* resistance.

6.4.1 SSR genotyping

The quality and quantity of DNA recovered from the sorghum leaf samples were sufficient for genotyping (Appendix 2). SSR analysis only necessitates a little amount of DNA (Semagn et al., 2006). The emphasis of this SSR genotyping study was to find accessions that have two same alleles for the marker alleles of the *Striga* resistant control variety N13, which flanked the targeted loci.

The genotyping results for the 92 accessions genotyped revealed the presence of resistance alleles in six accessions, each with one QTL, one accession with two QTLs, one accession with three QTLs, and 84 accessions with no homozygous resistance alleles. Detailed QTL type, number, and linkage group is shown in Table 6.2. These genotyping results allowed the selection of 8 accessions with *Striga* resistance QTLs for further evaluation in artificially *Striga* infested pot experiment.

6.4.2 Pot experiment

The results of SSR genotyping were tested in this work by evaluating chosen sorghum accessions in a *Striga*-infested pot experiment. To identify accessions that possess better resistance to *Striga* infestation and better agronomic trait, count of *Striga* germinated, vigorousity of *Striga*, days count to occurrence of *Striga*, and the grain output of the crop under infection circumstances have been used as the most crucial criteria considered among several other agronomic traits of the crop.

Table 6.3 illustrates the analysis of variance of 10 sorghum germplasms that were examined under *Striga hermonthica* infection. Sorghum accessions differed significantly in yield, yield components and *Striga hermonthica* parameters. In most of the accessions that exhibited *Striga* resistance in the genotyping data, the emergence of *Striga hermonthica* was delayed by roughly 15 days as contrasted to the appearance in the vulnerable check, Hugurtay (Table 6.4). Gebremedhin et al., (2000) revealed a prolonged appearance of *Striga* on resistant sorghum contrasted to a vulnerable genotype. Late *Striga* emergence could indicate late *Striga* attachement to the sorghum host. According to Rodenburg et al., (2006), genetic differences between sorghum genotypes influence the time of attachment of the parasite, with resistant genotypes demonstrating slower parasite attachment and *Striga* emergence than vulnerable genotypes. The parasitism and multiplication of *Striga* are affected by the timing of the first infection. Late *Striga* emergence significantly minimizes the amount of damage it does to host plants (Van Ast and Bastiaans, 2006; Frost et al., 1997). *Striga* species cause phytotoxic effects on their hosts by sucking up nutrients resulting in stunted growth and a reduction of sorghum output as described by Presse et al. (1996).

Influence of sorghum accessions on *Striga hermonthica* numbers differed statistically significant ($p < 0.05$) (Table 6.4). Accessions EG1075, EG1168, EG1239, and N13 (resistant control) supported fewer *Striga* emergence compared to the other entries. The majority of the sorghum accessions studied showed a decrease in *Striga* vigor and number (Table 6.4). This could be an indication for presence of genetic resistance in those accessions. Doggett (1988) reported that resistant cultivars host much less *Striga* individuals and also have a greater yields than susceptible varieties when cultivated under *Striga* invasion. Hugurtay, the susceptible check, produced a substantially ($p < 0.05$) larger quantity of *Striga hermonthica* emergence (Table 6.4). This could be due to the parasite's growth being aided by the synthesis of adequate strigolactones and haustorial initiation factors. In comparison to Hugurtay, N13 (the resistant check) showed considerably reduced parasite infection (Table 6.4). This reduction in number of the parasite's infection in N13 could be due to mechanical barrier that prevented attachment to the root of the host as revealed by Haussmann et al. (2004) and Grenier et al. (2007). Kavuluko et al. (2021) revealed that parasite development was stopped in N13 before it reached the host cortex, and the haustorium did not fully develop, resulting in the majority of infecting parasites failing to generate vegetative tissue. Grain yield differed significantly among the accessions (Table 6.4). Mean grain yield of 34.3 gram per plant was recorded for accession EG1168 followed by accessions EG1075, EG1239 with 23.4 and 20.9 gram per plant respectively. All these accessions have one to three *Striga* resistance QTL alleles. When contrasted with Hugurtay, the appearance of *Striga* was prolonged in these cultivars, which could explain the increased grain yields. The results support findings by Mohamed et al. (2014) who reported a reduction of *Striga* appearance and improved sorghum grain harvests on germplasms that have *Striga* resistance QTLs.

Overall, selecting host varieties with comparatively thick panicles and greater grain output, as well as low and weak *Striga* counts, is necessary for developing better sorghum cultivars with greater grain harvests and better *Striga* resistance. In the current study, accessions EG1168, EG1075, and EG1239 have relatively better panicle weight and grain yield with relatively fewer *Striga* counts (Table 6.4). Interestingly, Accessions EG1075, EG1168, EG1239 were sourced from Northern Red Sea region of Eritrea (Table 6.2) where *Striga* is not a serious problem. Hence these accessions can be an important source of *Striga* resistance and they can be further evaluated in Gash-Barka zone where *Striga* is a major constraint in sorghum production.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

7.1 General discussion

Sorghum is Eritrea's extensively produced grain crop at the moment. *Striga hermonthica* infestation, on the other hand, drastically limits its output. This infamous weed has infected much of the western low-land sorghum-growing area of the country, as well as some areas of the mid-land of Debub and Anseba zones. The majority of farmers in Eritrea are subsistence farmers who cannot afford high-input *Striga* management techniques. For such farmers, *Striga* resistant varieties could be the most realistic control option.

Farmers encountered difficulties growing sorghum in each study sub-zone as a result of several biotic and abiotic stressors. Farmers mentioned sorghum production barriers such as drought, *Striga* infestation, and a lack of agricultural inputs. These limitations lower the production potential of sorghum. *Striga* invasion was regarded as the second most sever constraint to sorghum yield, preceded by drought. According to the majority of respondents, Striga had contaminated their sorghum with invasion levels ranging from slight to significant. Based on farmers' knowledge and experience, favored sorghum landraces were identified and described. Farmers' preferences and justifications for sticking with the mentioned sorghum landrace varieties over introduced improved cultivars were reported. These included the capacity of the landraces to grow in challenging environment, *Striga* resistance, a reseanable grain yield, good plant heights and straw yields, grain size, and grain color, good cooking quality, and disease resistance.

Due to a lack of information and limited access to other technology, farmers utilized hand weeding to control *Striga*, the same way they control other weed species. Hand weeding may help to prevent further reproduction of *Striga* but it may not improve the yield of the crop. To mitigate these challenges the numerous technologies that are currently available should be made accessible to farmers, and it is critical to improve the connection between research and agricultural extension services.

To investigate the variability of sorghum germplasms from Eritrea, the current study conducted a series of experiments utilizing molecular and invitro approaches to look for *Striga* resistance in those land race sorghum accessions. The potential of different sorghum cultivars to enhance germination of *Striga* hermonthica seeds vary. As a result, cultivars that produce low levels of *Striga* germination stimulant should be identified. More than ten accessions were identified to cause less *Striga* germination in a study to detect low *Striga* germination stimulant production. When compared to the resistant control, germination in accession EG830 was considerably lower (Prob $\leq$ 0.05). EG830, EG1076, EG473, EG 1261, and EG546 were the five sorghum accessions with the lowest germination of *Striga* percentages, at 11.85%, 13.05%, 14.68%, 15.32%, and 15.74%, respectively. Their high level of resistance to *Striga* was indicated by their small percent level of stimulant production. *Striga* germination was poor in those accessions, which might mean germination stimulant production was little. The host plant's low amounts of germination stimulant may lead to lower *Striga* seeds germinating.

The variations observed in the SSR genotyping for the presence of *Striga* resistance QTLs indicate there exists a genetic variation in the sorghum landraces studied. This genetic variability is crucial for an efficient selection which enabled to select eight accessions that have 1 – 3 *Striga* resistance QTLs for further evaluation. Accessions that showed *Striga* resistance QTLs in the

laboratory delayed emergence of *Striga* compared to the susceptible check, hugurtay. Due to the fact that delayed *Striga* emergence reduces the potential damage the weed might do, it is one of the crucial traits for selection in breeding for *Striga* resistance. The number of emerged *Striga* was significantly lower in accessions EG1075, EG1168, and EG1239 with less *Striga* vigor indicating the potential of these accessions for *Striga* resistance.

RNA sequencing, which is a method for determining the order of the nucleotides that make up an RNA molecule, helps to learn more about which genes were expressed (turned on) or silenced (shut off) at specific periods in different types of cells. Gene expression analysis contrasting the resistant cultivar N13 and susceptible genotype hugurtay demonstrated that several genes were significantly differentially expressed, of which 155 transcripts were upregulated while 8 were down regulated when the cultivars were infested with *Striga*. The significantly differentially expressed genes between the resistant N13 and the susceptible Hurgutay samples are primarily engaged in binding catalytic or transport activities and the control of various molecular functions. Several genes were directly expressed in response to a stimulus and to conduct cell signaling. There was overrepresentation of genes such as SORBI_3004G065900, SORBI_3001G482800, SORBI_3001G482700, SORBI_3001G077400 and SORBI_3001G259700 as a response to *Striga* wounding.

Several transcription factors comprising WRKY, AP2/ERFs domain proteins that play a significant role in defense against *Striga hermonthica* parasitism were identified. These transcription factors activate jasmonate and ethylene signaling pathways to induce defense genes. These transcription factors were overexpressed more in the resistant cultivar N13 compared to the susceptible check Hugurtay. The importance of WRKY and AP2/ERF transcription factors in defense agaist *Striga* is well documented.

7.2 Conclusion

The participatory rural appraisal research identified farmers' sorghum production prospects, major production limitations, the use of indigenous knowledge in farming, farmers' perspectives and priorities of biological traits in cultivars presently cultivated in the Hamelmalo, Goluj, and Tesseney sub-zones. Drought and *Striga* infestation were the two critical challenges restricting sorghum yield and productivity among important sorghum production constraints that were identified and given priority. Eritrea's *Striga* infestation in the study area was considerably high in all the three sub-zones surveyed. This is mainly attributed due to intense pressure on land because of continuous mono cropping of sorghum and reduced usage of fallow as described in the group meetings. The most crucial qualities for choosing a sorghum variety were identified as high yield, drought tolerance and *Striga* resistance as mentioned by farmers in the research area. Only a small number of improved sorghum varieties were mentioned by farmers across the three sub-regions (Table 3.9). This is a concern that need to be addressed to improve sorghum production.

The study to identify sorghum accessions that produce low *Striga* germination stimulant producers indicated that accessions EG830, EG1076, EG473, EG 1261, and EG546 generated lower *Striga* germination percent than the commercial controls. These accessions could be useful as stand-alone *Striga* resistance sources or in hybridization activities. The identification of low *Striga* germination stimulant producing sorghum genotypes will play an important role to mitigate *Striga* challenge in the country.

The alternate hypothesis that says there exist genetically superior sorghum landraces that confer resistance to *Striga* among the Eritrean sorghum accessions was verified in the genotyping experiment. It

has been confirmed that eight sorghum accessions (EG1075, EG1168, EG1172, EG1235, EG1237, EG1239, EG2453, EG544) possessing 1-3 *Striga* resistance QTLs were identified from the sorghum landrace collections from Eritrea, rendering the opportunity to exploit the genetic potential for breeding to manage *Striga* infestation challenges. In particular, accessions EG1075, EG1168, and EG1239 which showed 1-3 *Striga* resistance QTLs in the genotyping analysis supported fewer *Striga* emergence with better grain yield in the pot experiment compared to the other entries evaluated. These accessions could be potentially useful germplasm in sorghum improvement progrogram for *Striga* resistance.

RNA sequencing helps to know the functions of genes and pathways in determing the resistance or succeptebility of the crop under study. Fifteen genes were directly expressed in the present study in response to a stimulus and to carry out cell signaling. The defferentially expressed genes which were observed in the resistant cultivar N13 have presumed functions in inhibiting *Striga* infection. Signalling genes and pathways played a crucial role in mediating defense against *Striga* in N13. These pathways were repressed or minimally expressed in Hurgutay, the succeptible genotype. Therefore, it can be concluded that N13 mainly uses signaling pathways to fight off *Striga* infection.

7.3 Recommendation

The following recommendations were made based on the findings of the present study:

- In Eritrea, effective solutions are needed to restrict the dramatic growth in soil *Striga* seed bank and the expansion of *Striga* to other agricultural land. Therefore, taking into account farmers' perspectives, sorghum improvement projects in Eritrea should concentrate on producing genotypes that contain farmers' chosen traits, with an emphasis on *Striga* and moisture stress resistance.

- There is a need to sensitize farmers about *Striga* biology, fecundity, life cycle, mobility/dispersal, dormancy and its control so as to reduce its spread and institute control measures.

- *Striga* infestation is considerably high in all the three sub-zones surveyed. Hence, a coordinated control measure should be devised, and the genetic potential of the available landraces identified in this study and improved cultivars might be used in the country's sorghum breeding program.

- Accessions EG47, EG1261, EG830, EG1076, EG54 and EG746 which produced low *Striga* germination stimulant compared to the commercial checks may be hybridized with the previously developed backcrosses which have *Striga* resistance QTL introgressed (Yohannes et al., 2015). Striga resistance in sorghum resulting from two mechanisms would be more resilient and consistent across different geographical areas than resistance based on only one mechanism.

- Further evaluations of the selected accessions (EG1168, EG1075, and EG1239) in sorghum growing ago-ecologies of Eritrea infested with *Striga* are needed. Since these accessions were collected in northern red sea zone, they can be tested in Gash-Barka zone where *Striga* is a major constraint in sorghum production.

- There is need to carry out further studies to better understand and functionally validate significant differentially expressed genes reported in this study that enhance *Striga* resistance.

- The identified transcripts in the present study could help to discover new SNP markers for further *Striga*-sorghum research.

