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SCREENING FOR STRIGA (*STRIGA HERMONTHICA* (DEL.) BENTH.) RESISTANCE GENE IN SORGHUM *(SORGHUM BICOLOR (*L.) MOENCH) GENOTYPES USING GEL BASED ASSAY AND *LGS1* MARKER IN ETHIOPIA

MSc THESIS

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ADDIS ABABA, ETHIOPIA

Screening for *Striga* (*Striga hermonthica* (Del.) Benth.) resistance gene in sorghum *(Sorghum bicolor* (L.) Moench) genotypes using gel based assay and *lgs1* marker in Ethiopia

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EXAMINERS' APPROVAL SHEET

We, the undersigned, members of the Board of Examiners of the final open defense by Mulatu Gidi Teferi have read and evaluated his thesis entitled **"Screening for *striga* (*Striga hermonthica* (Del.) Benth.) resistance gene in sorghum *(Sorghum bicolor* (L.) Moench) genotypes using gel based assay and *lgs1* marker in Ethiopia"** and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirement for the degree Master of Science in Biotechnology.

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ABSTRACT

Sorghum (*Sorghum bicolor* L.) is the most important cereal crop in the semi-arid parts of the world where it is the staple food crop for most of the population. In sub-Saharan Africa, the hemi-parasitic flowering weed, *Striga hermonthica* is the major biotic constraint for sorghum production. In countries such as Ethiopia and Sudan its yield losses can be up to 100% when the crop is heavily infested by witch weeds (*Striga* spp.). Overcoming sorghum grain yield losses caused by striga through resistance breeding has been hampered by a lack of reliable screening techniques. Striga resistant varieties offer the most practical control option. Therefore, the objective of this study was to screen striga resistant or susceptible sorghum germplasms using agar gel assay, low germination stimulant marker and root system architecture. The experiment was conducted in the University of California, Davis, USA. The treatments consisted of 59 sorghum genotypes (including 2 standard checks). These genotypes were screened in a completely randomized design (CRD) with four replications. The treatments were grouped according to their germination stimulant activity on *S. hermonthica*: high (Maximum Germination Distance (MGD) >10 mm); low (MGD <10 mm). From High Germination Stimulant (*LGS1*) or low germination stimulant (*lgs1*) genotyping and gel germination, we concluded that the SRN39, Gobiye, Birhan, Hora Doldy No- 02 and ESTL-101295 are likely resistant genotypes to *S. hermonthica.* Root System Architecture screening also revealed that the different sorghum genotypes studied had different root system architecture. Therefore, the conducted study showed the possibility to get sources of striga resistance or susceptible genotypes if larger numbers of Ethiopian sorghum genotypes are screened.

Keywords/Phrases: Agar gel assay, *LGS 1* marker, Resistance

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DEDICATION

This thesis is dedicated to my beloved Father Gidi Teferi Diba who played great role and passed away in my life time. Though I missed you at the harshest time, I remember your suffering in all my life. I wish rest in peace!

TABLE OF CONTENTS

Contents **Page**

LIST OF FIGURES

Figures **Page**

LIST OF TABLES

LIST OF APPENDICES

ABBREVIATIONS

AGA	Agar Gel Assay
CRF	Confined Research Facility
CSA	Central Statistical Agency
GAP	Good Agricultural Practices
FAO	Food and Agricultural Organization
IBC	Institute of Biodiversity Conservation
ISC	Integrated Striga Control
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IITA	International Institute of Tropical Agriculture
LGS1	Low Germination Stimulant 1
MGD	Maximum Germination Distance
NABRC	National Agricultural Biotechnology Research Center
RSA	Root System Architecture
$sddH_20$	sterile double distilled water
SSA	Sub-Saharan Africa

1. INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is the most important cereal crop in the semi-arid parts of the world where it is the staple food crop for most of the population. It is generally, although not universally, considered to have first been domesticated in East Africa, possibly in the Nile or Ethiopia. The domestication of sorghum has its origins in Ethiopia and neighboring countries, commencing around 4000–3000 BC (Zidenga, 2004; Dillon *et al*., 2007). It belongs to the *Poaceae* family and has a genome of ($2n = 2x = 20$) compared to the model grass species, rice (394 Mb). The genome size of sorghum is approximately 740 Mb (Paterson, 1995). Its wide adaptation to harsh environments, tolerance to stress conditions, availability of diverse germplasm collections and its small genome size make sorghum an important botanical model crop for many tropical grasses with complex genomes and employing C_4 photosynthesis (Menz *et al*., 2002).

Sorghum has a wide geographical distribution, being cultivated in the Americas and Asia, as well as its native Africa. It is a very good choice in Africa because it is adapted to low input agriculture, especially in areas of water scarcity. Over the continent as a whole, sorghum is the second most important cereal after maize occupying 22% of the total area planted to cereals (FAO, 2015). In Africa, the area under sorghum production is about 23.14 million ha and total production and average yield being 23.35 million metric tons and 1.01 ton/ha, respectively.

Ethiopia is the fourth top sorghum producing country in the world following the United States of America, Nigeria and Mexico (FAOSTAT, 2017). In Ethiopia, sorghum is the third most important crop after tef and maize, both in area coverage and production. It is grown in almost all regions of the country occupying an estimated total land area of 1.6 million ha. Out of the total cereals production of the country, sorghum accounts for 18.5% with a productivity of about 28 tons per hectare (CSA, 2018). The primary demand for sorghum and millets is for food in Africa, especially in the dry land regions (FAO, 2015). The crop is utilized in different forms, where the grain is used for human food and homemade beverages, and for feed. The juice from sorghum can be converted to alcohol using currently available, conventional fermentation technology (Reddy *et al*., 2007). Sorghum crop occupies second position (after maize) in grain based ethanol production in United States of America, and to weed biology as well as improvement of a wide range of other forage, turf and biomass crops (Kresovich *et al*., 2005). In sub-Saharan Africa, the hemi-parasitic flowering weed, *S. hermonthica* is the major biotic constraint for sorghum production.

In African countries such as Ethiopia, Botswana, Burkina Faso, Eritrea, Kenya, Mali, Mozambique, Niger, Nigeria, Senegal, Sudan and Tanzania, Striga infestation results in significant yield losses reaching up to 100% (Gebisa Ejeta and Gressel, 2007). Currently, integrated control packages based on the use of resistant genotypes in combination with cultural and/or chemical control methods seem to be the best option for striga management (Tesfaye Tesso and Gebisa Ejeta, 2011). However, resistance against these parasitic weeds is a complex phenomenon that is also dependent on the interaction between the parasite, the host and the environment.

The life cycle of striga comprises a series of steps, such as stimulation of seed germination, initiation of an attachment organ (haustorium), penetration of the host root, connection to the host xylem and subsequent growth and development (Parker and Riches, 1993) (Fig.1)

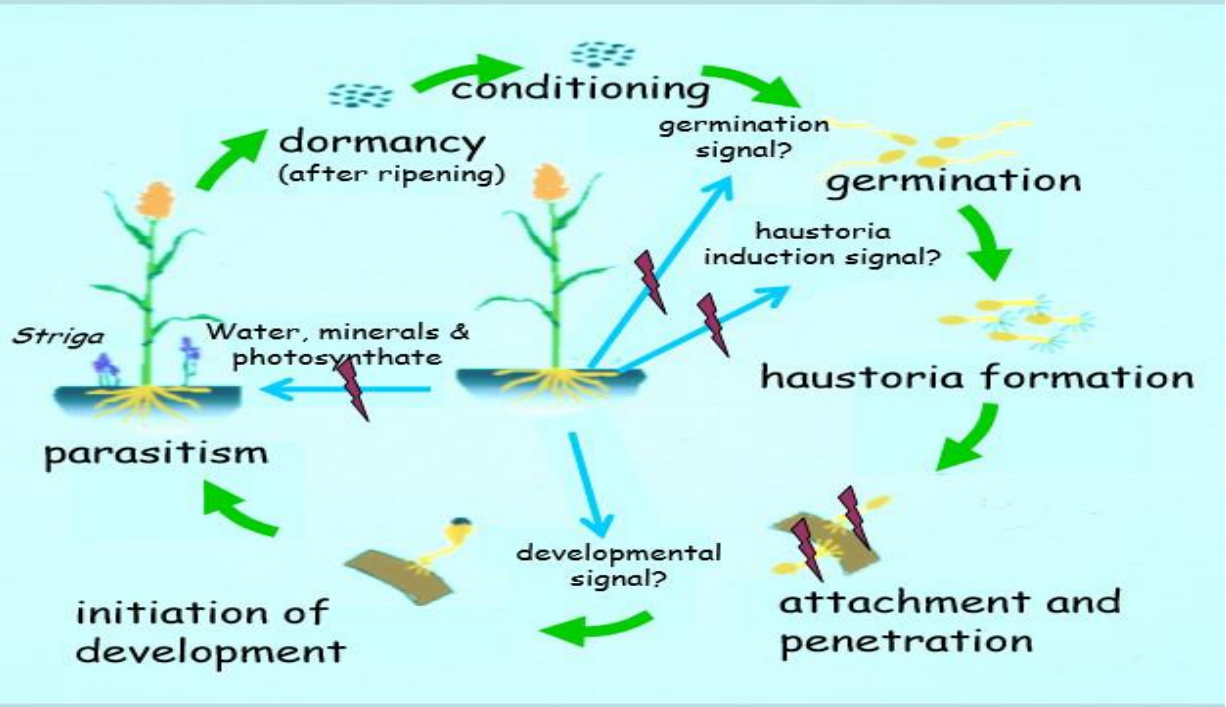


Figure: **1.** Striga life cycle (Source: Gebisa Ejeta *et al*., 1993).

To allow germination, striga seed dormancy needs to be broken under warm and moist conditions (a process called "pre-conditioning" in the lab) and later exposure to specific exogenous signaling molecules exuded by the roots of their host (Bouwmeester *et al*., 2003). Roots of striga hosts and some non-hosts may produce and release different germination stimulants (Sato *et al*., 2003). However, the strigolactones are known to be the most potent stimulant, eliciting germination at nanomolar or even picomolar concentrations in seeds of many striga species (Bouwmeester *et al*., 2003). Following germination, the striga radicle grows (possibly chemotropically) towards the host root, where striga stops growing upon perception of a second host-derived signal and forms a haustorium, the multicellular attachment and penetration organ. The haustorium initiation represents the switch from the autotrophic to the parasitic mode of life (Babiker, 2007).

In the present study pre-attachment resistance in 59 sorghum genotypes have been characterized based on the presence of either the germination stimulant (*lgs1*) or high germination stimulant (*LGS1*) using *LGS1* molecular marker, gel based assay and root system architecture to overcome the problem of striga infestation in sorghum productivity and production. Striga resistance is broadly categorized as pre- and post-attachment resistance (Yoder and Scholes, 2010). Pre-attachment resistance concerns disruption of one or more of the events starting at striga germination to haustorium attachment. The most commonly described mode of pre-attachment resistance is low germination stimulation (*lgs*). Post-attachment resistance encompasses interruption of sorghum root penetration and/or striga developmental stages. Interruption of striga penetration of the host root can be due to the production of mechanical barriers, hypersensitive response, antibiosis and occlusion and sealing off of the host vessels (Mohammed *et al*., 2003).

Of these pre-attachment and post-attachment resistance mechanisms, low germination stimulation (*lgs*) is the best documented (Hess *et al*., 1992). Germination of striga is induced by strigolactones present in the root exudates of its hosts. One gene associated with striga pre-attachment resistance is at the *LGS1* locus. *LGS1* codes for an enzyme annotated as a sulfotransferase (Daniel Gobena, *et al*., 2017). Functional loss of this gene results in a change of the dominant strigolactone (SL) in root exudates from 5-deoxystrigol, a highly active striga germination stimulant, to orobanchol, a SL with opposite stereochemistry (Xie *et al*., 2013). Although not all sorghum lines showing field resistance to striga had low striga germination stimulant activity, all low-stimulant sorghums that were field-tested showed striga resistance (Gebisa Ejeta, 2007). Low striga germination stimulant activity has been an important resistance trait in sorghum improvement but less so in other crop hosts of striga such as maize, millet, and rice (Perez-Vich *et al*., 2013). Genetic studies have shown that inheritance of low striga germination stimulant activity in sorghum is through a mutant allele (*lgs*) expressed in homozygous recessive individuals. Monitoring striga germination stimulation by sorghum is relatively easy to assay. Introducing breeds with lower striga germination stimulation has the advantage of lowering striga infection and reducing further seed bank buildup (Haussmann *et al*., 2004).

