

**EVALUATION OF PRE-ATTACHMENT AND POST-ATTACHMENT  
*Striga hermonthica* (del.) BENTH. RESISTANCE IN PEARL MILLET  
(*Pennisetum glaucum* (L.) )**

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**DECLARATION**

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


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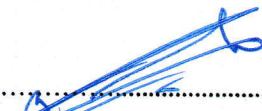
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## **DEDICATION**

I dedicate this work to my late best friend Anselme Desire Akotan.

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**ABBREVIATIONS AND ACRONYMS**

<b>\$</b>	Dollar
<b>%</b>	Percentage
<b>°C</b>	Degree Celsius
<b>ANOVA</b>	Analysis Of Variance
<b>CDR</b>	Completely Design Randomized
<b>DAI</b>	Day After Infection
<b>FAO</b>	Food and Agriculture Organization
<b>GFF</b>	Glass Fiber Filter
<b>HAI</b>	Hour after infection
<b>ICRISAT</b>	International Crops Research Institute for the Semi-Arid Tropics
<b>KU</b>	Kenyatta University
<b>MGD</b>	Maximum Germination Distance
<b>PTL</b>	Plant Transformation Laboratory
<b>SSA</b>	Sub-Saharan Africa

## ABSTRACT

*Striga* species are parasitic weeds, one of the most devastating biological constraint threatening agriculture especially production of cereals in sub-Saharan Africa (SSA), causing approximately 30% to near crop failure, thus endangering the livelihood of more than 300 million poor smallholder farmers. One way of combating *Striga* is having natural host resistance which is cost effective and efficient for tolerance to phytopathogenic damages. Such resistance can either be due to failure of the host to stimulate germination of the parasite (pre-germination resistance) or blockage of the parasite penetration by the host (post-germination resistance). From field evaluations, wild pearl millet (*Pennisetum glaucum*) accession 29 AW was shown to get less *Striga* attachments compared to the cultivated variety, SOSAT-C88 P10. However, the mechanism of resistance was not established. The aims of this study were to investigate pre- and post-attachment *Striga* resistance in pearl millet using SOSAT-C88 P10, a cultivated susceptible pearl millet and 29 AW. Pre-germination resistance was determined using an agar-gel assay which measured the maximum germination distance (MGD), whereas post-germination resistance was determined using rhizotron based-approach as well as histological analyses. A high *Striga* germination activity was observed in SOSAT-C88 P10 (MGD=  $35.94 \pm 2.88 > 10$  mm) coupled with elevated percentage of germinated *Striga* seeds (>85.04%) compared to 29 AW (MGD=  $7.96 \pm 2.75$  mm <10 mm) with few sprouts (8.14 %) of the parasite. Moreover, macroscopic screening showed that there were highly significant differences between SOSAT-C88 P10 and 29 AW in terms of number of *Striga* attachments, *Striga* dry weight and the length of *Striga* seedlings (P-value < 0.05). SOSAT-C88 P10 had many *Striga* attachments, with much longer *Striga* seedlings with significant weight, while, 29 AW had very few *Striga* seedlings attachment, smaller and slow growth seedlings with lower biomass. Further histological analyses revealed that the high number of attachments of *Striga* on SOSAT-C88 P10 compared to 29 AW was due to biochemical or physiological barriers at host endodermis layer. This work therefore demonstrated that there are multiple forms of *Striga* resistance (pre- and post-attachment) in wild pearl millet 29 AW. These findings will be useful for future resistance breeding of pearl millet.

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background information

*Striga* is a flowering witchweed which belongs to Orobanchaceae family. It is one of the most challenging pests to agriculture globally, particularly, in sub-Saharan Africa (SSA) and parts of Asia. The genus *Striga* has approximately 30 species amongst which five are considered socio-economically important (Ejeta, 2007a; Atera *et al.*, 2011). These include *Striga hermonthica*, *S. asiatica*, *S. gesnerioides*, *S. aspera*, and *S. forbesii*.

In Africa alone, about \$ 117 million of economic losses have been reported due to *Striga* infestation perennially. This has compromised the livelihood of over 300 million small-scale poor farmers, with some forced to abandon their farms due to crops failure especially when compounded by poor edaphic factors (Ejeta, 2007a; Atera *et al.*, 2012). *Striga* seeds are very tiny and tons of them are viable in soil for over 14 years, coupled with underground pathogenicity, this makes the management difficult (Kountche *et al.*, 2016).

Although research efforts have led to the development of many conventional control measures employed by farmers, these strategies are yet to be successful because of the complexity of *Striga* parasitism. Conventional management methods used include: hand-pulling and burning, weeding, intercropping as well as the use of herbicide and resistant varieties of which the resistance is breached over time due new parasite ecotypes (Atera *et al.*, 2012, Kountche *et al.*, 2013a).

Pearl millet (*Pennisetum glaucum* L. R. Br.) is a diploid species belonging to the Poaceae family and widely grown in Arid and Semi-Arid zones in Africa and parts of Asia. Unlike other cereals, pearl millet nutritional value especially its high iron content makes it a staple food for the marginalized in these dry regions (Shukla *et al.*, 2015). It is capable to adapt and grow in difficult agro ecological zone and its fairly tolerate drought (Kountche *et al.*, 2013a). Recently, genomics studies unraveled the sustainability of pearl millet to an increased 40° C (Varshney *et al.*, 2017) untolerated by other cereals like rice, maize and sorghum, ranking it as climate smart crop (Kountche *et al.*, 2016).

*Striga* parasitism in sorghum, rice, maize and cowpea, among other crops have been documented, however, there have been less focus on understanding the mechanism of defense to *Striga* in pearl millet. Wilson *et al.* (2004) demonstrated the resistance to *Striga* in four wild relatives (PSs 202, 637, 639, and 727) of pearl millet under field conditions, however, the mechanism of resistance remained unknown. Remarkably, Kountche *et al.* (2013b) had shown in six pearl millet landraces Ms 141; 239; 029; 197; 017 and KBH the resistance to *Striga* in the fields.

*Striga* resistance in pearl millet is not well documented, with no existing finding that have shown pre-attachment resistance in millet, particularly, pre- and post-attachment resistance in a single genotype. To-date, the observed *Striga* resistance in host plants are

of two types: pre-attachment resistance and post-attachment resistance. In the former type of resistance, the host plant exudates reduce germination stimulant, inhibits or reduce germination and haustorial initiation, whereas the post-attachment resistance occurs after haustorial formation, and is referred as abiosis which involves production of cytotoxic compounds (Timko and Scholes, 2013). Laboratory based techniques for screening for *Striga* resistance are well known in describing these types of resistance because they have the advantage that mirror the field based resistance by assessing the individual mechanism of *Striga* resistance (Hausmann *et al.*, 2000; Kountche *et al.*, 2016).

In this study, two pearl millet genotypes were used: SOSAT-C88 P10 a susceptible cultivar and 29 AW, a wild relative millet. SOSAT-C88 P10 also known as LCIC-MV1, is an opened-pollinated improved variety derivative of landraces Souna and Sanio cross from Mali. It had been released in Nigeria for its high yielding characteristics and is one of the most popular pearl millet variety in Burkina Faso and Niger (Wilson *et al.*, 2008; Izge *et al.*, 2013). Besides, SOSAT-C88 P10 has an early maturity attribute, diseases resistance in particular to downy mildew and root-knot nematode, making it farmers preferred variety (Omanya *et al.*, 2007; Izge *et al.*, 2013). Despite its preferred traits, SOSAT-C88 P10 is susceptible to *Striga hermonthica*. Unlike SOSAT-C88 P10, 29 AW, a wild relative of pearl millet exhibits resistance to *Striga* in the field.

## 1.2 Problem statement

Pearl millet is one of the crops that are fairly tolerant to drought, the only cereal which resists increased temperatures of over 42° C and has high nutritional values (ref). Also known as a witchweed, *Striga* is an obligate root hemi parasite threatening grain crops (Estep *et al.*, 2011), including pearl millet leading to yield losses of between 20-80% in the fields and, in the worst cases, up to 100% of the yield is destroyed (Atera *et al.*, 2011; Kountche *et al.*, 2013b).

To date, the mechanism of *Striga* resistance in pearl millet has not been investigated (Kountche *et al.*, 2013b). As there is no complete host-resistance, continuous search of alternative approaches to subvert parasitism during infestations is necessary (Parker, 2012), especially the identification of genes with resistant alleles may help to sustain the resistance stability over time in crop plant (Kountche *et al.*, 2013b). Therefore, understanding the basis underlying *Striga* resistance in pearl millet will help in improving farmers preferred cultivars through molecular breeding

## 1.3 Justification

In Africa, by 2005 losses in 25 countries due to *Striga* infestation had reached 60% (De Groot *et al.*, 2008) . This endangered the livelihood of more than 100 million smallholder famers with 1 billion \$ annual loss estimated in Africa (Waruru, 2013). The manifestation is attributed to *Striga* ability to produce and scatter widely, high amount

of long-lived tiny seeds forming persistent seed bank storage in soil for over 10 years and causing damages underground before its emergence (Atera *et al.*, 2011).

