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Faculty of Agricultural Sciences
Institute of Plant Production and Agroecology in the Tropics and Subtropics
Section Agroecology in the Tropics and Subtropics
Prof. Dr. J. Sauerborn

# UNDERSTANDING THE ROLE OF PLANT GROWTH PROMOTING BACTERIA ON SORGHUM GROWTH AND BIOTIC SUPPRESSION OF *Striga* INFESTATION

Dissertation

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LENARD GICHANA MOUNDE

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**Examination Committee**

| | |
|---|---|
| Supervisor and Reviewer: | Prof. Dr. Joachim Sauerborn |
| Co-Reviewer: | Prof. Dr.Otmar Spring |
| Additional Examiner: | PD. Dr. Frank Rasche |
| Head of the Committee: | Prof. Dr. Dr. h.c. Rainer Mosenthin |

# Dedication

This thesis is dedicated to my beloved wife Beatrice and children Zipporah, Naomi and Abigail.

# Author's Declaration

I, Lenard Gichana Mounde, hereby affirm that I have written this thesis entitled "Understanding the Role of Plant Growth Promoting Bacteria on Sorghum Growth and Biotic suppression of *Striga* infestation" independently as my original work as part of my dissertation at the Faculty of Agricultural Sciences at the University of Hohenheim.

No piece of work by any person has been included in this thesis without the author being cited, nor have I enlisted the assistance of commercial promotion agencies. This thesis has not been presented into other boards for examination.

Stuttgart, 15th September 2014 Lenard Gichana Mounde

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# List of Abbreviations

| | |
|---|---|
| ABA | Abscisic acid |
| ACC | Aminocyclopropane-1-carboxylate |
| ACN | Acetonitril |
| AMF | Arbuscular mycorrhiza fungi |
| APS | Agricultural production systems |
| AS | Attached *Striga* seeds |
| BEH | Bridged ethylene hybrid |
| C | Carbon |
| $CaCl_2$ | Calcium chloride |
| CFU $ml^{-1}$ | Colony forming units per milliliter |
| $CO_2$ | Carbon dioxide |
| Da | Dalton |
| DAP | Diammonium phosphate |
| DMBQ | 2,6-dimethoxybenzoquinone |
| DNA | Deoxyribonucleic acid |
| DT | Dead *Striga* tubercles |
| EAGA | Extended agar gel assay |
| FA | Formic acid |
| FAO | Food and Agriculture Organization of the United Nations |
| FGFP | Fiber glass filter paper |
| GA | Gibberellic acid |
| GLM | Generalized linear model |
| GP | Germination percentage |
| GR24 | Synthetic *Striga* germination stimulant |
| GS | Germinated seeds |
| h | Hours |
| Ha | Hectare |
| HR | Hypersensitive response |
| HRGP | Hydroxyproline-rich glycoproteins |
| $H_2O_2$ | Hydrogen peroxide |
| IAA | Indole acetic acid |

| | |
|---|---|
| ISR | Induced systemic resistance |
| K | Potassium |
| LC-ESI-MS/MS | Liquid chromatography-electrospray ionization-mass spectrometry/ mass spectrometry. |
| LB | Luria –Bertani |
| LCOs | Lipochitooligosaccharides |
| LTQ | Lysine tyrosylquinone |
| min | Minutes |
| mM | Millimole |
| *m*/*z* | mass-to-charge ratio |
| N | Nitrogen |
| NaCl | Sodium chloride |
| NCBI | National Center for Biotechnology Information |
| ng/ml | Nanograms per milliliter |
| $^{O}C$ | Degrees Celcius |
| P | Phosphorus |
| PCD | Programmed cell death |
| PGPR | Plant growth promoting rhizobacteria |
| PVC | Polyvinyl chloride |
| PVP | Polyvinylpolypyrrolidone |
| RCD | Randomized complete design |
| RL | Radicle length |
| SAS | Statistical analysis software |
| SDW | Sterile distilled water |
| SDS-PAGE | Sodium dodecyl sulphate polyacrylamide gel electrophoresis |
| sp. | Species(pl.) |
| SRS | Sterile Ringer solution |
| Std. Dev. | Standard deviation |
| SXSg | Sorgoleone and hydroquinone sorghum xenognosins |
| TS | Total seeds |
| UPLC | Ultra performance liquid chromatography |
| VI | Vigor index |

# Chapter 1: General introduction

## 1.1 *Striga*

Witchweeds (*Striga* spp.) are parasitic weeds of great agricultural significance, parasitizing the roots of their hosts (Sauerborn *et al*., 2007). There are over 50 species of *Striga,* but the most economically important ones are *S. hermonthica, S. asiatica, S. aspera, S. forbesii* (Plate 1.1) parasitizing cultivated legume and cereal crops like cowpea (*Vigna unguiculata*), finger millet (*Eleusine corocana*), maize (*Zea mays*), pearl millet (*Pennisetum glaucum*), rice (*Oryza sativa*), sugarcane (*Saccharum officinarum*) and sorghum (*Sorghum bicolor*) (Elzein & Kroschel, 2004; Scholes & Press, 2008). *Striga,* like all other root parasitic weeds, drain essential organic and inorganic resources from their hosts leading to poor crop development and low yield (Watson *et al*., 1998). In Africa, about 50 million ha in 30 countries are infested by *Striga* spp. causing grain loss of cereals (Gressel *et al*., 2004). Estimated yield losses of maize, sorghum, millets (pearl and finger) and upland rice are between 30 and 90% (van Ast *et al*., 2005; Ejeta, 2007; Sauerborn & Müller-Stöver, 2009). Although improved cultural practices, herbicide use, and growing resistant varieties have been used to control the parasite (Scholes & Press, 2008), the *Striga* problem still remains unsolved to date. The parasite, therefore, is ranked as the leading biotic constraint to cereal production in Africa (Ejeta & Butler; 1993) where it has caused considerable loss in crop yield quantity and quality (Westwood *et al*., 2012). The stunted and chlorotic appearance which appears as distorted "bewitched" curls of the infected crops before *Striga* emergence is what led to this weed to be called "witchweed".

## 1.2 Sorghum

Sorghum, a major *Striga* host, is an important cereal crop in Africa with an estimated 23 million hectares under cultivation (FAO, 2012). This scale of production is more than half of the global production of 40 million hectares. Despite this large acreage, total and average production per hectare is below the global average, making it unable to meet the growing demand for human food, animal feed, fuel and building materials in Africa (Jamil *et al*., 2011). *Striga* has been identified as one on the major biotic constraints (Guo *et al*.,

2011) while poor soil fertility, soil degradation and nutrient reduction are the major abiotic constraints (Palé *et al*., 2009) responsible for this underproduction.


**Plate 1.1** Common *Striga* species (a) *Striga hermonthica* (purple witchweed), (b) *S. forbesii* (pink witchweed), (c) *S. asiatica* (red witchweed) (Photos by Lenard Mounde)

Crop damage by *Striga* is worse under conditions of nutrient limitation and insufficient fertilizer use (Gacheru & Rao, 2001; Phoenix & Press, 2005). Nitrogen (N) and phosphorus (P) inavailability in soils, low fertilizer use due to high prices (Bekunda *et al*., 1997; Bagayoko *et al*., 2000) are limiting sorghum production in Africa. Generally, the magnitude of yield loss is largely influenced by the level of *Striga* infestation, soil fertility, agro-climatic conditions, land use system, the plant species and the host genotype (Oswald & Ransom, 2004). The parasite causes damage even before emergence from the soil and currently, there is no universally accepted and adopted control method for *Striga* (Oswald, 2005). While some sorghum cultivars have demonstrated some level of resistance through low strigolactone and haustorial initiation factors production, parasite avoidance, physical barriers to parasite attachment or penetration, and antibiosis (Ejeta *et al*., 1999), total *Striga* control has not been realized in many sorghum fields. Plate 1.2 shows *Striga*-free and *Striga* infested sorghum crop in the field.

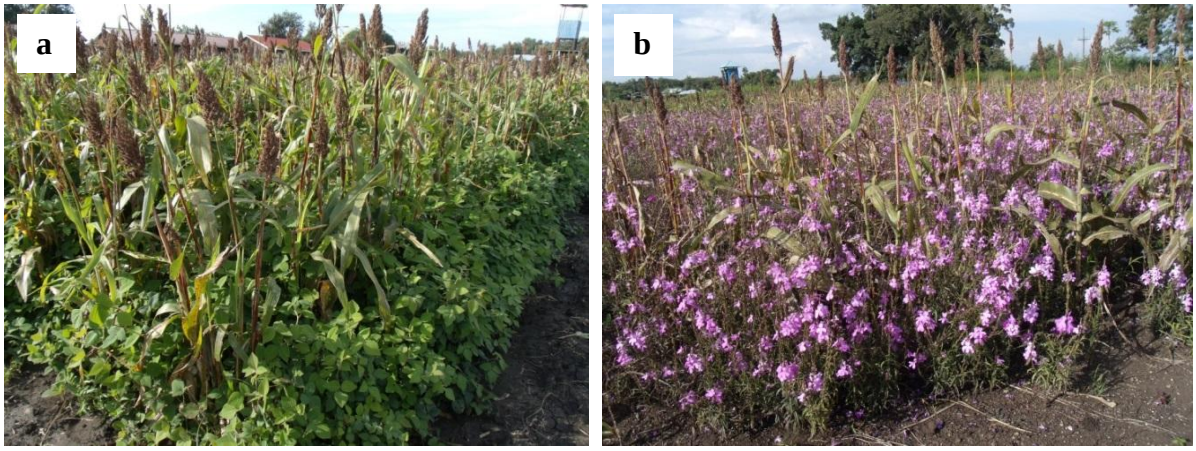


**Plate 1.2** *Striga*-free and *Striga* infested sorghum crop (a) *Striga*- free sorghum crop under "push and pull" control strategy and (b) a sorghum crop under heavy *Striga hermonthica* infestation in Kenya (Photos by Lenard Mounde)

## 1.3 Plant growth promoting rhizobacteria

Plant growth promoting rhizobacteria (PGPR) from a number of genera: *Acetobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Beijerincka, Burkholderia, Enterobacter, Herbaspirillum, Klebsiella, Paenibacillus, Pseudomonas* and *Stenotrophomas* (Dobbelaere *et al*., 2003) are promising components for integrated solutions to agro-environmental problems because inoculants possess the capacity to promote plant growth (Adesemoye *et al*., 2008) and reduce the population of deleterious microbes in the rhizosphere (Lazarovits & Nowak, 1997). Mechanisms of promoting plant growth include; (i) aiding in the acquisition and uptake of mineral nutrients (ii) preventing pathogenic organisms (e.g. by synthesizing antibiotics); or by (iii) directly stimulating plant growth by either providing plant hormones such as auxin or cytokinin, or lowering plant ethylene levels through the action of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Glick *et al*., 1999). Most plants use similar defence responses to parasitic plant infection as those used in response to pathogens (Westwood *et al*., 1998; Joel & Portnoy., 1998; Goldwasser *et al*., 1999).

One of the reasons that have hampered effective control of *Striga* is the complex parasite-host interaction which remains poorly understood to date (Ejeta & Butler, 1993; Runo *et al*., 2012). Understanding host-parasite interaction is paramount in developing appropriate strategies for *Striga* management (Runo *et al*., 2011). There are only few studies done on *Striga* control using PGPR compared to other biological control methods e.g use arbuscular

mycorrhiza fungi (AMF), *Fusarium* and insects (Watson, 2013). However, this group of bacteria is generating great interest as effective candidates for biocontrol of parasitic weeds. For instance, the use of ethylene producing *Pseudomonads* and *Bradyrhizobium japonicum* isolates have been reported to induce *Striga* germination in the absence of host plants (Berner *et al*., 1999; Ahonsi *et al*., 2002a). Given that a vast majority of host plants of *Striga* are colonized by PGPR, attachment of a parasitic plant to a host plant could lead to a tripartite system within which nutrients, water and carbohydrates flow from one associate to another. The flow of substances within such a system could be more complex if the root parasites are themselves colonized or parasitized by the PGPR, a phenomenon commonly referred to as hyperparasitism. Defence mechanisms employed by plants against *Striga* weeds have been reported (Yoder & Scholes, 2010) but it is not clear if some of these mechanisms can be elicited by PGPR. Whether these parasites are themselves colonized by PGPR or not is not completely understood, perhaps due to few studies that have been done on this area.

Currently, attention is increasingly focusing on Gram-positive members of the aerobic, spore-forming genus *Bacillus* as potential candidates for *Striga* control. The main reason behind this shift of focus is that *Bacilli* spp. are prevalent in many soils, in immediate contact with plant roots, form endospores and produce a broad spectrum of antibiotics which offer protection against root pathogens compared to Gram negative bacteria (Kim *et al*., 1997). Among the antibiotics produced include lipopeptide surfactins, iturin and fengycin which are very effective in suppressing fungi activities and growth of a wide range of plant pathogens (Toure *et al*., 2004). *Bacillus* spp. also produce phytohormones, indole acetic acid (IAA) (Idris *et al*., 2007) and cytokinins (Tsavkelova *et al*., 2006; Aslantas *et al*., 2007 ) which have a positive effect on plant growth (Idris *et al*., 2007). Phytohormones generated by bacteria can be taken up by plants leading to an increase in hormone levels in these plants (Patten & Glick., 1996; Barazani & Friedman, 1999). In addition, a number of *Bacillus* strains fix nitrogen; solubilize phosphate and control plant diseases (Kloepper *et al*., 2004) and nematodes (Oostendorp & Sikora, 1990). Their heat-resistant spores make it easier for the bacteria to be formulated into stable biofertilizer or biocontrol products (Deng *et al*., 2011).

Strains, particularly those belonging to the *Bacillus subtilis* and *B. amyloliquefaciens* group (FZB13, FZB14, FZB24, FZB37, FZB38, FZB42, FZB44 and FZB45) possess plant-growth-promoting, higher crop yields and biological activities against some soil-borne

fungal diseases (Grosch *et al*., 1999). For instance *B. subtilis* Bsn5 exhibits *in-vitro* antibiosis and produces lipopeptides (Deng *et al*., 2011) which controls pathogens. *B. amyloliquefaciens* FZB42 and *B. subtilis* GBO3 produce volatiles acetoin and 2, 3-butanediol synthesis, which contribute to IAA synthesis (Idris *et al*., 2007; Zhang *et al*., 2007) thus promoting plant growth. Additionally, *B. subtilis* GB03 has been found to increase photosynthetic efficiency and chlorophyll content in *A. thaliana* through the modulation of endogenous signaling of glucose and abscisic acid sensing (Zhang *et al*., 2008). In another study, Minaxi *et al*. (2012) reported that *Bacillus subtilis* solubilized P, exhibited 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, produced ammonia and IAA.

Since *Striga* infection lowers IAA levels in hosts (Press *et al*., 1999) and auxins such as IAA are thought to inhibit *Striga* germination (Miché *et al*., 2000), *Bacillus* strains could offer growth benefits to sorghum and suppressive effect on *Striga* due to their IAA producing ability. Auxins are also associated with strong inhibition to *Striga* attachment and haustorium development because of their antagonistic nature with cytokinins and benzoquinone, both of which favour attachment and haustorium development (Keyes *et al*., 2000). Little is known about other suppressive abilities of *Bacillus subtilis* on *Striga* although Leclère *et al*. (2005) suggested that *B. subtilis* produces lipopeptides which compete with the parasite over binding sites with strigolactones hence reducing *Striga* germination. In a study involving *Orobanche aegyptiaca* and *O. cernua* treated with *B. subtilis*, Barghouthi and Salman (2010) also noticed significant reduction in radicle elongation in the presence of a synthetic stimulant GR24.

*Burkholderia* sp. strain PsJN which was originally designated *Pseudomonas* sp. strain PsJN (Frommel *et al*., 1991), has been found to promote plant growth in potato (*Solanum tuberosum*) (Frommel *et al*., 1991), grapevines (*Vitis vinifera*) (Ait Barka *et al*., 2000), tomato (*Solanum lycopersicum*) (Compant *et al*., 2005) in addition to disease control in grapevines (Ait Barka *et al*., 2002) and tomato (Sharma & Nowak, 1998). The PGPR has been found to induce larger roots, stronger stems and vascular system (Nowak, 1998), enhance accumulation of chlorophyll and phenolics (Nowak *et al*., 1998), cytokinins (Lazarovitis & Nowak, 1997) and resistance to pathogens (Salles *et al*., 2006) in plants. The strain is also known to have ACC deaminase activity (Blaha *et al*., 2006; Weilharter *et al*., 2011) which reduces the level of the growth inhibitory hormone ethylene (Ashrafuzzaman *et al*., 2009). The strain also produces IAA and siderophores (Weilharter

*et al*., 2011), which are plant growth enhancing compounds. Mineralization of IAA by *Burkholderia phytofirmans* PsJN has been found to be crucial for efficient root colonization of *Arabidopsis* (Poupin *et al*., 2013).

Mechanisms of *Burkholderia phytofirmans* in disease or weed suppression are not clearly understood although antibiotic compounds phenazine and pyrrolnitrin (El Banna & Winkelmann, 1998), antifungal lipopeptides and antibiosis (Kang *et al*., 1998; Heungens & Parke, 2000) have been identified in some strains. There has been a lot of research efforts regarding the genomics, growth promoting and disease control mechanisms, among other aspects, of *Burkholderia phytofirmans* PsJN in the recent past (Sessitsch *et al*., 2005; Compant *et al*., 2008; Theocharis *et al*., 2012; Kim *et al*., 2012; Zúñiga *et al*., 2013). This formed the basis of the current investigation on this strain together with *Bacillus* spp.

Although there are numerous studies on plant growth promotion and biological control of diseases, weeds, nematodes and parasitic weeds using PGPR, little is known about the potential of some *Bacillus subtilis, B. amyloliquefaciens* and *Bourkhoderia phytofirmans* strains in sorghum growth promotion and biotic suppression of *Striga* infection.

## 1.4 Research hypothesis

This study hypothesized that;

i) Extended agar gel assays (EAGA) as proposed by Mohamed *et al*. (2010a) and root chamber experiments as described by Linke *et al*. (2001) incorporating *Striga*, sorghum and PGPR will provide some understanding on the tripartite interaction between PGPR-sorghum roots-*Striga* interactions and provide a generic working model system to assess sorghum growth promotion and *Striga* weed suppression.

ii) PGPR can reduce *Striga* infection by directly suppressing underground development stages or indirectly by maintaining sorghum biomass in spite of *Striga* infection.

iii) PGPR have a suppressive effect on *Striga* germination and radicle growth while having a germination and vigor enhancing effect on sorghum seeds.

iv) PGPR will inhibit *Striga* germination and radicle elongation through production of phytotoxic compounds.

## 1.5 Study objectives

The main objective of the study was to assess the effect of *B. subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 and *Burkholderia phytofirmans* strain PsJN on growth promotion of sorghum plants and suppression of *Striga* development, thus providing a basic understanding on the sorghum-PGPR-*Striga* interaction. Specific objectives were to;

- apprehend the tripartite interaction between sorghum, *Striga* and PGPR by reviewing the state-of-the-art knowledge in published literature.
- identify a suitable media for delivering *B. subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 and *Burkholderia phytofirmans* strain PsJN to sorghum rhizosphere and understand their effect on sorghum and *Striga* development.
- investigate the effect of *Bacillus* strains (*B. subtilis* Bsn5, *B. subtilis* GBO3, and *B. amyloliquefaciens* FZB42) and *Burkholderia phytofirmans* strain PsJN on sorghum and *Striga* germination, sorghum vigor and *Striga* radicle elongation.
- determine if *B. subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 and *Burkholderia phytofirmans* PsJN can offer any protection against *Striga* and promote early growth of sorghum in root chamber experiments.

## 1.6 Outline of thesis

The overall objective of this thesis was to gain insights into the tripartite interactions among sorghum, *Striga* and plant growth promoting rhizobacteria with emphasis on the role of PGPR on sorghum growth and biotic suppression of *Striga* development. To achieve this objective, a comprehensive literature review on the current knowledge on tritrophic interaction between sorghum, *Striga* and PGPR is presented in Chapter 2. Research gaps are described and future research directions recommended. Chapter 3 presents all the procedures and data analysis of all laboratory experiments conducted in an effort to seek answers on the role of *Bacillus subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 and *Burkholderia phytofirmans* PsJN on sorghum growth

promotion and *Striga* suppression. Chapter 4 presents results obtained in Chapter 3. A detailed discussion of results is provided in Chapter 5 followed by a general conclusion and future research perspectives in Chapter 6.

# Chapter 2: Tripartite interactions between sorghum, witchweeds (*Striga* spp.) and plant growth promoting rhizobacteria

## 2.1 Introduction

Plant-plant parasitism is a well-established phenomenon where some species of flowering plants have evolved over the years and developed parasitic associations with other members of the plant kingdom (Kuijt, 1969). Watling & Press (2001) and Runo *et al*. (2012) have estimated that these parasitic plants are in the range of 4000 species, grouped in 13 families and occurring in all ecosystems except in aquatic systems. Today, many Agricultural Production Systems (APS), mainly in Subsaharan Africa (SSA) and the Meditterranean region are experiencing parasitic weed problems (Atera *et al*., 2011). Those parasites that are of great agricultural significance belong to the genera *Alectra, Orobanche* and *Striga*, parasitizing mainly the roots of their hosts (Sauerborn *et al*., 2007) where they have demonstrated high efficacy in obtaining organic and inorganic resources from the affected hosts. *Striga* and *Orobanche* demonstrate a close phylogenetic relationship and are among the estimated 90 genera in the *Orobanchaceae* family, a plant family with more than 2,000 species (Westwood *et al*., 2010). The main difference between both genera is that *Orobanche* lacks chlorophyll and depends fully on its host plant for water, inorganic and organic nutrients, *Striga* derives only a part of its carbon, but fully for water, from its host except *Striga gesnerioides,* which like *Orobanche* doesn't fix its own carbon. *S. gesnerioides* has poorly developed leaves with low chlorophyll content which don't fix net carbon even on exposure to light (Graves *et al*., 1992). That explains why *Orobanche* infects both the phloem and xylem while *Striga* attacks the xylem only **(**Irving & Cameron, 2009). *Orobanche* and *S. gesnerioides* are therefore collectively known as holoparasites attacking solely dicots. *Striga* (except *S. gesnerioides*), on the other hand, are hemiparasites whose hosts belong mainly to Poaceae family.

The *Striga* problem is aggravated by poor soil fertility and moisture limitations that are present in many small holder farms in sub-Saharan Africa. In addition, the majority of the affected farmers are resource poor with little capacity to mitigate these challenges or invest in alternative crops. As a result of the huge economic impact and food security threat, research is ongoing to find long lasting management options to fight this parasite. PGPR

might be possible biocontrol agents for *Striga* due to their ability to colonize roots of host crop and promote their growth. It is envisaged that the sorghum-PGPR-*Striga* interaction could have an influence on the parasitic association between *Striga* and sorghum crops especially if colonization is established and functioning before or after invasion of host roots by the parasitic plants.