Policy brief

In terms of area and crop harvest, sorghum is the most important cereal in Eritrea and is cultivated on about 200,000 hectare annually (MoA, 2020). Production is however seriously hampered by the parasitic weed Striga hermonthica. As shown in this study in interviews with farmers from the Eritrean subzones of Golij, Tesenei, and Hamelmalo using semi structured questionnaire and focused group discussions, Striga infestation and drought stress were ranked as the highest production constraints that need immediate intervention. Therefore the findings of this study should play the role of increasing sorghum productivity in Eritrea and contribute towards food security as illustrated in the social impact pathway shown here below (Figure 7.1).

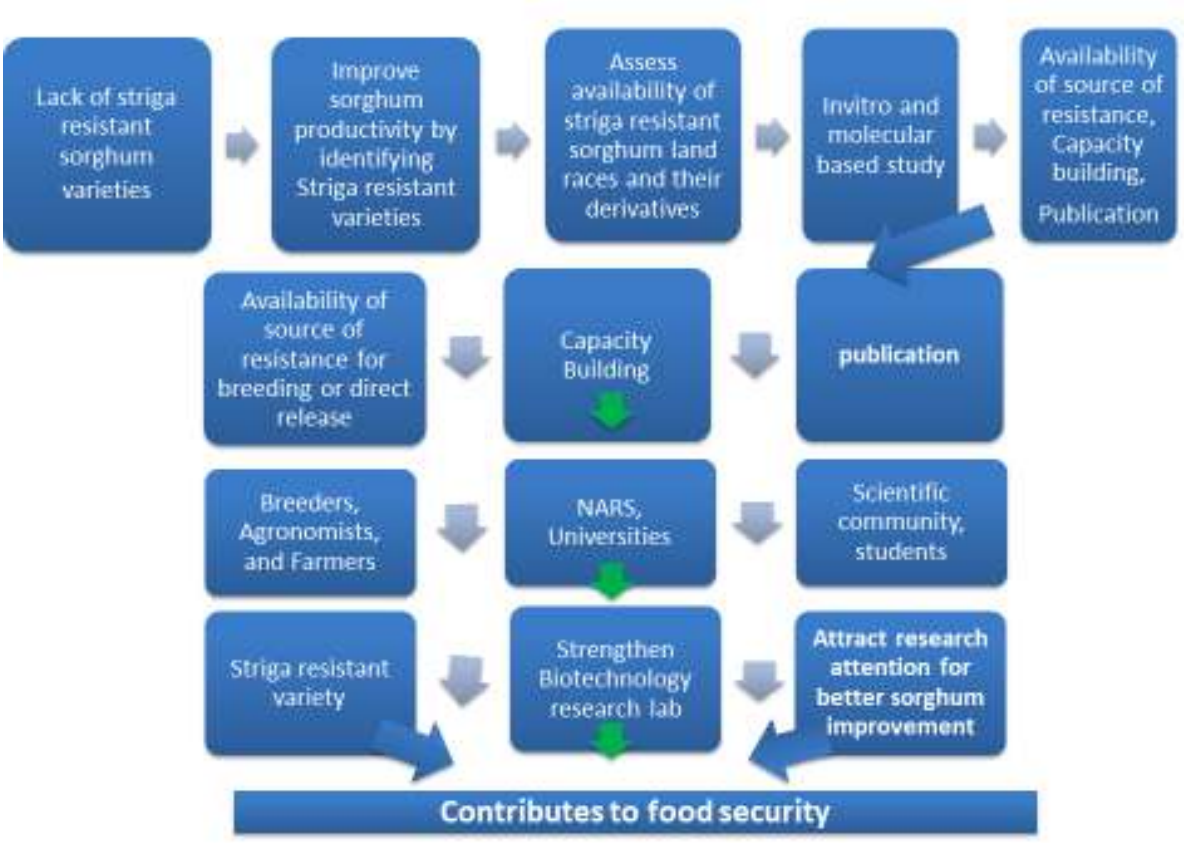


Figure 7.1: Suggested social impact path way of the findings of this study for increasing sorghum productivity in Eritrea

The key policy recommendations are:

- Sorghum improvement program in Eritrea should concentrate on developing cultivars that contain farmers' preferred traits, with an emphasis on *Striga* and drought resistance (Action; National Agricultural Research Institute and Hamelmalo Agricultural College should take the lead).

- *Striga* infestation is considerably high in all the three sub-zones surveyed. There is also a tendency of expanding infestation rates in terms of area and intensity. Therefore, a coordinated control measure should be devised, and the genetic potential of the available landraces and improved cultivars should be identified and used in the country's sorghum breeding program (National Agricultural Research Institute and Hamelmalo Agricultural College to take lead).

- Consistent farmers' sensitization about *Striga* biology, fecundity, life cycle, mobility/dispersal, dormancy and its control is needed so as to reduce its spread and institute control measures. Besides, only a small number of improved sorghum varieties were being used by farmers across the three sub-regions indicating that there might be a gap either in the availability of improved varieties or lack of awareness about improved varieties. This need to be addressed to improve sorghum production (MOA extension services, NGOs).

- Accessions EG1075, EG1168, and EG1239 which have 1-3 *Striga* resistance QTLs and supported fewer *Striga* emergence and had higher grain yields should be used in the sorghum improvement program for *Striga* resistance. Additionally, accessions EG830, EG1076, EG473, EG 1261, and EG546 with lower percent of *Striga* germination stimulant producers than the commercial controls could be included (National Agricultural Research Institute and Hamelmalo Agricultural College can take the lead).

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APPENDICES

Appendix 1: Household survey questionnaire

Date of interview _____

Name of interviewer_____

Location of household

01 zoba _____ 02 Sub-zoba _____

03 Kebabi _____ 04 Village _____

Household structure

05 Name of the respondent _____

06 Age _____ 07 Sex Male ☐ Female ☐

08 Household size _____

Household income per annum

09 sources of income: Farm proportion (%) _____

Off farm employment proportion (%) _____

Remittance from relatives proportion (%) _____

Loan and others proportion (%) _____

Total proportion (%) 100%

Crops and area cultivated by farmers

10 which of these crops do the household grow (Rank from 1= most important to 6= least important)

Ranking (1 to 6)	1	2	3	4	5	6
Sorghum	☐	☐	☐	☐	☐	☐
Maize	☐	☐	☐	☐	☐	☐
P.Millet	☐	☐	☐	☐	☐	☐
Ground nuts	☐	☐	☐	☐	☐	☐
Sesame	☐	☐	☐	☐	☐	☐

11. Total land areas cultivated _____ (Tsimdi)

12. Own farm area cultivated _____ (Tsimdi)

13. Farm area rented from owners _____ (Tsimdi)

14. Rent paid to land owners _____ (Nakfa)

15. Area cultivated against collateral _____(Tsimdi)

16. Value of collateral _____(Nakfa)

17. Land sharecropped _____ (tsimdi)

18. Percent of harvest given to owner _____ %

Major production constraints

19. Which of these are constraints to agricultural production (sorghum) for your household?

Ranking as follows		1	2	3
1= most important	Drought	☐	☐	☐
2= somewhat important	Striga infestation	☐	☐	☐
3= least important	Access to good seed	☐	☐	☐
	Access to farm labor	☐	☐	☐
	Access to credit	☐	☐	☐
	Access to land	☐	☐	☐
	Irrigation	☐	☐	☐
	Crop protection	☐	☐	☐
	Access to fertilizer	☐	☐	☐
	Access to market	☐	☐	☐

Sorghum and seed system

20. Total area of sorghum on average annually _____(tsimdi)

21. Total sorghum seed required _____ (kg)

22. Yield of sorghum _____ (kg/tsimdi)

23. What do you regard as characteristics of a good variety of sorghum?

Ranking as follows		1	2	3
1= most important	High yield	☐	☐	☐
2= somewhat important	Good adaptation to area	☐	☐	☐
3= least important	Striga/disease resistance	☐	☐	☐
	Drought tolerance	☐	☐	☐
	Plant height	☐	☐	☐
	Grain size	☐	☐	☐
	Grain color	☐	☐	☐
	Suitable for food preparation	☐	☐	☐
	Tillering capacity	☐	☐	☐
	Others/specify	☐	☐	☐

24. Name the varieties of sorghum you cultivate ☐

25. Have you heard about improved varieties? Yes ☐ No ☐

26. Are the improved varieties better than the local ones? Yes ☐ No ☐

27. What are the reasons that you do not use the improved?

Ranking as follows		1	2	3
1= most important	High cost	☐	☐	☐
2= somewhat important	Importation	☐	☐	☐
3= least important	Availability of seed	☐	☐	☐
	Questionable quality	☐	☐	☐
	Lack of clean seed source	☐	☐	☐
	Lack of information	☐	☐	☐
	Limited credit	☐	☐	☐
	Others specify_____	☐	☐	☐

28. What are the sources of new sorghum varieties?

Close relatives	☐
Neighbor farmer	☐
Traders	☐
Farmers associations	☐
Extension services	☐
Seed Company	☐
Other s/specify	☐

29. What would you regard as characteristics of good seed of sorghum?

Ranking as follows		1	2	3
1= most important	High germination	☐	☐	☐
2= somewhat important	large grain size	☐	☐	☐
3= least important	No admixture with other seeds	☐	☐	☐
	Chemical treatment applied	☐	☐	☐
	Good packing	☐	☐	☐
	Other /specify	☐	☐	☐

30. Have you ever been unable to keep your own sorghum seed from one year to next?

Yes ☐ No ☐

31. What was the reason for loss over past 10 years?

Ranking as follows		1	2	3
1= very severe	Drought	☐	☐	☐
2= medium	Striga infestation	☐	☐	☐
3= minimal	No harvest	☐	☐	☐
	Insects	☐	☐	☐
	Eaten up	☐	☐	☐

Other /specify ☐

32. How often did you loss the seed during last 10 years? ☐

33. If so where did you get new seed? 34 What are the major sources of seed? ☐

Self saved seed _____ (kg) Acquired seed _____ (kg)

35. From whom did you get the seed last season/year?

Close relative ☐ _____ (kg)

Neighbor farmer ☐ _____ (kg)

Traders ☐ _____ (kg)

Farmers associations ☐ _____ (kg)

Government ☐ _____ (kg)

Relief projects ☐ _____ (kg)

Seed Company ☐ _____ (kg)

Other s/specify ☐ _____ (kg)

36. How do you get sorghum seed from others last season (tick box)

Gift ☐ _____ (kg)

Exchange for seed/grain ☐ _____ (kg)

Exchange of labor ☐ _____ (kg)

Exchange for other items ☐ _____ (kg)

Bought for cash ☐ _____ (kg)

Borrowed ☐ _____ (kg)

Relief seed ☐ _____ (kg)

Other s/specify ☐ _____ (kg)

37. How far did you need to travel to get the seed from other providers?

With in village ☐

Neighboring village ☐

>10 km or > 3hours walk ☐ specify_____

Local market place ☐

38. What percent of total sorghum seed you need have you got? % _____

39. Are you satisfied with the quality of seed that you plant? Yes ☐ No ☐

40. What proportion of total harvest do you intend for home use from the last season harvest?

(%) _____

41. What proportion of total harvest do you intend for sale the last season harvest?

(%) _____

42. Percent of total harvest intended for seed saving? % _____

43. Do you use fertilizer for sorghum crop? Yes ☐ No ☐

44. Do you get fertilizer when you need it? Yes ☐ No ☐

45. Do you get the recommended dose of fertilizer? Yes ☐ No ☐

46. Is your sorghum crop infested with Striga? Yes ☐ No ☐

47. What proportion of your sorghum area is infested with Striga? % _____

Appendix 2: DNA concentration and absorbance ratio of 93 sorghum samples used for genotyping

Sample ID	DNA Conc. (ng/µl)	A260	A280	260/280
1	**73.5**	1.469	**0.763**	1.93
2	**32.6**	0.652	**0.412**	1.58
3	**15.9**	0.318	**0.153**	2.08
4	**22.3**	0.446	**0.211**	2.11
5	**554.8**	11.096	**5.65**	1.96
6	**150.3**	3.006	**1.531**	1.96
7	**23.4**	0.469	**0.251**	1.87
8	**28.4**	0.568	**0.29**	1.96
9	**27**	0.54	**0.265**	2.04
10	**32.1**	0.643	**0.32**	2.01
11	**19.7**	0.395	**0.199**	1.99
12	**46.5**	0.931	**0.488**	1.91
13	**23.1**	0.462	**0.248**	1.86
14	**96.4**	1.928	**1.22**	1.58
15	**24**	0.48	**0.238**	2.02
16	**32.6**	0.651	**0.335**	1.94
17	**22**	0.44	**0.234**	1.88
18	**37.9**	0.757	**0.377**	2.01
19	**27.9**	0.557	**0.28**	1.99
20	**50.5**	1.011	**0.515**	1.96
21	**23.5**	0.47	**0.229**	2.05
22	**31**	0.62	**0.314**	1.97
23	**37.8**	0.755	**0.392**	1.93
24	**28.9**	0.579	**0.297**	1.95
25	**360.2**	7.204	**3.793**	1.9
26	**303.4**	6.068	**3.182**	1.91
27	**22.2**	0.445	**0.225**	1.98
28	**33.1**	0.662	**0.328**	2.02
29	**33.3**	0.666	**0.323**	2.06
30	**92**	1.84	**0.953**	1.93
31	**219.3**	4.386	**2.305**	1.9
32	**19**	0.38	**0.167**	2.28
33	**33.9**	0.678	**0.341**	1.99
34	**41**	0.82	**0.416**	1.97
35	**39.5**	0.79	**0.392**	2.02
36	**26.1**	0.521	**0.243**	2.15
37	**45.7**	0.915	**0.455**	2.01
38	**118.5**	2.37	**1.255**	1.89
39	**39.1**	0.783	**0.391**	2
40	**132.3**	2.645	**1.24**	2.13
41	**187.9**	3.758	**1.759**	2.14
42	**55.8**	1.117	**0.516**	2.17
43	**38.6**	0.771	**0.348**	2.22
44	**176.1**	3.523	**1.909**	1.85
45	**60.7**	1.213	**0.614**	1.98
46	**214.6**	4.292	**2.038**	2.11
47	**55.1**	1.102	**0.533**	2.07
48	**282.5**	5.649	**2.664**	2.12
49	**129.4**	2.588	**1.259**	2.06
50	**74.2**	1.484	**0.755**	1.97
51	**55.3**	1.106	**0.538**	2.06
52	**72.1**	1.443	**0.718**	2.01
53	**57.4**	1.149	**0.584**	1.97
54	**154**	3.08	**1.62**	1.9
55	**99.2**	1.984	**1.015**	1.95
56	**50.2**	1.003	**0.458**	2.19
57	**53**	1.06	**0.495**	2.14
58	**150.8**	3.016	**1.632**	1.85
59	**48**	0.96	**0.444**	2.16
60	**122.9**	2.458	**1.14**	2.16
61	**37.6**	0.752	**0.361**	2.08
62	**71**	1.421	**0.774**	1.84
63	**25.4**	0.508	**0.229**	2.22
64	**109.4**	2.188	**1.042**	2.1
65	**24.6**	0.492	**0.244**	2.02
66	**32.8**	0.656	**0.407**	1.61
67	**24.4**	0.488	**0.231**	2.11
68	**34.4**	0.687	**0.334**	2.06
69	**18**	0.36	**0.159**	2.27
70	**25.8**	0.516	**0.233**	2.22
71	**42.3**	0.847	**0.391**	2.16
72	**30.6**	0.612	**0.278**	2.2
73	**70**	1.399	**0.684**	2.05
74	**223.5**	4.471	**2.307**	1.94
75	**65.5**	1.311	**0.645**	2.03
76	**17.8**	0.357	**0.153**	2.33
77	**16.9**	0.357	**0.183**	1.85
78	**447.4**	0.357	**4.714**	1.9
79	**19.4**	0.357	**0.202**	1.93
80	**439.2**	0.357	**4.676**	1.88
81	**43.6**	0.357	**0.429**	2.03
82	**92.1**	0.357	**0.948**	1.94
83	**26.6**	0.357	**0.263**	2.02
84	**144.3**	0.357	**1.528**	1.89
85	**16.7**	0.357	**0.161**	2.08
86	**49.1**	0.357	**0.551**	1.78
87	**331.3**	0.357	**3.453**	1.92
88	**53.6**	0.357	**0.585**	1.83
89	**44.8**	0.357	**0.471**	1.9
90	**22.7**	0.357	**0.204**	2.22
91	**177.7**	0.357	**2.001**	1.78
92	**45.7**	0.357	**0.491**	1.86
93	**287.9**	0.357	**3.072**	1.87

