ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

INSTITUTE OF BIOTECHNOLOGY

Screening and Identification of Potential *Striga* [*Striga hermonthica (Del.)*] Suppressing Rhizobacteria Associated with Sorghum [*Sorghum bicolor* (L.) Moench] in Northern Ethiopia

By

Urgesa Tsega Tulu

A Thesis Submitted to the School of Graduate Studies, Addis Ababa University for the Partial Fulfillment of the Requirement for the Degree of Masters of Science in Biotechnology

Addis Ababa, Ethiopia

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Approval Sheet

As thesis research Advisor, we hereby certify that we have read and evaluated this thesis prepared under our guidance by Urgesa Tsega Tulu entitled "**Screening and Identification of Potential *Striga* [*Striga hermonthica (Del.)*] Suppressing Rhizobacteria Associated with Sorghum [*Sorghum bicolor* (L.) Moench] in Northern Ethiopia**", we recommended to be submitted as fulfilling the thesis requirement.

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As members of the examining committee of the final MSc. Open Defense Examination, we certify that we have read and evaluated the thesis prepared by Urgesa Tsega Tulu and examined the candidate. We recommended the Thesis to be accepted as fulfilling the Thesis requirement for the degree of Master of Science in Biotechnology.

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ABSTRACT

*Sorghum (*Sorghum bicolor *(L.) Moench) is one of the globally important cereal crops well adapted to Sub-Saharan Africa (SSA) agro-ecologies. However, the productivity of sorghum is hindered by both abiotic and biotic factors including drought,* Striga*, insect pests, poor soil fertility and diseases. Among the constraints,* Striga *(genus), also called witch weed is the most important production problem in the area. Out of* Striga *species,* Striga hermonthica *can cause a complete yield loss making it the most economically important weed. Control methods including cultural, chemical, biological and breeding for resistance have been practiced for years. To date, none of these strategies have become practically effective to eradicate* Striga*. Some of the existing control methods mentioned are not easily accessible for small holder farmers, while still some are not environmentally friendly. Therefore, this thesis focused on an alternate control of* Striga hermonthica *that employ rhizobacteria whose application is scanty in the study area. Hence, study was designed with the objective of identifying potential* Striga *suppressing rhizobacteria associated with sorghum. Soil samples were collected from three* Striga *infested sorghum fields with different levels of* Striga *infestation in northern Ethiopia. In order to activate the microbes, the sample was mechanically homogenized and various sorghum varieties grown on it in the greenhouse for four weeks. One hundred seventeen rhizobacteria were isolated from the root surface of the sorghum and these were then screened for their* Striga *suppressive traits, namely hydrogen cyanide and indole acetic acid production ability. Accordingly, 40.2% and 46.8% were capable of producing hydrogen cyanide and indole acetic acid, respectively. Twenty two isolates (18.8%) could produce both hydrogen cyanide and indole acetic acid in common. These elites were considered for further* in vitro *effect on* Striga *seed germination, in the presence and absence of susceptible host plant, and all of them, with varying ranges, showed a statistically significant germination inhibition. Finally, shortlisted most effective isolates RI6, RI9, RI19, RI23, RI26, RI38, RI54 and RI72 were morphologically and biochemically identified to belong to the genera of* Pseudomonas, Klebssiella, Bacillus *and* Entrobacter*. The result of the study demonstrated the existence of promising soil born bacteria that could be exploited as a bioherbicides to control* Striga *infestation on sorghum provided that broader samples from various parts of the country are explored.*

Key words/phrases: Biotic factor, Germination inhibition, Rhizosphere, Striga infestation, susceptible host

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ACRONYMS AND ABBREVIATIONS

AATF	African Agricultural Technology Foundation
ACC	1-aminocyclopropane-1-carboxylic acid
AGA	Agar Gel Assay
ANOVA	Analysis of Variances
CFU	Colony Forming Units
CSA	Central Statistical Agency
DRB	Deleterious Rhizosphere Bacteria
EIAR	Ethiopian Institute of Agricultural Research
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organization
FAOSTAT	Food and Agricultural Organization Statistical Database
GPS	Global Positioning System
GR-24	Germination Regulator-24
HCN	Hydrogen Cyanide
IAA	Indole acetic acid
ICRSAT	International Crop Research in Semi-Arid Temperature
ISC	Integrated *Striga* Control
LB	Luria Broth
LGS1	Low Germination Stimulant 1
NABRC	National Agricultural Biotechnology Research Center
PGPR	Plant Growth Promoting Rhizobacteria
SSA	Sub Saharan Africa
VIGS	Viral Induced Gene Silencing

1. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) belongs to the *Poaceace* family and it is one of the globally important cereal crops. It is widely grown in the semi-arid tropics, where frequent drought is experienced. It is the fifth in line of production after maize, rice, wheat and barley and feeds over 500 millions of people in the developing world (Shapiro and Wortmann, 2006; FAO, 2012; Spallek *et al*., 2013). 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 2.8 tons per hectare (CSA, 2018).

However, due to abiotic and biotic constraints, its potential productivity is reduced. Among the abiotic factors are low soil fertility, drought and salinity. Agriculturally important biotic constraints include the hemi-parasitic weed *Striga*, panicle diseases, stem borers and insects (Wortmann *et al*., 2006). In Ethiopia, sorghum production challenges associated to both biotic and abiotic constraints vary from region to region. However, drought and *Striga* are the most important problems across the country (Rich *et al*., 2004).

The genus *Striga*, also commonly called ‘Witch weed’, is one of the greatest biological constraints to food production in arid and semi-arid regions of Sub-Saharan Africa (SSA) and other parts of the world where sorghum is widely grown (Spallek *et al*., 2013). *Striga* is under the family *Orobanchaceae* that contains the highest number of parasitic species (Atera *et al*., 2011). Approximately, more than 30 *Striga* species have been described and most parasitize cereals including sorghum, millet and maize grown in most semi-arid and tropical regions of the world (Atera *et al*., 2011; Spallek *et al*., 2013). Complex host-parasite interactions, production of large number of seeds with prolonged viability (about 800,000 seeds per plant which remain viable in the soil for up to 20 years), and special germination requirements make *Striga* the most problematic weed (Mourik, 2007: Atera *et al*., 2011; Hayelom Berhe, 2014).

Several control measures have been suggested for weeds in general and *Striga* in particular. The most widely investigated and developed control approaches include cultural, chemical, biological, genetic or breeding for resistance and combination of more than one of these (Gebissa Ejeta, 2007; Ayongwa *et al*., 2011; Nzioki *et al*., 2016). Many of these methods are either not practically successful or not economically feasible for low income farmers in SSA (Bozkurt *et al*., 2014).

Research on breeding for *Striga* resistance has been widely conducted. More than 80 resistant sorghum varieties have been selected by the International Crop Research in Semi-Arid Temperature (ICRSAT) in India of which some high yielding *Striga* reistant introduced and registered in Ethiopia by EIAR (Gebissa Ejeta, 2007; Hayelom Berhe, 2014). However, effective resistance against *Striga* is difficult to develop mainly because of the complex nature of the parasitic weed and the numerous environmental effects and genetic diversity of parasitic plants (Rubiales, 2003; Aly, 2012). In addition, resistant breeds come with some undesirable agronomic characteristics such as low farmers preferences and low durability of the resistance gene (Hayelom Berhe, 2014; Hailegebrial Kinfe *et al*., 2018). The use of chemical herbicides on the other hand is risky and hazardous; do not differentiate between the crop and the parasite in their mode of action.

Small-scale farmers particularly in Northern region of Ethiopia need easy accessible and effective *S. hermonthica* management strategies that are compatible with their production practices. The use of microorganisms as an alternative to control weed has been gaining momentum throughout world (Hayelom Berhe, 2014). Advantages of weed biological control include low cost of production, high specificity, safe and environmental friendly (Charudattan, 2001). Studies have indicated the use of rhizosphere bacteria as a weed management strategy (Flores-Vargas and O'Hara, 2006; Kremer and Kennedy, 1996).

Soil borne bacteria have potentials to perturb early stages of *Striga* and *Orobanche* growth by reducing their incidence by 90 to 100 percent (Hassan *et al*., 2009). Ahonsi *et al*. (2002) also showed that soil-borne fluorescent *Pseudomonad* strains suppressed the germination of *S. hermonthica* and *Orobanche* seeds. Moreover, a few pathogenic bacteria were found to be effective to control *S. hermonthica* and replace commercial chemical herbicides (Pilgeram and Sands, 2010). Mounde (2014) showed the significant suppression of the key stages of *Striga* development by *Bacillus* strains. Neondo (2017) identified microbes that were potent against *S. hermonthica* and proposed their use in reduction of *S. hermonthica* seed bank in infested soils.

The communication between microbes and *S. hermonthica* depends on signal transduction, the expression of pathogenicity and virulence factors of the microbe (Neondo, 2017). Thus, inoculation of microbes such as rhizobacteria could minimize competition of cereal crops with the weeds and may reduce the use of chemical herbicides, and could benefit agriculture contributing to increased crop yields. However, isolation, characterization and utilization of specific microbial agents capable of causing *S. hermonthica* seed decay has not been much exploited in Ethiopia. Therefore, the aim of this study was to identify specific rhizobacteria that have potential of suppressing *Striga* infestation on sorghum.

2. OBJECTIVES OF THE STUDY

2.1 General Objective

The main objective of this study was to screen and identify potential *Striga* suppressing rhizobacteria associated with sorghum in Northern Ethiopia.

2.2 Specific objectives

- To isolate *Striga* suppressive rhizobacteria associated with sorghum and screen for selected traits
- To evaluate the effect of selected bacteria on *Strig*a germination
- To identify effective *Striga* suppressive bacteria for future use as biological control

3. LITERATURE REVIEW

3.1 Origin and Distribution of *Striga*

Striga originates along a region between Ethiopia and Sudan (Atera *et al*., 2011). This parasite weed is generally native to SSA but has been observed in more than 40 countries (Gebissa Ejeta, 2007). Out of more than 30 species of *Striga* described, nine species are found outside Africa and three species: *S. curvilflora*, *S. multiflora* and *S. parviflora* are present in the Australian continent (Berne*r et al*., 1995; Spallek *et al*., 2013).

S. hermonthica is widely spread in semi-arid areas and is found in northern tropical Africa, from West Africa (Senegal) to Eastern Africa (Ethiopia, Uganda and Kenya), and the Democratic Republic of Congo, and extends from the western Arabian region and southwards into Angola, Namibia, Madagascar and Tanzania (Parker and Riches 1993; Atera *et al*., 2011). Nigeria, Sudan, Ethiopia, Mali and Burkina Faso are heavily affected counties in Africa (Berhane Sibhatu, 2016).

Striga asiatica is the most widely distributed and is found throughout African tropical parts from portions of southern (including Madagascar), central, and western Africa and Australia (Cochrane and Press, 1997). It is also native to Asia including the Philippines, Cambodia, Indonesia, China, Malaysia, Thailand, Vietnam, Mauritius, India and the Arabian Peninsula. *S. asiatica* has been introduced to the US. *S. gesnerioides* is endemic to Africa, Arabia and Asia and it has been introduced to the United States (Mohamed *et al*., 2007).

3.2 Economic Importance of *Striga*

Among *Striga* species described, five *Striga* species, *S. hermonthica*, *Striga asiatica*, *S. gesnerioides Striga aspera Striga forbesi*, are currently of economic importance, with *Striga* *hermonthica* causing the most serious damage to Sub-Saharan cereal production (Parker, 2009). The most devastating *Striga* species to staple crops in SSA are *S. hermonthica, S. asiatica,* and *S. gesnerioides*. (Spallek *et al*., 2013; Hayelom Berhe, 2014). Most *Striga* species parasitize grass species, but *S. gesnerioides* has evolved the capacity to parasitize dicotyledonous plants (Spallek *et al*, 2013)

S. hermonthica is particularly harmful to sorghum, maize and millet, but it is also increasingly being found in sugarcane and rice fields (Atera *et al*., 2011). Depending on *Striga* seed density, soil fertility, rainfall distribution, variety grown and degree of *Striga* infestation, the parasitic weeds damage ranges from 20-80% of staple food crops in the semi-arid tropics of Africa and Asia. The situation in Sudan is even worse, where yield losses in cereal crops heavily infested by *S. hermonthica* may reach up to 100% yield loss (Gebissa Ejeta, 2007; Atera *et al*., 2011).

The annual crop losses due to *Striga* are estimated at US$ 7 billion in SSA and particularly in Ethiopia, Mali and Nigeria, it is estimated at US$75 million, US$87million and US$1.2 billion, respectively (AATF, 2011). Yield losses due to *Striga* can reach up to 100 percent in susceptible cultivars under a high infestation level and when compounded by drought conditions (Haussmann *et al*., 2000).

3.3 The Parasitic Life Cycle of *Striga*

Striga species are annual plants completing most of their life cycle underground. The life cycle of *Striga* can be divided into three stages (Figure 1): germination, haustorium development and establishment of parasitism and its maintenance until seeds are set (Spallek *et al*., 2013)).

Striga seed germination is elicited when ripened seeds are pre-conditioned by exposure to warm moist conditions for several days, known as conditioning or preconditioning, followed by exogenous chemical signals produced by host roots and some non-hosts (germination stimulant) (Gebissa Ejeta, 1993; Babiker *et al*., 2007). After germination the radicle elongates towards the root of the host, develops an organ of attachment, the haustorium, that helps to penetrates into the host vascular tissue and establish parasitism. This follows the deprivation of water, mineral nutrients and carbohydrates of host plant, causing drought stress and wilting (Berner *et al.*, 1997; Musselman, 1980).