#### **1.4 Null hypotheses**

- i. There is no pre-attachment *Striga* resistance in pearl millet.
- ii. There is no post- attachment *Striga* resistance in pearl millet.

#### **1.5 Objectives**

##### **1.5.1 General objective**

The main objective of this study was to determine the mechanisms of *Striga hermonthica* resistance in Pearl millet.

##### **1.5.2 Specific objectives**

- i. To determine the mechanisms of pre-attachment *Striga* resistance in pearl millet
- ii. To determine the mechanisms of post-attachment *Striga* resistance in pearl millet

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Classification and ecology of pearl millet

Pearl millet (*Pennisetum glaucum* L.) accounts for 50% of cultivated millet species. *P. glaucum* is a cross pollinated species with 85% outcrossing, diploid. Its origin over the decades had been subject of controversial debate among scholars, while Ethiopia and Sudan are believed to be the origin due to their diversity presence (Vavilov, 1940-1950). However, morphological variability and B-chromosome investigation indicated that pearl millet originated from Central Africa; Nigeria and Sudan respectively (Burton *et al.*, 1968; Muntzing, 1958). Later, archeological, geographical distribution and phylogeny analysis showed that pearl millet had been domesticated in sub-Saharan Africa (Brunken, 1977; Oumar *et al.*, 2008; Hu *et al.*, 2015), and adopted in India.

Pearl millet belongs to the Poaceae family and *Pennisetum* genus, the largest with over 140 species in the tribe Paniceae with pearl millet being the most important species. The genus *Pennisetum* contains four taxa (Meredith, 1955), which include *P. albicauda*, *P. nigritarun*, *P. echinurus* and *P. americanum*, the latter has three sub-species (ssp.), *ssp. Americanum*, *ssp. Monodii* and *ssp. Stenostachyum* (Brunken, 1977). There are 4 races of pearl millet: the typhoides, nigritarun, globossum and leonis grown in India;

western Sudan to Northern Nigeria; Benin, Ghana, Niger Central Nigeria and Togo, Senegal and Mauritania respectively (Mathur *et al.*, 2012)

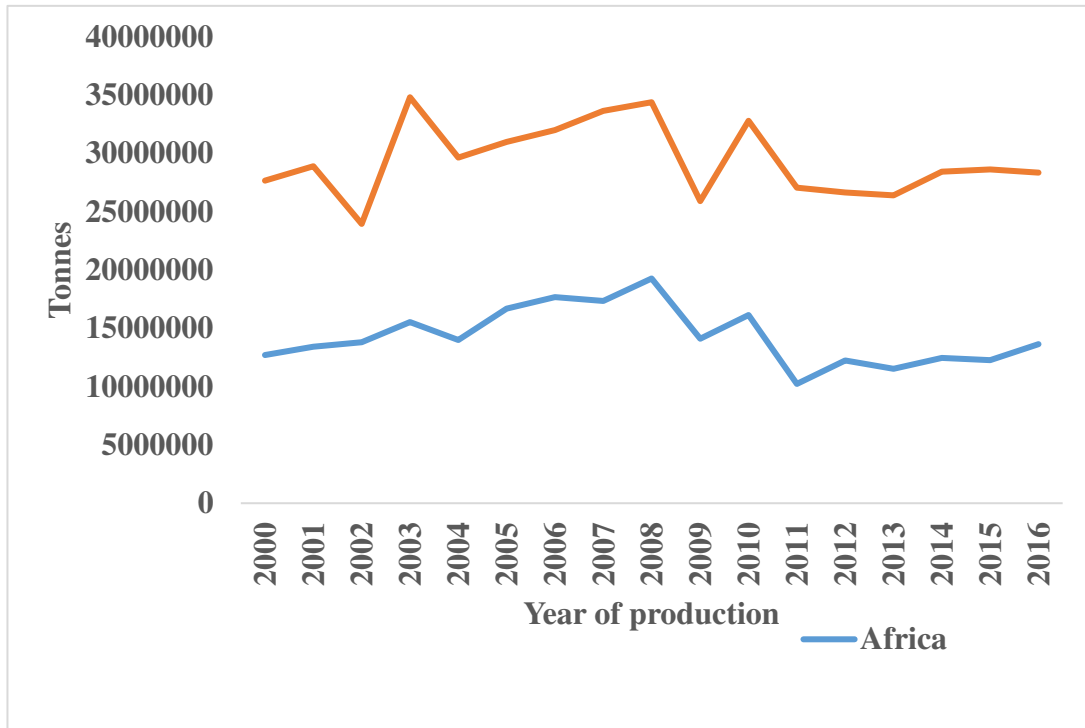
## **2.2 Constraints to pearl millet production of**

In 2017, FAOSTAT showed that pearl millet production dwindled in Africa as well as globally (Figure 2.1) which can be attributed to abiotic and biotic conditions. Pearl millet is affected by an array of biotic stresses which includes fungi, viruses, bacteria, insects and parasitic weed. Several pearl millet diseases have been reported but only five are considered economically important. These are downy mildew (DM), rust, blast, ergot, and smut (Thakur *et al.*, 2011).

Downy mildew is the most economically important fungal disease of pearl millet, caused by an obligate oomycete *Sclerospora graminicola* (Sacc.), 40%, 50-70%, 60% and 20% yield losses in India, Nigeria, Mozambique and globally respectively (Kumar, 2011; PDM, 2015). Fungal infection by *Puccinia substriata* and *Pyricularia grisea* Sacc., the most devastating foliar diseases causing rust and blast respectively dwindles the fodder and grain produce of pearl millet. *Puccinia substriata* occurrence in Africa, Asia and USA has effects adding to the 70% global loss perennially (PDM, 2015). In Uganda rust been shown to reduce pearl millet yield to 73.58%, whereas losses attributed to blast were estimated at 62.2% (Lubadde *et al.*, 2014). Ergot and smut are mainly panicles and tissue specific diseases which occur wherever pearl millet is grown

especially in Africa, India and USA, and which are elicited by *Claviceps fusiformis* Loveless and *Moesziomyces penicillariae* Bref. Vanky.

Weeds are amongst the major biotic stresses threatening agriculture worldwide. They compete with crops for nutrients, water, sunlight and space, among other factors. In sorghum and millets species, damages on yields due to weed have been estimated at 50-94% (Mishra, 2016). In pearl millet, most weeds affecting the production are autotrophic such as grasses, broadleaf species, sedges; and *Striga* species, specifically *Striga hermonthica* are the most virulent parasitic weed, which can sap up 80% of pearl millet Carbon (C), 66% C in sorghum, 40% in maize, while *Striga gesnerioides* has been estimated to draw up to 99% C from cowpea (Press and Graves, 1991). *Striga* (witch weed) are obligate root parasitic weed which belongs to Orobanchaceae family, with four *Striga* species namely, *Striga asiatica*, *Striga gesnerioides*, *Striga aspera* and *Striga hermonthica* being the most economically important with the latter notoriously the most threat to cereals. *Striga* affects 40% of arable Savanna region with an estimated \$ 13 million losses and may even results in complete crop failure in some cases. Kountche *et al.* (2013b) reported a varied 30-100% yield losses annually associated with *Striga*. Other millet constraints are pests which include insects and birds like red-billed known as *Quelea quelea* the most devastating wild bird in the world with damages estimated at US\$ 8 million per annum across Africa (PDM, 2015).



**Figure 2.1:** Production/yield quantities trends of pear millet (FAOSTAT, 2017)

### 2.3 Economic importance of pearl millet

In the fields, not only does the production of Pearl millet constrains limited to biotic factors only but also a myriad of abiotic elements like edaphic factors, drought and rising temperatures across the globe. The semi-arid areas in Africa and Asia which are dependent on rains are adversely affected. Although pearl millet is fairly tolerant to drought, salinity and poor edaphic conditions compared to other crops in the semi-arid areas with about 40% cultivation.

According to Varshney *et al.*, (2017), pearl millet tolerates high temperature ( $> 42^{\circ} \text{C}$ ), thus can be used as a crop for climate change adaptation. Therefore, Pearl millet ranks top as preferable crop in a number of countries in the semi-arid tropics where agriculture is dependent on rain while more than 90 Million farmers. In these regions including SSA and parts of Asia use it both as cash crop and staple food due to its high nutritional profile, besides utilization as forage crop for cattle (ICRISAT, 2013; Okech *et al.*, 2016). The nutritional content provides energy, micronutrients, protein (11-12%) with remarkable amino acid profile compared to other cereals (Amarender *et al.*, 2013; Shukla *et al.*, 2015).