Appendix 3: RNA concentration and absorbance ratio of 24 sorghum samples used in gene expression analyses.

Sample ID	RNA conc.(ng/µl)	A260	A280	260/280
1	80.2	2.004	0.973	2.06
2	68.2	1.704	0.841	2.03
3	234.7	5.867	2.809	2.09
4	364.1	9.102	4.331	2.1
5	178	4.451	2.139	2.08
6	126.3	3.158	1.522	2.07
7	105.3	2.632	1.283	2.05
8	149.5	3.738	1.782	2.1
9	185.1	4.628	2.21	2.09
10	104.8	2.619	1.238	2.12
11	175.2	4.381	2.066	2.12
12	190.5	4.763	2.275	2.09
13	122	3.049	1.472	2.07
14	158.6	3.965	1.89	2.1
15	89.6	2.24	1.136	1.97
16	141.6	3.54	1.71	2.07
17	83.4	2.084	0.988	2.11
18	285.1	7.127	3.416	2.09
19	153.8	3.846	1.849	2.08
20	250.3	6.257	3.005	2.08
21	178.4	4.461	2.127	2.1
22	252.6	6.315	3.034	2.08
23	118.3	2.958	1.427	2.07
24	56.3	1.407	0.672	2.09

Appendix 4: Significant genes obtained when comparing various experimental conditions of the Resistant and Susceptible cultivars

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Hurgutay vs Mock	1	SORBI_3001G035000	23.27002305	1.441232321	0.299203002	4.826689711	1.39E-06	0.002361461
Hurgutay vs Mock	2	SORBI_3001G051600	81.72439451	1.098534432	0.209835312	5.242370793	1.59E-07	0.001018294
Hurgutay vs Mock	3	SORBI_3001G052300	34.8194549	1.278682324	0.277946982	4.590911523	4.41E-06	0.005598184
Hurgutay vs Mock	4	SORBI_3001G063600	80.77170657	0.90368825	0.232929693	3.877321314	0.000105613	0.03059104
Hurgutay vs Mock	5	SORBI_3001G064900	78.91145005	0.920719256	0.219891017	4.18587451	2.84E-05	0.015862112
Hurgutay vs Mock	6	SORBI_3001G079500	374.985196	0.869956923	0.214016134	4.062826367	4.85E-05	0.022244623
Hurgutay vs Mock	7	SORBI_3001G095700	104.8248986	1.352246289	0.344857469	3.919042186	8.89E-05	0.028830636
Hurgutay vs Mock	8	SORBI_3001G149500	227.4071427	0.829952891	0.22248923	3.721471778	0.000198065	0.041713789
Hurgutay vs Mock	9	SORBI_3001G192800	104.5484632	0.908623113	0.2404548	3.777456166	0.000158438	0.036341114
Hurgutay vs Mock	10	SORBI_3001G267600	15.62299593	1.493192337	0.370409475	4.014456009	5.96E-05	0.024692371
Hurgutay vs Mock	11	SORBI_3001G372500	111.5831851	0.739547761	0.195753144	3.780573267	0.000156468	0.036341114
Hurgutay vs Mock	12	SORBI_3001G381800	47.66797134	0.863922748	0.227372226	3.79219356	0.000149322	0.036341114
Hurgutay vs Mock	13	SORBI_3001G393500	45.16458888	1.066573869	0.268116179	3.969740458	7.20E-05	0.026410115
Hurgutay vs Mock	14	SORBI_3001G420100	484.4030345	0.987648627	0.229736654	4.333922512	1.46E-05	0.011893202
Hurgutay vs Mock	15	SORBI_3001G440200	70.13361846	0.894328306	0.228801921	3.907734035	9.32E-05	0.028830636
Hurgutay vs Mock	16	SORBI_3001G464900	346.3996886	0.819865241	0.213997094	3.835291758	0.000125415	0.03371927
Hurgutay vs Mock	17	SORBI_3001G489500	23.50984799	1.231543128	0.289901528	4.233692832	2.30E-05	0.014063482
Hurgutay vs Mock	18	SORBI_3002G184600	117.9182865	1.326479361	0.339808529	3.904244616	9.45E-05	0.028830636
Hurgutay vs Mock	19	SORBI_3002G338500	87.78345924	1.570902273	0.389723647	4.00352119	6.24E-05	0.025054303
Hurgutay vs Mock	20	SORBI_3002G343500	104.2461036	0.766488914	0.176899273	4.331464038	1.48E-05	0.011893202
Hurgutay vs Mock	21	SORBI_3002G367900	55.85451277	0.83050043	0.218079291	3.809583501	0.000139201	0.035065027
Hurgutay vs Mock	22	SORBI_3002G405600	216.2893883	0.852517369	0.208913234	4.080727593	4.49E-05	0.02136168
Hurgutay vs Mock	23	SORBI_3002G418900	131.4231407	1.516325044	0.357322266	4.253239606	2.11E-05	0.013534309
Hurgutay vs Mock	24	SORBI_3003G043600	97.96196773	0.964240156	0.243286276	3.962224053	7.43E-05	0.026498653
Hurgutay vs Mock	25	SORBI_3003G102200	52.27702953	1.087972775	0.285638137	3.814599064	0.000136404	0.035047698
Hurgutay vs Mock	26	SORBI_3003G252400	133.367848	0.810917977	0.219119491	3.700368073	0.000215287	0.043901458
Hurgutay vs Mock	27	SORBI_3003G268900	56.0414856	1.418916719	0.355805703	3.978077639	6.95E-05	0.026251215
Hurgutay vs Mock	28	SORBI_3003G385000	41.96203092	1.556694077	0.392273516	4.020676166	5.80E-05	0.024692371

Appendix 4 continued…….

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Hurgutay vs Mock	29	SORBI_3003G396000	230.2843559	1.056618918	0.218792253	4.827017934	1.39E-06	0.002361461
Hurgutay vs Mock	30	SORBI_3003G416500	248.9508208	0.80984625	0.220853043	3.675388483	0.000237488	0.047671957
Hurgutay vs Mock	34	SORBI_3004G025000	130.468282	1.334249746	0.334402957	3.993794072	6.50E-05	0.025314174
Hurgutay vs Mock	35	SORBI_3004G052000	74.39154796	1.07580049	0.252312347	4.264347003	2.00E-05	0.013534309
Hurgutay vs Mock	36	SORBI_3004G065900	227.0811882	1.238306217	0.250468164	4.94070287	7.78E-07	0.002000058
Hurgutay vs Mock	37	SORBI_3004G165600	33.54192731	1.392226492	0.332416229	4.18316773	2.87E-05	0.015862112
Hurgutay vs Mock	38	SORBI_3004G244100	91.49555756	1.004912842	0.248198795	4.052008092	5.08E-05	0.022495499
Hurgutay vs Mock	39	SORBI_3004G244300	82.58149669	0.959666143	0.262679732	3.653765767	0.000258422	0.049551461
Hurgutay vs Mock	40	SORBI_3004G268900	26.11094386	1.169945947	0.312985073	3.726872449	0.000193871	0.041510907
Hurgutay vs Mock	41	SORBI_3004G280900	20.41241104	1.377711379	0.360710177	3.834178444	0.000125985	0.03371927
Hurgutay vs Mock	42	SORBI_3004G292500	36.81114263	1.204296417	0.397413206	3.709911281	0.000207332	0.042961174
Hurgutay vs Mock	43	SORBI_3004G305000	51.47313786	1.410682414	0.374684204	3.775078205	0.000159957	0.036341114
Hurgutay vs Mock	31	SORBI_3005G037000	22.62606825	1.71424494	0.397388663	4.255173489	2.09E-05	0.013534309
Hurgutay vs Mock	32	SORBI_3005G047500	44.79911018	0.881908873	0.234245478	3.764853102	0.000166647	0.036912316
Hurgutay vs Mock	33	SORBI_3005G064200	48.76671893	0.936217556	0.214123079	4.378247927	1.20E-05	0.011822917
Hurgutay vs Mock	51	SORBI_3006G011300	262.9237559	1.069529503	0.283013199	3.783448591	0.00015467	0.036341114
Hurgutay vs Mock	52	SORBI_3006G025300	105.9539337	1.005772287	0.208792669	4.815201578	1.47E-06	0.002361461
Hurgutay vs Mock	53	SORBI_3006G069200	84.72160614	1.60779936	0.401978543	4.094398451	4.23E-05	0.020914218
Hurgutay vs Mock	54	SORBI_3006G095600	149.7644259	1.406752327	0.334814124	4.176271189	2.96E-05	0.015862112
Hurgutay vs Mock	55	SORBI_3006G146500	130.1714144	1.325738706	0.305576209	4.357545604	1.32E-05	0.011893202
Hurgutay vs Mock	56	SORBI_3006G205600	28.45365065	1.561186575	0.411518524	3.835562585	0.000125277	0.03371927
Hurgutay vs Mock	57	SORBI_3006G259000	102.3358942	0.895999632	0.230429438	3.873795877	0.000107153	0.03059104
Hurgutay vs Mock	44	SORBI_3007G011200	144.268494	1.027966633	0.274137525	3.748855613	0.000177643	0.038681079
Hurgutay vs Mock	45	SORBI_3007G051800	72.43990603	1.578054083	0.399895717	3.916171734	9.00E-05	0.028830636
Hurgutay vs Mock	46	SORBI_3007G156700	19.74332932	2.767405231	0.361374263	7.297607611	2.93E-13	3.76E-09
Hurgutay vs Mock	47	SORBI_3007G219100	38.57989332	-0.973503415	0.234359414	-4.143158955	3.43E-05	0.017603182
Hurgutay vs Mock	48	SORBI_3008G036000	44.91822327	1.972687787	0.401999248	4.98874998	6.08E-07	0.00195182
Hurgutay vs Mock	49	SORBI_3008G129000	101.8125581	0.984520088	0.25791602	3.817178103	0.000134987	0.035047698

Appendix 4 continued…….