In 1991, ICRISAT provided accessions SRN39 and IS9830 as varieties exhibiting field resistance due to low germination stimulant production (Bantilan *et al*., 2004). Indeed, in the past decade or so, several sorghum varieties with striga resistance based on *LGS1* have been introduced, such as SRN39 and IS9830 in Sudan, and Abshir, Gobiye and Birhan in Ethiopia (Tefera Hailu *et al*., 2012). The present study focused on pre-attachment resistance in Ethiopian sorghum lines. Germination is the most important component of pre-attachment resistance, and as described below, low germination has successfully been introduced into sorghum varieties to obtain striga resistance (Gebisa Ejeta, 2007). However, it is difficult to establish a uniform level of infestation at an appropriate intensity level for steady and reproducible results. Several mechanisms of resistance to striga have been proposed (Doggett, 1988).

One of the best characterized mechanisms is unusually low production by host plant roots of root exudate compounds that stimulate striga seed germination (Vasudeva, 1987). Sorghum varieties that exhibit this characteristic are resistant because striga seeds do not germinate unless they are exposed to a specific stimulant from the host root (Vasudeva, 1987). Striga is an obligatory parasite that survives only a few days after germination unless it successfully parasitizes a host root (Worsham and Egley, 1990). So, in order to more effectively screen for the capacity of the host plant to induce striga germination, the agar gel assay is an important technique for assessing striga pre-attachment resistance. Since plants are unarguable important to humans and indeed, to most life on earth by providing foods, fuel shelter etc., their growth and survival depends on the environment.

Root-system architecture (RSA) defines the spatial organization of primary roots and root- and stem-derived branches (lateral roots, adventitious root), which play a fundamental role in plant adaptation to adverse soil conditions by improving plant growth and productivity (Smith and Smet, 2012). Several reports suggest the RSA of different cereal crops depends on the crop genotype itself, the environment they grow in and genotype by environment (GXE) interactions. For example, it has been suggested that cereal crops with the deepest main root length, few lateral roots and long lateral roots have a better chance to survive under drought (Lynch, 2013).

Within the environment there is an interaction between plants and every other organism in soil, including parasitic plants. As a mature parasite, striga produces up to 500,000 seeds (Dixon and Parker, 1984). The interaction of these seeds with a host root is necessary for parasitism. However, not all of the seeds in the soil are equally effective in developing successful interactions. Those seeds which are most effective on sorghum are found in the upper 5 to 30 cm of the soil profile (Robinson and Kust, 1962; Babiker *et al*., 1987), while parasitism of corn occurred mostly in the top 10-cm of the soil profile (Bebawi *et al*., 1984).

Therefore, genotypes with contrasting root system architecture could have different levels of resistance or susceptibility to striga. It can be hypothesized that the sorghum genotype with the deepest main root, few lateral roots and if present, long lateral roots will be resistant to *Striga hermonthica* because most of the parasite seeds are found in the upper soil (Robinson and Kust, 1962). The present study focused on pre-attachment resistance in Ethiopian sorghum genotypes.

1.1. Objectives

1.1.1. General Objective

To screen the striga resistance or susceptibility of 59 selected Ethiopian sorghum genotypes

1.1.2. Specific objectives

To screen striga resistance or susceptible of 59 sorghum genotypes using a gel based assay

To determine striga resistant or susceptible of 59 sorghum genotypes using LGS1 markers

To characterize root system architecture of the 59 sorghum genotypes

2. LITERATURE REVIEW

2.1. Sorghum Origin and Domestication

Sorghum originated in Africa (Ethiopia, Sudan, East Africa), where the greatest diversity of both wild and cultivated species occurs (Acquah, 2007). The presence of wild and cultivated sorghums in Ethiopia reveals that Ethiopia is the primary center of origin and diversity for this crop (Firew Mekibeb, 2006). There are also suggestions that cultivated sorghum was domesticated by selections from a wild progenitor, subspecies *verticilliflorum*, about 5000-7000 years ago (Purseglove, 1972). A balance of farmer selection for cultivated traits and natural selection for wild characteristics has generated improved sorghum types, wild types and intermediate types (Doggett, 1970). Numerous varieties of sorghum were produced through the practice of disruptive selection, whereby selection for more than one level of a particular character within a population occurs. These results from a balance of farmer selection for cultivated traits and natural selection for wild characteristics, generating improved sorghum types, wild types and intermediate types (Olembo *et al*., 2010). There are also suggestions that sorghum was domesticated in Ethiopia and parts of the Congo between 5,000 and 7,000 and with secondary centers of origin in India, Sudan, and Nigeria (Acquah, 2007). Ethiopia is often regarded as the center of domestication of sorghum because of the greatest genetic diversity in the country for both cultivated and wild forms (Masresha Fetene *et al.*, 2011).

2.2. Taxonomy, Ecology and Botanical Description of Sorghum

Sorghum was first described by Linnaeus in 1753 under the name of *Holcus*. Moench later separated the genus sorghum from the *Holcus* and gave it the binomial of *Sorghum bicolor*. The current formal taxonomic concept of the sorghum genus and species agrees with the one established by Moench. All the different names given by the various taxonomists are hence taken as synonym to [*Sorghum bicolor* (L.) Moench]. Sorghum belongs to the *Poaceae* family and is herbaceous annual grass of tropical origin that is planted from seed, stores an appreciable amount of sugar with modest water requirements. Sorghum can tolerate a wide range of soil conditions, from heavy clay soils to light sand, with pH ranging from 5.0 to 8.5. Sorghum is tolerant of arid and saline growing conditions, and reaches maturity in 90 to 180 days. It is considered a crop with universal value because it can be grown in tropical, sub-tropical, temperate, and semi-arid regions of the world. It is adaptable to existing cropping systems, can serve as a secondary crop and is used as a source of forage and silage for livestock production systems (Smith and Frederickson, 2000).

Sorghum is an annual and predominantly self-pollinated cereal with the degree of spontaneous crosspollination, in some cases, reaching up to 30% depending on panicle types (Poehlman and Sleper, 1995; Dje *et al*., 2004). The sorghum plants have an extensive root system that can penetrate 1.5 to 2.5 meters into the soil and extend one meter away from the stem. The large amount of root material contributes to the build-up of soil organic carbon after removal of the aerial parts of the plant, and can alleviate concerns about depletion of soil organic matter resulting from the removal of Stover (Wilhelm *et al*., 2004).

Sorghum has culms that may stand 0.6-4.5 m tall, depending on the type and variety. It may produce two or more tillers. The stalk is solid. The center of the stem can be dry or juicy, insipid or sweet to taste. A dry stalked variety has leaves with a white or yellow midrib, while a juicy stalked variety has a dull green midrib because of the presence of the juice instead of air spaces in the pithy tissues. The number of leaves on the plant varies between 7 and 24 depending on the variety. The sorghum inflorescence is a panicle that may be loose or dense. It is usually erect but may curve to form a "gooseneck". The panicle has a central rachis, with short or long primary, secondary, and mature tertiary branches, which bear groups of spikelet. The length and closeness of the panicle branches determine the shape of the panicle, which varies from densely packed conical or oval to spreading and lax. A fully developed panicle may contain 2,000 grains, each one usually partly covered by glumes (Acquah, 2007).

2.3 Diversity of sorghum

In Ethiopia, sorghum is grown under a wide range of environmental conditions; this has given rise to a wealth of genetic variability in terms of grain color, quality, plant height, disease and pest resistance, adaptation to wide array of temperature and moisture regime. Such highly variable genetic resources are very useful for sorghum improvement program (Ayana Adugna and Endashew Bekele 1998; Ayana Adugna *et al.*, 2000; Adugna Abdi *et al.*, 2002; Firew Mekbib, 2008). It is the best crop grown in eight major agro ecologies and twenty sub agro ecologies that cover about 66% of the total land mass of the country (Geremew Gebeyehu *et al.*, 2004). The sorghum growing environment are so diverse in the range of both biotic (weeds such as striga, diseases, insect pests and birds) and abiotic (drought, soil fertility and frost) stresses that highly constrain sorghum productivity and production. Evaluation of germplasm based on needs and requirements aid in the better utilization of available resources.

Ethiopia has provided a good base for sorghum improvement programmes worldwide. Haile Mengesha *et al.,* (1989) noted that 1446 and 3018 sorghum germplasm was collected by Rockefeller foundation and ICRISAT, respectively from Ethiopia until June 1986. Aberra Deressa *et al.,* (1995) reported that over 10,000 indigenous sorghum germplasm accessions were collected from different sorghum producing regions of the country and evaluated for some agronomic and taxonomic characteristics by the Ethiopian sorghum improvement program now called Institute of Biodiversity Conservation (IBC). This shows that the traditional sorghum growing ecologies are diverse and evaluating the diverse germplasm can help to identify valuable landraces which can be incorporated in to a crop improvement program. Selected accessions have been found very useful and used in various breeding for striga resistance, drought tolerance, disease and insect pest resistance, etc. to transfer some of the desirable traits.

Many studies show that Ethiopia has a potential source of sorghum accessions that can be used as a source of striga resistance, however, most of the efforts made in developing resistant varieties in Ethiopia were confined to exotic germplasm (Wondemu Bayu *et al.*, 2001). No or little attempt has been made to use the local genetic pool, despite the fact that Ethiopia is the center of diversity of the crop and the farmers' knowledge of the existence of striga resistance/tolerance sorghum landraces. Traditional landraces and wild relatives evolve through centuries of introgression, and natural and human selection and acquire resistance to specific pests, diseases and environmental stresses, which can be used as sources of resistance. The germplasm has been screened for resistance to striga, a biotic stresses (drought and temperature stress) and for capability of emergence at higher soil temperatures.

Some germplasm accessions were identified as resistant to striga and were used in breeding programs (Rao *et al*., 2004). As indicated by Gebisa Ejeta *et al.* (2007) selected African landraces of sorghum collected from African countries including Ethiopia were used to initiate crosses and testing in large plot trials with data collected accordingly. Based on field evaluation data, some of the populations collected showed resistance to striga. Therefore, plant genetic resources are invaluable input for sustaining and improving agricultural productivity. The collection and conservation of genetic diversity of important food crops like sorghum has been given significant focus due to concerns over loss of genetic resources. Nevertheless, only small fractions of the total available collection have been fully utilized by breeders, whose main attention has been on introduced exotic germplasm.

2.4. Economic Importance of Sorghum

Globally, sorghum is the fifth most important cereal crop in the world, after wheat, maize, rice and barley (FAOSTAT 2013). It is cultivated in wide geographic areas in the Americas, Africa, Asia and the Pacific. Even though sorghum has become important in the agricultural production of developed countries, it is still primarily a developing country crop, with 90% of the world's acreage found in Africa and Asia. Sorghum is crucially important to food security in Africa as it is uniquely drought resistant among cereals and can withstand periods of high temperature (Taylor, 2002). Sorghum grain output for Africa has been increasing from time to time with much of the increase coming from expanded area under production. This implies that most nations in sub-Saharan Africa continue to depend on sorghum as principal source of food and sorghum is replacing less drought hard crops as environments became too harsh to grow drought susceptible crops (http://www.asareca.org).

Sorghum is also among the leading staple cereal crops in Eritrea, Ethiopia, Tanzania, Kenya, Uganda and elsewhere in the region. Besides providing food for humans, it is also used for alcoholic beverages and as an important feed crop for chickens and other livestock (http://www.asareca.org). In dry land areas of Ethiopia, it ranks third in the country following teff and maize in the total production and second to teff in its *injera* (pancake like bread) making quality (Berhane Sibhatu, 1982). Grain sorghum is gluten free and is a good substitute for cereal grains such as wheat, barley, and rye for individuals with celiac disease (Delserone, 2008). Sorghum is an attractive biofuel crop for use on marginal lands. It is a wide range of adaptation, drought resistance, and salinity tolerance (Reddy *et al.*, 2007; Shoemaker and Bransby, 2010).