Conditioning, germination, parasitic contact (attachment) and penetration are mediated by elegant systems of chemical communication between host and parasite (Sato *et al*., 2003). After several weeks of growth, the parasite emerges above the soil surface and starts to flower and produces seeds (Kroschel, 2002; Rich and Gebissa Ejeta, 2007).

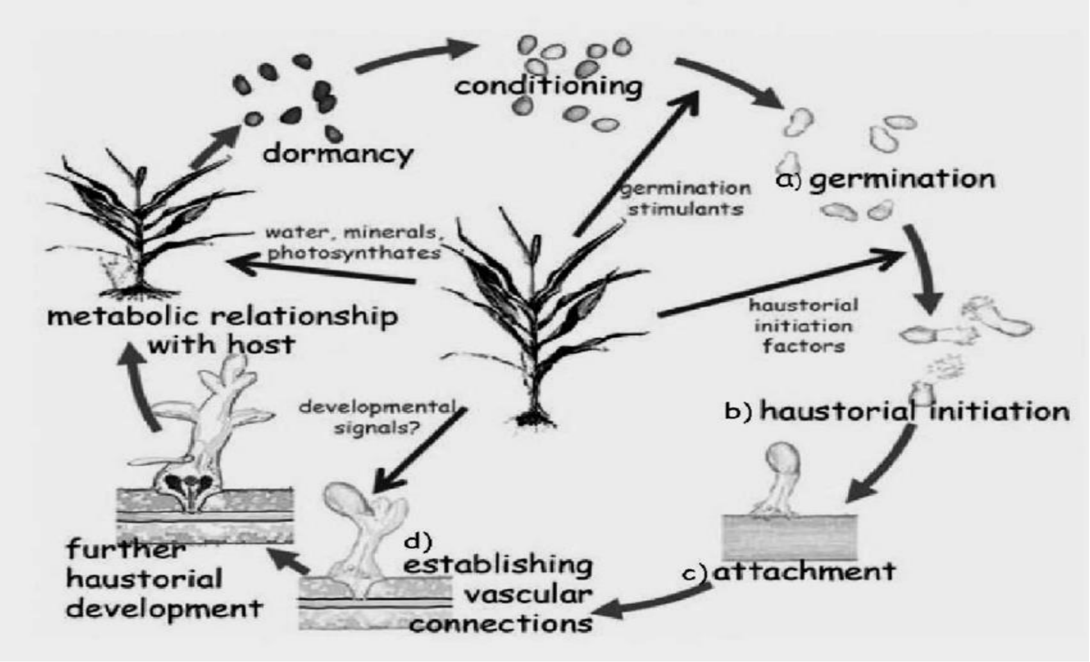


Figure 1. Generalized *Striga* life cycle (Rich and Gebissa Ejeta, 2007)

3.4 Management and Control Options of *Striga*

Striga parasitism control strategy generally include cultural, chemical, resistance breeding and biological (Hayelom Berhe, 2014; Berhane Sibhatu, 2016) and genetic engineering and/or mutation breeding (Pixley *et al*., 2019) each of which has been described below.

3.4.1 Cultural and Mechanical Control Methods

Several cultural and mechanical practices have been used as *Striga* control strategies (Nzioki *et al*., 2016) including crop rotation (Sunda *et al*., 2013), soil fertility management (Fasil Reda and Verkleij, 2007), push and pull technology, intercropping (Van Mourik *et al*., 2011; Sunda *et al*., 2013; Watson, 2013), and hand-pulling (Ransom, 2000) and application of fertilizers (Gworgwor and Weber, 2003). It has been noted that weed seedling often stays below ground for over a month, during which time it is totally parasitic on the crop and does considerable damage before the farmer even sees the weed. This made conventional hand or mechanical weeding less effective. Besides, this method has got acceptances by few poor farmers due to socioeconomic and financial constraints (Berhane Sibhatu, 2016).

3.4.2 Chemical Control Methods

Today there are both synthetic and natural compounds investigated for their ability to induce suicidal seed germination of parasitic weeds *Striga*. As it has been reviewed in Hayelom Berhe (2014), strigol analogue chemicals like ethylene can induce germination of *Striga* seeds in the absence of a suitable host and therefore reduce *Striga* seed bank in the soil in a process of suicidal germination. In the United States, the *S. asiatica* eradication program using ethylene gas caused 90% germination when injected into the soil (Gebissa Ejeta and Gressel, 2007). Furthermore, Nijmegen 1 and GR 24, synthetic analog structurally related to the natural stimulant Strigolactone, are the most potent for many species of *Striga* and *Orobanche* at low concentration (Wigchert *et al.* 1999; Khan *et al.*, 2008). However, the instability of these chemicals in soil, and the high cost of producing large quantities of these compounds, have so far hindered their use in agriculture (Hayelom Berhe, 2014).

3.4.3 Resistance Breeding

One of the promising new approaches to *Strig*a control is the use of resistant cultivars. During the previous few decades, research efforts were dedicated to identifying germplasm with resistance to *Striga*. As a result, a few cultivars with a good trait of *Striga* resistance have been screened mainly in sorghum and pearl millet (Hess and Gebissa Ejeta, 1992; Kountche *et al*., 2013). *Strig*a resistant cultivars have been bred in a number of crops though a complete resistance to *Striga* has not been found in all host crops. Studies have indicated that, in *Strig*a infested areas, cultivation with resistant crops results in fewer *Striga* plants and higher crop yield than a susceptible genotype (Mamudu *et al*., 2019).

Improved breeding strategies have resulted in the development of appropriate breeding populations and deployment of target selection for resistance to *Strig*a. More than 82 lines with *Striga* resistance have been selected by Purdue University and ICRISAT (Gebissa Ejeta, 2007; Hayelom Berhe, 2014). Three *Striga*-resistant varieties, locally called Gubiye, Abshir, and Birhan, have been reported to be officially released for wide cultivation in *Striga*-endemic regions of Ethiopia (Gebissa Ejeta, 2005; Kountche *et al.*, 2016). The *Striga*-resistant sorghum variety locally named Hormat has been also developed and released in India by ICRISAT (Asfaw Adugna, 2007).

Long term stability of the released *Striga*-resistant varieties has been analyzed by many researchers and does not look promising. High genetic variability has been reported to be maintained within populations from generation to generation, especially in *S. hermonthica* (Huang *et al*., 2012). Furthermore, the ability of this *Striga* species to quickly evolve new races and to break resistance has been recorded (Rich and Gebissa Ejeta, 2008).

Pyramiding of resistance genes into cultivars decreases the chance of resistance breakdown. Virulent *S. hermonthica* races would be less likely to emerge if multiple mutations were required to overcome host resistance genes (Rich and Gebissa Ejeta, 2008).

3.4.4 Application of Genetic Engineering

Neither conventional breeding nor other weed management strategies have provided efficient *Striga* control. Application of the modern biotechnological methods such as genetic engineering in the field of *Striga* resistance research provides solutions for *Striga* control. This technique targets genes responsible for the production of root exudates that stimulate seed germination, attachment and haustorium development in sorghum (Pixley *et al*., 2019).

Mutant low germination stimulant 1(LGS1) allele causes resistance to *Striga* by reducing germination stimulant activity in sorghum and changing stereochemistry of strigolactones, hormones that control plant architecture and below-ground signaling to mycorrhizae. These hormones are also required to stimulate parasite germination (Daniel Gobena *et al*., 2017; Bellis *et al*., 2020). The LGS1 allele contains gene that codes for sulfotransferase and whose functional loss alters the strigolactone biosynthetic pathway in the root exudate to reduce production of the *Striga* germination (Daniel Gobena *et al*., 2017; Pixley *et al*., 2019). The mutation at LGS1 locus does not knock out strigolactones in root exudates rather it changes the relative abundance of certain types while other essential functions of strigolactones remain intact (Daniel Gobena *et al*., 2017).

Tomilov *et al*. (2008) and Alakonya *et al*. (2012) successfully silenced RNAi gene in *Cuscuta pentagona* and *Triphysaria versicolor* in preventing haustorial establishment and development on tobacco and lettuce, respectively. In addition, Kirigia *et al*. (2014) silenced a gene in *S. hermonthica* using viral induced gene silencing approach (VIGS). Studies have shown that there are many putative parasitism genes that were upregulated during haustoria development in *S. hermonthica* (Yang *et al*., 2015). However, determination of which genes to target to prevent attachment in *Striga* is ongoing (Pixley *et al*., 2019).

3.4.5 Biological Control

The limitations of chemical herbicides encouraged researchers to look for alternative systems of weed control (Boyette *et al*., 1991). Biological control is considered as a potential cost effective, safe and environmentally beneficial alternative as a means of reducing weed populations in crops (Charudattan, 2001). Majority of microbes used as bio-herbicides are fungal pathogens, though there are increasing number of bacterial strains being explored and developed as bio-control of weed as well (Bailey *et al*., 2011). A virulent strain of *Xanthomonas campestris* was shown to control common cocklebur (*Xanthium strumarium* L.) which is an important weed in soybean, cotton and peanut production (Boyette and Hoagland, 2013).

Of the bacteria used as potential biological control of weed, *Pseudomonas fluorescens* and *Xanthomonas campestris* have been widely investigated for their use as bio-herbicides (Harding and Raizada, 2015). In contrast to *Xanthomonas* ssp. not all *Pseudomonas ssp*. are phytopathogens. *Pseudomonas chlororaphis* and *P. fluorescens* strains have been also used as biocontrol agents, while several strains of *Pseudomonas aeruginosa* and *Pseudomonas stutzeri* show strong plant growth-promoting activities (Shen *et al*., 2013). Several *P. putida* strains were also used to control velvetleaf and *S. hermonthica*, *P. fluorescens* strains to control *S. hermonthica*, broomrape and wild radish (Stubbs and Kennedy, 2012). Furthermore, strains belonging to the genera *Burkholderia*, *Aeromonas*, *Chryseomonas*, *Agrobacterium* and *Vibrio* spp., were tested for potential use as bioherbicides (Li and Kremer, 2006).

3.5 Striga-rhizobacteria Interaction

Rhizosphere is the narrow region of soil that is directly influenced by living roots, and the primary site of interaction between plants and microorganism (Berendsen *et al*., 2012). The microbe-plant interaction in the rhizosphere can be beneficial, neutral or deleterious on the basis of their effects on plant growth (Glick, 1995). The potential of using bioherbicides to control weeds such as *S. hermonthica* has received increasing attention (Charudattan, 2001; Gafar *et al*., 2016) for the purposes briefly discussed below.

3.5.1 Germination Induction

Bacterial effect on *Striga* seed could be either germination induction, in the absence of host plant or inhibition (Berner *et al*., 1999; Ahonsi *et al*., 2002). For example, bacterial strains of *Pseudomonas syringae* pv. glycinea induced early germination of *Striga* seeds and reduced subsequent establishment of *Striga*. The main growth regulators hormones acting as germination promoters produced by *P. syringae* are indoleacetic acid (IAA) and ethylene (Babalola *et al*., 2007). *P. syringae* strains stimulated more germination of *S. hermonthica* seeds as compared to ethylene gas (Berner *et al*., 2003).

This bacterium can be highly applicable in inducing pseudal germination of *Striga* seeds but its use in agriculture is limited because of its pathogenesis. The bacterial stimulation of *Striga* germination through the action of ethylene and a procedure testing how some ethylene-producing bacteria stimulate *Striga* germination has been developed (Babiker *et al.*, 1993; Berner *et al.*, 1999)

Furthermore, some other bacteria produce growth-regulators like auxins, cytokinins and gibberellins which are necessary in priming *Striga* seeds prior to germination thus reducing the preconditioning period and promotes germination (Hoagland *et al*, 2004; Joel *et al*., 2007). Although little is known about the mechanism of action of IAA prior to *Striga* seed germination, the hormone is critical in establishing the orientation of xylem differentiation between host and parasite (Hoagland *et al*, 2004; Delavault *et al*., 2017).

3.5.2 Germination Inhibition

Some microorganisms colonizing the root surface have growth inhibition effect on parasitic weeds like *Striga*. Soil bacteria including *Pseudomonas sp*., *Enterobacter sakazakii* and *Klebsiella oxytoca* have been evaluated for their potential to inhibit *S. hermonthica* seed germination (Babalola and Odhiambo, 2008). Other studies have also shown that *P. fluorescens* and *Pseudomonas putida* isolates significantly inhibit germination of *S. hermonthica* seeds (Babalola *et al*., 2002; Babalola *et al*., 2007). Furthermore, an *in vitro* evaluation of the effect of *Azospirillum* cells on *Striga* seed in the presence of GR24 demonstrated unsuppressed germination but shortened radicles. It has also been suggested that phytohormones such as IAA or lipophilic compounds released by the bacteria caused suppressed germination, radical growth and cell differentiation. (Miche *et al*., 2000).

3.6 Modes of Action of Bio-herbicidal Rhizobacteria

Many *Pseudomonas* strains are characterized as deleterious rhizobacteria. This is a group of non-parasitic pathogens which excrete exopolysaccharides and allelochemicals in the form of phytotoxins, phytohormones, cyanide, siderophores and that can negatively affect the metabolism of plants (Li and Kremer, 2006). Soil bacteria or endophytes may produce host-specific phytotoxic secondary metabolites. For example, bacterial pathogens like *Agrobacterium spp*. and *Pseudomonas savastanoi* pv. savastanoi produce auxins, which cause tumor and gall formation, and *Enterobacter sp*. strain produces IAA and seedlings of lettuce and radish inoculated with this strain showed reduced biomass production (Carvalho *et al*., 2007).