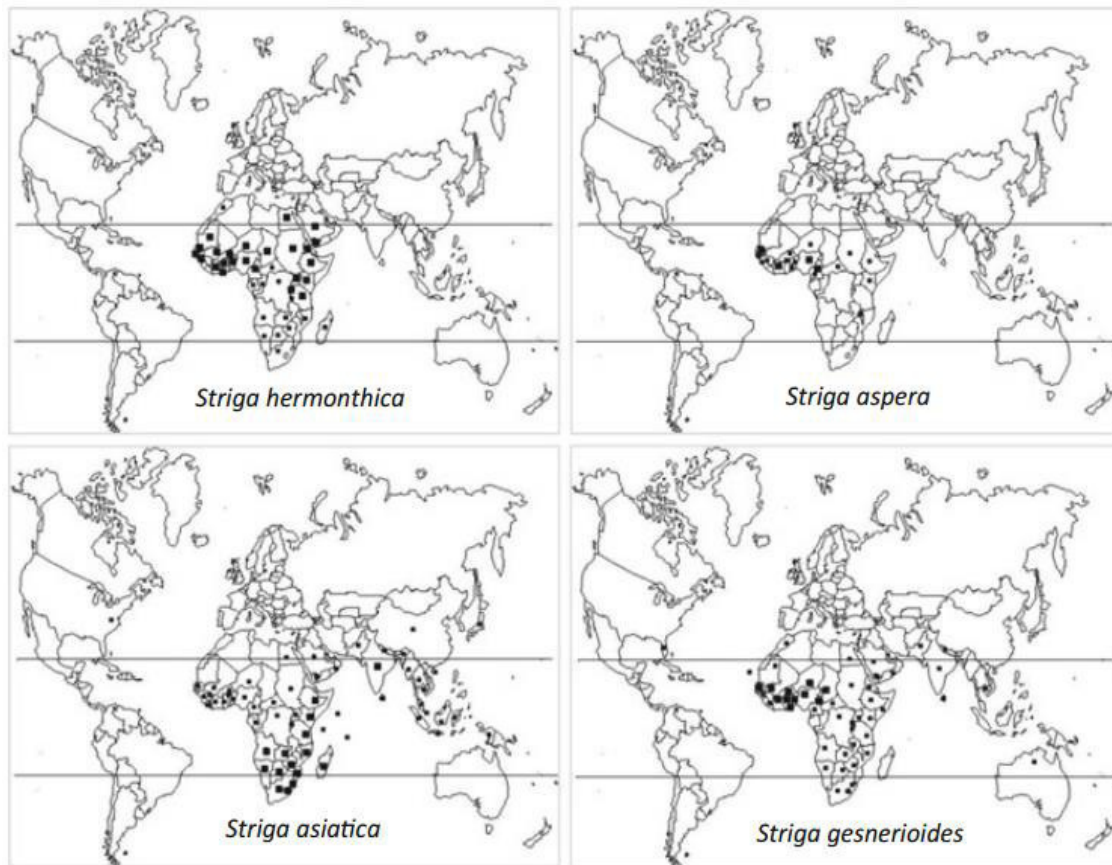
To-date, bioenergy production has been given much attention as a source of renewable energy to substitute fossils sources. Many annual and perennial plants are being researched on like oilseed plants, grasses and sweet sorghum, among others. Even though little research has been done on ethanol production from pearl millet, investigations by Wu *et al.* (2006) and Wilson *et al.* (2007) indicated that pearl millet is a potential source for biofuel production, with a yield 8% less than ethanol production from maize. They also reported that in the process of Ethanol production, 85% fermentation could be reached in pearl millet 12 hr earlier than in maize, suggesting pearl millet a better bioenergy producer especially in dry regions where maize production is limited by rains.

## 2.4 Classification, host range and distribution of *Striga*

*Striga*, a member of Orobanchaceae family, is the greatest threat to agricultural production, especially for cereals, and amongst the 28 species of the genus *Striga*, four



**Figure 2.2:** Economically important *Striga* species (a) *S. hermonthica* in Sorghum; (b) *S. asiatica*; (c) *S. gesnerioides*; (d) *S. aspera* (Adapted from Parker, 2013)



**Figure 2.3:** World distribution of four economically important *Striga* species (Parker, 2013). The black dots represent areas where *Striga* infestation occurrence are of economic importance (Figure 2.2) with a wide host range found worldwide (Figure 2.3) namely; *S. hermonthica* (Del) Benth and *S. asiatica* (L.) Kuntze parasitize cereals, *S. gesnerioides* (Willd.) Vatke affects legumes (cowpea) and tobacco and *S. aspera* (Willd.) Benth (Parker, 2012).

## **2.5 Economic impact, biology and life cycle of *Striga***

### **2.5.1 Economic impact**

The four widely spread *Striga* Species (Figure 2.3) namely: *S. asiatica* (L.) Kuntze, *S. gesnerioides* (Willd.) Vatke, *S. aspera* and *Striga hermonthica* (Del.) Benth., the latter being the most devastating, have an equally wide spread host range including grasses and legumes (Berhane, 2016), hence the prevalence biotic constrain to agricultural production in Asia and especially Africa with 25 nations documented by 2005 (De Groot *et al.*, 2008 ) having *Striga* infestation.

*Striga hermonthica* and *Striga asiatica* are the most prevalent *Striga* species in pearl millet fields, causing havoc to the poor farmers partly due to lack of measures to handle *Striga*. . In sub-Saharan Africa, it has been reported that 50 million hectares of cereals and legumes plantations have been infested by *Striga* species with yield losses valued at over US\$ 10 billion annually (Ejeta, 2007a; Scholes and Press, 2008; Westwood *et al.*, 2012), thus putting livelihood of more than 300 million people in jeopardy. In Kenya, surveys by Atera *et al.* (2011) indicated that more than 70% of sorghum and maize yields were lost due to *Striga* infestation resulting into the abandonment of the *Striga*-prone fields. These loses depend on the severity of the attack, susceptibility of host, environmental and control measures (Berhane, 2016) taken by the farmers.

### 2.5.2 Life cycle of *Striga*

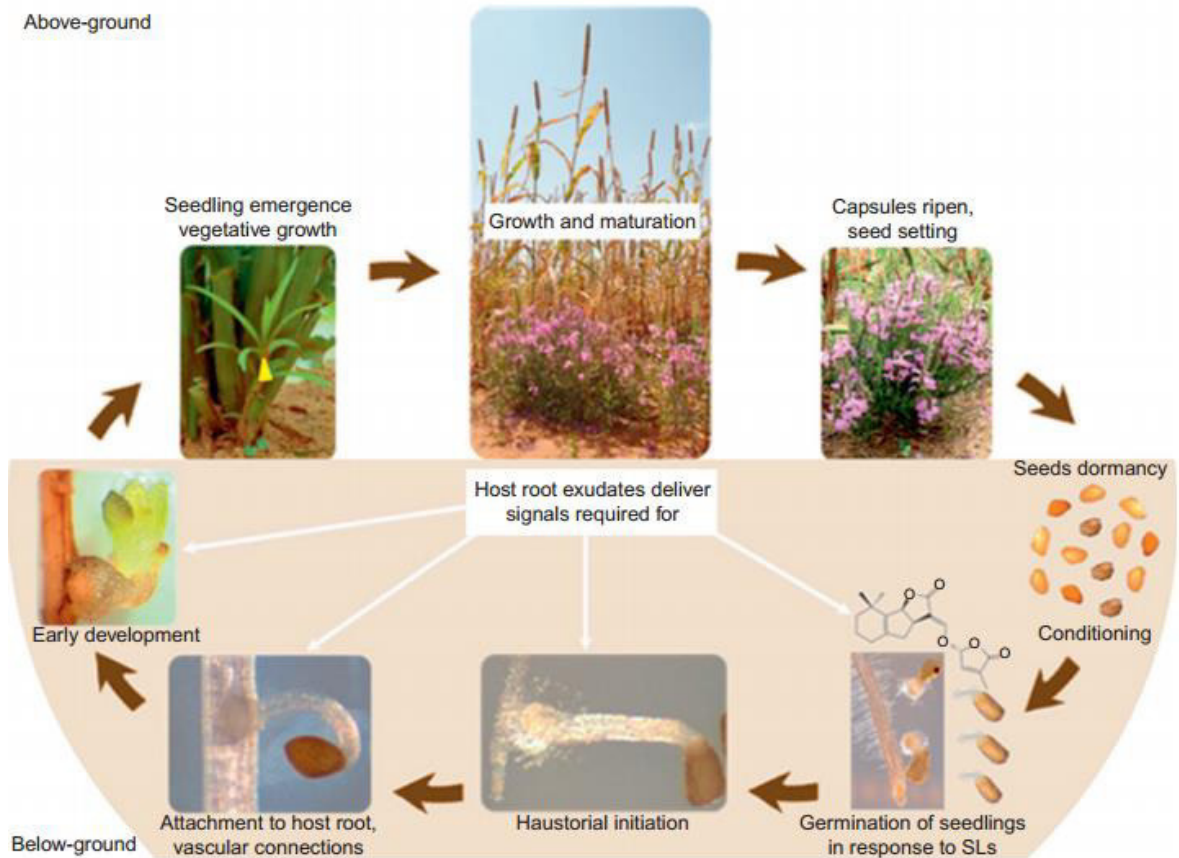
*Striga* is an annual and hemiparasitic weed with some of its species being autogamous, *Striga asiatica*, on the other hand, *Striga hermonthica* is allogamous viz. requiring pollinators which contribute to its high genetic diversity. *Striga* life cycle (Figure 2.4) starts from the germination, haustorium initiation and host recognition and parasitism with (Kountche *et al.*, 2016) constituting a huge *Striga* seeds genbank underground, which can survive for over 14 years.