2.5. Sorghum Production Constraints

The potential productivity of sorghum is reduced due to a number of abiotic and biotic stresses. Among the abiotic factors are low soil fertility (nutrient deficiency) and drought. Important biotic constraints include the parasitic weed striga (principally *S. hermonthica* and *S. asiatica*), foliar and panicle diseases, stem borers, and shoot fly (Wortmann *et al*., 2006). Among the major sorghum diseases anthracnose, smuts and rusts account for the most substantial yield losses in Ethiopia. Sorghum production constraints vary from region to region within Ethiopia. In Ethiopia, drought and *Striga* weed have been found to be the most important constraints in the northern and north-eastern parts of the country (Rebka Gebretsadik *et al*., 2014)

The Striga *spp*. (witch weeds also part of *Orobanchaceae*) are hemi-parasite but, although they have chlorophyll and a basal photosynthetic activity, basically also behave as hollo-parasites (Parker and Patrick, 1993). In Ethiopia, about 30% of low altitude (<1500 m.a.sl) areas where sorghum is the predominant staple crop are infested with striga (Watson *et al*., 2007) which causes yield losses ranging from 50 to 100% (Temam Husseni, 2006; Tesfaye Tesso *et al*., 2007). Recently, few to high yielding striga resistant sorghum varieties have been introduced and released in the country (Asfaw Adugna, 2007; Gebisa Ejeta, 2007). These varieties when deployed along with moisture conservation practices and soil amendment inputs can dramatically reduce striga infestation and increased sorghum yield by up to 40%.

2.6. Sorghum-Striga Interaction

One of the obstacles to effective management of striga is the complex parasite-host interaction which remains poorly understood to date (Gebisa Ejeta and Butler, 1993; Runo *et al*., 2012). In some host-parasite systems the level of resistance of host genotypes, or the virulence of parasite genotypes, may be the main determinant of the interactions between species (Grech *et al.,* 2006). Sorghum and *Striga hermonthica* have a long co-evolutionary history (Welsh and Mohamed, 2011). In order to beat the severity of the parasitic weed, it is important to have an idea of how sorghum and striga interact.

The life cycle of the parasite is highly harmonized with that of the host, right from germination to maturity (Park and Patrick, 1993). Accordingly, the chemical cross talk that controls striga germination and the development of physical connections with the host are well understood (Palmer *et al*., 2004). In this situation the array of signal exchanges between striga and its hosts leading to successful parasitism is a fascinating biological phenomenon. Specific chemical signals produced by host plants are required to induce germination of parasitic seeds and elicit attachment organ formation (Patrick and Gebisa Ejeta, 2008).

2.7. Origin, Occurrence and Distribution of Striga

As it is shown in Hayelom Berhe (2014), plants belonging to the genus *Striga* comprise obligate root parasites of cereal crops that inhibit normal host growth. Striga are generally native to semiarid, tropical areas of Africa, but have been recorded in more than 40 countries. It is believed that *Striga hermonthica* and *Striga asiatica* originated in the Nubian hill of Sudan and Semien Mountains of Ethiopia. These areas are also known to be the origin of sorghum and pearl millet which are readily infected by the witch weed. *Striga gesnerioides* may have originated in West Africa. Over the years, striga has spread to other parts of sub-Saharan Africa human through the activities (Atera *et al*, 2013). Striga generally prefer infertile soils in open grasslands and savannah in semi-arid tropical areas. Their seeds are well adapted to hot, dry conditions, remaining dormant until rain (Zerihun Sarmiso, 2015).

Striga hermonthica is particularly harmful to sorghum, maize, millet, but is also increasingly being found in sugarcane and rice fields. Mesfin Abate (2016) has also reported that *Striga hermonthica* is the only species that cause economic losses in sorghum. Hayelom Berhe (2014) has shown that upland rice is becoming more and more important for African agriculture, not least because it can sustain more people per crop area than can maize or sorghum. Crops previously unaffected by striga are now showing serious infestation. Striga is, therefore, fast becoming a pandemic of serious proportions in Africa because of its tremendous geographic spread and its economic impact on millions. The enzyme systems of the parasite thrive under low soil fertility and moisture stress conditions, where most soils have been diminished of fertility through removal of organic matter and limited use of manure.

2.7.1. General characteristics of *Striga hermonthica*

Striga hermonthica is a flowering root parasitic plant, and it leads a hemi-parasitic way of life. It is deemed to be one of the most ubiquitous parasitic weed of food crops (Koua, 2011). It is thought to have co-evolved with wild relatives of sorghum during domestication in the Sudano-Ethiopian region of Africa (Mohamed *et al.*, 1998). *S. hermonthica* causes severe constraints in cereal crop production in the SSA by parasitizing the roots of the host crop. It has a wide host range including many food and fodder crops, although the major damage caused by this parasite is on staple crops of the rural poor in the African savanna sorghum [*Sorghum bicolor* (L.) Moench] is among the best host (Gebisa Ejeta, 2007a). The life cycle of striga is closely linked to that of its hosts. They are released from dormancy through conditioning or preconditioning under specific temperature and moisture conditions (Gebisa Ejeta, 2007b).

Striga hermonthica is herbaceous plant (Koua, 2011) with a hairy, hard and quadrangle-shaped stem (Csurhes *et al*., 2013) and narrow leaf (Kagot, 2013). Its height does not exceed 1 meter. It has purple flowers though they are sometimes white. The seeds usually develop in small capsules which up on maturity, burst open to release the seeds for dispersal. One seed capsule can contain between 250 and 500 minute seeds. A single *S. hermonthica* plant therefore, has the capacity to produce over 500,000 seeds. If conditions for germination are not conducive the seeds can remain viable in the soil for up to 20 years (Kagot, 2013). The root system in striga is vestigial (Fig. 2), in which the germinated seed radical produces haustorium instead of characteristic angiosperm root in order to interact with the host (Koua, 2011).

Figure 2: Pictures of *S. hermonthica*, Photo: Marco Schmidt 2009

2.7.2 Geographical distribution of *Striga hermonthica*

The geographical distribution and infestation level of striga are increasing, particularly in SSA. There are some factors causing the increasing of striga distribution and infestation. From the factors, trade and transport of contaminated seeds, cattle movement between fields, dispersal of striga seeds through wind and surface water flows and lack of knowledge and means to control striga. Future climate change may further influence the geographic distribution and invasive potential of striga as habitats suitable for striga growth might expand and/or shift to new areas (Cotter, 2012). As it is shown in Koua (2011), striga is mainly distributed in tropical arid and semi-arid zones with 400-1000 mm of annual rainfall.

The origin of *S. hermonthica* is thought to be in the Nuba Mountains of Sudan and partly Ethiopia. The most severely affected countries are Mali, Upper Volta, Niger, Nigeria, Cameroon, Chad, Sudan, Ethiopia, and India. In some regions of these countries where striga is common, crop yields may regularly be reduced by 60-70%. Serious crop losses also occur widely in parts of the Gambia, Senegal, Mauritania, Togo, Ghana, Kenya, Tanzania, Uganda, Botswana, Swaziland and Mozambique and more locally elsewhere in Africa, Asia, Australia, and the USA (Ayensu *et al.*, 1984).

2.8. Striga species

Witch weeds (*Striga* spp.) are root-parasitic plants belonging to the family *Orobanchaceae* are considered the most serious biotic factor. It is a parasitic weed that invades farmlands and infests cereals. It damages crop plants by attaching itself to their roots and feeding on their nutrients, causing the crops to be stunted, discolored and twisted. It has over run an estimated 22 to 40 million hectares of African Cropland, causing damage in excess of US $3 billion per year. Striga is a major cause of food insecurity and rural stagnation in Africa (AATF, 2006). As it is shown in Hayelom Berhe (2014), striga species (witch weed), are root parasitic flowering plants, which are common in sub-Saharan Africa (SSA) causing severe constraints to crop production. It survives by diverting essential nutrients, which can be taken up by cereal crops such as sorghum, maize etc. Striga is the Latin word for" witch"; striga is known as witch weed because plants diseased by striga display stunted growth and an overall drought-like phenotype. It has given different local names, some of these are; in Kenya, farmers refer to it as Kyongo (Luo), Oluyongo (Loyal), Imato (Teso).

In Ethiopia it is known as, Akenchira(Amharic), Metselem(Tigrigna) (Hayelom Berhe, 2014) and, Atikur, (personal communication). Striga species are annual (IITA, 1997), obligate hemi-parasite plants that attach to the root of their host to obtain water, nutrients and carbohydrates (Hayelom Berhe, 2014). Crop yield loss due to striga attacks can vary depending on striga seed density, soil fertility, rainfall distribution, the cereal host species and variety (Hayelom Berhe, 2014). They are chlorophyllous, but require a host to complete their life cycle (IITA, 1997).

As indicated in Larsson (2012), globally there are 30 to 35 different species of the genus *Striga* and of these about 23 species can be found in SSA.

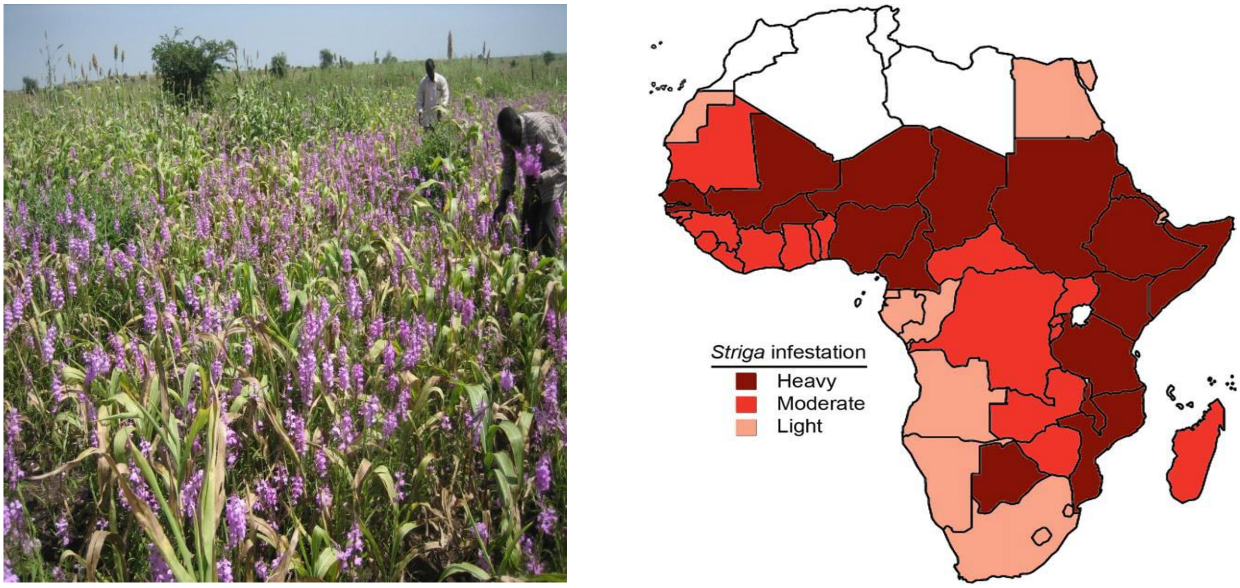
Striga species are one of the most troublesome and damaging weed species in the world. Especially, those that can infest agricultural crops are of great economic importance and the most important striga species are purple witch weed (*Striga hermonthica* and asiatica witch weed (*Striga asiatica* (L.) Kuntze). Of these two striga species *Striga hermonthica* is the most damaging parasitic weed (Atsbha Gebrelsaie *et al*., 2016) and it is an obligate chlorophyll-bearing root parasite, which means that the weed is dependent on its plant host during its entire life cycle, germination flowering reproduction (Larsson, 2012).