3.6.1 Production of Phytotoxin and Other Secondary Metabolites

Secondary metabolites produced by microbes have comparably shorter life spans and are biodegradable than conventional halogenated chemical structures. Rhizobacteria for biological control of weeds likely metabolize phytotoxins at root surfaces where they're readily absorbed by the plant. It's not known how widespread phytotoxin production is among weed biocontrol rhizobacteria, but evidence is accumulating showing that phytotoxins play a causal role in deleterious activity (Kao-Kniffin *et al*., 2013). Metabolites such as phaseolotoxin, tabtoxin, and coronatine were produced by *Pseudomonas* sp. and found to exhibit good herbicidal activity (Sexena, 2014). Within the rhizosphere of plants, the metabolites produced are often can be phytotoxic at beyond physiologic concentrations and these include the indole acetic acid (IAA), auxins and hydrogen cyanide. Other herbicidal compounds prevent the germination of seeds through inhibition or arrestment (Kao-Kniffin *et al.,* 2013)

Many rhizobacterial genera are known to produce IAA and auxin-related compounds. The best examples are the genera of *Acetobacter, Agrobacterium, Arthobacter, Azospirillum, Azotobacter,* *Bacillus, Klebsiella, Pseudomonas and Xanthomonas* (Idris *et al*., 2007; Spaepen *et al.,* 2008; Ali *et al*., 2010; Spaepen and Vanderleyden, 2011; Saha *et al*., 2012).

In addition, Cyanide was identified as secondary metabolite produced by many rhizosphere bacteria and having growth inhibition effects to suppress weeds (Kremer and Souissi, 2001). It is produced by a wide range of plants, bacteria and algae and it is proved to be accountable for growth reduction of weeds (Lakshmi *et al.*, 2015). The production of this toxic chemical could be a common trait of many Rhizosphere *Pseudomonas spp*. Cyanide is a potential inhibitor of enzymes involved in various plant metabolic processes (Reeth *et al.*, 2014). Other herbicidal compounds prevent the germination of seeds through inhibition or arrestment (Kao-Kniffin *et al.*, 2013).

3.6.2 Antibiotic Production

Many antibiotics are produced intracellularly by rhizobacteria and secreted through cell membranes into the surrounding vicinity. Some of these are toxic compounds that inhibit seed germination. Herbicidin is an antibiotic compound obtained from *Streptomyces saganonensis* that inhibits several monocots and dicots (Mallik, 2001). Other antibiotics known to have herbicidal activities include blasticidin and 5-hydroxylmethyl-blasticidin. These antibiotics are highly selective for dicots, inhibiting the maximum amount as 98% of the plants examined (Scacchi *et al*., 1992). Moreover, nigericin, hydantocidin, and geldanamycin, phytotoxic metabolites obtained from *Streptomyces hygroscopicus* were tested for herbicidal activity and selectivity on several annuals and perennials at the pre and post emergency stages (Heisey, 1990; Singh *et al*., 2003).

Methoxyhygromycin is an antibiotic produced by *Streptomyces* sp. and it has a unique structure and weed suppressive abilities (Lee *et al*., 2003). Isolation of bialaphos from *Streptomyces viridochromogenes* and *Streptomyces hygroscopicus* helped in the production of glufosinate herbicide (Hoerlein, 1994). Furthermore, thaxtomin A could be a metabolite isolated from *Streptomyces acidiscabies* and has shown high selectivity for common monocot and dicot weed control in rice crops and turfgrass landscapes (Kao-Kniffin, 2013). Tagetitoxin from *Pseudomonas syringae* pv. Tagetis, a symptom of apical chlorosis in infected plants caused by the phytotoxin tagetitoxin, is also useful as a natural herbicide due to its impact on chloroplasts (Lydon *et al*., 2011).

4. MATERIALS AND METHODS

4.1 Description of Study Area

The experiment was carried out in Microbial Biotechnology and *Striga* Bioassay Laboratories at National Agricultural Biotechnology Research Center (NABRC), Holeta. NABRC is located at 9º3'N latitude and 38º30'E longitude 34 km away from Addis Ababa, in the central part of Ethiopia, West Shoa Zone of Oromia Regional State.

4.2 Source of Experimental Materials

4.2.1 Soil Sample

Soil samples were collected from three sorghum growing fields in Northern regions of Ethiopia during 2018 main crop season. These were Artuma Fursi (site 1), Kewet (site 2) and Qaftay Humera (site 3) districts with medium, low and high *Striga* infestation, respectively. In each site, 50 kg of soil sample was collected from four random spots in four quadrants after locating the fields using Global Positioning System (GPS) coordinates and recording the altitude for each site (Table 1 and Figure 2). Soil samples were collected using a sterile shovel at a depth of 20-30 cm and put into a labeled clean plastic bag and transported to the greenhouse facility at National Agricultural Biotechnology Research Center, Holeta. The sample was then air dried, homogenized by mortal and pistil and sieved with 4 mm diameter sieve mesh.

Table 1. List of soil samples used in the study based on their collection Regional States and zones

Site code	Region	Zone	Woreda/ district	Kebele	Altitude (m)	Longitude (North)	Latitude (East)
E19	Amhara	Semien Shoa	Kewet	Abayatir	1373	09.55.11.0	040.01.42.3
E19	Amhara	Semien Shoa	Kewet	Abayatir	1371	09.55.09.51	040.01.41.9
E19	Amhara	Semien Shoa	Kewet	Abayatir	1376	09.55.09.9	040.01.42.2
E19	Amhara	Semien Shoa	Kewet	Abayatir	1375	09.55.11.5	040.01.42.4
E29	Amhara	Oromia	Artuma Fursi	Jara Kichini	1453	10.30.53.3	039.58.47.7
E29	Amhara	Oromia	Artuma Fursi	Jara Kichini	1457	10.30.54.2	039.58.47.7
E29	Amhara	Oromia	Artuma Fursi	Jara Kichini	1458	10.30.54.4	039.58.47.9
E29	Amhara	Oromia	Artuma Fursi	Jara Kichini	1457	10.30.53.3	039.58.47.2
E40	Tigray	West Tigray	Qafta Humera	Maykedira	635	14.10.26.8	036.36.11.3
E40	Tigray	West Tigray	Qafta Humera	Maykedira	635	14.10.27.4	036.36.11.3
E40	Tigray	West Tigray	Qafta Humera	Maykedira	634	14.10.27	036.36.10.1
E40	Tigray	West Tigray	Qafta Humera	Maykedira	633	14.10.27.1	036.36.10.3

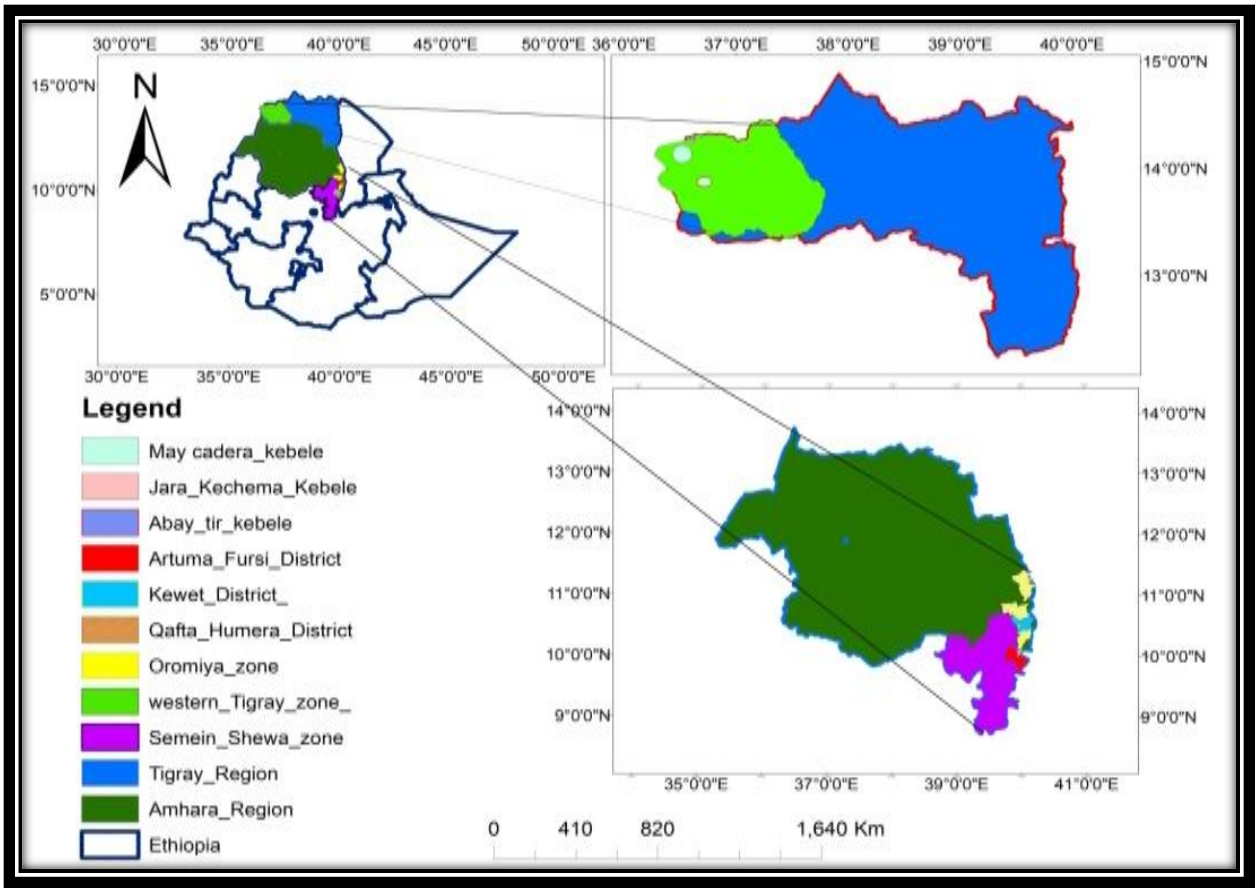


Figure 2. Map showing soil sample collection areas.

4.2.2 Sorghum Seeds

Seeds of different sorghum germplasm that are known to be *Striga* susceptible, *Striga* resistant, drought tolerant, widely used, released and local land races were used in this study. The seeds were stored at the National Agricultural Biotechnology Research Center cold room by the national Integrated *Striga* Control (ISC) project under Ethiopian Institute of Agricultural Research (Table 2).

Table 2. Sorghum genotype selection for greenhouse planting and isolation of bacteria

Code	Name	Source	Character	Selection criteria
G1	ETSL101847	Tigray	Local land race	Land race and widely used
G2	ETWS 90754	Amhara	Wild type	Wild type
G3	ETWS 91242	Beneshangul	Wild type	Wild type
G4	Framida	Purdue University	*Striga* resistance	*Striga* resistant and widely used
G5	ETSL100046	Land race	LGS	Land race and LGS
G6	ETSL101853	Land race	HGS	Land race, widely used and HGS
G7	Misikir	MI_Drought_Score	Drought tolerant	Drought tolerant
G8	S35	ICRISAT	Stay green	Stay green or Drought tolerant
G9	Shanqui red	China	*Striga* susceptible variety	HGS and model for *Striga* susceptible variety
G10	SR5-Ribka	IBC	*Striga* resistant and fusarium compatibility	*Striga* resistant and fusarium compatibility
G11	SRN39	Purdue University	Striga resistance	*Striga* resistant and widely used
G12	Teshale	ICRISAT	Best released susceptible varieties	Widely used

Note: LGS=low germination stimulant; HGS=high germination stimulant, G=genotype

4.2.3 Striga Seeds

S. hermonthica seeds used for this experiment were collected from *S. hermonthica*-infested sorghum in farmers' fields in Ethiopia Tigray Region Central Zone Abergele District Titay Hagum Kebele during 2018 main cropping season [altitude (m): 1466; latitude (East): 13.25'51.8''; longitude (North): 038.59'50.3''].

4.3 Greenhouse Experiment

Seeds of the sorghum that were well matured and with good morphological characteristics were selected and surface sterilized in 1.5% bleach for 30 minutes. The seeds were then allowed to germinate for about 30 hours in incubator set to 30 °C. In order to activate microbes, the seedling was transferred to a pot and grown on the soils collected from various sorghum growing sites in greenhouse. The management including watering and weeding was made accordingly until it set three leaves or ready for harvesting rhizosphere soil sample.

4.4 Rhizosphere Soil Collection

Rhizosphere soil sample collection was made following the method described in Barillot *et al*. (2013). After setting the third leaf, the sorghum was uprooted and vigorously shaken by hand for five min until non-adhering soil was completely removed. Rhizosphere soil was collected by removing the sorghum's above soil parts with sterile blade and shaking roots for 10 min in 50 ml falcon tubes containing 35 ml sterile distilled water to remove the adhering soil. The soil suspensions were then incubated to homogenize soil content on a shaker (300 rpm, 90 min and 25 °C) before being centrifuged at room temperature for 10 min to concentrate soil particles in the pellet.