## 2.2 Interaction between *Striga* and sorghum

In order to have a clear understanding of the tripartite interaction between *Striga*, sorghum and PGPR, it is important to have an idea of how *Striga* and sorghum interact in the absence of PGPR. Already the chemical cross talk that controls *Striga* germination and the development of physical connections with the host is well understood (Palmer *et al*., 2004). In this situation, the life cycle of the parasite is highly harmonized with that of the host, right from germination to maturity (Park & Riches, 1993). However, there are some development stages of the parasite that do not require the presence of the host. According to Joel *et al*. (1995) the life cycle of *Striga* can be divided into two phases; the independent phase and parasitic phase.

### 2.2.1 Independent phase

The independent phase is also referred to as non-parasitic phase. It begins when the *Striga* seed germinates and culminates when the radicles attach to host roots. The parasite can develop independently from the host provided the right chemical signals to trigger their germination and attachment are present, either artificially or from non-hosts (Bouwmeester *et al*., 2007). These stimulants are a special class of chemicals called strigolactones. Many of these chemicals have been isolated and identified. They can trigger germination in both *Striga* and *Orobanche* almost in equal measure even at very low concentrations (Cook *et al*., 1966). They include, alectrol (Hauck *et al*., 1992), sorgolactone (Hauck *et al*., 1992; Awad *et al*., 2006), orobanchol (Yokota *et al*., 1998), 5-deoxy-strigol, strigol (Sato *et al*., 2005; Awad *et al*., 2006) and sorgomol (Jamil *et al*., 2013).

The amount of strigolactones secreted by plants of the same variety is inversely correlated to the fertility status of the soil. Ayongwa *et al*. (2006); Yoneyama *et al*. (2007) and Jamil *et al*. (2012) demonstrated this fact when they observed that host sorghum plants deficient in N and P, secreted more strigolactones to the rhizosphere compared to well-nourished

plants. Recently, Jamil *et al*. (2013) reported that diammonium phosphate (DAP) fertilizer can reduce *S. hermonthica* emergence and increase sorghum grain yield if applied close to the root zone by reducing secretion of strigolactones into the rhizosphere. This relationship between plant nutritional status or soil fertility and strigolactone production could be one of the reasons why there is a huge *Striga* problem in many developing countries. These areas are frequently characterized by poor soils and the farmers have little financial resources to invest in expensive inputs such as fertilizers or plant alternative crops (Boone *et al*., 1995). In addition, there are other chemicals that stimulate *Striga* germination although they are not present in root exudates of the host crop. Examples are ethylene, cytokinins, jasmonates, amino acids and polyols (Babiker *et al*., 1993; Galindo *et al*., 2004).

Some chemicals released by plant roots for defence against other competing plants, parasites or pathogens have also been found to trigger parasite seed germination. Examples of these chemicals are sorgoleone and hydroquinone sorghum xenognosins (SXSg). The chemicals are distinct from strigolactones but can trigger *Striga* germination (Lynn & Chang, 1990; Keyes *et al*., 2001). Sorghum-*Striga* interaction after germination is influenced by other secondary metabolites released by the host, consequently leading to the parasitic mode of living. The processes influenced by these metabolites include *Striga* radicle protrusion, orientation towards the host and haustorium development (Dube & Oliver, 2001).

### 2.2.2 Parasitic phase

After germination, *Striga* must attach to a suitable host within 5 to 7 days. Otherwise they will exhaust their energy reserves and die. This requires them to shift from the independent to the dependent (parasitic phase). After attachment, host-derived secondary metabolites like flavonoids, quinines positively influence formation of the haustorium which is a physiological connection between the parasite and the vascular vessels of host plants (Riopel & Timko, 1995). Some of these compounds are phenolic in nature such as 2,6-dimethoxybenzoquinone (DMBQ) (Kim *et al*., 1998). DMBQ is released from host cell walls under the influence of chemicals released by the *Striga* radical e.g hydrogen peroxide and was isolated from sorghum roots through peroxidase-mediated oxidation of sorghum cellular components (Chang & Lynn, 1986; Lynn & Chang, 1990). In other words, *Striga* provokes the host to produce a signal necessary for its development in a process termed

semagenesis (Keyes *et al*., 2007). It is not clearly understood how host benzoquinones induce haustorial development, but the downregulation of a gene for one *Striga* expansin protein, accompanied by upregulation of genes for two expansins, *saExp1* and *saExp2* has been suggested as a possible mechanism (O'Malley & Lynn, 2000; Torres *et al*., 2005). The involvement of auxin in modifying cell shape has been implicated too (Tomilov *et al*., 2005).

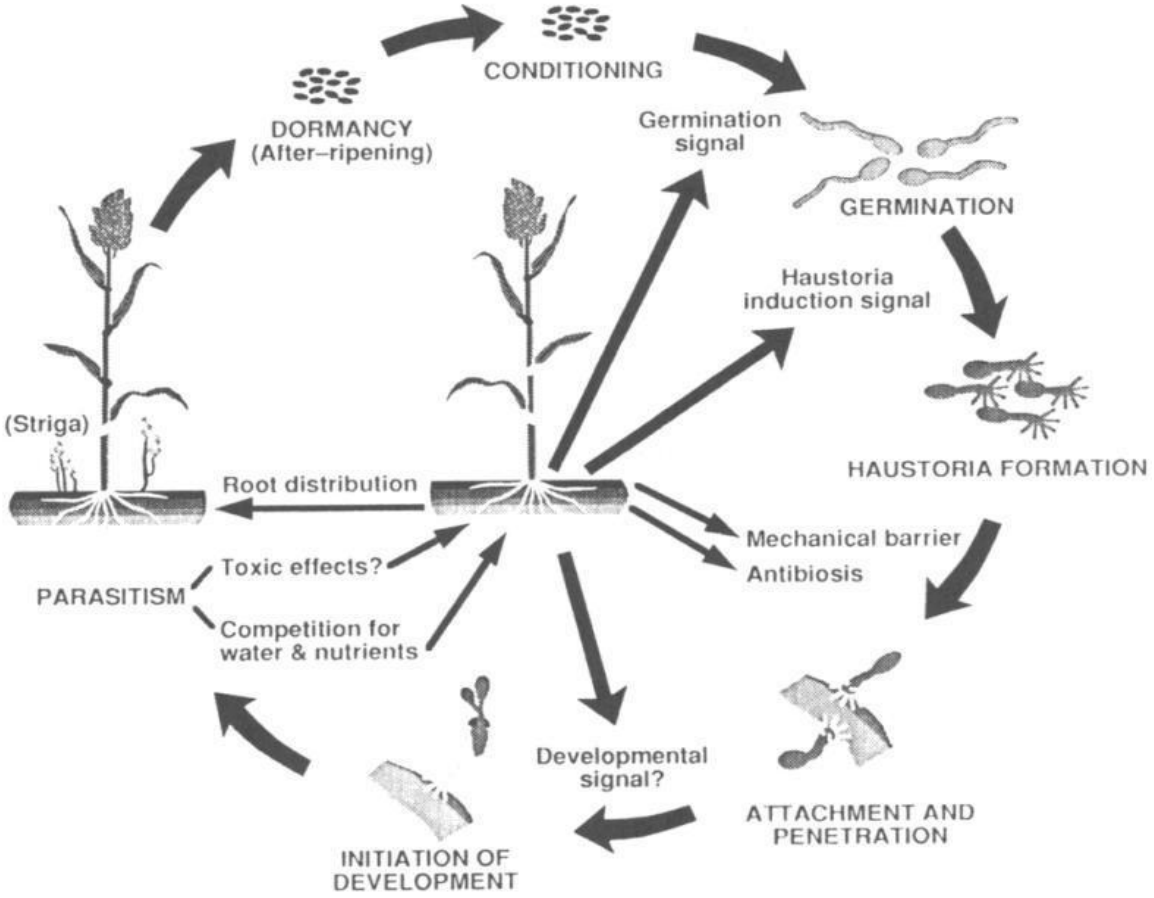


**Figure 2.1** Generalized illustration of *Striga* life cycle. Source: Ejeta & Butler, 1993

Haustorium penetration into host root takes place under the aid of hydrolytic enzymes produced by the parasite, eventually forming a haustorial connection with the host (Losner-Goshen *et al*., 1998). Cell wall-degrading enzymes are involved whereby they alter sorghum cell walls at the point of infection (Olivier *et al*., 1991). *Striga* shoots will emerge after about one month and take another 30 to 40 days before flowering (Stewart & Press, 1990). Flowering and seed production will then follow consequently signaling the start of another life cycle. A generalized illustration of *Striga* life cyle is shown (Figure 2.1). The soil seed bank then continues to increase after each successful life cycle (Bouwmeester *et*

*al*., 2003). This continuous addition of seeds into the soil is one of the factors that have made *Striga* control challenging to date. Moreover, the seeds have a long viability (>10 years) in the soil (Hearne, 2009).

### 2.2.3 Impact of *Striga* on sorghum

Plants capable of supporting parasite from germination to seed production are considered hosts (Timko *et al*., 2012). As the host crop support the parasite, the crop faces a lot of negative consequences. After successful connection of *Striga* to its host, the parasite becomes a sink for metabolites and water from the host (Joel *et al*., 2007). The haustorium is the organ that the parasite uses to withdraw water, mineral nutrients and assimilates from the host. Dörr (1996) reported that all parasitic weeds are capable of acquiring resources from the host xylem because of the luminal contact with the xylem of their hosts. The parasite is a strong sink for water and solutes because of two reasons. The first reason is its unique ability to maintain high stomatal conductance (open stomata) at all times (Jiang *et al*., 2003). The second reason is its ability to accumulate high amounts of osmotically active compounds such as mineral ions (e.g. potassium), sugars and sugar alcohols (Irving & Cameron, 2009). Open stomata lowers water potentials in parasites leading to elevated transpiration which in turn creates a high demand for water from the host even when the host is under severe water stress (Smith & Stewart, 1990). Osmotically active substances create a high negative water potential enabling solute flow from the host to the parasite. The water loss impact on crops is worsened if the crop is growing under water stress conditions.

*Striga* is a xylem-invading parasite. Then the question arises. How does it obtain carbon from sorghum? Press & Whitaker (1993) found out that it can do so from the xylem sap or use other apoplastic means. Westwood (2013) describes apoplastic loading as a process involving exporting sugars from cells around the vein into the apoplastic space and then rapidly re-importing them into the phloem companion cells. It is an active process with an energy cost implication. Before emergence, *Striga* seedlings depend totally on their hosts for carbon because of their inability to access light (Graves *et al*., 1990). The parasite derives between 35 to 65% of its C from sorghum (Graves *et al*., 1989; Pageau *et al*., 1998) and the amount withdrawn from the host depends on the concentration of N in the soil. Cechin & Press (1993) reported that the higher the concentration of N in the soil, the lesser

the C taken by *Striga*. No explanation is available yet on this relationship but may partly explain the severity of *Striga* infection in N poor soils in *Striga*- dominant areas.

A decreasing and increasing parasite dependence on the host for C and N, respectively, as the parasite matures has been observed too. Aflakpui *et al*. (2005) reported a 100% *Striga* dependence of C on host but only 59% of N before emergence but N dependence increased to nearly 100% as the parasite matured. Total *Striga* dependence on host for N has been explained by the parasite's low nitrate reductase and glutamate synthetase activity which in turn reduces N assimilation (Press *et al*., 1986). So the parasite has to obtain all its N requirements in fully reduced forms such as ammonium or amino acids (Westwood, 2013). More evidence has shown that, although enhancing N fertilization e.g ammonium nitrate in sorghum-*Striga* association led to higher accumulation of nitrogen in leaves and higher photosynthetic rate of the parasite, there was decreased biomass production because of its low N assimilation capacity (Cechin & Press, 1993). This may explain why *Striga* grows poorly in soils rich in N (Igbinnosa & Thalouram, 1996). It has been revealed that *Striga* infection can induce an increase of nitrates and free amino acids in sorghum xylem sap (Pageau *et al*., 2003) to make it easier for the parasite to withdrawal these resources from the crop (Nemec, 1995).

In general, it is estimated that between 20 and 80% of all *Striga* biomass is taken from their hosts (Tenakoon & Pate, 1996; Teˇsˇitel *et al*., 2010). This huge variation takes care of species differences and life cycle stages of the parasite. This resource withdrawal has a direct impact on crop performance and yield. For instance, while carbon withdrawal ranged between 28 and 35% (Press *et al*., 1987a), sorghum shoot yield reduction varied between 77 and 86% depending on *Striga* species (Press *et al*., 1987b). This shows that host yield reduction cannot be explained only by the carbon withdrawn by the parasite. This argument is made strong when the amount of resources removed *Striga*-infected crops has been found to be more than *Striga* biomass, sometimes even 30 times more (Press *et al*., 1996). The loss of crop biomass is a clear indicator that not all the resources the host loses are taken up by the parasite (Parker *et al*., 1984) suggesting that the parasite has more detrimental effects on their hosts in addition to simple draining of resources (Press *et al*., 1996). The loss of crop biomass inflicted by *Striga* infection indicates the involvement of other mechanisms other than source/sink-based relations. Some of these mechanisms include disruption of photosynthesis and metabolism, hormonal imbalances, toxins among

others. Specifically, the percent of carbon gained by *S. hermonthica* from sorghum was estimated to be around 40% (Press & Graves, 1991).

Besides the withdrawal of carbon from the host, *Striga* can also negatively influence host photosynthetic process. Although capable of fixing carbon (Cameron *et al*., 2008), high respiratory rates of *Striga* makes it to utilize more carbon than they synthesize. So the net carbon gain is small (Press, 1989) creating a huge demand for the resource from their hosts. Gurney *et al*. (1995) has reported already on lower photosynthetic rates in leaves of *Striga*/infected sorghum plants. This can be directly or indirectly through reduced leaf sizes in hosts due to *Striga* infection (Press & Stewart, 1987). A direct effect is linked to reduction of carbon fixation where it has been estimated that over 80% of sorghum growth reduction is due to *Striga*-induced reduction in photosynthesis (Graves *et al*., 1989) and reduced leaf expansion (Aflakpui *et al*., 2002). There are two possible ways by which this disruption can take place. First, is the reduction of plant size and total leaf area. This is one of the responses of sorghum to *Striga* infection (Walting & Press, 1997). Short plants, leaves and reduced leaf area minimize light capture and reduce the surface area for photosynthesis respectively. The consequence is that the overall amount of fixed carbon is lowered. The second mechanism is a direct effect linked to reduction of carbon fixation. Gurney *et al*. (1995) attributed lower photosynthetic rates in leaves of *Striga*-infected sorghum to their reduced biomass. *Striga* infected plants also tend to close their stomata due to lower stomatal conductance (Frost *et al*., 1997). However, studies by Walting and Press. (1998) on wild grass (*Achnatherum hymenoides*) did not find any involvement of *Striga* on the photosynthetic process of the host.

The other issue that needs further discussion and research is whether sorghum, being a C4 plant requires open stomata to concentrate more $CO_2$. C4 plants are known to have other mechanisms of concentrating $CO_2$ through its Kranz anatomy. A study conducted by Watling and Press (1997) demonstrated that, in terms of growth, sorghum responds positively to increased $CO_2$ better than *Striga* which is a C3 plant. Elevated $CO_2$ levels were even detrimental to the parasite. However, no significant reduction of its negative impact on sorghum was observed. Infected sorghum showed similar growth in both ambient and elevated $CO_2$ conditions. These results show that increased carbon still doesn't offer solution to the *Striga* threat. The photosynthetic capacity of emerged *Striga* can also be reduced by competition for light posed by host plants and nearby plants in addition to its cellular and physiological limitations as described by Tuohy *et al*. (1986);

Press *et al*. (1986) and Salle *et al*. (1987). However, there is likelihood that this competition could have little effect on *Striga* performance. For instance, Dörr (1997) demonstrated that *Striga* can grow to maturity even in darkness as long as the host is exposed to light.

Disruption of host`s hormone stability has also been suggested as another cause of *Striga* damage to cereals. Generally, there is a sharp increase of abscisic acid (ABA) and cytokinins in *Striga* after attachment to hosts (Lechowski & Bialczyk, 1996; Taylor *et al*., 1996). ABA, being a drought-stress hormone is thought to be stimulated in the parasite because of the low water potential experienced by *Striga* (Jiang *et al*., 2010). However, parasites are less sensitive to ABA than hosts hence will have open stomata despite high ABA content in cell sap (Jiang *et al*., 2003). Furthermore, high cytokinins content in *Striga* leaves antagonizes ABA and keeps the stomata open (Lechowski, 1997; Westwood., 2013).

Elevated levels of ABA in sorghum leaf tissue and xylem sap after the infection process have also been reported (Frost *et al*., 1997) although no explanation on the role of this hormone in host crops has been offered (Jiang *et al*., 2010). Several authors have suggested that elevated levels of ABA in the host crop may play a role in reducing photosynthesis through regulating stomatal opening (Frost *et al*., 1997). Minimal stomata opening by a host can be beneficial to *Striga* because of a shift in water use from the host to the parasite (Westwood, 2013). High ABA content in hosts also lowers defence responses, especially those associated with salicyclic acid, in infected host roots (Vieira Dos Santos *et al*., 2003; Grifitts *et al*., 2004). These effects can have a negative consequence on the amount of carbon fixed. Increased root hydraulic conductivity as stimulated by ABA could also lead to more water loss from the host to the parasite (Jiang *et al*., 2004).

It has been argued that host plants might also be affected by toxins produced by *Striga* species. This is only possible if there will be a parasite-to-host movement (reverse flow) of materials. There is evidence that the movement is possible. Okonkwo (1966) showed that radiolabbed $CO_2$, urea and sulphur applied on *Striga senegalensis* also moved into the sorghum host. There is more recent evidence of reverse flow in *Orobanche* (Aly *et al*., 2011) suggesting the possibility of toxic material moving from *Striga* to sorghum. Musselman (1980) and Parker (1984) suggested that stunted growth in *Striga*-infected cereals was due a toxin released by the parasite and translocated to the cereal crop. Ransom *et al*. (1996) also reported that *Striga*- produced toxins could lead to enzymatic and

hormonal disruptions which will lead to poor water uptake and photosynthesis by the host. Iridoid glucosides (iridoid glucosides (mussaenosidic acid, mussaenoside, gardoside methyl ester, bartsioside, isoaucubin, melittoside, aucubin and eurostoside), caffeoyl phenylethyl glycosides (calceolarioside A and verbascoside), shikimic acid and trigonelline, all known for their toxicity on cereal herbivorous pests (Adler *et al*., 1995) have been isolated in *Striga hermonthica* and *S. asiatica* parasitic to sorghum (Rank *et al*., 2004). However, little is known about possible toxicity of these metabolites on *Striga*-infected sorghum plants.

In general, the overall impact of *Striga* on their hosts include; the loss of water, mineral nutrients and photosynthates often leading to stunted growth, chlorosis and even death, followed by poor economic yield (Parker, 1991). It is rare for host crops, especially domesticated varieties, to exhibit complete resistance to *Striga* infection. However, some wild relatives of crop species tend to demonstrate some resistance and tolerance to infection (Scholes & Press, 2008; Hearne, 2009). Improved cultivars that are resistant or tolerant to *Striga* are also available in the seed market. In the following text, existing host resistance responses to *Striga* infection is reviewed.

### 2.2.4 Sorghum resistance responses to *Striga* infection

Resistance is defined as the ability of a plant, whether host or non-host, to endure parasite attack in a manner that prevents establishment and growth of the parasite (Timko & Scholes, 2013). Plants can respond to *Striga* infections at all stages of growth, before or after the parasite attaches (Rodenburg *et al*., 2010).

#### *2.2.4.1 Pre-attachment mechanisms*

Pre-attachment resistance includes all mechanisms that allow sorghum to avoid or prevent *Striga* attachment. The mechanisms include; absence or reduced production of germination stimulants, interference with haustorial formation and development and mechanical barriers to infection (Timko & Scholes, 2013). Even when attachment does occur, it doesn't result in parasitism unless a functional haustorium is established.

According to Reda *et al*. (1994), low germination stimulant production is among the well-known resistance mechanisms that have been used for breeding sorghum against *Striga*. It has so far produced encouraging results (Haussmann *et al*., 2000a). This trait has also been

observed in some wild and cultivated varieties of sorghum (Rich *et al*., 2004) and is controlled by a single recessive gene with additive gene action (Volgler *et al*., 1996). Ejeta (2007) noticed low *Striga* germination in fields planted with genotypes characterized by low strigolactone production. This mechanism has since remained the only comprehensively used trait for breeding resistant varieties in sorghum (Mohamed *et al*., 2010b)**.** However, Olivier and Leroux (1992) suggested that low production of germination stimulant could only partly explain the resistance demonstrated by resistant sorghum varieties although the possibility of other unknown compounds inhibiting *Striga* germination could not be ruled out. Yoneyama *et al*. (2010) reported that the type of strigolactones secreted by resistant genotypes could be more crucial in *Striga* prevention rather than the overall amount of secreted stimulants. This may not be a solution to *Striga* control because some parasites can still respond to these other strigolactones.

Sorghum may also respond to *Striga* infection by inhibiting germination inspite of producing strigolactones. Weerasuriya *et al*. (1993) reported the presence of a germination inhibitor in some sorghum cultivars that hampered *Striga* germination although the inhibitor was never identified. Later on, Rich *et al*. (2004) observed inhibited *Striga* germination in some wild accessions of sorghum following low germination stimulant production and low haustorial initiation activity. *Striga* seeds exposed to these plants were not induced to germinate by low levels of xenognosin activity, suggesting that either the biosynthesis of xenognosin and germination stimulants are co-regulated or are inhibited by the same host factors. Mohammed *et al*. (2010a) also observed *Striga* germination inhibition in sorghum under EAGA experiment but the cause(s) of the inhibition was not established.

In some cases, *Striga* germination and attachment may take place but the seedling fails to form haustorium. Reda *et al*. (1994) confirmed that even if some sorghum varieties induce high *Striga* germination, few haustoria are formed. This strategy is important in *Striga* control because if germinated *Striga* doesn't form haustoria and connect to the sorghum root, no parasitism will take place. The seeds will exhaust their energy reserves and die shortly afterwards. The low haustorial initiation may be attributed to the host producing inhibitors (Rich *et al*., 2004) or low amounts of haustorial inducing factor (Gurney *et al*., 2003). Low production of haustorial initiation factors is said to be controlled by a single dominant gene (Ejeta, 2007).

Some resistant sorghum cultivars have demonstrated a hypersensitive response that is characterized by necrosis at the infection point (Mohamed *et al*., 2010a). This is a defence strategy because once the host cells are dead it becomes difficult for the parasite to attach since living cells are required.