The major agricultural striga species are *Striga hermonthica* (Del.) Benth and *Striga asiatica* (L.) Kuntze infecting cereals (maize, sorghum, millet and upland rice), and *Striga gesnerioides* (Willd.) Vatke legumes and tobacco. Other species such as *Striga forbesii* (Benth.) and *Striga aspera* (willd.) Benth have been reported to have sporadic effects on cereal crops (Atera *et al*., 2013). The greatest diversity of striga species occurs in grassland. However, *Striga hermonthica* mainly occurs in farmland infecting grasses. The parasite devastating effect is accomplished prior to its emergence from the soil and it may cause yield losses in cereals ranging from 15% under favorable conditions to 100% (Atera *et al*., 2013).

2.9. Economic importance of striga weeds

Food security in SSA is degrading significantly and this is due to infestation of striga in areas considered the "food baskets" for the continent. Agricultural production sector in SSA is the major source of food, foreign exchange and most importantly the main source of family income through direct and indirect employment (AATF, 2006). However, striga threatens the same source of livelihood (AATF, 2006). Striga infestation has contributed to low food production in Africa (FAO, 2006). Striga is perceived to be the major biological constraint to crop production in SSA and the most damaging or severing pest encountered by farmers growing sorghum, millet and maize in SSA (Rasha *et al.*, 2009). The economic loss due to striga is enormous (Runo *et al.*, 2012).

Striga infests two-thirds of the arable land of Africa and constitutes the biggest single biological cause of crop damage in Africa (Gebisa Ejeta, 2007b). The prize for controlling striga is progress in food security, economic development and well-being of millions of people living in Africa (Atera *et al.*, 2013). It is important to note that striga weed is pervasive and therefore time is of essence in its control. It is estimated that striga seeds have infested about 100 million hectares of land in Africa (Scholes and Press, 2008) and the infestation causes 30 % to 50 % loss of Africa's agricultural economy in 40 % of its arable land (Amudavi *et al.*, 2007; Hearne, 2009). The weed is responsible for about 26 % loss of sorghum and millet in African countries (Gressel *et al.*, 2004). FAO (2006) estimated that cereal yields in SSA increased by 29 % between 1961 and 2005 against a population increase by 216 % within the same period. According to Gebisa Ejeta, (2007a), the striga problem in many African countries is pandemic and seems to be getting worse. This implies that much effort should be put in cereal production in SSA to feed the exponentially growing human population. The consequences of striga infestation are severe rendering small scale farmers helpless and often bewildered in SSA.



A B

Figure: 3 A) Striga infestation in sorghum fields, B) Striga infestation in SSA is most severe in the most food insecure areas. Adapted from a report by Gressel *et al*., (2004).

2.10. Striga Control and Management

Appropriate agricultural technologies such as replenishing soil fertility, use of certified seeds, utilizing Good Agricultural Practices (GAPs), reducing weed soil seed banks and reducing disease and pest pressure on the crops have been recommended for the management of the weed (Bruce, 2010). Several institutions both private and government have been involved or have supported in conducting research on striga. These institutes have recommended control options to farmers in Africa geared towards reducing infestation and damage (AATF, 2011). These options include; intercropping of cereals and legumes, crop rotation, the use of trap crops that stimulate suicidal germinations, application of manure and nitrogen fertilizers and the use of resistant crop varieties (AATF, 2011).

Trap cropping can also be used as control method. This involves planting a species in an infested field that will reduce the striga seeds to germinate but will not support attachment of the parasite. This method has been used in sorghum fields by planting *Celosia argentea* and in maize fields by planting *Desmodium uncinatum* (Radford, 2003). Imposed ‟suicidal germination‟ in the fields not yet planted with crops can be used as a control measure. This method involves inducing seeds present in the soil to germinate by injecting ethylene gas in the soil. Ethylene gas mimics the natural physiological response tied to host recognition. Since no host roots will be available, the seedlings will fail to attach and therefore dies. However, this method is relatively expensive and not affordable to the subsistence farmers in developing countries (Olupot *et al*., 2003).

The use of such resistant cultivars is considered an effective and affordable component of integrated striga control strategy (Yoder and Scholes, 2010). Resistant cultivars have the ability to prevent or limit striga attachment and growth, hence capable to grow and produce in areas that are infested with striga. Integrated Striga Control (ISC) is a control option where a wide range of technologies are combined into a program for the control of striga as opposed to the application of a single method in the control. ISC is recommended for effective management of striga. Franke *et al.* (2006) observed that ISC approach reduced the soil striga seed bank by 46% and improved crop productivity by 80%. The major objective of ISC is to reduce striga densities in the soil to avoid new plants emerging in the subsequent seasons.

3. MATERIALS AND METHODS

3.1. Description of the study area

Experiments were conducted in the Laboratory of University of California, Davis, USA. The center is located at the western part of the United States at 38º 33’ N latitude, 121 º 44’’ W longitudes.

3.2. Source of plant materials

A total of 59 sorghum genotypes from Ethiopian were identified for use in the PROMISE project (Appendix **Table 1**) and include local landraces, wild varieties, drought tolerant varieties from Ethiopia, striga resistant varieties including *lgs1* ((SRN39) negative control), the line carrying a *lgs1* allele or low striga germination stimulant activity) from Purdue University and dual purpose, Stay green, best released varieties and Malt type from ICRISAT, Shanqui Red (line carrying *LGS1* allele/ has high striga germination stimulant activity) and Teshale (positive control) variety. Striga (*Striga hermonthica*), Abergelle type seeds were collected from the Tigray region, Ethiopia.

3.3. *LGS1/lgs1* genotyping of *Sorghum bicolor* germplasm

3.3.1. DNA extraction

Sorghum leaf tissue (one leaf per sorghum genotypes with four biological replications) was collected in a 1.2 ml micro tube from each plant. Sterile forceps were used to push the one leaf (20 milli gram) per sample to the bottom of the appropriate well of a 96-well plate with one metal bead on ice. Two hundred fifty micro liter of extraction buffer without SDS (200 mM Tris-Cl pH 7.5, 250 mM NaCl, and 25 mM EDTA) was added to each sample tube and the tubes were sealed with tape using a blue roller. The tissue was disrupted with tissue lyser (Tissue Lyser II, Qiagen), with 2 cycles of 30 Hz for 2 minutes. Two hundred fifty micro liter of extraction buffer with 2x SDS (200 mM Tris-Cl pH 7.5, 250 mM NaCl, 25 mM EDTA, 1% SDS) was added and centrifuged at 2250 rcf for 10 minutes. Two hundred micro liter of isopropanol per well was added to 0.8 ml storage plates while plates were being centrifuged. Then 200 µl of supernatant per sample was transferred into the wells (containing isopropanol) and mixed by pipetting. The plate with PCR plate adhesive was sealed and incubated at RT for 10 minutes and centrifuged at 2250 rcf for 35 minutes. The DNA was washed by 150 µl of 70% ethanol (nuclease-free) and poured off. Then, the spin plates were pulsed to ~ 100 rcf. After the DNA was air dried for 20-30 minutes in the flow hood, the DNA was resuspension in 150 µl Nuclease free water and shaked for five minutes. Finally, plates with PCR adhesive were sealed and stored at -20 °C until needed for PCR work.

3. 3.2. PCR of the *LGS1* and *lgs1* alleles

DNA from individual plants was used for PCR-based genotyping to determine if striga germination stimulant activity of a given genotype is determined by the *lgs1* locus. To determine PCR amplicon size for 59 sorghum genotypes, 2 μL genomic DNA, 1 μL Dream Taq Red, 1 μL LGS1 (F+R) primer mix, 1 μL Positive Control (F+R) primer mix, 1 μL Dream Taq buffer with loading dye, 1 μL DMSO and 10 μL sddH$_2$o was used in each PCR tube (20 μL tolal reaction) in the wells. The PCR conditions were; 95 °C for 2 minute (initial denaturation), 95 °C for 30 second (denaturation), 65 °C for 30 seconds (annealing), 72 °C for 1 minute (extension) for 35 cycles, 72 °C for 5 minutes (final extension) and 4 °C holding temperature.

3. 3.3. Gel electrophoresis

The PCR product was separated in a 3% agarose gel using electrophoresis with a 0.5× TBE buffer (Xin *et al*., 2003) at 100 V for 3 hrs. Two micro liter of 6xloading dye with gel red on gel, visualized under UV light and subsequently photographed. To determine the size of the amplicon, 100 bp size ladder was used. Two and half micro liter of the contents from each PCR tube was run in the wells of the 3% agarose gel. The amplicon size of the negative (resistant) control (*lgs1*) sorghum genotypes showed band size of 333 bp and in the positive (susceptible) controls (*LGS1*) sorghum genotypes showed band size of 208 and 333 bp (Patrick J.Rich, 2017).

3.4. Gel germination assays of *Sorghum bicolor* germplasm and association with the *LGS1/lgs1* alleles

3.4.1. Surface Sterilization of Striga Seeds

Striga seeds were surface sterilized following the procedure described by Amusan *et al.* (2011). Striga seed clean and free from other plant material was prepared and transferred to 50 ml flask. Seeds were then rinsed by adding 25 ml 75% (v/v) ethanol and agitated for 2 min by sucking the solution with a sterile glass pipette equipped with a plastic bulb. This mixture was then poured out. The seeds were again washed by adding 25 ml of activated Metricide solution as before, for 2 min by sucking seeds and Metricide solution in and out of the pipette under the surface of the liquid. Bubbles from the surface of the Metricide solution were removed and floating seeds and debris were discarded. This step was followed by 2 times of rinsing in sddH_2Owith each rinse lasting 2 minutes.

3.4.2. Preconditioning of Striga Seeds

Surface sterilized striga seeds were preconditioned by incubation in 25 ml of sddH_2O and 25 µl of a 0.015% Benomyl [methyle-1-(butylcarbomyl) - 2- benzimidazolecarbamate] in a 50 ml flask. The flasks were then placed in a 29 °C incubator for 5 days. Aluminum foil was used to enclose the flasks. The Benomyl solution was changed after first and second day. After 5 days, (sddH_2O and Benomyl), 30 ml sterilized (0.7% w/v) agar cooled to 50 °C was poured over a drop of striga seeds to embed it and allow the conditioning to finish in agar. The plates were stacked in a Petri dish bag and then placed in a 29 °C incubated for 5 days in the dark at 29 °C before use (Patrick J. R, and Daniel Gobena, 2016).

3.4.3. Sorghum Seed Surface Sterilization and Pre-germination

Sorghum seeds (10 similar size and color per each of 59 sorghum genotypes) with four replications were surface sterilized and pre-germinated according to the method described by Amusan *et al*. (2011). After seeds were deglumed, they were soaked in freshly prepared 30% bleach /0.2% Tween 20 solution in sddH_2O for 30 minutes. After this treatment, seeds were rinsed 3 times in sddH2O before soaking overnight in 5% (w/v) of Captan slurry (active ingredient: (2, 4, 5, 6-tetrachloro-1, 3- benzenedicarbonitrile, 75%), a non-systemic fungicide. Sterilized seeds were pre-germinated on sterile Petri plates containing filter paper thoroughly wetted with 5 ml of sddH2O. Sorghum was germinated in covered plates in the dark at 30 °C for 24 hours before use in the agar gel assay and root system architecture. After emergence of both the radicle and the plumule only healthy germinated seeds were transferred to the plates with embedded striga seeds for gel germination assay.

3.5. Root system architecture analysis of *Sorghum bicolor* germplasm

3.5.1. Sorghum Seed Surface Sterilization

Ten seeds of similar size and color per each of 58 sorghum genotypes were added to an individual falcon tube. Then, 25 mL 30% of commercial bleach (Clorox) was added to each falcon tube and closed tightly and placed on a shaker. After 20 minutes the vigorously shaken falcon tube with seeds was taken to the laminar flow hood. The bleach solution was removed carefully. Then 25 ml sterile milli-Q water was added into falcon tubes, closed and placed on a shaker for 2 minutes. This step was repeated five times. Finally, seeds were left in 10 ml of sterile water before finishing all batches. Fifteen milli liter of sterile water was poured into each germination pouch and one seed was transferred into an individual pouch. Then after, three pieces of tape were placed to close the pouch, two on both sides and one in the middle. Seed germination pouches for characterizing root system architecture were labeled and placed in a box with Styrofoam separators in a way that roots were not exposed to light. Finally, the boxes were transferred to the greenhouse.