Germination of *Striga* seeds is promoted by the breakage of seeds dormancy known as seeds conditioned or preconditioned through warm moisture conditions offer by rains and hosts root exudate. After *Striga* seeds are conditioned, they require stimulant to trigger the germination, otherwise they will fall into a second period of dormancy. The *Striga* seeds germination stimulants which include dihydrosorogoleone, sesquiterpene, ethylene and strigolactones are some of the most potent and studied inducers.

Once the seeds germinated, the radicle grow by chemotropism towards the host roots, where they differentiate into haustorium, after they reach touch the roots. A haustorium is a parasitic organ that attaches and establishes vascular connection, metabolic and osmotic link with the host (Atera, 2011; Spallek *et al.*, 2013). Haustorial initiation factors (HIFs) like Kinetin and quinones such as 2,6-dimethoxy-1,4-benzoquinone (DMBQ) mediate haustorium formation. HIFs, especially quinones are used by *Striga*

species to locate a nearby and viable host. Besides chemicals signals, a mechanotropism response is also needed for a suitable development of haustorium.

Germinated *Striga* seeds last few days because they are endogenous storage lipids-dependent, which are depleted during germination and radicle elongation process. For example, 37% of *S. asiatica* seeds weight are storage lipids and this is used for physiological needs during germination. Thus, penetration into the host by haustorium formation is very important for rapid uptake of host nutrients and water (Rich *et al.*, 2007). No phloem-to-phloem connection has been investigated to-date, however, once xylem-to-xylem connection is established, the leaves cotyledons are free from the seed coat within a day (Ejeta, 2007b). Upon vascular connections, scale leaf pairs sprout along *Striga* stem, and within 6 weeks, it emerges above ground, followed by flowering and mature seeds production within 6 and 2 weeks after emergence and pollination respectively.



**Figure 2.4:** Life cycle of *Striga* (Kountche *et al.*, 2016)

## 2.6 Host plant defense

A myriad of pathogens invade a particular plant species, which are always met with resistance or tolerance by the host plant. Resistance in plant is the ability of the host to overcome the attack from a pathogen in order to prevent its ingress and growth, however, tolerance is the ability of the host to repair damages caused by the attacks by host responses (Timko and Scholes, 2013). A complete resistance to pathogenic infection has been reported, and wild relatives of some crops have been shown to exhibit

partial to full resistance or tolerance to parasitism because of their exposure to a mixture of pathogens in nature, whereas in cultivated species, the pattern of resistance is less due to genetic losses which is attributed to bottleneck effects.

Generally, plant response to pathogens by using innate immune system, the widely known model is ‘zig-zag’ (Jones and Dangl, 2006). Two types of innate immune systems exist in plant species: microbial/pathogen-associated molecular pattern (MAMP/PAMP) and effector-triggered immunity (ETI). The first type MAMP/PAMP surveillance mechanisms are recognized by pattern recognition receptors (PPRs) that leads to pathogen-triggered immunity (PTI) referred as to basal immunity, which halts survival and growth of parasites. As a counter-measure to PTI, plant pathogens have evolved number of effector proteins to suppress PTI through a subset of the effector known as resistance avirulence (Avr) proteins. Plants species have developed resistance genes (R) to recognize and inactivate parasitic effectors, which, leads to the second class ETI (Ahmed *et al.*, 2016). It is known to be a full long-term immune response mediated by programme cell death (PCD) reactions called hypersensitive response (HR). The effector-triggered immunity is also known as systemic acquired resistance (SAR) because it results from previous exposure of the host to pathogens (Walters *et al.*, 2008).

## **2.7 Pathogenic resistance in host-plants: nature and genetic**

Resistance to parasitic weed has been revealed to be either quantitative/polygenic regulated by many genes or qualitative/monogenic, governed by major genes.

Monogenic resistance (resistance mediated by one or two genes) is particular to a pathogenic race and can be overcome over time with evolution of new races. Unlike quantitative resistance which is partial defense to the host-plant, qualitative resistance provides a complete resistance (Das *et al.*, 2016; French *et al.*, 2016).

For instance, a single nuclear gene in sorghum and sorghum SRN-39-derived population (Vogler *et al.*, 1996) is reported to regulate both low germination stimulant and haustorial initiation (Ejeta, 2007b). Likewise, in cowpea B301, Suvita-2 and IT82D-849, resistance to *Striga gesnerioides* has been shown to be monogenic or qualitative (Touré *et al.*, 1997). However, like ETI, qualitative resistance is likely to be overcome over a period of time with the occurrence of new pathogen races as it is a race-specific resistance.

Unlike other types of resistance, quantitative/polygenic resistance is effective against several pathogens, reliable and has long term resistance inheritance (Das *et al.*, 2016). In contrast to previous investigation that showed monogenic resistance in sorghum to *Striga*, Haussmann *et al.* (2001) revealed that resistance to *Striga* in sorghum is under the control of many genes, thus appears to be quantitative as confirmed by Mohamed *et al.* (2003, 2010), indicating that the polygenic *Striga* resistance is governed by two major genes HR1 and HR2 in Sorghum.

## 2.8 Host-plant resistance mechanisms to parasitic *Striga*

Host-plant resistance has been categorized into two types namely: pre-attachment resistance and post-attachment resistance.

Pre-attachment resistance to *Striga* has been reported in some crops, for example, in Uganda, based on rhizotron screening techniques, Samejima *et al.* (2016) indicated that Umgar, a rice variety conferred resistance to *Striga*. Similarly, NERICA1 and CG14 have been also reported to exudate less strigolactones, thus less infection to *Striga* parasitism in comparison to NERICAs 7, 8, 11 and 14, therefore are a pre-attachment resistant to *Striga* based on strigolactones profiling studies.

In the latter type of resistance, the host-plant displays a number of defense responses such as abiosis, mechanical barriers, program cell death expressed in form of hypersensitive reaction (HR) and incompatible responses. Sorghum cultivars Framida, Dodds, SARs 16, 19, 33 and wild genotypes P47121 exhibited post-attachment resistance to *Striga asiatica* (Hausmann *et al.*, 2000; Mohamed *et al.*, 2003) through a HR, which has been reported in pearl millet cultivar SR-EC (Kountche *et al.*, 2016). However, reports on sorghum accession SRN39 indicated that it displayed incompatible response to *Striga hermonthica* (Amusan *et al.*, 2011), whereas, histological analysis on maize inbred lines Z.DiploBC<sub>14</sub>-19-4-1#-3-1-B-1-B-B (Z.DiploBC<sub>14</sub>), a *Striga* resistant, and 5057, a *Striga* susceptible genotype showed that Z.DiploBC<sub>14</sub> exhibited physical barriers and incompatible responses to *Striga* infection ( Amusan *et al.*, 2008).

Phenotypic screening rhizotron-based showed that Umgar, NERICAs 5, 13, 4, 18 (upland rice varieties) and Nipponbare (lowland rice variety) are post-resistant to *Striga hermonthica*.

## 2.9 Technologies for *Striga* control

### 2.9.1 Conventional methods for *Striga* management

Many control measures have been developed and employed by farmers so far to manage *Striga* infestation. These include hoeing, tap crops and catch crops, push-pull technology, intercropping, polycultures, use of manure, fertilizer and herbicides (Teka, 2014). Some of the strategies and their limitation are presented in Table 2.1.

**TABLE 2.1:** *Striga* control options and their limitation

Strategies	Advantages	Setback
Manual weeding	Reduction of <i>Striga</i> seeds bank, easy implementation	Yield benefit is not immediate, labor intensive
Crop rotation	Increase soil fertility, reduction of <i>Striga</i> seeds bank	Benefit accrument requires time, costly as per family food requirement
Hand pulling	Reduction of <i>Striga</i> seeds bank if done before flowering	Inappropriate disposal, increase seeds bank
Trap crop (allelopathic effect)	Reduction of <i>Striga</i> incidence, increase yield, provide livestock fed	Crop uneconomical to farmers without livestock

**Source:** Atera *et al.* (2013)

### **2.9.2 Approaches for screening for *Striga* resistance in crops**

A number of techniques have been developed for screening crops for *Striga* resistance *in vitro* as well as under the field conditions. These screening methodologies (Table 2.2) aimed at direct selection or indirect selection of resistant genotypes among cultivated varieties and their wild relatives for resistance breeding against the witchweed *Striga*.