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Hurgutay vs Mock	50	SORBI_3008G185400	57.00187913	0.766078259	0.194490609	3.941201176	8.11E-05	0.028150413
Hurgutay vs Mock	64	SORBI_3009G036100	44.29429358	1.331241181	0.291734082	4.555379963	5.23E-06	0.005598184
Hurgutay vs Mock	65	SORBI_3009G245000	40.69159289	-1.190355647	0.314434606	-3.773087862	0.000161239	0.036341114
Hurgutay vs Mock	66	SORBI_3009G247900	173.0101387	0.914582416	0.24933106	3.670452445	0.000242122	0.047854386
Hurgutay vs Mock	58	SORBI_3010G076700	103.5900136	1.240223421	0.288494233	4.295320284	1.74E-05	0.013182632
Hurgutay vs Mock	59	SORBI_3010G102000	16.5561011	1.784475897	0.374064253	4.696673509	2.64E-06	0.003774629
Hurgutay vs Mock	60	SORBI_3010G166500	101.9861989	0.850588079	0.218420024	3.899231228	9.65E-05	0.028830636
Hurgutay vs Mock	61	SORBI_3010G209100	33.91679178	1.485592677	0.372227845	3.921570419	8.80E-05	0.028830636
Hurgutay vs Mock	62	SORBI_3010G209200	17.78589859	1.988610973	0.378251834	5.131919204	2.87E-07	0.001228184
Hurgutay vs Mock	63	SORBI_3010G210600	20.19109043	1.892723686	0.408291851	4.562942735	5.04E-06	0.005598184
Hurgutay vs Mock	67	SORBI_3K013000	87.96494091	0.860099501	0.234774084	3.663283513	0.000249003	0.048468747
Hurgutay vs N13	86	ENSRNA049481228	220.2666681	-1.535799719	0.404570678	-3.798028866	0.000145851	0.029183942
Hurgutay vs N13	68	ENSRNA049484836	159.375125	-1.361115363	0.325642302	-4.186720871	2.83E-05	0.011234907
Hurgutay vs N13	1	SORBI_3001G035000	21.64257104	1.795742851	0.36155197	4.929100431	8.26E-07	0.001763154
Hurgutay vs N13	2	SORBI_3001G040200	280.5535497	0.93324771	0.262108656	3.565712552	0.000362869	0.0469144
Hurgutay vs N13	3	SORBI_3001G050800	13.82736432	1.428284597	0.396506038	3.587336344	0.000334073	0.045191645
Hurgutay vs N13	4	SORBI_3001G063600	78.31801852	1.025427459	0.285178521	3.595085073	0.000324286	0.044814402
Hurgutay vs N13	5	SORBI_3001G077400	52.88330056	1.480230368	0.29591682	4.981064946	6.32E-07	0.001735267
Hurgutay vs N13	6	SORBI_3001G095700	102.0273881	1.617058764	0.340521261	4.721248232	2.34E-06	0.002524564
Hurgutay vs N13	7	SORBI_3001G119000	19.50348755	1.708643049	0.448302558	3.803579961	0.00014262	0.02883775
Hurgutay vs N13	8	SORBI_3001G138200	31.17187278	1.596889651	0.339967389	4.714075397	2.43E-06	0.002524564
Hurgutay vs N13	9	SORBI_3001G138600	14.61960055	1.926202848	0.434330727	4.39358791	1.11E-05	0.005354272
Hurgutay vs N13	10	SORBI_3001G143100	9.064785571	2.335999033	0.488824897	4.793355983	1.64E-06	0.002250392
Hurgutay vs N13	11	SORBI_3001G192800	99.58160199	1.089969764	0.296217044	3.670875034	0.000241722	0.040618457
Hurgutay vs N13	12	SORBI_3001G235900	70.93477644	1.03722006	0.284536207	3.650567798	0.000261661	0.042595343
Hurgutay vs N13	13	SORBI_3001G247300	52.24484833	1.773549599	0.477163766	3.5611307	0.000369261	0.047088725
Hurgutay vs N13	14	SORBI_3001G259700	17.36873067	1.691180646	0.451264783	3.735453274	0.000187377	0.035287568
Hurgutay vs N13	15	SORBI_3001G267600	14.14449334	2.322336527	0.376574828	6.040399911	1.54E-09	1.48E-05

Appendix 4 continued…….

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Hurgutay vs N13	16	SORBI_3001G304700	153.0695508	1.284854101	0.334297214	3.853339202	0.000116518	0.026734874
Hurgutay vs N13	17	SORBI_3001G333400	68.56079306	1.317362188	0.326053344	3.987932322	6.67E-05	0.018828117
Hurgutay vs N13	18	SORBI_3001G353200	43.2453762	0.998115767	0.211378788	4.716726491	2.40E-06	0.002524564
Hurgutay vs N13	19	SORBI_3001G383700	181.8193388	1.059415215	0.294973977	3.592129458	0.000327987	0.045002136
Hurgutay vs N13	20	SORBI_3001G383800	15.22510158	1.687936823	0.350217539	4.797086898	1.61E-06	0.002250392
Hurgutay vs N13	21	SORBI_3001G386000	184.3605715	0.879605779	0.19250566	4.572788568	4.81E-06	0.003605716
Hurgutay vs N13	22	SORBI_3001G393500	43.69898518	1.213522504	0.298401271	4.05287369	5.06E-05	0.015674643
Hurgutay vs N13	23	SORBI_3001G440200	65.48917041	1.182017643	0.242277536	4.874145724	1.09E-06	0.002099165
Hurgutay vs N13	24	SORBI_3001G441100	8.527395111	1.54510314	0.41336365	3.677490549	0.00023554	0.040618457
Hurgutay vs N13	25	SORBI_3001G449100	5.711432601	1.807385157	0.467196325	3.760878308	0.000169318	0.032524255
Hurgutay vs N13	26	SORBI_3001G464900	323.3434143	1.086400408	0.274109854	3.964043501	7.37E-05	0.020221812
Hurgutay vs N13	27	SORBI_3001G481100	32.43744833	1.640684422	0.378969656	4.33704751	1.44E-05	0.006765757
Hurgutay vs N13	28	SORBI_3001G482700	77.60969954	1.298125199	0.35290996	3.669600371	0.00024293	0.040618457
Hurgutay vs N13	29	SORBI_3001G482800	10.16735185	1.632886487	0.446066701	3.680878603	0.000232432	0.040618457
Hurgutay vs N13	30	SORBI_3001G489500	22.64090374	1.364137223	0.370495965	3.680112461	0.000233131	0.040618457
Hurgutay vs N13	31	SORBI_3001G501100	50.30538069	1.496152463	0.31237817	4.770169552	1.84E-06	0.002357212
Hurgutay vs N13	32	SORBI_3001G505300	28.73927442	1.255433502	0.31231868	4.015731732	5.93E-05	0.017513181
Hurgutay vs N13	33	SORBI_3001G508466	40.20230237	1.110189074	0.308431359	3.584590278	0.000337608	0.045350404
Hurgutay vs N13	34	SORBI_3001G526000	61.393376	1.467251727	0.284963199	5.14110228	2.73E-07	0.001049316
Hurgutay vs N13	35	SORBI_3002G113100	104.4509492	1.291429922	0.342553411	3.851776722	0.000117264	0.026734874
Hurgutay vs N13	36	SORBI_3002G195400	18.86919101	1.965383325	0.474002269	4.116583012	3.85E-05	0.013429915
Hurgutay vs N13	37	SORBI_3002G197600	16.59376886	1.501217544	0.357128708	4.176144744	2.96E-05	0.011234907
Hurgutay vs N13	38	SORBI_3002G207300	378.0172344	1.001871089	0.21856745	4.585360723	4.53E-06	0.003605716
Hurgutay vs N13	39	SORBI_3002G269300	47.57408319	1.362535139	0.38567583	3.526817804	0.000420586	0.049665297
Hurgutay vs N13	40	SORBI_3002G269400	45.5389993	1.347113411	0.352548681	3.819953832	0.000133477	0.028770961
Hurgutay vs N13	41	SORBI_3002G328400	110.6587556	1.370756751	0.374861985	3.625408242	0.000288505	0.042728302
Hurgutay vs N13	42	SORBI_3002G330300	142.4771459	0.924090605	0.23787003	3.884729745	0.000102444	0.024909399
Hurgutay vs N13	43	SORBI_3002G333400	4.524471613	1.940488177	0.520313153	3.667369706	0.000245058	0.040618457

Appendix 4 continued…….

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Hurgutay vs N13	44	SORBI_3002G337100	31.69537792	1.72602304	0.405440029	4.239486568	2.24E-05	0.009156219
Hurgutay vs N13	45	SORBI_3002G343500	102.3469949	0.790490209	0.217368969	3.634720388	0.000278282	0.042728302
Hurgutay vs N13	46	SORBI_3002G362900	153.4365374	0.933046099	0.241843134	3.857106856	0.000114737	0.026734874
Hurgutay vs N13	47	SORBI_3002G367900	53.14646261	1.01330284	0.274582013	3.686753444	0.000227133	0.040618457
Hurgutay vs N13	48	SORBI_3003G037500	49.27012426	1.752863064	0.359505432	4.943017365	7.69E-07	0.001763154
Hurgutay vs N13	49	SORBI_3003G043600	88.95141684	1.413863482	0.301114688	4.708366678	2.50E-06	0.002524564
Hurgutay vs N13	50	SORBI_3003G083200	75.63674297	1.386871823	0.363253977	3.807558392	0.000140346	0.028770961
Hurgutay vs N13	51	SORBI_3003G092900	19.53274245	1.088198673	0.300119977	3.629142656	0.000284364	0.042728302
Hurgutay vs N13	52	SORBI_3003G102200	48.85529613	1.345985327	0.384207017	3.538035799	0.000403115	0.048868583
Hurgutay vs N13	53	SORBI_3003G150400	5.215576972	1.79918608	0.489505729	3.624812953	0.000289171	0.042728302
Hurgutay vs N13	54	SORBI_3003G202200	169.0059921	1.274798401	0.299160322	4.269283297	1.96E-05	0.008379036
Hurgutay vs N13	55	SORBI_3003G214700	34.21459051	-0.944373015	0.263925985	-3.571138302	0.000355433	0.046763803
Hurgutay vs N13	56	SORBI_3003G217500	7.464020329	-1.772517475	0.476688088	-3.625666987	0.000288217	0.042728302
Hurgutay vs N13	57	SORBI_3003G234000	40.65027206	1.521512319	0.430283445	3.614595155	0.000300817	0.043122387
Hurgutay vs N13	58	SORBI_3003G252400	130.5954249	0.885574876	0.218307689	4.054614157	5.02E-05	0.015674643
Hurgutay vs N13	59	SORBI_3003G272200	25.25159366	1.569333555	0.449725364	3.540583911	0.000399243	0.048868583
Hurgutay vs N13	60	SORBI_3003G277700	147.867437	1.13314336	0.309764987	3.665243943	0.000247103	0.040618457
Hurgutay vs N13	61	SORBI_3003G288100	8.302623574	1.959089125	0.476197854	4.094560051	4.23E-05	0.014008355
Hurgutay vs N13	62	SORBI_3003G291600	85.22316301	1.073874465	0.264603351	4.063886813	4.83E-05	0.015674643
Hurgutay vs N13	63	SORBI_3003G311800	12.90691254	1.541797013	0.397460303	3.89122736	9.97E-05	0.024881497
Hurgutay vs N13	64	SORBI_3003G313800	19.85434206	1.476195047	0.384993987	3.823460329	0.000131592	0.028770961
Hurgutay vs N13	65	SORBI_3003G356000	146.9016197	-1.315292689	0.315519182	-4.174769904	2.98E-05	0.011234907
Hurgutay vs N13	66	SORBI_3003G361100	123.5605415	1.389502369	0.296904727	4.677521971	2.90E-06	0.002583021
Hurgutay vs N13	67	SORBI_3003G385000	39.80464202	1.758544191	0.492051417	3.636097955	0.000276799	0.042728302
Hurgutay vs N13	69	SORBI_3003G416500	226.2868394	1.188199937	0.247153582	4.806125981	1.54E-06	0.002250392
Hurgutay vs N13	70	SORBI_3003G416800	19.61551774	1.265854514	0.356673772	3.535471997	0.000407047	0.048868583
Hurgutay vs N13	71	SORBI_3003G428700	8.490429405	1.881336281	0.380509016	4.851175465	1.23E-06	0.002143234
Hurgutay vs N13	72	SORBI_3003G430400	7.192743839	1.691213506	0.466779462	3.564965023	0.000363905	0.0469144

Appendix 4 continued…….

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Hurgutay vs N13	73	SORBI_3003G436800	101.4776463	1.436378636	0.397049342	3.621563274	0.000292828	0.042938444
Hurgutay vs N13	81	SORBI_3004G006500	8.641276198	1.478286021	0.415369385	3.557036216	0.000375062	0.047088725
Hurgutay vs N13	82	SORBI_3004G025800	19.3375365	-1.073164855	0.292675073	-3.641544517	0.000271007	0.042728302
Hurgutay vs N13	83	SORBI_3004G052000	73.87403865	1.084471434	0.284858008	3.807938855	0.00014013	0.028770961
Hurgutay vs N13	84	SORBI_3004G065900	216.4655817	1.418270181	0.341067495	4.147867238	3.36E-05	0.012396709
Hurgutay vs N13	85	SORBI_3004G086800	122.0378949	1.137074649	0.252785178	4.502653908	6.71E-06	0.004158446
Hurgutay vs N13	87	SORBI_3004G114400	262.8156018	1.650629932	0.468019093	3.559421355	0.000371673	0.047088725
Hurgutay vs N13	88	SORBI_3004G157700	43.67038627	0.867531297	0.244265855	3.550219844	0.00038491	0.048011221
Hurgutay vs N13	89	SORBI_3004G232500	17.05196314	1.483909455	0.418685854	3.582506131	0.000340314	0.04539642
Hurgutay vs N13	90	SORBI_3004G243500	16.05429803	1.462197784	0.35391298	4.106616993	4.01E-05	0.013772035
Hurgutay vs N13	91	SORBI_3004G244100	90.09185094	1.031447285	0.27859608	3.699629611	0.000215914	0.039127347
Hurgutay vs N13	92	SORBI_3004G244300	79.57972248	1.100346433	0.303995998	3.614608034	0.000300802	0.043122387
Hurgutay vs N13	93	SORBI_3004G292900	54.53827633	1.690682828	0.463462901	3.644481431	0.000267931	0.042728302
Hurgutay vs N13	94	SORBI_3004G293500	184.4469976	1.22140497	0.344736021	3.52628064	0.00042144	0.049665297
Hurgutay vs N13	95	SORBI_3004G297600	78.13537862	1.167345437	0.326032817	3.568382809	0.000359191	0.0469144
Hurgutay vs N13	96	SORBI_3004G299600	12.39714154	1.982650175	0.500986491	3.66493361	0.000247403	0.040618457
Hurgutay vs N13	97	SORBI_3004G319300	52.76169748	0.825131929	0.22416971	3.675314013	0.000237557	0.040618457
Hurgutay vs N13	98	SORBI_3004G333300	12.36625122	1.350804885	0.389278497	3.547562913	0.000388813	0.048185204
Hurgutay vs N13	74	SORBI_3005G019400	20.04521693	1.461560971	0.38740042	3.777274985	0.000158554	0.031078132
Hurgutay vs N13	75	SORBI_3005G047500	42.01964743	1.103301121	0.268553924	4.098381251	4.16E-05	0.014008355
Hurgutay vs N13	76	SORBI_3005G064200	45.82142139	1.171089608	0.255189844	4.581774523	4.61E-06	0.003605716
Hurgutay vs N13	77	SORBI_3005G104200	59.51795269	1.740357923	0.399394331	4.424811293	9.65E-06	0.004879421
Hurgutay vs N13	78	SORBI_3005G165900	27.64188026	1.036860305	0.262458217	3.942962145	8.05E-05	0.021177623
Hurgutay vs N13	79	SORBI_3005G176100	16.61716786	1.373725483	0.327703383	4.177036482	2.95E-05	0.011234907
Hurgutay vs N13	80	SORBI_3005G182500	22.41664077	1.73671696	0.436041011	3.92236934	8.77E-05	0.022457228
Hurgutay vs N13	115	SORBI_3006G011400	7.973075416	1.933268021	0.520784851	3.81605729	0.000135601	0.028770961
Hurgutay vs N13	116	SORBI_3006G025300	110.5795672	0.828573783	0.215083108	3.849618252	0.000118302	0.026734874
Hurgutay vs N13	117	SORBI_3006G028000	5.456116015	2.131809592	0.520262068	3.825210545	0.00013066	0.028770961
Hurgutay vs N13	118	SORBI_3006G041700	5.211918525	1.851995329	0.496172	3.647629104	0.000264671	0.04272329

Appendix 4 continued…….