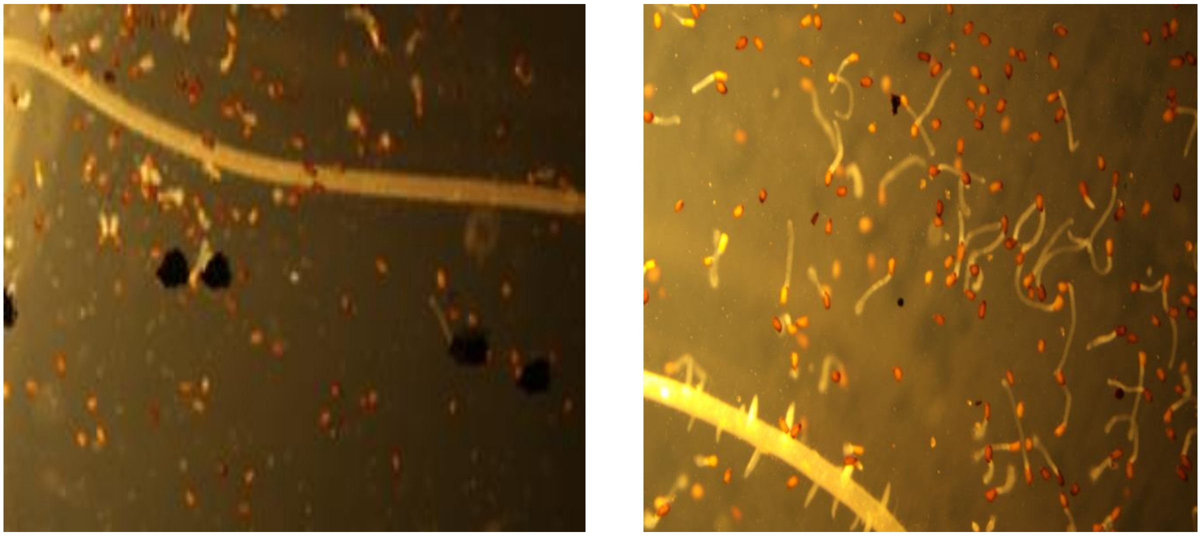
4. DATA COLLECTION

4.1 Gel germination assays of *Sorghum bicolor* germplasm and association with the *LGS1/lgs1* alleles

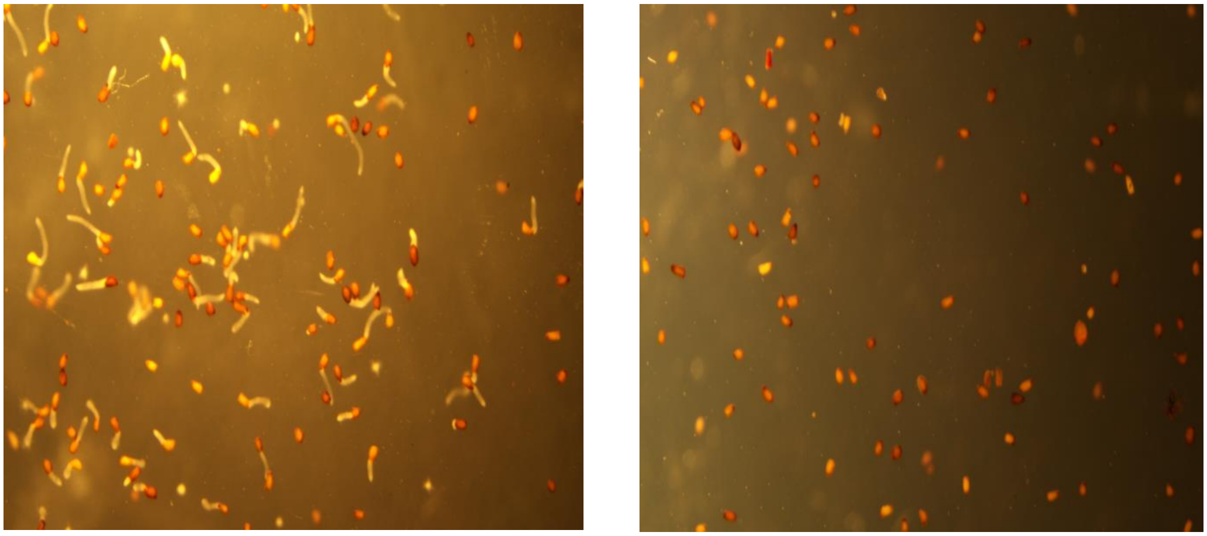
4.1.1 Maximum Germination Distance (MGD)

The plates with striga and sorghum were observed under a Zoom Stereomicroscope (SZ series) at 10× magnification after ten days of sorghum planted in agar with striga embedded to determine germination stimulant activity. A transparent ruler was used to measure the distance of germinated striga seeds from the sorghum main or lateral root. Only the region located 2 cm from the kernel toward the root apex was examined.

The maximum germination distance (MGD, measured as distance from the sorghum root to a striga seed located the furthest from the sorghum root) of three striga seeds was recorded for each plate. The sorghum genotypes used as controls were Teshale (as a positive control) and SRN39 (as negative control) (Fig. 4). The averages of the three furthest germinated striga seeds (in mm) from the sorghum root were measured. MGD (Maximum germination distance) is the most important information recorded from the agar gel assay and it is considered as an established measure of striga germination stimulant activity. Plates having a MGD $\geq$ 10 mm are called as having high striga germination stimulant activity, while those with a MGD < 10 mm are called as having low striga germination stimulant activity, according to the threshold established by Hess *et al.,* (1992).



A) Resistant genotype (SRN 39) B) Susceptibe genotype (Teshale)



C) With GR 24 D) Blank (without sorghum or GR 24)

Figure 4: Germination status of preconditioned striga embedded in agar gel, (A) SRN 39 (resistant genotype) that has low striga germination stimulant genotype, (B) Teshale (susceptible genotype) that has high striga germination and C) With GR 24 D) Blank (without sorghum or GR 24).

5. DATA ANALYSIS

All data were subjected to descriptive statistics and analysis of variance (ANOVA) using the Genstat (version- 15) Statistical package. Duncan's Multiple Range Test (DMRT) was used to separate means at P≤0.05.

6. RESULTS

6.1. *LGS1/lgs1* genotyping of *Sorghum bicolor* germplasm

From the 59 sorghum genotypes screened using *LGS/lgs*1 marker, SRN39 (negative control) (genotype 16), Gobiye (genotype 14), Birhan (genotype 15), Framida (genotype 17) (Holy Doldy No-2 (genotype 32) and ETSL 101295 (genotype 57) are sorghum genotypes potentially resistant to *Striga hermonthica* due to the presence of the *lgs1* allele and band showed at only 333 bp. The remaining sorghum genotypes are likely susceptible to *Striga hermonthica* due to the presence of the *LGS1* allele and band showed at both 208 and 333 bp (Fig. 5).

Figure 5: PCR result of the 59 sorghum genotypes screened using *LGS/lgs1* marker.

6.2. Gel germination assays of *Sorghum bicolor* germplasm and association with the *LGS1/lgs1* alleles

The results described in Figure 6 below show that SRN39(negative control) (5.86 mm MGD), Gobiye(7.42 mm MGD), Birhan(3.43mm MGD), Framida(2.92 mm MGD), S35(9.60 mm MGD), 2001 Pawe Coll #068 (4.35 mm MGD) and ETSL100016 (4.67 mm MGD) have greater than 10 mm MGD indicating striga pre-attachment resistance. SRN 39 (resistant control) and Teshale (susceptible control) were used to determine sorghum resistant or susceptible to *Striga hermonthica.*

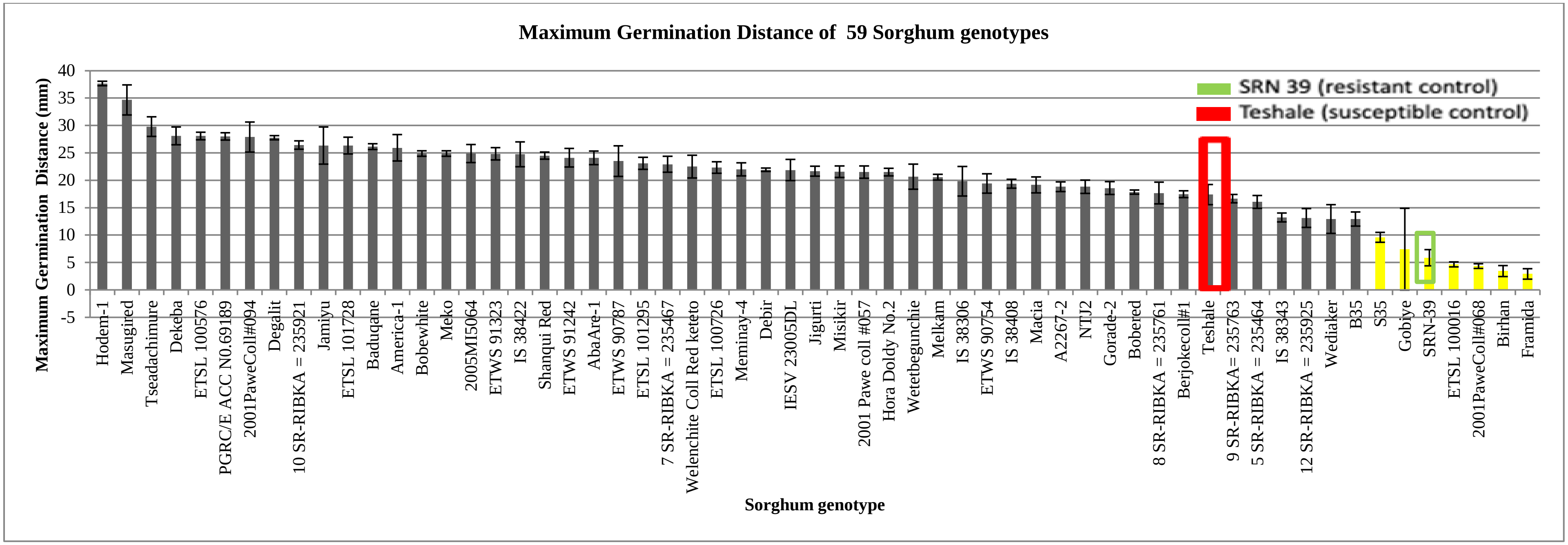


Figure 6: Summary of MGD for the 59 sorghum genotypes tested. The values represent the mean of the 4 replicates and error bars represent standard deviation whereas yellow bars indicate sorghum genotypes resistant to *Striga hermonthica*.

From 59 sorghum genotypes screened using lgs1 and gel germination assay, two graphs below (Fig.7), it can be concluded that there was correlation of striga susceptibility/resistance with the *LGS1/lgs1* alleles of some sorghum genotypes. Sorghum genotypes like SRN 39 (negative control), Gobiye, Birhan and Framida are resistant in both gel based assay and *lgs1* allele. However, not all the resistant genotypes have the *lgs1* locus low MGD presence was observed in S35, Pawe Coll #068 and ETSL 100016 but not *lgs1*. Furthermore, several genotypes with the *lgs1* allele did NOT demonstrate resistance including Hora Doldy No-2 and ETSL 101295. Genotypes with a low MGD and pre-attachment resistance may have a different strigolactone profile or other resistance mechanisms.