Host crops can also avoid *Striga* infection by minimizing contact with parasitic seeds. It has been revealed that parasitic attachment can be stopped if contact between sorghum and *Striga* seeds is minimized during the formative stages of development (van Ast *et al.,* 2005). The ability of a deeper root system to delay infection or reduce *Striga* attachment host crops is well known (van Delft *et al*., 2000). Although not followed by significant increases in crop yields, a combination of deep sorghum planting, the use of pre-germinated seedlings and superficial soil cultivation can keep *Striga* parasitism to minimum levels, both in pot and field experiments (van Ast *et al*., 2005). When *Striga* sheds its mature seeds, they tend to remain on the upper soil layers, usually up to 5 cm deep, if the soil is not disturbed. If the soil is disturbed through deep tilling, the seeds get evenly distributed and move down the horizon up to a maximum of 15 cm (van Delft *et al*., 1997). The implication of this scenario therefore is that *Striga* infection can be reduced if (i) soil tillage is reduced, (ii) the crop is planted deeply and (iii) the crops develop a deeper root system. It is also possible that *Striga* seeds in deeper soil layers may take long to emerge, thus delaying maturation of the parasite.

*2.2.4.2 Post-attachment mechanisms*

Post attachment resistance mechanisms are employed by host plants after haustorial formation and as the parasite tries to penetrate host roots to form vascular connections. The mechanisms include; secretion of phytotoxic compounds such as phenolics and phytoalexins (abiosis), physical barriers to parasite penetration e.g lignification and suberization of cell walls and hypersensitive response (HR) causing programmed cell death (PCD) at the parasite infection points (Timko & Scholes, 2013).

Some cultivated varieties and wild accessions of sorghum have exhibited HR through death of *S. asiatica* haustorial cells before connecting to the host vascular tissues (Mohamed *et al*., 2003). Reduced nutrient flow to the haustorium or accumulation of phenolic compounds at the sites of infection has also been found to cause inhibition of haustorial development (Arnaud *et al*., 1998; 1999). Olivier *et al*. (1991) reported that sorghum accumulated phenolic compounds at the host-*Striga hermonthica* interface as a defence

strategy while Gurney *et al*. (2003) reported the presence of a compound released by a resistant wild relative of maize, *Tripsacum dactyloides,* which inhibited haustorial formation in *S. hermonthica.* This compound was speculated to have been taken up by the parasite together with water and assimilates and inhibited haustorial formation.

Plants can also employ post-attachement resistance responses soon after the parasite has established vascular connections with the host. Death of parasite tubercles is the main indicator of these strategies. This can happen either at the root cortex or endodermis where further parasite development will be hindered. Resistance to vascular penetration of *Striga* at the endodermis has been observed in sorghum (Maiti *et al*., 1984; Haussmann *et al*., 2004).

Toxins have been touted as another mechanism that resistant sorghum cultivars use against *Striga.* This mechanism is called abiosis, where compounds that are injurious to *Striga* haustorial development have been reported to be released by sorghum plants. For instance, pectone epitones (Arabinogalactan) proteins JIM5 and JIM7, hydroxyproline-rich glycoproteins (HRGP), lignins and phenolic compounds caused death of *Striga* cells at the point of infection on sorghum root cells (Neumann *et al*., 1999). Accumulation of a compound suspected to phenolic in nature were also observed by Arnaud *et al*. (1999) on resistant sorghum variety (*Framida*) conductive tissues which reduced parasite growth and belated emergence even after successful haustorial connection. It was suggested that a toxin may have contributed to this incompatibility although this was not verified. Abiosis has also been reported in *Tripsacum dactyloides* (Gurney *et al*., 2003) and rice (Gurney *et al*., 2006) parasitized by *S. hermonthica.*

## 2.3 Interaction between sorghum and plant growth promoting rhizobacteria

The amount of carbon and nitrogen-rich compounds released by plants contribute to a huge population of rhizosphere organisms, interacting either in a beneficial or deleterious manner (Choudhary &Johri, 2009). These organisms include bacteria (McLellan *et al*., 2007; Belimov & Wenzel, 2009; Karthikeyan *et al*., 2009).

### 2.3.1 Growth promotion

Crop yields are positively influenced by PGPR through a number of direct or indirect mechanisms (Ryu *et al*., 2005; Lugtenberg & Kamilova, 2009). Several PGPR strains play crucial roles in cell elongation, increasing ACC deaminase activity and plant growth promotion (Sgroy *et al*., 2009). *Bacillus* spp. has been found to increase yield in sorghum (Broadbent *et al*., 1977) and enhanced uptake of $NO^{3-}$, $K^{+}$, and $H_2PO_4$ followed by higher crop yields in sorghum inoculation with *Azospirillum brasilense* (Okon & Kapulnik, 1986). Besides fixing nitrogen, *A. brasilense* also increase crop yield through improved root development that leads to increased rates of water and mineral uptake (Okon *et al*., 1998). Other PGPR that have had positive growth effect on sorghum include; *Azoarcus* ssp. (Stein *et al*., 1997), and *Herbaspirillum* spp. (James *et al*., 1997). PGPR also produce metabolites such as lipo-chitooligosaccharides (LCOs) which stimulate sorghum growth (Dakora *et al*., 2002). Photosynthetic efficiency and chlorophyll content in *A. thaliana* has been shown to be improved by *B. subtilis* GB03 through the modulation of endogenous signaling of glucose and ABA sensing (Zhang *et al*., 2008). The same effect might be expected in sorghum because *A. thaliana* is a model plant. Recently, *Pantoea* sp., *Bacillus* sp., and *Pseudomonas* sp. have been found to increase germinability and seedling vigor of sorghum (Malleswari & Bagyanarayana, 2013) while foliar application of *Pseudomonas putida* and *Pseudomonas fluorescens* promoted growth and yield of forage sorghum (Afshar *et al*., 2011).

### 2.3.2 Disease and pest control

Different mechanisms have been used to control diseases and pests. Seed coating of some plants with PGPR strains have also led to ISR in treated plants against rhizosphere pathogens and parasites (van Loon *et al*., 1998; Ryu *et al*., 2004). *Bacillus* spp., *Brevibacterium laterosporus, Pseudomonas fluorescens* and *Serratia marcescens* are associated with ISR has been reported in sorghum against *Pythium ultimum*, the aetiological agent for root rot (Idris *et al*., 2008). It has been proposed that volatiles such as 2, 3-butanediol and acetoin (Ryu *et al*., 2003) and lipopeptide biosurfactants (Ongena *et al*., 2007) produced by *Bacillus* spp. can be involved in biocontrol.

## 2.4 Interaction between *Striga* and plant growth promoting rhizobacteria

It is well known that the rhizosphere is a complex system where microfauna and microflora interact with soil borne pathogens and influence the outcome of pathogen infection (Raaijmakers *et al*., 2009). Interaction between PGPR and *Striga* can occur at any of the stages of the parasite's life cycle, from germination to seed set. During this time, crucial exchange of signal molecules and biochemical cross-talk occur between PGPR and parasites (Cardoso *et al*., 2011). Parasitic weeds can be controlled either by preventing seed germination or enhancing germination in the absence of host plants, a phenomenon commonly referred to as inefficient germination (Rubiales & Fernández-Aparicio, 2012). In the following text, current knowledge on the effect of PGPR on *Striga* development in the absence of sorghum is reviewed. Three scenarios are expected when *Striga* seeds and PGPR interact. The first one is enhanced germination compared to seeds not in contact with PGPR. The second scenario is no change in germination. The third scenario is inhibited germination. This review will focus on the first and third scenario.

### 2.4.1 Promotion of *Striga* germination

The identification and application of bacteria in inducing *Striga* germination in the absence of sorghum plants has gained a lot of interest by many researchers in recent times. For instance, (Berner *et al*., 1999) and Ahonsi *et al*. (2002b) noticed that some strains of *Pseudomonas syringae* pathovar *glycinea* produced ethylene that highly stimulated *Striga* germination. This bacterium can be highly applicable in inducing ineffective germination of *Striga* seeds but its use in agriculture is limited because it causes disease to some crops. Most bacteria stimulate *Striga* germination through the action of ethylene (Babiker *et al*., 1993; Hassan *et al*., 2010a) and a procedure testing how ethylene-producing *P. syringae* pv. *glycinea* stimulates *Striga* germination has been developed (Berner *et al*., 1999). Other bacteria produce growth-regulators like auxins, cytokinins and gibberellins (GA) (Joel *et al*., 1995). GA is necessary in priming *Striga* seeds prior to germination thus reducing the preconditioning period (Joel *et al*., 1991) and promotes germination (Hsiao *et al*., 1988). Inhibition of GA biosynthesis, therefore, may inhibit seed preconditioning in soil (Joel, 2000). Although little is known about the action of IAA prior to *Striga* seed germination,

the hormone is critical in establishing the orientation of xylem differentiation between host and parasite (Bar-Nun *et al*., 2008).

### 2.4.2 Inhibition of *Striga* germination

Just as it has seen how some PGPR promote *Striga* germination, there are some PGPR that may inhibit *Striga* seed germination. For instance, an *in-vitro* experiment involving *Azospirillum* cells in the presence of GR24 and *Striga* seeds demonstrated unsuppressed germination but shortened radicles (Miche *et al*., 2000). This is among the few studies that have focused on the effect of PGPR on *Striga* germination inhibition at *in-vitro* level. The authors suggested that phytohormones especially IAA or lipophilic compounds released by the bacteria caused reduced germination, radical growth and cell differentiation. Keyes *et al*. (2000) reported that *Striga* seed germination can be inhibited by auxin-like compounds. Many rhizosphere inhabiting bacteria genera are known to produce IAA and auxin-related compounds e.g *Acetobacter, Agrobacterium, Arthobacter*, *Azospirillum, Azotobacter*, *Bacillus, Klebsiella, Pseudomonas* and *Xanthomonas* (Frankenberger & Arshad, 1995; Idris *et al*., 2007; Ghosh *et al*., 2008; Spaepen *et al*., 2008; Ali *et al*., 2009).

## 2.5 Interaction between sorghum, *Striga* and plant growth promoting rhizobacteria

Interaction between sorghum, *Striga* and PGPR is a life-long process for the three biological entities. It is for this reason that efforts that can stop germination and attachment are crucial since they will prevent the weed from progressing into the parasitic phase. Some strains inhibited *Striga* germination and promoted sorghum growth while others inhibited germination but no corresponding growth enhancement in the host crop (Bouillant *et al*., 1997).

Results from a study by Hassan *et al*. (2009) revealed that some bacterial isolates have both detrimental and positive effects on *Striga* on sorghum. This is evident from delayed and reduced *Striga* infection that was observed after *Pseudomonas putida*, *A. brasilense* and other isolates were inoculated on sorghum. Suppressive effects were more pronounced on tolerant and resistant sorghum crop cultivars, suggesting that a combination of genetic resistance and PGPR could be one of the best management options for *Striga.* There were huge reductions in *Striga* germination, haustorium initiation and attachment (Hassan *et al*.,

2011a). In another study conducted to test the effects of mycorrhiza fungi and plant growth promoting bacteria on *Striga* control in sorghum, *Striga* germination attachment and emergence were reduced considerably followed by enhanced crop performance (Hassan *et al*., 2011b). This confirmed results from previous studies by Gworgwor & Weber (1992) and Lendzemo (2004) which found out that AMF improves crop performance through increased P uptake and competition with *Striga* on the utilization of strigolactones. Table 2.1 provides a quick overview of some bacteria that have been found to influence *Striga* development, either positively or negatively.

## 2.6 Conclusion

This literature review attempts to draw together information and understand the tripartite interactions on *Striga*, sorghum and a spectrum of PGPR. Significant gaps in the current understanding of basic aspects of sorghum, *Striga* and PGPR relations have been identified. For instance, while the structural chemistry of strigolactones is known and its low production is genetically controlled, no explanation is available to help understand if there is activation of responsible genes under enhanced crop nutrition. The depressed uptake of C by *Striga* under high N fertilization has not been elucidated too. Contradictions still exist on the role of *Striga* in the photosynthetic process of their hosts. There is evidence that wild grass' (*Achnatherum hymenoides*) photosynthetic process is not affected by *Striga* infection while conventional sorghum cultivars are. Certainly more studies are required to ascertain if the difference is genetically induced.

While low germination stimulant production and low haustorial initiation activity remain key defence strategies by hosts under *Striga* threat, there is limited information on the cause(s) of the inhibition. Several inhibitors have been identified in *Orobanche*-infected plants but unfortunately, none in sorghum under *Striga* infection. Some authors also suggested that abiosis occurs in *Striga*-infected cereals. Unidentified phytotoxic substances, mainly produced in *in vitro* experiments are increasingly being suspected to be responsible for low *Striga* germination and radical elongation too. However, no experimental evidence has been provided to proof this mechanism nor any toxin identified. Since some toxins have been characterized in crops infected by *Orobanche* and considering the phylogenetic closeness of *Striga* and *Orobanche*, more studies are required to characterize these "toxic" compounds and elucidate their mode of action in *Striga*-sorghum associations. For instance, the role of lipophilic, phenolic and lipopeptide compounds on

germination, radicle elongation and haustorial initiation needs further investigation. These studies might also establish compounds that cause HR on the sorghum-*Striga* interface.

Other compounds which might be playing a role in the stimulation and inhibition of *Striga* germination, in addition to the possibility of breakdown or chemical modification of *Striga* germination elicitors by some rhizosphere-inhabiting organisms have been suggested, but not identified yet. Finally, there is limited information on *Striga* influence on PGPR, PGPR-induced disease control and growth promotion in sorghum.

**Table 2.1** Plant growth promoting bacteria influencing *Striga* development exposed to synthetic stimulants or sorghum root exudates

| Bacterium | Parasite/source of germination stimulant | PGPR action on parasite | Reference |
|---|---|---|---|
| a. Bacteria promoting *Striga* seed development | | | |
| *Pseudomonas syringae* pathovar *glycinea* (Psg) | *Striga hermonthica*, *S. aspera*, *S. gesnerioides* | Promotes germination | Berner *et al*.,1999 |
| *Bradyrhizobium japonicum* and *Pseudomonas syringae* | *S. hermonthica* | Promotes germination | Ahonsi *et al*., 2003 |
| *Enterobacter spp.* QUBC20 | GR24 | Promotes germination | Bargouthi *et al*., 2000 |
| *Klebsiella* spp | GR24 | Promotes germination | Frankenberger & Arshad, 1995. Hassan *et al*.,2010 |
| *Pseudomonas syringae* pv *glycinea* | *Striga* | Promotes germination | Ahonsi *et al*. (2002a) |
| *Serratia marcescens* QUBC6 | GR24 | Promotes germination | Bargouthi *et al*., 2000 |
| b. Bacteria hindering *Striga* seed development | | | |
| *Azospirillum brasilense.* | *S. hermonthica* | Inhibits germination | Bouillant *et al*., 1997 |
| *P. fluorescens* and *P. putida* | *S. hermonthica* | Inhibits germination | Ahonsi *et al*., 2002 |
| *A. brasilense* | *S. hermonthica* | Inhibits germination and radicle elongation | Miche *et al*., 2000 |
| *A. brasilense*, *Pseudomonas putida*, or combination of *A. amazonas* and *P. putida* | *S. hermonthica* | Inhibit germination, disrupt haustorium development and reduce emergence. | Hassan *et al*.,2009 |
| *A. brasilense; P. putida* and other isolates | *S. hermonthica* | Reduce germination, haustorium initiation and attachment | Hassan *et al*.,2011a |
| *Glomus* and *Paraglomus* spp. alone or with *Flavobacterium*, *Azotobacter* or *Bacillus* sp. | *S. hermonthica* | Reduce germination, attachment and emergence, delay emergence | Hassan *et al*., 2011b |
| Fluorescent *Pseudomonads* and *P. pudica* | | Reduce emergence | Ahonsi *et al*., 2002b |

Source: Lenard Mounde

# Chapter 3: Materials and Methods

## 3.1 Source of experimental materials

### 3.1.1 Sorghum and *Striga* seeds

*Sorghum bicolor* variety, Sorgho Malienne 335 (CSM 335) from Mali and *Striga hermonthica* seeds collected from a sorghum field in Nafadji, Mali in 2007 were used in this study. Sorgho Malienne 335 CSM 335 was selected for its known susceptibility to *Striga* infection (Haussmann *et al*., 2000b).

### 3.1.2 Bacteria strains

Four Plant Growth Promoting Rhizobacteria (PGPR) strains were used in this study. They were, three *Bacilli* (*B. subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42) and one *Burkholderia (Burkholderia phytofirmans* PsJN). *Bacillus* strains were obtained from the *Bacillus* Genetic Stock Center; Ohio State University, Ohio, USA where they are designated 3A35, 3A37 and 10A6 respectively, while *Burkholderia phytofirmans* PsJN was obtained from Austrian Institute of Technology, Austria.

## 3.2 Experimental preliminaries

### 3.2.1 Surface sterilization of *Striga* seeds

*Striga* seeds were surface sterilized according to the method described by Amusan *et al.* (2011) with minor modifications. Seeds were soaked in 75% ethanol for 2 min and then bathed in 1% sodium hypochlorite solution containing 3–5 drops of polyoxyethylene 20 sorbitan monolaurate (Tween® 20) for 5 min. This step was followed by 3 rinses in sterile distilled water with each rinse lasting 5 min. Seeds were then sonicated for 2 min with 25 ml glutaraldehyde (active ingredient glutaraldehyde, 2.5%, Carl Roth GmbH, Germany) to remove remaining particles adhering to their surfaces. After rinsing, seeds were poured into a methanol-sterilized nylon cloth (Φ100 μm) and rinsed 3 times with sterile distilled water. The nylon mesh allowed debris (<Φ100 μm) to pass, while clean seeds were left on the cloth. Seeds were air-dried in a laminar flow bench before use.

### 3.2.2 *Striga* seed preconditioning

Fiber glass filter paper (FGFP) discs (Whatman GF/A, Whatman International Ltd, England) measuring 9 mm in diameter were prepared using a cork borer. The discs were then heat-sterilized for 2 h at 150$^{o}$C in a dry heat oven (Heraeus Instruments, Germany). Two layers of 12 x 12cm heat-sterilized filter papers (Whatman GF/A9) were laid in a 12 x 12 cm sterile Petri dish moistened with 5.5 ml sterile deionized water. One hundred discs were placed on the filter paper in the Petri dishes (Plate 3.1). Approximately 200 surface sterilized *Striga* seeds were sprinkled on each disc in a laminar flow bench. The dishes were sealed with parafilm to prevent desiccation and recontamination. Aluminium foil was used to enclose the dishes to exclude light. The seeds were then preconditioned for 10 days in the dark at 30°C before use in relevant experiments.

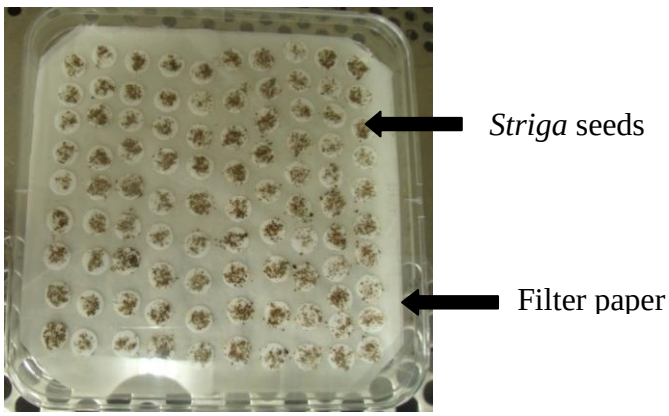


**Plate 3.1** *Striga* seeds on fiber glass discs during preconditioning (Photo by Lenard Mounde)

### 3.2.3 Sorghum seed surface sterilization and pre-germination

Sorghum seeds were surface sterilized and pre-germinated according to the method described by Amusan *et al*. (2011) with minor modifications. Seeds were soaked in 1% (w/v) sodium hypochlorite solution for 30 min. After this treatment, seeds were rinsed 3 times in sterile water before soaking overnight in 5% (w/v) Captan slurry (active ingredient: *N*-[trichloromethyl] thio-4-cyclohexene-1,1-dicarboimiide, 39%) (Sigma Aldrich GmbH, Germany), a non-systemic fungicide, before use. Sterilized seeds were pre-germinated on filter paper kept wet in sterilized Petri dishes at 30°C for 48 h before use in Petri dish bioassays. After protrusion of both radicle and plumule, seedlings for use in root

chamber experiments were kept for an extra day between two sheets of moist germination paper rolls (Rotilabo®-germ testing paper, Carl Roth GmbH; Germany) in a sterile glass beaker at 30°C to orient shoot and root growth in opposite direction. Thereafter, the seedlings were used in root chamber experiments.

### 3.2.4 *Striga* seed germination test

In order to test the germinability of *Striga* seeds for use in subsequent experiments, a germination test was conducted. Seeds preconditioned as described in 3.2.2 were used. Two pieces of sterile Whatman filter paper were placed in a sterile petri dish and moistened with 2 ml of sterile deionized water. FGFP (9 mm diameter) were placed on the moistened filter papers. Small amounts (50–100) of *Striga* seeds were aseptically dabbed on to the discs. Five discs representing different replications were put in one Petri dish (Plate 3.2).

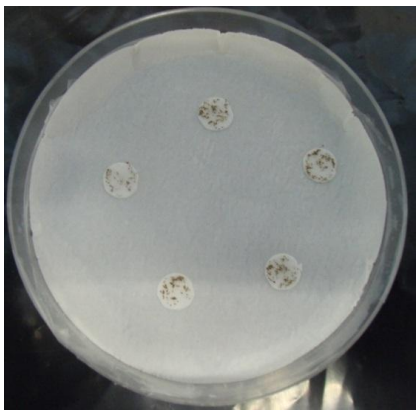

**Plate 3.2** Preconditioned *Striga* seeds on fiber glass discs used for germination test (Photo by Lenard Mounde)

100 µm of the germination stimulant (GR24) (Chiralix B.V, Nijmegen, The Netherlands) were added to the seeds. The Petri dish was sealed with parafilm and incubated in the dark at 30°C.

After 24–48 h, seeds on each disc were observed for germination using a binocular microscope (Axioplan, Carl Zeiss GmbH, Germany) fitted with a digital camera (Power Shot A640, Canon Inc., China). A *Striga* seed was considered to have germinated if it showed a protruded radicle through the seed coat (Prandi *et al*., 2011). A picture of the entire disc was taken. Total number of seeds (TS) and germinated seeds (GS) were counted

using the "Paint" counting tool for Windows Photo Viewer (Windows 7 Professional, Microsoft Corporation). Germination percentage (GP) was calculated using the following formula:

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

Mean germination percentage was obtained by calculating the average germination percentage from the five discs.