**Table 2.2:** Screening methods for *Striga* resistance

<b>Aim of the screening methodologies</b>	<b>Techniques for screening to <i>Striga</i></b>	<b>Advantages</b>	<b>References</b>
Identification of low production of germination stimulant for <i>Striga</i>	Pot screening	Screen for additive resistance mechanism, control over environmental factors, high germination percentage, inexpensive	<b>11, 4</b>
	Sandwich screening		
	Pasteur pipette screening		
	In-vitro agar gel assays	Simple, easy and quick, positive and strong correlation with field resistance, reliable and reproducible	<b>4, 5</b>
	Eplee bag	Easy assessment of seeds death causes, screen <i>in situ</i>	<b>9, 8</b>
	Cut-root assay	High germination rate, cost-efficient, easy to execute, more sensitive	<b>2, 1</b>
	Root-exudate technique	Straightforward method	<b>1</b>
Identification of anti-haustorial factors	Root-slope	Stimulate chemical factors that induce mechanical resistance	<b>9</b>
	Seed-pan	High attachment rate, good growth conditions for <i>Striga</i>	<b>10</b>
	Rhizotron screening platform	Technically simple method, robust, reliable, reproducible, Correlate with field resistance	<b>6, 3, 7</b>
Field resistance screening		Direct selection, reliable Complex resistance mechanism, matches with the reality	<b>5</b>

**Sources:** 1-Ayongwa *et al.* (2006); 2- Emechebe *et al.* (2004); 3- Mbuvi *et al.* (2017); 4- Omanyanya *et al.* (2000); 5- Omanyanya *et al.* (2004); 6- Rodenburg *et al.* (2015); 7- Rodenburg *et al.* (2017); 8- Van *et al.* (2005); 9- Van *et al.* (2011); 10- Vasudeva (1985); 11-Vasudeva *et al.* (1981)

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Experimental site

This study was done at Plant Transformation Laboratory (PTL), Kenyatta University (KU) in Kenya.

#### 3.2 Plant materials

Two pearl millet lines comprised of 29 Aw and SOSAT-C88 P10 were used in this study. These accessions were acquired from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT-Niger). Pearl millet 29 Aw is a wild genotype that exhibited resistance to *Striga* infection in the field. However, SOSAT-C88 P10 is a cultivated pearl millet line derived from a cross between Souna and Sanio. It is a farmer preferred variety in West Africa because of its high yielding ability, downy mildew resistant, drought tolerant but highly susceptible to *Striga* (CNEV, 2012; Moumouni *et al.*, 2015).

During this study two *Striga hermonthica* ecotypes from Kibos and Alupe were used to challenge pearl millet. Kibos and Alupe are two *Striga* prone regions in Western Kenya.

#### 3.3 Experimental design

All the experiments were conducted in a controlled environment in the laboratory using a completely randomized design (CRD). Four treatments were considered as shown in Table 3.1, and 5 plants per treatment were used for this study. The treatments comprised of combination between levels of two factors: *Striga* ecotypes and pearl millet lines.

**Table 3.1:** List of treatments

<i>Striga hermonthica</i> ecotypes	Pearl millet genotypes	Treatments
Alupe	29 Aw	T1: Sh-A Pg29AW GR24
Kibos		T2: Sh-K Pg29AW GR24
Alupe	SOSAT-C88	T3: Sh-A PgSosat GR24
Kibos		T4: Sh-K PgSosat GR24

**Legend:** A: Alupe; K: Kibos; Pg: *Pennisetum glaucum*; Sh: *Striga hermonthica*

### 3.4 Data collection and analysis

#### 3.4.1 Agar gel assay for pre-attachment resistance to *Striga*

The agar-gel assay developed by Hess et al. (1992), is one of the most efficient approach used in pre-attachment *Striga* resistance screening to score the maximum germination distance (MGD) between a host root and the furthest germinated *Striga* seeds.

##### 3.4.1.1 Surface sterilization of host seeds

Pearl millet seeds (20-30) were first washed with autoclave water for 20 minutes for the removal of any husks binding to the seeds before they were transferred to a clean tissue culture hood for surface sterilization. The seeds were surface sterilized in 70% Ethanol for 3 minutes followed by rinsing three times with sterile distilled water. Furthermore, the seeds were soaked in 20% commercial bleach containing drops of tween 20 for 20 minutes. Later, the seeds were rinsed three times with autoclaved distilled water before they were transferred on moist filter papers in sterile petri plates and incubated at 27° C in the dark for 3 days.

### **3.4.1.2 Determination of Maximum Germination Distance (MGD)**

The autoclaved agar water (8g agar plant/L) was dispensed in the 9 cm petri plates before it solidified. Preconditioned *Striga* seeds were pipetted into the dishes prior to pouring the agar gel, and three emerging radicle of germinated millet seeds were then planted at the edges of the plates and two seedlings were removed 12 hours later from the plates. The petri dishes containing the parasite and the host were incubated at 30° C in the dark for 4 days. After incubation, the maximum germination distance (MGD) was determined from photographs of the plates which were taken using a Leica microscope.

The MGDs were calculated by scoring the distance of the furthest germinated *Striga* seeds from pearl millet root in a plate using ImageJ software, and the average was tabulated. Six petri dishes were used for each pearl millet genotype, representing 2 plates replicated three times. The final MGD for each millet line is the mean of MGDs taken from 6 plates.

## **3.4.2 Macroscopic screening**

### **3.4.2.1 Germination of pearl millet**

Pearl millet seeds were germinated in pots filled with vermiculite. After 10 days, the seedlings were transferred into the micro observation chamber (120\*120\*17 mm) packed with vermiculite, wrapped with aluminum foil. The plants were watered twice daily with 5 ml with tap water for a period of 10 days.

### **3.4.2.2 Preconditioning of *Striga* seeds**

In order to break the dormancy, *Striga* seeds were preconditioned as described by Matusova *et al.* (2004). The seeds were surface sterilized in 1.5% sodium

hypochlorite for 10 minutes. The seeds were washed with autoclave water until the smell of sodium hypochlorite disappeared. They were spread on moistened glass fiber filter paper (GFFP-Whitman GFA) and placed in sterile petri dishes. The plates were sealed with parafilm, wrapped with aluminum foil and incubated at 30° C for 11 days. On the 11<sup>th</sup> day, 3 ml of GR24 (0.1 ppm) were applied to the preconditioned *Striga* seeds in each plate to germinate the seeds. Efficiency of the germinated *Striga* seeds was analyzed under a binocular microscope. The seeds for which the radicle protruded through the seed coat were considered germinated. A selected rate of germinated *Striga* seeds was used to infect pearl millet roots.

#### **3.4.2.3 Inoculation of pearl millet roots**

To screen pearl millet lines 29 Aw and SOSAT-C88 P10 for *S. hermonthica* resistance, 11 days after growing millet plants in rhizotron, preconditioned *Striga* seeds (0.075 mg) were used to infect pearl millet roots. The inoculation consisted in aligning *S. hermonthica* seeds on the host roots using small brushes. After the infection, the rhizotron were closed, wrapped with aluminum foil and the plants were daily watered for 21 days.

#### **3.4.2.4 Phenotypic resistance screening of pearl millet infected with *Striga***

In order to screen pearl millet for *Striga* resistance, *Striga* seedlings from inoculated pearl millet roots were harvested at 21 day after infection (DAI) as described by Mbuvi *et al.* (2017). Score on the number, length and biomass of *Striga* were determined using ImageJ software. These data were subjected to an analysis of variance (ANOVA) followed by Turkey Honest test for means separation.

Furthermore, a factorial analysis of variance was carried out among *Striga* ecotypes and pearl millet lines. These analyses were performed in R software (version 3.4).

### **3.4.3 Histological screening for *Striga* resistance in pearl millet**

The pattern of *Striga* ingress in pearl millet tissues was determined by sectioning host/parasite point of attachment as described by Yoshida *et al.* (2009). First of all, small pearl millet root tissues with an attached *Striga* seedling were collected at 3 and 9 DAI under aseptic microscopic conditions. The tissues were fixed in a Carnoy's fixative solution containing Ethanol: acetic acid (4:1). They were stained with 1% Safranin in 30% ethanol for an average of 5 minutes followed by a clean-up in Choral Hydrate (2.5g/ml) for 12 hours. Prior to pre-infiltrate in Technovit 1: Ethanol (1:1) for 1-2 hours, photographs of Safranin stained tissues were taken. They were infiltrated in a 100 % Technovit 1 for 15 minutes and 3 days. They were embedded in a mixed solution of Hardener 2 and Technovit 1 in a 1:15 ratio in a 1.5 ml Eppendorf tube lids. In order to enable the sticky resin to dry up, the lids were covered with aluminum foil, incubate for 30 minutes and mount onto histoblocks using Technovit 3040 medium.