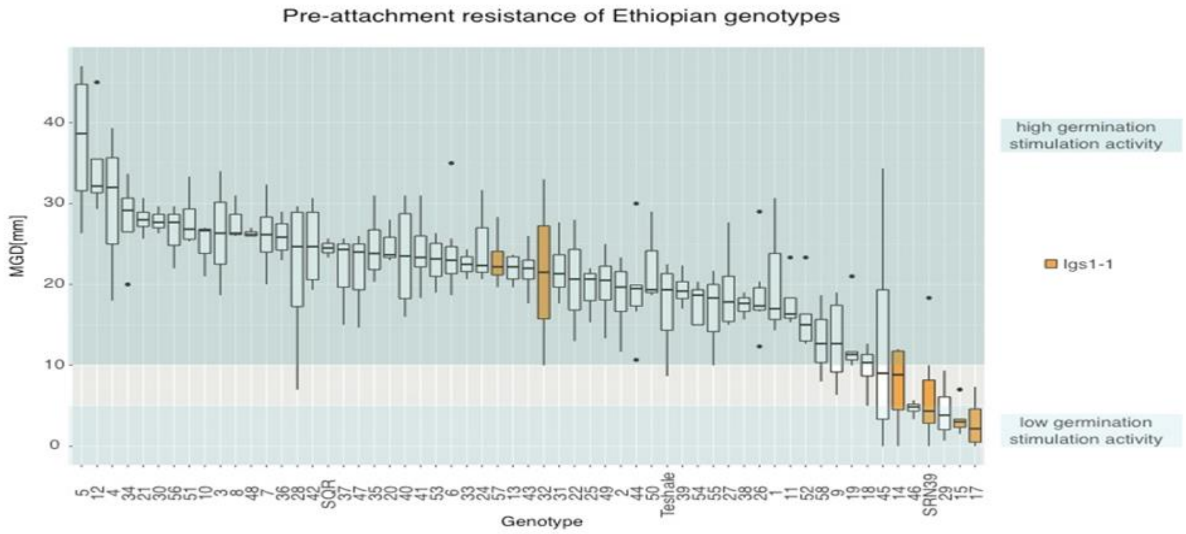


Figure 7: Correlation of striga susceptibility or resistance with the *LGS1/lgs1* Alleles

Table 1: Summary of the **ANOVA for MGD** of the 59 sorghum genotypes

Source	**DF**	**SS**	**MS**	**F vaue**	**Pr > F**
Genotype	58	11444.79050	197.32397	295.32	<.0001
Error	177	118.26577	0.66817		
Corrected Tot	235	11563.05628			

R-Square	**Coeff Var**	Root MSE	Plant mean
0.989772	**4.052435**	0.817416	20.17097

The above table shows that the analysis of variance showed significant variation ($P \leq 0.001$) among 59 sorghum genotypes screened for MGD (Table 1). This ANOVA table is the summary of the 59 sorghum genotypes treated with four biological replications.

Table 2: Mean of the Maximum Germination Distance (MGD) of the 59 Ethiopian sorghum genotypes screened using gel germination assay

No	Genotype	Mean
1	Masugired	31.08^{a}
2	ETWS 90787	30.59^{a}
3	Jamiyu	30.50^{a}
4	2001 Pawe coll #057	28.92^{b}
5	PGRC/E ACC N0.69189	28.67^{bc}
6	10 SR-RIBKA = 235921	27.75^{cd}
7	Hora Doldy No.2	27.08^{de}
8	Degalit	26.92^{de}
9	Bobewhite	26.42^{ef}
10	Baduqane	26.42^{ef}
11	SR-RIBKA = 235467	25.75^{fg}
12	ETSL 101728	25.67^{fg}
13	Bobered	25.50^{fg}
14	Hodem-1	25.17^{g}
15	Misikir	25.08^{gh}
16	Jigurti	25.50^{gh}
17	ETSL 100576	24.83^{gh}
18	Dekeba	24.00^{hi}
19	America-1	23.09^{ij}
20	Melkam	23.00^{ijk}
21	Meko	22.84^{jkl}
22	AbaAre-1	22.83^{jkl}
23	Shanqui Red	22.75^{jkl}
24	Debir	22.50^{j-m}
25	Teshale	22.17^{j-m}
26	ETSL101295	22.17^{j--m}
27	IESV 23005DL	22.00^{j-n}
28	Macia	21.92^{k-n}
29	IS 38320	21.84^{l-o}
30	Tseadachimure	21.83^{l-o}
31	2005MI5064	21.74^{n-p}
32	Meminay-4	21.42^{n-o}
33	Wediaker	20.92^{n-q}
34	ETWS 91323	20.83^{opq}
35	IS 38422	20.67^{pq}
36	ETWS 91242	20.59^{pq}
37	A2267-2	20.17^{qr}
38	IS 38306	19.84^{qrs}
39	IS 38408	19.34^{rst}
40	ETSL 100726	19.25^{rst}
41	Gorade-2	19.17^{rst}

42	Berjokecoll#1	19.00^{st}
43	9 SR-RIBKA= 235763	19.00^{st}
44	8 SR-RIBKA = 235761	18.25^{tu}
45	Wetetbegunchie	17.58^{uv}
46	NTJ2	17.17^{uvw}
47	Bobered	16.50^{vwx}
48	ETWS 90754	16.09^{wx}
49	5 SR-RIBKA = 235464	15.67^{x}
50	12 SR-RIBKA = 235925	13.42^{y}
51	S35	11.84^{z}
52	Gobiye	11.50^{z}
53	B35	11.17^{z}
54	Framida	5.42^{a}
55	ETSL 100016	4.67^{ab}
56	2001PaweColl#068	4.17^{bc}
57	Birhan	3.92^{bc}
58	IS 38343	3.84^{bc}
59	SRN-39	3.08^{c}

Sample size	59
CV	4.05
SE	0.81
P(5%)	<0.001

Key: CV= Coefficient Variation; SE= Standard error; P=P-value. Superscript values are means of combined data of four biological replicates each. Values within each column followed by same letter are not statistically different at $p \leq 0.05$ according to the Duncan’s Multiple Range Test

6.3. Root system architecture analysis of *Sorghum bicolor* germplasm

Main root length, number of lateral root and total lateral root length (in cm) were measured using ImageJ software for each seedling of 8 days old sorghum (after germination) for each genotype (Fig.8).

Hypothesis: Sorghum genotypes those have deep main root length, fewer number of lateral root and long lateral root length are resistant to *S. hermonthica* because of most striga seeds are found on upper soil surface.

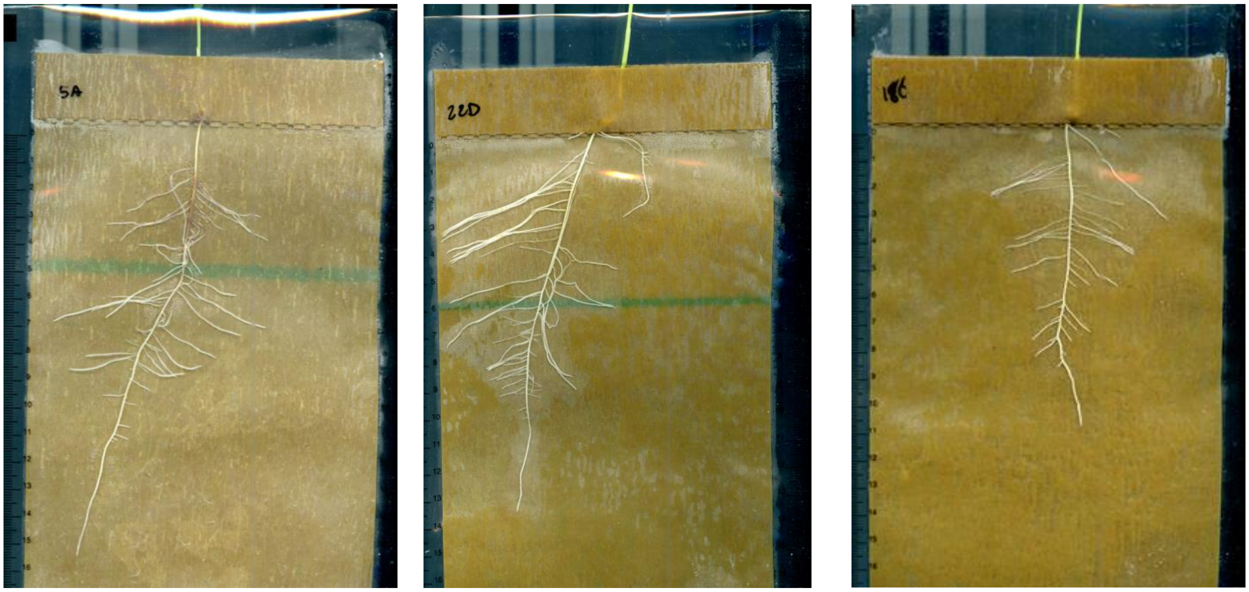


Figure 8: Quantification for RSA Analysis of 8 days old sorghum genotypes on pouch experiment (using Image j software).

The results in Figure 9 below show that ETSL 100016 (14.92 cm), Hodem -1 (14.52 cm), 2001pawe coll#094 (14.23 cm), Melkam(13.05 cm), Welenchite coll Red K. (12.26 cm) and Memaniya (12.01 cm) have deeper or longer main root length as compared to other genotypes. From these above genotypes might be resistant to striga. ETSL 100016 and Shanqui Red genotype have the longest (**14.92** cm) and shortest (**4.39 cm**) MRL, respectively.

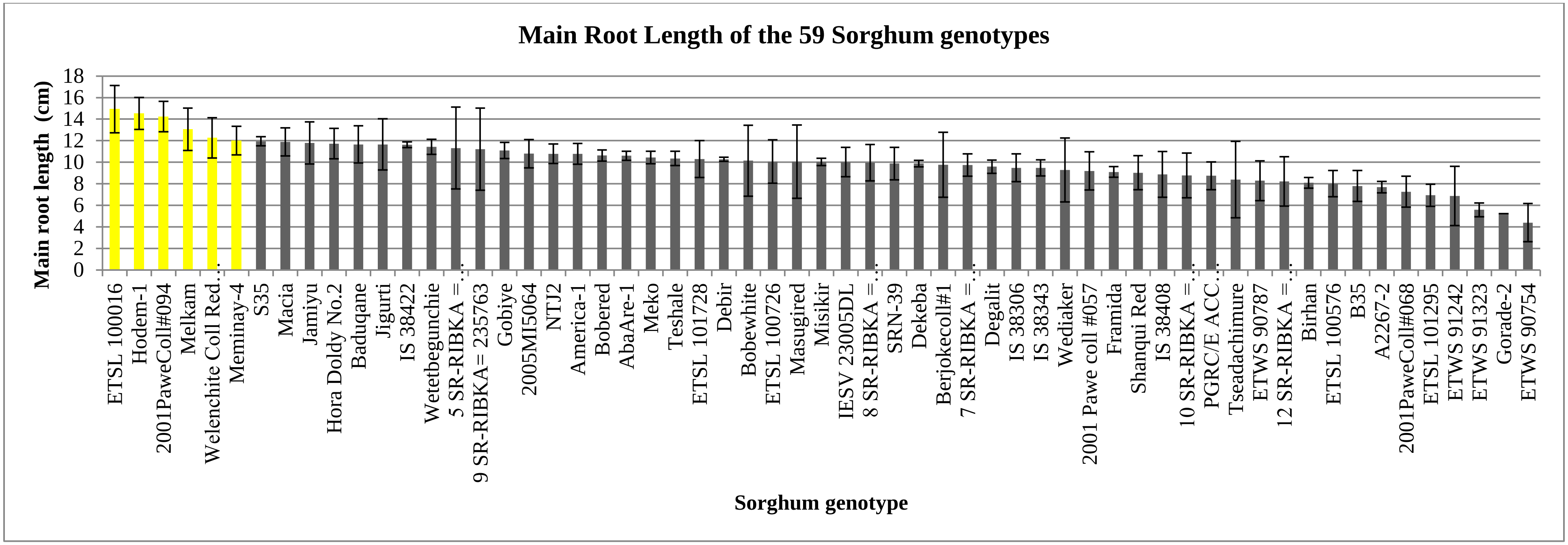


Figure 9: Summary of Main Root Length of 8 days old after germination of the 59 sorghum genotypes. The values represent the mean of the 4 replicates and error bars represent standard deviation whereas yellow bars indicate sorghum genotypes that have most extreme main root length and are resistant to *Striga hermonthica*.