## 3.3 Experimental details

### 3.3.1 Determination of suitable concentration of bacteria media for sorghum germination and vigor enhancement

The objective of this experiment was to determine the most suitable Luria-Bertani (LB) liquid media concentration for suspending bacteria inocula to be used in subsequent sorghum germination experiments. The media evaluated was Luria Bertani (LB) media which was prepared to concentrations of 100%, 50% and 10% and used as experimental treatments. The ingredients of each concentration were as follows: 100% LB (g/liter): Trypton, 10.0; Yeast extract, 5; NaCl, 10.0, pH 6.9), 50 LB% (g/liter): Trypton, 5.0; Yeast extract, 2.5; NaCl, 5.0, pH 6.5), 10% LB (g/liter): Trypton, 1.0; Yeast extract, 0.5; NaCl, 1.0, pH 6.3). 1g/lt of NaCl was equivalent to 17.1 mM. The experiment was set up in an *in-vitro* germination bioassay in Petri dishes lined with sterile FGFP. Using sterile forceps, surface sterilized sorghum seeds were immersed for 30 min. in individual media. Control treatment seeds were immersed in sterile distilled water (SDW, pH 6.2), for 30 min. Fifteen seeds from each treatment were placed in Petri dish and moistened with 2 ml of individual media or SDW. Each treatment was replicated three times and experiment laid out in randomized complete design (RCD) in growth chamber (Percival Scientific Inc. USA) at 30°C. The experiment was repeated once.

Seven days after sowing, the number of seeds germinated per Petri dish was recorded and GP calculated using the formular described in experiment 3.2.4. To soften seedlings' tissues and allow them to be stretched to their full length without breaking, Petri dishes were then frozen at -4$^{o}$C before measuring seedling root and shoot length (≥1 mm) of all germinated seedlings per replicate using Fitomed (Castellano *et al*., 2001). Seed vigor

index (VI) was calculated by multiplying germination (%) and seedling length (mm) using the formular:

*Vigor Index = Seedling length (Mean root length+ Mean shoot length) X % germination*

(Abdul- Baki & Anderson, 1973).

### 3.3.2 Determination of suitable bacteria media concentration for *Striga* germination and radicle growth

The objective of the experiment was to test if same concentration of LB media described in experiment 3.3.1 could have an effect on *Striga* germination and radicle elongation. About 200 preconditioned surface sterilized *Striga* seeds were dabbed gently onto moist sterile FGFP discs prepared as described above and laid on two layers of filter paper, moistened with 2 ml of deionized sterile water inside Petri dishes. 100 µm of 100% LB, 50% LB; and 10% LB were added to the seeds. Control seeds received 100 µm sterile distilled water (SDW). The seeds were left for 5 min before pipetting 100 µm of 1ppm synthetic germination stimulant (GR24) on to them. The treatments were replicated three times and sealed with parafilm. The experiment was laid in RCD in a growth chamber at 30°C in darkness. The experiment was repeated once.

After five days, seeds on each disc were counted and germination percentage calculated as described in experiment 3.2.4. Radicles were observed by a binocular microscope (Zeiss Binokular Stemi 2000 C, Carl Zeiss GmbH, Germany). Five radicles which appeared longer than the other radicles were randomly selected and measured using a Zeiss AxioVision Rel. 4.8, SP1 imaging software (Carl Zeiss Microimaging GmbH, Germany).

The best performing LB media concentration in terms of sorghum germination and vigor as well as *Striga* germination and radicle elongation was selected for PGPR inocula and cell culture supernatant preparation.

### 3.3.3 Plant growth promoting rhizobacteria inocula and cell culture supernatant preparation

Three *Bacillus* strains (*B. subtilis* Bsn5, *B. subtilis* GBO3, and *B. amyloliquefaciens* FZB42) and *Burkholderia phytofirmans* PsJN were assessed for sorghum germination promotion and *Striga* germination inhibition activity. Stock cultures were stored at -80°C

in 30% glycerol. The cultures were activated by culturing them in LB agar media (g/liter): Trypton, 10.0; Yeast extract, 5; NaCl, 10.0; Agar, 15.0) for 48 h at 30$^{o}$C.

To prepare each PGPR liquid inocula and cell culture supernatants, two loops of individual bacteria were incubated in 100 ml of 10% LB liquid medium in a 250-ml Erlenmeyer flask at 30°C (considered favourable for the PGPR growth) for 48 h in a rotary shaker (150 rpm) as described by Ait Barka *et al*. (2000) and Compant *et al*. (2005) with minor modifications. The liquid culture was then centrifuged at 7,500 g for 10 min at 4$^{o}$C (Sorvall® RC-5B Refrigerated Superspeed Centriguge, Du Pont Instruments GmbH, Germany). The supernatant fraction was poured into sterile flasks and was either used directly or stored at -15°C in subsequent experiments.

The biomass fraction (bacteria cells) of each bacterium was suspended in 10% LB liquid media before adjusting the bacterial concentration through serial dilutions and spread agar plate counting following a method described by Micklos and Freyer (2003). A standard curve was developed by corresponding colony forming units (CFU) values in each dilution against the spectrophotometer (UV-1600PC-Spectrophotometer, VWR International bvba, Leuven, Belgium) value at 600 nm. This curve was used to estimate bacterial concentrations in subsequent experiments. Each bacterial inoculum concentration was adjusted to over $10^{8}$ CFU ml$^{-1}$ with 10% LB liquid medium. To maintain the efficacy of bacteria, innocula were used immediately in relevant experiments.

### 3.3.4 Effect of plant growth promoting rhizobacteria inocula on sorghum seed germination and vigor index

The experiment was set up in an *in-vitro* germination bioassay as described in experiment 3.3.1. The treatments in the experiment were bacteria inocula prepared as described in 3.3.3. They were: *B. subtilis* Bsn5, *B. amyloliquefaciens* FZB42, B. *subtilis* GBO3 and *Burkholderia phytofirmans* PsJN. Control treatment seeds were treated with SDW, and blank 10% LB liquid media. Each treatment was replicated three times and experiment laid out in RCD in growth chamber at 30°C. The experiment was repeated once.

### 3.3.5 Effect of plant growth promoting rhizobacteria supernatant on sorghum seed germination and vigor index

The experiment was set up as described in 3.3.4 but inocula were replaced with cell culture supernatants prepared as described in 3.3.3. These supernatant treatments were: *Bacillus subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 and *Burkholderia phytofirmans* PsJN. Seeds for the control treatments were immersed in SDW and 10% LB liquid medium. All the supernatants, LB and SDW were filtered through a 0.22 µm sterile filter (GE Infrastructure, UK) to exclude any bacterial contamination. Fifteen seeds were placed in each Petri dish lined with sterile FGFP and moistened with 1 ml of each PGPR cell culture supernatant, SDW or 10% LB liquid medium according to treatments. Each treatment was replicated three times and experiment laid in RCD in growth chamber at 30°C. The experiment was repeated once.

Seven days after sowing, germination percentage and vigor index data from experiment 3.3.4. and 3.3.5. were collected and calculated using procedures described in 3.3.1.

### 3.3.6 Effect of plant growth promoting rhizobacteria inocula on *Striga* seed germination and radicle elongation

About 200 preconditioned surface sterilized *Striga* seeds were dabbed gently onto moist sterile FGFP discs prepared as described above and laid on two layers of filter paper, moistened with 2 ml of deionized sterile water inside petri dishes. 100 µm of *Bacillus subtilis* Bsn5, *B. amyloliquefaciens* FZB42, *B. subtilis* GBO3 and *Burkholderia phytofirmans* PsJN inocula prepared as described in experiment 3.3.3 were added to the seeds. Control seeds received blank 10% LB and SDW. 100 µm of 1 ppm synthetic germination stimulant (GR24) were then pipetted on to the *Striga* seeds on each disc. 20 µm of Rifampicin antibiotic (Carl Roth GmbH, Germany) were added to control treatments to prevent bacterial infection. The treatments were replicated three times, sealed with parafilm and laid in RCD in a growth chamber at 30°C for 7 days in darkness. The experiment was repeated once.

### 3.3.7 Effect of plant growth promoting rhizobacteria supernatants on *Striga* seed germination and radicle elongation

The experiment was set up as described in 3.3.6 but inocula were replaced with cell culture supernatants prepared as described in 3.3.3. 100 µm of filter sterilized *Bacillus subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 and *Burkholderia phytofirmans* PsJN cell culture supernatants were added to the seeds. 10% LB liquid medium and SDW were the control treatments. The treatments were replicated three times and sealed with parafilm. The experiment was laid in RCD in a growth chamber at 30°C in darkness. The experiment was repeated once.

After five days, germination percentage and radicle length data from experiments 3.3.6. and 3.3.7.were collected using methods described in 3.2.4 and 3.3.2. An extended agar gel assay (EAGA) was conducted using similar PGPR inocula and cell culture supernatants but replacing GR24 with sorghum seedlings to test if a similar trend of results could be obtained.

### 3.3.8 Effect of plant growth promoting rhizobacteria inocula on *Striga* seed germination and radicle elongation in presence of sorghum seedlings

An extended agar gel assay (EAGA) described by Mohammed *et al*. (2010a) with minor modifications was used. Approximately 4,500 surface-sterilized preconditioned *Striga seeds* were pipetted aseptically into empty sterile Petri dishes (12 x 12 cm). This was an approximate equivalent of 3 drops of settled seed in a *Striga*-water mixture after vortexing. 1 ml of 1 ppm GR24 was added. Three ml of individual *Bacillus subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 and *Burkholderia phytofirmans* PsJN prepared as described in experiment 3.2.6 were added to the seeds and kept for 30 min in dishes. Control treatments were; 10% LB liquid medium and SDW. After cooling to about 50$^{o}$C, 30 ml of 0.7% agar solution (7 g in 1000 ml water) were poured into dishes and the solution shaken gently to allow even distribution of the seeds. The gel was then left to solidify before pre-germinated sorghum seeds were sown into each dish. A two-day old seedling of sorghum variety CSM 335, prepared as described in 3.2.3 was sown at the edge of the center of any of the four sides of each Petri dish (Plate 3.3). Treatments were replicated 3 times and arranged in RCD in a growth chamber at 30°C. The experiment was repeated once.

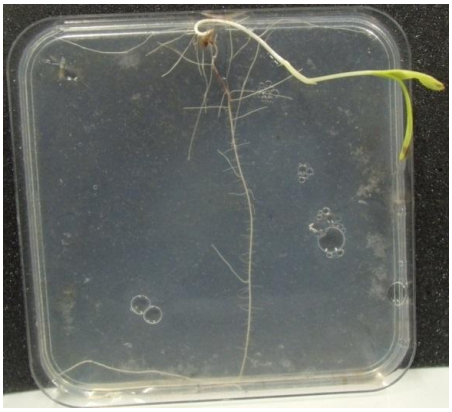

**Plate 3.3** Sorghum seedling growing on agar gel (Photo by Lenard Mounde)

### 3.3.9 Effect of plant growth promoting rhizobacteria supernatants on *Striga* seed germination and radicle elongation in presence of sorghum seedlings

The experimental set up was similar to the one described in experiment 3.3.8. The only difference was that *Bacillus subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 and *Burkholderia phytofirmans* PsJN supernatants prepared as described in 3.3.3 were used instead of bacteria inocula. Treatments were replicated 3 times and arranged in RCD in a growth chamber at 30°C. The experiment was repeated once.

After five days, sixteen (16) grids measuring 3 x 3 cm were made at the back side of the agar plate using a thick water-resistant marker pen (Plate 3.4) in both experiment 3.3.8 and 3.3.9.Three grids were selected, provided the sorghum root could be seen passing through the grid, to form a representative sample for the entire plate. A grid where a root was present increased the chances of *Striga* seeds having come into contact with sorghum root exudates. Total and germinated *Striga* seeds in each grid were counted with the aid of a binocular microscope and recorded.

After 7 days, seeds on selected grids were counted under a binocular microscope (Zeiss Binokular Stemi 2000 C, Carl Zeiss GmbH, Germany). Germination percentage and radicle length data from experiments 3.3.8 and 3.3.9 were collected using methods described in 3.2.4 and 3.3.2.

A cell culture supernatant producing the highest germination and radicle length inhibition was selected for isolation and identification of *Striga* germination and radicle length-inhibiting compounds.

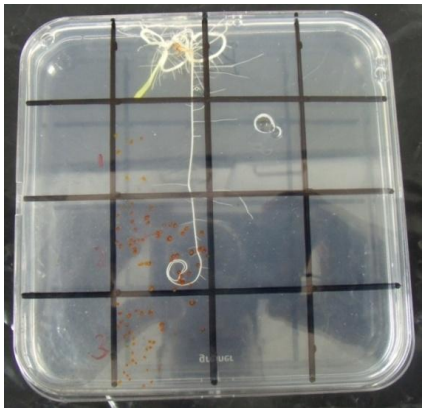

**Plate 3.4** Square grids at the back of extended agar gel assay Petri dishes (Photo by Lenard Mounde)

### 3.3.10 Determination of polarity of radicle elongation inhibition in *Bacillus subtilis* Bsn5 supernatant

The experiment was conducted with the aim of identifying the polarity of the inhibitors as a step towards isolating and identifying the compounds. Ethyl acetate (acetic acid ethyl ester; Carl Roth GmbH, Germany) was used to extract all compounds in *Bacillus subtilis* Bsn5 cell culture supernatant based on their solubility in the solvent.10 ml of *Bacillus subtilis* Bsn5 cell culture supernatant were mixed with equal volume of ethyl acetate in sterile 50 ml Falcon® tubes. The mixture was vortexed and let to settle for 10 min to allow separation into hydrophilic and hydrophilic fractions. The lipophilic fraction (ethyl acetate phase) settled on top and the hydrophilic fraction (water phase) at the bottom. Both fractions were then pipetted separately into sterile 50 ml Falcon® tubes before use.

A germination experiment was set up as described in 3.3.2 with the following treatments: *Bacillus subtilis* Bsn5 supernatant, ethyl acetate phase (1% and 100%), water phase (1% and 100%). The ethyl acetate phase was diluted with ethyl acetate while the water phase was diluted with SDW. The control treatments were SDW, 10% LB liquid media and 100% ethyl acetate. Three sterile FGFP discs (9 mm), which served as replicates, were laid on 90 mm sterile FGFP in Petri dishes. 100 µl of individual treatment solutions were pipetted on the discs in a laminar flow bench. The Petri dishes were left open for 1 h to let acetyl acetate evaporate from the discs since it is known to be toxic to the seeds. Thereafter, other discs containing preconditioned *Striga* seeds were placed on the treated discs before moistening with 2 ml of SDW. 65 µl of GR24 was added to each disc (Figure

3.1). The dishes were sealed with parafilm before incubation at 30°C for 5 days in the dark. The experiment was repeated once.

After five days, germination percentage and radicle length data were collected using methods described in experiments 3.2.4 and 3.3.2.

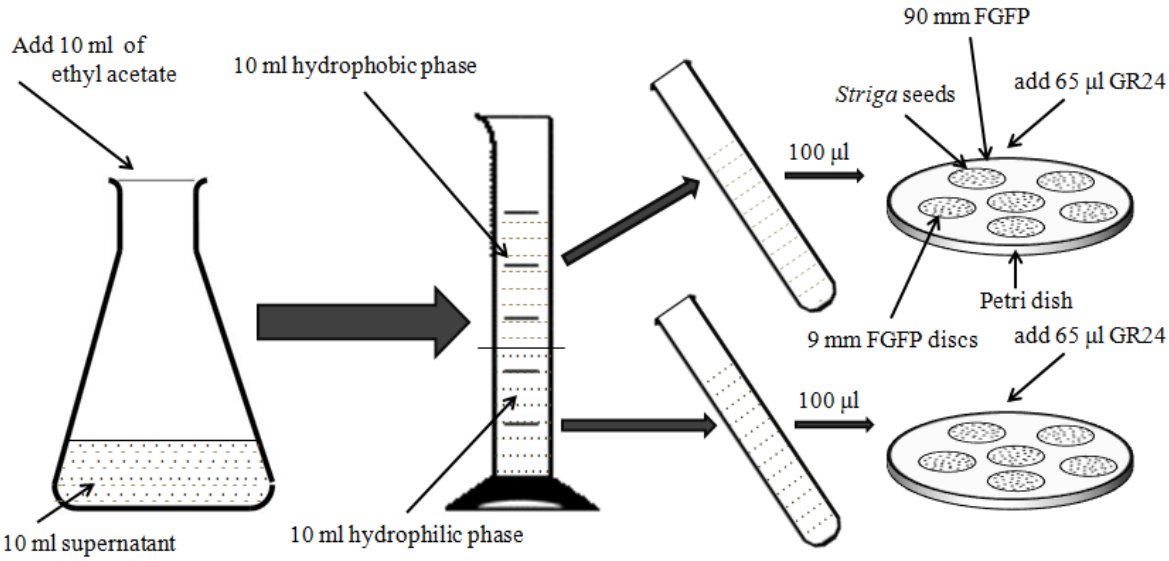


**Figure 3.1** Schematic diagrams showing the application of hydrophilic and hydrophobic fractions of *Bacillus subtilis* Bsn5 cell culture supernatant on *Striga* seeds

### 3.3.11 Determination of protein composition in *Bacillus subtilis* Bsn5 supernatant

The experiment was conducted by the Life Science Center, University of Hohenheim with the aim of identifying proteins which are produced by *Bacillus subtilis* Bsn5. Proteins (peptides) have been proposed as some of *Striga* germination and radicle elongation inhibitors (Dadon *et al*., 2004).

#### *3.3.11.1 Sample preparation*

*Bacillus subtilis* Bsn5 cell culture supernatant was filtered through a 0.22 µm sterile filter (Suppelco, Germany) and proteins were subsequently precipitated with acetone. Briefly, 20 ml cold acetone (-20°C) were added to 5 ml of cell culture supernatant and proteins were precipitated over night at -20°C. Samples were centrifuged at 14,000 rpm and supernatants

discarded thereafter. Protein pellets were dissolved in sodium dodecyl sulphate (SDS) sample buffer and applied to a 10 % Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Gel electrophoresis was stopped after proteins had migrated 2 cm into the separation gel. Proteins were visualized by colloidal Coomassie blue staining (Carl Roth GmbH, Germany).

*3.3.11.2 Mass spectrometry analysis*

Proteins were in-gel-digested using trypsin (Roche, Germany) according to Shevchenko *et al*. (1996). Tryptic peptides were purified using stage tips as described in Rappsilber *et al*. (2003). Nano-LC-ESI-MS/MS experiments were performed on an ACQUITY™ nano-UPLC system (Waters, USA) coupled to a LTQ-Orbitrap XL™ hybrid mass spectrometer (Thermo Fisher Scientific, Germany). Tryptic digests were concentrated on a precolumn (2 cm x 180 µm, Symmetry C18, 5 µm particle size, Waters, USA) and separated on a 25 cm x 75 µm BEH 130 C18 reversed phase column (1.7 µm particle size, Waters, USA). Gradient elution was performed from 1% ACN to 50% ACN in 0.1% formic acid (FA) within 90 min. The LTQ-Orbitrap was operated under the control of XCalibur 2.0.7 software. Survey spectra (m/z = 250-2000) were detected in the Orbitrap at a resolution of 60,000 at m/z = 400. Data dependent tandem mass spectra were generated for the seven most abundant peptide precursors in the linear ion trap. For all measurements using the Orbitrap detector, internal calibration was performed using lock-mass ions from ambient air as described in Olsen *et al*. (2005).

*3.3.11.3 MS data analysis*

Mascot 2.3 (Matrix Science, UK) was used as search engine for protein identification. Spectra were searched against the bacteria subset of the American National Center for Biotechnology Information (NCBI) protein sequence database downloaded as FASTA-formatted sequences fromftp://ftp.ncbi.nih.gov/blast/db/FASTA/nr.gz. Search parameters specified trypsin as cleaving enzyme allowing three missed cleavages, a 5 ppm mass tolerance for peptide precursors and 0.6 Da tolerance for fragment ions. Carbamido methylation of cysteine residues was set as fixed modification. Methionine oxidation was allowed as variable modification.

### 3.3.12 Effect of plant growth promoting rhizobacteria on sorghum growth

The experiment was carried out in root chambers under controlled conditions in a growth chamber. Root chambers are compartments (20 x 6 x 2 cm length, width and depth, respectively) having backs and sides made of PVC while the front is closed with transparent plexiglass allowing the observation of the host plant roots and the *Striga* underground stages. A strip (20 x 6 cm) of FGFP moistened with the benomyl solution was used to cover the plexiglass and placed in each chamber. The chambers were then filled with sterilized sand and moistened with SDW. One sorghum seedling (1-1.5 cm root length) was placed between the lid and the FGFP strip. Plate 3.5 shows the experimental set up in a growth chamber.

The seedlings were subjected to 8 bacterial treaments; four with *Striga* and an equal number without *Striga*. These treatments were: sorghum + *Striga* + *B. subtilis* Bsn5; sorghum + *Striga* + *B. subtilis* GBO3; sorghum + *Striga* + *B. amyloliquefaciens* FZB42; sorghum + *Striga* + *Burkholderia phytofirmans* PsJN; sorghum + *B. subtilis* Bsn5; sorghum + *B. subtilis* GBO3; sorghum + *B. amyloliquefaciens* FZB42 and sorghum + *Burkholderia phytofirmans* PsJN. Sorghum + 10% and sorghum + *Striga* were the control treatments. Each plant received 5ml of the prepared bacterial inoculum prepared as described in 3.2.6, blank 10% LB or SDW according to treatments.

A similar dose was given 2 weeks later. Each plant was fertilized with 20 ml of a multinutrient solution, prepared according to Pedas *et al*. (2005) (Table 3.1) at 5-day intervals until end of the experiment. Plants were regularly watered with SDW. Each treatment for plant height determination and leaf chlorophyll SPAD values were replicated twelve times while treatments for dry matter determination were replicated six times. The experiment was set in a RCD in a growth chamber at 25°C (12 h night) and 30°C (12 h day). Plant height and leaf chlorophyll SPAD determination experiment was repeated twice while dry matter measurement experiment was repeated once.

Sorghum plant height and leaf chlorophyll SPAD values were measured with the aid of a ruler and SPAD meter respectively at 7 day intervals for 28 days. Plant height was determined by measuring the length of each plant from the base of the stem to the ligule of the youngest fully expanded leaf according to methods described by Press and Stewart (1987). For each plant, SPAD measurements were taken at four locations on the youngest fully expanded leaf, two on each side of the midrib and then averaged using a SPAD-502

Chlorophyll Meter (Konica Minolta Sensing, Inc. Japan). Chlorophyll SPAD values are an accurate and non-destructive measurement of leaf chlorophyll concentrations (Ling *et al*., 2011). The values are proportional to the amount of chlorophyll present in the leaf through derived calibration curves.