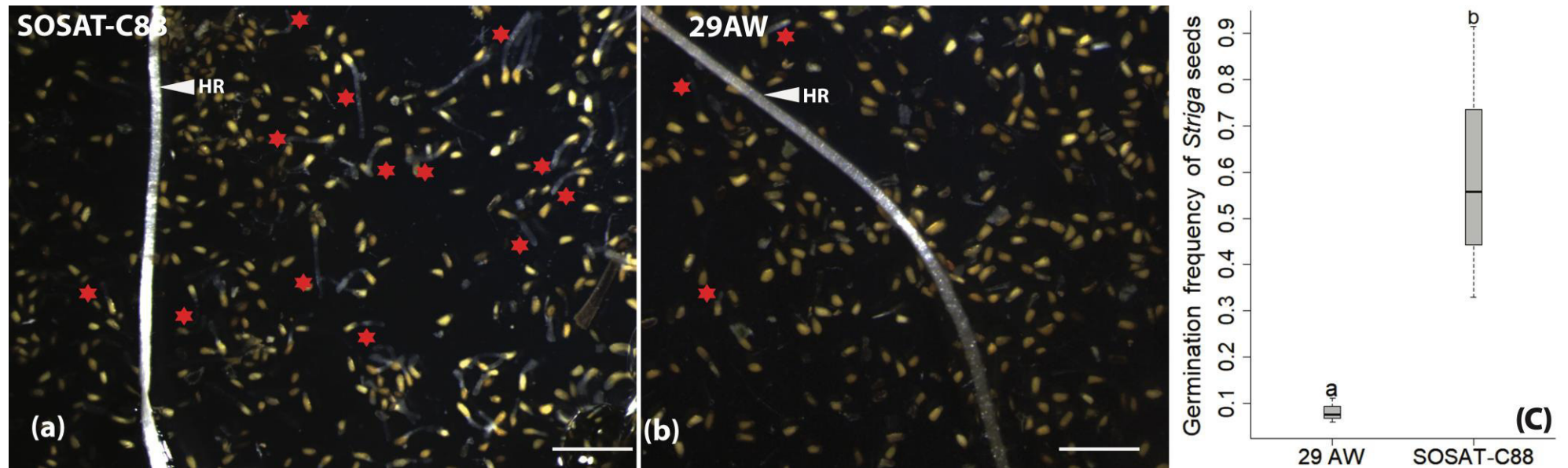
The sectioning of the tissues was done in a Leica RM 2145, tissues with 5 µm were transferred to microscopic slides and stained with 25 g Toluidine Blue O (BHD) in 100 mM Phosphate buffer (Ph7.0). The excess stain was rinsed with distilled water and dried at 65°C for 30 minutes, mounted on De-Pex. This was visualized and photographed on Olympus BX51 microscope.

## CHAPTER FOUR

### RESULTS

#### 4.1 Pre-attachment *Striga* resistance in pearl millet

In this study, the agar-gel allowed the scoring of MGD of two pearl millet lines, SOSAT-C88 P10 and 29 AW. Figure 4.1 indicates the parasite and the host embedded in agar, which shows germination of *Striga* stimulated by the host. Cultivated pearl millet had an average MGD of  $35.94 \pm 2.88$  mm compared to 29 AW ( $7.96 \pm 2.75$  mm). Additionally, it was observed that SOSAT-C88 had a higher percentage of germinated *Striga* seeds (>85.04%), while, 29 AW had an average of 8.14 % germinated *Striga* as shown in Figure 4.1(a) and Figure 4.1(b), respectively. This represented a significant difference (P-value < 0.05) as shown in Figure 4.1c. This suggests that 29 AW has higher *Striga* germination stimulation potential.



**Figure 4. 1:** Reduced amount of *Striga* germination stimulant activity in pearl millet. Red stars indicate host induced germinated *Striga*. (a) SOSAT-C88 and *Striga* growing in agar gel. The host induced germination of several *parasite* seeds that are far from the host root. (b) Wild pearl millet, 29 AW and *Striga* co-growth, showing little induction of germination of *Striga* seeds most of which are very near the host root. (c) Plots indicating germination frequency of *Striga* seeds induced by SOSAT-C88 and 29 AW. Data represent the means of six individual MGD (three replicates) of farthest germinated *Striga* seeds from each host root. The letters indicate significant differences at  $P \leq 0.05$ . HR: Host root. Scale bars, 2 mm.

## 4.2 Post attachment resistance of pearl millet against *S. hermonthica*

### 4.2.1: Rhizotron screening

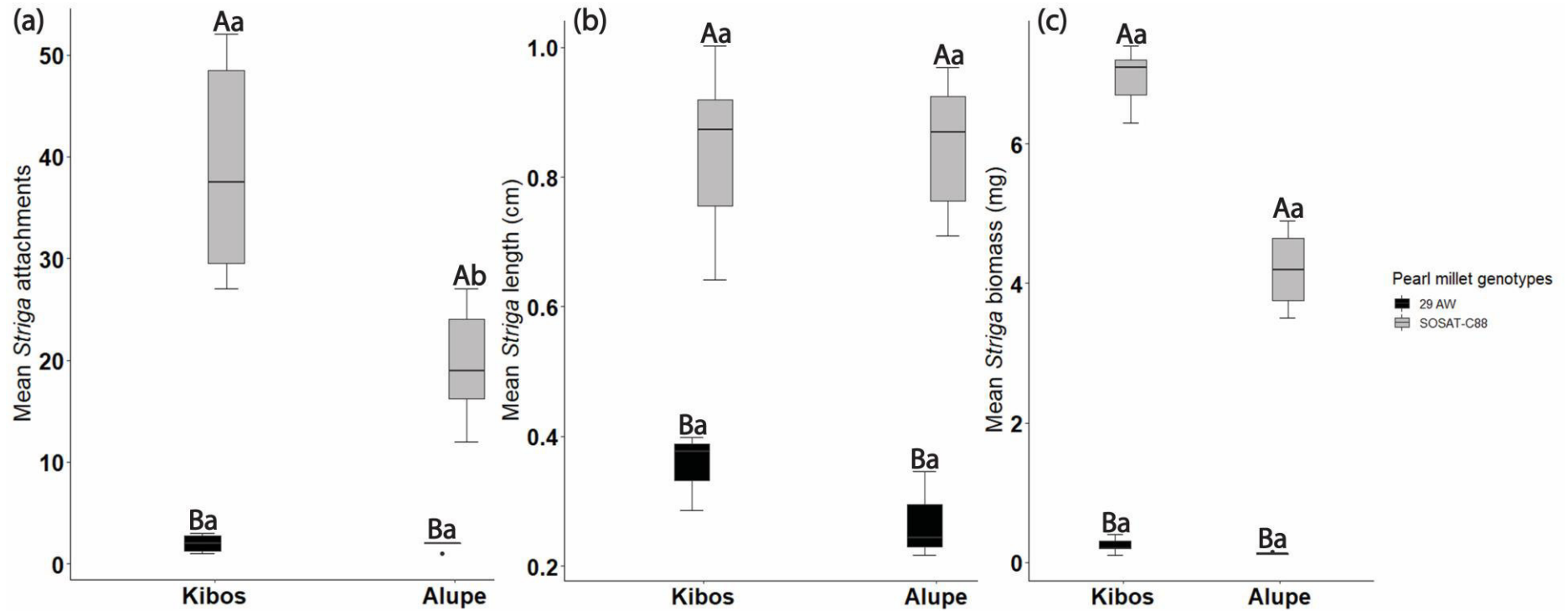
In the rhizotron-based assay, SOSAT-C88 showed numerous *Striga* attachments (Figure 4.2a) compared to the wild genotype 29 AW (Figure 4.2b). These observations indicate that SOSAT-C88 is highly susceptible to *Striga* while 29 AW, has resistance against the parasite.



**Figure 4.2:** Profiling *S. hermonthica* growth on the roots of selected pearl millet lines 21 dpi. The red arrows indicate growing *Striga* seedlings. *Striga hermonthica* from Kibos (a) growing on a susceptible cultivated pearl millet SOSAT-C88 roots. (b) Wild resistant pearl millet (29 AW) with a growing *Striga hermonthica* (Kibos). SOSAT-C88 exhibited a countless *Striga* (Kibos) attachment with vigorous parasite seedlings indicating its high susceptibility. On the other hand, 29 AW had very few parasite attachment with very tiny *Striga* seedlings showing a good pattern of resistance. Scale bars=2  $\mu$ m

An ANOVA showed significant differences between the two pearl millet lines in terms of the number of *Striga* attachments, the biomass, and the length of *Striga* seedlings (P-value < 0.05) (Figure 4.3a,b and c). The cultivated pearl millet SOSAT-C88 had more and vigorous *Striga* attachments for both *Striga* from Kibos and Alupe,  $38.9 \pm 4.5$  and  $19.69 \pm 4.5$ , (Figure 4.3a). Unlike SOSAT-C88, 29AW had very few and smaller parasite attachments ( $2 \pm 0.36$  and  $1.8 \pm 0.17$ ) (Figure 4.3a). Likewise, the length of the parasite were much longer on SOSAT-C88 ( $0.9 \pm 0.05$  and  $0.8 \pm 0.04$  cm) than 29 AW ( $0.35 \pm 0.03$  and  $0.27 \pm 0.04$  cm) (Figure 4.3b).