4.5 Rhizosphere Bacteria Isolation

Rhizosphere bacteria were isolated by serial dilution technique. One gram of each soil pellet was suspended, each in 90 ml sterile distilled water in 50 ml falcon tube and mixed thoroughly overnight using a mechanical shaker at 110 rpm, till completely dispersed. Then 100 µl aliquot was transferred with sterile pipettes to 9 ml sterile 0.85% saline solution in test tube. A subsequent serial dilution (up to 10^{-8}) was prepared. From each $10^{-4,}$ $10^{-5,}$ and 10^{-6} serial dilutions, 0.1 ml of aliquot was spread on nutrient agar on Petri-dishes (90 mm), for each dilution in triplicate. Plates were incubated at 28 °C for 24 hours. Representative types of bacterial colonies were further purified by sub-culturing on fresh medium and used for downstream works or stored in 35% glycerol at -80 °C (Babalola *et al*., 2007; Hassan *et al.*, 2011; Barillot *et al*., 2013).

4.6 Coding of Rhizobacterial Isolates

The isolates were given code for easy traceability of their origin (site of collection of soil and sorghum genotype from which rhizosphere sample was obtained). To this end, isolates from rhizosphere soils of site 1 were labeled E19, while isolates from rhizosphere soils of site 2 were labeled E29 and from site 3, E40. The sorghum genotypes were labeled G followed by sequential numeric order from 1 to 12 including B (blank soil where sorghum had not grown on). Full code of isolates was written soil code followed by sorghum genotype, each soil and sorghum genotypes (replicated three times). Examples of coded names of the isolates in this study include bacterial isolates such as E19G1 (site 1 soil and sorghum G1). To make it much easier, isolates were re-coded as RI followed by sequence of number (Appendix Table 1).

4.7 *In vitro* Screening of Rhizoabcterial Isolates for their Biocontrol Traits

Rhizobacteria isolates were first screened for production of HCN followed by screening HCN positive isolates for IAA production. Common producers of HCN and IAA isolates were selected for further evaluation of their effects on *S. hermonthica* germination inhibition *in vitro* (Table5).

4.7.1 Hydrogen Cyanide (HCN) Production

Hydrogen cyanide production by the bacterial isolates was tested qualitatively using the methods followed by Bakker and Schippers (1987) with slight modification in incubation period. The bacterial isolate cultures were streaked on Trypto Soya Agar amended with 4.4 g/l glycine.

Whatman filter papers were sterilized and soaked in 2% sodium carbonate in 0.5% picric acid solution was placed in the top of each plate. Plates were sealed with parafilm and incubated for 4 days at 28 °C. The change in the color of Whatman filter paper from yellow to light brown, brown or reddish brown was observed as an indication of weak, moderate or strongly hydrogen cyanide producers, respectively (Table 3 and Figure 4).

4.7.2 Indole Acetic Acid (IAA) Production

The ability to produce IAA of the isolate was detected from the culture of the bacterial isolates following the procedure described by Thakuria *et al*. (2004). Briefly, pure colonies from a 24-hr. culture were inoculated into nutrient broth supplemented with 2 % tryptophan and in the absence of tryptophan (control), and incubated at 28 °C for 48 hrs. Five milliliter culture was removed from each tube and centrifuged at 12,000 x g for 15 min. Two milliliter aliquot of the supernatant was transferred to a fresh tube. This was then treated with 2 ml salkowsky reagent (1ml 0.5 M FeCl in 50 ml $HClO_4$) and incubated at room temperature for 25 min. Development of pink color indicates positive result for IAA production (Table 4 and Figure 5).

4.8 *In vitro* Evaluation of the Effects of Selected Isolates on Striga Germination

Each common HCN and IAA producer isolates was evaluated for their *Striga* germination and seed decay activity in *Striga* bioassay laboratory using Agar Gel Assay (AGA) and Whatman filter paper. To do this, *S. hermonthica* seed germination test was conducted to determine its viability prior to use in the *in vitro* evaluations of the effects of rhizobacterial isolates on the seed in the absence and presence of susceptible host. However, the seed had to be exposed to the right environmental conditions for the optimum period of time to break dormancy and ready for germination. Hence, the *Striga* seed was conditioned by incubating at 29 ^{o}C for 10 - 14 days. In each case, *S. hermonthica* seed was treated with each isolate and germination percentage computed to see the germination inhibitory activity of the isolates (Berner *et al*., 1997; Rich and Daniel Gobena, 2016).

4.8.1 Determination of Germination Percentage of S. hermonthica

S. hermonthica seeds surface sterilization and pre-conditioning was made according to the protocol used by Rich Daniel Gobena (2016). First seeds were surface sterilized in 75% ethanol under hood in 50 ml flask for 2 minutes and rinsed three times with sterile double distilled water. This was followed by washing the seed with activated metricide (fungicide) for 2 minutes and rinsed three times with sterile double distilled water. Finally, 14.5 ml ddH_2O and 1.5 ml of Benomyl solution (conditioning solution) was added to flask. The flask was wrapped with aluminum foil and incubated at 30 °C for 10 days for pre-conditioning.

After 10 days of pre-conditioning, about hundred sterilized *S. hermonthica* seeds were transferred into a sterile glass fiber disc on a Petri plate lined with moist Whatman filter paper. Three glass fiber discs on each plate containing preconditioned *S. hermonthica* seeds were germinated by adding 20 µl of 0.1 ppm GR24 and incubated for 2 days at 28 °C (Gafar *et al*., 2015). Negative controls containing pre-conditioned *S. hermonthica* seeds were added with sterile distilled water. The numbers of germinated and non-germinated *S. hermonthica* seeds were counted using a binocular microscope fitted with a digital camera (Power Shot A640, Canon Inc., China). Germination percentage of *Striga* was determined by counting the total number of seeds on each disc and germinated *Striga* (Rich and Daniel Gobena, 2016; Neondo, 2017).

4.8.2 Evaluation of the Effects of Selected Isolates on Striga Seed Germination in the Absence of Host Plant

Isolates selected for their ability to produce HCN and IAA were evaluated for their ability to reduce *Striga* germination. *Striga* seed surface sterilization and pre-conditioning was done as described in section 3.6.1. About 100 pre-conditioned *Striga* seeds were added on a glass fiber discs placed in a Petri plates lined with double sterile filter papers and moistened with 3 ml of sterile ddH_2O. The experiment was replicated 3 times each (three glass fiber discs per Petri plate). The seeds on the disc were treated with 100 µl of three days old bacterial suspensions in broth. In control treatment, blank broth was added to discs containing pre-conditioned *S. hermonthica* seeds. The Petri plates were sealed with parafilm and incubated at 30 °C in the dark for 48 h.

After 48 h, 20 µl of 0.1 ppm GR24 was added to keep germination uniform except for the effect of isolates and further incubated overnight at 29 °C. The number of total *S. hermonthica* seeds and number of germinated/ inhibited per replicate was recorded under a stereomicroscope fitted with camera (Neondo, 2017). Germination percentage for each replicate was calculated using the formula described in Rich and Daniel Gobena (2016).

$$\text{GP} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \text{X } 100$$

4.8.3 Evaluation of the Effects of Selected Rhizobacterial Isolates on S. hermonthica Seed in the Presence of Susceptible Host

Striga conditioning was made as explained in section 3.6.1 but by this time it was embedded in agar (bacto agar) solution after 5 days. By using a glass Pasteur pipette, a drop of pre-conditioned *Striga* seeds were added to the center bottom of a sterile plate from conditioning flask. The seeds were treated with 0.5 ml of three days old of bacterial suspensions in broth and kept for 30 min. In control treatment, seeds were treated with blank nutrient broth media. Each treatment was replicated three times and arranged in RCD in incubator at 30 °C. 0.7% (g/l) agar solution was prepared and autoclaved for 15 min, and then allowed to cool in containment room water bath to 50 °C. The liquid agar was directly poured over the *Striga* under hood until the agar reaches the sides of the plate and the *Striga* seeds distributed evenly across the plate. Plates were allowed to cool for 10 minutes before covering and placed in a dark at 30 °C in incubator for 10 days from the conditioning start date of the *Striga* seed.

Simultaneous to *Striga* conditioning, surface sterilization was made to susceptible sorghum seeds called Teshale using 1.5% bleach (containing a drop of Tween-20) and agitated three times for 30 minutes. The bleach solution was then poured off and rinsed 2 times with sterile ddH_2O. The seeds were then soaked overnight to imbibe in 5 ml of a 5% (w/v) Captan solution. Next day the Captan slurry was poured off under a laminar flow hood and rinsed with 5 ml $sddH_2O$. Then, the seeds and water were poured into labeled sterile Petri dishes, each containing two Whatman filter paper (90 mm) circles and incubated until radicles emerged (Rich and Daniel Gobena, 2016).

Next day, the germinated sorghum was gently picked up with sterile forceps and planted 1 cm from the edge of the plate pointing toward the center of the plate in agar in which the *Striga* seeds were already embedded. The plates were incubated at 30 ^{o}C in incubator where they remained for 3 days.

After 3 days, 2 x 2.5 cm area measuring was made along the main sorghum root 2 cm from the kernel at the back side of the agar plate using a thick water-resistant marker pen. This area is with high probability of *Striga* seeds coming into contact with sorghum root exudates. Total and germinated *Striga* seeds in each area were counted under stereomicroscope and germination percentage computed using methods described in 3.6 (Mohammed *et al*., 2010; Mounde, 2015). Isolates producing the highest germination inhibition were selected for morphological and biochemical identification.

4.9 Morphological and Biochemical Characterization of Selected Isolates

The most efficient bacterial isolates with production of HCN and IAA and corresponding inhibitor of *S. hermonthica* indicated by low mean germination percentage were selected. These isolates were morphologically and biochemically characterized using the method followed by Hemraj *et al*. (2013) and Prescott and Harley (2002) as described below.

4.9.1 Morphological Characterization

The efficient bacterial isolates were characterized by growing on nutrient plate for 24 hr at 28 ºC. Best candidate of bacterial isolates were observed under stereomicroscope for colony size, shape, color, arrangement and gram reaction. For Gram staining, slide was cleaned with detergent and marked by codes of isolates. With the help of sterile wire loop, single colony of bacterial culture was made on clean glass slide and air dried and heat fixed. Then smear was covered with crystal violet for 1min and slide was washed with drop of distilled water. Smear was covered with 2 drops of iodine solution for 30 seconds and slide was washed with alcohol and then distilled water. The smear was covered with 1 drop of safranin for 1min and then washed by distilled water, air dried and observed under microscope.

4.9.2 Biochemical Characterization

Each efficient bacterial isolate was tested for sugar utilization, production of methyl red, indole and catalase. This would help to identify the isolates at genus level.

4.9.2.1 Sugar Utilization Test

The ability of the isolates to utilize carbohydrate sugars as a sole carbon source was determined in broth media containing specific sugar (glucose, fructose and sucrose) and Bromocrsol purple (0.4g/l). A 96 deep well ELISA plate filled with 1ml broth was inoculated by 0.1 ml of fresh culture in triplicate including control. The culture was incubated at 28 ºC for 24 hrs and observed for the formation of yellow color as positive results.

4.9.2.2 Methyl Red Test

Broth containing (5g of each Peptone, Glucose, Potassium phosphate and 1000 ml distilled water; pH=7) was prepared and steam sterilized using autoclave. In test tubes, 1.5 ml of the broth was poured and each was inoculated with test organism, and then incubated at 28 °C for 48 hrs. Four drops of methyl red indicator was added to each tube and gently shaken for 30 seconds. The tubes were kept for 15 minutes and observed for color change (where, positive test = bright red and negative test = yellow to orange)

4.9.2.3 Catalase Test

The nutrient agar slants were inoculated with test isolates. An inoculated nutrient agar slant was kept as control. The cultures were incubated at 28 °C for 24 hrs. A loop full of bacterial culture was kept on a clean slide with the isolate label. A drop of 3% hydrogen peroxide was added on a slide. The culture was then observed for the gas bubble formation.

4.10 Statistical Analysis

All the experimental units were arranged in CRD. Data on effects of selected isolates in *S. hermonthica* seeds germination was recorded. R software version 3.5.3 was used to perform analysis of variance (ANOVA) for all measured data. Tukey's test was used to compare and separate the means for significance at 5% (Mangiafico, 2017).

5. RESULTS

5.1 Bacteria Isolation

A total of 117 bacteria were isolated from rhizosphere of 12 sorghum varieties grown on soil collected from different sorghum growing regions in Ethiopia (Figure 3 and Appendix Table 1).