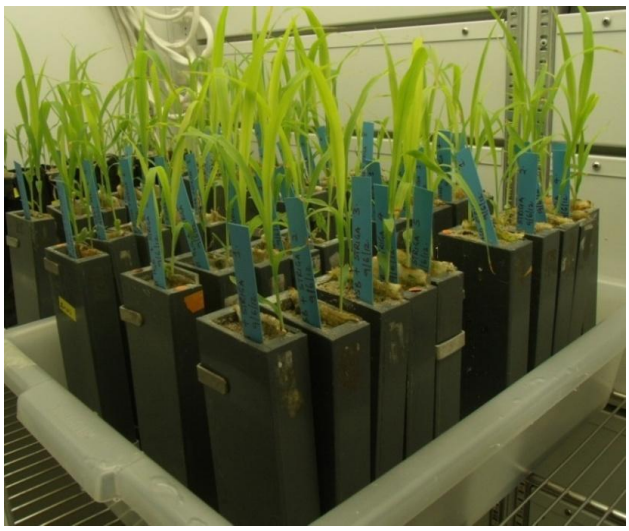

**Plate 3.5** Sorghum growing in root chambers in a growth chamber (Photo by Lenard Mounde)

After 28 days, plants were destructively harvested and dry weight calculated. Roots and shoots were separated, oven dried (Modell 700, Memmert GmbH, Germany) at 70$^{o}$C for 48 hrs before measuring the dry matter content. Shoot and root dry matter was summed up to give total dry matter content.

### 3.3.13 Effect of plant growth promoting rhizobacteria in *Striga* growth suppression

The experiment was done in root chambers under controlled conditions in a growth chamber as described in 3.3.12. Square grids measuring 3 x 3 cm were made on the transparent plexiglass with a water-insoluble marker pen. Three grids were then randomly selected along the host root for data collection. Total seeds (TS) in the selected grids were counted. Developmental stages of attached *Striga* on host roots were recorded at 7 day interval until the 28$^{th}$ day after sowing (DAS) using a binocular microscope. The stages of development were defined as GS, germinated seed; AS, attached seed; LT, live tubercles and DT, dead tubercles. GS and AS were expressed as percentages of the TS while LT and DT were expressed as a percentage of the AS.

**Table 3.1** Protocol for multi-nutrient solution used to fertilize plants in root chamber experiments

| Solution | Compounds per solution | Concentration (mol $L^{-1}$) | Mol-weight (g $mol^{-1}$) | Amount (g $L^{-1}$) | Amount (g ½L) |
|---|---|---|---|---|---|
| A | $KH_2PO_4$ | 0.20 | 136.09 | - | 13.61 |
| | $K_2SO_4$ | 0.20 | 174.27 | - | 17.43 |
| B | $MgSO_4 \cdot 7H_2O$ | 0.30 | 246.48 | - | 36.97 |
| | NaCl | 0.10 | 58.44 | - | 2.92 |
| N | $Mg(NO_3)_2 \cdot 6H_2O$ | 0.30 | 256.41 | - | 38.46 |
| | $Ca(NO_3)_2 \cdot 4H_2O$ | 0.90 | 236.15 | - | 106.27 |
| | $KNO_3$ | 0.60 | 101.11 | - | 30.34 |
| Iron | Fe(III)-EDTA-Na | 0.05 | 367.05 | - | 9.18 |
| Micro | $MnCl_2 \cdot 4H_2O$ | 0.0070 | 197.91 | 1.39 | - |
| | $ZnCl_2$ | 0.0007 | 136.28 | 0.10 | - |
| | $CuSO_4 \cdot 5H_2O$ | 0.0008 | 249.68 | 0.20 | - |
| | $H_3BO_3$ | 0.0020 | 61.83 | 0.12 | - |
| | $Na_2MoO_4 \cdot 2H_2O$ | 0.0008 | 241.95 | 0.19 | - |

**Note:** 1 ml from each solution were pooled together and dissolved in 1000 ml water

Source: Pedas *et al*. (2005)

### 3.3.14 Determination of phytohormone composition in bacterial cell culture supernatants

PGPR supernatants prepared as described in experiment 3.3.3 were used to investigate for the presence of phytohormones. This experiment was conducted by the Institute of Crop Physiology of Speciality Crops, University of Hohenheim. 25 ml of individual *B. subtilis* Bsn5, *B. subtilis* GBO3, and *B. amyloliquefaciens* FZB42 and *Burkholderia phytofirmans* PsJN supernatant were added to 0.1 M ammonium acetate. The concentration was adjusted to 0.01 M ammonium actetate ($p^H$ 7). The solutions were then purified using a combination of columns; Polyvinylpolypyrrolidone (PVP), DEAE Sephadex $^{TM}$ A-25 (Sigma Aldrich GmbH, Germany) and SepPark $C_{18}$ (Waters GmbH, Germany) as described by Jimenez *et al*. (2001). 10 ml of each supernatant dissolved in 0.01 M ammonium acetate were passed though PVP column to remove disturbing phenolics. 4 ml of the resultant solution were passed through a Sephadex anion exchanger column to bind acid hormones (ABA, IAA, GAs). Cytokinins were let to flow through to the next column, a conditioned SepPak $C_{18,}$ where the hormone was bound. Quantitative hormone analysis was performed on 1 ml of each sample in duplicates by Radio-Immuno-Assay (RIA) following a method described by Weiler (1980) and Bohner and Bangerth (1988) using polyclonal antibodies for Z/ZR (cytokinins), indoleacetic acid (IAA) and gibberellic acid (GA3). Given that GA3 antibody showed cross reactivity for GA1 and GA20, the results were presented as GAs.

## 3.4 Data analysis

Germination percentage, vigor index, radical length, attached seed, live and dead tubercle, hormone concentration data were analysed using generalized linear (GLM) models of SAS 9.3 for Windows statistical software (SAS Institute, Cary, USA) taking treatments as fixed effects while replications as random effects. Data from repeat trials in each experiment were combined after confirming homogeneity of variance before analysis. Non-normally distributed data were square root transformed before being subjected to ANOVA. Multiple comparisons among treatment means were calculated and mean separation executed through the Tukey's test at $p \leq 0.05$. A correlation analysis between individual hormones and germination percentage, vigor index, radicle length, attached seed, live and dead tubercle, was performed using correlation procedure (PROC CORR.) of SAS.

# Chapter 4: Results

## 4.1 Effect of Luria-Bertani media on sorghum germination and vigor

No significant differences ($p<0.05$) were detected on sorghum germination among different LB media concentrations (Table 4.1). Germination percentage in all treatments including SDW control ranged between 83% and 87%. Significant ($p<0.05$) differences were, however, detected on seedling vigor where an inverse relationship between media concentration and vigor was observed. The highest VI (>11000) was recorded in SDW and 10% LB treated seeds while the lowest (<4,000) in 50% and 100% LB treated seeds. There was a 71% and 80% vigor index reduction of seedlings exposed to 50% LB and 100% LB, respectively, compared to the seeds treated with 10% LB.

**Table 4.1** Effect of Luria-Bertani (LB) medium on sorghum germination and vigor in filter paper bioassays

| Treatment | Germination (%) | Vigor index (VI) |
|---|---|---|
| 10% Luria-Bertani + sorghum | 85.0 (9.2) a | 11,731.7 (103.0) a |
| 50% Luria-Bertani + sorghum | 86.7 (9.3) a | 3,356.7 (57.8) b |
| 100% Luria-Bertani + sorghum | 83.3 (9.1) a | 2,307.8 (47.4) b |
| Sterile distilled water + sorghum | 83.3 (9.1) a | 14,561.7 (119.5) a |
| Sample size (n) | 24 | 24 |
| Sample Mean. | 84.5 | 7989.4 |
| Std. Dev | 11.8 | 6724.8 |
| Pr>F | 0.9643 | ≤0.0001 |

Values are means of combined data of two trials with three replicates each. Means within each column followed by same letter are not statistically different at $p\leq0.05$ according to the Tukey-test. Values in parenthesis are square root transformed means.

## 4.2 *Striga* germination test

The average germination percentage was 71%.

## 4.3 Effect of Luria-Bertani media on *Striga* germination and radicle elongation

*Striga* germination and radicle lengths were significantly ($p<0.05$) greater in seeds treated with distilled water and 10% LB compared to 50% and 100% LB concentrations (Table 4.2). The greatest percentage of germination (over 60%) occurred in SDW and 10% LB

while the lowest (0%) in 50% and 100% LB concentrations. A similar trend was observed in radicle lengths where media concentrations of 10% LB and SDW produced higher radicle lengths compared to LB concentrations above 50%. Significant differences in radicle lengths were also observed among 10% media and SDW treatments where SDW induced higher lengths (3.5 mm) compared to 10% LB (1.1 mm).

10% LB, which showed the high sorghum vigor, sorghum germination and radicle elongation was selected for use in preparing liquid inocula and cell culture supernatants as described in 3.3.3 for subsequent experiments.

**Table 4.2** Effect of Luria-Bertani (LB) medium on *Striga* germination and radicle length in filter paper bioassays

| Treatment | Germination (%) | Radicle length (mm) |
|---|---|---|
| 10% Luria-Bertani + *Striga* + GR24 | 60.6 (7.8) a | 1.10 (1.0) c |
| 50% Luria-Bertani + *Striga* + GR24 | 0 (0) b | 0 (0) b |
| 100% Luria-Bertani + *Striga* + GR24 | 0 (0) b | 0 (0) b |
| Sterile distilled water + *Striga* + GR24 | 63.1 (7.9) a | 3.5 (1.9) a |
| Sample size (n) | 24 | 24 |
| Sample Mean. | 30.9 | 1.4 |
| Std. Dev | 31.9 | 1.4 |
| Pr>F | <0.0001 | <0.0001 |

Values are means of combined data of two trials with three replicates each. Means within each column followed by same letter are not statistically different at $p \leq 0.05$ according to the Tukey-test. Values in parenthesis are square root transformed means.

## 4.4 Effect of plant growth promoting rhizobacteria inocula on sorghum seed germination and vigor

The PGPR did not differ significantly in their effect on sorghum seed germination. Germination percentage in all PGPR inocula treatments and controls ranged between 86% and 96%. However, sorghum seedling vigor differed upon exposure to different PGPR inoculants. The highest VI (>18,000) was achieved in seeds inoculated with *Burkholderia phytofirmans* PsJN while the lowest (<11,000) was recorded in seeds inoculated with *Bacillus amyloliquefaciens* FZB42; *Bacillus subtilis* Bsn5 in 10% LB and 10% LB control medium. Results on sorghum seed germination and vigor index are summarized in (Table 4.3).

**Table 4.3** Effect of plant growth promoting rhizobacteria inocula on sorghum seed germination and vigor in filter paper bioassays

| Treatment | Germination (%) | Vigor Index (VI) |
|---|---|---|
| Sorghum + *Bacillus subtilis* Bsn5 | 86.7 (9.3) a | 10,101.3 (100.4) d |
| Sorghum + *Bacillus amyloliquefaciens* FZB42 | 92.2 (9.6) a | 10,424 (101.4) d |
| Sorghum + *Bacillus subtilis* GBO3 | 95.6 (9.8) a | 13,048 (113.8) c |
| Sorghum + *Burkholderia phytofirmans* PsJN | 93.3 (9.7) a | 18,215.7 (134.8) a |
| Sorghum + 10% Luria-Bertani | 88.9 (9.4) a | 10,528.2 (101.9) d |
| Sorghum + Sterile distilled water | 87.8 (9.4) a | 15,743.1 (125.1) b |
| Sample size (n) | 36 | 36 |
| Sample Mean. | 90.3 | 13,400.5 |
| Std. Dev | 7.8 | 3,671.8 |
| Pr>F | 0.2482 | <0.0001 |

Values are means of combined data of two trials with three replicates each. Means within each column followed by same letter are not statistically different at $p \leq 0.05$ according to the Tukey-test. Values in parenthesis are square root transformed means.

## 4.5 Effect of plant growth promoting rhizobacteria cell culture supernatants on sorghum seed germination and vigor

Different PGPR supernatants did not cause any variation on sorghum germination percentages but on seedling vigor (Table 4.4), *Burkholderia phytofirmans* PsJN showed the highest VI (>12,000) while and *B. subtilis* Bsn5 and *Bacillus amyloliquefaciens* FZB42 the lowest VI (<10,500). The effect of *Burkholderia phytofirmans* PsJN supernatant on vigor was, however, not different with *B. subtilis* GBO3 and control treatments.

## 4.6 Effect of plant growth promoting rhizobacteria inocula on *Striga* seed germination and radicle elongation

The germination percentage and radicle length of *Striga* as a function of PGPR treatments are presented in Table 4.5. There were significant differences ($p<0.05$) in the effect of PGPR inoculum on *Striga* germination compared with uninoculated controls. The highest germination percentage (>60%) were recorded in the SDW, followed by 10% LB (40%). Total germination inhibition (0% germination) occurred in all seeds exposed to bacteria suspended in 10% LB. Radicle elongation followed an almost similar pattern of inhibition like the one observed on germination. Highest radicle length inhibition (100% inhibition) occurred in seeds exposed to all PGPR treatments. The longest radicle lengths (3.5 mm) were recorded in SDW + GR24-treated controls.

**Table 4.4** Effect of plant growth promoting rhizobacteria cell culture supernatants on sorghum seed germination and vigor in filter paper bioassays

| Treatment | Germination (%) | Vigor Index (VI) |
|---|---|---|
| Sorghum + *Bacillus subtilis* Bsn5 | 90.0 (9.5) a | 10,241 (100.2) b |
| Sorghum + *Bacillus amyloliquefaciens* FZB42 | 88.9 (9.4) a | 10,125 (100.6) b |
| Sorghum + *Bacillus subtilis* GBO3 | 90.3 (9.5) a | 10,806 (103.9) ab |
| Sorghum + *Burkholderia phytofirmans* PsJN | 87.8 (9.4) a | 12,167.3 (110.1) a |
| Sorghum + 10% Luria-Bertani media | 96.7 (9.8) a | 11,381.9 (106.6) ab |
| Sorghm + Sterile distilled water | 92.2 (9.6)a | 11,488.4 (107.2) ab |
| Sample size (n) | 36 | 36 |
| Sample Mean | 91.0 | 11,035.0 |
| Std Dev | 7.9 | 1645.3 |
| Pr>F | 0.5566 | 0.0005 |

Values are means of combined data of two trials with three replicates each. Means within each column followed by same letter are not statistically different at $p \leq 0.05$ according to the Tukey-test. Values in parenthesis are square root transformed means.

**Table 4.5** Effect of plant growth promoting rhizobacteria inocula on *Striga* germination and radicle elongation in filter paper bioassays

| Treatment | Germination (%) | Radicle length (mm) |
|---|---|---|
| *Striga* + GR24 + *Bacillus subtilis* Bsn5 | 0 (0) c | 0 (0) c |
| *Striga* + GR24 + *Bacillus amyloliquefaciens* FZB42 | 0 (0) c | 0 (0) c |
| *Striga* + GR24 + *Bacillus subtilis* GBO3 | 0 (0 )c | 0 (0) c |
| *Striga* + GR24 + *Burkholderia phytofirmans* PsJN | 0 (0) c | 0 (0) c |
| *Striga* + GR24 + 10% Luria-Bertani | 40.0 ( 6.3) b | 1.1 (1.0) b |
| *Striga* + GR24 + Sterile distilled water | 63.1 (7.9) a | 3.5 (1.9) a |
| Sample size (n) | 36 | 36 |
| Sample Mean. | 17.0 | 0.8 |
| Std. Dev. | 25.4 | 1.31 |
| Pr>F | 0.0001 | 0.0001 |

Values are means of combined data of two trials with three replicates each. Means within each column followed by same letter are not statistically different at $p \leq 0.05$ according to the Tukey-test. Values in parenthesis are square root transformed means.

## 4.7 Effect of plant growth promoting rhizobacteria cell culture supernatants on *Striga* seed germination and radicle elongation

The effect of bacterial supernatants on the germination percentage and radicle length of seeds was significant (Table 4.6). The highest germination percentage were recorded in the positive controls exposed to GR24 only (66%) and 10% LB+ GR24 only (63%). These two percentages were not significantly different at $p<0.001$. The lowest germination (7.4 %) was observed in *Bacillus subtilis* Bsn5. In comparison with control treatment, germination was inhibited by 89% in *Bacillus subtilis* Bsn5, 67% in *B. subtilis* GBO3, 63% in *B.*

*amyloliquefaciens* FZB42 and 62% in *Burkholderia phytofirmans* PsJN. Significant differences were also observed on *Striga* seed radicle lengths under different bacterial supernatant treatments. *Bacillus subtilis* Bsn5 supernatant produced the lowest mean radicle lengths (0.12 mm). Radicle length were inhibited by 95% in *Bacillus subtilis* Bsn5, 91% in *B. amyloliquefaciens* FZB42 and 71% in both *Bacillus subtilis* GBO3 and *Burkholderia phytofirmans* PsJN compared to control.

**Table 4.6** Effect of plant growth promoting rhizobacteria cell culture supernatants on *Striga* germination and radicle length in filter paper bioassays

| Treatment | Germination (%) | Radicle length (mm) |
|---|---|---|
| *Striga* + GR24 + *Bacillus subtilis* Bsn5 | 7.4 (2.6) c | 0.1 (0.3) e |
| *Striga* + GR24 + *Bacillus amyloliquefaciens* FZB42 | 24.0 (4.8) b | 0.2 (0.4) d |
| *Striga* + GR24 + *Bacillus subtilis* GBO3 | 21.6 (4.6) b | 0.6 (0.8) c |
| *Striga* + GR24 + *Burkholderia phytofirmans* PsJN | 25.4 (5.0) b | 0.6 (0.8) c |
| *Striga* + GR24 + 10% Luria-Bertani | 63.1 (7.9) a | 1.2 (1.1) b |
| *Striga* + GR24 + Sterile distilled water | 66.4 (8.1) a | 2.2 (1.5) a |
| Sample size (n) | 36 | 36 |
| Mean | 34.7 | 0.8 |
| Std. Dev. | 23.1 | 0.7 |
| Pr>F | 0.0001 | 0.0001 |

Values are means of combined data of two trials with three replicates each. Means within each column followed by same letter are not statistically different at $p \leq 0.05$ according to the Tukey-test. Values in parenthesis are square root transformed means.

Generally, all supernatant-treated seeds germinated, but the radicles that emerged were shorter and thicker when compared to controls (Plate 4.1). Light microscopy of *Bacillus subtilis* Bsn5 supernatant-treated *Striga* radicles revealed a reduction in cell sizes at the radicle elongation zone compared to control treatments (Plate 4.2).

## 4.8 Effect of plant growth promoting rhizobacteria inocula on *Striga* seed germination and radicle elongation in presence of sorghum seedlings in extended agar gel assays

The germination percentage and radicle length of *Striga* as a function of PGPR inocula treatments are presented in Table 4.7. There was a significantly lower germination percentage (24%) accompanied by shorter radicle lengths (1.6 mm) of *Striga* seeds exposed to *Bacillus subtilis* Bsn5 compared to other PGPR and control treatments. The

highest germination percentage (60%) and radicle length (3.5 mm) was recorded in control seeds which were exposed to SDW + sorghum only.

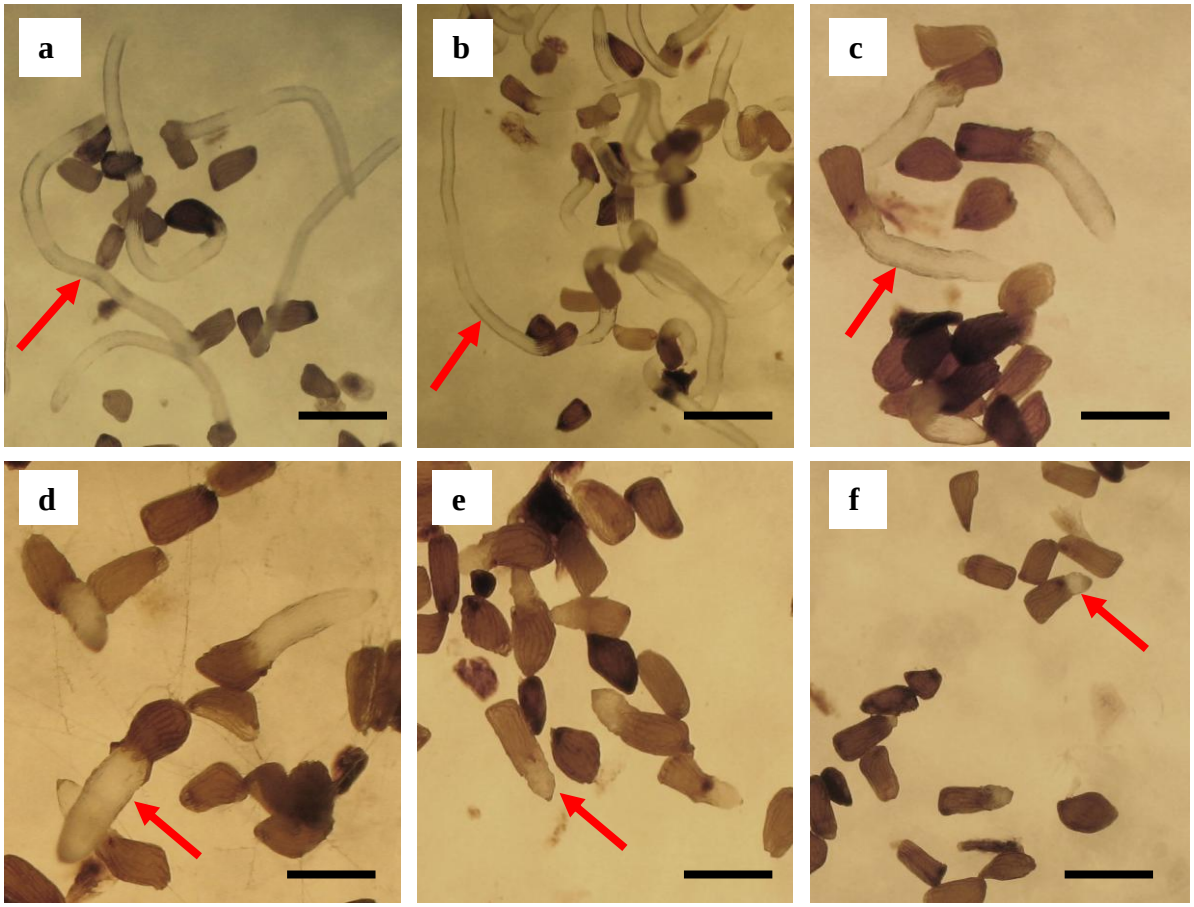


**Plate 4.1** *Striga* radicle length as influenced by plant growth promoting rhizobacterial supernatants in filter bioassays. Letters a, b, c, d, e and f represent treatments with 10% LB liquid medium, Sterile distilled water, *Burkholderia phytofirmans* PsJN, *Bacillus subtilis* GBO3 *Bacillus amyloliquefaciens* FZB42 and *Bacillus subtilis* Bsn5, respectively. The red arrows point to *Striga* radicles. Black bar = 500µm. (Photos by Lenard Mounde)