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Hurgutay vs N13	119	SORBI_3006G069200	85.45097452	1.720638151	0.470957884	3.730980607	0.000190736	0.035571321
Hurgutay vs N13	120	SORBI_3006G124000	33.74034689	1.364789326	0.375533894	3.617984592	0.000296906	0.043122387
Hurgutay vs N13	121	SORBI_3006G131900	42.76802221	1.365209944	0.351784306	3.863910963	0.000111586	0.026462392
Hurgutay vs N13	122	SORBI_3006G146500	127.8102147	1.411402089	0.360747922	3.932506315	8.41E-05	0.021821624
Hurgutay vs N13	123	SORBI_3006G154500	53.54795778	1.795039802	0.351532277	5.083078329	3.71E-07	0.00118893
Hurgutay vs N13	124	SORBI_3006G184400	42.19562162	1.585075186	0.445910724	3.557636751	0.000374206	0.047088725
Hurgutay vs N13	125	SORBI_3006G187900	127.0249372	0.924937548	0.216520514	4.269067755	1.96E-05	0.008379036
Hurgutay vs N13	126	SORBI_3006G210300	136.5011155	1.29710463	0.275865367	4.694982818	2.67E-06	0.00256084
Hurgutay vs N13	127	SORBI_3006G274700	115.2692444	0.827805985	0.219549125	3.763275668	0.000167702	0.032524255
Hurgutay vs N13	99	SORBI_3007G025800	38.03205763	1.211291039	0.317684963	3.806773054	0.000140792	0.028770961
Hurgutay vs N13	100	SORBI_3007G051800	76.75026383	1.529828871	0.41984748	3.611837258	0.000304035	0.043260852
Hurgutay vs N13	101	SORBI_3007G077100	68.97426992	1.169583421	0.290184807	4.027846829	5.63E-05	0.016894914
Hurgutay vs N13	102	SORBI_3007G077200	75.18779648	1.411127179	0.331014875	4.255990705	2.08E-05	0.008691043
Hurgutay vs N13	103	SORBI_3007G077300	99.15318826	1.36756064	0.306594169	4.45199988	8.51E-06	0.004879421
Hurgutay vs N13	104	SORBI_3007G143400	18.51845948	1.636688728	0.452970737	3.535906224	0.000406379	0.048868583
Hurgutay vs N13	105	SORBI_3007G156700	20.39332465	2.165931577	0.462209373	4.569862096	4.88E-06	0.003605716
Hurgutay vs N13	106	SORBI_3007G158500	8.047615633	1.62396078	0.42868494	3.712068663	0.000205572	0.037607965
Hurgutay vs N13	107	SORBI_3007G158600	12.64255925	1.943289399	0.483427412	3.97329063	7.09E-05	0.019734177
Hurgutay vs N13	108	SORBI_3007G188100	59.50710994	0.913287567	0.213202451	4.280275806	1.87E-05	0.008378126
Hurgutay vs N13	109	SORBI_3008G022100	5.70108061	1.824023559	0.524587336	3.635191803	0.000277774	0.042728302
Hurgutay vs N13	110	SORBI_3008G026900	29.44059608	1.097759282	0.293565186	3.738858439	0.000184858	0.035157753
Hurgutay vs N13	111	SORBI_3008G036000	45.9062114	2.306278749	0.409455808	5.537190635	3.07E-08	0.000196804
Hurgutay vs N13	112	SORBI_3008G109300	65.23844369	1.859358167	0.519267735	3.81282564	0.000137387	0.028770961
Hurgutay vs N13	113	SORBI_3008G123100	41.02156776	1.395145463	0.342001352	4.059790466	4.91E-05	0.015674643
Hurgutay vs N13	114	SORBI_3008G129000	97.28940281	1.165966644	0.300810608	3.873760729	0.000107169	0.025732529
Hurgutay vs N13	146	SORBI_3009G010400	98.93897428	1.198631114	0.308095566	3.886088648	0.000101872	0.024909399
Hurgutay vs N13	147	SORBI_3009G014900	19.44794227	-1.298697278	0.291214684	-4.425352162	9.63E-06	0.004879421
Hurgutay vs N13	148	SORBI_3009G028500	489.5738957	0.839852497	0.188940219	4.439971168	9.00E-06	0.004879421
Hurgutay vs N13	149	SORBI_3009G043600	88.48447161	1.625142083	0.400918347	4.039955547	5.35E-05	0.016300615

Appendix 4 continued…….

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Hurgutay vs N13	150	SORBI_3009G100500	287.4647101	0.928910942	0.258244713	3.59821164	0.000320413	0.044600074
Hurgutay vs N13	151	SORBI_3009G120800	80.93632571	1.322134652	0.318346029	4.132774902	3.58E-05	0.012989985
Hurgutay vs N13	152	SORBI_3009G136400	95.25012978	1.110567866	0.285144595	3.891412403	9.97E-05	0.024881497
Hurgutay vs N13	153	SORBI_3009G145200	8.87185048	-2.273827814	0.489455529	-4.529847152	5.90E-06	0.00404942
Hurgutay vs N13	154	SORBI_3009G152600	78.05400368	1.545937801	0.231307439	6.679565182	2.40E-11	4.60E-07
Hurgutay vs N13	155	SORBI_3009G173300	150.1577619	1.302488201	0.340255678	3.808034187	0.000140076	0.028770961
Hurgutay vs N13	156	SORBI_3009G183101	968.9423888	0.884742453	0.244964714	3.609725562	0.000306521	0.043293856
Hurgutay vs N13	157	SORBI_3009G187900	7.117137787	1.578880669	0.432371826	3.625990097	0.000287856	0.042728302
Hurgutay vs N13	158	SORBI_3009G208000	19.50569507	1.139041192	0.318559667	3.571242999	0.000355291	0.046763803
Hurgutay vs N13	159	SORBI_3009G217500	32.27970329	1.796589047	0.416664696	4.279222623	1.88E-05	0.008378126
Hurgutay vs N13	160	SORBI_3009G217600	37.19916639	1.55005732	0.432855732	3.587772267	0.000333515	0.045191645
Hurgutay vs N13	161	SORBI_3009G247900	165.9013966	1.093429522	0.24213254	4.51311529	6.39E-06	0.004090382
Hurgutay vs N13	162	SORBI_3009G254900	20.16145779	1.261611019	0.355315973	3.537795505	0.000403482	0.048868583
Hurgutay vs N13	128	SORBI_3010G023200	108.3937969	1.101225965	0.275585272	3.99046614	6.59E-05	0.018828117
Hurgutay vs N13	129	SORBI_3010G071000	47.33179307	1.112828899	0.302796905	3.667299334	0.000245126	0.040618457
Hurgutay vs N13	130	SORBI_3010G075300	86.76315246	2.234450746	0.507721207	4.551839569	5.32E-06	0.003783382
Hurgutay vs N13	131	SORBI_3010G076600	102.5160859	1.12106735	0.252357162	4.431419467	9.36E-06	0.004879421
Hurgutay vs N13	132	SORBI_3010G076700	103.865442	1.283103917	0.28997952	4.41792372	9.97E-06	0.004908322
Hurgutay vs N13	133	SORBI_3010G087900	28.62894942	1.8075655	0.485165252	3.722307738	0.00019741	0.036462046
Hurgutay vs N13	134	SORBI_3010G102000	16.50890994	1.95213852	0.410131967	4.673692298	2.96E-06	0.002583021
Hurgutay vs N13	135	SORBI_3010G105300	104.6234171	0.953133896	0.264388994	3.600283421	0.000317871	0.044569157
Hurgutay vs N13	136	SORBI_3010G117900	21.99666009	2.079993598	0.506717556	3.96014952	7.49E-05	0.020264913
Hurgutay vs N13	137	SORBI_3010G131950	67.21907099	1.386487742	0.312063131	4.438536127	9.06E-06	0.004879421
Hurgutay vs N13	138	SORBI_3010G135100	8.86843485	2.296911819	0.502536426	4.495153748	6.95E-06	0.004173137
Hurgutay vs N13	139	SORBI_3010G182966	12.47097514	1.518216526	0.413543071	3.634936866	0.000278049	0.042728302
Hurgutay vs N13	140	SORBI_3010G209100	33.94880233	1.624181699	0.399942045	3.989947159	6.61E-05	0.018828117
Hurgutay vs N13	141	SORBI_3010G209200	18.68738872	1.995220597	0.362292245	5.409154705	6.33E-08	0.000304092
Hurgutay vs N13	142	SORBI_3010G228000	62.01315194	1.280311471	0.282778099	4.518892159	6.22E-06	0.004090382
Hurgutay vs N13	143	SORBI_3010G234000	124.0771348	1.217001761	0.3074062	3.956350743	7.61E-05	0.020303753

Appendix 4 continued…….

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Hurgutay vs N13	144	SORBI_3010G246000	178.4594806	1.113270579	0.269656861	4.12430193	3.72E-05	0.013227916
Hurgutay vs N13	145	SORBI_3010G259300	10.10842175	1.759408107	0.457093752	3.791155403	0.000149948	0.029694359
Hurgutay vs N13	163	SORBI_3K013000	86.41312294	0.904438592	0.255861577	3.533460306	0.000410158	0.048936142
N13Stage1 vs Stage2	45	ENSRNA049470939	631.4696417	2.045826051	0.484674587	3.96298509	7.40E-05	0.019052201
N13Stage1 vs Stage2	80	ENSRNA049475729	583.4314251	1.549293714	0.485841137	3.222114318	0.001272484	0.041433273
N13Stage1 vs Stage2	107	ENSRNA049478834	1104.960029	1.548883395	0.446504669	3.491504705	0.000480308	0.028713147
N13Stage1 vs Stage2	93	ENSRNA049480363	909.5773077	-0.952816832	0.300196405	-3.272378527	0.001066467	0.038134271
N13Stage1 vs Stage2	75	ENSRNA049480683	1416.994008	1.41704712	0.464570591	3.053117605	0.002264772	0.049035422
N13Stage1 vs Stage2	91	ENSRNA049483158	267.1965312	1.516202049	0.317641924	4.704572854	2.54E-06	0.003001897
N13Stage1 vs Stage2	53	ENSRNA049484836	442.4761846	1.547722051	0.370776789	4.10473732	4.05E-05	0.015921132
N13Stage1 vs Stage2	1	SORBI_3001G003200	144.2442179	1.300421413	0.373477779	3.481485688	0.00049864	0.028713147
N13Stage1 vs Stage2	2	SORBI_3001G040200	246.5085178	0.937004994	0.3013112	3.115802757	0.001834449	0.046204908
N13Stage1 vs Stage2	3	SORBI_3001G064500	274.4422402	0.710280632	0.204162536	3.481383836	0.00049883	0.028713147
N13Stage1 vs Stage2	4	SORBI_3001G068301	328.4960925	1.566928413	0.470509447	3.32485002	0.000884661	0.037681565
N13Stage1 vs Stage2	5	SORBI_3001G070000	253.0676608	0.81692145	0.263005218	3.100273395	0.001933421	0.046204908
N13Stage1 vs Stage2	6	SORBI_3001G073900	155.5839152	0.915991709	0.286306099	3.198954769	0.001379268	0.041433273
N13Stage1 vs Stage2	7	SORBI_3001G078900	206.638349	1.084283965	0.294774874	3.678741806	0.000234387	0.025143376
N13Stage1 vs Stage2	8	SORBI_3001G197000	134.9320017	-0.732218013	0.214149427	-3.417102389	0.000632915	0.033012419
N13Stage1 vs Stage2	9	SORBI_3001G208100	320.8523364	1.023401902	0.326284288	3.130186963	0.001746951	0.046204908
N13Stage1 vs Stage2	10	SORBI_3001G217700	148.8735905	1.227369911	0.401648143	3.064290184	0.002181872	0.048123523
N13Stage1 vs Stage2	11	SORBI_3001G293800	162.5733804	1.180605261	0.356261985	3.289090982	0.001005115	0.037681565
N13Stage1 vs Stage2	12	SORBI_3001G327800	269.9234448	1.134564604	0.35934732	3.16078927	0.001573423	0.04420569
N13Stage1 vs Stage2	13	SORBI_3001G359300	138.5596092	1.446200893	0.440558623	3.286209437	0.001015455	0.037681565
N13Stage1 vs Stage2	14	SORBI_3001G372200	452.8108832	-0.783845197	0.24371691	-3.215713441	0.001301207	0.041433273
N13Stage1 vs Stage2	15	SORBI_3001G372500	228.891841	1.768766016	0.414425773	4.270438752	1.95E-05	0.012069785
N13Stage1 vs Stage2	16	SORBI_3001G410100	208.1145889	0.726079172	0.202349478	3.587430917	0.000333952	0.027258
N13Stage1 vs Stage2	17	SORBI_3001G420100	476.6804691	1.399697178	0.240174994	5.825574568	5.69E-09	1.34E-05
N13Stage1 vs Stage2	18	SORBI_3001G465100	303.0690233	0.727273336	0.216061478	3.366777482	0.00076052	0.035192703
N13Stage1 vs Stage2	19	SORBI_3001G473300	244.1646177	0.945174071	0.262142783	3.604094526	0.000313243	0.027258

Appendix 4 continued…….