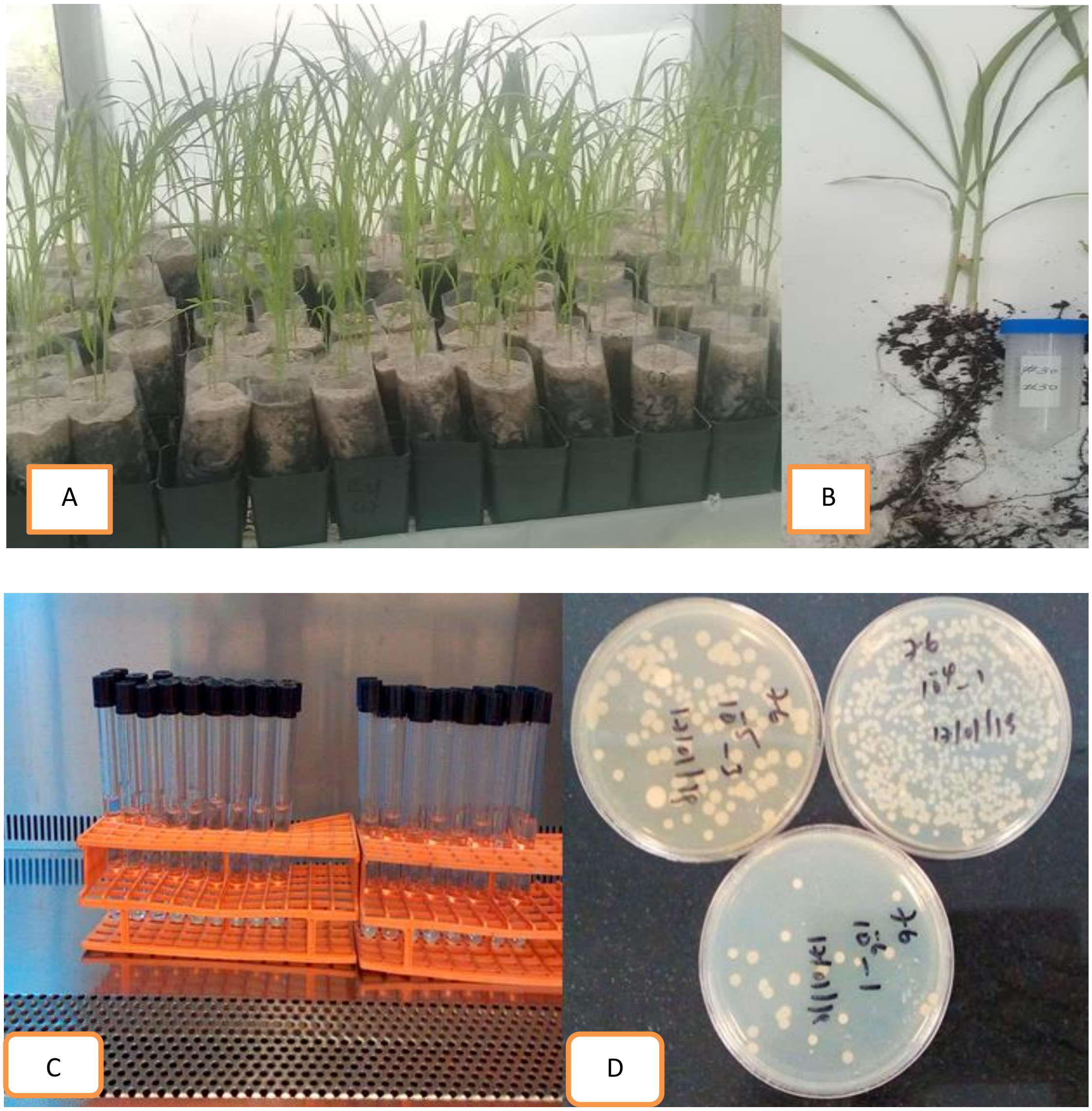


Figure 3. Rhizosphere bacteria isolation. A) Planting sorghum in greenhouse B) Rhizobacteria harvesting C) Serial dilution D) Bacteria isolation

5.2 *In vitro* Screening of rhizobacterial isolates for their Biocontrol Traits

5.2.1 HCN Production

In the present study, a total of 117 bacterial isolates were tested for their qualitative hydrogen cyanide production on a nutrient agar plate. From these, only 47 isolates produced HCN at different levels (low producers, medium producers and strong producers) and this accounts 40.2% of the total isolates (Table 3 and Figure 4).

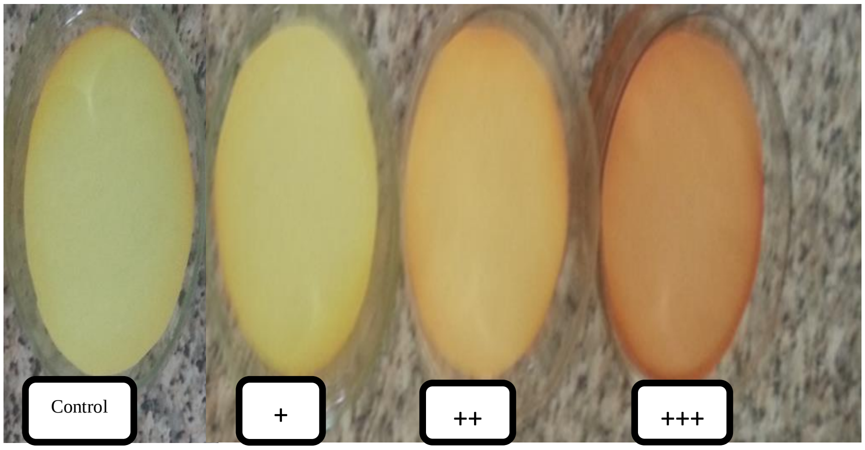
Table 3. HCN production by isolates from various sorghum varieties grown on soil collected from different sorghum growing regions in Ethiopia

No	Code	HCN production	No	Code	HCN production	No	Code	HCN	No	Code	HCN production
1	RI1	++	31	RI31	+	61	RI61	+	91	RI91	-
2	RI2	+	32	RI32	+++	62	RI62	-	92	RI92	++
3	RI3	+	33	RI33	++	63	RI63	+	93	RI93	-
4	RI4	-	34	RI34	-	64	RI64	-	94	RI94	-
5	RI5	-	35	RI35	-	65	RI65	-	95	RI95	-
6	RI6	+++	36	RI36	+	66	RI66	++	96	RI96	+++
7	RI7	-	37	RI37	++	67	RI67	-	97	RI97	-
8	RI8	-	38	RI38	+++	68	RI68	+++	98	RI98	-
9	RI9	++	39	RI39	-	69	RI69	-	99	RI99	+
10	RI10	+	40	RI40	-	70	RI70	-	100	RI100	-
11	RI11	++	41	RI41	++	71	RI71	+	101	RI101	++
12	RI12	-	42	RI42	-	72	RI72	+++	102	RI102	-
13	RI13	+++	43	RI43	-	73	RI73	++	103	RI103	_
14	RI14	_	44	RI44	-	74	RI74	-	104	RI104	-
15	RI15	_	45	RI45	+	75	RI75	-	105	RI105	-
16	RI16	+	46	RI46	-	76	RI76	-	106	RI106	-
17	RI17	-	47	RI47	_	77	RI77	+	107	RI107	-
18	RI18	_	48	RI48	-	78	RI78	-	108	RI108	-
19	RI19	+	49	RI49	-	79	RI79	+	109	RI109	-
20	RI20		50	RI50	+	80	RI80	-	110	RI110	+
21	RI21	_	51	RI51	+	81	RI81	-	111	RI111	-
22	RI22	++	52	RI52	-	82	RI82	-	112	RI112	-
23	RI23	+++	53	RI53	-	83	RI83	+++	113	RI113	-

Table 3. Continued

24	RI24	+	54	RI54	++	84	RI84	-	114	RI114	+++
25	RI25	+	55	RI55	-	85	RI85	_	115	RI115	-
26	RI26	++	56	RI56	-	86	RI86	-	116	RI116	++
27	RI27	+	57	RI57	-	87	RI87	+	117	RI117	-
28	RI28	+	58	RI58	-	88	RI88	-		Con.	-
29	RI29	-	59	RI59	-	89	RI89	-			
30	RI30	++	60	RI60	+++	90	RI90	-			

- = no production, +=low production, ++= moderate production, +++=strong production



Note: + = weak (light brown), ++ = moderate (brown), +++ = strong (reddish brown)

Figure 4. HCN Production

5.2.2 IAA Production

Forty seven isolates capable of producing HCN were again tested for IAA production, another weed suppressive trait of rhizobacteria. Accordingly, 6 (12.8%) isolates were strong producers, 9(19%) were moderate producers and 7(14.9%) were low or weak producers and 25(53.2%) were not producers of IAA at all indicated by +++, ++, +, -, respectively (Table 4 and Figure 5).

Table 4. IAA production by isolates from rhizosphere of various sorghum varieties grown on soil collected from different sorghum growing regions in Ethiopia

No	**Code**	**IAA**	**Decision**	**No**	**Code**	**IAA**	**Decision**	**No.**	**Code**	**IAA**	**Decision**
1	RI1	+++	Selected	18	RI30	-		35	RI71	_	
2	RI2	_		19	RI31	-		36	RI72	+++	Selected
3	RI3	++	Selected	20	RI32	_		37	RI73	-	
4	RI6	+	Selected	21	RI33	++	Selected	38	RI77	-	
5	RI9	+++	Selected	22	RI36			39	RI79	+	Selected
6	RI10	_		23	RI37	+++	Selected	40	RI83	++	Selected
7	RI11	++	Selected	24	RI38	+++	Selected	41	RI87	-	
8	RI13	-		25	RI41	+	Selected	42	RI92	++	Selected
9	RI16	-		26	RI45	-		43	RI96	+	Selected
10	RI19	+	Selected	27	RI50	+++	Selected	44	RI99	-	
11	RI22			28	RI51	-		45	RI110	_	
12	RI23	++	Selected	29	RI54	++	Selected	46	RI114	++	Selected
13	RI24	-		30	RI60	-		47	RI116	++	Selected
14	RI25	-		31	RI61	+	Selected		Con.	-	
15	RI26	+	Selected	32	RI63	-					
16	RI27	_		33	RI66	-					
17	RI28	-		34	RI68	-					

Notice: - = no production, += low production, ++= moderate production, +++= strong production

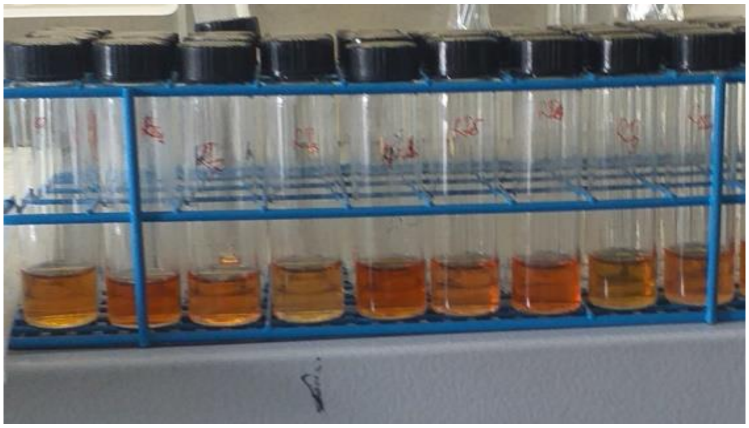


Figure 5. IAA production

Twenty two common producers of HCN and IAA rhizobacteria isolates were selected for further evaluation of their effects on *S. hermonthica* germination inhibition *in vitro.* Two isolates, namely RI38 and RI 72 were strong producers of both HCN and IAA whereas isolates RI19, RI61 and RI79 were weak producers of HCN and IAA in common (Table 5).

Table 5. Selected common HCN and IAA producing rhizosphere bacteria isolates

S/N	Code	HCN	IAA
1	RI1	++	+++
2	RI3	+	++
3	RI6	+++	+
4	RI9	++	+++
5	RI11	++	++
6	RI19	+	+
7	RI23	+++	++
8	RI26	++	+
9	RI33	++	++
10	RI37	++	+++
11	RI38	+++	+++
12	RI41	++	+
13	RI50	+	+++
14	RI54	++	++
15	RI61	+	+
16	RI72	+++	+++
17	RI79	+	+
18	RI83	+++	++
19	RI92	++	++
20	RI96	+++	+
21	RI114	+++	++
22	RI116	++	++

5.3 *In vitro* Evaluation of the Effects of Selected Isolates on Striga Germination

5.3.1 Determination of Germination Percentage of *S. hermonthica*

In this study, germination test for *S. hermonthica* resulted in 63% germination up on conditioning the seeds for 10 days and treating with GR-24, a synthetic germination stimulant (Figure 6).

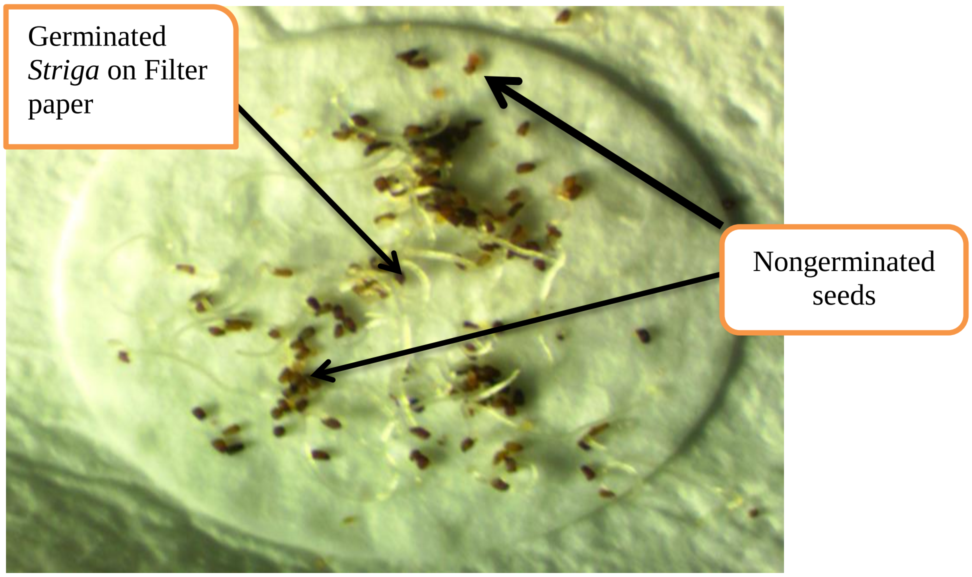


Figure 6. Germination test of *S. hermonthica*

5.3.2 In vitro Evaluation of the Effects of Selected Bacterial Isolates on GR-24 Induced Germination of S. hermonthica

In this study, 22 selected IAA and HCN producing bacterial isolates were evaluated for their effects on *Striga* germination/inhibition *in vitro*. The result of the assay showed that significant differences ($p<0.001$) were observed between some isolates on the effects of rhizobacteria isolates on GR-24 induced *S. hermonthica* germination in filter paper (Table 6 and Figure 7). Regardless of considerable variation, all isolates showed significant reduction in germination percentage compared to the control (broth treatment). But, the extent of germination inhibition varies from 9 to 59.7 mean germination percentage. Low mean germination percentage was observed in isolates RI6 (16%), RI9 (10%), RI19 (26%), RI23 (9%), RI26 (29.7%), RI38 (14%), RI54 (12%) and RI72 (18%) (Table 6).