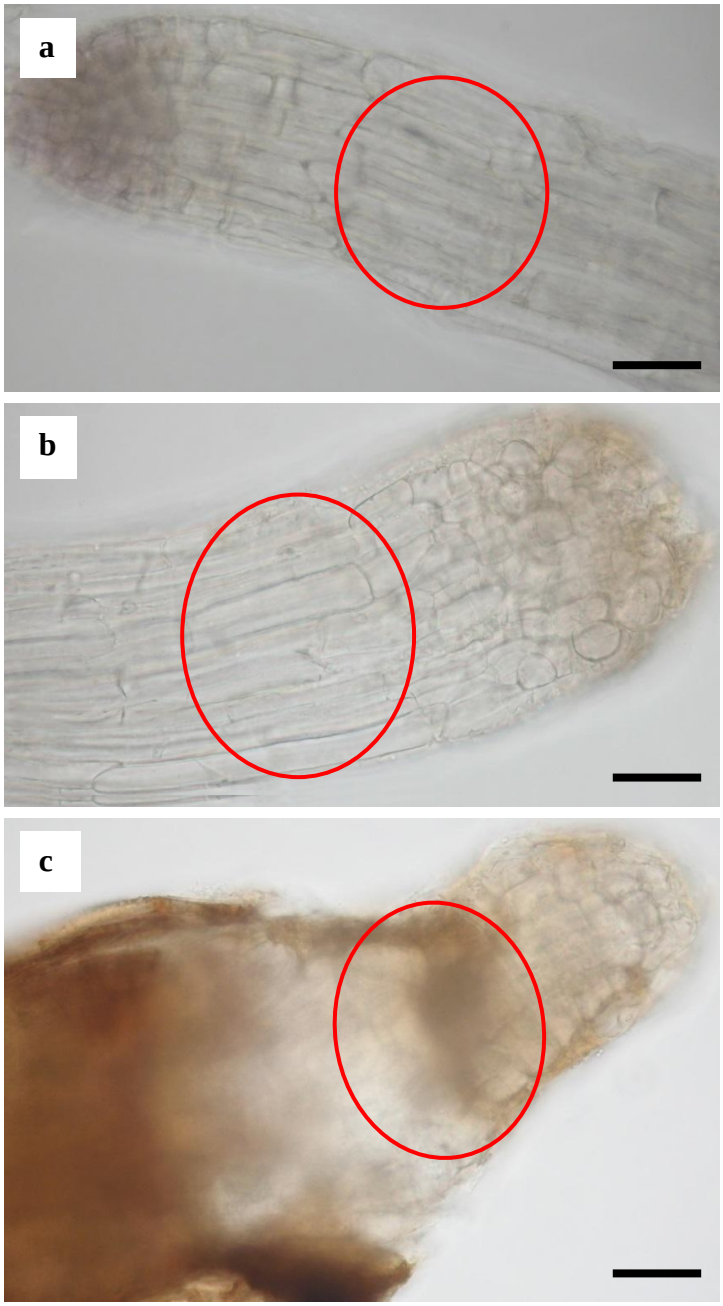


**Plate 4.2** *Striga* radicle morphology as influenced by plant growth promoting rhizobacterial supernatants in filter paper bioassays. Letters a, b and c represent treatments with Sterile distilled water, 10% LB liquid medium and *Bacillus subtilis* Bsn5, respectively. The red outlines show the zone of radicle elongation. Notice the elongated cells in a and b compared to the short ones in c. Black bar = 50µm. (Photos by Lenard Mounde)

**Table 4.7** Effect of plant growth promoting rhizobacteria inocula on *Striga* germination and radicle lengths in extended agar gel assays

| Treatment | Germination (%) | Radicle length (mm) |
|---|---|---|
| Sorghum + *Bacillus subtilis* Bsn5 + *Striga* | 23.9 (4.4) bc | 1.6 (1.3) b |
| Sorghum + *Bacillus amyloliquefaciens* FZB42 + *Striga* | 40.3 (6.2) ab | 2.7 (1.6) a |
| Sorghum + *Bacillus subtilis* GBO3 + *Striga* | 29.6 (5.2) b | 2.8 (1.7) a |
| Sorghum + *Burkholderia phytofirmans* PsJN + *Striga* | 34.8 (5.8) ab | 3.1 (1.8) a |
| Sorghum + 10% Luria–Bertani media + *Striga* | 39.0 (6.2) ab | 2.6 (1.6) a |
| Sorghum + Sterile distilled water + *Striga* | 59.9 (7.6) a | 3.5 (1.9) a |
| Sample size (n) | 36 | 36 |
| Sample Mean | 37.9 | 2.7 |
| Std. Dev. | 18.3 | 0.8 |
| Pr>F | 0.0345 | 0.0025 |

Values are means of combined data of two trials with three replicates each. Means within each column followed by same letter are not statistically different at $p \leq 0.05$ according to the Tukey-test. Values in parenthesis are square root transformed means.

## 4.9 Effect of plant growth promoting rhizobacteria supernatants on *Striga* seed germination and radicle elongation in presence of sorghum seedlings in extended agar gel assays

Effects of bacterial supernatants on the germination percentage and radicle length of *Striga* seeds were significant at $p<0.05$ (Table 4.8). The highest germination percentage (60%) was recorded in the controls exposed to sorghum only. The lowest germination (23%) was observed in *Bacillus subtilis* Bsn5 (24%) and *B. amyloliquefaciens* FZB42. Significant differences on radicle lengths under exposure to different bacterial supernatant treatments were observed too with LB treatment (5.2 mm) and *Bacillus subtilis* Bsn5 (2.5 mm) recording the highest and lowest lengths, respectively.

**Table 4.8** Effect of plant growth promoting rhizobacteria supernatants on *Striga* germination and radicle lengths in extended agar gel assays

| Treatment | Germination (%) | Radicle length (mm) |
|---|---|---|
| Sorghum + *Bacillus subtilis* Bsn5 + *Striga* | 23.9 (4.8) c | 2.8 (1.6) c |
| Sorghum + *Bacillus amyloliquefaciens* FZB42 + *Striga* | 23.5 (4.7) c | 2.5 (1.6) c |
| Sorghum + *Bacillus subtilis* GBO3 + *Striga* | 36.1 (5.7) bc | 4.2 (2.0) b |
| Sorghum + *Burkholderia phytofirmans* PsJN + *Striga* | 30.3 (5.5) bc | 4.1 (2.0) b |
| Sorghum + 10% Luria–Bertani media + *Striga* | 39.0 (6.2) b | 5.2 (2.3) a |
| Sorghum + Sterile distilled water + *Striga* | 59.9 (7.6) a | 4.8 (2.2) ab |
| Sample size (n) | 36 | 36 |
| Sample Mean | 35.4 | 3.9 |
| Std. Dev. | 17.3 | 1.3 |
| Pr>F | 0.0018 | <0.0001 |

Values are means of combined data of two trials with three replicates each. Means within each column followed by same letter are not statistically different at $p \leq 0.05$ according to the Tukey-test. Values in parenthesis are square root transformed means.

## 4.10 Effect of hydrophilic and hydrophobic fractions of *Bacillus subtilis* Bsn5 supernatant on *Striga* germination and radicle elongation

Significant differences (<0.05) were observed on the effect of different fractions of supernatant on *Striga* germination and radical elongation (Table 4.9). The highest germination percentage (63%) and radical length (2.9 mm) was observed in SDW control treatment. There was complete inhibition of seed germination after exposure to either unseparated Bsn5 supernatant or 100% water phase (hydrophilic fraction) of the supernatant. However, at 1% hydrophilic concentration, the inhibitory effect was reduced by almost 50%. The hydrophobic (ethyl acetate) fraction at both 100% and 1% concentration produced a germination percentage of >40 percent which was statistically similar to 10% LB and ethyl acetate controls.

**Table 4.9** Effect of different hydrophilic and hydrophobic fraction concentrations of *Bacillus subtilis* Bsn5 supernatant on *Striga* germination and radicle lengths in filter paper bioassays

| Treatment | Germination (%) | Radicle length (mm) |
|---|---|---|
| *Bacillus subtilis* Bsn5 supernatant + *Striga* + GR24 | 0 (0) e | 0 (0) e |
| 100% hydrophilic phase + *Striga* + GR24 | 0 (0) e | 0 (0) e |
| 1% hydrophilic phase + *Striga* + GR24 | 49.4 (7.0) b | 2.0 (1.4) d |
| 100% hydrophobic phase + *Striga* + GR24 | 41.0 (6.4) d | 2.5 (1.6) bc |
| 1% hydrophobic phase + *Striga* + GR24 | 45.6 (6.7) bc | 2.3 (1.5) cd |
| 10% Luria–Bertani media + *Striga* + GR24 | 42.5 (6.5) cd | 2.2(1.5) d |
| 100% Ethyl acetate + *Striga* + GR24 | 46.0 (6.8) bc | 2.6(1.6) ab |
| Sterile distilled water + *Striga* + GR24 | 63.0 (7.9) a | 2.9 (1.7) a |
| Sample size (n) | 36 | 36 |
| Sample Mean | 35.4 | 3.9 |
| Std. Dev. | 17.3 | 1.3 |
| Pr>F | 0.0018 | <0.0001 |

Values are means of combined data of two trials with three replicates each. Means within each column followed by same letter are not statistically different at $p \leq 0.05$ according to the Tukey-test. Values in parenthesis are square root transformed means.

## 4.11 Effect of hydrophilic fraction concentrations of *Bacillus subtilis* Bsn5 supernatant on *Striga* germination and radicle elongation

There were significant differences ($p<0.05$) in *Striga* germination and radicle length subjected to different concentrations of the hydrophilic phase of *Bacillus subtilis* Bsn5 (Table 4.10). No germination occurred on seeds treated with hydrophilic phases of ≥50% concentration. Among the treatments where germination occurred, 25% hydrophobic phase-treated seeds produced the lowest germination percentage (34%) while SDW yielded the highest (63%). Radicle lengths were also influenced by concentration of the hydrophilic phase. In hydrophilic treatments where *Striga* germination occurred, there was an inverse correlation between concentration and radicle lengths. There was 85% reduction of radicle length upon *Striga* seeds exposure to 25% hydrophilic phase (0.3 mm) in comparison to 1% hydrophilic phase (2.0 mm). SDW produced the longest radicles (2.9 mm).

**Table 4.10** Effect of *Bacillus subtilis* Bsn5 supernatant hydrophilic fraction concentration on *Striga* germination and radicle length in filter paper bioassays

| Treatment | Germination (%) | Radicle length (mm) |
|---|---|---|
| *Bacillus subtilis* Bsn5 supernatant + *Striga* + GR24 | 0 (0) e | 0 (0) d |
| 100% hydrophilic phase + *Striga* + GR24 | 0 (0) e | 0 (0) d |
| 75% hydrophilic phase + *Striga* + GR24 | 0 (0) e | 0 (0) d |
| 50% hydrophilic phase + *Striga* + GR24 | 0 (0 ) e | 0 (0) d |
| 25% hydrophilic phase + *Striga* + GR24 | 34.1 (5.8) d | 0.3 (0.6) c |
| 1% hydrophilic phase + *Striga* + GR24 | 49.4 (7.0) b | 2.0 (1.4) b |
| 10% Luria–Bertani media + *Striga* + GR24 | 42.5 (6.5) c | 2.2 (1.5) b |
| Sterile distilled water + *Striga* + GR24 | 63.0 (7.9) a | 2.9 (1.7) a |
| Sample size (n) | 48 | 48 |
| Sample Mean | 35.9 | 1.8 |
| Std. Dev. | 22.2 | 1.1 |
| Pr>F | <.0001 | <.0001 |

Values are means of combined data of two trials with three replicates each. Means within each column followed by same letter are not statistically different at $p \leq 0.05$ according to the Tukey-test. Values in parenthesis are square root transformed means.

## 4.12 Determination of protein composition in *Bacillus subtilis* Bsn5 cell culture supernatant

Twenty eight proteins were identified in *Bacillus subtilis* Bsn5 cell culture supernatant. Some of these proteins were also detected in other PGPR and the control 10% LB media. However, nine proteins, with their respective molecular weights, were identified to be produced by *Bacillus subtilis* only. They were; beta-1,3-1,4-glucanase (24 kDa), peptidase S8 (86 kDa ), N-acetylmuramoyl-L-alanine amidase (17 kDa); ChbA (22 kDa), peptidase G2 (87 kDa), serine protease (48 kDa); hypothetical protein(15 kDa); bacillopeptidase (155 kDa**)** spore germination protein Q (11 kDa).

## 4.13 Effect of plant growth promoting rhizobacteria inocula on sorghum growth in presence of *Striga* infection in root chamber trials

In the absence of PGPR inoculation, significant ($p<0.05$) differences in biomass content were realized between *Striga*-free and *Striga*-infected plants (Table 4.11). Total biomass in *Striga*- infected sorghum plants was 40% lower than non-infected plants. No significant

variation in sorghum chlorophyll content and plant height were measured between the two treatments.

Similarly, inoculated *Striga*-free plants showed significantly ($p<0.05$) higher plant heights, leaf chlorophyll SPAD values and total biomass (roots + shoots) compared to uninoculated *Striga*-free treatments. Roots of inoculated *Striga*-free plants were more branched, sturdier and developed more biomass than those of uninoculated *Striga*-infected treatments. *Bacillus amyloliquefaciens* FZB42, *B. subtilis* GBO3 and *Burkholderia phytofirmans* PsJN inoculated *Striga*-free sorghum showed a 75%; 142% and 158% increase in total biomass, respectively, compared to uninoculated *Striga*-free sorghum. However, there were no significant differences in total biomass observed between inoculated and uninoculated *Striga*-infected sorghum plants. Roots of inoculated *Striga*-free plants were more branched and sturdier than those of uninoculated *Striga*-infected treatments (Plate 4.3).

## 4.14 Effect of plant growth promoting rhizobacteria inocula on *Striga* development

Seed germination under different bacterial treatments were significantly different ($p<0.05$) (Table 4.12). The highest germination percentage (68%) was observed in control plants while the lowest was observed in plants inoculated with *B. amyloliquefaciens* FZB42 (54%) and *B. subtilis* GBO3 (50%). Percentage attachment was also significantly ($p<0.05$) different among treatments. *B. subtilis* GBO3 treated sorghum gave the lowest percentage (23%) compared to all other treatments. *Bacillus subtilis* Bsn5, *B. amyloliquefaciens* FZB42 and *Burkholderia phytofirmans* PsJN and control plants produced significantly higher parasite attachments. The number of successful attachments that developed into healthy tubercles was significant ($p<0.001$) among treatments. All the bacteria treatments recorded fewer healthy tubercle numbers with corresponding high tubercle death rates. At the end of the experiment, control plants recorded the highest tubercle survival rate (97%) of attached *Striga* compared with the average survival rate of 40–64% in PGPR treatments. Total number of *Striga* tubercle death in PGPR-treatments was in the range of 35 and 59% compared to <3% in control plants. Plate 4.4 show live and dead *Striga* tubercles on a sorghum root.

**Table 4.11** Effect of plant growth promoting rhizobacteria on sorghum shoot height, leaf chlorophyll SPAD values and total biomass dry weight after 30 days under controlled conditions in root chambers

| Treatment | Height (cm) | SPAD | RDW (g) | SDW(g) | TB (g) |
|---|---|---|---|---|---|
| Sorghum + LB | 24.6 bc | 23.1 d | 0.8 cd | 0.5 cd | 1.2 cd |
| Sorghum + *Striga* | 24.5 c | 22.9 d | 0.4 e | 0.3 e | 0.7 e |
| Sorghum + *Striga* + Bsn5 | 26.3 abc | 24.9 cd | 0.5 de | 0.3 e | 0.8 de |
| Sorghum + *Striga* + FZB42 | 28.9 a | 24.4 cd | 0.3 e | 0.3 e | 0.6 e |
| Sorghum + *Striga* +PsJN | 28.5 ab | 25.4 bcd | 0.5 de | 0.4 de | 0.9 de |
| Sorghum + *Striga* + GBO3 | 27.7 ab | 25.4 bcd | 0.4 e | 0.3 e | 0.7 e |
| Sorghum + Bsn5 | 28.8 ab | 27.9 ab | 0.9 bc | 0.6 c | 1.5 bc |
| Sorghum + GBO3 | 30.4 a | 26.5 abc | 1.7 a | 1.2 a | 2.9 a |
| Sorghum + PsJN | 29.8 a | 28.0 ab | 1.8 a | 1.3 a | 3.1 a |
| Sorghum + FZB42 | 28.9 a | 28.5 a | 1.3 ab | 0.8 b | 2.1 b |

Values are means of combined data of four trials with three replicates each for sorghum height and SPAD determination and two trials with three replicates each for root, shoot and total dry biomass determination. Means within each column followed by same letter are not statistically different at $p \leq 0.05$ according to the Tukey-test. LB = Luria-Bertani media; Bsn5 = *B. subtilis* Bsn5; FZB42 = *Bacillus amyloliquefaciens* FZB42; PsJN = *Burkholderia phytofirmans* PsJN; GBO3 = *Bacillus subtilis* GBO3; RDW = Root dry weight; SDW = Shoot dry weight; TB = Total biomass dry weight.

**Table 4.12** Effect of plant growth promoting rhizobacterial inocula on *Striga* germination and underground stages after 30 days under controlled conditions in root chambers

| Treatment | Germination (%) | Attachment (%) | Live tubercles (%) | Dead tubercles (%) |
|---|---|---|---|---|
| Sorghum + *Striga* | 67.9 a | 29.4 a | 97.0 a | 3.0 b |
| Sorghum + *Striga* + Bsn5 | 62.6 a | 34.8 a | 64.4 ab | 35.6 a |
| Sorghum + *Striga* + PsJN | 60.6 ab | 32.5 a | 55.3 b | 44.7 a |
| Sorghum + *Striga* + FZB42 | 54.4 b | 39.1 a | 40.9 b | 59.1 a |
| Sorghum + *Striga* + GBO3 | 49.7 b | 23.0 b | 54.9 b | 45.1 a |
| Sample number(n) | 45 | 45 | 45 | 45 |
| Sample Mean | 57.1 | 31.8 | 62.5 | 37.5 |
| Std. Deviation. | 13.9 | 11.5 | 40.9 | 40.9 |
| Pr>F | ≤0.05 | ≤0.05 | ≤0.001 | ≤0.005 |

Values are means of combined data of three trials with three replicates each. Means within each column followed by same letter are not statistically different at $p \leq 0.05$ according to the Tukey-test. Bsn5 = *B. subtilis* Bsn5; FZB42 = *Bacillus amyloliquefaciens* FZB42; PsJN = *Burkholderia phytofirmans* PsJN; GBO3 = *Bacillus subtilis* GBO3

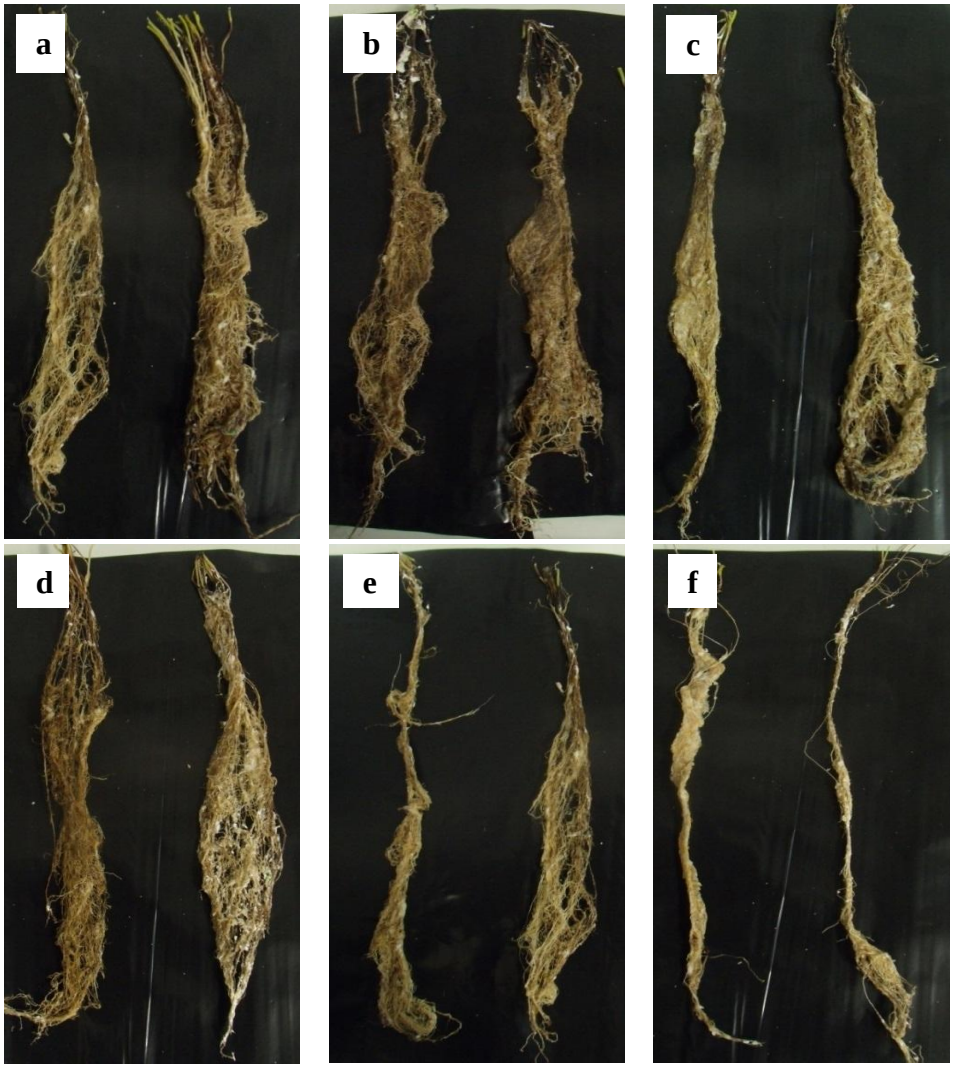


**Plate 4.3** Sorghum root development as influenced by plant growth promoting rhizobacterial inocula and *Striga* in root chamber trials. Letters a, b, c, d, e represent *Striga*-free treatments with *Burkholderia phytofirmans* PsJN, *Bacillus subtilis* GBO3, *B. amyloliquafaciens* FZB42, *Bacillus subtilis* Bsn5 and 10% LB liquid medium, respectively. Letter f represents uninoculated *Striga*- infested control (Photo by Lenard Mounde)

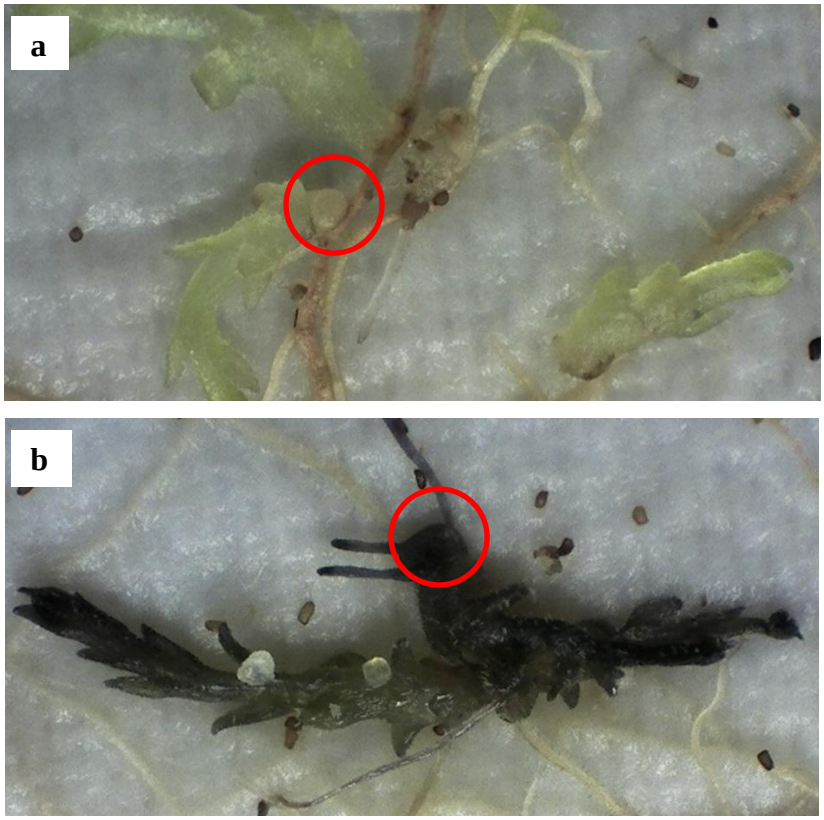


**Plate 4.4** *Striga* tubercles on sorghum roots under root chamber experimental set-up. Letters a and b represent live and dead tubercles in *Bacillus amyloquafaciens* FZB42-inoculated and noninoculated sorghum, respectively. The red outlines show the sorghum root-*Striga* interface (Photo by Lenard Mounde)

## 4.15 Determination of phytohormone composition in PGPR cell culture supernatants

All supernatants and the control (10% LB media) showed production of phytohormone cytokinins, IAA, GAs and ABA (Table 4.13). Individual hormonal quantities, however, differed with supernatants producing more cytokinins but less IAA in comparison with control media. GA and ABA quantities were not significantly different ($p<0.05$) among all treatments. There was a significant correlation ($r= -0.96$) between IAA and cytokinins. ABA and GA3 did not show significant correlation to either IAA or cytokinins. Sorghum plant height, SPAD values and biomass production did not show any significant correlation with all the phytohormones neither did *Striga* germination, attachment and tubercle death.