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
N13Stage1 vs Stage2	20	SORBI_3001G479500	282.0486168	1.516147686	0.478271408	3.144774478	0.001662149	0.044575816
N13Stage1 vs Stage2	21	SORBI_3001G489900	131.0239682	0.935977841	0.285018556	3.280062828	0.00103784	0.037681565
N13Stage1 vs Stage2	22	SORBI_3001G509200	202.9370201	1.009561652	0.326343405	3.090679325	0.001996992	0.046204908
N13Stage1 vs Stage2	23	SORBI_3001G516500	143.0470505	0.91987488	0.279266365	3.297028871	0.000977135	0.037681565
N13Stage1 vs Stage2	24	SORBI_3001G517700	223.1968955	1.515760479	0.432600527	3.492594679	0.000478352	0.028713147
N13Stage1 vs Stage2	25	SORBI_3001G519700	250.4149178	1.635912992	0.507113467	3.383057569	0.000716836	0.033834661
N13Stage1 vs Stage2	26	SORBI_3002G000500	137.6620536	1.36417261	0.433053685	3.146310958	0.001653441	0.044575816
N13Stage1 vs Stage2	27	SORBI_3002G046800	142.6005116	1.225175072	0.291074916	4.209694886	2.56E-05	0.012069785
N13Stage1 vs Stage2	28	SORBI_3002G047400	246.4438727	1.556202646	0.481850166	3.20962604	0.001329078	0.041433273
N13Stage1 vs Stage2	29	SORBI_3002G118000	319.3535562	-0.859558739	0.260656189	-3.296529538	0.000978873	0.037681565
N13Stage1 vs Stage2	30	SORBI_3002G230100	264.528841	1.170701191	0.306101363	3.83257986	0.000126806	0.019052201
N13Stage1 vs Stage2	31	SORBI_3002G242000	754.0329696	1.627687717	0.516618238	3.192253173	0.001411675	0.041433273
N13Stage1 vs Stage2	32	SORBI_3002G324400	207.0017048	1.874792547	0.487492916	3.798591293	0.000145521	0.019052201
N13Stage1 vs Stage2	33	SORBI_3002G363100	784.1670296	0.504595708	0.133322671	3.785521842	0.000153386	0.019052201
N13Stage1 vs Stage2	34	SORBI_3002G367700	137.1824412	0.917323479	0.267634848	3.430169113	0.000603205	0.033012419
N13Stage1 vs Stage2	35	SORBI_3002G373800	427.5473877	-0.670828361	0.216819251	-3.094263988	0.001973018	0.046204908
N13Stage1 vs Stage2	36	SORBI_3002G394400	133.9479	0.717174612	0.217298437	3.297878141	0.000974184	0.037681565
N13Stage1 vs Stage2	37	SORBI_3002G403600	408.2570463	1.5793074	0.483811538	3.243540278	0.001180541	0.041433273
N13Stage1 vs Stage2	38	SORBI_3002G408100	139.8558334	1.620334445	0.425436321	3.788663789	0.00015146	0.019052201
N13Stage1 vs Stage2	39	SORBI_3003G020600	175.6442459	1.066701928	0.341978765	3.105392115	0.001900269	0.046204908
N13Stage1 vs Stage2	40	SORBI_3003G034200	639.6250475	-1.553121695	0.367345751	-4.217036166	2.48E-05	0.012069785
N13Stage1 vs Stage2	41	SORBI_3003G044200	130.2036677	0.770838298	0.248225939	3.105062673	0.001902387	0.046204908
N13Stage1 vs Stage2	42	SORBI_3003G117000	186.9583059	-1.451656612	0.400033789	-3.625913441	0.000287942	0.027258
N13Stage1 vs Stage2	43	SORBI_3003G134300	166.6753441	0.933046041	0.250072814	3.716203852	0.000202238	0.022727748
N13Stage1 vs Stage2	44	SORBI_3003G151100	265.6001866	1.62794448	0.48077179	3.820609801	0.000133122	0.019052201
N13Stage1 vs Stage2	46	SORBI_3003G178700	264.0658902	1.598462908	0.445639707	3.582648614	0.000340128	0.027258
N13Stage1 vs Stage2	47	SORBI_3003G185900	157.4916832	1.488494472	0.448410674	3.325590105	0.000882316	0.037681565
N13Stage1 vs Stage2	48	SORBI_3003G234400	331.8049191	1.627282004	0.518661959	3.22276797	0.001269584	0.041433273
N13Stage1 vs Stage2	49	SORBI_3003G239900	536.0971934	-0.928411204	0.282610322	-3.284266156	0.001022483	0.037681565

Appendix 4 continued…….

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
N13Stage1 vs Stage2	50	SORBI_3003G290300	156.1383063	1.030174247	0.284217322	3.622007776	0.000292325	0.027258
N13Stage1 vs Stage2	51	SORBI_3003G296000	246.5835831	0.79344184	0.255639584	3.102539614	0.001918679	0.046204908
N13Stage1 vs Stage2	52	SORBI_3003G343400	122.7304561	0.905852607	0.288153738	3.100003838	0.001935181	0.046204908
N13Stage1 vs Stage2	54	SORBI_3003G403300	126.6712178	1.604007475	0.498963574	3.183077286	0.001457187	0.041433273
N13Stage1 vs Stage2	55	SORBI_3003G404200	164.1216754	0.805121063	0.214059889	3.761878975	0.000168642	0.019899715
N13Stage1 vs Stage2	56	SORBI_3003G419100	157.8404788	0.971562979	0.267214625	3.625902942	0.000287954	0.027258
N13Stage1 vs Stage2	67	SORBI_3004G006300	1248.922923	-0.651379907	0.19799373	-3.288782081	0.001006219	0.037681565
N13Stage1 vs Stage2	68	SORBI_3004G018400	423.3361638	-0.68149046	0.212447948	-3.216575398	0.001297304	0.041433273
N13Stage1 vs Stage2	69	SORBI_3004G030200	4143.687917	1.039028207	0.306923585	3.396693873	0.000682052	0.033316967
N13Stage1 vs Stage2	70	SORBI_3004G128900	188.2261143	1.191933953	0.384690954	3.093102143	0.001980759	0.046204908
N13Stage1 vs Stage2	71	SORBI_3004G201700	457.5052484	1.09671342	0.344961237	3.187938203	0.001432912	0.041433273
N13Stage1 vs Stage2	72	SORBI_3004G220300	2461.934209	-0.830984367	0.244658993	-3.401980005	0.000668995	0.033316967
N13Stage1 vs Stage2	73	SORBI_3004G249200	141.6418147	-0.705485033	0.19752381	-3.569216826	0.00035805	0.027258
N13Stage1 vs Stage2	74	SORBI_3004G271600	192.8120173	1.03667264	0.340562836	3.046207254	0.00231748	0.049720487
N13Stage1 vs Stage2	57	SORBI_3005G055900	242.9945958	1.16173959	0.360105059	3.206186684	0.001345067	0.041433273
N13Stage1 vs Stage2	58	SORBI_3005G101200	304.1541617	1.032639849	0.330227809	3.122583141	0.001792715	0.046204908
N13Stage1 vs Stage2	59	SORBI_3005G105000	171.9671284	0.593940764	0.193231136	3.072113739	0.002125487	0.047772855
N13Stage1 vs Stage2	60	SORBI_3005G111200	167.9649539	1.630767959	0.457704673	3.56066615	0.000369915	0.027281243
N13Stage1 vs Stage2	61	SORBI_3005G114800	190.4709562	-0.547221706	0.171049429	-3.198248288	0.001382652	0.041433273
N13Stage1 vs Stage2	62	SORBI_3005G162500	196.66683	0.505281236	0.158697055	3.184844165	0.00144832	0.041433273
N13Stage1 vs Stage2	63	SORBI_3005G167300	183.374341	0.529031091	0.139508823	3.792245453	0.000149291	0.019052201
N13Stage1 vs Stage2	64	SORBI_3005G179300	414.2670399	1.204837716	0.391994763	3.072708015	0.002121259	0.047772855
N13Stage1 vs Stage2	65	SORBI_3005G182400	125.362939	1.157717986	0.299762666	3.860902102	0.000112969	0.019052201
N13Stage1 vs Stage2	66	SORBI_3005G222700	269.5673546	0.686909662	0.194729809	3.518323238	0.000434283	0.027700215
N13Stage1 vs Stage2	87	SORBI_3006G064100	288.1965334	0.877556893	0.257244863	3.412600309	0.000643462	0.033012419
N13Stage1 vs Stage2	88	SORBI_3006G100900	158.3280607	1.652922075	0.534247533	3.152438909	0.001619127	0.044575816
N13Stage1 vs Stage2	89	SORBI_3006G124800	207.1644267	1.476126479	0.470926214	3.203002013	0.00136003	0.041433273
N13Stage1 vs Stage2	90	SORBI_3006G147300	266.0638745	1.422930427	0.459085048	3.110042664	0.001870603	0.046204908
N13Stage1 vs Stage2	76	SORBI_3007G004600	213.5429702	1.358278741	0.435685067	3.086317242	0.002026525	0.046432996

Appendix 4 continued…….

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
N13Stage1 vs Stage2	77	SORBI_3007G069400	159.8332882	1.06964971	0.340296073	3.147122311	0.00164886	0.044575816
N13Stage1 vs Stage2	78	SORBI_3007G071500	1181.949774	1.553564713	0.51212077	3.069161731	0.002146603	0.047792304
N13Stage1 vs Stage2	79	SORBI_3007G085000	232.6564412	-0.725304346	0.21170944	-3.424442565	0.000616062	0.033012419
N13Stage1 vs Stage2	81	SORBI_3007G191100	151.9094784	-0.538434288	0.152662509	-3.529811987	0.000415855	0.027401649
N13Stage1 vs Stage2	82	SORBI_3007G213700	428.6823689	1.292702079	0.366910669	3.528456107	0.000417991	0.027401649
N13Stage1 vs Stage2	83	SORBI_3008G001000	627.1836296	1.164475134	0.373148343	3.121505364	0.00179929	0.046204908
N13Stage1 vs Stage2	84	SORBI_3008G094000	332.971583	1.522368778	0.391321413	3.893303518	9.89E-05	0.019052201
N13Stage1 vs Stage2	85	SORBI_3008G105500	225.3397325	0.668224148	0.197397331	3.392828001	0.000691751	0.033316967
N13Stage1 vs Stage2	86	SORBI_3008G189500	202.876826	1.609750735	0.476939143	3.30986076	0.000933424	0.037681565
N13Stage1 vs Stage2	103	SORBI_3009G034200	182.5256659	1.258417077	0.380608203	3.297403621	0.000975832	0.037681565
N13Stage1 vs Stage2	104	SORBI_3009G056700	150.222344	1.334283347	0.374863674	3.569456446	0.000357723	0.027258
N13Stage1 vs Stage2	105	SORBI_3009G119200	887.2916991	1.585474367	0.442461027	3.535280711	0.000407342	0.027401649
N13Stage1 vs Stage2	106	SORBI_3009G123900	572.8774935	1.310928054	0.399563263	3.289444454	0.001003854	0.037681565
N13Stage1 vs Stage2	108	SORBI_3009G196800	351.3974307	1.637944173	0.427623278	3.831600386	0.000127312	0.019052201
N13Stage1 vs Stage2	109	SORBI_3009G219100	268.4593635	1.382771083	0.449674552	3.092540661	0.00198451	0.046204908
N13Stage1 vs Stage2	110	SORBI_3009G242100	336.9587811	1.358994628	0.405439801	3.343915271	0.00082605	0.037489944
N13Stage1 vs Stage2	92	SORBI_3010G019000	241.294607	0.632947092	0.176392039	3.58854649	0.000332527	0.027258
N13Stage1 vs Stage2	94	SORBI_3010G149300	198.6148094	0.951517305	0.26895068	3.53056393	0.000414675	0.027401649
N13Stage1 vs Stage2	95	SORBI_3010G161600	154.2557011	1.464870974	0.474587106	3.058371879	0.002225432	0.048629814
N13Stage1 vs Stage2	96	SORBI_3010G173100	725.348521	-0.607083803	0.174976666	-3.468133464	0.000524087	0.029448694
N13Stage1 vs Stage2	97	SORBI_3010G214600	173.265376	0.830814651	0.206954649	4.01802428	5.87E-05	0.019052201
N13Stage1 vs Stage2	98	SORBI_3010G246600	154.0324737	1.988473113	0.5149066	3.95391915	7.69E-05	0.019052201
N13Stage1 vs Stage2	99	SORBI_3010G248200	269.3221828	1.765847255	0.45234187	3.929965151	8.50E-05	0.019052201
N13Stage1 vs Stage2	100	SORBI_3010G248301	175.6110471	1.570084063	0.524131805	3.186309244	0.001441005	0.041433273
N13Stage1 vs Stage2	101	SORBI_3010G255300	130.2333865	1.529724494	0.481280355	3.189838967	0.001423521	0.041433273
N13Stage1 vs Stage2	102	SORBI_3010G276400	276.8207722	1.597804549	0.496295801	3.234114513	0.001220205	0.041433273
N13 vs Mock	2	ENSRNA049468934	639.8229127	-4.927368289	1.051431915	-4.686340805	2.78E-06	0.002340791
N13 vs Mock	21	ENSRNA049470939	631.4696417	-2.774129019	0.752454528	-3.686772977	0.000227116	0.04944094
N13 vs Mock	22	ENSRNA049471137	115.6218922	-5.379779841	1.146315447	-4.693105947	2.69E-06	0.002340791

Appendix 4 continued…….