Table 6. Effect of rhizobacteria isolates on GR-24 induced *S. hermonthica* germination in filter paper assay.

No	**Code**	**Mean Germination %**
1	RI1	42.7^{cde}
2	RI3	46^{c}
3	RI6	16^{jk}
4	RI9	10^{lm}
5	RI11	51^{b}
6	RI19	26^{i}
7	RI23	9^{m}
8	RI26	29.7^{hi}
9	RI33	45^{cd}
10	RI37	35^{fg}
11	RI38	14^{jkl}
12	RI41	40^{de}
13	RI50	34.7^{fg}
14	RI54	12^{klm}
15	RI61	43^{cde}
16	RI72	18i
17	RI79	42.7^{cde}
18	RI83	32.7^{gh}
19	RI92	51^{b}
20	RI96	43.7^{cd}
21	RI114	38.7^{ef}
22	RI116	33^{gh}
23	Broth	59.7^{a}

Sample Size (n)	**23**
Std. Dev.	**14.4**
CV	**8.95**
P(5%)	**<0.001**

Values are means of combined data of three replicates each. Means followed by same letter are not statistically different at p≤0.05 according to the Tukey-test.

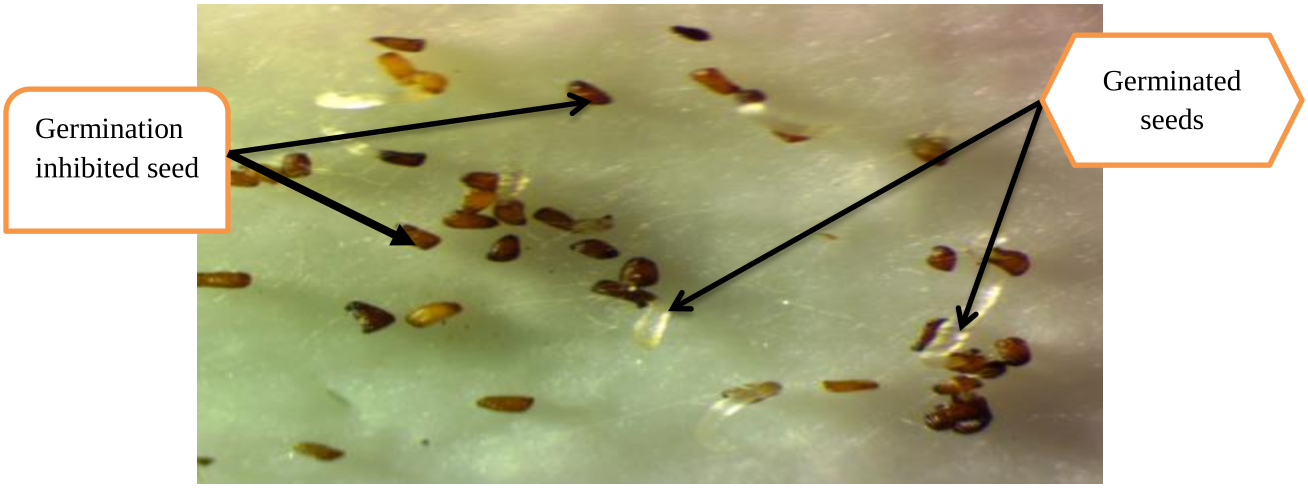


Figure 7. Glass Fiber Filter Paper *S. hermonthica* germination assay

5.3.3 Evaluation of the Effects of Selected Rhizobacterial Isolates on *S. hermonthica* Seed Germination in the Presence of Susceptible Host

This was an activity done as an alternative to the greenhouse evaluation to see whether there are similar or different trends compared to the evaluation using GR-24 as stimulant. The study indicated that germination of *Striga* in the presence of host plant was lower than that of GR-24 induced germination in all treatments. Regardless of this, there was a significant differences (p<0.001) in mean germination percentage among some isolates (Figure 8 and Table 7).

A few isolates showed increased germination of *S. hermonthica* seeds, while many of them showed a significant suppression of *S. hermonthica* seed germination (P<0.001) compared to control treatment (broth). The highest germination percentage (27%) were recorded in the control (blank broth treated seed), followed by isolates RI9 (24%) and RI96 (20%). Treatment of *S. hermonthica* seeds with isolates RI38, RI 54 and RI23 resulted in the lowest *S. hermonthica* seed germination of 0% 1%, and 2.7% respectively, which were significantly lower than any of the treatment. Germination inhibition followed an almost similar pattern with the treatments without the presence of host plant except that treatment with synthetic stimulant GR-24 caused elevated germination percentage as compared to treatment in the presence of host plant sorghum.

Table 7. Effects of rhizobacterial isolates on *S. hermonthica* seed germination in the presence of susceptible host plant

No	**Code**	**Mean Germination %**
1	RI1	11^{h}
2	RI3	9^{i}
3	RI6	16^{e}
4	RI9	24^{b}
5	RI11	18^{d}
6	RI19	6^{jk}
7	RI23	2.7^{l}
8	RI26	9^{i}
9	RI33	6^{jk}
10	RI37	14^{f}
11	RI38	0^{m}
12	RI41	17.7^{de}
13	RI50	12^{fgh}
14	RI54	1^{lm}
15	RI61	11^{gh}
16	RI72	7^{ij}
17	RI79	5^{k}
18	RI83	11^{h}
19	RI92	14^{f}
20	RI96	20^{c}
21	RI114	5.7^{jk}
22	RI116	13^{fg}
23	Broth	27^{a}
	Sample size(n)	**23**
	SE±	**1.1**
	CV	**9.8**
	P (5%)	**< 0.001**

Values are means of combined data of three replicates each. Means followed by same letter are not statistically different at p≤0.05 according to the Tukey-test

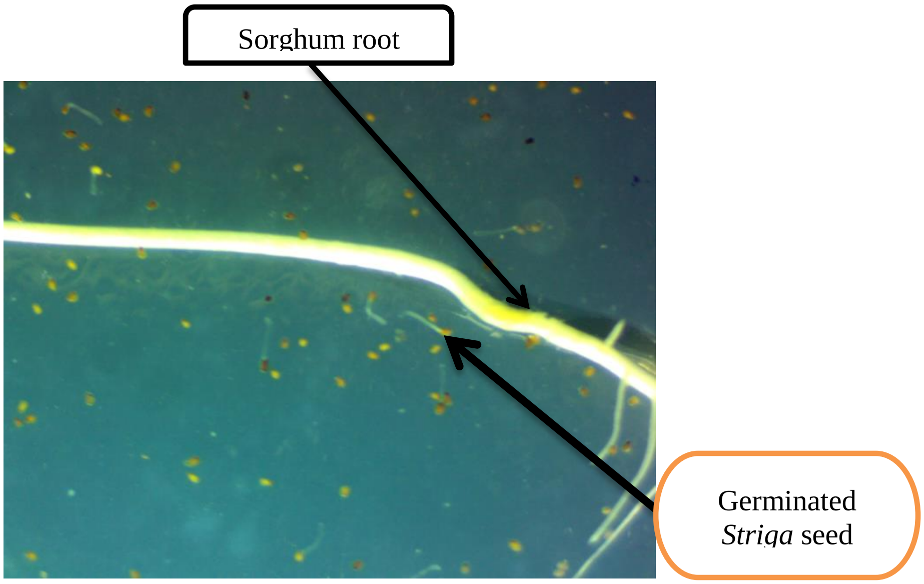


Figure 8. *Striga* seed germination using agar gel assay

Majority of the selected isolates did vary in their HCN and IAA production abilities. Some strong producers of HCN were comparably moderate and weak producers of IAA and vice versa. A few isolates, however, showed similarity in their HCN and IAA production (Table 8).

With regard to mean germination percentage in the absence of host plant, eight isolates, namely RI6, RI9, RI19, RI23, RI26, RI38, RI54 and RI72 showed significant inhibition of *S. hermonthica* germination as indicated by low mean germination percentage, 16, 10, 26, 9, 29.7, 14, 12 and 18, respectively. Majority of these isolates were correspondingly strong producers HCN and IAA (Table 8). Similarly, some isolates that have indicated high *Striga* germination inhibition in the absence of host plant also showed the reduced germination in the presence of host plant though not correspondently or in the same pattern (Table 8). There was also no consistent pattern in all isolates and parameters evaluated in association with various sorghum genotypes from where they have been isolated and the three soil types. But, many bacteria isolated from the soil of low *Striga* infested site (E29) potentially inhibited *Striga* germination in the absence of host plant.

Table 8. Summary of the results of HCN and IAA production test and mean germination percentages of each isolates in the presence or absence of susceptible host plant

Isolate code	**Soil site**	**Sorghum genotype**	**HCN production**	**IAA production**	**MG % (with host)**	**MG% (without host)**
RI1	E19	G1	++	+++	42.7^{cde}	11^{h}
RI3	E19	G3	+	++	46^{c}	9^{i}
RI6	E19	G6	+++	+	16^{jk}	16^{e}
RI9	E19	G9	++	+++	10^{lm}	24^{b}
RI11	E19	G11	++	++	51^{b}	18^{d}
RI19	E19	G6	+	+	26^{i}	6^{jk}
RI23	E19	G10	+++	++	9^{m}	2.7^{l}
RI26	E19	B	++	+	29.7^{hi}	9^{i}
RI33	E19	G7	++	++	45^{cd}	6^{jk}
RI37	E19	G11	++	+++	35^{fg}	14^{f}
RI38	E19	G12	+++	+++	14^{jkl}	0^{m}
RI41	E29	G2	++	+	40de	17.7^{de}
RI50	E29	G11	+	+++	34.7^{fg}	12^{fgh}
RI54	E29	G2	++	++	12^{klm}	1^{lm}
RI61	E29	G9	+	+	43^{cde}	11^{gh}
RI72	E29	G7	+++	+++	18i	7^{ij}
RI79	E40	G1	+	+	42.7^{cde}	5^{k}
RI83	E40	G5	+++	++	32.7^{gh}	11^{h}
RI92	E40	G1	++	++	51^{b}	14^{f}
RI96	E40	G5	+++	+	43.7^{cd}	20^{c}
RI114	E40	G10	+++	++	38.7^{ef}	5.7^{jk}
RI116	E40	G12	++	++	33^{gh}	13^{fg}
Control			-	-	59.7^{a}	27^{a}

MG=mean germination, - = no production of HCN and IAA, B= blank soil (no sorghum grown on)

5.4 Morphological and Biochemical Characterization and Identification of the Most Effective Rhizobacteria Isolates

Finally, upon *in vitro* evaluation, eight efficient rhizosphere bacteria isolates with different *Striga* suppressive effects were further morphologically and biochemically characterized for gram reaction, colony color, size, shape, margin, elevation, sugar utilization ability, catalase and methyl red test. Accordingly, 6 (75 %) of the rhizosphere bacteria inhibiting *Striga* germination were found to be gram negative, 2 (25%) gram positive, 4 (50%) glucose positive, 7 (87.5%) fructose positive, 2 (25%) sucrose positive, 6 (75%) methyl red positive, 6 (75%) catalase positive (Tables 9 and 10 and Appendix Figure 1).

Table 9. Morphological characterization and identification of the most effective rhizobacteria isolates

Isolates	Morphological characterization					
	Pigment	**Shape**	**Size**	**Elevation**	**Margin**	**Gram staining**
RI6	white	Circular	Medium	Raised	Entire	**-**
RI9	white	Circular	Medium	Raised	Entire	**-**
RI19	brown	Circular	Medium	Raised	Entire	+
RI23	white	circular	Medium	Raised	Entire	**-**
RI26	brown	Irregular	large	raised	flat	**-**
RI38	brown	Circular	Medium	Raised	Entire	+
RI54	white	Irregular	Large	raised	Mucoid	**-**
RI72	white	Circular	Medium	Raised	Entire	-

+= positive for a given test, - = negative for a given test under consideration

Based on the comparative analysis of various morphological and biochemical characteristics, the bacterial isolates are identified to fall under four genera: *Bacillus*, *Pseudomonas*, *Enterobacter* and *Klebsiella*. Among the bacterial genera, four were *Pseudomonas*, two *Bacillus*, one *Enterobacter* and one *Klebsiella* (Table 10).