**Table 4.13** Phytohormone production by plant growth promoting rhizobacteria in cell culture supernatants after 48 h in 10% Luria-Bertani liquid media

| Treatment | Cyt. (ng/ml) | IAA (ng/ml) | GA (ng/ml) | ABA (ng/1ml) |
|---|---|---|---|---|
| Bsn5 | 0.2 ( 0.5) a | 1.4 (1.2) b | 0.3 (0.5) a | 0.03 (0.2) a |
| FZB42 | 0.2 (0.5) a | 1.5 (1.2) b | 0.1 (0.4) a | 0.04 (0.2) a |
| GBO3 | 0.2 (0.5) a | 1.7 (1.3) b | 0.2 (0.4) a | 0.07 (0.3) a |
| PsJN | 0.15 (0.4) b | 1.9 (1.4) ab | 0.2 (0.4) a | 0.03 (0.2) a |
| 10% LB | 0.1 (0.3) c | 2.6 (1.6) a | 0.2 (0.4) a | 0.03 (0.2) a |
| Sample number (n) | 10 | 10 | 10 | 10 |
| Sample Mean | 1.8 | 1.8 | 0.2 | 0.04 |
| Std. Deviation. | 0.05 | 0.5 | 0.1 | 0.02 |
| Pr>F | 0.0087 | 0.0381 | 0.3927 | 0.4266 |

Values are means of data from 1 trial with 2 replicates each. Values in parentheses are square root transformed means. Means within each column followed by same letter are not statistically different at $p \leq 0.05$ according to the Tukey-test. Bsn5 = *B. subtilis* Bsn5; FZB42 = *Bacillus amyloliquefaciens* FZB42; PsJN = *Burkholderia phytofirmans* PsJN; GBO3 = *Bacillus subtilis* GBO3; Cyt. =Cytokinins; IAA=Indole acetic acid; GA=Gibberellins; ABA= Abscisic acid; ng /ml= nanograms per milliter.

# Chapter 5: General discussion and conclusion

The growth stimulating effect on sorghum and suppressive effect on *Striga* by a wide range of plant growth promoting rhizobacteria (PGPR) has been elaborately described in Chapter 2. The review presents a state-of-the-art knowledge on two- and three-way interactions involving sorghum roots, *Striga* and PGPR. However, the understanding of these interactions is still incomplete due to the difficulty of studying underground processes under controlled conditions. This was evident from the research gaps identified. Thus, developing novel methodologies to study rhizosphere interactions under both *in-vitro* and natural conditions was needed. It was proposed that Extended Agar Gel Assay (EAGA) and root chamber experiments as described by Mohamed *et al.* (2010a) and Linke *et al.* (2001), respectively, incorporating *Striga*, sorghum and PGPR would provide some understanding on the three-way interaction. The main focus was to investigate if successful colonization of sorghum roots by the selected PGPR takes place and if there are any effects on *Striga* infections. Finally, synthesis of the knowledge of these inter-relationships would potentially lead to the screening of PGPR colonizers of sorghum roots and device ways of delivering them to sorghum rhizosphere in soils where they are lacking.

The objective of this study was to evaluate the role of *Burkholderia phytofirmans* PsJN; *Bacillus subtilis* Bsn5; *B. subtilis* GBO3 and *B. amyloliquefaciens* FZB42 on sorghum and *Striga* growth under controlled conditions. To meet this objective, preliminary experiments to identify a suitable media for delivering bacteria to sorghum rhizosphere and to assess their effect on sorghum growth and *Striga* suppression were conducted. The selected media needed to meet two conditions; i) able to support the growth of bacterial strains and ii) have insignificant effect on germination of both *S. hermonthica* and sorghum seed. In this regard, Luria Bertani (LB) liquid media, commonly used in culturing *Bacillus* spp. and *Burkholderia* spp. in different concentrations was evaluated for their effect on sorghum germination and seedling vigor as well as *Striga* germination and radicle elongation. The ingredients of undiluted commercial media were; trypton (10 g $l^{-1}$), yeast extract (5 g $l^{-1}$) and NaCl (170 mM) (Carl Roth GmbH, Germany).

The study revealed that LB liquid media containing ≥85 mM NaCl, 5 g $l^{-1}$ trypton and 2.5 g $l^{-1}$ yeast extract had no significant effect on sorghum seed germination but decreased seedling vigor compared to 17 mM NaCl and sterile distilled water treatments. The observed differences in sorghum seedling vigor are attributed to seedling length; seedling

elongation as being more sensitive to salt stress than germination (Abdul-Baki & Anderson, 1973). There was no evidence from any published literature stating that trypton and yeast extract have any influence on sorghum seedling vigor hence NaCl concentration remained the only factor causing variation in sorghum seedling length. Ionic and osmotic stresses on plants growing in saline conditions are known to cause reduction in water uptake followed by root growth inhibition (Munns, 1993). In this study, there is a clear indication that water imbibed by sorghum seeds exposed to ≥ 85 mM NaCl concentration was enough to induce germination but hardly enough to sustain further seedling growth. These results showed that sorghum seeds can tolerate higher salt concentrations to germinate than to sustain root growth. Previous studies have shown that germination can occur under saline stress conditions provided the moisture content can allow seeds to achieve germination but root elongation is inhibited under the same stress (Meyer and Boyer, 1981). Resultsfrom this study are consistent with those of Patanè *et al.* (2009) who reported that sorghum seeds treated with >50 mM NaCl exhibited germination percentages that were not significantly different from those seeds treated with distilled water, but root length reduced by 30%. Additional studies have cited loss of cell turgidity attributed to salinity stress as one of the causes of reduced root elongation in sorghum (Gill *et al.*, 2003). Lin and Kao (2000) also observed that increasing concentrations of NaCl from 50 to 150 mM decreased root growth in rice (*Oryza sativa*) following the elevation of hydrogen peroxide ($H_2O_2$) levels in roots (Lin & Kao, 2001). $H_2O_2$ is known to damage DNA, protein, and membrane functions in plant cells (Patanè *et al.*, 2009) thus leading to reduced seedling growth.

This study further demonstrated that exposure of *Striga* seeds to ≥85 mM NaCl, 5g $l^{-1}$ trypton and 2.5 g $l^{-1}$ yeast inhibited germination by 100%. Similar to the sorghum bioassay results discussed already, the inverse relationship between *Striga* germination and same LB media concentrations can be explained by the composition of media. Ahonsi *et al.* (2002b) reported that *Striga* seeds preconditioned in 0.1% yeast extract produced the same level of germination as distilled water when both treatments were exposed to GR24. On the other hand, there is no published work that has linked trypton with *Striga* germination inhibition. Therefore, NaCl was the remaining factor that we associated with the observed variation in *Striga* seed germination. It is known that germination of parasitic weeds is affected by temperature and salinity (Kebreab & Murdoch, 1999; Hassan *et al.*, 2010b). However, temperature cannot be considered as a limiting factor in our study because it was uniform

in all treatments. After revealing the adverse effect of elevated (≥85 mM) NaCl concentration on sorghum, it was expected that the same inhibitory effect will apply to *Striga* seeds as well. Salinity stress has also impacted negatively on *Orobanche* seed germination in previous studies and given that *Striga* and *Orobanche* are phylogenetically related, it would be expected that the same inhibition takes place in *Striga*. Therefore, it was concluded that water imbibitions at ≥85 mM NaCl levels were responsible for the observed lack of germination in *Striga*. Abu-Irmaileh (1998) had reported that salinity effect caused by 77 mM NaCl was responsible for lack of germination in *Orobanche ramosa* seeds. Al-Khateeb *et al.* (2005) also observed complete lack of *Orobanche ramosa* attachment and emergence in tomato irrigated with water containing 75 mM NaCl. At 10% LB concentration, germination percentage similar to that induced by SDW indicated that salinity levels in 10% LB medium had insignificant detrimental effects on sorghum and *Striga* seeds. Although 10% LB reduced *Striga* germination by almost 20% in some experiments, this effect was insignificant to the reduction that was observed in >50% LB concentration. Based on these results, 10% LB liquid medium was found to be the most suitable media for use in PGPR inocula and cell culture supernatant preparation in subsequent experiments.

Furthermore, this study found that *Burkholderia phytofirmans* PsJN-treated sorghum seeds grew more vigorously compared to other *Bacillus* strains and controls. These findings were consistent with the expectations and are in agreement with a previous report by Kloepper *et al.* (1988). Seedling length was identified as the main indicator of differences in sorghum seedling vigor especially as germination percentages did not differ among treatments. It is known that during seed germination, ethylene is produced by many plant species but high concentrations of ethylene after germination can inhibit root elongation as reported for canola *(Brassica napus* L.) (Glick *et al.*, 1995; Hall *et al*., 1996) and mung bean *(Vigna radiata)* (Mayak *et al*., 1999). Results from the present study could be explained by the ability of *Burkholderia phytofirmans* PsJN to produce ACC deaminase which can neutralize the inhibitory effect of ethylene on sorghum root elongation (Compant *et al*., 2005; Ait Barka *et al*., 2002). The *Bacillus* strains evaluated here did not enhance vigor and this could be explained by their inability to produce the ACC enzyme. This concur with previous studies which have reported that ACC deaminase promotes root elongation in canola (Sun *et al.*, 2009), tomato (Mayak *et al*., 2004) and maize (Shaharoona *et al*., 2006).

This study further revealed that after 30 days in a growth chamber, sorghum plants that were not infected with *Striga* had improved total biomass yield compared to uninoculated sorghum plants. These results were attributed to enhanced plant growth promoting hormone levels triggered by PGPR inoculation. Cytokinins, IAA and GA detected in cell culture supernatants concur with the findings of Idris *et al*. (2009) who observed improved sorghum growth occasioned by IAA in growth media. Moreover, a synergistic effect between cytokinins and IAA contributing to plant growth promotion has also been observed. For instance, Hussain and Hasnain (2011) reported an increase in wheat (*Triticum aestivum*) growth following improvement of the plant's IAA and cytokinins pool by PGPR. Additionally, application of exogenous cytokinins has been found to increase plant height, NPK uptake and total biomass in rice (Zahir *et al.*, 2001). Cytokinins are known to boost chlorophyll production which is an indication of sorghum plants' improved capacity to fix carbon hence the increase in biomass observed in *Striga*-free sorghum plants. Ling *et al.* (2011) showed that healthy and actively metabolizing plants produced more chlorophyll to meet carbon demands for the plant. The results obtained in this study were expected because *Burkholderia phytofirmans* PsJN has been associated with improved chlorophyll content of plants such as potatoes (Nowak *et al.*, 1997) and *Arabidopsis* (Zhang *et al.*, 2007). Furthermore, in a previous study by Kim *et al.* (2012), *Burkholderia phytofirmans* PsJN-inoculated switchgrass (*Panicum virgatum* L.) cv. *Alamo* plants had significantly higher chlorophyll content coupled with increased biomass compared to controls after 30 days in a growth chamber. The authors concluded that *Burkholderia phytofirmans* PsJN's capacity to produce ACC deaminase activity which degraded ACC was the cause of the observed growth promotion in inoculated switchgrass plants. Moreover, the strain produces a 15-fold ACC deaminase activity that is required to promote sorghum growth (Sessitsch *et al*., 2005).

*Striga* has remained a major constraint to cereal production in regions where the parasite is prevalent. The parasite is known to be a strong sink for water and assimilates, besides interfering with photosynthetic activities of the host crop (Graves 1995; Press *et al.* 1987b; Graves *et al.*, 1989). Details of detrimental impact of *Striga* on sorghum are found in Chapter 2. This underscores the need for a lasting solution to the *Striga* menace. One of the hypotheses of this study was that inoculating *Striga* infected sorghum can alleviate the damaging effects of *Striga* and improve sorghum growth. However, contrary to this hypothesis, there was no difference between inoculated *Striga*-infected and uninoculated

*Striga*-infected sorghum plants with regard to total biomass production. This implies that the PGPR-generated growth promoting phytohormones did not offer any significant benefit to *Striga* infected plants. It is known that *Striga* reduces the levels of IAA (Press *et al*., 1999) and reduces cytokinin production and its export from the roots to the leaves due to the withdrawal of resources, especially N, from host plants (Van der Werf & Nagel, 1996). This could explain the observed poor growth of inoculated *Striga*-infected compared to inoculated *Striga* free sorghum.

A key stage in *Striga* development that has been a target for controlling the parasite is germination (Watson, 2013). In this study, filter paper germination bioassays demonstrated that PGPR can cause total inhibition (0% germination) of *Striga* germination. *Bacillus subtilis* Bsn5 induced a higher inhibition of *Striga* germination and radicle elongation compared to other PGPR and controls in both EAGA and filter paper germination bioassays. Although the exact inhibitor(s) were not identified in this study, hydrophilic compounds present in the *Bacillus subtilis* Bsn5 cell culture supernatant were associated with this inhibition. An inverse relationship between the concentration of the hydrophilic fraction and *Striga* radicle elongation can be explained by a decline in efficacy of the inhibitors due to dilution. A previous study by Dadon *et al.* (2004) on *Orobanche aegyptica* germination and radicle inhibition reported that peptides produced by *Azospirillum brasilence* and that could not be extracted by ethyl acetate were the cause of *Orobanche* germination inhibition. Thereafter, Nun *et al.* (2005) suggested that peptides could be competing with strigolactones over binding sites leading to low germination of *Orobanche*, but their theory is yet to be proved. Since *Orobanche* and *Striga* share many similarities in their response to germination stimulants (Bouwmeester *et al.*, 2003), and inhibitors (Matusova *et al.*, 2005), it is suggested that either one or a combination of the peptides identified in *Bacillus subtilis* Bsn5 cell culture supernatant could be responsible for *Striga* seed germination and radicle length inhibition in our study. This argument is strengthened by the findings of Leclère *et al.* (2005) who showed that *Bacillus subtilis* produces peptides and Kakinuma *et al.* (1969) who stated that some peptides produced by the bacterium are hydrophilic.

In a review of the three-way interaction between sorghum, *Striga* and PGPR in Chapter 2, the beneficial effects of PGPR on sorghum growth and their harmful effects to *Striga* were shown. Results from controlled experiments conducted in this study in roots chambers depict the ability of *B. subtilis* GBO3 and B. *amyloliquefaciens* FZB42 to suppress *Striga*

through reduced germination compared to other PGPR and control treatments. Improved sorghum growth has been attributed to the action of phytohormones. Therefore, results from this study were expected because *B. subtilis* GBO3 and B. *amyloliquefaciens* FZB42 are known to produce IAA (Idris *et al*., 2007) which would have resulted in the inhibited germination. It is also reported that *Bacillus subtilis*-induced inhibition of *Striga* seeds in *in-vitro* bioassays. However, the inhibitory effect of *Bacillus subtilis* appeared not to be effective in root chamber experiments. The fact that there was *Striga* germination inhibition in all PGPR treatments in our bioassays supports the argument that involvement of IAA in this inhibition was possible. It is also possible that the inhibitors were not as effective in root chambers as in bioassays because of change in experimental conditions. This has been a common challenge in biological control systems where a control agent can be effective under *in-vitro* conditions but fail to produce the same effect in a different set up e.g root chamber, green house or field experiments.

For *Striga* to transit from its independent to parasite phase in host roots, attachment, haustorial development and formation of tubercles are critical. In Chapter 2, we reviewed a wide range of resistance mechanisms that host plants employ when faced with the *Striga* challenge. Some of these mechanisms arise as a result of host plants being manipulated by external factors. One of such factors would be the action of PGPR. The high tubercle deaths observed in all PGPR-treated plants is a manifestation that inoculated sorghum plants were resisting *Striga* infection. Previously, it has been reported that inhibition of haustorial development can take place following antagonism between plant hormones. For instance, Keyes *et al*. (2000) found out that IAA antagonized cytokinins and benzoquinone, both of which favour attachment and haustorium development. Therefore, results obtained in the present study were expected because all the PGPR used are known IAA producers.

Furthermore, all PGPR inoculated plants caused a high number of tubercle deaths when compared with non-inoculated controls. These findings could be attributed to incompatible resistance and abiosis that may have occurred after *Striga* made vascular connection with sorghum. Studies done in the past have identified the endodermis of host roots as a major barrier to parasitic infection in a number of host-parasite associations including sorghum to *S. asiatica* and *S. hermonthica* (Amusan *et al.*, 2008) and rice (*Oryza sativa*). Additionally, vascular resistance to *Striga* infection has been reported previously by Maiti *et al.* (1984) and Haussmann *et al.* (2004) while Neumann *et al.* (1999) and Arnaud *et al.*, (1999) have reported abiosis and incompatibility on resistant sorghum varieties. It is known that

rhizosphere bacteria are capable of producing compounds, which if taken up by plants, can stimulate defence responses against deleterious pathogens (Lazarovits & Nowak, 1997). Moreover, the fact that most plants use similar defence responses to parasitic plant infection as those used in response to fungal and bacteria pathogens (Westwood *et al*., 1998; Joel & Portnoy., 1998; Goldwasser *et al*., 1999), strengthens the argument that defence mechanisms against *Striga* were activated in sorghum by PGPR.

In conclusion, this study presents a partial picture of the interactions among sorghum, *Striga* and PGPR through filter paper, EAGA and root chamber experiments. It has been shown that *Bacillus subtilis* Bsn5 has the potential to inhibiting *Striga* germination and radicle elongation through the action of hydrophilic inhibitor(s). Comparatively, sorghum seedling vigor is better enhanced by *Burkholderia phytofirmans* PsJN than *Bacillus* strains. *Burkholderia phytofirmans* PsJN; *Bacillus subtilis* GBO3 and *B. amyloquafaciens* FZB42 have shown potential in improving sorghum growth in the absence of *Striga* infection during the first one month. All the PGPR used in this study improved sorghum growth in the absence of *Striga* infection but did not cause the same benefits to *Striga*-infected sorghum plants. However, the PGPR did suppress tubercle formation in *Striga.*

## Limitations of study and recommendations for further research

In this study, some methodology limitations were identified. Firstly, the SDS-PAGE method used to purify samples for protein analysis has a lot of chances of small peptides getting lost during the process. So the proteins identified in the samples may not be a true reflection of the proteins present in cell culture supernatants. Secondly, the concentration of plant hormones were not determined after PGPR inoculation which makes it is difficult to conclude whether or not plant hormone concentration depended on the amount of hormones in external growth media. Thirdly, the control culture media (blank 10% LB) which was expected to have the least hormonal concentration, expressed same GA and ABA concentration but with a higher IAA content compared to the PGPR cell culture supernatants. This scenario could be explained by the chemical composition of the samples used for hormonal analysis. While cell culture supernatants were obtained after the bacteria had utilized the food and other chemical resources in the media, 10% LB had all the ingredients intact. That might explain the high binding ability of the control media.

Future research endeavors should focus on conducting more laboratory tests, pot trials under greenhouse conditions and field trials to confirm the efficacy of these strains in addition to investigating mechanisms of growth promotion and *Striga* suppression by the PGPR at different growth stages may be helpful in gaining more understanding on the role of PGPR on sorghum and *Striga* growth. Secondly, since inhibitor(s) has/have been detected in *Bacillus subtilis* Bsn5 bacterial growth medium, it is recommended that future studies should focus on isolating and identifying individual inhibitor(s). There is need to isolate, purify and characterize the inhibitors with special attention on their biological activity on *Striga* radicles. In addition, further screening of the PGPR for phytohormone and ACC deaminase activity among other mechanisms in inoculated plants at different stages of plant growth is necessary. This will help improve our understanding of mechanism(s) responsible for improved sorghum growth. Finally, because of limited understanding on the causes of reduced *Striga* attachment on host roots following PGPR inoculation, further investigations on the role of haustorium initiation inhibitors, interruption of haustorium inducing signals, disorientation of the radical from the host root, structural and chemical barriers at the *Striga* infection points is recommended. Such increased knowledge will be highly useful for the development of PGPR-based, cost-effective bio-herbicides used by smallholder farmers in *Striga*-prone areas of Sub-Saharan Africa

# Summary

Witchweeds (*Striga* sp.) are parasitic weeds of great agricultural significance, parasitizing the roots of their hosts. *Striga*, like all other root parasitic weeds, drain essential organic and inorganic resources from their hosts leading to poor crop development and low yield. In Africa, about 50 million ha in over 30 countries are infested by *Striga* spp. causing grain loss of cereals. Estimated yield losses of maize, sorghum, millets and upland rice are between 30 and 90%. The parasite, therefore, is ranked as the leading biotic constraint to cereal production in the continent.