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
N13 vs Mock	18	ENSRNA049476934	1793.83314	-2.66525067	0.666458679	-3.999123656	6.36E-05	0.024673577
N13 vs Mock	16	ENSRNA049476982	77.98925706	-3.261475531	0.836563005	-3.898660962	9.67E-05	0.029255109
N13 vs Mock	68	ENSRNA049480363	909.5773077	1.408056761	0.379624034	3.70908224	0.000208012	0.047934352
N13 vs Mock	84	ENSRNA049483597	15.87544485	-7.843012694	2.003808375	-3.914053257	9.08E-05	0.028495378
N13 vs Mock	82	ENSRNA049483650	10.16581475	-7.160823937	1.831958393	-3.908835465	9.27E-05	0.028495378
N13 vs Mock	83	ENSRNA049483655	14.54933748	-7.580329555	1.929325049	-3.929005929	8.53E-05	0.028467384
N13 vs Mock	81	ENSRNA049483662	44.35903721	-5.67165639	1.488475032	-3.810380603	0.000138753	0.038238101
N13 vs Mock	76	ENSRNA049484570	61.64782314	-5.526811881	1.365188586	-4.048387115	5.16E-05	0.022688075
N13 vs Mock	77	ENSRNA049484573	62.48107054	-5.54798959	1.381420378	-4.016148653	5.92E-05	0.024673577
N13 vs Mock	75	ENSRNA049484587	79.76671566	-6.76003546	1.477272881	-4.576023526	4.74E-06	0.003397492
N13 vs Mock	78	ENSRNA049484591	3832.956936	-5.770177048	1.126050241	-5.124262523	2.99E-07	0.000567116
N13 vs Mock	79	ENSRNA049484632	180.6855846	-7.707132719	1.40441535	-5.487787297	4.07E-08	0.000131305
N13 vs Mock	80	ENSRNA049484640	849.2009596	-6.338318863	1.621329648	-3.909333843	9.26E-05	0.028495378
N13 vs Mock	85	ENSRNA049485438	552.6762821	-6.050237345	1.017370563	-5.946935724	2.73E-09	1.06E-05
N13 vs Mock	89	ENSRNA049485441	196.6717669	-3.994705594	0.833260238	-4.794067221	1.63E-06	0.001681876
N13 vs Mock	86	ENSRNA049485539	606.8666921	-6.673555885	1.051811427	-6.344821623	2.23E-10	2.16E-06
N13 vs Mock	87	ENSRNA049485548	187.7692732	-4.186783892	0.797322166	-5.251056688	1.51E-07	0.000365918
N13 vs Mock	88	ENSRNA049485554	23527.64985	-5.751200982	1.514482417	-3.797469628	0.000146181	0.038238101
N13 vs Mock	30	ENSRNA049485566	224.709327	-6.119933799	1.611523374	-3.797607841	0.000146099	0.038238101
N13 vs Mock	29	ENSRNA049485569	433.6450027	-6.115471807	1.27617029	-4.792049975	1.65E-06	0.001681876
N13 vs Mock	28	ENSRNA049485571	137.6323073	-5.201225327	1.112258749	-4.676272795	2.92E-06	0.002356198
N13 vs Mock	27	ENSRNA049485573	55.90969732	-5.870582626	1.448632701	-4.052499036	5.07E-05	0.022688075
N13 vs Mock	31	ENSRNA049485587	56.30891162	-7.320314856	1.509335993	-4.85002338	1.23E-06	0.001405625
N13 vs Mock	1	SORBI_3001G034900	11.36176785	6.433119133	1.641421026	3.919237679	8.88E-05	0.028495378
N13 vs Mock	3	SORBI_3001G266300	46.62308685	-3.646222268	0.921061106	-3.958719181	7.54E-05	0.027520822
N13 vs Mock	4	SORBI_3001G291200	46.41322358	3.803967333	0.53184182	7.152441177	8.52E-13	1.65E-08
N13 vs Mock	5	SORBI_3001G346100	75.79028776	-1.787525335	0.436557724	-4.094591013	4.23E-05	0.020990645
N13 vs Mock	6	SORBI_3001G438500	22.69479277	-5.881508099	1.447894375	-4.062111297	4.86E-05	0.022413046
N13 vs Mock	7	SORBI_3001G538800	60.16015393	-1.265588406	0.332572766	-3.805448119	0.000141548	0.038238101

Appendix 4 continued…….

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
N13 vs Mock	8	SORBI_3002G061600	64.86944855	-1.542057977	0.357117693	-4.318066589	1.57E-05	0.008943711
N13 vs Mock	9	SORBI_3002G082600	55.83806294	2.056274636	0.477552042	4.305865029	1.66E-05	0.008943711
N13 vs Mock	10	SORBI_3002G124400	24.32649439	-3.133759115	0.766560707	-4.088076894	4.35E-05	0.021048995
N13 vs Mock	11	SORBI_3002G141100	7.41843208	-4.909965406	1.107649902	-4.432777359	9.30E-06	0.005808781
N13 vs Mock	12	SORBI_3002G185800	8.88328651	5.645050362	1.436417403	3.929951247	8.50E-05	0.028467384
N13 vs Mock	13	SORBI_3002G319900	8.822697961	5.821605112	1.495509926	3.892722483	9.91E-05	0.029519581
N13 vs Mock	14	SORBI_3002G357600	7.381440367	5.720734533	1.481817472	3.860620246	0.0001131	0.033170732
N13 vs Mock	15	SORBI_3002G376800	97.01796856	2.476290541	0.621110229	3.986877733	6.69E-05	0.024921581
N13 vs Mock	17	SORBI_3003G065100	6.353541963	5.820729322	1.455851225	3.99816219	6.38E-05	0.024673577
N13 vs Mock	19	SORBI_3003G107100	30.63139405	-1.800724962	0.487894924	-3.69080487	0.000223546	0.04944094
N13 vs Mock	20	SORBI_3003G142900	45.43736869	-3.651940324	0.9116158	-4.006008151	6.18E-05	0.024673577
N13 vs Mock	23	SORBI_3003G235500	87.00429879	2.151910086	0.57924227	3.715043253	0.000203169	0.047382424
N13 vs Mock	24	SORBI_3003G266500	9.163615584	3.129412512	0.842125805	3.716086709	0.000202332	0.047382424
N13 vs Mock	43	SORBI_3004G024100	26.63450885	2.862042962	0.764013749	3.746062117	0.000179632	0.044014419
N13 vs Mock	44	SORBI_3004G064900	25.03565048	3.40727068	0.756098565	4.506384269	6.59E-06	0.00440149
N13 vs Mock	45	SORBI_3004G096200	11.61009363	-7.388074106	1.553526395	-4.755679805	1.98E-06	0.001897437
N13 vs Mock	46	SORBI_3004G155900	25.28491667	-2.911190468	0.787402082	-3.697209515	0.000217982	0.04944094
N13 vs Mock	47	SORBI_3004G156300	48.47248649	3.843531914	0.723264983	5.314140744	1.07E-07	0.000296333
N13 vs Mock	48	SORBI_3004G311000	39.30707427	4.075081128	1.061289975	3.839743356	0.000123163	0.035583075
N13 vs Mock	25	SORBI_3005G003200	416.1728482	-3.213139943	0.745649803	-4.30918097	1.64E-05	0.008943711
N13 vs Mock	26	SORBI_3005G020700	112.2050743	-1.901530768	0.307873195	-6.176344024	6.56E-10	4.23E-06
N13 vs Mock	32	SORBI_3005G130100	193.1766055	-10.61200862	2.136801968	-4.966304216	6.82E-07	0.000880626
N13 vs Mock	33	SORBI_3005G152600	4.508088173	-6.060822506	1.595083749	-3.799689209	0.000144878	0.038238101
N13 vs Mock	34	SORBI_3005G154400	56.31245918	21.58624769	4.247957929	5.081558728	3.74E-07	0.000567116
N13 vs Mock	35	SORBI_3005G170100	5.922656303	5.555113954	1.473607733	3.769737244	0.00016342	0.041081965
N13 vs Mock	36	SORBI_3005G171300	8.997490821	-3.138741632	0.850576128	-3.690136048	0.000224134	0.04944094
N13 vs Mock	37	SORBI_3005G173100	56.08023673	21.59901563	4.247954697	5.084568262	3.68E-07	0.000567116
N13 vs Mock	38	SORBI_3005G183300	20.68354073	-5.609430584	1.422771012	-3.942609554	8.06E-05	0.028245467
N13 vs Mock	39	SORBI_3005G183500	15.32203329	-5.761758325	1.299283352	-4.434566421	9.23E-06	0.005808781

Appendix 4 continued…….

condition	No.	gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
N13 vs Mock	40	SORBI_3005G190800	20.40281343	-3.103162186	0.818363225	-3.791913041	0.000149491	0.038582698
N13 vs Mock	41	SORBI_3005G208200	58.43305053	21.68587604	4.247907612	5.105072432	3.31E-07	0.000567116
N13 vs Mock	42	SORBI_3005G222200	134.4625049	-1.414721175	0.305788694	-4.626466584	3.72E-06	0.002769219
N13 vs Mock	63	SORBI_3006G010100	14.41556375	-7.276296368	1.608721225	-4.523031246	6.10E-06	0.004214312
N13 vs Mock	64	SORBI_3006G160000	50.44157878	2.054745244	0.509027404	4.036610264	5.42E-05	0.023326925
N13 vs Mock	65	SORBI_3006G175700	19.32654569	-6.169198741	1.2148209	-5.078278403	3.81E-07	0.000567116
N13 vs Mock	66	SORBI_3006G194300	56.32442824	2.047226571	0.442374332	4.627815002	3.70E-06	0.002769219
N13 vs Mock	67	SORBI_3006G227000	21.04666551	-6.514095059	1.705515309	-3.819429252	0.000133761	0.037993215
N13 vs Mock	49	SORBI_3007G006200	55.96958523	3.619687398	0.862836948	4.195100137	2.73E-05	0.014269304
N13 vs Mock	50	SORBI_3007G016600	10.73331765	-4.110456281	0.947760403	-4.337020483	1.44E-05	0.008471748
N13 vs Mock	51	SORBI_3007G038432	31.12288715	-7.657011939	1.526527117	-5.015968504	5.28E-07	0.000729579
N13 vs Mock	52	SORBI_3007G054500	16.83804056	5.353587731	1.368879432	3.910927147	9.19E-05	0.028495378
N13 vs Mock	53	SORBI_3007G092500	55.79998737	2.801427047	0.749616856	3.737145218	0.000186121	0.045005394
N13 vs Mock	54	SORBI_3007G107000	99.77133013	-2.499057918	0.66492115	-3.758427474	0.000170985	0.042432674
N13 vs Mock	55	SORBI_3007G113400	59.20431717	-2.001378982	0.492062978	-4.067322825	4.76E-05	0.022413046
N13 vs Mock	56	SORBI_3007G226500	7.840731077	-6.299268321	1.326833335	-4.747595764	2.06E-06	0.001897437
N13 vs Mock	57	SORBI_3008G002800	16.22121098	6.892161236	1.744024616	3.951871536	7.75E-05	0.027796066
N13 vs Mock	58	SORBI_3008G049000	6.642000801	-6.345282677	1.588761387	-3.993855043	6.50E-05	0.024673577
N13 vs Mock	59	SORBI_3008G079400	32.06246839	-4.154831789	0.841582586	-4.936926997	7.94E-07	0.000960145
N13 vs Mock	60	SORBI_3008G124100	193.6231702	-3.449991963	0.837634518	-4.118731842	3.81E-05	0.019406063
N13 vs Mock	61	SORBI_3008G157200	263.9520744	-0.732482772	0.19869093	-3.686543566	0.000227321	0.04944094
N13 vs Mock	62	SORBI_3008G189900	11.89822472	2.751988994	0.721101653	3.816367611	0.000135431	0.037993215
N13 vs Mock	72	SORBI_3009G014900	28.82621348	1.496860126	0.396995669	3.770469667	0.000162941	0.041081965
N13 vs Mock	73	SORBI_3009G145700	5.53145488	5.493962796	1.394649109	3.939315461	8.17E-05	0.028245467
N13 vs Mock	74	SORBI_3009G215700	64.34071902	21.00620567	3.510427892	5.983944497	2.18E-09	1.05E-05
N13 vs Mock	69	SORBI_3010G131200	21.17287236	-3.073347338	0.705546551	-4.355980954	1.32E-05	0.008013332
N13 vs Mock	70	SORBI_3010G139301	16.2933499	4.119741553	1.103251516	3.734181639	0.000188327	0.045005394
N13 vs Mock	71	SORBI_3010G164500	271.9110732	-3.316835561	0.827876121	-4.006439462	6.16E-05	0.024673577

Appendix 5: ***Total sequencing reads, read file IDs and overall alignment rate obtained from each RNA-seq library.***