The below graph (Figure 10) shows genotypes like ETWS 90754 (11.50 cm), ETWS 90787 (20.00 cm), PGRC/E ACC No.6918 (20.75 cm), Tseadachimure (21.33 cm), ETSL 100576 (21.75 cm) and ETWS 91323 (22.00 cm) have fewer lateral roots as compared to other genotypes and are potential candidates for resistant genotypes due to RSA.

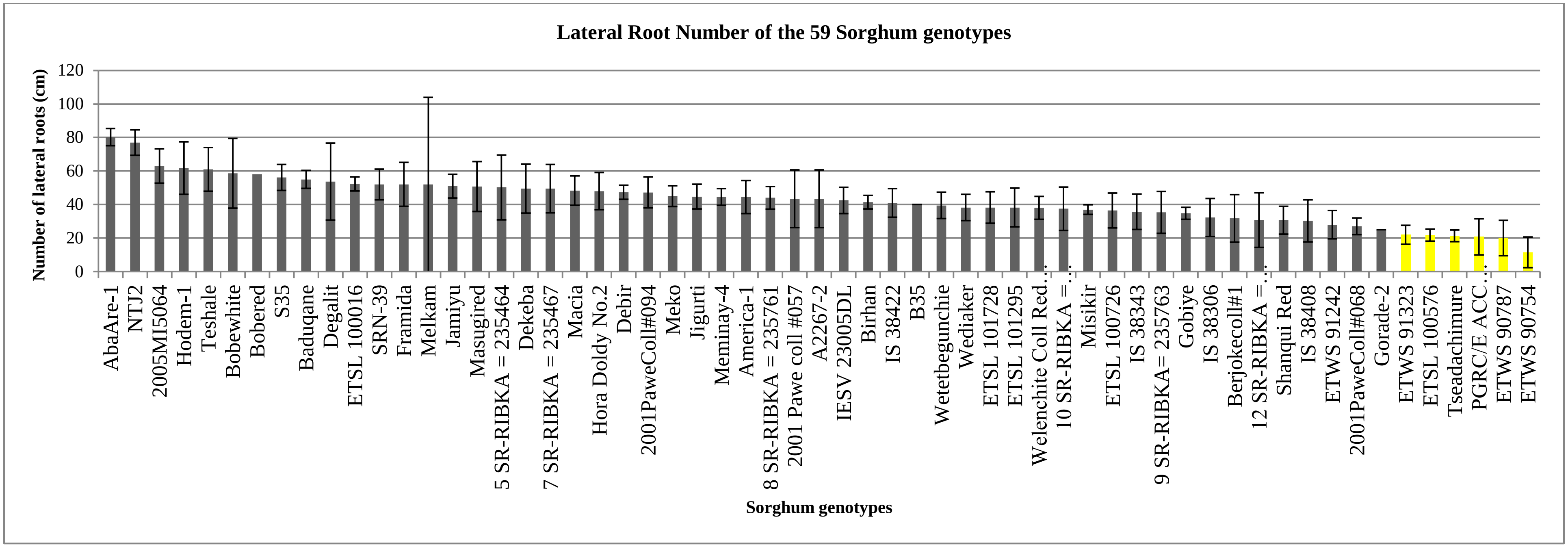


Figure 10: Summary of the Number of Lateral Root of 8 days old after germination of sorghum genotypes. The values represent the mean of the 4 replicates and error bars represent standard deviation whereas yellow bars indicate sorghum those with the lowest lateral root number per cm of primary root.

The results described in Figure 11 below demonstrate that genotypes NTJ (122.12 cm), Bobewhite (113.75 cm), AbaAre-1 (107.94 cm), Melkam (106.02 cm), Bobered (102.41 cm) and ETSL 100016 (101.42 cm) are good candidates for resistance to *Striga hermonthica* due to altered root system architecture as they have increased lateral root length.

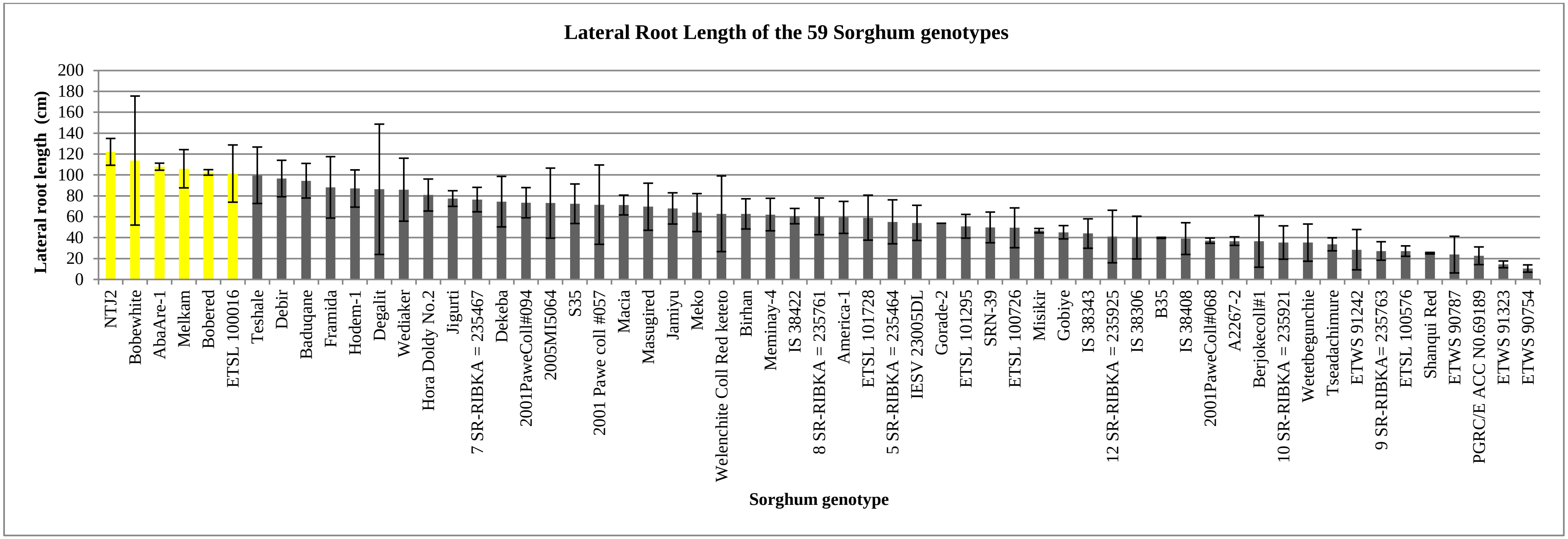


Figure 11: Summary of the Lateral Root Length of 8 days old after germination of sorghum genotypes. The values represent the mean of the 4 replicates and error bars represent standard deviation whereas yellow bars indicate the most extreme sorghum genotypes for lateral root length.

Table 3: Summary of the **ANOVA** for **RSA** of the 59 sorghum genotypes

Variables	Genotype	Mean Square	Error	CV	Grand mean	F	Pr>F
MRL	59	12.45	163	17.43	10.10	4.02	<.0001
#RL	59	652.49	163	23.93	42.79	6.22	<.0001
LRL	59	2477.23	163	32.45	61.70	6.88	<.0001

The analysis of variance showed significant variation (P≤ 0.001) among 59 sorghum genotypes screened for RSA (Table 3). This ANOVA table is the summary of the 59 sorghum genotypes treated with four biological replications.

7. DISCUSSIONS

In the current study, screening of various sorghum genotypes grown in Ethiopia for screening striga (*S. hermonthica*) resistance was conducted in the presence of control checks SRN 39 (negative control) and Teshale (positive control) using agar gel assay (AGA), low germination stimulant (lgs1) marker and root system architecture (RSA). This method helps to overcome the limitations experienced during field evaluation in establishing uniform environment to study host parasite interaction, as it happens in a controlled environment, laboratory. The method also allows observation of host-parasite interaction at various stages of striga life cycle (Mohamed *et al*., 2010)

The results from this study show that, from *LGS1/lgs1* genotyping, we concluded that the SRN39 (lines carrying *lgs1* allele), Gobiye, Birhan, Hora Doldy No- 02 and ESTL-101295 are likely resistant genotypes to *S. hermonthica* due to the presence of the *lgs1* allele. The other genotypes (from 59) are likely susceptible genotypes, at least at the pre-attachment stage, due to the presence of the *LGS1* allele (Fig.5). However, next to the low stimulant production (i.e. due to the presence or absence of *LGS/lgs1* allele**)**, there are a number of other resistance mechanisms to striga that have been suggested (Gebisa Ejeta *et al*., 1993). These include i) mechanical barriers to parasite ingress, ii) chemical defense (antibiosis) in which the crop plants may produce chemical compounds that discourage subsequent development of striga seedlings and iii) hypersensitivity where the host cells surrounding the endophytic part of the haustorium die and preclude further development of the parasite. Among all crops, more progress in breeding for resistance has been made in sorghum (Aba *et al*., 2001).

Germination is the first committed step of the striga life cycle as it occurs only in response to specific secondary metabolites, generally believed to be strigolactones (Bouwmeester *et al*.,

2007) in the root exudates of the host plant. There are variants in sorghum with low striga germination stimulant activity and such resistance is a viable target for controlling the parasite (Patrick R, and Gebisa Ejeta, 2008). Among the germplasm screened in this study, it shows, based on the agar gel assay, that low striga germination stimulant activity (MGD<10mm) escape from yield loss due to the parasitic weed. Although, the agar gel assay, requiring some investment of time and effort, gives a good indication of the presence or absence resistance trait. Therefore, screening for low striga germination stimulant activity by the agar gel assay could be a valuable and effective tool in screening sorghum genotypes resistant to striga.

According to Tokuma Legesse (2016), screening pre-attachment striga resistance among different genotypes for maximum germination distance (MGD) of the resistant standard check variety, SRN-39 (MGD = 1.4mm) and other resistant checks variety, Birhan had MGD value of 1.1mm. Both of these sorghum genotypes were previously reported to have low striga germination stimulant activity (Satish *et al*., 2012) indicating the reliability of the approach used in this study and confirmation for the level of resistance of those varieties.

As shown in fig.6 above, Framida (2.92 mm MGD), Birhan (3.43 mm MGD), ETSL 100118(4.35 mm MGD), ETSL100016 (4.67 mm MGD), SRN 39 (5.86) (control that have low MGD <10 mm MGD) and S35 (9.60 mm MGD) are sorghum genotypes likely resistant to *S. hermonthica* because they have less than 10 mm MGD and the other sorghum genotypes are susceptible to *S. hermonthica* because they have greater than 10 mm MGD value. Hodem-1 (37 mm MGD) genotype has the most extreme MGD value and is thus an extremely susceptible genotype. There was also a significant difference among the 59 sorghum genotypes screened using agar gel assay.

Different sorghum genotypes have different root system architecture. At this stage of development (8 days old after germination), sorghum genotypes like Melkam and ETSL 100016 are the only sorghum genotype with a long main root and high lateral root length combined. Thus these genotypes may have the capacity to overcome environmental stress due to striga parasitism. ETSL100016 also displays pre-attachment resistance due to its low MGD in the presence of striga. From the perspective of root system architecture only, sorghum genotypes like ETSL100576 and ETWS 91242 might be less susceptible, despite their high maximum germination distance and presence of the *LGS1* locus.

Generally, the study about root system architecture of cereals like sorghum is difficult; because different sorghum genotypes have different genetic variation and also different adaptation mechanism to environmental condition. This is indicated that sorghum resistance to *S. hermonthica* can be achieved through different resistance mechanisms.

As some reports indicate, different resistance mechanisms have been described by authors (Haussmann *et al.*, 2000b, Gebisa Ejeta, 2000) from different sources of resistance, i.e., low production of the germination stimulant (SRN 39, IS 9830, Framida, 555, SAR lines, IS 15401) low haustorial initiation stimulant (accession P-78 of Sorghum drummondii) mechanical barriers (N 13, Framida) antibiosis (SRN 39, N 13) and hypersensitivity (SAR 16, SAR 19, SAR 33, Sorghum versicolor). So, from our result it can be suggested that it is better if the characterization of root system architecture of sorghum occurs at later developmental stages, for example two-week-old plants because of the result obtain from this study was 8 days old stage.