Plant growth promoting rhizobacteria (PGPR) are promising components for integrated solutions to agro-environmental problems because inoculants possess the capacity to promote crop growth and reduce the population of deleterious microbes in the rhizosphere. Although there are numerous studies on crop growth promotion and biological control of diseases, weeds, nematodes and parasitic weeds using PGPR, little is known about the potential of some *Bacillus subtilis*, *B. amyloliquefaciens* and *Burkholderia phytofirmans* strains in sorghum growth promotion and resistance against *Striga* infection. The main objective of the study was to assess the effect of *B. subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 and *Burkholderia phytofirmans* PsJN on growth promotion of sorghum crop and suppression of *Striga* development, thus providing a basic understanding on the sorghum-PGPR-*Striga* interaction.

This study opens with an elaborate review of the state-of-the-art knowledge on the tripartite interactions between *Striga*, sorghum and different species of PGPR. Prior to this, bipartite relationship between sorghum and *Striga*, PGPR-sorghum and PGPR-*Striga* are reviewed with a focus on understanding *Striga* impact on sorghum, sorghum defence responses to infection, plant growth and disease suppression benefits by PGPR on sorghum, and the effect of PGPR on *Striga* development. Knowledge gaps in both bipartite and tripartite relationships are described, and future research recommendations given. A key recommendation from the review is to conduct experiments under controlled environmental conditions using *Bacillus subtilis, B. amyloliquefaciens* and *Burkhoderia phytofirmans* strains in order to understand their relationship with sorghum and *Striga* at bipartite and tripartite levels.

Petri dish bioassays and root chamber experiments under controlled conditions were conducted at the Institute of Plant Production and Agroecology in the Tropics and

Subtropics, University of Hohenheim between 2012 and 2014. *B. subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 and *Burkholderia phytofirmans* strain PsJN inocula and their corresponding cell culture supernatants were evaluated for their growth promotion potential on sorghum and suppressiveness on *Striga* development. Sorghum root exudates and synthetic stimulant GR24 were used to induce *Striga* seed germination. *Bacillus subtilis* Bsn5 supernatant, which showed the greatest inhibitory activity on *Striga* germination and radicle elongation, was separated by ethyl acetate into lipophilic and hydrophilic phases. The purpose of this extraction was to try and identify the polarity of the inhibitor. Protein composition by mass spectrometry (MS) was also done on the supernatant with a view of establishing the presence of peptides because peptides have been associated with *Orobancheceae* germination and radicle inhibition in previous studies. In addition, determination of plant growth hormones in bacteria supernatants was also conducted using Radio-Immuno-Assay (RIA) in order to relate PGPR hormone production and sorghum growth enhancement.

*Burkholderia phytofirmans* PsJN significantly (<0.05) induced a higher vigor index (VI) on sorghum seedlings (>18,000) compared to other PGPR and control treatments. The lowest VI (7626) was recorded in seeds inoculated with *Bacillus amyloliquefaciens* FZB42. Complete *Striga* germination inhibition (0% germination) occurred in seeds exposed to all PGPR inocula suspended while the highest germination (>60%) occurred in control treatments (10% Luria Bertani (LB) + GR24 and sterile distilled water (SDW) + GR24). The effect of bacterial supernatants on the germination percentage and radicle length of *Striga* seeds was also significantly (<0.05) different among treatments. The least germination (7.4 %) was observed in *Bacillus subtilis* Bsn5 + GR24 while the highest (66 %) was observed in SDW + GR24 control. *Bacillus subtilis* Bsn5 supernatant produced the lowest mean radicle lengths (0.1 mm) while the highest radicle lengths were observed in SDW + GR24 (2.2 mm). Therefore, *Bacillus subtilis* Bsn5 supernatant was selected for further investigation of compounds causing inhibition of *Striga* germination and preventing radicle elongation. The supernatant was separated into hydrophilic and hydrophobic fractions using ethyl acetate. Each fraction was then prepared in 1%, 25%, 50%, 75% and 100% concentrations before being evaluated for their inhibitory activity in *Striga* germination and radicle elongation. The highest germination percentage (63%) and radical length (2.9 mm) was observed in SDW + GR24 control treatment. The ethyl acetate (lipophilic) fraction at both 100% and 1% concentration + GR24 produced a germination

percentage of >40% which was similar to 10% LB + GR24 and ethyl acetate + GR24 controls. There was complete inhibition of *Striga* seed germination after exposure to either *Bacillus subtilis* Bsn5 supernatant + GR24 or 100% hydrophilic fraction of the supernatant + GR24. However, at 25% and 1% concentration + GR24, *Striga* germination percentage increased to 34% and 49%, respectively. Light microscopy examination of *Striga* radicles exposed to *Bacillus subtilis* Bsn5 supernatant + GR24 revealed that stunting of the radicles was due to reduction in cell sizes at the radicle elongation zone. Extended agar gel assays (EAGA) experiments showed a similar trend of results with *B. subtilis* Bsn5 showing the highest inhibitory activity on *Striga* germination and radicle elongation compared to other PGPR and control treatments.

Results from root chamber experiments demonstrated significant ($p<0.05$) differences in biomass production between *Striga*-free and *Striga*-infected sorghum. Total biomass yield in uninoculated *Striga*-free plants was 40% higher than uninoculated *Striga*-infected sorghum plants. *Bacillus amyloliquefaciens* FZB42, *B. subtilis* GBO3 and *Burkholderia phytofirmans* PsJN inoculated *Striga*-free sorghum showed a 75%; 142% and 158% increase in biomass yield, respectively, compared to uninoculated *Striga*-free sorghum. There were no significant differences in biomass yield observed between inoculated and uninoculated *Striga*-infected plants. All PGPR supernatants and 10% LB media showed production of phytohormones cytokinin, IAA, GAs and ABA. Cytokinin content in PGPR supernatants was significantly (>0.05) higher than blank 10% LB control media. There was a significant negative correlation ($r= -0.96$) between IAA and cytokinins. However, there was no significant positive correlation between any phytohormone and sorghum plant height, SPAD values, biomass production, *Striga* germination, attachment and tubercle death.

Finally, this study shows that *Bacillus subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 and *Burkholderia phytofirmans* PsJN might accelerate sorghum growth and suppress key stages of *Striga* development under laboratory conditions. Greenhouse and field experiments are recommended to better understand these interactions under natural conditions where other biotic and abiotic factors come into play. These findings could contribute to a better understanding of sorghum and beneficial bacteria interactions and provide novel information of the long-term effects of a PGPR on sorghum development, opening new avenues for *Striga* control and sustainable, ecofriendly sorghum production.

# Zusammenfassung

Pflanzen der Gattung *Striga* sind parasitäre, die Wurzeln ihres Wirtes befallende Unkräuter mit einer großen landwirtschaftlichen Bedeutung. *Striga* entzieht ihrem Wirt essentielle organische und anorganische Ressourcen. Dies führt zu einem verschlechterten Wachstum und zu geringeren Erträgen bei der Wirtspflanze. Über 50 Millionen Hektar landwirtschaftlicher Nutzfläche in über 30 Ländern Afrikas sind von *Striga* befallen. Dies führt zu Ertragsverlusten bei Mais, Sorghum, Hirse und Reis von geschätzten 30 bis 90 Prozent, je nach Ackerfrucht und Befallsstärke. Deswegen wird *Striga* auch als maßgebliches biotisches Hemmnis bei der Getreideproduktion des Kontinents gewertet.

Ein vielversprechender Bestandteil für eine integrative Lösung zur Kontrolle von *Striga* könnten pflanzenwachstumsfördernde Bakterien (plant growth promoting rhizobacteria, PGPR) sein, die im Allgemeinen im Wurzelraum verschiedenster Pflanzen zu finden sind. Bodenimpfungen mit diesen Bakterien zeigten Wirksamkeit bei der Unterstützung des Wachstums von Feldfrüchten sowie eine Reduktion der Populationen von schädlichen Mikroorganismen in der Rhizosphäre. Obwohl sich schon eine Vielzahl von Studien mit der Unterstützung des Pflanzenwachstums und der biologischen Kontrolle von Krankheiten, Unkräutern, Nematoden und Parasiten durch PGPR befasst haben ist relativ wenig über das Potential einiger Bakterienstämme (*Bacillus subtilis, B. amyloliquefaciens* und *Burkholderia phytofirmans*) bei der Unterstützung des Wachstums von Sorghum und der Resistenz gegen *Striga*- Infektionen bekannt. Das vorranginge Ziel der hier vorgestellten Studie war es die Auswirkungen von *B. subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 und dem *Burkholderia phytofirmans* Stamm PsJN auf das Wachstum von Sorghum und die Entwicklung von *Striga* zu erfassen, um damit ein grundlegendes Verständnis für die Interaktionen zwischen Sorghum-*Striga*-PGPR zu erhalten.

Zu Beginn dieser Arbeit steht eine ausführliche Literaturübersicht zum aktuellen Stand des Wissens auf dem Gebiet der dreiteiligen Interaktionen zwischen *Striga,* Sorghum und verschiedenen Arten von PGPR. Zuerst werden dafür die zweiteiligen Interaktionen zwischen Sorghum und *Striga*, PGPR und Sorghum sowie zwischen PGPR und *Striga* erörtert. Dies soll einen Einblick darüber verschaffen wie *Striga* Sorghum beeinflusst und wie die Verteidigungsmechanismen von Sorghum gegen eine solche Interaktion aussehen. Gleichzeitig wird die Unterstützung diskutiert, die PGPR bei Pflanzenwachstum und bei

der Unterdrückung von Krankheiten in Sorghum leisten kann. Abschließend wird beleuchtet wie sich PGPR auf die Entwicklung von *Striga* auswirken. Sowohl für die zweiteiligen als auch für die dreiteiligen Interaktionen werden Wissenslücken aufgezeigt und Vorschläge für zukünftige Forschungsansätze gegeben. Eine der grundlegenden Empfehlungen dieser Übersicht ist es Experimente unter kontrollierten Umweltbedingungen durchzuführen, die es erlauben Rückschlüsse auf die Wechelwirkungen zwischen den oben genannten PGPR Stämmen und Sorghum sowie *Striga* bei zweiteiliger und dreiteiliger Interaktion zu schließen.

Zwischen 2012 und 2014 wurden am Institut für Pflanzenproduktion und Agrarökologie der Tropen und Subtropen an der Universität Hohenheim sowohl Labore Experimente als auch versuche in Wurzelgefäßen unter kontrollierten Bedingungen durchgeführt. Inokulate (und die zugehörigen Überstände der Zellkulturen) von *B. subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 und *Burkholderia phytofirmans* Stamm PsJN wurden auf ihr Potential als Wachsstumspromotoren in Sorghum und ihrer Wirkung auf die Entwicklung von *Striga* hin bewertet. Sowohl Wurzelsekrete von Sorghum als auch das synthetische Keimstimulanz GR24 wurden benutzt, um eine Keimung von *Striga* Samen zu induzieren. Während der Versuche zeigten die Überstände der *Bacillus subtilis* Bsn5 Kulturen den größten inhibitorischen Effekt sowohl auf die Keimung von *Striga* als auch auf die Verlängerung der Keimwurzel. Deswegen wurde der Überstand durch Hinzugabe von Essigsäureethylester in eine hydrophobe und eine hydrophile Phase gespalten, um die Polarität dieser Inhibierung aufzeigen zu können. Die Proteinzusammensetzung des Überstandes wurde mit Hilfe eines Massenspektrometers (MS) untersucht um das Vorhandensein von Peptiden abschätzen zu können. Peptide wurden in früheren Studien mit der Keimung von verschiedenen Orobanchearten, insbesondere im Hinblick auf die Verkürzung der Keimwurzel, in Verbindung gebracht. Mit Hilfe eines Radioimmunassays (RIA) wurden Pflanzenwachstumshormone im Überstand bestimmt, um die Produktion dieser Hormone durch PGPR mit den Auswirkungen auf die Verbesserung des Wachstums von Sorghum in Verbindung setzen zu können.

Durch die Behandlung mit *Burkholderia phytofirmans* PsJN konnte ein signifikant ($<0.05$) höherer Vitalitäts Index (VI > 18000) an Sorghumkeimlingen erreicht werden als in den Kontrollbehandlungen oder in Behandlungen mit anderen PGPR. Den niedrigsten VI erreichten Keimlinge in der Behandlung mit *Bacillus amyloliquefaciens* FZB42 (VI 7626). Komplette Keimungsunterdrückung von *Striga* wurde bei allen PGPR Inokulaten erreicht,

wenn die Samen in 10 prozentiger Luria Bertani Lösung suspendiert wurden. Die höchsten Keimprozente (>60%) wurden in den zwei Kontrollversuchen (10% Luria Bertani (LB) + GR 24, sowie in sterilem destillierten Wasser (SDW) + GR24) beobachtet.

Ebenso konnte ein signifikanter Effekt (<0.05) des bakteriellen Überstandes auf die Länge der Keimwurzel von *Striga* bei den verschiedenen Behandlungen festgestellt werden. Die niedrigsten Keimprozente (7.4%) wurden bei der Behandlung mit *Bacillus subtilis* Bsn5 + GR 24 beobachtet, die höchsten (66%) bei der Kontrollbehandlung mit SDW + GR24. Die Überstände aus den *Bacillus subtilis* Kulturen ergaben die niedrigste Durchschnittslänge bei Keimwurzeln (0.1 mm), während die höchste durchschnittliche Länge (2.2 mm) bei SDW + GR24 beobachtet wurde. Aus diesem Grund wurde der Überstand von *Bacillus subtilis* Bsn5 für die weiterführenden Untersuchungen herangezogen, die Einblicke zu den ursächlichen Bestandteilen der Unterdrückung der Keimung von *Striga* sowie der Verhinderung der Elongation der Keimwurzel liefern sollten.

Der Überstand wurde mit Hilfe von Essigsäureethylester in eine hydrophile und eine hydrophobe Fraktion aufgetrennt. Jede Fraktion wurde dann zu Konzentrationen von 1%, 25%, 50%, 75% und 100% aufbereitet und auf ihre inhibitorische Aktivität auf die Keimung von *Striga* und die Elongation der Keimwurzel getestet. Die höchsten Keimprozente (63%) und Keimwurzellänge (2.9 mm) wurde bei den Kontrollbehandlungen mit SDW + GR24 beobachtet. Beide Essigsäureethylester Fraktionen von 100% und 1%, jeweils + GR24, zeigten Keimprozente von >40%, vergleichbar zu den Kontrollen mit 10% LB und Essigsäureethylester, auch jeweils + GR24. Eine komplette Inhibierung der Keimung von *Striga* Samen zeigte sich bei der Exposition sowohl zum gesamten Überstand von *Bacillus subtilis* Bsn5 + GR24 oder zur 100 % hydrophilen Fraktion des Überstandes (+ GR24). Allerdings zeigte sich auch eine Erhöhung der Keimprozente von *Striga* bei den Konzentrationen von 25% und 1% + GR24 (jeweils auf 34% und 49%). Lichtmikroskopische Untersuchungen der Keimwurzel zeigten das bei der Behandlung mit dem Überstand der *Bacillus subtilis* Kulturen + GR24 eine Reduktion der Zellgröße im Bereich der Elongationszone ausschlaggebend für die kürzere Keimwurzel ist. Ähnliche Ergebnisse zeigten sich auch während Extended Agar Gel Assays (EAGA), wo *Bacillus subtilis* Bsn5 die höchste inhibitorische Aktivität auf die Keimung und Keimwurzelelongation von *Striga*, verglichen zu anderen PGPR und den Kontrollversuchen, hatte.

Signifikante Unterschiede ($p<0.05$) bei der Biomasseproduktion konnten bei den Versuchen in Wurzelgefäßen zwischen *Striga* freien und *Striga* infizierten Sorghumpflanzen. Die Gesamtbiomasse nicht beimpfter Striga freier sorghumpflanzen war 40% höher als bei gleich behandelten mit *Striga* befallenen Pflanzen. Unter Abwesenheit von *Striga* zeigten Behandlungen mit *Bacillus amyloliquefaciens* FZB42, *B. subtilis* GBO3 und *Burkholderia phytofirmans* PsJN eine Zunahme der Biomasse um jeweils 75, 142 und 158%, verglichen mit den nicht beimpften Pflanzen. Bei *Striga* befallenen Pflanzen konnte kein signifikanter Unterschied in der Biomasseproduktion zwischen den Behandlungen mit PGPR oder gänzlich ohne festgestellt werden.

Es konnte die *in-vitro* Produktion von Phytohormonen (Cytokinin, Auxin, Abscisinsäure und Gibberellinsäure) sowohl in den Überständen der PGPR als auch im 10% LB Medium festgestellt werden. Der Gehalt an Cytokinin war in den PGPR Überständen signifikant ($>0.05$) höher als in den Kontrollen mit 10% LB Medium. Es konnte eine signifikante negative Korrelation ($r= -0.96$) zwischen Auxin und Cytokinin festgestellt werden. Allerdings gab es keine signifikante positive Korrelation zwischen einem der Phytohormone und Faktoren wie Sorghum Wuchshöhe, SPAD Werten und Biomasse Produktion oder der Keimung, Anheftung oder Absterben der Keimwurzel von *Striga.* Abschließend zeigt diese Studie auf, das unter Laborbedingungen, Behandlungen mit *Bacillus subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 und *Burkholderia phytofirmans* PsJN die Entwicklung von Sorghum beschleunigen und Schlüsselstadien bei der *Striga* Entwicklung unterdrücken können. Um diese Erkenntnisse weiter unter natürlichen Bedingungen verstehen zu können werden Gewächshaus- und Freilandversuche empfohlen, da hier weitere biotische und abiotische Faktoren ins Spiel kommen. Die hier vorgestellten Ergebnisse tragen zu einem besseren Verständnis der komplexen Interaktionen zwischen Sorghum und nutzbringenden Mikroorganismen bei. Gleichzeitig konnten neue Erkenntnisse zu den mittelfristigen Auswirkungen von PGPR auf die Entwicklung von Sorghum gefunden werden, die neue Möglichkeiten für die Bekämpfung von *Striga* in einer nachhaltigen, umweltfreundlichen Sorghumproduktion aufzeigen.

# Curriculum Vitae

Lenard Gichana Mounde, MSc.

**Personal information**

| | |
|---|---|
| Date/Place of birth | 4th July 1974/ Kisii- Kenya |
| Nationality | Kenyan |
| Gender | Male |
| Marital status | Married |
| Current address | Ebinger Weg 7<br>70567 Stuttgart, Germany<br>Tel. (Office): +49 711 459 26302<br>(Mobile): +4915210517552<br>Email: moundel05@yahoo.com<br>LG.Mounde@uni-hohenheim.de |
| Permanent address | Pwani University,<br>Mombasa-Malindi Highway<br>P.O Box 195-80108 Kilifi,<br>Kenya<br>Tel: +254707972983 |
| Profession | Horticulturalist/ Plant Pathologist |

**Education and training**

| | |
|---|---|
| 1st October 2011 – 9th Dec. 2014 | Doctoral fellow. Institute of Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim, Germany.<br>Title of thesis: *Understanding the Role of Plant Growth Promoting Bacteria on Sorghum Growth and Biotic Suppression of Striga Infestation.* |
| Aug. 2006 – Sept.2009 | Graduate student. Department of Horticulture. Jomo Kenyatta University of Agriculture and Technology, Kenya.<br>Title of thesis: *Distribution, Characterization and In-vitro Chemical Screening of Phytophthora Species Causing Citrus gummosis in Kenya.* |

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|---|---|
| April 1993 – Dec. 1996 | Undergraduate student. Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology, Kenya.<br>Title of thesis: *Effect of Artificial Chilling and GA3 Application on Flowering of Alstromeria flowers*<br>Qualification: Bsc. Horticulture. |
| Jan.1988 – Dec.1991 | High school student. Maseno National School, Kenya |
| Jan.1980 – Nov.1987 | Primary school pupil. Esani Primary School, Kenya. |

**Working experience**

| | |
|---|---|
| June 2008 – to date | Assistant Lecturer<br>Pwani University,<br>P.O Box 195-80108 Kilifi,<br>Kenya |
| Jan. 2005 – July 2008 | Agricultural Training officer<br>Kilifi Institute of Agriculture, P.O. Box 195 Kilifi, Kenya |
| Nov. 1997 – Dec. 2004 | Agricultural Training officer<br>Animal Health and Industry Training Institute (AHITI), Ndomba. P.O Box 32 Kirinyaga, Kenya |
| May 1997 – Oct. 1997 | Teaching instructor.<br>Omoyo Secondary school, P.O Box 35 Gesima, Kenya. |

**Seminars, workshops and conferences**

| | |
|---|---|
| $10^{th}$ – $15^{th}$ June 2013 | Leadership Development workshop in Windeck-Rosbach, Germany |
| $3^{rd}$ – $8^{th}$ June 2013 | Ethics in Food Security and Development Research and Action workshop in Berlin, Germany. |
| $12^{th}$ – $17^{th}$ Mar. 2013 | Working within Political Contexts workshop in Berlin, Germany. |
| $2^{nd}$ – $4^{th}$ Dec. 2011 | Learning Intercultural Competence workshop.<br>Windeck- Rosbach, Germany |
| $8^{th}$ – $11^{th}$ Dec. 2008 | $8^{th}$ Workshop on Sustainable Horticultural Production in the Tropics. Masinde Muliro University of Science and Technology, Kakamega, Kenya.<br>Title of paper: *Occurrence and distribution of citrus gummosis in Kenya* |
| $2^{nd}$ – $5^{th}$ Dec. 2009 | $9^{th}$ Workshop on Sustainable Horticultural Production in the Tropics. Arusha, Tanzania.<br>Title of paper: *Morphological characterization of Phytophthora species causing citrus gummosis in Keny*a. |

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|---|---|
| 8th–11th Dec. 2010 | 10th Workshop on Sustainable Horticultural Production in the Tropics. Jomo Kenyatta Univeristy of Agriculture and Technology, Thika, Kenya.<br>Title of paper: *Efficacy of selected fungicides in in-vitro control of Phytophthora gummosis*. |

**Personal skills and competences**

| | |
|---|---|
| Languages | Fluent in spoken and written English, Kiswahili and Kisii<br>Basic knowledge in German |
| Social skills and competences | Good personal commitment, efficiency and drive for results<br>Self driven and analytical<br>Innovative and creative |
| Organisational skills and competences | Good communication, presentation and interpersonal skills.<br>Ability to work effectively and respect for diversity<br>Good management and negotiation skills<br>Ability to work under pressure and meet set deadlines |
| Technical skills and competences | Ability to prepare reports and publications |
| Computer skills and competences | Good in Ms Office computing skills<br>Proficient in ICT skills |
| Other skills and competences | Athletics, soccer and gardening. |

**Professional affliations**

1. Council for Tropical and Subtropical Research (ATSAF e.V)

2. Horticultural Association of Kenya (HAK).

**Publications**

**Mounde L.G.,** Ateka, E.M., Kihurani, A.W., Wasilwa, L. and Thuranira, E.G. (2012). Morphological characterization and identification of *Phytophthora* Species causing citrus gummosis in Kenya. *African Journal of Food, Agriculture, Nutrition and Development*, 12(7), 7072-7087.

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Stuttgart, 15th September, 2014 Lenard Gichana Mounde

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