Sample ID	Sample Name	Treatment	Striga infection stage	Replicate number	Read_File	Total reads	alignment rate
T10	Hugurtay	Mock	Stage1	Rep II	10_S6_L001_R1_001.fastq.gz	1,267,925	64.29%
T11	N13	Striga	Stage1	Rep II	11_S7_L001_R1_001.fastq.gz	1,052,853	51.84%
T12	N13	Mock	Stage1	Rep II	12_S8_L001_R1_001.fastq.gz	2,113,957	59.75%
T13	Hugurtay	Striga	Stage2	Rep II	13_S5_L001_R1_001.fastq.gz	1,158,131	42.76%
T14	Hugurtay	Mock	Stage2	Rep II	14_S6_L001_R1_001.fastq.gz	2,181,830	55.80%
T15	N13	Striga	Stage2	Rep II	15_S7_L001_R1_001.fastq.gz	1,714,746	69.80%
T16	N13	Mock	Stage2	Rep II	16_S8_L001_R1_001.fastq.gz	1,353,570	65.86%
T17	Hugurtay	Striga	Stage1	Rep III	17_S9_L001_R1_001.fastq.gz	1,528,644	50.67%
T18	Hugurtay	Mock	Stage1	Rep III	18_S10_L001_R1_001.fastq.gz	1,599,895	60.54%
T19	N13	Striga	Stage1	Rep III	19_S11_L001_R1_001.fastq.gz	1,900,632	55.82%
T1	Hugurtay	Striga	Stage1	Rep I	1_S1_L001_R1_001.fastq.gz	884,317	61.92%
T20	N13	Mock	Stage1	Rep III	20_S12_L001_R1_001.fastq.gz	1,968,230	53.55%
T21	Hugurtay	Striga	Stage2	Rep III	21_S9_L001_R1_001.fastq.gz	1,670,597	63.20%
T22	Hugurtay	Mock	Stage2	Rep III	22_S10_L001_R1_001.fastq.gz	2,314,929	68.52%
T23	N13	Striga	Stage2	Rep III	23_S11_L001_R1_001.fastq.gz	2,754,129	61.14%
T24	N13	Mock	Stage2	Rep III	24_S12_L001_R1_001.fastq.gz	1,986,660	65.05%
T2	Hugurtay	Mock	Stage1	Rep I	2_S2_L001_R1_001.fastq.gz	668,883	70.37%
T3	N13	Striga	Stage1	Rep I	3_S3_L001_R1_001.fastq.gz	1,734,670	64.16%
T4	N13	Mock	Stage1	Rep I	4_S4_L001_R1_001.fastq.gz	1,618,849	55.96%
T5	Hugurtay	Striga	Stage2	Rep I	5_S1_L001_R1_001.fastq.gz	1,639,489	61.71%
T6	Hugurtay	Mock	Stage2	Rep I	6_S2_L001_R1_001.fastq.gz	1,840,473	66.51%
T7	N13	Striga	Stage2	Rep I	7_S3_L001_R1_001.fastq.gz	1,761,029	68.53%
T8	N13	Mock	Stage2	Rep I	8_S4_L001_R1_001.fastq.gz	2,384,624	69.75%
T9	Hugurtay	Striga	Stage1	Rep II	9_S5_L001_R1_001.fastq.gz	1,278,915	51.25%
					Total	40,377,977	14.5875
					Mean	1,682,416	0.6078125

Appendix 6: SSR genotyping results for *Striga* resistance in sorghum accessions from Eritrea

	QTL A				QTL B						QTL I				QTL J1				QTL J2			
Sample ID	Xtxp 208		Xtxp 302		Xtxp 201		Xtxp 050		Xtxp304		Xtxp 145		Xtxp 057		Xtxp 065		Xtxp 303		Xtxp 015		Xtxp 225	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
N13	260	260	237	237	184	184	300	300	243	243	244	244	241	241	130	130	153	153	217	217	163	189
N13	260	260	237	237	184	184	300	300	243	243	244	244	241	241	130	130	153	153	217	217	163	189
EG_1075	257	257	198	198	184	184	298	298	243	243	238	246	247	247	133	133	165	165	213	213	161	177
EG_1076	257	257	198	198	198	198	298	306	243	243	244	244	251	251			153	153			139	139
EG_1157	257	260	180	180	216	216	300	314	213	213	194	194	243	243	125	133	155	155	213	213	165	169
EG_1168	260	260	237	237	184	184	300	300	243	243	244	244	241	241			153	153			139	139
EG_1172	254	254	210	210	202	202	298	306	252	252			225	225	133	133			217	217	163	173
EG_1208	257	257	195	195	212	212	300	300	213	213			247	247	133	133	155	155	215	215	163	163
EG_1224			186	186	202	202	298	318	213	213	212	212	247	247			155	155	169	169		
EG_1233	257	257	195	195	206	206	294	294	216	216	212	212	247	247	133	133	155	155	215	215	163	169
EG_1235	260	260	180	180	206	206	296	316	216	249	244	244	241	241	133	133	155	155	215	215	163	169
EG_1237	257	257	213	213	184	198	300	300	258	258	238	246	247	247	133	133	165	165	215	215	163	177
EG_1239	260	260	180	243	184	184	300	312	213	213	228	238	239	239	130	130	153	153	213	213	161	181
EG_1246			162	162			308	316	213	213	238	244	247	247	133	133	165	165	213	213	163	173
EG_1256	257	257	180	180	202	202	306	306	237	237	238	238	247	247	133	133	149	149	217	217	161	171
EG_1257	257	257	186	186	202	202	318	318	216	216			247	247	122	122	165	165	169	207	165	169
EG_1258	257	257	180	180	220	220	294	294	213	213			247	247			165	165	221	221	181	181
EG_1261	257	260	186	186	206	206	310	310	237	237	236	236	249	249	133	133	171	171	215	215	161	177
EG_2161	257	257	213	213	184	184	298	306	240	240	240	240	241	247	133	133	153	165	213	213	163	177
EG_2453	254	254	201	201			298	306	243	243	244	244	241	241	128	133	149	165	213	213	163	171
EG_2456	260	260			184	184	312	312	252	252	230	230	247	247	133	133	153	153	213	213	165	165
EG_2457	257	257	210	210	182	202	308	308	237	237	238	238	241	241	131	131	149	149	213	221	163	177
EG_469	257	257	195	195	184	184	306	306	225	225	226	226			133	133	149	149	213	213	165	171
EG_473	257	257	186	186	184	184	310	310	237	237	244	244	249	249			149	149				
EG_480	257	257	212	212	184	184	304	314	237	237	238	238	243	243	133	133	147	147	213	213	163	171

Appendix 6 continued……

	QTL A				QTL B						QTL I				QTL J1				QTL J2			
Sample ID	Xtxp 208		Xtxp 302		Xtxp 201		Xtxp 050		Xtxp304		Xtxp 145		Xtxp 057		Xtxp 065		Xtxp 303		Xtxp 015		Xtxp 225	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
EG_494	257	257	213	213	202	202	302	302	237	237	236	236	235	235	133	133	147	149	213	213	163	177
EG_497			165	165			308	308	234	234	236	236	243	243	128	128	149	149	213	213	161	169
EG_519	257	257	213	213	184	198	306	314	237	237	236	236	247	247	133	133	153	165	213	213	165	189
EG_526	257	257	212	212	202	202	302	302	237	237	236	236	235	235	133	133	147	149	213	213	163	177
EG_532	257	257	198	198	202	202	306	314	234	234	236	236	247	247	133	133	149	165	215	215	163	171
EG_537	257	257	198	198	202	202	306	306	234	234	236	236	247	247	133	133			213	213	163	171
EG_538	257	257	213	213	184	198	298	314	237	237	236	236	247	247	133	133	165	165	213	213	161	177
EG_540	257	257	198	198	202	202	306	306	228	228	226	226	241	241	133	133	149	155	213	213	163	171
EG_544	257	257	213	213	184	184	300	310	245	245	244	244	247	247	128	128	153	153	201	201	161	161
EG_546	254	257	180	180	202	202	298	314	249	249	238	244	243	243	128	128	147	165	213	213	161	161
EG_547															133	133	149	165	213	213	163	177
EG_554	257	257	198	198	202	202					246	246	247	247	125	133	149	149	213	213	161	171
EG_555	257	257	213	213	184	198	306	314	258	258	228	228	247	247	133	133	149	165	213	213	163	171
EG_557	257	257	213	213	184	198	306	314					205	205	128	128			213	213	161	171
EG_584	257	257	210	210	206	206	306	306	237	237	236	236	239	239	133	133	149	155	213	213	161	169
EG_711	257	257	180	180	202	202	306	306	243	243	236	246	247	247			149	149				
EG_717	260	260	180	180	198	198	300	308	245	245	244	244	247	247	133	133	153	153	213	213	163	173
EG_723	257	257	180	180	206	206	306	306	240	240	240	240	251	251	133	133	147	149	213	213	163	177
EG_724	257	257	195	195	202	202	300	316	233	255	236	236	247	247	133	133	153	153	213	213	163	177
EG_726	257	257	219	219	184	184	298	306	249	249	238	238	247	247	133	133	153	165	207	207	163	163
EG_732	260	260	195	195	198	198	300	300	225	225	226	226	243	243	133	133	149	149	213	213	161	171
EG_735	257	257	213	213	198	198	306	306	237	237	238	238	247	247	133	133	147	147	213	213	163	189
EG_736	257	257	213	213	198	198	306	306	237	237	236	236	247	247	133	133	147	147	213	213	165	189
EG_746	257	257	213	213	184	198	306	306	255	255	244	244	243	243	133	133	153	165	213	213	161	173

Appendix 6 continued……

	QTL A				QTL B						QTL I				QTL J1				QTL J2			
Sample ID	Xtxp 208		Xtxp 302		Xtxp 201		Xtxp 050		Xtxp304		Xtxp 145		Xtxp 057		Xtxp 065		Xtxp 303		Xtxp 015		Xtxp 225	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
EG_750	257	257	195	195	198	198	298	306	225	225	226	226	243	243	133	133	149	149	213	213	171	171
EG_756	257	257	180	180	206	206	314	314	261	261	244	244	247	247	128	133	165	165	213	213	163	171
EG_779	257	257	180	180	184	202	300	316	258	258	226	244	247	247	133	133	165	165	213	213		
EG_782	257	257	210	210	184	184	300	308	255	255	230	230	241	241	133	133	149	155	213	213	161	171
EG_783	257	257	225	225			306	306	237	237	238	238	243	243	133	133	149	149	213	227	163	171
EG_786	257	257	186	186	184	184	310	310	237	237	238	238	249	249	133	133	149	149	221	221	163	171
EG_787							308	310	237	237	236	236	247	249	133	133	149	149	223	223	171	171
EG_789	257	257	180	180	206	206	296	314	216	216			243	243			165	165	201	201		
EG_791	257	257	180	180	206	206	296	314	216	216	238	238	247	247	133	133	165	165	207	207	165	171
EG_794	257	257	180	180	206	206	306	306			238	238	247	247	133	133	149	149	223	223	161	171
EG_797			150	150	262	262	310	310	243	243	238	238	249	249	128	128	149	149	221	221	161	177
EG_801	260	260	219	219	184	198	306	306	258	258	238	238	247	247	133	133	153	153	213	213	161	171
EG_806	257	257	180	180	206	206	310	310	237	237	238	238	249	249	133	133	149	149	221	221	161	171
EG_812	257	257	180	180	202	202	306	306			216	246	239	249	128	133	149	149	221	221	161	171
EG_813	257	257	180	180			306	310	243	243	244	244	249	249	133	133	149	149	221	221	161	171
EG_815	257	257	195	195	206	206	314	314			238	238	235	247	133	133	165	165	207	213	165	171
EG_830	257	257	216	216			314	314			240	240	241	241	128	128			213	213	161	169
EG_836	257	257	180	198	202	202	306	306	234	234	236	246	247	247	133	133	149	149	213	221	161	169
EG_843	257	257	195	195	202	202	306	314	233	233	236	236	243	243			149	149				
EG_845	257	257	210	210	202	202	298	306	258	258	240	240	239	243	128	133	149	149	213	221	161	171
EG_846	257	257	186	186			310	310	243	243	244	244	243	243	130	130	149	149	223	223	161	171
EG_849	257	257	180	180	202	202	306	306	237	237	236	236	249	249			165	165				
EG_850	257	257	210	210	184	198	300	300	255	255	238	238	247	247	133	133	165	165	213	213	163	177
EG_855	257	257	180	195	198	202	298	306			238	246	247	249	133	133	153	153	213	213	161	161

Appendix 6 continued……

	QTL A				QTL B						QTL I				QTL J1				QTL J2			
Sample ID	Xtxp 208		Xtxp 302		Xtxp 201		Xtxp 050		Xtxp304		Xtxp 145		Xtxp 057		Xtxp 065		Xtxp 303		Xtxp 015		Xtxp 225	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
EG_857	257	257	180	195	198	198	304	304	231	231	234	246	247	247	125	133	149	153	215	215	163	163
EG_858	257	257	212	212	198	198	298	298	255	255	236	236	247	247	133	133	153	165	213	213	161	171
EG_859	257	257	195	195	202	202	298	306	243	243	244	244	247	247	125	133	149	153	213	213	163	173
EG_864	257	257	195	195	202	202	298	298	258	258	234	234	247	247	133	133	155	155	213	213	161	171
EG_870	257	257			206	206	296	314	213	213	212	212	247	247	133	133	165	165	169	215	163	169
EG_873	257	257	180	180			306	306	231	231	234	234	247	247	128	128	149	149	223	223	161	171
EG_875	257	257	180	180	206	206	314	314	213	213	212	212	247	247	133	133	165	165	207	207	165	169
EG_881	257	257	210	210	184	184	306	306	225	225	226	244			130	130	149	149	215	215	165	171
EG_883	257	257	210	210	202	202	300	300	225	225	226	226	239	239	128	128	149	149	215	215	161	169
EG_885	257	257	210	210	202	202	306	306	258	258	226	226	239	239	128	133	149	149	213	213	161	169
EG_889	257	257	210	210	202	202	298	306	258	258	230	230	247	247	133	133	149	165	215	215	161	161
EG_890	257	257	198	210	202	202	298	298	255	255	226	226	239	239	128	128	149	149	213	213	161	169
EG_893	257	257	210	210	202	202	300	300	255	255	226	226	239	239	128	128	149	149	213	213	161	169
EG_896	257	257	195	195	202	202	300	300	225	258	226	226	243	243	133	133	149	149	213	213	163	163
EG_898	257	257	180	180	184	202	300	312	216	216			241	241	133	133	155	155	213	213	161	181
EG745	257	257	216	216	184	184	298	306	255	255	236	240	241	241	133	133	165	165	213	213	161	173
H-35-1									213	213			243	243			155	155			163	163
Hamelmalo							296	296	213	213	212	212	247	247	128	128	155	155	215	215	165	169
ICSV_111	260	260	201	201	194	194	300	300					205	205	128	128			217	217	165	169
IS9830	257	257	195	195	232	232	294	294	258	258	216	216	247	247	133	133	155	155	215	215	163	177
Kibra	257	257	198	219	206	206	306	314	243	243	252	252	247	247			149	149				