In addition, biomass, which averaged  $6.99 \pm 0.40$  (Kibos) and  $4.2 \pm 0.21$  (Alupe) mg dry weights of the parasite in SOSAT-C88, and  $0.25 \pm 0.04$  (Kibos) and  $0.15 \pm 0.15$  (Alupe) mg in 29 AW (Figure 4.3c) were significantly different. Furthermore, the ANOVA showed that *Striga* from Kibos was more aggressive than *Striga* from Alupe in terms of the number of parasite attachments and on *Striga* dry weights (P-value < 0.05). The interaction effect between pearl millet lines and *Striga* ecotypes were also significant on the number of infecting parasite as well as *Striga* biomass (P-value < 0.05).

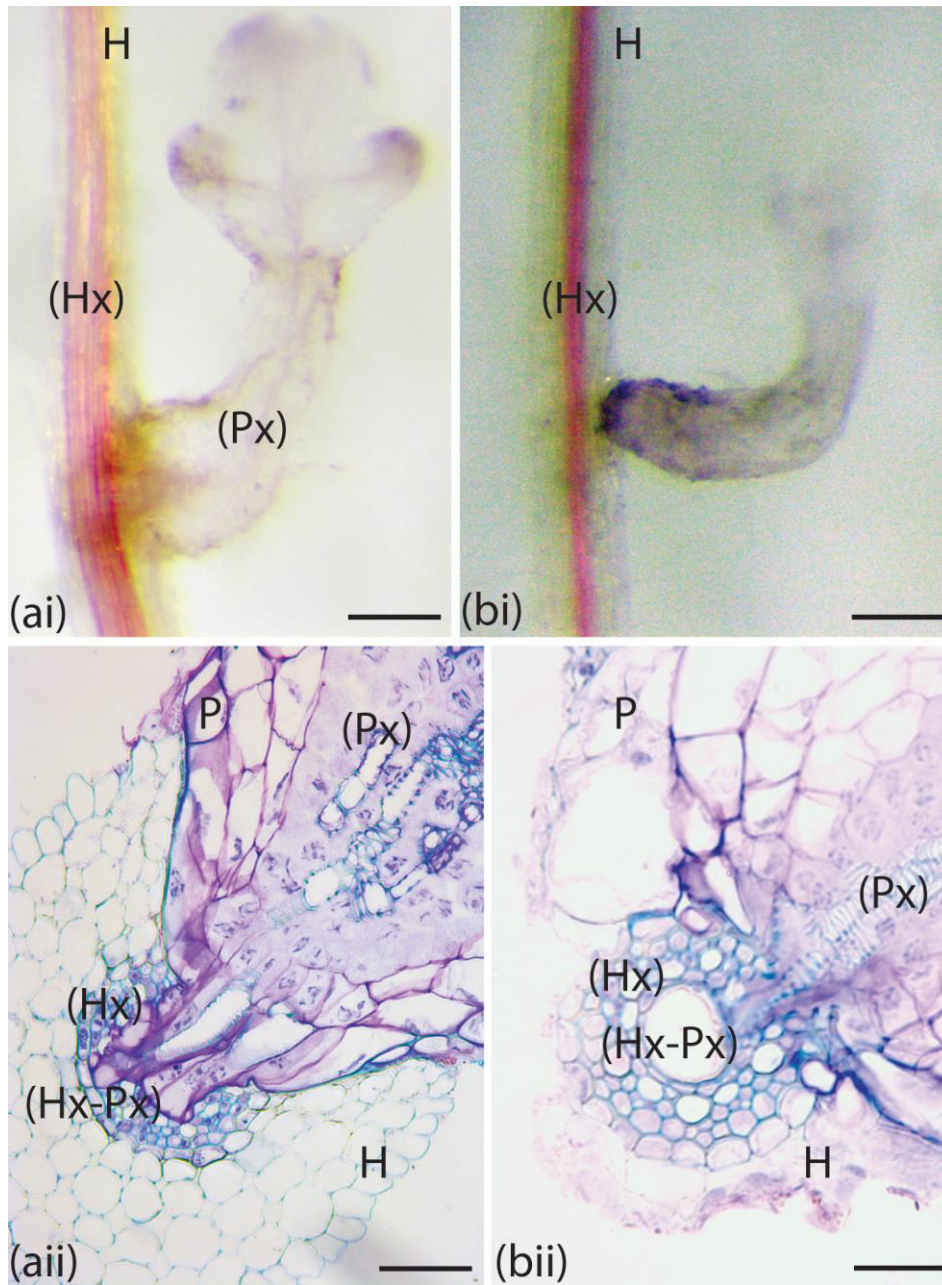


**Figure 4.3:** Evaluation of post attachment resistance against *S. hermonthica* in cultivated pearl millet SOSAT-C88 and wild pearl millet 29 AW, 21 days after infection with *Striga* collected from Kibos. (a) Mean number of *S. hermonthica* attachments; (b) Mean of *S. hermonthica* seedlings length; (c) Mean of dry weights of parasite. Data indicate the mean of three replicates representing fifteen individuals of each host per *Striga* ecotypes. The letters indicate significant differences at  $P \leq 0.05$ .

### 4.3 Efficiency of *Striga* attachment on pearl millet

The establishment of a vascular connection between *Striga* and a host is considered as an indicator of a successful infection. Staining using Safranin-O showed no evidence of vascular connection between host and parasite in both genotypes at 3 Dai. However, at 9 DAI, 74 % of *Striga* had established vascular connection in SOSAT-C99, whereas, the percentage of the parasite establishment in 29 AW averaged 22%. These findings indicate that SOSAT-C99 was more susceptible than 29AW. Further evidence for inefficient vascular formation in 29 AW compared to SOSAT-C99

However, on 9 dpi, the parasite had successfully invaded SOSAT-C88 central cylinder, thus indicating a successful parasitism (Figure 4.4 ai and aii). On the other hand, *Striga* was unable to penetrate 29 AW cells. Compared to 3 dpi, the parasite had penetrated host endodermis, but could not progress further due probably because of some barriers at the layer (4.4 bi and bii).



**Figure 4.4:** Vascular connection between *P. glaucum* and *S. hermonthica* from Kibos, 9 days post infection (dpi).

## CHAPTER FIVE

### DISCUSSION, CONCLUSION AND RECOMMENDATIONS

#### 5.1 Discussion

##### 5.1.1 Low germination stimulant production as a mechanism of defense to *Striga* parasitism

This study was carried to quantify pre-germination resistance in pearl millet against *Striga hermonthica*. There are several pre-attachment *Striga* resistance mechanisms that have been described in sorghum (Rich *et al.*, 2004). These include reduced amount of germination stimulant activity, inhibition or reduced germination and haustorial initiation. The low amount of germination stimulant activity is one of the well-studied *Striga* resistance mechanisms. Genotypes with this trait are expected to trigger the germination of few *Striga* seeds in the close proximity of the host root.

Many germination stimulants have been reported, however, Strigolactones (SLs) are the well-characterized host chemicals to induce *Striga* germination. In pearl millet for example, orobanchol, *epi*-orobanchol, orobanchyl acetate and 5-deoxystrigol were identified during *Striga* parasitism (Jamil *et al.*, 2014), whereas, sorgolactone, 5-deoxystrigol and sorgomol had been reported to be the major SLs in sorghum (Gobena *et al.*, 2017). Strigolactones play an important role in plants development and serve as perception signals for arbuscular mycorrhizal fungi (AM), which, live in association with host plants and improve its performances during water and nutrient related stresses. SLs play also a major role in host shoot and root branching. The production of SLs is believed to be affected by nutritional deficiency, in particular,

the absence of phosphate. However, the factors that affect significantly the release of SLs is still unclear.

This study showed that SOSAT-C88, a cultivated pearl millet, highly stimulated *Striga* germination (Figure 4.1a), followed by a high frequency of germinated *Striga* seeds *in vitro* (Figure 4.1c). Unlike SOSAT-C88, wild pearl millet (29 AW) exhibited a very low germination stimulation activity (Figure 4.1b), and less frequency of germinated seeds (Figure 4.1c). This resistance trait was previously found only in sorghum genotypes (Jamil *et al.* 2011; Robert, 2013). The results showed similarity with the recent investigation by Gobena *et al.* (2017), who described this mechanism of defence in sorghum SRN39 and 555 with an MGD of  $0.1 \pm 0.2$  and  $0 \pm 0$  mm, respectively. Previously, Haussmann *et al.* (2000) demonstrated the existence of reduced *Striga* germination activity in sorghum 555 and IS9830, and an average of 4.6 mm and 0.4 mm MGD were observed in these two genotypes. Moreover, Jamil *et al.* (2011) reported the reduced amount of SLs production in rice ‘NERICA 1’ and ‘CG14’, which, showed very few germinated *Striga*. A similar study by Yoneyama *et al.* (2015) where low production of germination stimulants was believed to cause pre-attachment resistance in maize ‘KST 94’ to *Striga*.