Table 10. Biochemical characterization of the most effective rhizobacteria isolates

Isolates	Biochemical tests					
	Glucose	**Fructose**	**Sucrose**	**Catalase**	**Methyl red**	**Tentative identification**
RI6	+	+	_	+	+	*Pseudomonas sp.*
RI9	**-**	**-**	_	+	+	*Pseudomonas sp.*
RI19	**-**	+	_	+	+	*Bacillus sp.*
RI23	+	+	+	-	-	*Klebsiella sp.*
RI26	+	+	-	+	+	*Pseudomonas sp.*
RI38	**-**	+	-	+	+	*Bacillus sp.*
RI54	**-**	+	+	-	-	*Entrobacter sp.*
RI72	+	+	_	+	+	*Pseudomonas sp.*

- = no sugar utilization, catalase and methyl red negative, += sugar utilization, catalase and methyl red positive

6. DISCUSSION

The potential of growth suppressive effects of rhizobacteria and their possible use as biological control option in management of *S. hermonthica* have been investigated to be agriculturally important to boost crop productivity (Atera *et al*., 2012; Neondo, 2017). A group of microorganisms with potential as biological control agents of weeds are the deleterious rhizosphere inhabiting bacteria (DRB) characterized as nonparasitic rhizobacteria colonizing plant root surfaces and being able to suppress plant growth (Kremer and Kennedy 1996; Raaijmakers et *al*., 2009; Mendes *et al*., 2013). Rhizosphere is the zone at the interface of soil-plant root that harbors the most complex microbial communities (Raaijmakers *et al*., 2009; Schlaeppi and Bulgarelli, 2015). The deleterious activity toward weed seed viability and seedling growth by most microorganisms under study for biological control is due to the production of phytotoxins. The common metabolites produced in the rhizosphere of plants that can be phytotoxic at higher than physiologic concentrations include the auxins and hydrogen cyanide (Kremer, 2007).

HCN producing rhizobacteria have been known to act as biocontrol agents against weeds (Schippers *et al*., 1990). In this study, 117 rhizobacteria isolates were tested for HCN production and 47(40.2%) were capable of producing HCN at different levels. Out of which, 11(23.4%) isolates were strong producers, 15(31.9%) were moderate producers and 21(47) % were low or weak producers of hydrogen cyanide. Heydari *et al*. (2015) conducted a similar study on weed germination inhibition potential of rhizosphere *Pseudomonas* and obtained 37 percent capability of HCN production of isolates and this capacity was different among the strains. Kremer and Souiss (2001) also reported that rhizobacteria such as *Pseudomonads* are known for their ability to produce HCN, but the quantity produced varies widely among species and strains of the bacterium.

It has been reported by Knowles (1976) and Schippers *et al.* (1990) that glycine is a direct precursor of HCN found in root exudates though several factors significantly influence its production across bacteria. For example, the level of HCN produced in root-free soil by *P. putida* and *A. delafieldii* generally increased with higher amounts of supplemental glycine, with *P. putida* typically generating more HCN at a given glycine level (Castric, 1977; Owen and Zdor, 2001). Studies have shown that HCN is a potential inhibitor of enzymes involved in metabolic processes like respiration, CO_2 and nitrate assimilation, and carbohydrate metabolism. Hence, this gas is known to negatively affect root metabolism and root growth (Schippers *et al*., 1990; Grossmann, 2010). Furthermore, cyanide interacts with the protein plastocyanin, which inhibits the photosynthetic electron transport (Kremer and Soussi, 2001).

Many authors reported about the potential of cyanogenic rhizobacteria for weed suppression. Trognitz *et al. (*2016) indicated production of HCN by rhizosphere bacteria and the role they play in biological control of weed. Cyanide producing rhizobacteria are specific in their actions and they do not generally negatively affect the host plants. A major group of rhizobacteria producing secondary metabolite hydrogen cyanide and with potential for biological control is the *Pseudomonas* (Kremer and Kennedy, 1996; Kamei *et al*., 2014). Lakshim *et al*. (2015) tested rhizosphere bacteria *Pseudomonas spp* for the ability to reduce weed growth and they were proved to produce HCN. Begonia and Kremer (1994) proved that *Pseudomonads* isolated from rhizoaphere of velvet leaf were able to reduce velvetleaf viability and emergence significantly.

The production of the IAA phytohormone is another common trait of rhizobacteria (Arshad and Frankenberger, 1991; Damam *et al*., 2016). Indole-3-acetic acid (IAA) is the major naturally occurring auxins which influences the root and shoot growth of the plant, stimulating ethylene production, cell division and differentiation. The rate of production of ethylene is directly proportional to the concentration of IAA (Shaik *et al*., 2016; Vargas *et al*., 2017). It has been noted that 80% of rhizospheric bacteria produce IAA by metabolizing L - tryptophan (Patten and Glick, 1996). Rhizosphere-inhabiting soil microbes synthesize and release auxins as secondary metabolites because of rich supplies of substrates exuded from plant roots. Some microbes produce auxins in the presence of enough precursor molecules such as tryptophan (Arshad and Frankenberger, 1991; Kremer, 2006).

The current study has shown that 46.8% of the tested isolates were capable of producing IAA. Similar study has been conducted by Idris *et al*. (2009) on growth promotion of rhizobacterial isolates from the rhizosphere of sorghum and grasses in Ethiopia and South Africa and found 73% production of IAA in tested isolates in the presence of tryptophan. The authors further noted the tendency of decreasing IAA concentration in the absence of tryptophan. The lower number of IAA producers in this study than the previous report could be due the difference in sorghum varieties from where the rhizobacteria isolated and other factors in the soil.

Similarly, Boyette and Hoagland (2015) reported the effects of rhizobacteria on plants and their use as bioherbicides to control weeds. Rhizosphere microorganisms mediated suppression of plant growth during interaction is linked to the secretion of secondary metabolites from microorganisms (Barazani and Friedman, 1999).

Bandurski *et al*. (1995) detected *Enterobacter sp*. and found significant amount of IAA secretion due to the presence of an increased activity of tryptophan deaminase, an enzyme which produces

IAA from its precursor molecule tryptophan. The negative effect of IAA is associated with the elevated levels of IAA production **(**Sarwar and Frankenberger, 1994). For example, Patten and Glick (2002) demonstrated the role of accumulated production of IAA by *P. putida* and its effects in inhibition on plant growth. The increased IAA production stimulates biosynthesis of ethylene by the enzyme aminocyclopropane-1-carboxylate (ACC) (Kende, 1993). Park *et al*. (2015) further showed the inhibition of lettuce plant growth and enhanced ethylene synthesis by IAA producing *Enterobacter sp.*

In the current study, *S. hermonthica* seed viability test resulted in 63% germination percentage up on conditioning the seed in benomyl solution for 10 days and treating the seed with a synthetic germination stimulant GR-24. This agrees with the report by Rich and Daniel Gobena (2016) which suggested that germination percentage of *Striga* seed has to be at least 30% for downstream application of the seed. Furthermore, Hassan *et al*. (2011) indicated 55-57% germination induction by GR-24 in seed condition in water.

Isolation of weed inhibiting rhizobacteria was made from sorghum rhizosphere and mechanism involved in weed inhibition of *Striga* seed germination inhibition was undertaken to identify potential *Striga* suppressive rhizobacteria associated with host plant sorghum. *In vitro* evaluation of the effects of inoculation of bacterial isolates on inhibition of *S. hermonthica* was studied under laboratory bioassay. This technique was developed for the selection of bacteria inhibitory to the germination of *S. hermonthica* seeds in such a way that adequate contact between the bacteria and *S. hermonthica* seeds was ensured without the bacterial culture medium itself inhibiting *S. hermonthica* seed germination**.** The study focused on germination inhibition at early stage of *S. hermonthica* and generated information to develop reliable and accessible Striga control strategies to small holder farmers.

There was considerable variation in inhibition of *Striga* germination by bacterial isolates obtained from sorghum rhizosphere grown on soil collected from different sites. The lowest (9%) and highest (59.7%) germination percentage was observed in RI23 and broth (control treatment), respectively. Ahonsi *et al*. (2002) conducted a similar study on rhizobacterial strains for suppression of germination of *S. hermonthica* and found a wide range of results (13–50% germination of *S. hermonthica* seeds). Our study indicated the potential of rhizosphere bacteria in inhibiting at early stage of *S. hermonthica* development. This helps to reduce much of the damages caused by *Striga* before emerging above the ground (Figure 1).

The study showed variations in the inhibition of *Striga* germination by isolates obtained from sorghum rhizosphere grown on soil collected from different sites. For example, many of bacteria isolated from soil E19 (Amhara Region, Oromo Special Zone) significantly reduced germination percentage of *S. hermonthica*. Soil E19 was obtained from site 1, where there was low *Striga* infestation (Table 1). The low infestation of *Striga* in the field from where soil E19 was obtained may be associated to *Striga* germination inhibition by the bacterial population and other factors in the vicinity. Ciotola *et al*. (1995) reported that some soils are known to be suppressive to *Striga*, and their suppression was linked to microbial population.

On the other hand, isolate RI11 from the same soil E19 but isolated from rhizosphere of *Striga* resistant SRN-39 did not significantly reduce *Striga* germination. The enhanced germination of *Striga* by this isolate may be again explained by the nature of microbiome of the soil. This is consistent with the finding of Ahonsi *et al*.(2002) in which few isolates increased germination of *S. hermonthi*ca seeds, others had no effect on the seed germination, while some showed a significant suppression of *S. hermonthica* seed germination compared with the check (no bacterium). Furthermore, Berner *et al.* (1996) suggested that both inhibition and promotion of

*Strig*a germination can be attributed to microbial action and this can be achieved by manipulation of ethylene biosynthesis, ethylene action, or by promotion of ethylene metabolism or that of its immediate precursor ACC (1-aminocyclopropane-1-carboxylic acid). Generally, there was no consistent pattern in *Striga* germination inhibition of the soils collected from three sites. However, many of the isolates collected from site 1(E19) were resulted in low mean germination percentage regardless of sorghum variety from where the bacteria were isolated.

The use of weed management strategies involving chemical herbicide generally alter soil structure going alongside with changes in the microbial community (Trognitz *et al.*, 2016). Using soil microorganisms to control weeds is an alternative method to herbicides that may reduce dependence on chemical herbicides and increase the use of environmentally sound practices that are easily available to small holder farmers. The soil microbiome plays an important role in the establishment of weeds and invasive plants to which they are associated and build up close relationship. For example, sorghum seedlings [*Sorghum bicolor* (L) Moench] of different genotypes differ in associations with soil microorganisms (Funnell-Harris, 2008).

Evaluation of the effects of isolates from various sorghum varieties grown on soil collected from different sorghum growing regions in Ethiopia was also conducted in the presence of host plant called Teshale variety (sorghum) using Agar Gel Assay (AGA). 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).

In this study, the lowest (0%) and highest (27%) mean germination of *S. hermonthica* seeds was obtained in the presence of host plant. This is much higher than the finding of Ahonsi *et al*. (2002) who obtained the lowest (13%) reduction in germination percentage of *S. hermonthica* inoculated with bacterial isolates in the presence of host plant sorghum. Similarly, Hassan *et al*. (2011) studied the use of rhizobacteria to control *S. hermonthica* and observed the inhibition of *Striga* germination by bacterial isolates. The germination inhibition of bacterial isolates could be associated to a direct effect of the isolates on the seed or indirectly via production of chemicals that are toxic to seeds, inhibitors/ promoters of ethylene biosynthesis or its action (Babiker *et al*., 1993; Babiker, 2007; Hassan *et al*., 2009).

Furthermore, the study demonstrated that the control (broth) treatment in the presence of host plant resulted in highest mean germination percentage (Table 7), but it was still lower than GR-24 induced germination percentage of seeds in the absence of host. Babalola *et al*. (2006) reported the effectiveness of GR-24 in *S. hermonthica* seed germination stimulatory activity.

Morphological and biochemical characterization of the most effective shortlisted isolates *in vitro* evaluation finally resulted in four different genera of bacteria: *Pseudomonas, Bacillus, Klebssiella* and *Eentrobacter* (Table 10). A variety of rhizobacteria, including *Bacillus (*Idris *et al*., 2007), *Pseudomonas (*Qessaoui, 2019), *Azospirillum* (Roesch *et al*. 2008) species are commonly found in the rhizosphere of crops. The study indicated that many isolates were strong producer of HCN and IAA and deduced to belong to the *Pseudomonas* genera. This agrees with the report by Kamei *et al*. (2014) that HCN production is found to be a common trait of *Pseudomonas* (88.89%) and *Bacillus* (50%) in the rhizospheric soil and plant root nodules.

Babalola (2002) indicated that *Pseudomonas* is among the common plant root inhabiting soil bacteria. Babalola and Odhiambo (2008) also showed *S. hermonthica* seed germination inhibition of rhizobacetria genera including *Pseudomonas sp*., *Klebsiella oxytoca* and *Enterobacter sakazakii.*

7. CONCLUSION

The results of this study have revealed that there are novel rhizobacteria with a great potential of inhibiting *Striga* seed germination resulting in reduction of parasitic infestation on sorghum. This potential can be exploited by isolating and characterizing rhizospheric bacteria associated with sorghum and evaluating their *Striga* suppressive effects. The suppression effects of rhizobacteria on *Striga* seed could be associated with microbial production of phytotoxic secondary metabolites and inhibitory chemicals such as HCN and IAA that could induce a biocontrol effect. Many of the isolates with most effective *Striga* suppression were obtained from low *Striga* infested field (E19) indicating that rhizospheric bacteria could contribute to the reduction in parasitic infestation. It has been also shown that, regardless of the level of inhibition, all rhizobacterial isolates suppressed *Striga* germination up on *in vitro* evaluation in the presence and absence of host plant sorghum. The most effective *Striga* suppressive isolates were identified to be fallen under four bacterial genera and majority of them were belong to the *Pseudomonas* genus. The isolates are good candidates for addressing *Striga* associated constraints in sorghum production where there is a low input for small holder farmers in our country.