CONCLUSION

Sorghum is the most important crop for food security in harsh environments in Ethiopia, where it is difficult to grow other food crops. Striga infestation, drought, declining soil fertility, lack of access to improved varieties and other production inputs, and bird damage identified by farmers as the most important constraints limiting sorghum production. Yield losses in sorghum due to *S. hermonthica* can be severe among subsistence farmers in Ethiopia who are unable to afford or with little access to inputs that can lessen the problem. Currently resource poor farmers in Ethiopia and across Africa require striga management options like resistant varieties that are environmentally friendly and cost effective.

So, this study was aimed to screen 59 sorghum genotypes identified in PROMISE project from different sorghum growing regions in Ethiopia under control environment (in laboratory) because of some difficulties in field tests and most pot studies for striga resistant variety evaluation. To meet this objective, screening of striga resistance sorghum genotypes using gel germination assay, low germination stimulant and root system architecture were done. In this study of the 59 sorghum genotypes screened, sorghum genotype like Framida, Gobiye, Birhan, Hora Doldy No- 02 and ESTL-101295 are likely to be resistant genotypes to *Striga hermonthica* in both low germination stimulant (lgs1) and gel based assay.

Depending on this information, from sorghum genotypes screened and resistant to striga were be used as production input for farmers. And also, this is potential use to plant breeders and pathologist with the goal of improving strength and durability of striga resistance in improved varieties.

RECOMMENDATION

In this study there were only 59 sorghum genotypes identified under PROMISE project and only one striga seed collection (Abergelle type) from Humera, Tigray region were used. However, this is amount of sorghum genotypes and striga seed are not enough to have sufficient information on screening sorghum genotypes to get striga resistant varieties. Based on this information, we recommended that;

- As striga populations are genetically diverse, different environment adaptation and for durable resistance, it would be desirable to include more striga collection locations to have striga resistance reported in this study with further post-attachment resistance mechanisms.
- It is also better to use more striga resistance mechanisms plus more molecular markers other than *LGS1* marker.
- So in the future, more sorghum genotypes should be screened using different approaches for striga resistance or host parasite interaction in order to better understand the mechanism underlying the effect of striga weed on sorghum crop.

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APPENDICES

Appendix 1: List of sorghum germplasm samples tested for *LGS1*, in gel germination and RSA genotyping

No	New ETSL No. or genotype	Name	Region/Origin
1	ETSL 101865	Wetetbegunchie	Amhara
2	ETSL 100312	Gorade-2	Amhara
3	ETSL 101851	Jamiyu	Tigray
4	ETSL 101862	Tseadachimure	Tigray
5	ETSL 101888	Hodem-1	Tigray
6	ETSL 100305	AbaAre-1	Tigray
7	ETSL 100308	Baduqane	Oromia
8	ETSL 101847	Degalit	Tigray
9	ETSL 101866	Wediaker	Tigray
10	ETSL 101844	Bobewhite	Benishangoul Gumuz
11	ETSL 101843	Bobered	Benishangoul Gumuz
12	ETSL 101857	Masugired	Oromia, west hararghe
13	ETSL 101845	Debir	Tigray
14	Gobiye	Gobiye	Purdue University
15	Birhan	Birhan	Purdue University
16	SRN-39	SRN-39	Purdue University
17	Framida	Framida	Purdue University
18	S35	S35	ICRISAT

19	B35	B35	ICRISAT
20	Meko	Meko	ICRISAT
21	Dekeba	Dekeba	ICRISAT
22	Melkam	Melkam	ICRISAT
23	Teshale	Teshale	ICRISAT
24	2005MI5064	2005MI5064	Advanced breeding lines
25	Macia	Macia	ICRISAT
26	NTJ2	NTJ2	ICRISAT
27	A2267-2	A2267-2	ICRISAT
28	ETSL 100107	2001 Pawe coll #057	2016 AN SMILL# 1459
29	ETSL 100118	2001PaweColl#068	Land race
30	ETSL 100143	2001PaweColl#094	Land race
31	ETSL 100316	Meminay-4	Land race
32	ETSL 100046	Hora Doldy No.2	Land race
33	IS 38320	Welenchite Coll Red keteto	Land race
34	P9501B	PGRC/E ACC N0.69189	Land race
35	IS 38422	IS 38422	Land race
36	ETSL 100307	America-1	Land race
37	ETSL 101853	Jigurti	Land race
38	ETSL 100309	Berjokecoll#1	Land race
39	ETWS 90754	ETWS 90754	Amhara/Tekle dingay/
40	ETWS 90787	ETWS 90787	Beneshangul/Mandura

41	ETWS 91242	ETWS 91242	Beneshangul/Bambasi/
42	ETWS 91323	ETWS 91323	Oromia/Mondi/
43	IESV 23005DL	IESV 23005DL	Selected based on 2015 MI Drought score
44	IS 38408	IS 38408	Selected based on 2015 MI Drought score
45	IS 38343	IS 38343	Selected based on 2015 MI Drought score
46	ETSL 100016	ETSL 100016	Selected based on 2015 MI Drought score
47	Misikir	Misikir	Selected based on 2015 MI Drought score
48	ETSL 101728	ETSL 101728	Selected based on 2015 MI Drought score
49	IS 38306	IS 38306	Selected based on 2015 MI Drought score
50	ETSL 100726	ETSL 100726	Selected based on 2015 MI Drought score
51	ETSL 100576	ETSL 100576	Selected based on 2015 MI Drought score
52	ETSL 101295	ETSL 101295	Selected based on 2015 MI Drought score
53	235464	-	Source is IBC

54	235467	-	Source is IBC
55	235761	-	Source is IBC
56	235763	-	Source is IBC
57	235921	-	Source is IBC
58	235925	-	Source is IBC
59	Shanqui Red	-	

Appendix 2: Summary of the LGS1/lgs1 allele presence or absence in 59 sorghum genotypes

S/N	Genotype name	LGS1 or lgs1	
		208	**333**
1	Wetetbegunchie	1	1
2	Gorade-2	1	1
3	Jamiyu	1	1
4	Tseadachimure	1	1
5	Hodem-1	1	1
6	AbaAre-1	0	0
7	Baduqane	1	1
8	Degalit	1	1
9	Wediaker	1	1
10	Bobewhite	1	1
11	Bobered	1	1
12	Masugired	1	1

13	Debir	1	1
14	Gobiye	0	1
15	Birhan (resistant)	0	1
16	SRN-39(Resistant)	0	1
17	Framida	1	1
18	S35	1	1
19	B35	1	1
20	Meko	1	1
21	Dekeba	1	1
22	Melkam	1	1
23	Teshale (suseptable)	0	1
24	2005MI5064	0	1
25	Macia	1	1
26	NTJ2	1	1
27	A2267-2	1	1
28	2001 Pawe coll #057	1	1
29	2001PaweColl#068	0	0
30	2001PaweColl#094	1	1
31	Meminay-4	1	1
32	Hora Doldy No-2	1	0
33	Welenchite Coll Red keteto	1	1
34	PGRC/E ACC N0.69189	1	1
35	IS 38422	1	1

36	America-1	1	1
37	Jigurti	1	1
38	Berjokecoll#1	1	1
39	ETWS 90754	1	1
40	ETWS 90787	1	1
41	ETWS 91242	0	1
42	ETWS 91323	0	1
43	IESV 23005DL	1	1
44	IS 38408	1	1
45	IS 38343	1	1
46	ETSL 100016	1	1
47	Misikir	0	0
48	ETSL 101728	1	1
49	IS 38306	0	0
50	ETSL 100726	1	1
51	ETSL 100576	1	1
52	5 SR-RIBKA = 235464	1	1
53	7 SR-RIBKA = 235467	0	0
54	8 SR-RIBKA = 235761	1	0
55	9 SR-RIBKA= 235763	0	1
56	10 SR-RIBKA = 235921	0	1
57	ETSL101295	1	0
58	12 SR-RIBKA = 235925	1	1

59	Shanqui Red (line carrying LGS1 allele (have high Striga germination stimulant activity))	1	1

Appendix: 3 List of the Maximum Germination Distance of the 59 Sorghum genotypes.

S/N	Sorghum genotype	Maximum Germination Distance (mm)(genotype mean)
1	Framida	2.92
2	Birhan	3.43
3	2001PaweColl#068	4.35
4	ETSL 100016	4.67
5	SRN-39	5.86
6	Gobiye	7.42
7	S35	9.60
8	B35	12.93
9	Wediaker	12.95
10	12 SR-RIBKA = 235925	13.11
11	IS 38343	13.20
12	5 SR-RIBKA = 235464	16.07
13	9 SR-RIBKA= 235763	16.67
14	Teshale	17.40
15	Berjokecoll#1	17.45
16	8 SR-RIBKA = 235761	17.67
17	Bobered	17.83

18	Gorade-2	18.58
19	NTJ2	18.83
20	A2267-2	18.83
21	Macia	19.17
22	IS 38408	19.39
23	ETWS 90754	19.42
24	IS 38306	19.83
25	Melkam	20.56
26	Wetetbegunchie	20.67
27	Hora Doldy No.2	21.50
28	2001 Pawe coll #057	21.50
29	Misikir	21.56
30	Jigurti	21.67
31	IESV 23005DL	21.87
32	Debir	21.92
33	Meminay-4	22.00
34	ETSL 100726	22.33
35	Welenchite Coll Red keteto	22.50
36	7 SR-RIBKA = 235467	22.92
37	ETSL 101295	23.09
38	ETWS 90787	23.50
39	AbaAre-1	24.10
40	ETWS 91242	24.11

41	Shanqui Red	24.50
42	IS 38422	24.75
43	ETWS 91323	24.83
44	Bobewhite	24.89
45	Meko	24.89
46	2005MI5064	24.89
47	America-1	25.92
48	Baduqane	26.17
49	ETSL 101728	26.33
50	Jamiyu	26.34
51	10 SR-RIBKA = 235921	26.45
52	Degalit	27.78
53	2001PaweColl#094	27.89
54	PGRC/E ACC N0.69189	28.00
55	ETSL 100576	28.08
56	Dekeba	28.09
57	Tseadachimure	29.78
58	Masugired	34.67
59	Hodem-1	37.67

Appendix 4: Protocol: DNA Extraction w/ 96-well plate (as of 12.14.18) gDNA Extraction:

1. Collect tissue into 1.2 ml collection microtubes, for each plant.

2. Add one silica bead per tube.

3. Add 250 µl of extraction buffer without SDS (200 mM Tris-Cl pH 7.5, 250 mM NaCl, 25 mM EDTA).

4. Seal tubes with cap strips using the blue roller.

5. Disrupt tissue with tissue lyser (e.g. TissueLyserII, Qiagen), with 2 cycles of 30 Hz for 2 minutes.

6. Pulse spin plates to remove liquid from lids, before adding 250 µl of extraction buffer with 2x SDS (200mM Tris-Cl pH 7.5, 250 mM NaCl, 25 mM EDTA, 1 % SDS).

7. Centrifuge at 2250 rcf for 10 minutes.

8. While plates are spinning, add 200 µl of isopropanol per well to 0.8 ml storage plates (Thermo Scientific, Cat. # AB-0765).

9. Using a multichannel pipette, transfer 200 µl of supernatant per sample into the wells (containing isopropanol). Notice plate orientation. Pipette up and down several times to ensure mix.

10. Seal plate with PCR plate adhesive and incubate at RT for 10 minutes.

11. Centrifuge at 2250 rcf for 35 minutes.

12. Pour off supernatant and blot plates with paper towel (whilst upside down).

13. Wash DNA with 150 µl of 70% EtOH (nuclease-free), then pour off.

14. Pulse spin plates to ~ 100 rcf (upside down on paper towel) to remove excess liquid.

15. Air dry for 20-30 minutes in the flow hood.

16. Resuspend in 150 µl Nuclease-free water. Shake gently; leave to stand for 5 minutes.

17. Seal plates with PCR plate adhesive and store at 4 ºC

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