With regard to pre-germination resistance, these findings indicate that wild pearl millet 29 AW exhibited a fair level of pre-attachment resistance against *Striga hermonthica* in the laboratory conditions. Even though the types of germination stimulants involved in this resistance was not investigated, these observations suggest that 29 AW could be a good source of *Striga* resistance in pearl millet as there is no previous study that has described this resistance in pearl millet.

### **5.1.2 Evaluation of the mechanism underlying the post-attachment *Striga* resistance in pearl millet**

In this study, macroscopic screening followed by histological analyses were carried-out to quantify the pattern of post-germination *Striga* resistance in pearl millet, and further to investigate the mechanism underlying this resistance in pearl millet. A resistant line is characterized by less parasite attachment, smaller length, and light dry weights as compared to a *Striga* susceptible genotype. This could be due to the post-attachment resistance, which occurs once the parasite haustorium is developed and attempts to invade the vascular system of the host. This can lead to the activation of host resistance mechanisms such as biosynthesis of cytotoxic compounds, also known as abiosis; prevention of the parasite invasion and growth by the formation of mechanical barriers, and programmed cell death (PCD) in form of hypertensive reaction (HR).

The results revealed the existence of post-attachment *Striga* resistance in wild pearl millet (29 AW), and confirmed the susceptibility of SOSAT-C88 P10 to *Striga* based on the number of parasite seedlings attachment, the length and the biomass which were evaluated (Figure 4.3a, b and c). The numerous, vigorous and lengthy parasite seedlings observed on SOSAT-C88 were an indication that *Striga* plants had sufficiently absorbed host nutrients, which could explain the faster growth of the parasite. However, the hindrance growth of *Striga* plants on wild pearl millet 29 AW suggested that the parasite did not get sufficient nutrients from the host. Fewer attachments and slow growth of parasite seedlings on a host have been associated to defence reaction to *Striga* (Gurney *et al.*, 2006; Amusan *et al.*, 2011). Previous studies

reported this resistance related to parasite attachments in many crops. In wild Sorghum accessions (WSE-1, WSA-1 and WSA-2), Mbuvi *et al.* (2017) demonstrated that these genotypes exhibited a high level of *Striga* resistance as they only supported very few *Striga* plants. Similarly, Cissoko *et al.* (2011) and Mutinda *et al.* (2018) showed that not many *Striga* could grow on rice, NERICA (1-5, 10, 12, 13, 17, CG14, WAB56-50 and WAB181-18), and on maize cultivar KSTP94, respectively.

The susceptibility and resistance traits observed on SOSAT-C88 and 29 AW were consistent regardless of *Striga* ecotypes. This may be due to the wide host range of the parasite (Huang *et al.*, 2012). The results also confirmed the aggressiveness of *Striga hermonthica* from Kibos compared to *Striga* from Alupe for both pearl millet genotypes. This could be due to the coevolution of sorghum and *Striga hermonthica* in Kibos, a region prone to *Striga* infestation, and the high genetic diversity of *Striga* from Kibos (Scholes & Press, 2008; Huang *et al.*, 2012). This may also explain the aggressiveness of *Striga* from Kibos to pearl millet as this crop is a close relative of sorghum.

The primary cause of the susceptibility of SOSAT-C88 to *Striga* may be explained by its hybrid nature, given the inheritance of resistance to *Striga* in the host, SOSAT-C88's progenitors may have lost the genes regulating host resistance during the process of domestication. However, the resistance trait observed in wild pearl millet 29 AW may have been contributed by the long term exposure of this genotype to plant pathogens (Yoshida *et al.*, 2009; Zhang *et al.*, 2017; Woolhouse *et al.*, 2002).

In addition to macroscopic features measured, which allowed the determination of *Striga* resistance in pearl millet, histological analyses indicate that SOSAT-C88 had penetrated more host endodermis than the wild type 3 DAI, and established vascular connection on 9 dpi (4.4 ai and aii). In wild pearl millet (29 AW) however, the obstruction of *Striga* haustorium was caused by endodermal cells, followed by the parasite expansion of host endodermis on day 3, and complete parasite growth blockage on day9 (Figure 4.4 bi and bii). The prevention of *Striga* invasion in host endodermis is still unrevealed, however, the molecules regulating *Striga* and its hosts association seem to be the catalyst of the resistance. Thus, the resistance can be accredited to biosynthesis of cytotoxic compounds (abiosis) and physiological barriers, which, could lead to the deposits of compounds that obstruct haustorium penetration (Yoshida and Shirasu, 2009).

The accumulation of these substances are darkly stained blue by toluidine blue (Maiti *et al.*, 1984), indicating the existence of physiological barriers-mediated resistance in wild pearl millet 29 AW (Figure 4.8 bii). These substances are believed to soften or dissolve host tissues, even though the chemicals involved in this process are unknown, cellulase assay were negative as demonstrated by Rogers and Nelson (1962). In addition to degrading enzymes of the host tissues, Dam and Bouwmeester (2016) demonstrated the role of secondary metabolites in the defense of hosts to *Striga* parasitism.

Several previous studies revealed physiological barriers as an important post-germination resistance mechanism to *Striga* in host plants. The results from this study are in consistence with previous studies. Cissoko *et al.* (2011) reported in rice

resistant cultivars against *S. hermonthica* from Kibos and *S. asiatica*. The authors showed that CG14, NERICA1 and 10 were resistant to *Striga* due to endodermal obstruction of *Striga* haustorium by the host. Additionally, in NERICA 10, *Striga* could penetrate the endodermis, but, with few vascular connection due to deposits of defense materials, restricting the parasite size to be smaller.

Likewise, resistance in wild sorghum line WSE-1 was attributed to deposition of secondary metabolites as described by Mbuvi *et al.* (2017). Mutinda *et al.* (2018) also described this resistance mechanism in maize variety KSTP94 and *S. hermonthica* association. Several other authors reported this type of resistance in many other *Striga* hosts. The failure of *Striga hermonthica* at the host endodermis cell layer was also reported in Cowpea with 80% of *Striga* seedlings failure penetrate host cells (Hood *et al.*, 1998), and in a non-host *Phytospermum japonicum* (Yoshida & Shirasu, 2009). Similar reports were made by Gurney *et al.* (2006) in rice variety Nipponbare and *Striga hermonthica* interaction.

These observations suggest that wild pearl millet 29AW has a post-germination resistance against *Striga*, this resistance may be due to biochemical or mechanical barriers released by the host. In addition to these barriers, there could be other post-attachment resistance involved in the interaction between *Striga* and pearl millet. However, this study demonstrated that biochemical or mechanicals barriers are important, but, not exclusive, post-germination *Striga* resistance mechanism in *Striga* and pearl millet association.

## 5.2 Conclusion

- i. This study demonstrated the existence of multiple *S. hermonthica* resistance forms in wild pearl millet 29 AW. The agar gel assay showed that the susceptible cultivated pearl millet (SOSAT-C88 P10) induced germination of *Striga* seeds even very far away from the host root as measured by MGD. Additionally, high percentage of germinated *Striga* was observed. On the other, Wild pearl millet (29 AW) together embedded with *Striga* in agar indicated that this line only triggered germination of very few *Striga* seeds very near host root. This suggests the existence of a pre-germination resistance to *Striga hermonthica* as a result of low amount of strigolactones activity.
  
- ii. In addition to pre-germination resistance, macroscopic screening revealed vigorous, long and numerous *Striga* seedlings attached SOSAT-C88 P10, while, few tiny *Striga* were growing on wild pearl millet, 29 AW. The parasite ingression in the wild host was prevented, and histological analyses revealed that biochemical or mechanical barriers as the mechanism of the post-attachment resistance to *S. hermonthica* in pearl millet 29 AW.

## 5.3 Recommendations based on the findings from this study

- i. Wild pearl millet 29 AW should be included in crop improvement programs because of its multiple forms of resistance to *Striga hermonthica* regardless of *Striga* ecotypes
  
- ii. An efficient and rapid phenotyping technique should be adopted by breeder for screening for *Striga* resistance in pearl millet genotypes for the discovery of novel sources of *Striga* resistance pearl millet

- iii. While screening for *Striga* resistance, pearl millet genotypes which exhibit pre- and post-attachment resistance to *Striga* should be mostly preferred because this may confer a sustainable resistance.

#### **5.4 Suggestion for prospective research**

- i. Investigate strigolactone profile in pearl millet during *Striga* infection
- ii. Pearl millet 29 AW-*Striga* systems should be dual-RNA sequenced to identify resistance candidate genes in the host, and effectors protein in the pathogens that are involved in the parasitism. This will help in the understanding of molecular mechanisms underlying *Striga* resistance in pearl millet.
- iii. Genome wide association mapping of pearl millet accessions should be carried out to find out genetic players involved in the resistance to *Striga hermonthica* in the host germplasm. The resistance traits will be further introgressed to susceptible farmer preferred varieties.

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