8. RECOMMENDATION

This study has indicated that there is a huge opportunity that microbe particularly those with ability to inhibit *S. hermonthica* seeds can be used to control *S. hermonthica* infestation. It is also witnessed that *in vitro* evolution of host-parasite interaction is fundamentally important to evaluate the tripartite interaction between host plant (sorghum), parasite weed (*Striga*) and microbes (rhizobacteria) to generate effective package to *Striga* prone environment for small holder farmers. So in the future, more sorghum associated rhizobacteria should be isolated, purified and evaluated for host parasite interaction in order to better understand the mechanism underlying the suppressive effects of microbes on *Striga* weed.

The present study gives significant inputs to the application of rhizosphere bacteria isolates for use in controlling *Striga* infestation on sorghum where this problem is sever. However, in order to develop effective *Striga* suppressive isolates, a broader screening for *Striga* suppressive traits both *in vitro* and *in vivo* needs to be conducted to fully understand the role of each trait at each stage of *Striga* life cycle as well as their mode of action. To do this a broader soil sampling from various parts of the country should be made to study the real diversity of the microbiome of the soil. The diversity profile of the isolates should also be studied using molecular methods such as 16s rRNA sequencing to generate reliable information for weed scientists.

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10. APPENDICES

Appendex 1: General Protocols used in conducting the experiments

1.1 Filling Pot with Soil to Growth Sorghum on Soil in Greenhouse

- Plastic pots of 400g soil holding capacity was be prepared
- The pot was washed and sterilized with 70% ethanol
- Each pot was labeled with the code or number given to respective genotypes
- 20% of the total weight of pot was filled with three soil types collected from different parts of Ethiopia.
- 80% of the pot total weight was filled with clean sand
- In each pot, 20% of the upper half i.e. 20g was filled with Ethiopian soil by mixing the remaining volume with 180 sand

1.2 Germination of Sorghum Seeds

- ➢ Select 30 seeds of each sorghum genotype with good quality, without immature characteristic and not damaged
- ➢ Put them in glass vials by labeling them individually
- ➢ Add approximately 20ml of 1.5% bleach(NaOCl) mixed with a drop of Tween 20 solution under hood
- ➢ Soak seeds for 3o minutes with occasional shaking
- ➢ Pour off the bleach and wash the contents with 5ml sterile ddH2O three times to remove traces of bleach

- Pour off the the water and damp the seeds on sterilized whatman filter paper placed in petridish
- Soak the the filter paper with sterile water and allow the seeds to germinate

1.3 Growing the Sorghum in Greenhouse

- Using alcohol sterilized forceps; each germinated seed was carefully picked and planted in the pot already filled with sandy soil.
- The plant was grown in greenhouse with temperature regulated and management like watering will be done based on the plants requirement
- The seedling was allowed to grow until at least the plant develops three leaves

1.4 Collection of Rhizosphere Soil Sample

- To collect Rhizosphere, the upper half containing the Ethiopian soil was used
- The root section grown in the con portion containing 10% soil sand mixture was considered
- The contents of the pots was discarded carefully on to 70% alcohol cleaned large greenhouse tray put on a trolley and the plants will pulled out of the bulk soil.
- The roots were shaken gently to remove the loose attached soil and cut using sterile scissor.
- The plant roots were shaked and centrifuged
- The resulting pellet was used to culture on petridish
- Serial dilutions was prepared by transferring 1ml of diluted suspension to 9ml of sterile distilled water and this was continue till eight fold dilutions
- 0.1 microliter of $10-^{3}$, 10^{-4} and 10^{-5} dilution was inoculated on nutrient medium
- Purification of each sample was made by streak plate method
- Two copies of each purified sample was stored at -80°C until further use

1.5 Culture Media Preparation

- 28 g of nutrient agar powder was suspended in 1 litre of distilled water.
- Brought to the boil to dissolve completely.
- Autoclaved at 121 0C for 15 minutes
- Dispensed 30ml of this to sterile petridish
- For purification of the colony, the same step was followed and same amount of the materials prepared
- Then finally pure isolates were stored under culture medium containing 30% glycerol
- Labeled the tube with the organism isolate name and date.
- Stored the tube in the freezer and record its location

Appendix Table 1. Bacteria isolates obtained from soils collected from different sorghum growing areas in Ethiopia during cropping season

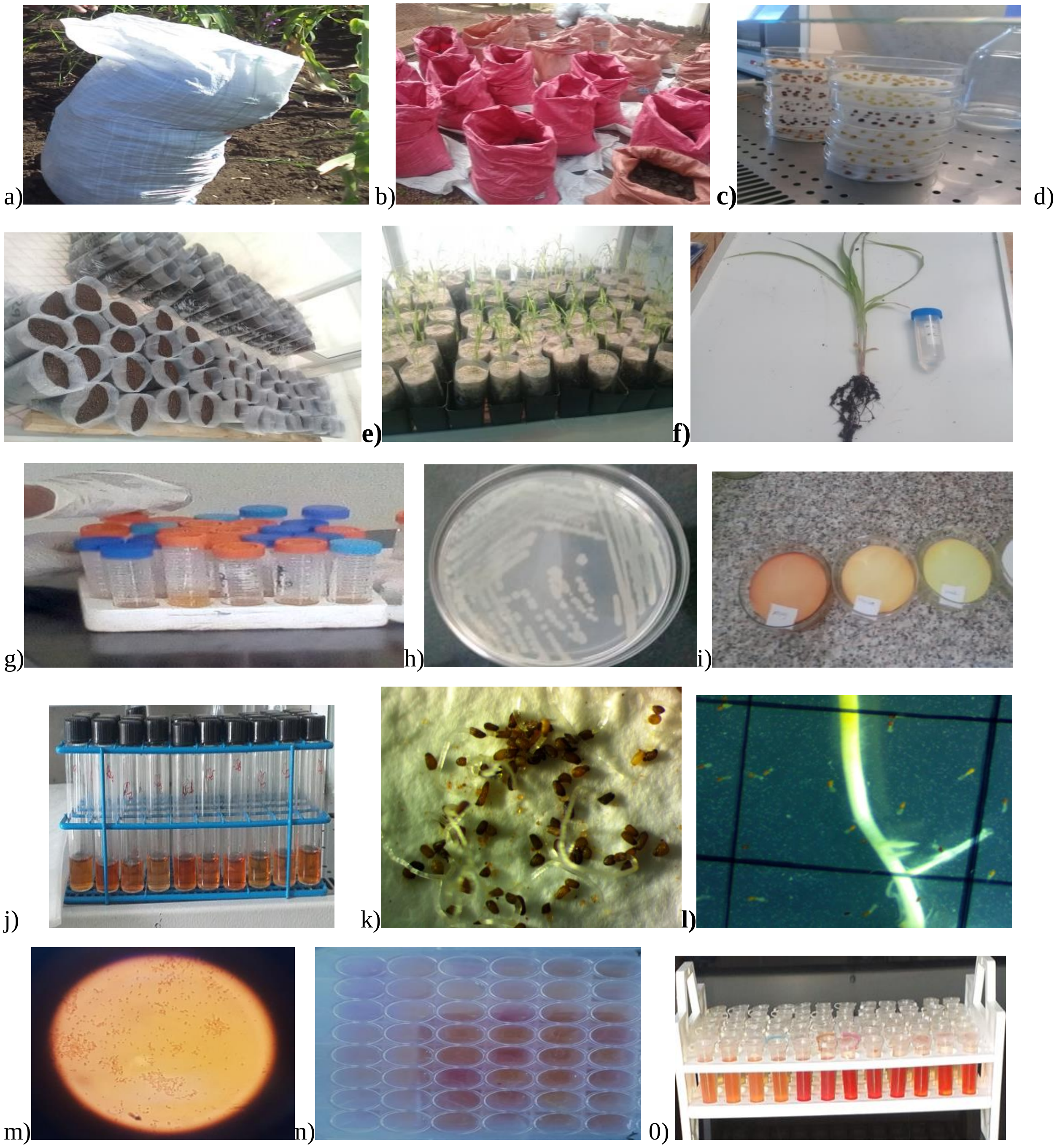
S/N	Isolate	Code	S/N	Isolate	Code	S/N	Isolate	Code	S/N	Isolate	Code
1	E19G1	**RI1**	31	E19G5	**RI31**	61	E29G9	**RI61**	91	E40B	**RI91**
2	E19G2	**RI2**	32	E19G6	**RI32**	62	E29G10	**RI62**	92	E40G1	**RI92**
3	E19G3	**RI3**	33	E19G7	**RI33**	63	E29G11	**RI63**	93	E40G2	**RI93**
4	E19G4	**RI4**	34	E19G8	**RI34**	64	E29G12	**RI64**	94	E40G3	**RI94**
5	E19G5	**RI5**	35	E19G9	**RI35**	65	E29B	**RI65**	95	E40G4	**RI95**
6	E19G6	**RI6**	36	E19G10	**RI36**	66	E29G1	**RI66**	96	E40G5	**RI96**
7	E19G7	**RI7**	37	E19G11	**RI37**	67	E29G2	**RI67**	97	E40G6	**RI97**
8	E19G8	**RI8**	38	E19G12	**RI38**	68	E29G3	**RI68**	98	E40G7	**RI98**
9	E19G9	**RI9**	39	E19B	**RI39**	69	E29G4	**RI69**	99	E40G8	**RI99**
10	E19G10	**RI10**	40	E29G1	**RI40**	70	E29G5	**RI70**	100	E40G9	**RI100**
11	E19G11	**RI11**	41	E29G2	**RI41**	71	E29G6	**RI71**	101	E40G10	**RI101**
12	E19G12	**RI12**	42	E29G3	**RI42**	72	E29G7	**RI72**	102	E40G11	**RI102**
13	E19B	**RI13**	43	E29G4	**RI43**	73	E29G8	**RI73**	103	E40G12	**RI103**
14	E19G1	**RI14**	44	E29G5	**RI44**	74	E29G9	**RI74**	104	E40B	**RI104**
15	E19G2	**RI15**	45	E29G6	**RI45**	75	E29G10	**RI75**	105	E40G1	**RI105**
16	E19G3	**RI16**	46	E29G7	**RI46**	76	E29G11	**RI76**	106	E40G2	**RI106**
17	E19G4	**RI17**	47	E29G8	**RI47**	77	E29G12	**RI77**	107	E40G3	**RI107**
18	E19G5	**RI18**	48	E29G9	**RI48**	78	E29B	**RI78**	108	E40G4	**RI108**
19	E19G6	**RI19**	49	E29G10	**RI49**	79	E40G1	**RI79**	109	E40G5	**RI109**
20	E19G7	**RI20**	50	E29G11	**RI50**	80	E40G2	**RI80**	110	E40G6	**RI110**
21	E19G8	**RI21**	51	E29G12	**RI51**	81	E40G3	**RI81**	111	E40G7	**RI111**
22	E19G9	**RI22**	52	E29B	**RI52**	82	E40G4	**RI82**	112	E40G8	**RI112**
23	E19G10	**RI23**	53	E29G1	**RI53**	83	E40G5	**RI83**	113	E40G9	**RI113**
24	E19G11	**RI24**	54	E29G2	**RI54**	84	E40G6	**RI84**	114	E40G10	**RI114**
25	E19G12	**RI25**	55	E29G3	**RI55**	85	E40G7	**RI85**	115	E40G11	**RI115**
26	E19B	**RI26**	56	E29G4	**RI56**	86	E40G8	**RI86**	116	E40G12	**RI116**
27	E19G1	**RI27**	57	E29G5	**RI57**	87	E40G9	**RI87**	117	E40B	**RI117**
28	E19G2	**RI28**	58	E29G6	**RI58**	88	E40G10	**RI88**			
29	E19G3	**RI29**	59	E29G7	**RI59**	89	E40G11	**RI89**			
30	E19G4	**RI30**	60	E29G8	**RI60**	90	E40G12	**RI90**			

Appendix Table 2. Summary of the results of all parameters evaluated in this study

Isolate code	**Soil site**	**Sorghum genotype**	**HCN**	**IAA**	**MG % (with host)**	**MG% (without host)**	**Methyl red**	**Catalase**	**Sugar utilization**			**Gram reaction**
									Glu	**Fru**	**Suc**	
RI6	E19	G6	+++	+	16^{jk}	16^{e}	+	+	+	+	+	-
RI9	E19	G9	++	+++	10^{lm}	24^{b}	+	+	-	-	-	-
RI19	E19	G6	+	+	26^{i}	6^{jk}	+	+	-	+	+	+
RI23	E19	G10	+++	++	9^{m}	2.7^{l}	-	-	+	+	+	-
RI26	E19	B	++	+	29.7^{hi}	9^{i}	+	+	+	+	+	-
RI38	E19	G12	+++	+++	14^{jkl}	0^{m}	+	+	-	+	+	+
RI54	E29	G2	++	++	12^{klm}	1^{lm}	-	-	-	+	+	-
RI72	E29	G7	+++	+++	18i	7^{ij}	+	+	+	+	+	-
Cont.			-	-	59.7^{a}	27^{a}						

MG=mean germination percentage, Glu=glucose, Fru=fructose, Suc=sucrose, Cont=control

Appendix 2: Overall steps underwent in experimental set up in the study



Appendix Figure 1. Pictorial representation of the overall steps underwent in the study (a) sample collection (b) sample processing (c) sorghum germinating (d) soil preparation (e) sorghum growing (f) rhizosphere soil sampling (g and h) bacteria isolation (i and j) Testing for HCN and IAA production (k and l) Evaluation of bacterial effects on *Striga* (m , n and o) Morphological and biochemical test